Prevalence of avian coccidiosis in Peshawar Khyber Pakhtunkhwa, Pakistan: its control using medicinal herbs and iron nanoparticles of *Verbena officinalis* including *in-silico* molecular docking

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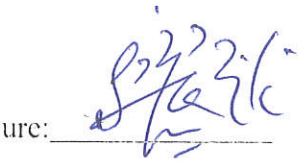
Student / Author Signature: 

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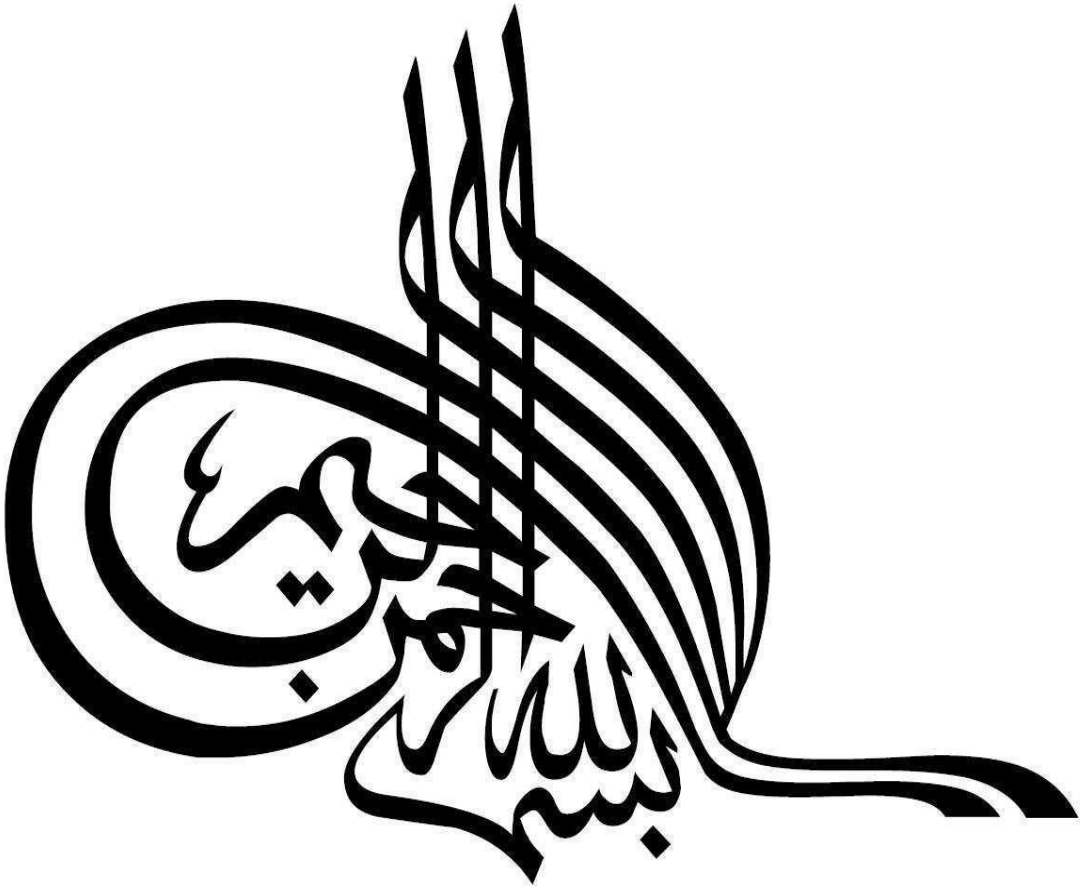

In the name of Allah,
the Most Beneficent,
the Most Merciful

Dedicated to Papa, Ammi, Mama & Nimra

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List of Abbreviations

µg/mg	Microgram per milligram
µl	Microliter
2D	Two dimension
3D	Three dimension
A	Angstrom
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Amino transaminase
Au	Arbitrary unit
B	Baluchistan
BEC	Bio ethics committee
BS	Binding Score
C	Chloroform
Ca	Calcium
CDPK	Calcium dependent protein kinase
CL	Class limit
cm^{-1}	Wave number
Coef	Coefficient
COVID	Corona Virus Infectious Disease
DB	Total number of dead birds
DB	Total number of dead chicks in a cage
Df	Degree of freedom
DPI	Day Post Infection
DPT	Day Post Treatment
E^+	*Eimeria* Positive Cases
EA	Ethyl Acetate
EDTA	Ethylene diamine tetra acetic acid
EtCDPK3	*Eimeria tenella* Calcium dependent protein kinase
eV	Electron volt
FCG	Feed consumed per group
FCR	Feed Conversion Ratio
FT-IR	Fourier transmission Infrared Spectroscopy
FeNP	Iron oxide nanoparticle
GC-MS	Gas Chromatography and Mass Spectroscopy
GDP	Gross domestic production
GSH	Glutathione
IBD	Infectious bursal disease
IC_{50}	50% Inhibitory concentration
IC_{90}	90% Inhibitory concentration
IN	Initial number of birds in a cage
LC_{50}	50% lethal concentration
Long	Longitude

M%	Percent mortality
m/z	Charge to Mass ratio
M	Methanolic extract
MF	Molecular formula
MFW	Mean final weight
MIW	Mean initial weight
MW	Molecular weight
MWG	Mean Weight Gain
N	Total number of birds
NARC	National Agricultural Research Center
ND	Newcastle disease
OPG	Oocysts per gram
P%	Percent cases
PARC	Pakistan Agricultural Research Center
PDB	Protein Drug Bank
PVC	Private Veterinary Clinics
RT	Retention time
S%	Survival percentage
S	Suspected cases
SAM	S-Adenosylmethionine
SEM	Scanning electron microscopy
SOD	Superoxide dismutase
SPI	Sporulation inhibition
SQX	Sulfaquinoxaline
TLC	Thin Layer Chromatography
TR	Total Residue
U/l	Units per liter
U/mg	Unit per milligram
UV VIS	Ultra Voilet Visible Spectroscopy
VIF	Variance inflation factor
VRI	Veterinary Research Institute
VSG	Variant surface glycoprotein
WDB	Weight gain of dead birds
WSB	Weight gain of surviving birds
XRD	X-rays diffraction
Λ	Lambda

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GENERAL ABSTRACT

Coccidiosis is an economically important disease that leads to $ 3 billion in annual loss globally, caused by several *Eimeria* species. It is prevalent throughout Pakistan, according to the poultry research institute's annual reports, but only a small number of cases were published online. The current thesis can be summarized into four study schemes.

In the first scheme of the current research, the prevalence of coccidiosis was reported for the first time in Peshawar, Pakistan. The postmortem data of coccidiosis effected farms and coccidiosis-associated risk factor data were statistically analyzed using the chi square test, binary logistic regression, and graphically presented in maps using arc-GIS to evaluate the impact of *Eimeria* spp. on poultry. The village, age, and year-wise distribution of coccidiosis in relation to humidity and temperature revealed 25.99% of the towns with cocci-positive farms, with many cases in between 16 to 30-day-old broiler chicks, i.e., 64.49%. The postmortem reports revealed 23.37% of farms had severe modes of infection. The survey of cocci-associated risk factors in 2020, concludes that vaccination ($P = 0.008$), disinfection ($P = 0.04$), footwear ($P = 0.02$), flooring ($P = 0.03$), and feeder hygiene ($P = 0.03$) have significantly affected the coccidial emergence.

In the second scheme of the current study, five pharmaceutically active herbs were used against avian coccidiosis *in-vitro* for sporulation inhibition (SPI) assay to calculate the inhibitory concentration (IC_{50}) and revealed *V. officinalis & P. glabrum* with maximum SPI % of 81.04% and 72.47% and minimum IC_{50} of 0.14, and 12 mg/ml, respectively. The same plants were selected for *in-vivo* anticoccidial activity in *E. tenella*-infected chicks. The mean weight gain, oocyst count, diarrhea, biochemical tests, hematology, and histopathology of all groups were analyzed using the Tukey test. The active herb was characterized by antioxidant assay, FT-IR, UV-Vis spectroscopy, and GC-MS analysis. The GC-MS' identified phyto-compounds of *V. officinalis* were docked with S-Adenosyl methionine (SAM) synthetase, revealed the best binding of strychane, 1-acetyl-20a-hydroxy-16-methylene with the lowest binding score (-6.4 Kcal/mol), suggested their anticoccidial potential in poultry.

As in the previous study scheme, *V. officinalis* was found effective against *E. tenella.* For this reason, various fractions of *V. officinalis* were tested for SPI and anticoccidial

assay. In the first step, fractions F1 to F9 were tested in the SPI, with F1, F3, F5, and F8 exhibiting good maximal (> 70%) inhibitory activity at 40 mg/ml concentration and the lowest IC_{50} of 16.83, 8.59, 10.65, and 10.32 mg/ml, respectively. The active fractions were *in-vivo* tested for trail of anticoccidial activity with F3 and F8 fraction as highly effective in case of higher concentration with significant mean weight gain at 14^{th} post-treatment, respectively, with FCR < 1. All the biological parameters were analyzed using the Tukey test ($P < 0.05$). The active functional groups and purity and composition of active fractions was characterized via UV-Vis and FT-IR spectroscopy, and GC-MS. The ligands of active fractions were docked against S-adenosyl methionine synthetase, resulting in the best binding with a-sitosterol, 1,2 benzenedicarboxylic acid, mono(2 -ethylhexyl) ester, and 3,9-epoxypregnane-11,14,18-triol-20-one, 16-cyano-3-methoxy-, 11-acetate with lowest binding energy, which suggests its best compatibility with the target protein and can be used as an inhibitory substance against avian coccidiosis to alter the methylation of DNA, which will interfere with the gene expression of parasite.

In the fourth study scheme, the iron oxide nanoparticles of *Verbena officinalis* were synthesized and characterized to confirm their purity, size, and crystalline structure using XRD, FT-IR, and SEM. The *in-vivo* antiprotozoal iron nanoparticle of *V. officinalis* was found to be effective against *E. tenella*-infected chicks. Which revealed significant weight gain in iron nanoparticle-treated groups with maximum weight gain at 15 mg/ml dosage with the lowest FCR of 1.0 ($P < 0.05$), least oocyst count (0.53×10^3/g), and cured from diarrhea. The means of all studied parameters were analyzed statistically using ANOVA (tukey test) to confirm the significant difference ($P < 0.05$) between the treated and untreated groups. The higher concentration of nanoparticle treated chicks showed normal hematological, biochemical, and histological profile.

CHAPTER: 01

GENERAL INTRODUCTION

Coccidiosis is one of the most economically important diseases that causes billions of dollars economic loss to both developed and underdeveloped countries (Mesa *et al*., 2021). The causative agent of coccidiosis is an apicomplexan parasite belong to the genus *Eimeria* (Lan *et al*., 2017). It create economic loss to the poultry farmers at the grass root level and in a larger image, it has a negative impact on the state's budget. Therefore, to overcome this disease, scientists need to backtrack the emergence of coccidiosis in the poultry sector with the help of some important parameters.

The human population largely depends on poultry products for food. Consequently, this field is growing rapidly to meet demand (Uddin *et al*., 2014). There is a severe need for chicks in the present era, both in fresh and frozen form (Hamid *et al*., 2018). Poultry meat is one of the basic food items that fulfill global protein requirements (Fontana *et al*., 2015). In developing countries, the poultry sector has been proven helpful in alleviating poverty by addressing malnutrition in the form of meat and eggs (FAO, 2011). Domestic poultry accounts for more than 50% of the gross poultry farming (Mohammed *et al*., 2021).

1.1.1. Role of Poultry Sector

A recent study revealed that more than 30% of south Asian and African residents were suffering from malnutrition due to low protein consumption, whereas chicks' egg and meat has sufficient amount of protein, i.e. 1/3 of meat content (Farrell, 2013) and other essential nutrients such as vitamins and minerals that make it more valuable, it also fulfill the carbohydrates and lipids needs if one can't get sufficient amount from green food (Marangoni *et al*., 2015). To eliminate the poverty and other social problems regarding diet deficiency, poultry industry is growing at a fast rate (Mottet & Tempio, 2017). The chicks' meat is a rich source of long chain (n3 series) fatty acid; a structural component of human nervous system. Furthermore, it is helpful in relieving cardiovascular diseases and also acts as antidepressant (Mancinelli *et al*., 2022). The world's population is increasing at a high rate and is estimated to be 10 billion by 2050, the food security will be primary problem then, to manage it, and the modern poultry industry has taken several steps by adoption of technology (Kleyn & Ciacciariello, 2021). The backyard poultry practice helps the lower class of society in uplifting them directly, plus it makes the cheapest poultry products available to rest of society at the doorstep (Kumar *et al*., 2021).

1.1.2 Poultry Industry in Pakistan

In Pakistan, the poultry industry plays an important role by contributing 1.3% shares to the national GDP (Hussain *et al*., 2015), providing 1 million tons of meat to the population annually (Rashid *et al*., 2019). The poultry sector fulfills the protein requirement in the developing world by producing 60 million metric tons of meat annually (Gueye, 2005). It provides an edible proteinaceous diet for the consumers. Beyond this basic need it also helps the nation by providing income resources. Being an agricultural country, Pakistan's government enhances this industry through different research institutes like VRI's, NARC and PARC. To encourage the livestock sector, the government waved off the sales and income taxes on this particular sector (Hussain *et al*., 2015).

1.1.3. Overview of World Poultry Industry

The poultry industry accounts for 78% of total global egg production, with China being the leading nation, whereas India, Brazil and China have increased the exports of poultry meat by 217%, 112% and 67% respectively (Scanes *et al*., 2007). The scenario of current climate change has affected the food cycle of many countries, leading to food starvation. To prevent this, different regions have increased their production by a certain ratio, i.e. Asia (68.83%), Australia and New Zealand (68.49%), Africa (67.73%), Europe (65.82%), and America (47.67%) (Castaneda & Gonzalez, 2015). Nahed and colleagues (2022) reported that the top seven European countries spend £7.0 to £13 billion on the treatment of avian coccidiosis. Safe production of poultry feed is under constant threat from coccidian parasites due to its ability to tolerate environmental stress and antibiotic resistance (Blake & Tomley, 2014). The overall direct and indirect losses due to coccidiosis in a single city of Indonesia were $ 3,371,549,813,512 in 2019 (Pawestri *et al*., 2020).

1.1.4. Impact of Universal Factors on Poultry

The poultry industry was hugely affected by the COVID pandemic. Due to the tight lockdown schedule, the shipment of feed, vaccines, feeders and other poultry relevant stuff was turned slowdown, due to which farmers faced many difficulties and eventually lead to severe loss (Attia *et al*, 2022). Secondly, during COVID, the common people faced the fear of food insecurity because the available farm owners were unable to deliver the chicks' meat and eggs to society (Debata *et al*., 2020). The chicks need a proper environment both

inside and outside the farm. The farmer can control the inside environment but was unable to make the outer environment suitable for birds. Therefore the phenomenon of climate change across the globe has badly affected poultry production and almost led the sector to huge economic loss, whereas the birds face severe environmental stress that makes them susceptible to the secondary infections (Ahaotu *et al*., 2019).

1.2. Avian Coccidiosis

This is one of the major issues faced by the poultry industry worldwide, affecting large economies (Price, 2012). Although much research has already been done in this area, it is still of major concern and has adverse effects on intensive poultry performance (Gussem, 2007). It can cause both clinical and subclinical infection. The clinical infection does appear with severe clinical signs and ends in mortality, whereas the subclinical infection is a moderate type of infection which cannot be seen apparently but lead to weight loss and poor FCR (Silva *et al*., 2021; Walaa *et al*., 2018). It is endemic in tropical and subtropical regions throughout the world (Abebe, & Gugsa, 2018).

Table 1.1: Phylogenetic classification of *E. tenella* (Ruggiero *et al*., 2015)

Kingdom	Chromista
Phylum	Miozoa
Infraphylum	Apicomplexa
Class	Conoidasida
Order	Eucoccidiorida
Family	Eimeridae
Genus	*Eimeria*
Species	*E. tenella*

1.2.1. Economic Loss Due to Avian Coccidiosis

In terms of economy, the poultry sector spent $ 3 billion on controling and prevention of coccidiosis (Mohsin *et al*., 2021), with 51.38% mortality (Mohammed *et al*., 2015) worldwide. Only China spent approximately $ 73-88 million on the production of anticoccidial drugs (Zhang *et al*., 2013). The United States of America spent $ 70 million on management strategies to increase feed consumption efficiency annually (Ruff, 1999). Pakistan, as a poor economy and security state, spent very little budget ($ 45,405) on the prevention and therapeutic measures of avian coccidiosis (Rashid *et al*., 2019).

1.2.2. Clinical signs of Avian Coccidiosis

The apparent clinical signs of coccidial infection are blood in feces, low weight gain by birds as its capability of reabsorption of nutrients is disturbed, in addition the factor of high morbidity signals towards the occurrence of infection (Hector *et al*., 2020). The microscopic examination of fecal material revealed the presence of *Eimeria* oocysts (Bachaya *et al*., 2012), isolated by salt floatation method for further research (Levine, 1985).

1.2.3. Etiology

The protozoan causing avian coccidiosis belongs to the genus *Eimeria* (Price, 2012), found in specific loci of the digestive tract (Nahed *et al*., 2022). It has almost 1700 species, infecting diverse types of host including birds, terrestrial animals and aquatic life (Clark *et al*., 2017; Osorio *et al*., 2020; Silva *et al*., 2021). Though this genus is species rich, but the interesting thing is each *Eimeria* sp is host and site specific as well (Clark *et al*., 2017).

Nine different species of *Eimeria* infect chicks (*Gallus gallus*), at different intestinal loci, such as *E. tenella* infecting cecum, *E. acervulina* resides in the duodenum, *E. maxima* and *E. necatrix* cause infection in the upper middle part of intestine, and *E. brunetti* causes eimeriosis by attacking the rectum of host (Hernandez *et al*., 2020). *E. tenella, E. acervulina, E. brunetti, E. maxima and E. necatrix* are deadly pathogenic (Bindari *et al*., 2021), *E. mivati* and *E. mitis* are causing mild infection (Schwarz *et al*., 2009; Williams *et al*., 2009; Price, 2012; Castaneda and Gonzalez, 2015), whereas *E. praecox, E. hagani* and *E. mivati* are lesser pathogenic (Fadunsin *et al*., 2019; Das., 2021; Bawm *et al*., 2021). Several biologists claimed seven species were involved in the chicks' pathogenicity by killing them or decreasing the productivity which results in nutrient malabsorption (Acharya & Acharya, 2017; Iouknane *et al*., 2018; Nonkookhetkhong and Chalalai, 2021). As for the morphology of *E. tenella* concerned, it's oocyst is 22.80 µm long and 19.65 µm wide on average (Kostadinovic *et al*., 2019).

1.2.4. Infection Duration of *Eimeria* Species

Just like the variation in pathogenic sites (Iouknane *et al*., 2018), each *Eimeria* sp has a different lifecycle period as *E. tenella* and *E. maxima* took 7 days to complete the

lifecycle, *E. acervulina*, *E. brunetti* and *E. necatrix* have prepatent period of 5, 6 and 8 days respectively (Bussiere *et al*., 2018), so *Eimeria* spp. can be traced from both factors i.e. site of infection and lifecycle period.

1.2.5. Transmission of Coccidiosis

The *Eimeria* oocysts spread with contaminated feed, water, flooring material, Coccidiosis transmission through feco-oral route by ingestion of sporulated oocysts (Abebe and Gugsa, 2018). In this regard, the coccidian parasite is distantly related to other apicomplexans and exhibits an endogenous and exogenous mode of life cycle (Blake and Tomley, 2014). This infection comes as a result of two types of consequences, i.e. clinical infection which ends in a morbid situation whereas the chronic type of infection leads to mortality (Ali *et al*., 2014).

1.2.6. Climate Favoring Coccidiosis

The climatic conditions influence the chicks' life. In this regard, the factor of temperature greatly affect the spread of the *Eimeria* population, it can bear harsh summer weather, but as the temperature limits exceed, it will eventually lead to the oocyst rupture (Attree *et al*., 2021). Further, the humid weather favors the sporulation process in *Eimeria* spp. (Yunus *et al*., 2009). Usually, avian coccidiosis emerges in all seasons but is highly prevalent in the humid season (Sultana *et al*., 2009) and that is why it is mostly reported in the rainy season (Akintunde and Adeoti, 2014).

1.2.7. Diagnosis of Avian Coccidiosis

The diagnostic techniques play an important role in the curing process, and are as old as the emergence of coccidiosis. In old age, when the farmers faced economic loss in the form of low weight gain, they report it in the form of physical conditions of birds, bloodshed in fecal material. The infection of coccidiosis can be confirmed by microscopic examination of stool, which is a simple technique that diagnoses the emergence of coccidiosis in birds with less expense but it may sometimes confused with other parasites in same sample (Jamil *et al*., 2016). The microscopic study involves certain characteristics such as developmental stages during the sporulation period, and the presence or lack of micropyle (Iouknane *et al*., 2018). To avoid the confusion, the veterinarian adopted precise

and advance technique of DNA extraction and polymerase chain reaction (PCR) that clears all the ambiguities (Fatoba and Adeleke, 2018), PCR is not only confirming the genus but specific species within the genus using specific markers (primers). The commonly conserved reference genes that are generally used for the identification, e.g. ITS-1 (Gyorke *et al*., 2013). Furthermore, the multi-species infections caused by different *Eimeria* species at different intestinal sites can be diagnosed by studying the life cycle and parasite forms using specific primer via PCR (Conway and McKenzie, 2007).

1.2.8. Co-Infections & Low Immunity

The coccidiosis emerges in all age chicks but the low immunity birds are the most vulnerable to it especially early few weeks after hatching (Chookyinox *et al*., 2009). During coccidiosis the birds become weakened and susceptible to any kind of viral, bacterial and fungal co-infection, which again damages poultry farm production (Gerhold, 2015).

1.2.9. Immunogenicity of *Eimeria*

The site of infection within the gastrointestinal tract determines the degree of pathogenicity of *Eimeria* spp. and immune response, particularly the immunological status of intestinal tissues. In the colon, the parasite causes destruction of epithelial tissue, which eventually is excreted in the form of bloody diarrhea, reduced nutrient reabsorption capacity and weight gain (Mohsin *et al*., 2021). The toxicity level for all seven *Eimeria* spp. is different; like *E. tenella*, which develops deep in the intestinal mucosa of cecum and cause extensive damage characterized by physical lesions, while others are less destructive but severely affect the growth of birds. Clinical signs of avian coccidiosis range from acute to asymptomatic form (Yang *et al*., 2019).

1.2.10. Pathogenicity

The *Eimeria* spp. has specialized organelles such microneme, rhouptries and dense granules that produce certain complexes which help in its survival and propagation within the host cell. Actually, these products lead to various pathogenic reactions that harm the host (Li *et al*., 2020). Other than epithelial lining of intestine, *Eimeria* spp. invades numerous types of immune cells like macrophages, lymphocytes and erythrocytes (Sharma *et al*., 2013). The *Eimeria* invasion involves four main steps. Initially it attaches to the host

cell and produces various complexes that help in penetrating to the intestinal cells. The parasites' movement is facilitated by the junction and before entry it creates a protective parasite-phorous vacuole around itself to cope the hosts' defense system (Li *et al*., 2020).

1.2.11. Pathogenecity Variation

All the *Eimeria* species have different types of infection depending upon the locus, such as *E. brunetti* multiplies in the ileum and colon and damages the tissues. *Eimeria acervulina* attacks the duodenum and upper jejunum, whereas *E. maxima* and *E. necatrix* infect the midgut. *E. necatrix's* oocysts matures in ilium, whereas *E. tenella* is found in ceca. The factors like high host density, physical contact with contaminated fecal material contribute to the spread of infection and morbidity. When *Eimeria* parasite invades the intestine and damages the epithelium, so the host may therefore experience diarrhea, malabsorption, and inadequate weight gain (Bozkurt *et al*., 2013).

1.2.12. Host Immunity against *Eimeria* Infection

The sub-microtitre dose of various coccidial strains has negligible impact on birds, leading to the development of natural immunity against parasites (Haq *et al*., 2011). The inflammation due to sporozoites penetration in the intestinal epithelium triggers the host immune response, leading to extensive infiltration of macrophages, granulocytes, and lymphocytes into the lamina propria. Macrophages regulate the CD^{+4} T cells to stimulate some efficient lymphocytic immune responses. The lymphocytes and macrophages are sources of cytokine synthesis that drive immune responses (Elmusharaf and Beynen, 2007). In this regard, *E. maxima* is highly immunogenic, so only a few oocysts are required to fully induce immunity (Dalloul and Lillehoj, 2006).

1.2.13. Prevalence of Avian Coccidiosis

Avian coccidiosis is prevalent in all parts of the world because it exists where poultry farming is common (Yan *et al*., 2021). Previous studies in South America, Asia, Europe, and the Middle East suggest that more than half of the world's bird flocks were thought to be infected with *Eimeria* spp. The mortality rate varies by country, such as Romania is reported with 92% mortality, Argentina (88.41%), Jordan (78%), Pakistan (71.9%), Ethiopia (70.9%), Turkey (54.3%), Nigeria (52.9%), and India (39.6%)

(Mohammed and Sunday, 2015). The prevalence also depends on several relevant factors such as species, susceptible animals, and the environment (Zhang *et al*., 2013). The prevalence of subclinical coccidiosis at the farm level is 75%, with *E. acervulina* infection (Shirzad *et al*., 2011). A Romanian study reported 92% cases in broiler chicks (Gyorke *et al.*, 2013). *E. tenella*, as the most pathogenic species, causes significant damage with 100% mortality (Usman *et al*., 2011). The global prevalence of coccidiosis is summarized in Table 1.2.

Table 1.2: Overview of coccidiosis prevalence globally

Location	Samples investigated	Positivity (%)	Sample Positivity for *Eimeria* spp.									Reference
			E. tenella	*E. necatrix*	*E. maxima*	*E. mitis*	*E. brunetti*	*E. praecox*	*E. mivati*	*E. acervulina*	*Mix* spp.	
Afghanistan	2792	20.95	+	+	+		+			+		Shahraki *et al*., 2018
Bangladesh	13	100	+	+	+	+	+	+		+		Alam *et al*., 2021
China	318	97	+	+		+						Geng *et al*., 2020
China	171	87.7	+	+	+	+	+			+		Huang *et al*., 2017
Colombia	245	96.3	+		+					+	+	Mesa *et al*., 2021
India	674	30.12	+	+	+	+	+	+	+	+	+	Das, 2021
India	490	86.2									+	Singh *et al*., 2021
Iran	200	64	+	+	+					+	+	Hadipour *et al*., 2021
Iran	200	64	+	+	+					+		Jabrani *et al*., 2019
Iran	130	21.53	+	+	+					+	+	Yakhchali, and Fakhri, 2017
Java	699	25.04	+	+	+	+	+	+		+		Hamid *et al*., 2018
Mayanmar	122	33.6			+			+			+	Bawm *et al*., 2021
Nigeria	597	32.83									+	Fadunsin *et al*., 2019
Nigeria	120	49									+	Auta *et al*., 2021
Poland	20	100	+	+	+		+		+	+		Balicka *et al*., 2021
Portugal	350	65	+	+	+	+	+	+	+	+		Lozano *et al*., 2021
Zhejiang	310	30.7	+	+	+	+				+		Lan *et al*., 2017

1.2.13.1. Prevalence of Avian Coccidiosis in Pakistan

In the history of Pakistan's poultry industry, only little data about coccidiosis is documented, although its emergence is very frequent. The chronological study of avian coccidiosis in Pakistan is illustrated in table 1.3 and graphically it is presented in figure 1.1. The occurrence of coccidiosis was recorded or published during the period of years 2003 to 2020 and ranged from 19 to 67%. Further, most of the documented reports were published from the province of Punjab, Pakistan, whereas very little data has been reported from Khyber Pakhtunkhwa, Baluchistan and Kashmir.

The data did not showed a single case reported or published from the province of Sindh, Pakistan. In most of these studies, mixed species infection was found during microscopic examination and molecular study of the sample collected in the form of fecal material or gut contents of chick, i.e. it might contain two or more species responsible for coccidiosis or in some studies, and the prevalence is reported only, on the basis of physical clinical signs. In this study, the species of *Eimeria* reported was *E. tenella*, *E. maxima*, *E. mitis*, *E. acervulina*, *E. brunette*, *E. necatrix* and *E. praecox* from different study areas, whereas *E. tenella* is prevalent throughout the studies across Pakistan.

All these seven species are common throughout the world for avian coccidiosis. *E. hagani* and *E. mivati* are also found in other regions of the world in poultry (Soulsby, 1986) but the data (Table 1.3) from Pakistan concludes the absence of these two species. The limitation of these studies is that the disease prevalence was assessed on single farms and the percent of the infected birds were considered as the prevalence of coccidiosis, whereas in the current study, the whole flocks throughout the district were assessed for a period of three years and then the cumulative data were presented to showed the prevalence of the coccidiosis.

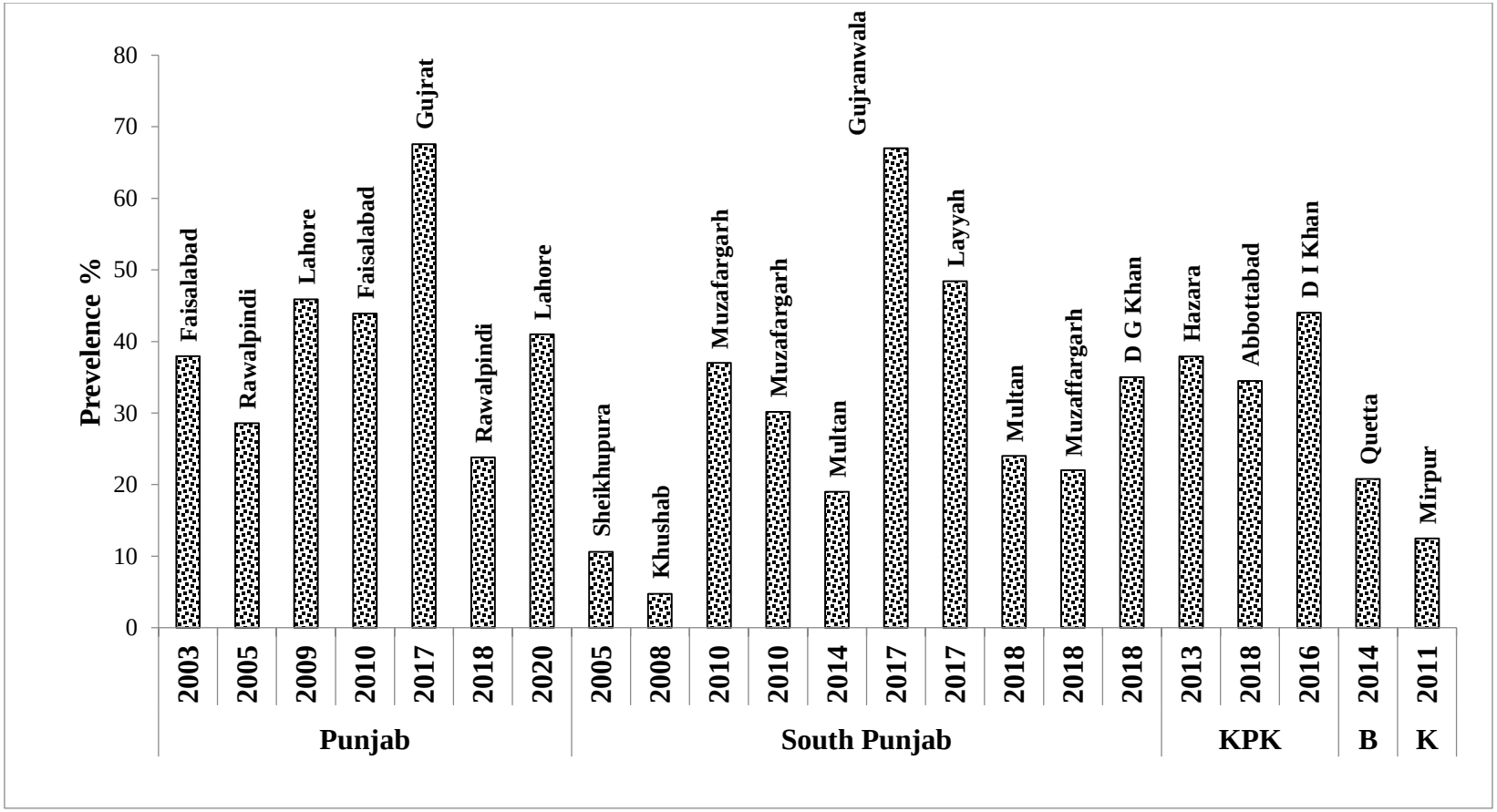


Figure 1.1: Overview of coccidiosis Prevalence in various regions of Pakistan

Table 1.3: Chronological overview on epidemiology of avian coccidiosis across Pakistan

S .no	Location	Year	Sample	Abundance (%)	*Eimeria* spp.	Reference
01	Faisalabad	2003	Gut	37.95	*E. tenella, E. acervulina, E. necatrix, E maxima, E brunette, E. mitis* & *E. praecox*	Ayaz *et al*., 2003
02	Rawalpindi	2005	Gut	28.57	*E. maxima, E. tenella, E. mitis* & *E. necatrix*	Khan *et al*., 2006
03	Sheikhupura	2005	Feces	10.6	-	Mustafa and Ali , 2005
04	Khushab	2008	Gut	4.75	-	Abbas *et al*., 2015
05	Lahore	2009	Feces	45.9	-	Sultana *et al*., 2009
06	Muzafargarh	2010	Gut	37	*E. maxima, E tenella, E. mitis* & *E. necatrix*	Bachaya *et al*., 2012
07	Muzafargarh	2010	Feces	30.18	*E. tenella, E. maxima, E. mitis* & *E. necatrix*	Bachaya *et al*., 2015
08	Faisalabad	2010	Gut	43.89	*E. tenella, E. maxima, E. acervulina* & *E. necatrix*	Awais *et al*., 2012
09	Mirpur	2011	Feces	12.49	*E. tenella* & *E. maxima*	Shamim *et al*., 2015
10	Hazara	2013	Gut	37.91	-	Amin *et al*., 2014
11	Multan	2014	Feces	19	-	Rashid *et al*., 2019
12	Quetta	2014	Gut	20.8	-	Ali *et al*., 2014
13	D.I Khan	2016	Feces	44	*E. tenella, E. maxima, E. mitis* & *E. necatrix*	Jamil *et al*., 2016
14	Gujranwala	2017	Feces	67	-	Akram *et al*., 2018
15	Gujrat	2017	Feces, Gut	67.57	*E. tenella, E. acervulina, E. necatrix, E. mitis, E maxima, E brunette* & *E. praecox*	Naveed and Faryal, 2019
16	Layyah	2017	Feces, Gut	48.4	-	Mahboob *et al*., 2017
17	Rawalpindi	2018	Feces	23.8	-	Yousaf *et al*., 2018
18	Abbottabad	2018	Feces	34.48	*E. acervulina, E. maxima, E. mitis & E. tenella*	Sohail *et al*., 2019
19	Multan	2018	Feces, Gut	24	*E. tenella & E. acervulina*	Khan, 2019
20	Muzafargarh	2018	Feces, Gut	22	*E. tenella & E. acervulina*	Khan, 2019
21	D.G Khan	2018	Feces, Gut	35	*E. tenella & E. acervulina*	Khan, 2019
22	Lahore	2020	Gut	41	*E. tenella, E. maxima, E. mitis, E. acervulina, E. brunette* & *E. necatrix*	Ullah *et al*., 2020

1.2.14. Life Cycle of *Eimeria*

This *Eimeria* parasite have a complex monoxenous life cycle i.e. it infect one host (Silva *et al*., 2021; Girard *et al*., 2016), with feco-oral transmission (Bozkurt *et al*., 2013). The poultry farms capacity in Pakistan are ranging from less than 1000 birds (mini farm) to greater than or equal to multiple 10,000 chicks (Yunus *et al*., 2008). There are almost 8000 genes in its genome, to control its life processes (Reid *et al*., 2014).

In the external environment the non sporulated oocyst act as non-infective stage, but when it meets the suitable conditions it become infective and survive for long period without host. The overall life cycle is complex and takes 4 to 6 days till oocyst release. Although the basic life cycle is the same for all species, there are species-specific differences in various host, place of development, prepatent and patent periods, and virulence (Bozkurt *et al*., 2013). The pre-infectious period often lasts 4 to 5 days after infection. After infection, maximum oocyst formation occurs after 6 to 9 days (Allen and Fetterer, 2002).

1. The non infectious, unsporulated oocysts exists in the environment for long time as it survive the the harsh environment (Kiani *et al*., 2007).
2. The oocyst undergoes sporulation when it meet suitable conditions of humidity, temperature and oxygen and become infective in estimated time of 24 hours (Dubey, 2019, Burrell *et al*., 2020; Reperant *et al*., 2021).
3. The life cycle of *Eimeria* begins when healthy or susceptible chick consumes sporulated oocysts with feed or water (Genova and Knoll, 2021). And undergoes schizogony and gametogony (Castaneda and Gonzalez, 2015). Each oocyst releases four sporocysts.
4. The sporocyst releases two sporozoites, which are motile and invades the gut cells (Osorio *et al*., 2020). The excystation is assisted by trypsin, bile, and CO_2.
5. These free sporozoites attack the intestinal cells and reside their by developing parasitoporous vacuoles (Castaneda and Gonzalez, 2015) this stage now called as tropozoite.
6. The trpozoites undergo asexual reproduction (schizogony) to form schizonts (Dubey *et al*., 2017).

7. The schizonts go through cytoplasmic division to form merozoites I. At the 5th day of cycle merozoite I become mature and raised to merozoite II.
8. The merozoites may follow the reinfective cycle and again convert into tropozoite.
9. The merozoites II matures into male and female gametes (Silva *et al*., 2021).
10. The male and female gametes undergo gametogony to form oocysts (Reperant *et al*., 2021).
11. The mature oocysts are shed into the environment with feces, which are non infcctive but become infective when meets the suitable condtions and the cycle repeats (Wiedmer *et al*., 2020).

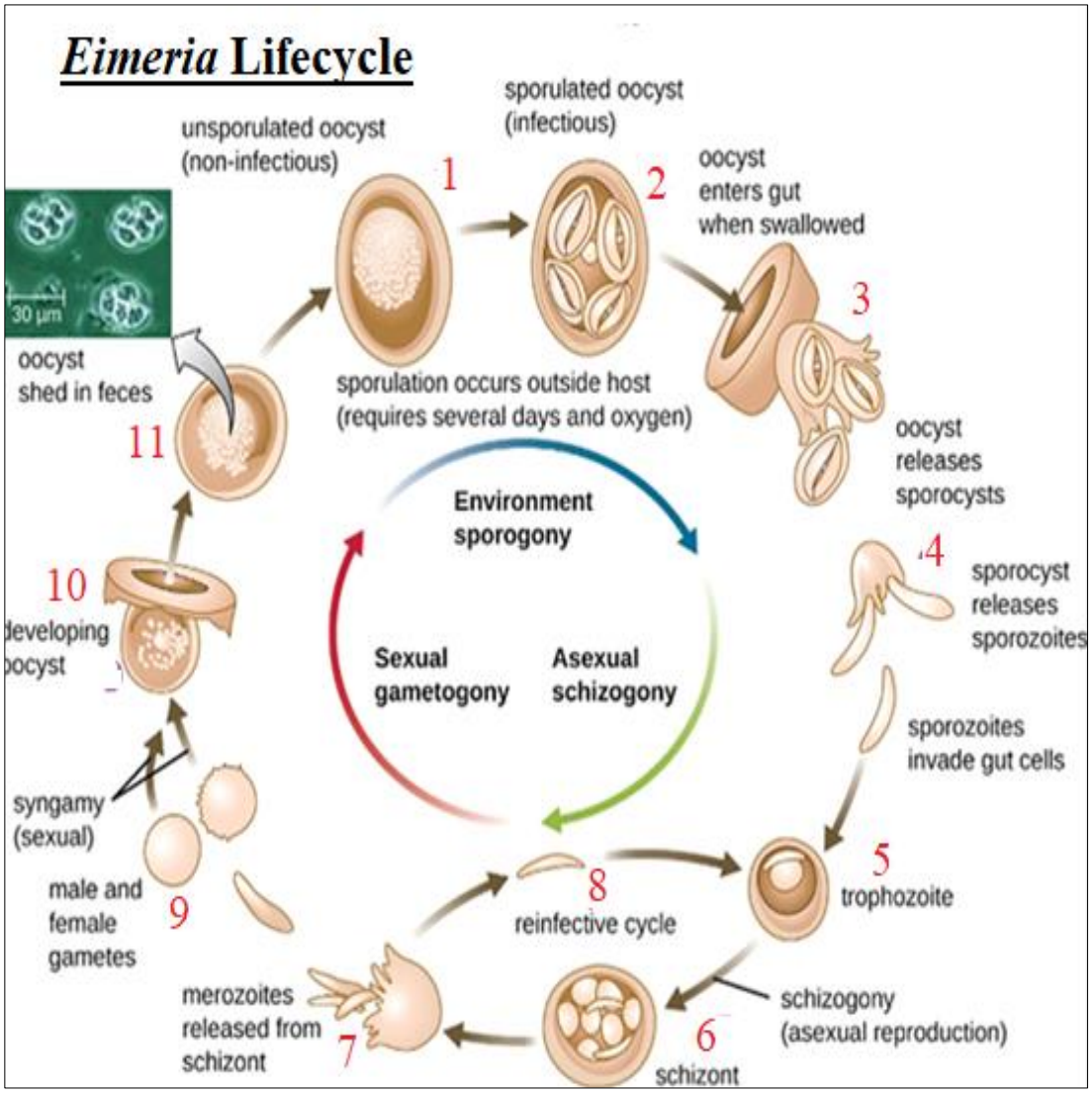


Figure 1.2: The life cycle of *Eimeria* spp.

1.2.16. Loses due to Coccidiosis

Coccidiosis leads to severe economic loss because of high consumption of feed in the case of infected birds, with no proper outcome, reflected in the form of poor FCR value (Nonkookhetkhong and Chalalai, 2021). The annual loss due to avian coccidiosis is almost three billion US dollars in the form of production losses and preventive measures (Yan *et al*., 2021). The survey conducted by the scientific team at the Royal Veterinary College, UK), reported that avian coccidiosis leads to 10 billion pounds annual loss (Attree *et al*., 2021).

1.3. Management Factors of Coccidiosis

The poultry farm's management parameters include geographic location of farm (Abbas *et al*., 2011), seasonal variations, age (Rashid *et al*., 2019) and density of chicks, sanitation of farms, ventilation, biosafety measures, disinfection of farms, vaccine and antibiotic usage and some other relevant parameters (Abbas *et al*., 2015). Coccidiosis is mostly common in rural areas because of poor hygiene, high density, birds' age, poor vaccination, lack of awareness (Khawaja *et al*., 2005) and poor socio-economic level (Islam *et al*., 2012).

1.4. Drug Resistance Factors

1.4.1. Birds' Age

The age factors often contribute to resistance to the immune system. The young birds with fewer parasites are generally considered less susceptible to coccidiosis, and even if parasites infect chicks, the damage is not severe often (Vermeulen *et al*., 2001).

1.4.2. Parasite Genetic Factors

The host genetic makeup influences the parasite dissemination, lesion severity, and weight gain. Even the genomes of different breeds influence drug resistance rather than the inheritance of specific resistance genes (Vermeulen *et al*., 2001). As coccidians multiply quickly, and one sporulated oocyst can produce >2.1 million sporocysts, causing the emergence of resistance in a short time period (Sundar *et al*., 2017). *Eimeria* spp. exhibits huge diversity in its genetic makeup, which is the possible reason for its varying pathogenic consequences. The complete genomic analysis of apicomplexan parasite may help in its

control, as there is difference in the genome which will ultimately reflect in the form of VSG (Variant surface glycoprotein), and such different VSGs will help in its narrow escape or drug resistance (Alam *et al*., 2021).

1.4.3. Operational Factors

The on-farm operational factors such as drug composition, serving, persistence in the host, kinetics clearance, application technique, therapeutic dosage, geographical use of treatments, under-dosing, use of poor quality and long half-life drugs (Sundar *et al*., 2017). The alternative coccidiosis control methods have a significant impact on both bird health and parasite drug resistance (Price, 2012). If all the operational features are carefully handled according to standard protocol, it would undoubtedly help birds to escape coccidiosis, otherwise the parasite would develop resistance and remain predominant in the population (Sundar *et al*., 2017).

1.5. Concurrent Infections

If operational factors are not properly managed, birds will become weakened and co-infected with some pathogens such as viruses (Reo Virus, Marek Virus, Newcastle Virus, and infectious Bronchitis Virus) and enteriobacteria, and the consequences of such infections become worse by complicated clinical signs. The severity and spread of an infectious disease is influenced by management factors such as: poor hygiene, entry of other animal farms without adoption of safety measures, the use of in-feed medicines, rearing of the new flock on the same contaminated farm without applying disinfection measures (Vermeulen *et al*., 2001).

1.6. Strategies to Control Avian Coccidiosis

Research of the last few decades has addressed the preventive techniques and treatment strategies based on the formulation of coccidiostat and anticoccidial drugs and the development of proper animal husbandry techniques (Acharya and Acharya, 2017). In this aspect, the conventional and integrated control measures have been developed with the passage of time.

1.6.1. Conventional Control Measures

The conventional control measures include anticoccidial drugs to treat coccidial infections. These agents are mainly classified as coccidiostat and coccidiocidal (Castaneda and Gonzalez, 2015). To prevent or arrest the oocysts in static condition, coccidiostats are fed to birds with water or feed with no noticeable clinical signs. In some cases, discontinuing coccidiostat treatment may allow the parasite to continue its life cycle and disseminate into the environment. Therefore, anticoccidial drugs are prescribed to avoid such situations (McDougald *et al*., 2008). Instead of targeting the infective and resistant oocyst stages, medications are being investigated that can readily target both the sexual and asexual (intra-host) stages of the *Eimeria* parasites (Castaneda and Gonzalez, 2015).The drugs developed against coccidiosis essentially aim to target specific signaling pathways within the parasite physiology that affect its metabolism. The chemosynthetic drugs that can interfere with metabolic pathways and alter a parasite's metabolism are halofuginone, clopidol, decoquinate, and amprolium. Several other anticoccidial drugs are commercially available in the form of dietary supplements, e.g. sulfaquinoxaline (Muthamilselvan *et al*., 2016).

The other class of anticoccidial drugs is ionopores that block ion transport channels and impair the osmotic balance of the parasite (Khater *et al*., 2020). The polyether ionophores (monensin, lasalocid, salinomycin and narasin) disrupt the osmotic equilibrium of the parasite (Dowling, 1992; Tewari and Maharana, 2011). However, an imbalance in the ionic gradient of the cell membrane occurs, resulting in energy depletion due to mitochondrial damage. Theses ionopore residues are also responsible for cardio-toxicity, muscle degeneration, and neuronal disorders (Noack *et al*., 2019).

The synthetic medicines in current use have harmful side effects on chicks as their ingredients remain in the chicks' meat as residues and affect human health (Sundar *et al*., 2017). However, the low dose of these drugs can lead to drug resistance (Acharya and Acharya, 2017).

1.6.2. Integrated Control Program

Due to the side effects of chemical-based drugs, scientists have turned to alternative parasite control strategies such as herbal products, vaccines, pre- and probiotics, and immune-modulators (Abbas *et al*., 2012).

1.6.2.1. Immuno-Prophylaxis

Consecutive 2 to 3 time infection of chicks with low dose can strengthen their immune system and develop strong immunity. Farmers may intentionally expose chicks to non-infectious parasitic doses in order to develop protective immunity. These kinds of experiments serve as the basis for commercial vaccines. The non-infectious doses can be served in two forms: live-attenuated and live-non-attenuated (Price, 2012). Vaccination can be the best alternative to all kinds of coccidiocidal drugs to prevent infection, but paying high cost makes it available only in large-scale facilities (Chapman *et al*., 2002).

1.6.2.2. Biosecurity and Management Practices

Biosecurity and biosafety are the key factors that can play a vital role in the management and control of any kind of parasitic or viral infection. In this scenario, in addition to preventive measures (such as chemical treatments or vaccines), the biosecurity programs and management techniques also play an important role in controlling avian coccidiosis (Graat *et al*., 1998; Hafez, 2008). The following procedures are commonly used to reduce and prevent the spread of oocysts.

1.6.2.2.1. Standard Stock Density

The commercial poultry farms are more susceptible to parasitic (coccidiosis) outbreaks than free-range poultry practice due to their high stocking densities and intensive farming practices. The standard stocking density for single bird is 0.08 square meters (Chapman, 2014) and exceeding this rule may result in failure leading to severe infections. For ease, small business owners are advised to maintain a stocking density of 1 ft^2 per chick (Drouin and Toux, 2000; Elson, 2010).

1.6.2.2.2. Intensive Farming

One of the main reasons for infection transmission is birds' lifestyle, especially the intensive way of farming is adopted in the present era, but in all three types of farming, it is only way in which the birds are more vulnerable to any kind of infection (Yousaf *et al*., 2018), beside this the farm location, lacking knowledge regarding biosafety and improper medication, bad internal environment (littering, humidity, temperature, ventilation) become the reason of parasitic infection in birds (Carrisosa *et al*., 2021). The inappropriate

feeding routine makes the birds weak and become susceptible to severe types of infection or serving feed in contaminated feeders increases the chance of coccidiosis (Attree *et al.*, 2021).

1.6.2.2.3. Poultry Farm Hygiene

The clean and fresh environment promotes the growth of chicks with significant weight gain and good FCR, so regular litter changes avoid contaminated feces. The feeders and water tanks should be cleaned and disinfected with sunlight, heat, or spray to prevent parasite infestation. The litter should be changed between flocks. Otherwise, the wet litter can contain parasitic oocysts, which can eventually infect the next flock. It has been reported that the new bedding probably contains certain types of bacteria that can digest oocysts (Chapman, 2014; Drouin and Toux, 2000).

1.6.3. Natural Alternatives for Coccidiosis Control

1.6.3.1. Acid based drugs

The acids have been reported to have antiprotozoal and antibacterial activity due to their low pH. Many acids such as hydrochloric acid, acetic acid, butyric acid, anacardic acid, and formic acid help in coccidial suppression (Zaman *et al*., 2012), but are toxic to the host. The acid in digestive system aids in the breakdown, digestion of food and nutrient reabsorption, and improves poultry performance in terms of weight gain and immunity. However, overdose of these acidic formulations can lead to morbidity (Owings *et al*., 1990).

1.6.3.2. Vaccination

The approach of controling coccidiosis through vaccination is effective, but complicated by cost factors and multiple *Eimeria* species infection make it more difficult. However, attenuated vaccines somehow elicit an immune response and may be used as an alternative vaccine. However, given the high cost of production, this seems out of reach for farmers (Mohsin *et al*., 2021).

1.6.3.3. Probiotics and Prebiotics

Dietary supplements are supplied with live beneficial microorganisms known as probiotics, which birds use as food to improve their gut micro-biome, whereas prebiotics

interfere with microbial activity in the colon. It is a non-digestible nutritive factor used as a growth promoter (Fuller, 1989). Prebiotics and probiotics reduce the enteric pathogens and promote the immune responses in poultry (Muthamilselvan *et al*., 2016).

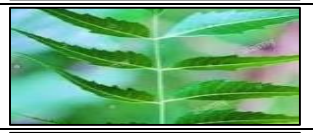
1.6.3.4. Essential Oils

The *Eimeria* infection suppresses the nitric oxide production by macrophages, which is required to trigger an innate immune response against the parasite. In this scenario, the essential oil prevents the parasite from suppressing nitric oxide (Abbas *et al*., 2012). The fusion of the two main ingredients of essential oil, carvacrol and thymol, plays an important role in the fight against coccidiosis (Silva *et al*., 2009). The antioxidant potential of essential oils helps in relieving the inflammation caused by *Eimeria* infection. The essential oils from coconut, oregano, cloves, peppermint and many other plants have been used to combat avian coccidiosis (Hafeez *et al*., 2020; Mohsin *et al*., 2021).

1.6.3.5. Medicinal Plants and Derivatives

The herbal extracts of medicinal plants with antioxidant and antiprotozoal properties restore balance and improve the health of coccidiosis-infected chicks (Allen *et al*., 1998). The neem fruits containing salinomycin significantly reduced the incidence of coccidiosis in terms of low oocyst burden and did not adversely affect the host health (Nidaullah *et al*., 2010). Due to the shortcomings and side effects of synthetic drugs, compounds in the composition of edible flora have re-emerged as alternative products to combat coccidiosis (Yang *et al*., 2019). The plant extracts used against coccidiosis are mentioned in table 1.4 whereas the plant-derived products are mentioned in table 1.5.

Table 1.4: Plant extracts used against different *Eimeria* species to control coccidiosis

S. no	Plant	Family	Solvent	*Eimeria* spp.	Host	Image	Reference
01	*Artemisia annua*	Asteraceae	Essential oil	*E. tenella*	Chicks		Dragan *et al*., 2014
02	*Azadirachta indica*	Meliaceae	Ethanol	*Eimeria* spp.	Goat		Affian *et al*., 2017
03	*Salvadora persica*	Salvadoraceae	Methanol	*E. papillata*	Worm		Dkhil *et al*., 2019
04	*Areca catechu*	Arecaceae	CO_2	*E. tenella*	Chicks		Wang *et al*., 2018
05	*Allium sativum*	Amaryllidaceae	Saline	*E. papillata*	Mice		Dkhil *et al*., 2011
06	*Moringa olifiera*	Moringaceae	Ethanol	*Eimeria* spp.	Chicks		Sahar *et al*., 2018
07	*Punica granatum*	Lythraceae	Hexane, Methanol, water	*E. tenella*	Chicks		Ahad *et al*., 2018
08	*Matricaria chamomilla*	Asteraceae	Essential oil	*E. bateri*	Quails		Ahmadov *et al*., 2016
09	*Thymus serpyllum*	Lamiaceae	Essential oil	*E. schachdagica*	Ducks		Ahmadov *et al*., 2016

10	*Camellia sinensis*	Theaceae	Powder from	*Eimeria* spp.	Chicks		Abbas *et al*., 2017
11	*Moringa olifiera*	Moringaceae	Ethanol	*Eimeria* spp.	Chicks		El Banna *et al*., 2016
12	*Butyrospermum paradoxum*	Sapotaceae	Water	*Eimeria* spp.	Chicks		Nwosu *et al*., 2011
13	*Pinus radiate*	Pinaceae	Methanol	*Eimeria* spp.	Chicks		Abbas *et al*., 2017
14	*Trachyspermum ammi*	Apiaceae	Methanol	*Eimeria* spp.	Chicks		Abbas *et al*., 2019
15	Palm	Arecaceae	Water	*E. papillata*	Mice		Metwaly *et al*., 2015
16	*Acacia concinna*	Fabaceae	Ether	*E. acervulina, E. Maxima, E. tenella*	Chicks		Hernandez *et al*., 2019
17	*Artemesia annua*	Asteraceae	Water	*E. tenella*	Chicks		Wiedosari and Wardhana, 2018
18	*Saccharum officinarum*	Poaceae	Methanol	*E. tenella, E. mitis, E. necatrix, E. brunette*	Chicks		Abbas *et al*., 2015
19	*Nectaroscordum tripedale*	Alliaceae	Ethanol	*E. tenella*	Chicks		Habibi *et al*., 2016

20	*Ageratum conyzoides*	Asteraceae	Ethanol	*E. tenella*	Chicks		Arlette *et al*., 2019
21	*Vernonia amygdalina*	Asteraceae	Ethanol	*E. tenella*	Chicks		Arlette *et al*., 2019
22	*Carica papaya*	Caricaceae	Water	*E. tenella*	Chicks		Dakpogan *et al*., 2018
23	*Salvadora persica*	Salvadoraceae	Methanol	*E. paillata*	Mice		Thagfan *et al*., 2017
24	*Cnidium monnieri*	Apiaceae	Water	*E. tenella*	Chicks		Song *et al*., 2020
25	*Taraxacum mongolicum*	Asteraceae	Water	*E. tenella*	Chicks		Song *et al*., 2020
26	*Galla rhois*	Anacardiaceae	Methanol	*E. tenella*	Chicks		Lee *et al*., 2012
27	*Morinda lucida*	Rubiaceae	Acetone	*E. tenella*	Chicks		Fadunsin and Ademola, 2014
28	*Ruta pinnata*	Rutaceae	Methanol	*E. ninakohlyakimovae*	Caprine spp.		Lopez *et al*., 2019
29	*Artemisia absinthium*	Asteraceae	Methanol	*E. tenella*	*In-vitro*		Ahmadov *et al*., 2019

30	*Pentaclethra macrophylla*	Asteraceae	Methanol	*E. magna, E. flavescens, E. stiedae, E. intestinalis*	Rabbit		Cedric *et al*., 2018
31	*Vernonia amygdalina*	Asteraceae	Nil	*Eimeria* spp.	Goat		Adediran *et al*., 2014
32	*Azadirachta indica*	Meliaceae	Ethanol	*Eimeria* spp.	Chicks		Kostadinovic and Lević, 2018
33	*Artemisia annua*	Asteraceae	Ethanol	*Eimeria* spp.	Chicks		Kostadinovic and Lević, 2018
34	*Brucea javanica*	Simaroubaceae	Ethanol	*E. tenella*	Chicks		Lan *et al*., 2016
35	*Cinnamomum verum*	Lauraceae	Powder form	*E. tenella*	Chicks		Qaid *et al*., 2021
36	*Tulbaghia violacea*	Amaryllidaceae	Acetone	*Eimeria* spp.	Chicks		Naidoo *et al*., 2008
37	*Echinacea purpurea*	Asteraceae	Ethanol	*E. acervulina*	Chicks		Orengo *et al*., 2012
38	*Boesenbergia pandurata*	Zingiberaceae	Essential oil	*E. tenella*	*In-vitro*		Jitviriyanon *et al*., 2016
39	*Ocimum basilicum*	Lamiaceae	Essential oil	*E. tenella*	*In-vitro*		Jitviriyanon *et al*., 2016

40	Grapefruit	Rutaceae	Ethanol	*Eimeria* spp.	Lamb		Fonseca *et al*., 2016
42	*Rosmarinus officinalis*	Lamiaceae	------	*Eimeria* spp.	*In-vitro*		Lahlou *et al*., 2021
43	*Thymus vulgaris*	Lamiaceae	------	*Eimeria* spp.	*In-vitro*		Lahlou *et al*., 2021
44	*Ageratum conyzoides*	Asteraceae	Ethanol	*E. tenella*	*In-vitro*		Nweze and Obiwulu, 2009
45	*Berberis lycium* Royle	Berberidaceae	Methanol + dichloromethane	*Eimeria* spp.	Chicks		Malik *et al*., 2016
46	*Artemisia vestita*	Asteraceae	Methanol	*E. tenella*	Chicks		Ahad *et al*., 2017
47	*Astragalus membranaceus*	Fabaceae	Nil	*E. papillata*	Mice		Tawab *et al*., 2020
48	*Artemisia annua*	Asteraceae	Nil	*E. tenella*	Chicks		Hady and Zaki, 2012
49	*Artemisia annua*	Asteraceae	Water	*Eimeria* spp.	Chicks		Kostadinovic *et al*., 2019
50	*Acacia concinna*	Fabaceae	Ether	*E.cervulina, E. maxima, E. tenella*	Chicks		Hernandez *et al*., 2019

51	*Allium Sativum*	Amaryllidaceae	Water	*E. tenella*	*In-vitro*		Udo and Abba, 2018
52	*Camellia sinensis*	Theaceae	Methanol	*Eimeria* spp.	Chicks		Zhang *et al*., 2020
53	*Rosmarinus officinalis*	Lamiaceae	Water	*Eimeria* spp.	*In-vitro*		Aouadi *et al*., 2021
54	*Trachyspermum ammi*	Apiaceae	Crude form	*E. tenella, E. necatrix, E. brunetii, E. mitis* and *E. maxima*	Chicks		Abbas *et al*., 2019
55	*Musa paradisiaca*	Musaceae	Methanol	*E. tenella*	Chicks		Anosa and Okoro, 2011
56	*Artemisia sieberi*	Asteraceae	Ethanol	*Eimeria* spp.	Chicks		Arab *et al*., 2006
57	*Allium Sativum*	Amaryllidaceae	Water	*Eimeria* spp.	Chicks		El-Khtam *et al*., 2014
58	*Nectaroscordum tripedale*	Amaryllidaceae	Ethanol	*E. tenella*	*In-vitro* & Chicks		Habibi *et al*., 2016
59	*Azadirachta indica*	Meliaceae	Nil	*Eimeria* spp.	Ram		Jack *et al*., 2020
60	*Garcinia kola*	Clusiaceae	Methanol	*E. tenella*	Chicks		Shetshak *et al*., 2021

61	*Bidens pilosa*	Asteraceae	Water	*Eimeria* spp.	Chicks		Chang *et al*., 2016
62	*Dichroa febrifuga*	Asteraceae	Chloroform	*E. tenella*	Chicks		Zhang *et al*., 2012
63	*Curcuma longa*	Zingiberaceae	Powder form	*E. tenella*	Chicks		Abbas *et al*., 2010
64	*Piper sarmentosum*	Piperaceae	CO_2	*E. tenella*	Chicks		Memon *et al*., 2021

Table 1.5: Review of the botanical derivatives and synthetic compounds used against coccidiosis

S. No	Compound	Solvent	*Eimeria* spp.	Host	Reference
01	Allicin	Water	*E. tenella*	*In-vitro*	Alnassan *et al*., 2015
02	Cinnamon essential oil	Water	*E. stiedae*	Rabbit	Sorour *et al*., 2018
03	Clove essential oil	Water	*E. stiedae*	Rabbit	Sorour *et al*., 2018
04	Triglycerides	Nil	*E. bovis, E. zuernii*	Calves	Sato *et al*., 2004
05	Decoquinate	----	*E. tenella*	Chicks	Bo *et al*., 2020
06	Toltrazuril,	----	*E. tenella*	Chicks	Ramadan *et al*., 1997
07	Halofuginone	----	*E. tenella*	Chicks	Ramadan *et al*., 1997
08	Monensin	----	*Eimeria acervulina*	Chicks	Willis and baker, 1981
09	Lasolocid	----	*Eimeria acervulina*	Chicks	Willis and baker, 1981
10	Multienzyme	----	*Eimeria* spp.	Chicks	Bozkurt *et al*., 2014
11	Prebiotic	----	*Eimeria* spp.	Chicks	Bozkurt *et al*., 2014
12	Probiotic	----	*Eimeria* spp.	Chicks	Bozkurt *et al*., 2014
13	Herbal essential oil	----	*Eimeria* spp.	Chicks	Bozkurt *et al*., 2014
14	Toltrazuril,	Water	*E. ninakohlyakimovae*	*In-vitro*	Odden *et al*., 2019
15	Diclazuril	Water	*E. ninakohlyakimovae*	*In-vitro*	Odden *et al*., 2019
16	Decoquinate	Water	*E. ninakohlyakimovae*	*In-vitro*	Odden *et al*., 2019
17	Sulphonamide	Water	*E. ninakohlyakimovae*	*In-vitro*	Odden *et al*., 2019

1.6.3.6. Nanotechnology

The phenomenon of drug resistance contributes to the epidemic of coccidiosis, paving the way for the introduction of new anticoccidiosis drugs that can br tolerated and survive in thre market for a long time. Initially, no products were developed that could ward off parasite resistance and strengthen the immune system. In recent decades, nanotechnology has been explored for veterinary applications, not only for diagnostic purposes but also for the development of pharmaceuticals and prophylactic agents through green synthesis. The minute size of the nanoparticles has proven to be useful in biological applications with their unique physicochemical characteristics, as they enable controlled

drug release, targeted drug delivery and immune-modulation. To confirm the reliability of nanotechnology, the green synthesized nanoparticles of trace elements have shown positive responses in antibacterial, anti-parasitic and antioxidant assays (Maddawy *et al.,* 2022).

Scientists have been working on the introduction of nanotechnology in the form of plant-based nanomaterial synthesized by an efficient and eco-friendly green synthesis method. Initially, the iron-based nanoparticles (Fe NPs) were biosynthesized using crude plant extracts as an available and affordable resource for producing crystalline magnetite nanoparticles (Saif *et al*., 2016). The magnetite nanoparticles are eco-friendly, harmless and have negligible cytotoxicity (Senapati *et al*., 2005; Nayak *et al*., 2022).

1.6.3.6.1. Applications of Iron Oxide Nanoparticles

Nanoparticles are used in many fields due to their broad spectrum. Among the recently appearing nanoparticles, iron oxide-based particles have attracted attention. Regarding nanomaterials' applications, iron oxide nanoparticles are needed due to their beneficial properties as environmental catalysts and their affordability. The nanoparticles are effective due to their nano size and environment friendly synthesis method. One of the main properties that enhance the efficiency of nanoparticles is their high surface-to-volume ratio (Ramimoghadam *et al*., 2014). Besides diagnostic and therapeutic purposes, magnetic resonance imaging (MRI) nanoparticles are utilized in drug delivery to cells and tissues (Tartaj *et al.,* 2005).

1.6.3.6.2. Green Synthesis

In the early days of science, synthetically produced nanoparticles were commonly used, but they had many side effects. For this reason, researchers have developed an efficient, affordable, and harmless method for synthesizing plant-based nanoparticles utilizing various plant parts. All plant parts contain essential phytochemicals that bind metals such as iron through nano-technological 'green' approaches (Demirezen *et al*., 2019).

The herbal medicines have been used in society for centuries because they are readily available, inexpensive, have few side effects, are long-lasting and do not rapidly develop resistance. Furthermore, it is a better preventive option for the control of coccidiosis (Naidoo *et al*., 2008).

1.7.1. Importance of Medicinal Herbs

The residents of remote areas strongly believe in using home remedies, especially herbal products, because there is no specific treatment. This is not only because of its availability but also because of its rich composition of bioactive ingredients that can alleviate many diseases (Lavinas *et al.,* 2019).

According to Kashani and his co-researchers (2012), the bioactive or secondary metabolites in the fractions of these herbal extracts have certain pharmacological effects. But among them, the activity and structure of some important groups of natural compounds have been described by some scientists, such as flavonoids, terpinoids and alkaloids are some compounds commonly used to fight against several diseases in today's therapeutic world (De Andrade *et al.,* 2012; Sarala *et al*., 2011). The most challenging aspect that creates hurdles in the use of these natural compounds is that these compounds are available in lesser amount and the purification of these compounds is very expensive, which is why the drug formulation process has become slower in third world countries such as Pakistan (Chan *et al.,* 2011; Tang *et al.,* 2012).

1.7.2. Medicinal herbs

The biological activities validate the use of secondary herbal metabolites in modern therapeutics. For this purpose, certain types of activities are commonly practiced, like computational simulations, rapid *in-vitro* bioassays, are less time and money consuming as compared to the *in-vivo* bioassays, but *in-vivo* assays are more reliable and accurate with supporting evidence (Sarker, 2005). The *in-vitro* bioassays are result specific and extensively used to obtain desired goals (Valgas *et al.,* 2007).

1.7.2.1. *Mentha arvensis*

Mentha arvensis (Lamiaceae) is a cosmopolitan herb (Thawkar *et al*., 2016), with antibacterial (Naseem *et al*., 2022), antioxidant activity (Biswas *et al*., 2014), anti-candid potential (Santos *et al*., 2012) and is also used to treat gastrointestinal infections in humans (Londonkar and Poddar, 2009). The essential oil of mint exhibits antifungal activity (Scartazzini *et al*., 2019; Makkar *et al*., 2018). Dengue infection is prevalent across Asia and Africa and several controlling strategies have been adopted for it. Among them, the natural extract has also been tried in one way or another, *M. arvensis* was applied as a

repellent to both *Aedes albopictus* and *Aedes egypti* (Wu *et al*., 2019; Manh and Tuyet, 2020).

The cytometric analysis of *M. arvensis* showed the inactivation of different bacterial strains under the cold condition (Pereira *et al*., 2018). *M. arvensis* extract is proven to be an anti-candid remedy that commonly inhabit various human organs (Santos *et al*., 2012). In daily life, people use different synthetic preservatives with side effects, but the introduction of *M. arvensis* oil extracts for preservation to prevent fungal infection or insect infestation is quite safe (Kumar *et al.,* 2009). The drug resistant *Staphylococcus aureus* is hard to eliminate but the use of alcoholic extract of *M. arvensis* has reduced the lethal concentrations of various antibiotics (Coutinho *et al*., 2009). The *M. arvensis* essential oil soothes asthma patients (Sharma *et al*., 2018).

1.7.2.2. *Polygonum glabrum*

Polygonum glabrum, commonly known as dense flower knotweed, is a perennial herb found along the river banks in South Asian countries (Raja and Ramya, 2017; Malik *et al*., 2018). Its medicinal properties are due to high content of zinc, copper, iron and vitamins (Bhati and Jain, 2016). The b-hydroxyfriedalanol extracted from *P. glabrum* has been shown to be effective against HIV (Said *et al*., 2015). It exhibits anti-leishmanial (Rahman *et al*., 2015), antifungal, antibacterial (Palani *et al*., 2014) and antioxidant properties (Babitha *et al*., 2012).

1.7.2.3. *Verbena officnialis*

Verbena officinalis (Verbenaceae) is a cosmopolitan and perennial herb commonly known as vervian (Akerreta *et al*., 2007). It is mainly found in Europe and Asia and is common in the agricultural lands in northern and western Pakistan (Waheed *et al*., 2016). The basic composition of *V. officinalis* is enriched with sterols, triterpenic acids, caffeoyl derivatives, flavonoids, phenolic acid, and iriods and glycosides (verbenalin, verbascoide, luteolin, kaempferol, ursolic acid) (Guarrera *et al*., 2005; Casanova *et al*., 2008). More than 25 different compounds were reported from the GC-MS analysis of *V. officinalis* (Zoubiri and Baaliouamer, 2011).

Verbena officinalis is used to treat hysteria, anxiety, and insomnia (Williams *et al*., 2006; Waheed *et al*., 2016), nervous disorders (antidepressant and anticonvulsant), liver,

and gallbladder diseases (Shamsardakani *et al*., 2010) and human chorio-carcinoma suppression (Kou *et al*., 2013). The essential oil of *V. officinalis* exhibits antifungal properties (Elshafie *et al*., 2015), anti-rheumatism and anti-thyroid (Akerreta *et al*., 2007; Guarrera *et al*., 2005), anti-analgesic agent (Agelet and Valles, 2011; Calvo, 2006), antifungal and antibacterial agent (Hernandez *et al*., 2000; Ahmed *et al*., 2012; Pavela, 2009), mosquito repellent (Zoubiri and Baaliouamer, 2011) and anticancerous (Encalada *et al*., 2015). The n-hexane, chloroform and ethyl acetate fractions of *V. officinalis* are active melanoma inhibitors (Nasir *et al*., 2022).

1.7.2.4. *Parthenium hysterophorus*

Parthenium hysterophorus is a parasitic plant found across the world except the coldest regions (Million *et al*., 2021), often causing skin irritation (Kushwaha and Maurya, 2012). In addition to its notorious nature, its vital phytochemicals enable the man to get something best out of the available useless parasitic weeds. It is mainly enriched with lactones, parthinin, hysterin and hymenin, phenols, saponins, free amino acids and glucose (Kushwaha and Maurya, 2012), flavonoids, glycosides, terpinoids, steroids and carbohydrates (Krishnavignesh *et al*., 2013).

Whereby it exhibits antibacterial (Kumar *et al*., 2014), anti-meningitis, anti-respiratory capability (Fazal *et al*., 2011) and anticancer agent (leukemia and prostate cancer) (Kumar *et al*., 2013). The constituents of *P. hysterophorus* extracted in different solvents were subjected to anti-mosquito effects, showed reduced egg-laying ability in *Aedes egypti* (Kumar *et al*., 2011). The green synthesized zinc oxide nanoparticles of *P. hysterophorus* were found most active against *Bacillus sp.* and *Enterobectoer sp.* with maximum zone of inhibition (Datta *et al*., 2017).

1.7.2.5. *Fumaria officinalis*

The genus *Fumaria* is a perennial, cosmopolitan herb, consisting of 46 species, containing beneficial contents (alkaloids, carbohydrates, phenolic compounds, glycosides, terpenoids, phytosterols, proteins, amino acids, saponins, steroids and tannins) (Snafi and Esmail, 2020), which can be used in various fields of life such as research, medicine and food stuff (Gupta *et al*., 2012).

Due to its rich composition, it is used as painkillers (Sharma *et al*., 2014), antioxidant (Sengul *et al*., 2009; Safari *et al*., 2018; Fatima *et al*., 2019), and anti-epileptic (Sharma *et al*., 2014). Further, it has a safeguard effect on male reproductive structures compared with fluoxetine (Sharef *et al*., 2020). The green synthesized silver nanoparticles of *F. officinalis* have a huge zone of inhibition to different bacterial strains and fungal growth (Cakic *et al*., 2018). Further, the crude extract of *Fumaria* enhances the urine volume by more than 100% compared to the normal saline (Paltinean *et al*., 2017).

1.7.3. Characterization of Herbs using Advance Techniques

The presence of bioactive components can be assessed by certain spectroscopic techniques (Beretta and Facino, 2010). The crude herbal extract can be purified to isolate specific compounds by different fractionation methods (Sticher, 2008). Several methodologies have been described for the purification and quantification of active organic metabolites from crude extracts, but column chromatography is the gold standard procedure using the principle of polarity (Wall *et al*., 1996); Abdulhamid *et al.*, 2017).

Further, these compounds can be identified by thin layer chromatography (TLC), using the paper stationary phase (Abdulhamid *et al.*, 2017). The FT-IR spectroscopy is used to identify the existence of functional groups in phyto-componds in the infrared region (Grube *et al.*, 2008). The use of UV-Vis spectroscopy is complicated because the bands in the UV-Vis region determines the probability of different compounds (Gunasekaran, 2003), therefore some advanced technique such as GC-MS to mark the presence of compounds at different retention times, which is actually present in the herbal extract (Karpagasundari and Kulothungan, 2014). GC-MS is the premium technique that uses mass-to-charge ratio to detect the presence of different compounds (Krishnakumari and Nagaraj, 2012).

1.7.4. Computational Identification of Natural Material against Diseases

In the present era, the *in-silico* simulation approach is an important tool to confirm drug-parasite interactions after *in-vitro* or *in-vivo* trials of drugs (Moine *et al*., 2015). The *in-silico* drug formulation uses the genomic information contained in the genome and protein databases (Egner *et al*., 2005). The computational *in-silico* techniques utilize the

structures of the phyto-compounds and ligands to confirm their interactions (Mura and McAnany, 2014).

The common online data bases used for *in-silico* docking include Autodock Vina (Trott and Olson, 2010), Autodock (Morris *et al*., 2009) and GOLD. These software finds certain interactions between the target molecule and the desired ligand, including electro-statistic interactions, hydrogen bonding and van der waals interactions or other non-covalent interactions (Rarey *et al*., 1996). Some drugs that do not exhibit non-covalent interactions are classified as covalent drugs (Singh *et al*., 2011). An important tool in the mechanism of drug formulation is the ligand-receptor interaction, which can be determined by a computational method called molecular docking (Kellenberger *et al.,* 2004).

1.8. *Eimeria* Invasion and Target Protein Selection

There are certain important compounds such as proteins or enzymes that play a vital role in parasite persistence. To manage parasitic infections, these specific macromolecules are targeted with drugs. In this scenario, several proteins have been studied *in-silico* as substrates to overcome the issue of coccidiosis. Some of the targets studied in the *Eimeria* spp. are type ii fatty acid, CDPK protein and SAM Synthetase. The CDPK mechanism is explained because any parasitic infection requires the parasite to enter the host cell through the cell membrane, which needs some secretory channels in the cell membrane (Dubremetz *et al*., 1998).

Parasites need some important components for invasion, and one of them is calcium. The calcium concentration severely affects the intracellular transport function (Moreno and Docampo, 2003). The apicomplexan parasites have a special receptor for sensing calcium signals, known as (CDPK) calcium-dependent protein kinase (Han *et al*., 2013). CDPK consists of several domains including an N-terminal variable region, a kinase domain containing the active site responsible for phosphorylation of target proteins, another domain "calmodulin-like domain", which is responsible for calcium binding (Ikura, 1996; Harper and Harmon, 2005; Han *et al*., 2013).

The *Eimeria* CDPKs are distinct from calmodulin-dependent protein kinase and protein kinase C, which play important roles in mammalian Ca^{2+} signaling (Ishino *et al*., 2006). It receives the Ca^{2+} signaling and initiates a cascade of intercellular pathways and

plays an efficient role in the lifecycle of parasites (Lourido *et al*., 2010). The CDPK's are found in the majority of the living genera except bacteria, fungi, helminthes and animals, especially *Eimeria* host (Ishino *et al*., 2006). The *Eimeria* has a special type of calcium dependent protein kinase, designated as EtCDPK3. It is studied at various stages of *Eimeria* life cycle, which proves its involvement in host cell invasion (Han *et al*., 2013).

Historically, various natural compounds have been reported as inhibitors of CDPK *in-silico, w*hich provides the evidence that CDPK inhibition simply prefers to inhibit the propagation of *Eimeria* spp. Therefore, if CDPK is blocked by natural compounds, this could restrict the penetration of *Eimeria* into the host cell by disturbing its life cycle, and thus its propagation in the chicks will be stopped (Aljedaie *et al*., 2021). Previously, *Eimeria* invasion was controlled by targeting CDPK synthetase, as *Eimeria* invasion depends upon the calcium concentrations of the attacked cell (Aljedaie *et al*., 2021).

1.8.1. S-Adenosyl methionine synthetase (*E. tenella*) as Target Protein

To date, no such protein has been found in the host of the apicomplexan parasites (Kieschnick *et al*., 2001), confirming that CDPK is a suitable drug target. Therefore, in a current study, the S-Adenosylmethionine (SAM) synthetase was adopted as a target for certain naturally available compounds described by the GC-MS technique in certain fractions of *V. officinalis.* SAM synthetase is a methyl donor that assists DNA methylation, switching genes on and off to control gene expression (Reytor *et al*., 2009). SAM synthetase also plays an important role in cellular metabolism. Its inhibition can inhibit the SAM synthetase synthesis and halt cellular metabolic activities and ultimately lead to the death of the organism (Lu and Markham, 2002). It is conserved in all organisms (Shah *et al*., 2023). Previously, the SAM synthetase from *E. maxima* was used as a drug target to control avian coccidiosis (Maheswari and Revathi, 2017).

1.9. Control Drug

The reason for choosing sulfaquinoxaline as a control drug is that it is one of the drugs of choice in the poultry industry (Ojimelukwe *et al*., 2018). It is a synthetic compound used against many infectious diseases, including coccidiosis; it has low adsorption in the environment, and can be easily detected in water. The main drawback of this drug is that it acts as a contaminant in the environment (Urbano *et al*., 2021). This is

when feed to birds or other animals for therapeutic purposes, it can't be completely digested and released to the environment in feces, and ultimately, land animals consume it or consumed by aquatic fauna when the manure is wasted in aquatic bodies like lakes or rivers etc, which may incorporate its essential cellular pathways (Boudriche *et al*., 2019). It replaces para aminobenzoic acid in folic acid synthesis pathway, ultimately inhibiting nucleic acid synthesis and parasites are unable to propagate (Lakkanatinaporn *et al*., 2004). The two dimensional structure of sulfaquinoxaline is shown in figure 1.3.

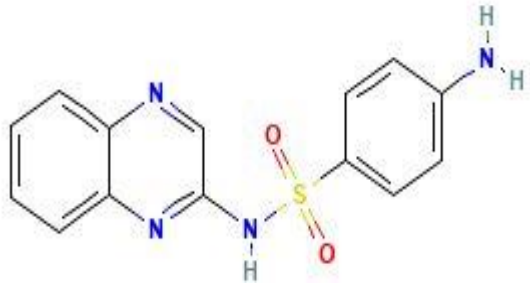
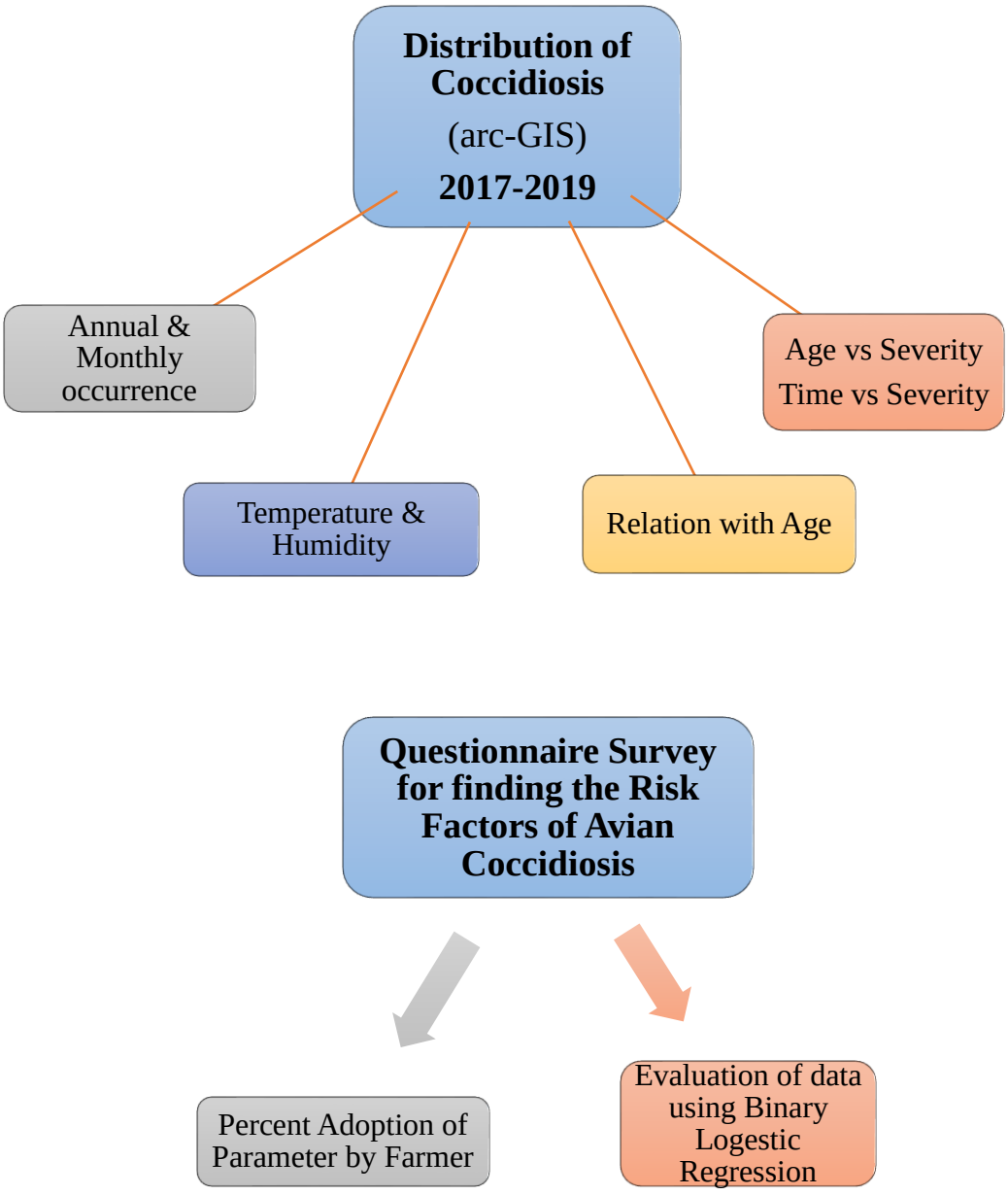


Figure 1.3: 2D structure of sulfaquinoxaline, retrieved from pubchem.ncbi.nlm.nih

Chapter: 02

Prevalence of Avian Coccidiosis and its Associated Risk Factors in District Peshawar Khyber Pakhtunkhwa, Pakistan

GRAPHICAL ABSTRACT



ABSTRACT

In this study, coccidiosis was reported for the first time in Peshawar, Pakistan. The data from 338 farms from 54 towns.regarding the prevalence of cocsidiosis during 2017-2019 was evaluated statistically using Excel and chi square test. The coccidiosis-associated risk factors was observed in 752 farms from 59 villages in 2020, and evaluated by binary logistic regression (ANOVA). The postmortem data of coccidiosis-effected farms and coccidiosis-associated risk factor data were studied to evaluate the impact of *Eimeria* spp. on poultry. The data was statistically analyzed by different statistical tools using Microsoft excel; the data on associated risk factors was analyzed using binary logistic regression (ANOVA) and graphically presented in maps using arc-GIS.

The 25.66% towns of district Peshawar were found affected by avian coccidiosis, with a high prevalence in villages Bara and Spersung, with 6.51% of infected farms in each village during the studied tenure. The impact of bird's age on coccidiosis showed that 16 to 30-day-old broiler chicks were most affected (64.49%) on average, with 27.52%, 88.99%, and 33.48% in 2017, 2018, and 2019, respectively. The month-wise occurrence showed variability in coccidiosis prevalence, with a higher rate in March, April, September, October, and November, with 9-13% cases in 2017. January, March, April, May, June, September, October, and December, with 10 - 15% cases in 2018 and April, June, July, November, and December, with 8 -14% cases in 2019. The severity based infection revealed 23.37% of farms with severe and 41.42% with moderate modes of infection. The prevalence of coccidiosis showed direct relation with humidity and inverse relation with temperature.

The survey for associated risk factors reported 33 out of 59 villages were found with coccidiosis in 2020. Seeking a preventive measures, the survey of different predictors was analyzed using binary logistic regression in 2020 and concluded that vaccination ($P = 0.008$), farm disinfection ($P = 0.04$), use of special footwear ($P = 0.02$), change of bedding ($P = 0.03$), and hygiene of the feeder ($P = 0.03$) have affected the coccidial emergence significantly.

MATERIALS AND METHODS

2.1. Study Area and Data Collection

The current study area 'Peshawar' (Lat: 33° 44 to 34° 15N; Long: 71° 22 to 71° 42E) (Begum *et al*., 2021), surrounded by district Charsadda in N-E, district Nowshehra in E, district Khyber in SW, and district Mohmand in NW. Peshawar city located about 160 km from Islamabad capital territory in west with 1257 km^2 area and consist of 227 villages and 130 neighborhood villages councils. The three years (2017-2019) data of coccidiosis was obtained from VRI and PVC's through with ethical approval. The coccidiosis-effected villages under study are presented in the form of dots on the map figure 2.1. The investigation reports includes data on case positivity for coccidiosis, lesion score or case severity, flock size, chicks' age, time of postmortem, and farm locality. The age-wise data were studied by making groups of bird's based on age in days i.e. G1 < 5, G2 = 6-10, G3 = 11-15, G4 =16-20, G5 = 21-25, G6 = 26-30, G7 = 31-35, G8 36-40, and G9 = >40.

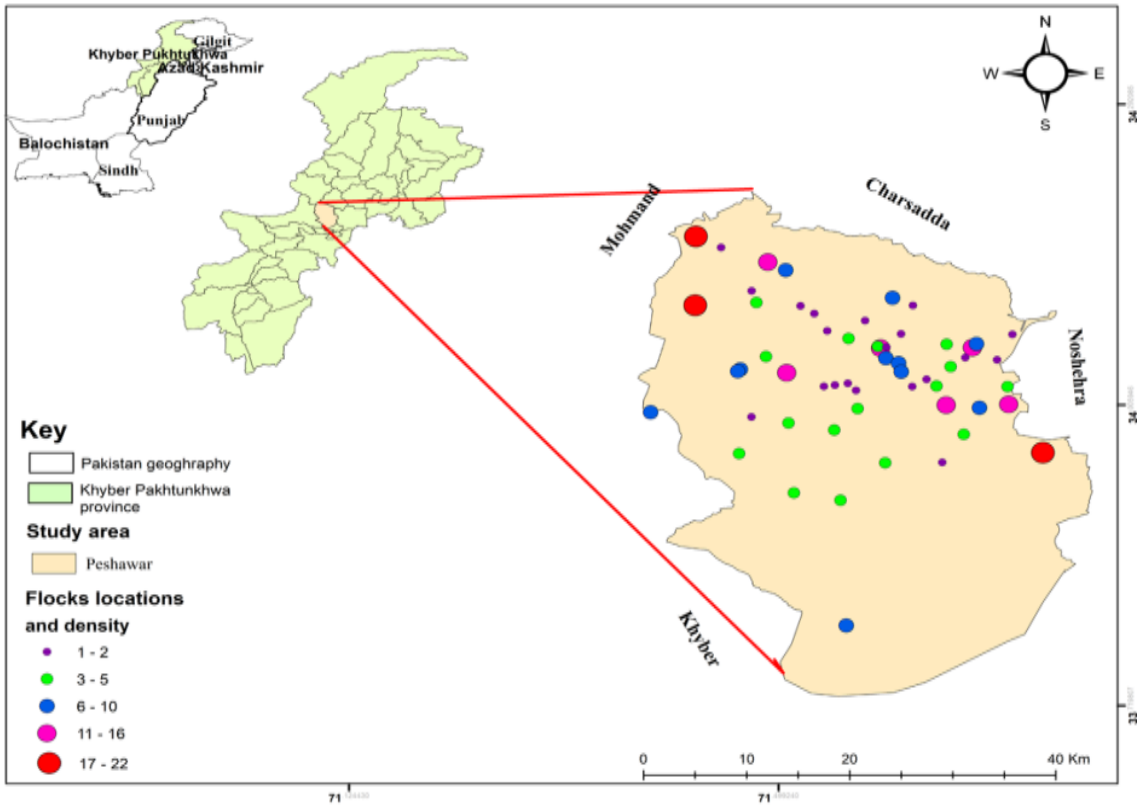


Figure: 2.1. Study area map of district Peshawar showing the occurrence and density of coccidiosis

2.2.1. Risk Factors Associated with Coccidiosis

The survey for finding risk factors associated with coccidiosis was conducted in 2020, visited 752 poultry farms from 59 villages of district Peshawar. Questionnaire performa was filled from farmers. In the current study, the information about certain factors like biosafety (disinfection of farm and biosafety kit), environmental conditions (temperature, humidity, asphyxia and ventilation), medication (vaccination and antibiotics) and farm hygiene (bedding/flooring and feeder's cleanliness) and feeding habit/ feeding formula was assessed and the data was recoded from the poultry farms. To test the effect of risk factors on avian coccidiosis, a linear regression model was adopted (Silva *et al*., 2020).

Regression equation: $P(1) = \exp(Y')(1+ \exp(Y'))$

The poultry farms surveyed during the study were represented graphically in the form of GIS-based maps using arc-GIS showed the cocci positive and negative farms (Silva *et al*., 2020). The risk factors or management parameters required for poultry farming were analyzed, and each parameter was assigned with a single score.

2.3.1. Statistical Analysis

The data was analyzed by using statistical tools like arc-GIS, to locate the actual location of farms along with prevalence. The data of percent coccidiosis, lesion scoring or severity level vs bird's age, effect of temperature and humidity on coccidiosis was analyzed and graphically represented using Microsoft office. The data associated with risk factors of coccidiosis in poultry was analyzed by binary logistic regression test using Minitab (18.1) to find out the effective parameters which can help in the prevention of coccidiosis. For ease, the farms surveyed were divided into ten categories on the base of size, i.e., ≤ 1, ≤ 1.5, $\leq 2, \leq 2.5, \leq 3, \leq 3.5, \leq 4, \leq 4.5, \leq 5$ and ≤ 5 ($\times$ 1000 Ft^2)

Questionnaire Sample

This questionnaire is designed to conducted survey purely based on research purpose, which is the requirement of academic degree.

Data_____

Farm dynamics

1. Location _____ Lat: _____ Long:_____
2. Shed Size_____ ft^2
3. Shed Height _____ ft

Biosafety

4. Disinfection of farm a: Yes ☐ b: No ☐
5. Special Shoes Using by farmer a: Yes ☐ b: No ☐
6. Safety Kit a: Yes ☐ b: No ☐

Breed information and feeding Habit

7. Variety of chick a: Ross ☐ b:Hub bird ☐ c: Other ☐
8. Number of Chicks _____
9. Feeding a: According to Formula ☐ b: No ☐
10. Feeding / day a: 3 times ☐ b: 2 times ☐ c: Random ☐

Environmental Conditions

11. Temperature a: Maintained ☐ b: Not maintained ☐
12. Humidity a:Normal ☐ b: High ☐
13. Suffocation a: Yes ☐ b: No ☐
14. Ventilation a: Good ☐ b: Bad ☐

Medication

15. Vaccination a: Yes ☐ b: Partial ☐ c: No ☐
16. Antibiotics a: Yes ☐ b: No ☐

Farm Hygiene

17. Bedding/ floor a: Dry ☐ b: Wet ☐
18. Feeders a: Clean ☐ b: Dirty ☐

(Ali *et al*., 2014; Bachaya *et al*., 2015; Wondimu *et al*., 2019)

RESULTS

In the current study, 338 broiler flocks were found affected with coccidiosis during the period of three consecutive years i.e. 2017-2019. Which were distributed in 59 villages of district Peshawar, The affected villages represented the rural area of the studied area, sharing boundaries with districts Mohmand, Chardadda and Naushehra.

Table 2.1: Annual temperature and humidity of district Peshawar for the period (2017-2019)

Month	Temperature (°C)			Humidity (%)		
	2017	2018	2019	2017	2018	2019
Jan	15.18	15.04	13.80	67.19	50.94	60.83
Feb	19.01	20.68	15.37	49.91	50.6	65.13
Mar	26.86	28.52	22.78	47.73	50.73	56.33
Apr	32.38	32.26	28.62	44.61	49.26	59.10
May	40.19	35.72	34.77	37.55	43.96	41.52
Jun	39.64	37.08	35.76	43.61	36.41	32.83
Jul	35.71	35.88	36.44	61.76	59.36	56.48
Aug	33.28	34.13	33.66	65.58	64.79	64.79
Sep	30.76	32.42	35.89	57.87	58.36	61.52
Oct	30.14	28.49	26.89	46.19	57.84	56.07
Nov	21.08	19.17	21.48	60.86	60.38	62.26
Dec	16.82	15.12	15.46	51.21	62.50	56.81

2.4.1. Density of *Eimeria* Effected Poultry Parms in District Peshawar

The farms affected by coccidiosis were plotted on a map of Peshawar district on the basis of farm density or disease burden. For ease, all the 59 *Eimeria*-infection positive villages were distributed into five categories, represented in different colors, i.e. villages with ≤ 2, 3-5,6-10, 11-16, and 17-22 farms consisting of 35.59%, 28.81%, 20.75%, 10.17% and 6.78% cases respectively as represented in figure 2.1. The data further showed an inverse relation between the number of villages and the density of coccidiosis affected poultry farms because the maximum number of villages with a lowered number of cases and the same pattern is followed for other categories also.

2.4.2. Monthly Occurrence of Coccidiosis

Collectively, throughout the occurrence of coccidiosis, the cocci-positivity rate observed is higher in the months of April, May, November, and December., whereas if that data is further dissected, the pattern of positivity will like, 2017, lesser cases is observed in the months of June and July, which represented the hottest season, whereas higher percent is observed in the months of April, May and November. In the year 2018, different patterns were observed throughout the year, a less percent of coccidiosis was observed in February, August and September and the higher percent *Eimeria* infection was observed in the month of June as compared to 2017 and 2019. Similarly, 2019 also showed variable distribution of positive *Eimeria* cases. The months of low occurrence was March and May to August as shown in table (2.2) whereas higher rate was observed in the October to February. Overall, study suggests large number of cases occurred in winter season with low temperature whereas the scenario for the summer season was opposite with less abundance of cases of coccidiosis (Fig. 2.2).

Table 2.2: Monthly distribution of suspected cases vs *Eimeria* infected chicks (n)

Months	**2017**			**2018**			**2019**		
	S	**E^+ (n)**	**P %**	**S**	**E^+ (n)**	**P %**	**S**	**E^+ (n)**	**P %**
Jan	251	8.0	3.2	280	14	5.0	156	10	6.4
Feb	240	8.0	3.3	250	1	0.4	147	9.0	6.1
Mar	205	10	4.9	241	16	6.6	240	7.0	2.9
Apr	101	10	10	285	11	3.9	300	17	5.7
May	109	8.0	7.3	251	13	5.2	401	6.0	1.5
Jun	104	2.0	1.9	221	13	5.9	352	12	3.4
Jul	300	1.0	0.3	216	9	4.2	453	12	2.6
Aug	281	9.0	3.2	208	0	0.0	367	8.0	2.2
Sep	276	11	4.0	227	2	0.9	355	4.0	1.1
Oct	402	16	4.0	258	11	4.3	146	8.0	5.5
Nov	307	23	8.0	242	8	3.3	161	11	6.8
Dec	273	9.0	3.3	224	11	4.9	109	10	9.1

(S= Suspected cases, E^+ = *Eimeria* Positive Cases, P% = Percent cases)

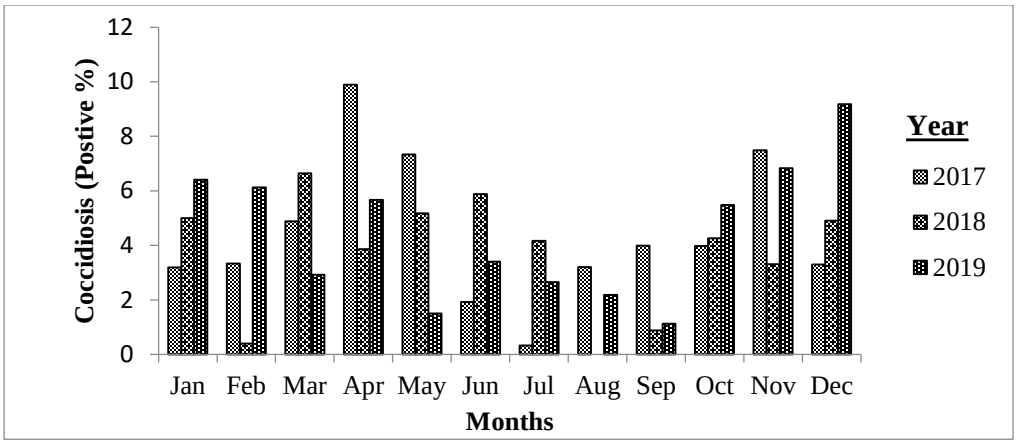


Figure 2.2: Graphical presentation of percent *Eimeria* infected flocks

2.4.3. Temperature and Humidity vs Avian Coccidiosis

The impact of environmental stresses or conditions upon the density of coccidiosis occurrence in the year 2017 was measured by plotting the month-wise positive cases against temperature and humidity. The humidity showed direct correlation with the density of coccidiosis in poultry farms because both have similar patterns, indicated in the form of regression type polynomial trendlines i.e. months with high humidity rate have a higher number of cases, like September to February. Whereas the months that represent the summer season had a low number of cases with lowered humidity. In contrast, coccidiosis has an inverse relation with temperature as indicated in the polynomial trendlines (Fig. 2.3a) i.e. with the increase in temperature, the density of coccidiosis decreases, which suggests that the lifecycle or survival of *Eimeria* spp. oocyst is highly favored by humid environment. The month-wise density of coccidiosis cases in the year 2018 has a similar indirect correlation with temperature as in the previous year (Fig. 2.3b), but the humidity factor was little bit different i.e. humidity raised throughout the year, but the overall pattern of coccidiosis trend line is straight and showed that it is not greatly affected by humidity, might be some preventive measure taken in this year to control the overwhelming impact of humidity on coccidiosis emergence. The figure 2.3c, showed the correlation of humidity and temperature with coccidiosis in poultry sector in 2019 in district Peshawar. The observation of the trendlines showed direct relation between humidity and coccidiosis, whereas an inverse relation was noticed between the cases abundance of coccidiosis in each month with the temperature.

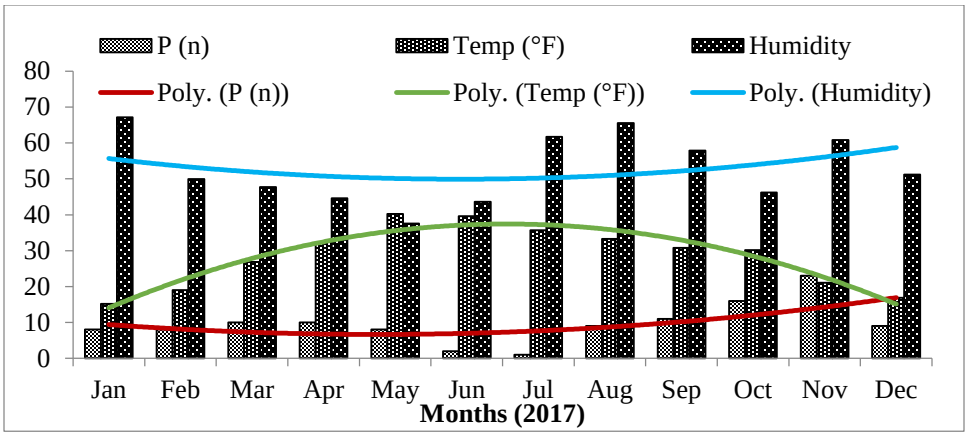


Figure 2.3(a): Correlation of poultry coccidiosis cases with temperature and humidity in 2017

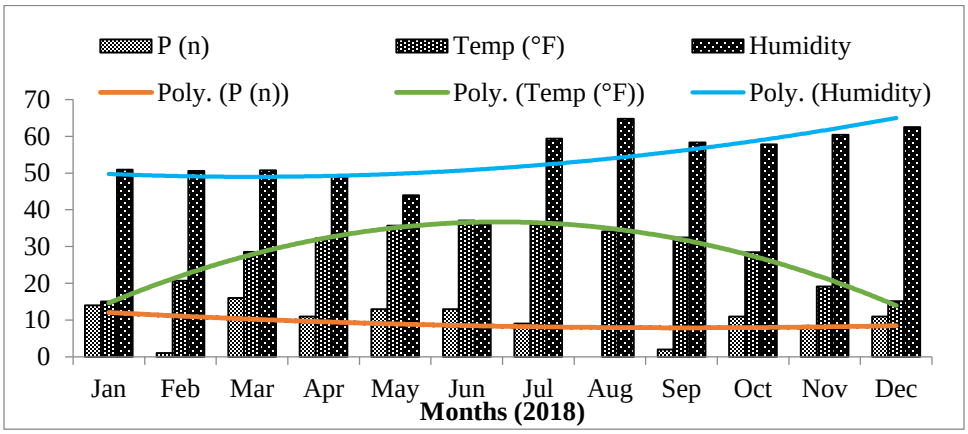


Figure 2.3(b): Correlation of poultry coccidiosis cases with temperature and humidity in 2018

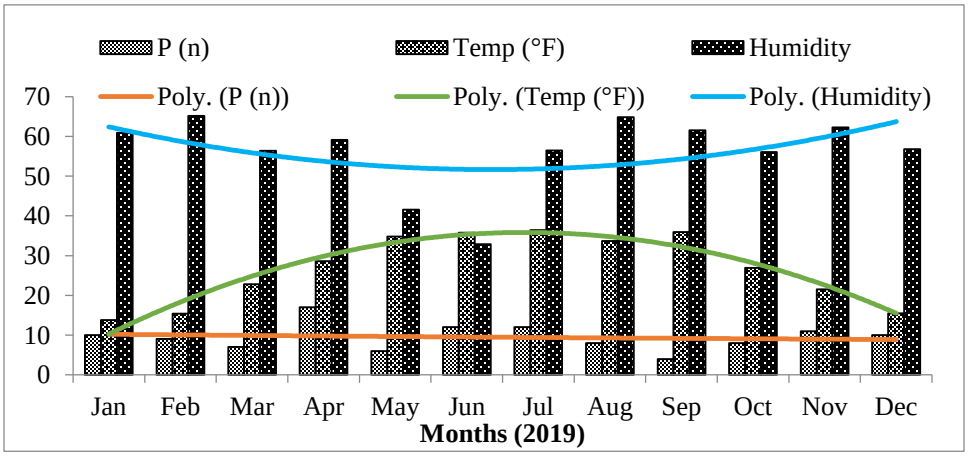


Figure 2.3(c): Correlation of poultry coccidiosis cases with temperature and humidity in 2019

2.4.4. Age-wise Prevalence

The age-wise data showed that birds between 15 to 30 days were more infected by *Eimeria* species in all three years with higher prevalence in age in G4 in 2018 and 2019, which was decreased with the growing age. Whereas in 2017 the cases were observed high in same middle age groups. In 2017, the postmortem reports of poultry farms showed high morbidity in chicks within the age groups from G2 to G7 with higher numbers in G4. The pictorial representation of age-wise distribution showed higher values in the age group G4 (n=31) in year the 2018 postmortem reports, and a gradual decrease in coccidial occurrence was reported in the groups of age above G4, In G1, no case is reported, whereas in G2, G8 and G9, small number of cases were observed (Fig. 2.4). In 2019 the rise of coccidial prevalence observed from G1 to G4, and then the number of deaths remain the same in G4, G5 and G6. Throughout the study, three groups (G4, G5 and G6) of adult age were observed with higher rate of morbidity due to *Eimeria* spp. infection in age-wise distribution, whereas low age and mature birds were least affected by *Eimeria* spp. infection. For ease the age-wise groups were symbolized as G1 = < 5 days, G2 = 6 to 10 days, G3 = 11 to 15 days, G4 = 16 to 20 days, G5 = 21 to 25 days, G6 = 26 to 30 days, G7 = 31 to 35 days, G8 = 35 to 40 days and G9 = >40 days

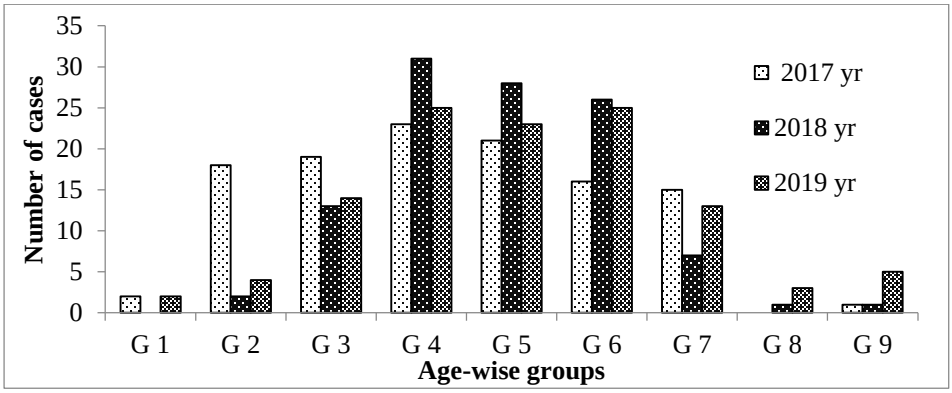


Figure 2.4: Age-wise distribution of *Eimeria* spp. infected flocks

2.4.5. Severity Based Annual distribution of Coccidiosis

During the analysis of postmortem reports data various severity levels were observed in the *Eimeria*-infected farms. Mainly the cases can be divided into the subtypes i.e. mild, moderate and severe. The mortalities due to mild level infection was found more prevalent in 2017 and decreased in 2018 and 2019 (Fig. 2.5a), whereas moderate level

infection was found more abundant throughout the study and greater number of farms were affected by this type of infection.

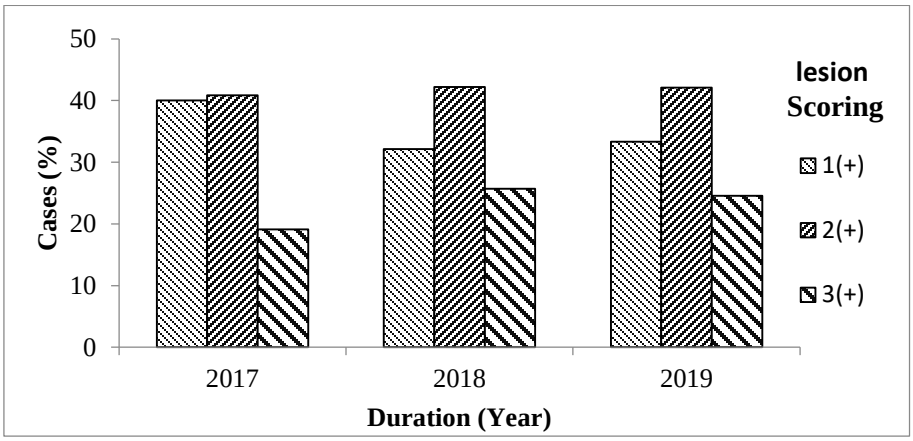


Figure 2.5(a): Annual distribution of *Eimeria*-infected flocks, based severity

2.4.6. Age-wise Distribution of Coccidiosis Based on Severity

The farms that reported coccidial emergence were studied keenly by plotting the cases based on disease severity against the age of birds. It has shown a similar prevalence pattern, such as group G4 was observed with high number of low severity cases and declines with the increase in age. The groups G4, G5 and G6 were reported with an abundance of moderate level of cases. The high level severity was also observed in middle-aged broiler chicks with p = 0.327 & 0.196, > 0.05 (Fig. 2.5b). Hence, it proves null hypothesis hypothesis that age groups and severity levels was independent and therefore the chi square correlation showed a relationship between the variables of age and disease severity levels.

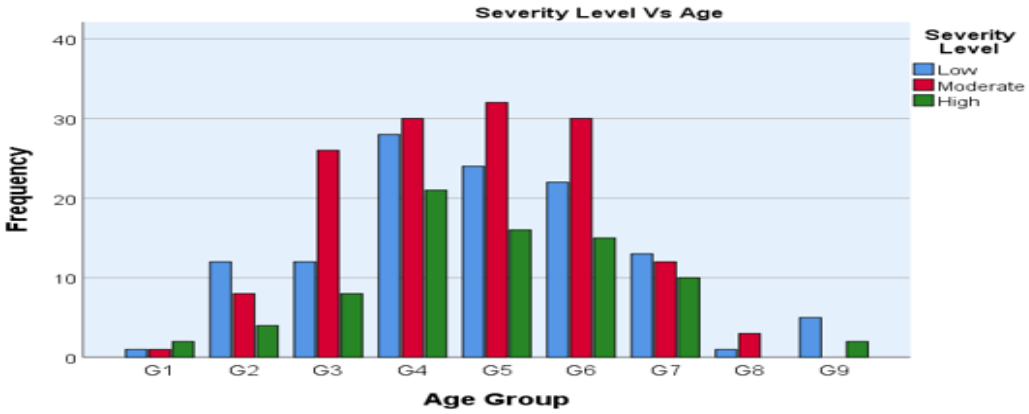


Figure 2.5(b): Age-wise distribution of *Eimeria* spp. infected flocks based severity

2.4.7. Month-wise Severity Based Distribution of Coccidiosis

The table 2.3 showed the month-wise occurrence of coccidiosis on the basis of lesion score or case severity in all the three years of study. In the year 2017, both the mild and mid types of severity was 40% with maximum emergence in the months of March, and September to November, whereas the high level severity cases were 20% with a high number of cases in the months of February and November. The coccidiosis density of the year 2018 showed 32% mild level severity, which was lowered then previous year, in the same year 42% mid-level severe cases with maximum emergence in January, June, November & December and 25% high level severity with maximum cases in the months of April and May, which is higher than previous year. The year 2019 showed same density as in 2018 in case of mid-level severity, whereas a slight rise in mild level severity and decline in high level severity was observed in 2019 as compared to 2018. The overall analysis showed a decrease in mild level severity, mid-level severity remained the same throughout the study and the high level severity showed increase throughout the study.

Table 2.3: Month-wise distribution of percent coccidiosis infection cases of 2017 to 2019

Month	2017			2018			2019		
	+	++	+++	+	++	+++	+	++	+++
Jan	3.48	2.61	0.87	5.51	5.51	1.84	3.51	3.51	1.75
Feb	0.87	2.61	3.48	0.00	0.00	0.92	2.63	4.39	0.88
Mar	6.09	2.61	0.00	4.59	6.42	3.67	1.75	3.51	0.88
Apr	3.48	2.61	2.61	1.83	3.67	4.59	4.39	7.90	2.63
May	1.74	2.61	2.61	3.67	3.67	4.59	0.88	3.51	0.88
Jun	0.87	0.87	0.00	0.92	7.34	3.67	1.75	6.14	2.63
Jul	0.87	0.00	0.00	6.42	0.92	0.92	2.63	3.51	4.39
Aug	1.74	4.35	1.74	0.00	0.00	0	2.63	0.88	3.51
Sep	6.09	2.61	0.87	0.92	0.00	0.92	0.88	2.63	0.00
Oct	6.09	6.96	0.87	4.59	3.67	1.83	5.26	0.88	0.88
Nov	6.09	9.57	4.35	0.92	5.51	0.92	2.63	1.75	5.26
Dec	2.61	3.48	1.74	2.75	5.51	1.83	4.39	3.51	0.88

(+ moderate, ++ mild, +++ severe)

The distribution of various levels of *Eimeria* infected farms in 2017, illustrates that a large number of farms were affected by a moderate type of infection as shown in figure 2.5a. This abundance was recorded higher in the months of October and November, the

abundance of mild level infection recorded was significantly high ($P < 0.05$) in March, September, October and November and as for the most severe type of infection concerned it was observed in the month of November also.

A moderate level of infection was observed in the most farms in the months of January, March, April, May, June, October, November and December as shown in Table 2.3. The severe level of infection was recorded from March to June with higher ratio. The data for the year 2019 elaborates that the months of April, June and, October to December had high rates (2.63% – 5.26%) of emergence of all three levels of coccidiosis. Overall, the cases of second level infection were more prevalent. On the basis of severity, the spatial data showed that, in total of 59 villages, three were severely affected, such as Bara (33.51 E, 71.46 N) with 22 farms, Urmar (33.96 E, 71.73 N) and Warsak (34.166 E, 71.42 N) affected from coccidiosis with 18 farms in each as indicated in figure 2.1 with bigger size dots of red color.

2.5.1. Associated Risk factors of Avian Coccidiosis

The risk factors of endemic diseases were measured using the tool of questionnaire with some of important informations. In the study, the total of 752 poultry farms belonging to 59 towns of the provincial capital city of Peshawar were studied. The pi-chart represented the richness of farms in each village. For ease, the villages were divided into 9 statistical classes with the increasing interval of 5 farms per village.

The highest number of farms were found in a few villages, i.e. single village exists in the two upper classes, i.e. 36 to 40 (2%) and 41 to 45 (2%) farms. 31% of villages under the study were found with the mildest number of farms (6 to 10), 25% of the villages fall in the class of 11-15 farms, the two classes were found with 15% villages in each class i.e. villages with 1 to 5 farms and villages with 16 to 20 farms. Similarly, two classes were found with 5% of villages in each class, i.e. villages with 21 to 25 farms and 26 to 30 farms. The pi-chart concludes that only 4% of villages in this study showed the richness in poultry sector and fulfill the requirements like meat and eggs of the growing population and in return contributes much revenue to the GDP district Peshawar (Fig. 2.6).

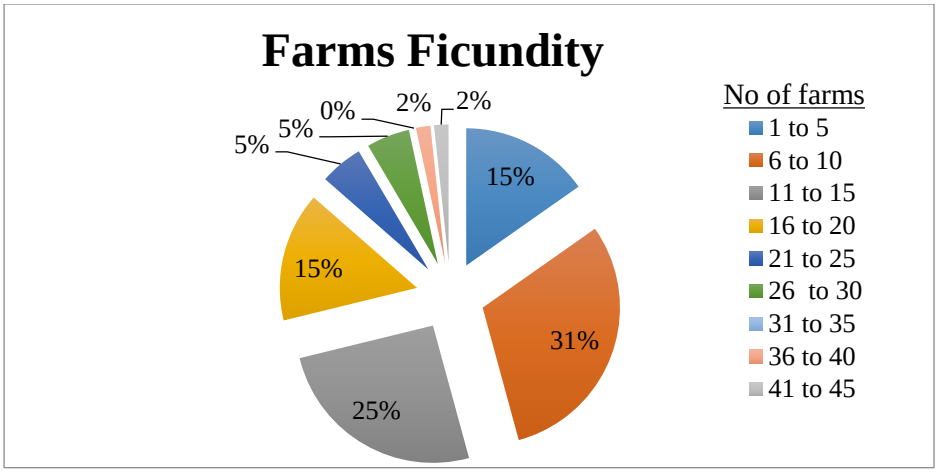


Figure 2.6: Pi chart showing richness of poultry farms in villages the study area

Table 2.4: Percent scoring of the farms regarding the essential parameter

Score (%)	Number of farms	Farms (%)
0 – 20	3	0.39
21 – 40	39	5.19
41-60	123	16.36
61 – 80	330	43.88
81 – 100	257	34.18

The farms in which many of the management parameters adopted were scored higher and vice versa. The risk factors showed that more than 70% of poultry farms in district Peshawar had scored above than 60% in 2020, which is 2/3 parts of whole study, and indicates the awareness level of farmers during rearing birds. Whereas the rest of the farms with mild score needs to be investigated for failing to meet or fulfill the requirements for progressive farming (Table 2.4).

2.5.2. Effect Risk Factors on Management of Poultry Farms

To ensure a safe internal environment of poultry farms, various parameters were assessed, table 2.5a elaborates the administration of vaccine and antibiotics, use of biosafety kit, foot gear, and disinfectant, ensuring the hygiene regarding bedding of the farm, or cleanliness of feeders, plus adoption of standard feeding formula, maintenance of the required temperature, humidity level, asphyxia extant and ventilation was assessed during the survey. To evaluate the risk factors of coccidiosis in poultry farms in district

Peshawar the farms were divided into several categories based on size. The usage of antibiotics in each farm size was (above mentioned order) 50.65%, 58.54%, 64.39%, 63.64%, 58.78%, 63.83%, 68.33%, 52.55%, 50% and 66.67% in $\leq 1, \leq 1.5, \leq 2, \leq 2.5, \leq 3, \leq 3.5, \leq 4, \leq 4.5, \leq 5$ and ≤ 5 ($\times$ 1000 Ft^2) respectively, which reflects the usage of antibiotics is highly adopted in densely populated or large size poultry farms. But overall usage of antibiotics was more than 50%, which indicates the awareness or economic level of the farmers.

The primary health care factor is vaccination of birds against the deadly pathogenic diseases like Newcastle disease and infectious bursal disease, three types of responses were noticed during survey i.e. vaccinated, partially vaccinated and not vaccinated. The farms size ≤ 1, ≤ 1.5, $\leq 2, \leq 2.5, \leq 3, \leq 3.5, \leq 4, \leq 4.5, \leq 5$ and ≤ 5 ($\times$ 1000 Ft^2) were vaccinated 62.34%, 54.88%, 55.61%, 59.59%, 50.38%, 55.32%, 61.67%, 60%, 50% and 66.67% respectively. The non-vaccinated farms in the same groups were 15.58%, 14.63%, 10.73%, 11.11%, 12.21%, 8.51%, 6.67%, 7.5%, 0% and 0% respectively, which showed that overall 8.6% farms were not vaccinated on average, whereas the rest of farms were under the process of vaccination.

The vaccination in the study inspected is categorized into three parts, which showed that more than half of the farms were fully vaccinated, 20 to 50% of farms were partially vaccinated and less than 20% of farms did not't received any kind of vaccine. Similarly, the farms inspected for the availability of antibiotics, showed that more than half of the farmers were practicing antibiotics in their poultry farms. Disinfection of poultry farms is the basic preventive measure that is being adopted before the rearing of birds on farm. In a current study, the adoption of disinfectants in the poultry farms was gauged and concluded that on average 67% of farms have been disinfected to avoid farm pre-available contaminations. Furthermore, this preventive measure has been adopted 50% in low-sized farms.

Biosafety kit is the important tool to keep the bird safe from man-carrying diseases, especially the workers who usually keep in touch with birds for various purposes. The use of biosafety kit was not common in almost all types of sheds except the large farms (Control sheds) with more area; it is the possible reason for infection in the farms. The use of dedicated foot wear on the farms by farmers showed 50:50 results in low area farms,

whereas the increased farm size showed an increased use of special footwear to ensure a safe environment. The use of disinfectants showed a similar pattern as footwear does. The adoptability of biosafety equipment in farms size ≤ 1, ≤ 1.5, $\leq 2, \leq 2.5, \leq 3, \leq 3.5, \leq 4, \leq 4.5, \leq 5$ and ≤ 5 ($\times$ 1000 Ft^2) was 4%, 4.88%, 19.02%, 10.10%, 19.85%, 36.17%, 35%, 60%, 100% and 66.67% respectively, with average of 35.5%. In short, the adoptability of biosafety equipment is recorded in a low percentage as compared to large-sized farms.

Footgears plays key role in the transmission of disease because of carrying oocysts from one place to another with ease. Therefore, the use of separate or decontaminated foot gear is essential to avoid parasites spreading. The 66.79% use of footgear was noticed on average, quite higher than biosafety kit. The reason for avoiding biosafety kits might be the cost or expenditure that farmers were unable to bear on small-sizef farms.

To avoid the spread of pathogenic infections, the bedding must be examined to evade unavoidable circumstances. During the survey of poultry farms in district Peshawar, 81.78% of (average) farms were found with fresh and dry bedding. The feeding parameters like feeders cleanliness, along with the adoption of feeding formula were observed during the survey. Overall, the average of 78.92% of farms were found with clean utensils were no debris or chick fecal materials, whereas in the 84.47% of farms the feed was served to chicks following the standard feeding formula according to age.

The floor material was found dried in more the 70% of the farms in each farm size category, which helps in well grooming of birds. The inspection of the water and food utensils was also found satisfying with more than 80% results of cleanliness. In very less number of large size (5500 ft^2) farms or control sheds the flooring material was found in bad situation showed its best management.

The environment of the farms was examined by assessing certain parameters like indoor temperature, and humidity. The normal or required temperature was maintained in 84.6% (average) farms, which helped in both the development of chicks and inhibition of parasites. The humidity plays a key role in the sporulation of oocysts. Therefore, it is necessary to maintain the normal humidity level, otherwise a high humid environment favors the sporulation of *Eimeria* oocysts. The maintenance of humidity in the surveyed farms of ≤ 1, ≤ 1.5, $\leq 2, \leq 2.5, \leq 3, \leq 3.5, \leq 4, \leq 4.5, \leq 5$ and ≤ 5 ($\times$ 1000 Ft^2) was

92.21%, 88.31%, 76.09%, 85.86%, 81.68%, 70.21%, 88.33%, 92.50%, 62.5% and 100% respectively with an average 83.77%. The freshness of farms was investigated by assessing the fresh air in two ways, either the farms were asphyxiated or not; and the ventilation of farms was also examined; 28.36% (average) of farms were asphyxiated, whereas the ventilation of 78.9% was found satisfactory.

Table 2.5(a): Percentage of farm management parameters adopted in different size of poultry farms

Parameter	Response	Size wise Classes of Poultry Farms (≤, × 1000 Ft^2)									
		1	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**
Antibiotics	**Yes**	50.65	58.54	64.39	63.64	58.78	63.83	68.33	52.50	50.00	66.67
	No	49.35	41.46	35.61	36.36	41.22	36.17	31.67	47.50	50.00	33.33
Vaccination	**Yes**	62.34	54.88	55.61	59.59	50.38	55.32	61.67	60.00	50.00	66.67
	Partial	22.08	30.49	33.66	29.29	37.40	36.17	31.67	32.55	50.00	33.33
	No	15.58	14.63	10.73	11.11	12.21	08.51	06.67	07.50	0.00	0.00
Disinfectant	**Yes**	53.25	58.54	57.56	64.64	61.07	70.21	70.00	65.00	100	66.67
	No	46.75	41.46	42.44	35.35	38.93	29.79	30.00	35.00	0.00	33.33
Biosafety kit	**Yes**	04.00	04.88	19.02	10.10	19.85	36.17	35.00	60.00	100	66.67
	No	96.00	95.12	80.98	89.90	80.15	63.83	65.00	40.00	0.00	33.33
Foot gears	**Yes**	46.75	41.46	53.66	54.55	66.41	85.11	78.33	75.00	100	66.67
	No	53.25	58.54	46.34	45.45	33.59	14.89	21.67	25.00	0.00	33.33
Bedding	**Dry**	84.42	80.49	72.68	79.80	79.39	70.21	83.33	92.50	75.00	100
	Wet	15.58	19.51	27.36	20.20	20.61	29.79	16.67	07.50	25.00	0.00
Feeders	**Clean**	90.91	80.49	86.83	85.86	85.49	82.97	88.33	80.00	75.00	33.33
	Dirty	09.09	19.51	13.17	14.14	14.50	17.02	11.67	20.00	25.00	66.67
Feeding Formula	**Yes**	81.82	78.05	80.00	81.82	86.25	85.11	86.67	90.00	75.00	100
	No	18.18	21.95	20.00	18.18	13.74	14.89	13.33	10.00	25.00	0.00
Temperature	**Maintained**	93.51	92.68	80.00	81.82	82.45	74.47	86.67	92.50	62.50	100
	Not	06.49	07.32	20.00	18.18	23.66	25.53	13.33	7.500	37.50	0.00
Humidity	**Normal**	92.21	88.31	76.09	85.86	81.68	70.21	88.33	92.50	62.50	100
	High	07.79	17.07	23.90	14.14	18.32	29.79	11.67	7.500	37.50	0.00
Asphyxia	**Yes**	20.78	28.05	76.59	25.25	19.08	25.53	28.33	10.00	50.00	0.00
	No	79.22	71.95	23.41	74.75	80.92	74.47	71.67	90.00	50.00	100
Ventilation	**Good**	77.92	79.22	76.10	73.74	80.92	74.47	71.67	92.50	62.50	100
	Bad	22.08	25.61	23.90	26.26	19.08	25.53	28.33	7.50	37.50	0.00

Table 2.5(b): Percent scoring of Farms in different area wise classes

Shed Size	**Percentage of farms in each class**				
(Ft^2 × 1000)	**0 to 20 %**	**21 to 40%**	**41 to 60%**	**61 to 80%**	**81 to 100%**
≤ 1	0.00	0.40	1.73	5.72	2.39
≤ 1.5	0.13	0.80	2.00	5.05	2.93
≤ 2	0.13	1.86	5.19	12.10	7.98
≤ 2.5	0.00	0.80	2.13	5.85	4.39
≤ 3	0.13	0.53	2.66	7.98	6.12
≤ 3.5	0.00	0.53	1.06	1.99	2.66
≤ 4	0.00	0.27	0.93	2.66	4.12
≤ 4.5	0.00	0.00	0.53	1.99	2.79
≤ 5	0.00	0.00	0.13	0.40	0.53
≤ 5.5	0.00	0.00	0.00	0.13	0.26

The whole number of farms were divided into 10 classes for ease; i.e., ≤ 1, ≤ 1.5, ≤ 2, ≤ 2.5, ≤ 3, ≤ 3.5, ≤ 4, ≤ 4.5, ≤ 5 and ≤ 5 (× 1000 Ft^2). In this graph, the farms percent were plotted in the dimensions farm size and score each category secured. The farms of 5.72% in the first category of 1k ft^2 farms, 5.05% in 1.5k ft^2, 12.10% in 2k ft^2, 5.85% in 2.5k ft^2 and 7.98% 3k ft^2 farms were scored between 61 to 80% respectively, whereas 2.39%, 2.93%, 7.98%, 4.39%, 6.12%, 2.66%, 4.12%, 2.79% scored between 81 to 100% in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, and 8th category farms respectively. The rest of the farms in all categories scored less than 60%. Very low numbers of farms were in the 0 to 20% scoring category.

2.5.4. Evaluation of Risk Factor using Binary Logistic Regression

Several risk factors were sorted out to determine the reason of coccidiosis occurrence in the total of 752 poultry farms with an overall 7.2% infection in the district Peshawar Khyber Pakhtunkhwa, Pakistan using binary logistic regression. The factors including vaccination, disinfection of farms, special foot gear and changing the floor material, hygiene of feeders and feeding chicks according to the regular diet formula showed significant ($P < 0.05$) association with the occurrence of coccidiosis. Whereas, the parameters like antibiotics, biosafety kit, and environmental factors (temperature, humidity, asphyxia, and ventilation) were not significantly ($P > 0.05$) associated with coccidiosis infection in recruited poultry farms.

In these positive farms it was further disclosed that certain parameters were not adopted during practicing poultry farming and lead to the occurrence of coccidiosis. In 45.61% of positive farms, the antibiotics were not served to the chicks. Furthermore, 21.05% of farms were not vaccinated against any kind of infectious disease.

The disinfection of farms before rearing chicks is a necessary preventive measure but 87.72% of cocci-positive poultry farms were not disinfected. The biosafety and internal environmental conditions play a key role in keeping the birds healthy and when it is not maintained or controlled it may lead to the occurrence of severe infectious disease, i.e. in 87.72% of cocci-positive poultry farms, the proper biosafety kits or any designated suits were used, whereas in 49.12% of farms, the specific foot wears were not used, which might cause the transmission of *Eimeria* spp. and the bedding was found wet with higher humidity, where both have a role in the developmental stages or lifecycle of the parasite. The fresh air matters a lot and provides enough amount of oxygen to birds. For this purpose, best ventilation is necessary. The survey indicates 61.40% of the cocci positive farms have bad ventilation systems, resulting in creating asphyxia. The *Eimeria* spp. infection can cause low weight gain due to inability of absorbing essential nutrients, so to avoid this case, the captive birds must be fed as per necessity or according to age. In this regard, 50.65% of the cocci positive poultry farms have not followed the feed formula and were fed randomly.

Table 2.5(c): Analysis of various risk factors to assess its effect on the occurrence of coccidiosis using binary logistic regression

Predictors	Cases (%) due to the inadaptability	Coef (3.604)	VIF	Odd Ratio (95% CL)	P
Antibiotics	45.61	-0.407	1.05	0.6657 (0.3337-1.3278)	0.249
Vaccination	21.05	-0.608	1.63	0.5444 (0.2275-1.3027)	0.008*
Disinfectant	50.87	0.352	1.05	0.4935 (0.2477-0.9836)	0.044*
Biosafety kit	87.72	-0.394	1.11	0.6745 (0.2010-2.2636)	0.511
Special shoes	49.12	-0.846	1.15	0.4291 (0.2086-0.824)	0.020*
Bedding	49.12	-1.103	2.16	0.3320 (0.1228-0.8977)	0.033*
Feeders	29.82	-0.873	1.18	0.4178 (0.1855-0.9413)	0.039*
Temperature	35.09	-0.544	1.26	0.5804 (0.2541-1.3260)	0.203
Humidity	49.12	0.251	2.68	1.2852 (0.4205-3.9283)	0.660
Asphyxia	61.40	0.76	12.34	0.4663 (0.0437-4.9705)	0.517
Ventilation	61.40	-1.16	12.59	0.3140 (0.0287-3.4311)	0.354
Feeding formula	59.65	-2.355	1.11	0.0949 (0.0469-0.1922)	0.00*

DISCUSSION

The current study is the first of its nature, in which the postmortem data (2017-2019) of avian coccidiosis from the countryside of Peshawar, Pakistan is studied, comprising the severity, incidence and abundance of in the poultry sector. To find out the extrinsic reason for the coccidiosis emergence in poultry farms, the associated risk factors were studied in the year 2020 including farm hygiene, feed, and medication etc. which provides the possible reason for the prevalence of avian coccidiosis in the previous years of the same catchment area included in the current study.

The average maximum temperature of district Peshawar is 40°C with a minimum range of 25°C in the summer season, whereas in winter both the extremes range in between 4 to 18°C. The geographical location of district Peshawar receives heavy rainfall in the months of the winter season, in this aspect both the temperature and precipitation factors lead to the emergence of coccidiosis due to favorable conditions for sporulation (Begum *et al*., 2021). Similarly, the Gawhatti state (Lat: 26.12°N; Long: 91.71°E) of India has the same climatic condition as Peshawar. That's why high coccidial emergence was reported in October (Buragohain *et al*., 2013). In contrast, Quetta city (Lat: 30.17°N; Long: 66.97°E) with somewhat cold tempperature than Peshawar has reported with higher prevalence in the months of June and July (Ali *et al*., 2014), means that the cities with opposite climatic condition have huge influence on the parasite dissemination. The Jammu and Kashmir (Lat: 33.27°N; Long: 75.34°E) is also reported with high avian coccidiosis in July because the overall climatic condition is different from Peshawar and there feel a little bit mild type temperature and humidity which favors *Eimeria* oocysts sporulation (Sharma *et al*., 2015).

The occurrence of avian coccidiosis was found high during the months of April, May, November and December during the tenure of the current study, whereas the rest of months there were low number of cases. The study of Fadunsin *et al*., (2020) supports the current data by reporting maximum numbers of cases in the same months as the current study, whereas the months with high temperature and low humidity have lesser number of cases. The current study reports large number of cases from rural area of Peshawar, and low percent of avian coccidiosis is recorded there. The possible reason for which might be

that poultry farming is usually practiced in countryside (Cervantes *et al*., 2020), where the infection rate is less than 5% (Lawal *et al*., 2016) may be due to a healthy environment and low expenditure. That's why the chance of infection is low.

The postmortem study states that unhygienic farms play a significant (P = 0.014) role in the dissemination of severe forms of disease with higher rate in young age chicks (Tadesse and Teshome, 2018; Ayana *et al*., 2017). A postmortem study revealed, poultry sector faced a huge economic loss in 2019, due to avian coccidiosis (Rashid *et al*., 2019).

The month-wise or geographic difference in coccidiosis prevalence in different villages of district Peshawar, Morgoglione *et al*., (2020) described such variance is due to biotic (age) and abiotic (hygiene, medication and environmental conditions) factors. Tamrat (2020) reported that the coccidiosis occurrence is tightly associated with hosts' age. This is the only reason that the maximum number of cases in current study was reported in the middle aged broilers, i.e. 15 to 30 days, prior to this, the chance of coccidiosis is low due to low farm density, prepatent period of *Eimeria* species which is from 5 to 7 days in case of different species.

The humid environment favors the development of *Eimeria* oocysts, so that its propagation will be higher in the month with more precipitation. That's why the current study area (Peshawar) has low coccidiosis cases in summer as compared to winter because the temperature exceed high levels in summer, left with no humid situation (Olabode *et al*., 2020). Jansen and coworkers (2020) mentioned that the coccidial occurrence is closely linked with humidity and precipitation. That's why the winter season in the current study area is reported with maximum number of cases. The possible reason for this is maximum oocyst sporulation, which is infective for birds otherwise the ingestion of unsporulated oocyst does not have any effect upon the host.

Good management plays a vital role in the health issues of poultry birds. If one has a farm of a million dollars' worth but fails to maintain it, the farm will eventually face huge economic loss. In this regard, the intensive mode of poultry farming encourages the severe prevalence of coccidiosis due to mismanagement of hygienic conditions, temperature, humidity, ventilation of the room. Therefore such farms house coccidiosis and other such diseases (Mohammed and Sunday, 2015). Olabode *et al*., reported the low rate of coccidiosis at an early age, similar to the findings of current research, which might be due

to the intensive care as the low age chicks covers a little farm area, which is easy to handle. Furthermore, the stocking density in this age is very low and the chicks don not experience densely population place (Olabode *et al*., 2020).

The bedding of the farm plays an effective role in farming. In this regard, when the bedding contents were ingested by chicks in the cocci positive farms, it will certainly parasitize the healthy birds. In this regard, Gazoni *et al*., (2020), reported the prevalence of *E. maxima* at a higher rate, with the bed scarping diagnosed in the intestinal mucosa during microscopic examination, but the good management of bedding in the current study. According to the recent study of Carrisosa (2021), the non-commercial poultry practices or small-scale farmers were unaware of biosafety or biosecurity concept of parasite dissemination from infected farms through certain carrying agents, which leads to severe type of coccidial infection in the poultry farm. Similar to this research the low level adoption of biosafety parameters in current study was also reported, which is the possible reason of infection the catchment area of veterinary research institute, Peshawar.

According to the observations of Wondimu *et al*., (2019), in all three types of poultry farming, the intensive way of bird keeping requires a lot of attention, if not handled carefully and the bird's population or the floor remains unhygienic or any other mishap like poor ventilation and asphyxia was experienced by the birds inside, it will have bad consequences that facilitates the coccidial emergence, in the light of Wondimu's study the current study concludes that maximum of the cases reported were practicing intensive way of farming with huge number of birds. The cases of coccidial infection from the poultry farms in the respective areas were certainly reported due to the appearance of prominent signs of blood traces in feces, that has led to the destruction of intestinal linning of the infected birds and cause malabsorbtion of nutrients which appear in the form of severe weight loss and high feed consumption with poor FCR (Olabode *et al*., 2020). When the birds do not absorb sufficient amount of nutrient from the feed due to coccidiosis, it will not only cause low protein (weight loss) production but will effect egg production severely (Irvine, 2006). The birds requires a suitable environment for best growth and nourishment. That's why the flooring of the farm must be clean, fresh and dry. The literature revealed that fresh and dry bedding contain such microbes that digest the *Eimeria* oocysts (Osorio *et al*., 2020).

CONCLUSION

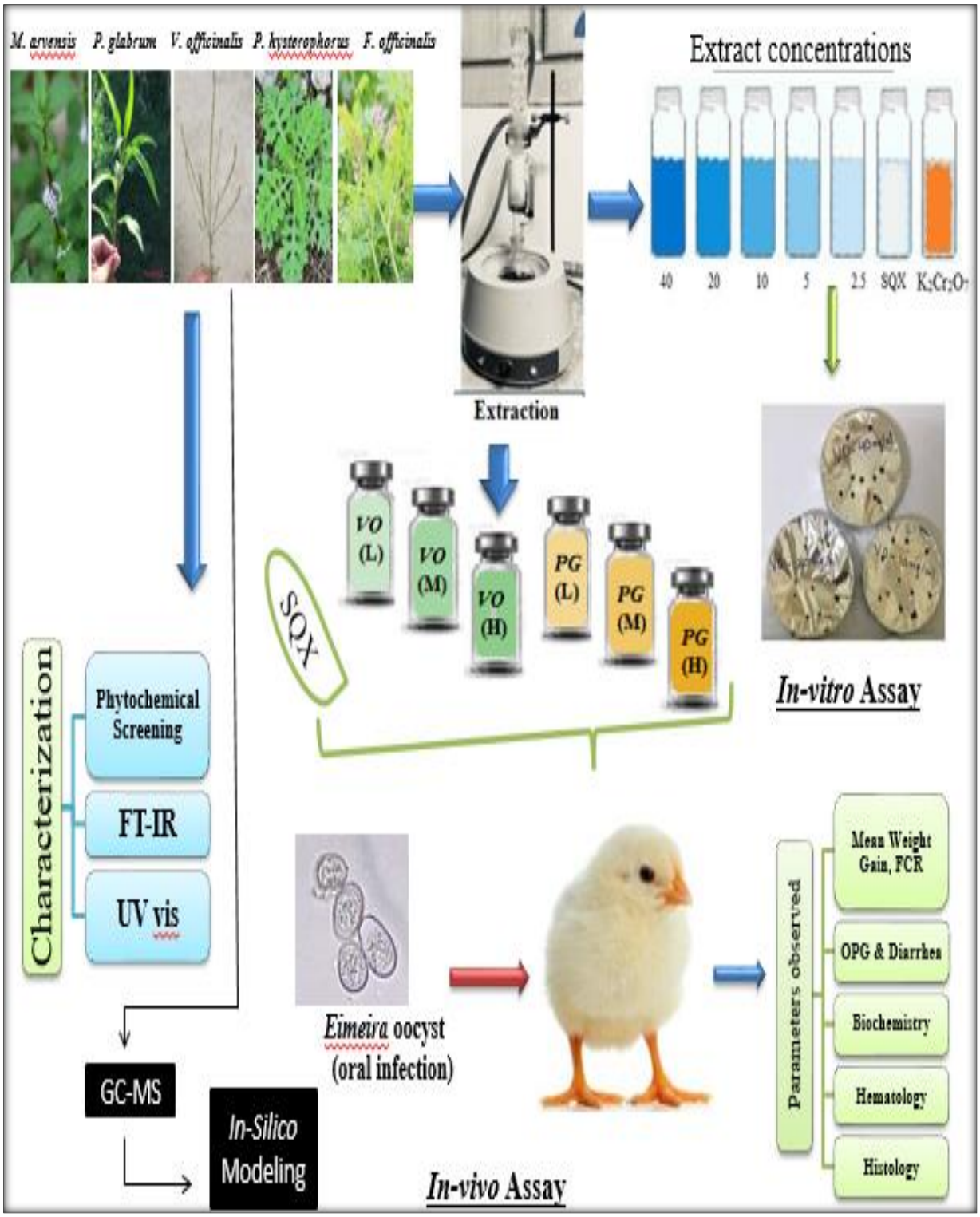
The present study focuses on the prevalence of coccidiosis in Distric Peshawar, Pakistan during the study period from 2017 to 2019 including survalene in 2020. The maximum prevalence was reported in the year 2017 and in the months with low temperatue and high humidity. The prevalence of coccidiosis and other parasitic diseases can be reduced to get more yield considering associated risk factors like management system, provision of supplements or vaccination, improve hygienic conditions, adoption of biosecurity and biosafety protocols and the forecast informations like humidity , weather onditions etc.

Further studies are recommended the formulation of new cost effective drugs to replace currently used resistant once.

Chapter: 03

Anticoccidial activity of medicinal herbs: Characterization and *In-silico* modeling of *V. officinalis* as a potential drug source

GRAPHICAL ABSTRACT



ABSTRACT

In the current study five pharmaceutically active herbs were used against avian coccidiosis. The aqueous and methanolic extracts of whole plants were applied *in-vitro* for a sporulation inhibition (SPI) assay to calculate the inhibitory concentration (IC_{50}). The percent SPI of the methanolic extract of *V. officinalis* and *P. glabrum* showed maximum percent sporulation inhibition of 81.04% and 72.47% and minimum IC_{50} of 0.14 and 12 mg/ml, respectively, followed by *F. officinalis, P. hysterophorus,* and *M. arvensis* with less than 50% SPI and IC_{50} of 36, 38.52, and 37.19 mg/ml against *E. tenella* despite its medicinal value.

Polygonum glabrum and *V. officinalis* were selected for *in-vivo* anticoccidial activity, for which nine groups of 14-day-old broiler chicks were infected with *E. tenella,* and three groups were treated with different concentrations of methanolic extracts of *V. officinalis* and *P. glabrum* post-infection. The mean weight gain, oocyst count, diarrhea, biochemical tests, hematology, and histopathology of all groups were analyzed. Further, the one-way Anova "tukey" test was used for comparison of the effectiveness of all herbs with that of the activity of sulfaquinoxaline; therefore, it is concluded that both of the effective herbs were subjected to *in-vivo* anticoccidial activity against the *E. tenella*-infected broiler chicks.

The anticoccidial active herbs were characterized by antioxidant assay, phytochemical screening, FT-IR, UV-Vis spectroscopy, and GC-MS analysis. The GC-MS' identified phytocompounds of *V. officinalis* were docked with S-Adenosyl methionine (SAM) synthetase. The *in-vivo* experiment showed that *V. officinalis* had significantly high anticoccidial potential with a significant hematological profile, like drug-treated controls. The histology of treated chicks also showed recovery in the studied tissues. The antioxidant assay showed that *V. officinalis* has 4.19 U/mg superoxide dismutase and 33.96 µM/mg glutathione quantities. The characterization confirmed the presence of a large number of organic compounds. Strychane, 1-acetyl-20a-hydroxy-16-methylene has the best binding with the target protein, with the lowest binding score (-6.4 Kcal/mol), suggesting its anticoccidial potential in poultry.

MATERIALS AND METHODS

3.1. Bioethical Approval

This study was approved by the Quaid-i-Azam University Bioethics Committee and received protocol number BEC-FBS-QAU2022-418.

Table 3.1: Identification and classification of the medicinal experimented herbs used for anticoccidial activity

S. No	Plant	Local name	Classification		Pictogram	Reference
1	*Mentha arvensis*	Corn Mint	Kingdom	Plantae		Kundalic *et al.*, 2009
			Phylum	Spermatophyta		
			Class	Dicotyledonae		
			Order	Lamiales		
			Family	Lamiaceae		
			Genus	*Mentha*		
			Species	*Arvensis*		
2	*Polygonum glabrum*	Dense flower knotweed	Kingdom	Plantae		Raja and Ramya, 2017
			Phylum	Tracheophyta		
			Class	Magnoliopsida		
			Order	Caryophyllales		
			Family	Polygonacae		
			Genus	*Polygonum*		
			Species	*Glabrum*		
3	*Verbena officinalis*	Vervain	Kingdom	Plantae		Munir, 2002
			Phylum	Tracheophyta		
			Class	Asterids		
			Order	Lamiales		
			Family	Verbenaceae		
			Genus	*Verbena*		
			Species	*Officinalis*		
4	*Fumaria officinalis*	Fumitory	Kingdom	Plantae		Snafi and Esmail, 2020
			Phylum	Spermatophytina		
			Class	Magnoliopsida		
			Order	Ranunculales		
			Family	Papaveraceae		
			Genus	*Fumaria*		
			Species	*officinalis*		
5	*Parthinum hysterophorus*	Carrot grass	Kingdom	Plantae		Kohli and Daizy, 1994
			Phylum	Tracheophytes		
			Class	Asterides		
			Order	Asterales		
			Family	Asteraceae		
			Genus	*Parthinum*		
			Species	*hysterophorus*		

3.2.1. Plants Sampling and Extraction

The aerial parts of selected herbs (Table 3.1) were collected from district Mardan, Pakistan (34.3410°N, 72.2897°E). The flora were washed, shade dried (27-37°C), grounded, and passed through 60 hole/cm mesh to obtain fine powder. The crude methanolic and aqueous extracts were prepared from respective plant powder using soxhlet extraction apparatus (Shanghai^{R} Heqi, China) by taking 30 gm powder in filter paper and 300 ml solvent at 65°C for 6 hours. The extracts were concentrated by evaporating solvents using a rotary evaporator (RE-5299). The crude extracts were stored in the refrigerator at 4°C. The stock solutions of each extract were prepared by dissolving 1 gm of crude extract in 100 ml of distal water, which is further diluted into 5, 10, and 15 mg/ml using standard dilution formula i.e. $V_1=C_2\times V_2/C_1$ (Shah *et al*., 2017). Whereas V_1: total volume, V_2: required volume, C_1: stock solution concentration and C_2: required Concentration. The percent extract yield of each experimented herb was calculated in grams by using the standard formula (Nwonuma *et al*., 2019).

$$\text{Percent Yield} = \frac{\text{Actual dry Yield (g)}}{\text{Plant Powder used (g)}} \times 100$$

3.2.2. Quantitative Analysis, FT-IR and UV Visible Spectroscopy

The presence of phenols, flavonoids, steroids, glycosides, alkaloids, tannins, plobatannins, anthocynins, leucoanthocynins, couramins, and anthraquinones in the herbal extracts was evaluated by different phytochemical tests mentioned in table 3.2 (Roghini and Vijayalakshmi, 2018). The functional groups of the organic compounds were characterized by FT-IR spectroscopy (Bruker Platinum ATR) in the range of 4000 to 500 cm^{-1} in the selected plants extract (Ullah *et al*., 2020). The proximate analysis of the respective plants was conducted using a spectrophotometer (Cecil CE-7400) with 2 mm wide slit using 10 mm cell under UV-Vis light from 200 to 800 nm wavelength to calculate the band gap with the help of absorption spectra. The samples were diluted with same solvent (Jain *et al*., 2016).

Table 3.2: Phytochemical tests for qualitative analysis of the essential ingredients

Phyto-compound	Test	Reaction/ Reagent	Result
Phenol		1 ml extract + Lead acetate	Ppt formation
Flavonoids	Alkaline test	2 ml extract + 1 ml NaOH	Yellow Colour
Steroids	Salkvoski test	1 ml extract + 1 ml chloroform + few drop H_2SO_4	Brown ring
Terpenoids	Salkvoski test	0.5 ml extract + 2 ml chloroform + Conc H_2SO_4	Brown Colour
Glycosides		2 ml extract + 3 ml chloroform + 10% NH_3	Pink Colour
Alkaloids	Wagner test	1 ml extract + 1 ml wagner's reagent	Redish brown ppt
Tannins	$FeCl_3$ test	1 ml extract + 5% $FeCl_3$	Greenish black
Plobatannins	Ppt test	1 ml extract + 2 ml 1%HCl + heat	Red ppt
Anthocynins	NaOH test	1 ml extract + 1 ml NaOH + 5min 100°C	Blue green colour
Leuco anthocynins		Isoamyl alcohol	Organic layer into red
Couramins		1 ml extract + 1 ml 10%NaOH	Yellow Colour
Anthraquinnones	Ppt test	1 ml extract + 10% NH_3	Pink ppt

3.2.3. GC-MS Analysis of *V. officinalis*

The phytochemical investigation of methanolic crude extract of *V. officinalis* was carried out with Thermo MS DSQ (version: 2.0.7) equipment. The conditions followed were according to standard protocol, TR 5-MS capillary column with 30 Mts, ID: 0.25 mm, Film thickness: 0.25 mm. Flow rate of mobile phase (Carrier gas: He) was set at 1.0 ml/min dimensions. In GC part temperature was 40° C, gradually raised to 250° C at 5° C/min. The sample injection volume was 1 ml and run at the range of 50-650 m/z. The mass spectrum was analyzed using the Main EI-MS Library (mainlib) data base with 242,464 reference spectra and Replicate spectra Library (replib) with 33,782 reference spectra (Kanthal *et al*., 2014).

3.3. Antioxidant Potential

The antioxidant potential of the active (*in-vitro*) plant extracts was analyzed by estimating the level SOD and GSH along with total protein concentration. The SOD level was determined by standard protocol (Mathew *et al*., 2011) using nitroblue tetrazolium reagent at 560 nm with the help of spectroscopy. The GSH was determined using the method defined by Jollow and coworkers (Jollow *et al*., 1974) with the wavelength of 412 nm. The total protein content of the plant extracts was estimated by the standard Bradford assay (λ = 595 nm).

3.4. *In-vitro* bioassay

3.4.1. Parasite Sampling and Identification

The confirmed oocysts of *E. tenella* suspension and infected chicks' ceca (Fig. 3.1a, b) was obtained from poultry research institute (District: Rawalpindi, 33.4389°N, 73.0437°E) in 2% $K_2Cr_2O_7$. The oocyst suspension was filtered with fine cloth gauze, following the salt floatation method, sodium chloride salt was added to suspension till it become supersaturated, and the suspension was centrifuged at 3000 rpm for 10 minutes, precipitating the organic debris to harvest pure *E. tenella* oocysts and reconfirmed under microscope. The species was further identified with COCCIMORPH software (400X magnifications); the software was downloaded from website "coccidia.isb.usp.br/coccimorph" (Eassa *et al*., 2019).

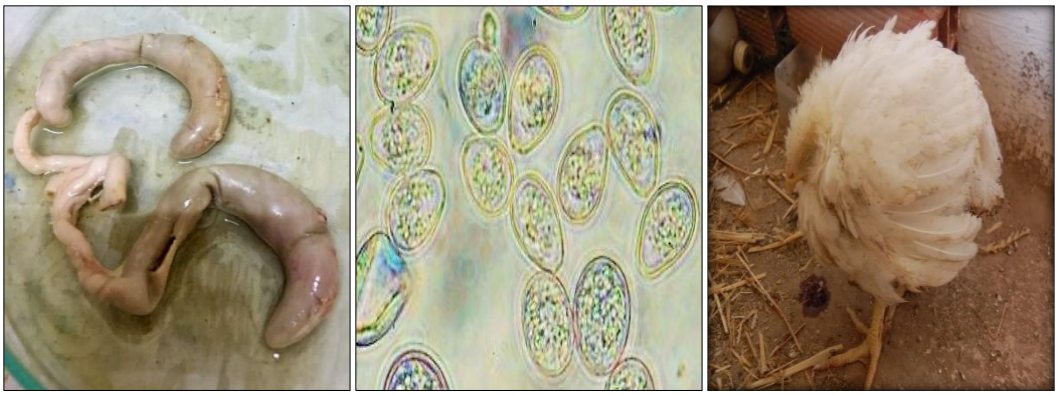


Figure 3.1: (a) *E. tenella* infected ceca (b) *E. tenella* sporulated oocysts (c) and *Eimeria* infected host

3.4.2. Sporulation Inhibition Assay

The *in-vitro*, anticoccidial potential of the *F. officinalis*, *P. hysterophorus*, *P. glabrum*, *V. officinalis* and *M. arvensis* was measured by inhibition of oocyst sporulation. The experiment was carried out in triplicate in petri dishes, provided with 3 ml of various concentrations (40, 20, 10, 5, and 2.5 mg/ml), inoculated with 1500 unsporulated oocysts, and incubated at room temperature. The petri dishes were covered with perforated aluminum foil to maintain oxygen supply. In parallel, $K_2Cr_2O_7$ (Potassium dichromate) treated group was kept as negative control and sulfaquinoxaline (SQX) treated group as a positive control, which inhibits sporulation. The percent sporulated and unsporulated oocysts were counted with a neubauer chamber after 48 and 72 hours of incubation under a stereo microscope (40X), to calculate the percent SPI using the formula (Abbas *et al*., 2015).

$$Percent\ sporulation(sp)\ inhibition = \frac{Sp\ \%\ of\ control - Sp\ \%\ of\ extract}{Sp\ \%\ of\ control} \times 100$$

The sporulation inhibition data were analyzed with probit analysis that cause 50% SPI "IC_{50} and IC_{90} values" to calculate the exact concentration of plant extract (Monzote *et al*., 2014).

3.5. *In-vivo* Anticoccidial Bioassay

3.5.1. Oocysts Sporulation

The unsporulated oocysts were settled down and separated in fresh 2% $K_2Cr_2O_7$ solution for sporulation at 21 to 32°C to become infective in the presence of oxygen and

moisture (Molan *et al*., 2009). The sporulating oocysts (Fig. 3.1b) were identified under microscope (Olympus-CX41) (Eassa *et al*., 2019).

3.5.2. Animals and Treatment

One hundred and fifty "Ross 308" chicks (1 day old) were purchased from commercial hatchery (District: Rawalpindi) and reared in an animal house facility (Department of Zoology, Quaid-i-Azam University, Islamabad) provided with suitable environmental conditions. Initially, the temperature was maintained in the range of 85 to 90°F and later on reduced by 5°F weekly. The chicks were fed with coccidiostat-free feed and water *ad libitum*, supplemented with vitamins, and vaccinated against Newcastle and infectious bursal diseases (Williams, 2006).

The *in-vivo* trial of the methanolic herbal extract was conducted which remains effective through *in-vitro* assay, for this purpose 135 healthy chicks (14 days old) were divided into nine groups each containing 15 individuals. All the groups except C1 were infected with (microtitre = 7.5×10^3 to 10×10^3 sporulated oocyst/ml) *E. tenella* orally. All groups were found infected after 4 to 7 days, and different parameters like weight loss, oocyst count, and diarrhea were observed to ensure the occurrence of coccidiosis. The first three groups were designated as control C1 (normal), C2 (Infected and Untreated), and C3 (Infected and SQX treated), the three infected groups (E1, E2, and E3) were treated with 5, 10, and 15 mg/ml concentration of *V. officinalis* and the groups (E4, E5 and E6) was treated with the 5, 10, and 15 mg/ml of *P. glabrum* respectively (Akhtar *et al*., 2012). The treated groups were fed for five days from age 21 days with a dose of 1 ml/bird/day as per prescribed SQX dose (Kaingu *et al*., 2017).

3.5.3. Evaluation of Anticoccidial Activity

Birds Performance or Physical Factors

The average weight of an individual from each group was measured on daily bases with the help of an electronic scale to calculate the MWG (Mean Weight Gain) post-infection and post-treatment, the post-infection and post-treatment FCR of all groups were estimated by calculating the feed consumed by individuals of each group on daily basis (Youn and Noh, 2001). The following formulae were used to calculate MWG and FCR.

$$\mathrm{MWG} = \mathrm{MFW} - \mathrm{MIW} + \mathrm{WDB}$$

$$\text{FCR} = \frac{Total\ Feed\ Consumed}{MWG}$$

MFW- Mean Final Weight, WDB- Weight of Dead Birds,

3.5.3.1. Fecal Examination

The number of oocysts was analyzed by taking the fecal sample in both chambers of the McMaster slide with the help of a dropper after mixing 1 gm of feces in 14 ml saturated sucrose solution from each group and observed at 4X magnification under the (Olympus-CX41) microscope (Zhang *et al*., 2013).

$$OPG = (Oocysts\ in\ Chamber\ 1 + \ Oocysts\ in\ Chamber\ 2) \times 50$$

The presence of blood in feces is one of the major clinical signs of avian coccidiosis, which showed the severity of the disease, to assess the effectiveness of methanolic plant extracts on the infected chicks bloody diarrhea was observed on daily basis with a naked eye and graded from low to high and presented in the heat map, which indicates its severity both pre and post-treatment (Habibi *et al*., 2016).

3.5.3.2. Sample Collection and Processing

The blood was collected from the jugular vein of individual from each group, after then the same individuals was euthanized and dissected for tissue collection and other anatomical observations.

3.5.3.2.1. Hematology

The blood samples were collected in EDTA tubes from each group, thrice at the interval of 7 days i.e. 21, 28, and 35 days age, for hematological analysis like hemoglobin level, red blood cells, white blood cells, granulocytes, lymphocytes, monocytes, and platelets of all groups both post-infection and post-treatment for comparison (Campbell, 2008).

3.5.3.2.2. Biochemical Analysis

The blood was collected in the anticoagulant-free tubes from each group and centrifuged at 2500 rpm for 15 min to obtain serum for the estimation of macromolecules at the age 21st, 28th, and 35th day. The protein content was estimated by using "Lowery protein estimation method" at the wavelength of 750 nm. The carbohydrate level of each

group was assessed by the phenol sulfuric acid method at the absorption of 480 nm using a spectrophotometer (Shah *et al*., 2017). In addition, the liver performance of each group was analyzed by estimating AST (Aspartate Aminotransaminase) and ALT (Alanine Aminotransferase), post-infection and post-treatment using an AMP diagnostics kit at the wavelength of 340 nm, respectively with a chemistry analyzer (Motenu-MTN 658C) (Al Mathal, 2010).

3.5.3.3. Histology

To investigate the effect of methanolic extract of *V. officinalis* and *P. glabrum* on the anatomy of *E. tenella* infected groups the ceca, liver, and kidney tissues were observed both post-infection and post-treatment using a standard protocol (Deepa *et al*., 2020) and studied under the microscope with noticeable variations were photographed (Olympus-CX41 with Tucsen Camera) with 10X/0.25 resolution.

3.6. Molecular Modeling

3.6.1. *In-Silico* Preparation of Protein Target

The 3D crystallographic structure of the SAM synthetase was acquired from the Protein Drug Bank in PDB format (PDB ID: 1FUG). Prior to preparation the ligands found in protein structure were removed via PyMOL software 2.1.0 (Schrodinger, 2015), water molecules was removed to reduce interference using AutoDock Tools and polar hydrogen molecules and Kollman United Atom charges were assigned to protein molecule. The protein were then converted into the PDB format with partial charges ('Q') and AutoDock 4 atom types ('T') (PDBQT) file format and saved for later use (Trott and Olson, 2010).

3.6.2. *In-silico* Preparation of Ligand Molecule

The phytocomponds of *V. officinalis* methanolic extract identified with GC-MS (Table 3.4) were used as the ligand for molecular docking with SAM synthetase. The 3D structures of all 22 compounds were constructed and saved in .sdf format, using Chemdraw (11) then converted into PDB format via PyMOL software and minimized by computing Gasteiger charges and finally converted to PDBQT file format using AutoDock Tools (Trott and Olson, 2010).

3.6.3. Molecular Docking Simulation

The prepared protein and ligand were called out via AutoDock Tools to compute suitable grid maps protein–ligands combination. During the docking process, AutoDock 4.2 was used; the center grid parameters were set to 26.536, 17.719, and 58.288 for the x-, y-, and z-axis. The grid center was kept at 26 for x-, y-, and z-axis in angstrom with spacing of 1.00 and located at the center of the active site. A configuration file that consists of the grid box properties was created and saved as .txt file format. Then, docking was carried out using AutoDock Vina (1.5.6) by inserting command lines in the Command Prompt application to generate the output score and the best fit model (mode 1) was selected from the 22 different conformations generated for each ligand (Trott and Olson, 2010). Interacting amino acid residues that were found in the binding site were visualized using LigPlot+ software. Amino acid residues exhibiting hydrogen bonding and hydrophobic interactions with each ligand molecule were summarized in Table 3.9 (Laskowski and Swindells, 2011).

3.7. Statistical Analysis

The IC_{50} concentration of aqueous and methanolic extracts of *F. officinalis*, *P. hysterophorus*, *M. arvensis*, *V. officinalis*, and *P. glabrum* against *E. tenella* were calculated with probit analysis to find out the exact concentration to cause 50% SPI using Minitab (version 19). Further, the one-way ANOVA test was applied on percent SPI, MWG, FCR, OPG, hematological and biochemical parameters to check HSD using Statistix software (version 9), and comparisons were made authentic by Tukey test to confirm the significance among the groups with $P < 0.05$ and presented in the form of means and standard deviation (Fadunsin and Ademola, 2014).

RESULTS

3.8.1. Plants Extract Percent Yield

The extract of all experimented herbs were made for the anticoccidial efficacy in both aqueous and methanolic solvents. For each reaction 300 ml solvent and 30 gm herbal powder was used. The reaction was run for 6 hours with 25 to 28 cycles per reaction. For every herb the reaction was repeated 4 times to get sufficient amount of yield. The percent yield of methanolic extracts of *M. arvensis*, *P. hysterophorus*, *P. glabrum*, *V. officinalis*, *and F. officinalis* was 4.25%, 3.47%, 3.25%, and 2.91%, 2.69% respectively (Table 3.3).

Table 3.3: Percent yield of crude extract of experimental herbs (A: Aqueous; M: Methanolic)

S. No	Plant	Solvent	Solvent/ reaction (ml)	Powder/ reaction (gm)	Number of reactions	Extract (gm)	Percent Yield
1	*M. arvensis*	A	300	30	4	4.3	**3.50 %**
		M	300	30	4	5.1	**4.25 %**
2	*P. glabrum*	A	300	30	4	2.7	**2.25 %**
		M	300	30	4	3.9	**3.25 %**
3	*V. officinalis*	A	300	30	4	2.5	**2.08 %**
		M	300	30	4	3.5	**2.91%**
4	*F. officinalis*	A	300	30	4	4.40	**3.6%**
		M	300	30	4	3.23	**2.69%**
5	*P. hysterophorus*	A	300	30	4	4.16	**3.47%**
		M	300	30	4	3.26	**2.71%**

3.8.2. Phytochemical Screening

3.8.2.1. Qualitative Analysis

The phytochemical screening of the aqueous and methanolic extracts of all experimented herbs has been conduct, adopting different screening tests for each components, represented in the form of heat map chart (Fig. 3.2). In current study, the presence of sixteen different compounds were tested in the extracts of selected plants, belong to different classes, first 14 compounds were micro compounds whereas the rest of four belong to macro molecules. The compounds found in both type of extracts of *V. officinalis* are phenols, flavonoids, alkaloids, anthracynins, leuco anthocynins, couramins, whereas the macromolecules found in the same plant are cellulose and starch,

anthraquinones are the only compounds found only in aqueous extract of *V. officinalis*. Seven group of compounds (phenols, steroids, terpenoids, leuco anthocynins, couramins, carbohydrates and starch) are commonly present in both aqueous and methanolic extracts of *P. glabrum*. The methanolic extracts of *M. arvensis* has flavonoids, glycosides, anthracynins, leuco anthracynins, couramins, anthraquinones, protein, cellulose and starch in integral composition. Both type of *M. arvensis* extracts are containing phenols and Alkaloids and terpenoids in the aqueous extract. The phytochemical screening of *F. officinalis* showed the presence of flavonoids, steroids, glycosides, alkaloids, anthracynins, plobatannins, couramins, anthraquinones, cellulose and starch in the aqueous extract, in addition, terpenoids, plobatannins, couramins, anthraquinones, and cellulose are found in the methanolic extract of the same plant. *P. hysterophorus* reflects the presence of terpenoids, tannins, plobatannins, leuco anthocynins, couramins, anthraquinones and carbohydrates in both types of extracts. Further, the compounds found only in aqueous extracts are flavonoids, whereas steroids and glycosides are detected in the methanolic extract of *P. hysterophorus*.

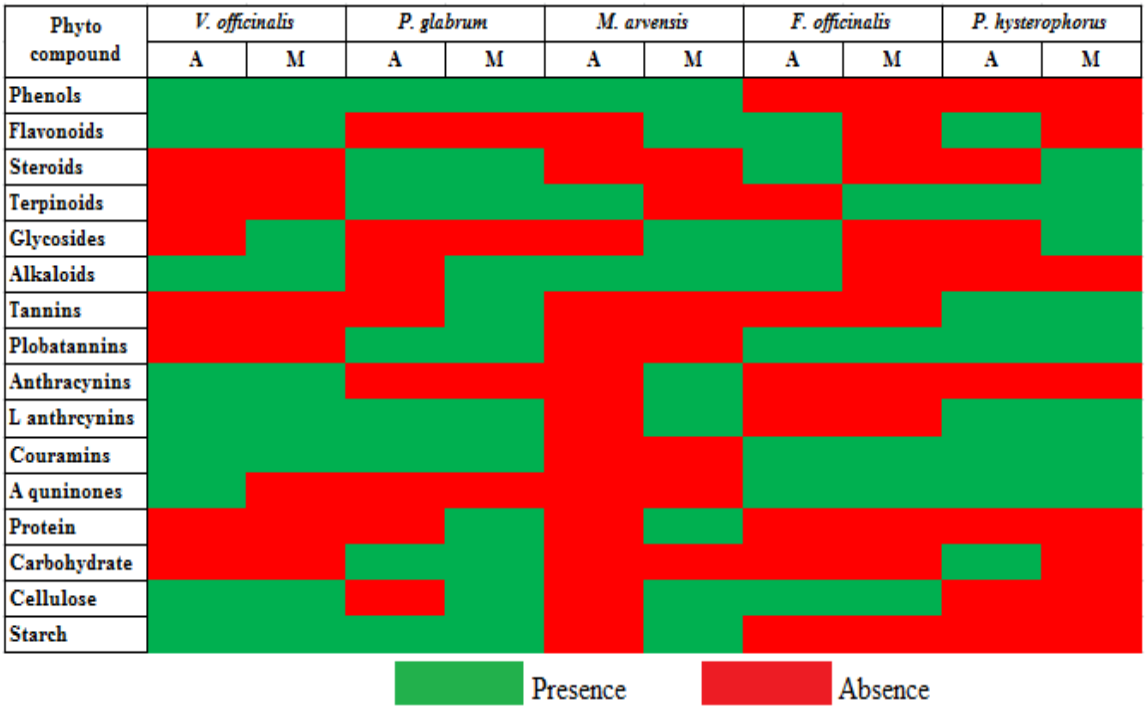


Figure 3.2: Heat map represented the presence or absence of the components in the aqueous (A) and methanolic (M) extracts of the selected plants

3.8.2.2. FT-IR Spectroscopy

The FT-IR spectra of both *V. officinalis* and *P. glabrum* showed several bands in the wavelength range 4000-500 cm^{-1} and most of them are common in both with the exception that more bands appeared in *V. officinalis* (Fig. 3.3a). Each wave number was studied in detail with reference ranges in the literature (Pavia *et al*., 2003). The region of the broad absorption band at 3550-3000 cm^{-1} was observed in all the respective herbal extracts which might be assigned to the presence of an alcoholic or phenolic group; the broadness of the band might be attributed to the presence of possible hydrogen bonding. It is further supported by another common absorptions band at 1113 cm^{-1} which indicates the presence of C–O functionality. At the same time; we cannot neglect the possible presence of the amine group as well, as its absorption band also appears above 3100 cm^{-1} which is again present in both herbs and might have been overlapped by the O–H broad absorption. The C–N absorption band also appears in almost the same region in which C–O absorbs. All herbs that share an absorption band below 3000 cm^{-1} may be assigned to the C–H sp^3 absorption. The band at above 2500 cm^{-1} may be attributed to the presence of thiols (S–H) functionality. The absorption band appearing at 1660 cm^{-1} may be due to the presence of C=O or C=N moiety. Absorption wave bands at 1440 and 1414 cm^{-1} may be assigned to the $–CH_2-$ bending. The wavelength absorption band at 1020 cm^{-1} may be due to the C–F stretch and the final absorption wavelength band near 600 cm^{-1} may be due to the C–Cl or C–I stretch (Fig: 3.3b).

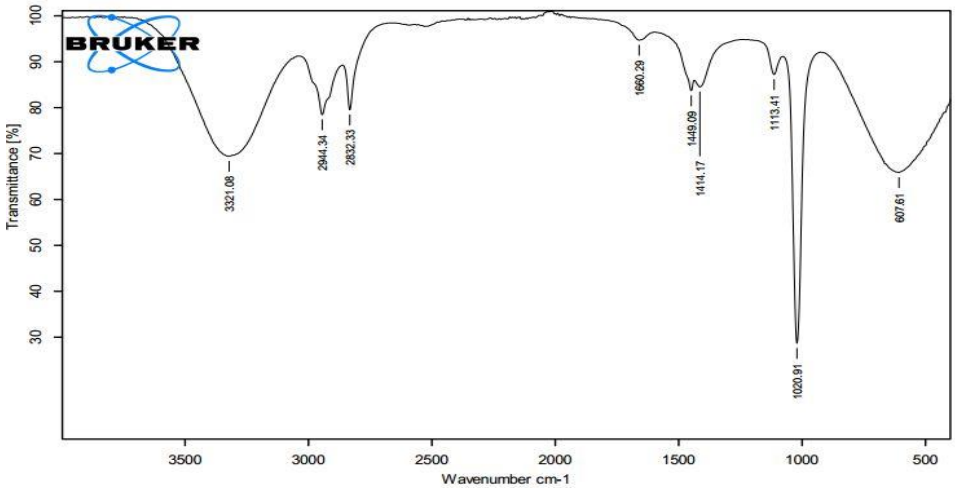


Figure 3.3a: FT-IR spectra of *V. officinalis* aqueous extract

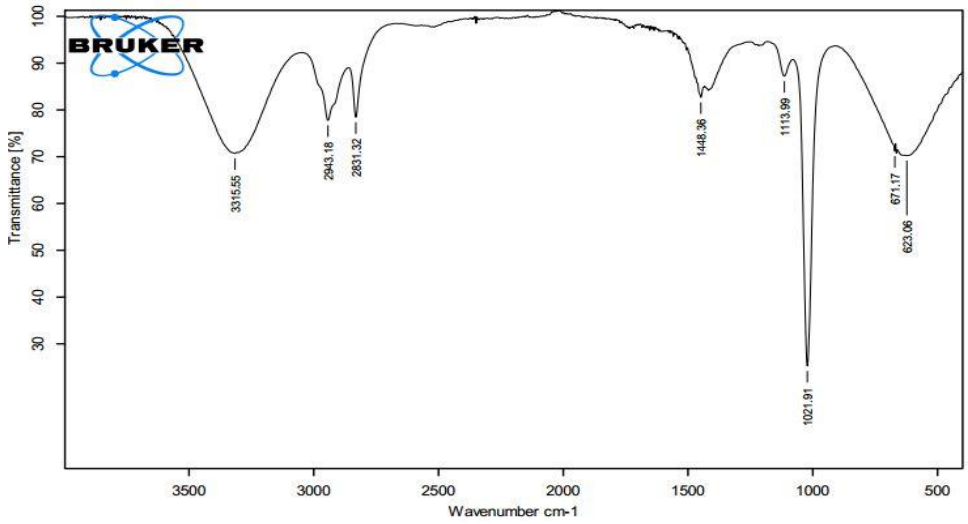


Figure 3.3b: FT-IR spectra of *P. glabrum* aqueous extract

3.8.2.3. UV-Vis Spectroscopy

The UV-Vis analysis was performed for the identification of the phytochemical composition of *V. officinalis, and P. glabrum,* to identify the compounds with σ & π bonds, lone pair of electrons, chromophores, and aromatic rings. The qualitative spectrum was selected on the bases of sharp peaks and proper baseline falling in the range of 200 to 800 nm wavelength.

The UV-Vis profile of *V. officinalis* methanolic extract was observed in the range of wavelength of 200 to 800 nm. The profile showed various peaks at 226.13, 289.68, 332.71, 410.11, and 665.29 nm with the absorption of 3.78, 1.52, 1.82, 0.54, and 0.18 respectively. It showed the indication of hetero atoms (S, N, and O), Unsaturated alkenes in the UV region with π-π* transition, and carbonyls in the visible region with n-π* transition.

The last peak of 665.29 nm showed the presence of N=O chromophores (Fig. 3.4a). The profile of *P. glabrum* showed the peak of 395 nm with an absorption of 2.4 in the UV region, which indicates the presence of aromatic compounds with π-π* transition, and the rest of the two peaks in the visible region at 610 and 670 nm with the absorption of 0.18 and 0.54 respectively, which indicates the presence of carbonyls with n-π* transition (Fig. 3.4b).

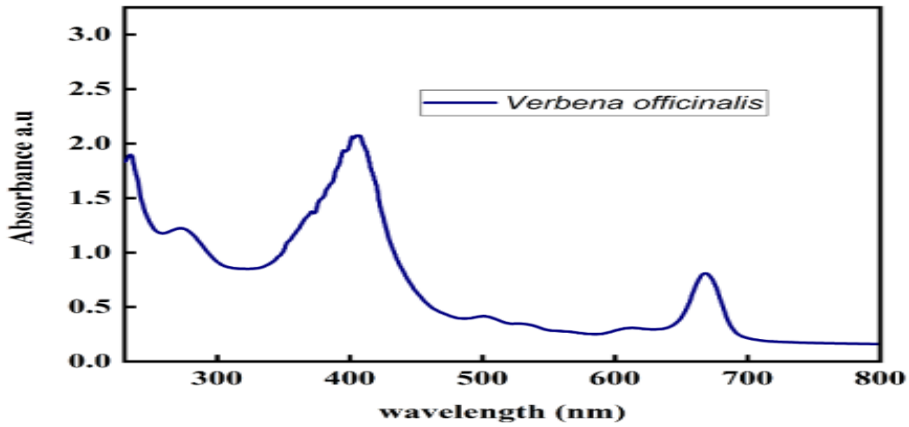


Figure 3.4(a): Absorption curves of methanolic extract of *V. officinalis*

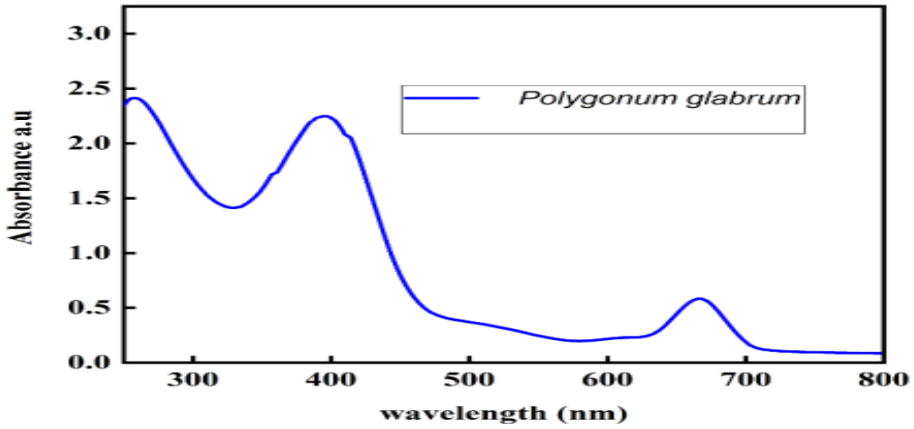


Figure 3.4(b): Absorption curves of methanolic extract of *P. glabrum*

3.8.2.4. GC-MS of *V. officinalis*

The GC chromatogram (Fig. 3.5) of methanolic herbal extracts of *V. officinalis* specified twenty one peaks, which refers to the presence of organic compounds. GC-MS is suitable technique used for detection of volatile compound, includes alkanes (Aliphatic hydrocarbons), carboxylic acid, phenol, esters, ether and triterpenes. Retention time (minutes), percent peak area, molecular formula, molecular weight can be used for identification of phytochemical components. Out of the whole compound detected 7 compounds area present in abundance covering high peak area percentage i.e. Hexadecane (4.84%), Sulfurous acid, Octadecane (3.34%), Squalene (4.75%), Hexacosane (8.11%), Octacosane (8.47%), Tetratetracontane (5.95%), and Pentacosane (14.18%) was reported as

a major constituents in methanolic herbal extract of *V. officinalis* whereas the rest of compounds mention in table 3.4, was present in minor quantity.

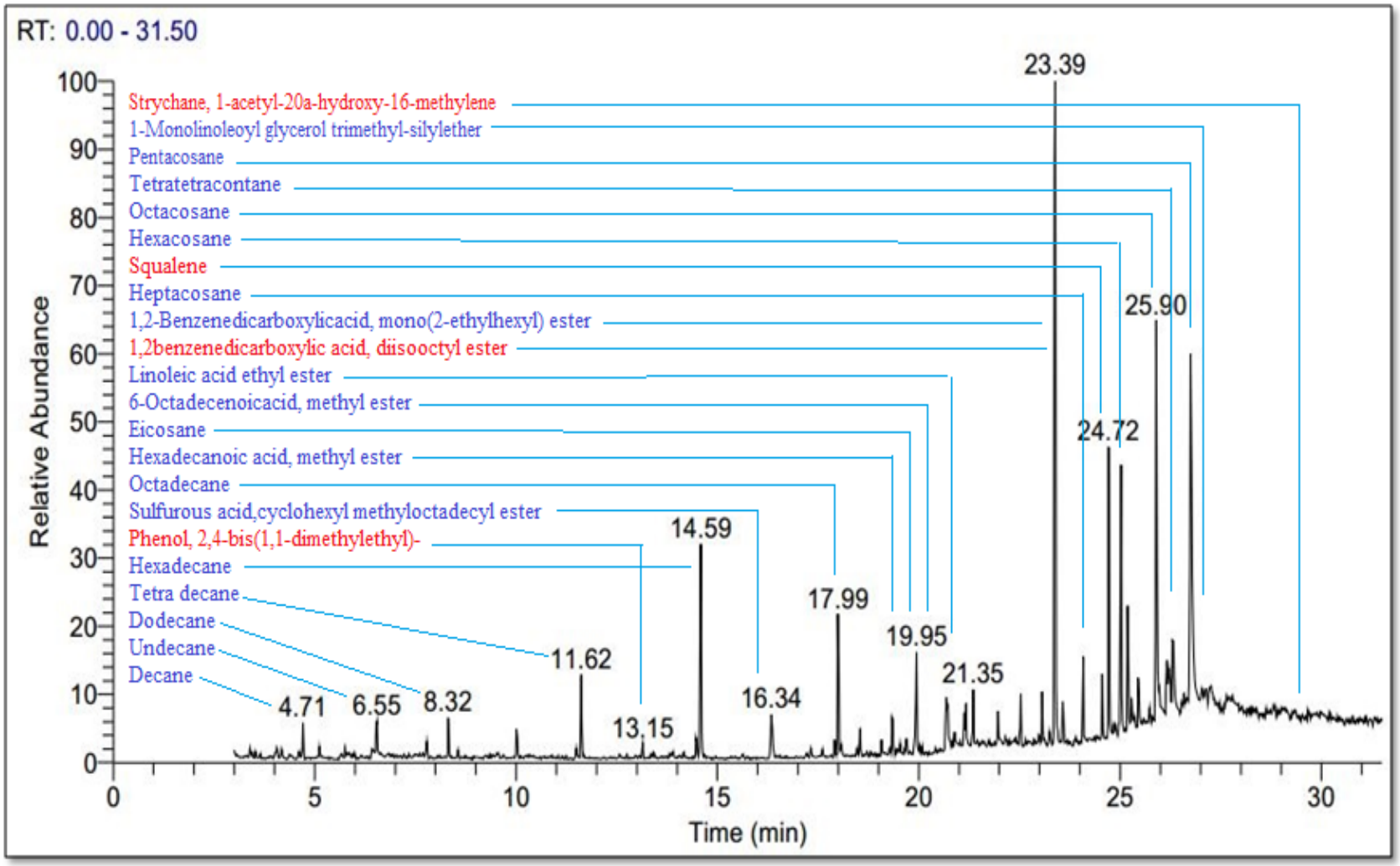
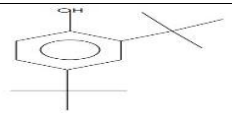
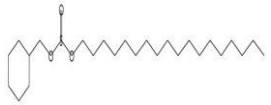
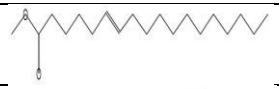
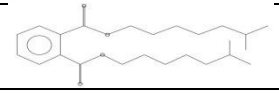
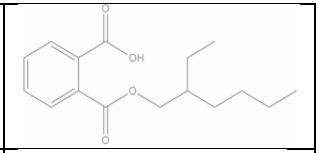
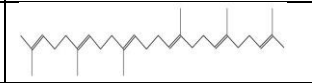
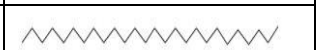
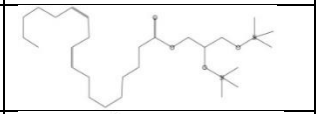
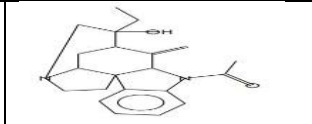


Figure 3.5: GC-MS chromatogram of crude extract of *V. officinalis*

Table 3.4: GC-MS analysis of methanolic crude extract of *V. officinalis*

P. No	RT	P. Area %	MF	MW	Compound	Nature	Structure
1	4.71	0.64	$C_{10}H_{22}$	142	Decane	Aliphatic hydrocarbon	
2	6.52	2.29	$C_{11}H_{24}$	156	Undecane	Aliphatic hydrocarbon	
3	8.32	0.69	$C_{12}H_{26}$	170	Dodecane	Aliphatic hydrocarbon	
4	11.62	1.63	$C_{14}H_{30}$	198	Tetradecane	Aliphatic hydrocarbon	
5	13.14	0.21	$C_{14}H_{22}O$	206	Phenol, 2,4-bis(1,1-dimethylethyl)-	Phenol	
6	14.59	4.84	$C_{16}H_{34}$	226	Hexadecane	Alkane	
7	16.34	1.31	$C_{25}H_{50}O_3S$	430	Sulfurous acid, cyclohexyl methyl octadecyl ester	Ester	
8	17.99	3.34	$C_{18}H_{38}$	254	Octadecane	Aliphatic hydrocarbon	
9	19.34	1.98	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester	Ester	
10	19.95	2.43	$C_{20}H_{42}$	282	Eicosane	Aliphatic hydrocarbon	
11	20.70	2.69	$C_{19}H_{36}O_2$	296	6-Octadecenoic acid, methyl ester	Fatty acid ester	
12	21.15	2.70	$C_{20}H_{36}O_2$	308	Linoleic acid ethyl ester	Ester	
13	23.39	16.49	$C_{24}H_{38}O_4$	390	1,2benzenedicarboxylic acid, diisooctyl ester	Ester	

14	23.39	16.49	$C_{16}H_{22}O_4$	278	1,2-Benzene dicarboxylic acid, mono(2-ethylhexyl) ester	Ester	
15	24.08	1.34	$C_{27}H_{56}$	380	Heptacosane	Aliphatic hydrocarbon	
16	24.72	4.75	$C_{30}H_{50}$	410	Squalene	Triterpene	
17	25.02	8.11	$C_{26}H_{54}$	366	Hexacosane	Aliphatic hydrocarbon	
18	25.90	8.47	$C_{28}H_{58}$	394	Octacosane	Aliphatic hydrocarbon	
19	26.28	5.95	$C_{44}H_{90}$	618	Tetratetracontane	Aliphatic hydrocarbon	
20	26.77	14.18	$C_{25}H_{52}$	352	Pentacosane	Aliphatic hydrocarbon	
21	27.73	1.63	$C_{27}H_{54}O_4Si_2$	498	1-Monolinoleoyl glycerol trimethyl-silyl ether	Ether	
22	29.05	1.73	$C_{21}H_{26}N_2O_2$	338	Strychane, 1-acetyl-20a-hydroxy-16-methylene	Ester	

P. No: Peak number, RT: Retention time, MF: Molecular Formula, MW: Molecular weight

3.9. Antioxidant Potential

As for the biochemical composition of plant extracts concerned, the SOD, GSH, and Protein contents were estimated. The level SOD and GSH is recorded high in *V. officinalis* than *P. glabrum*. The amount of protein content is almost the same in *V. officinalis* and *P. glabrum* as shown in the figure 3.6.

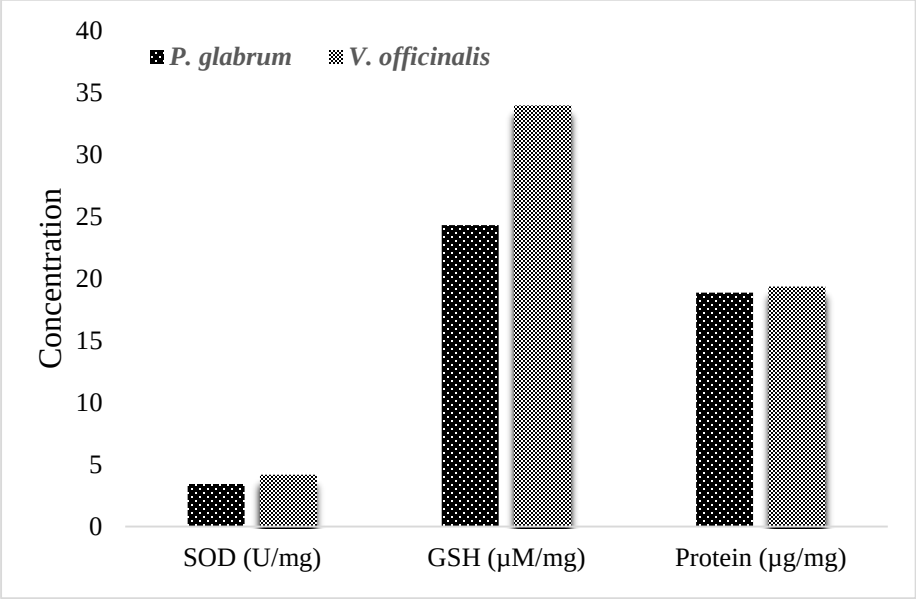


Figure 3.6: Antioxidant activities of *P. glabrum* and *V. officinalis*

3.10. *In-vitro* Anticoccidial Assay

3.10.1. Sporulation Inhibition Assay

The unsporulated *E. tenella* oocysts was used for *in-vitro* SPI -assay. In this regard five medicinally important herbs (*M. arvensis, V. officinalis, P. glabrum, F. officinalis* and *P. hysterophorus*) belong to different families and same geographical location was selected for the anticoccidial activity against *Eimeria tenella*.

In this regard the SPI of various concentrations of the methanolic and aqueous extracts of the said herbs was conducted along with the control groups treated with $K_2Cr_2O_7$ and SQX. The $K_2Cr_2O_7$ treated ensures maximum sporulation, whereas the SQX treated group showed maximum inhibition rate, both of the control groups ensures the stability of environmental conditions for experimental trail. In terms of sporulation inhibition the results can be summarized as *V. officinalis* > *P. glabrum* > *P. hysterophorus* > *M. arvensis* > *F. officinalis*, further the results of inhibiting sporulation of *E. tenella* oocysts as a result

of methanolic herbal extracts can be elaborated as *V. officinalis* showed more than 80% inhibition i.e. 81.04±0.96 %, *P. glabrum* came with results of more than 70% inhibition rate i.e. 72.47±2.65%, whereas the rest of three plants showed less than 50% sporulation inhibition i.e. 47.59±1.41, 47.26±1.40 and 44.56±1.13 treated with 40 mg/ml respectively after the incubation time of 48 hours (Table 3.5), further the sporulation rate of oocysts in the $K_2Cr_2O_7$ treated groups was recorded more than 90% i.e. this groups has shown overall inhibition of less than 10%, whereas the SQX treated groups has shown more than 90% inhibition of the oocysts to sporulate.

Adding to it, the common phenomenon found in case of all extract is that the rate sporulation inhibition decreases with the decreasing amount of the extracts i.e. 40 > 20 > 10 > 5 > 2.5 mg/ml respectively. Further, the sporulation inhibition of all the extract treated groups were re-observed after 72 hours of incubation, this time all groups showed somewhat lowered sporulation inhibition rate but this decreasing rate varied among all the plants, for instance the SPI at 40 mg/ml the methanolic extract of *V. officinalis* dropped with 4.44% to 76.60±1.33%, the SPI of methanolic extract of 40 mg/ml of *P. glabrum* is declined by 6.5% to 65.97±0.27%, the methanolic extract of 40 mg/ml of *M. arvensis* is reported with 13.2% declining rate to 34.06±0.58%,

The same concentration of the methanolic extract of *F. officinalis* has shown 29.94±0.92% inhibition, which almost decrease by 14.62%, and *P. hysterophorus* by 13.72% to 33.87±0.58%, so the declining rate of sporulation inhibition from after 24hr of first observation can be summarized as *F. officinalis* > *P. hysterophorus* > *M. arvensis* > *P. glabrum* > *V. officinalis*, the low declining sporulation inhibition rate of *V. officinalis* defines its stable and long lasting effect against the *Eimeria tenella* inhibition and control.

3.10.2. Probit Analysis

The $K_2Cr_2O_7$ and SQX treated groups were run as a control for comparative analysis. To analyze the potential of the selected plant extracts, the IC_{50} was estimated with probit analysis, which showed activity of *V. officinalis* at 48 hours in both methanolic and aqueous extracts shown in table 3.6 and Fig. 3.7, Though *P. glabrum* interrupted the sporulation process but the rate of inhibition was found lower than that of *V. officinalis* extract. The inhibitory activity of *M. arvensis, F officinalis* and *P. hysterophorus* is not remarkable so that's why the IC_{50} value signals that much amount of crude extract is required to inhibit oocysts sporulation.

Table 3.5: Sporulation Inhibition percent of aqueous and methanolic extracts of selected plants after incubation period of 48 and 72 hours (A: Aqueous; M: Methanolic)

Plant	E	IP (hr)	2.5 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml	40 mg/ml	$K_2Cr_2O_7$	SQX
M. arvensis	A	48	8.71±0.45^{o}	10.37±1.10^{o}	17.55±0.62^{mn}	21.54±0.45^{klm}	41.10±0.44^{ef}	2.03±0.76^{p}	95.73±1.05^{a}
		72	9.77±0.71^{p}	9.59±0.09^{p}	14.63±1.19^{n}	21.69±0.62^{jk}	30.97±1.59^{h}	5.83±0.45^{q}	97.03±0.79^{a}
	M	48	6.59±0.70^{pq}	12.11±1.35^{op}	15.58±1.43^{no}	30.15±1.13^{ijk}	47.26±1.40^{f}	2.68±0.89^{q}	97.27±0.19^{a}
		72	3.94±0.80^{n}	10.46±1.27^{m}	11.47±0.28^{m}	29.46±0.32^{hij}	34.06±0.58^{gh}	7.13±0.25^{mn}	97.03±0.79^{a}
V. officinalis	A	48	25.16±3.13^{jk}	41.86±0.61^{e}	57.12±0.89^{d}	66.15±1.22^{c}	75.11±0.79^{b}	2.77±0.81^{p}	96.10±2.27^{a}
		72	21.10±0.78^{kl}	38.26±1.14^{f}	52.89±0.48^{d}	63.30±0.09^{c}	72.87±0.60^{b}	4.0±0.81^{q}	97.10±0.17^{a}
	M	48	27.30±2.39^{k}	43.18±1.03^{fg}	58.14±0.94^{e}	67.59±0.71^{cd}	81.04±0.96^{b}	2.3±0.35^{q}	96.53±1.29^{a}
		72	24.62±0.46^{jk}	40.15±0.99^{ef}	52.87±0.65^{d}	61.89±0.86^{c}	76.60±1.33^{b}	5.45±0.78^{mn}	97.27±0.06^{a}
P. glabrum	A	48	21.28±1.89^{klm}	30.07±2.05^{hi}	39.98±1.04^{ef}	59.15±2.60^{d}	64.87±1.63^{c}	2.17±0.61^{p}	96.47±2.41^{a}
		72	10.30±1.32^{p}	23.69±2.05^{ijk}	29.85±1.40^{h}	46.17±0.39^{e}	51.35±1.11^{d}	4.77±0.76^{q}	97.83±0.55^{a}
	M	48	25.05±2.39^{kl}	34.82±2.43^{hi}	44.21±4.03^{fg}	62.48±1.7^{de}	72.47±2.65^{c}	3.12±1.26^{q}	97.71±0.46^{a}
		72	11.90±3.06^{m}	24.77±1.98^{jk}	31.45±1.99^{hi}	51.58±0.72^{d}	65.97±0.27^{c}	5.27±1.50^{mn}	97.60±0.27^{a}
F. officinalis	A	48	19.51±3.28^{lmn}	26.54±1.59^{ij}	31.33±1.37^{hi}	36.89±1.68^{fg}	42.35±0.24^{e}	2.0±0.61^{p}	94.20±0.87^{a}
		72	13.66±0.72^{no}	18.17±0.57^{lm}	24.34±1.22^{ij}	29.93±0.16^{h}	34.35±0.45^{g}	4.03±1.19^{q}	98.22±1.56^{a}
	M	48	9.87±0.70^{op}	18.91±1.35^{mn}	24.46±1.43^{klm}	35.76±1.13^{hi}	47.59±1.41^{f}	4.38±0.89^{q}	93.10±0.19^{a}
		72	10.53±0.62^{kl}	13.42±1.06^{ij}	20.64±0.27^{gh}	25.90±0.49^{fg}	29.94±0.92^{e}	4.20±0.46^{mn}	96.73± 0.93^{a}
P. hysterophorus	A	48	5.64±0.45^{op}	15.76±1.10^{n}	22.74±0.62^{jkl}	33.40±0.44^{gh}	44.40±0.44^{e}	3.37±0.76^{p}	96.10±1.05^{a}
		72	3.78± 0.71^{no}	11.13±0.09^{lm}	15.36±1.19^{ij}	25.22±0.62^{i}	32.67 ±1.59^{gh}	4.23±0.45^{q}	97.27±0.79^{a}
	M	48	9.87±0.70^{op}	18.91±1.35^{mn}	24.46±1.43^{klm}	35.76±1.13^{hi}	47.59±1.41^{f}	4.38±0.89^{q}	93.10±0.19^{a}
		72	4.28±0.80^{n}	12.12±1.27^{m}	18.83±0.27^{l}	25.80±0.32^{jk}	33.87±0.58^{gh}	4.73±0.25^{mn}	97.87±0.79^{a}

E: extract solvent, A: aqeous, M: methanol, IP: incubation period, SQX: sulfaquinoxaline

Table 3.6: Inhibitory concentration (IC_{50}) of aqueous and methanolic plant extracts against *E. tenella* oocysts

Plant	Extract	IP (hr)	IC_{50} (mg/ml) (UCL-LCL)	IC_{90} (mg/ml) (UCL-LCL)	P
M. arvensis	A	48	45.14±4.2 (38.41-55.91)	84.61±9.13 (70.30-108.62)	0.139
		72	59.81±8.56 (47.27-85.76)	114±18.60 (88.18-172.19)	0.613
	M	48	39.22±3.04 (34.16-46.61)	73.63±6.68 (62.86-90.38)	0.157
		72	49.27±5.10 (41.26-62.83)	91.83±10.97 (74.97-121.65)	0.024
V. officinalis	A	48	16.16±1.35 (13.59-19.02)	46.88±3.69 (40.75-55.80)	0.00
		72	18.19±1.42 (15.55-21.23)	49.06±3.85 (42.68-58.34)	0.00
	M	48	14.69±1.19 (12.41-17.17)	41.82±3.03 (36.74-48.99)	0.00
		72	16.83±1.35 (14.30-19.70)	46.91±3.64 (40.85-55.67)	0.00
P. glabrum	A	48	23.41±1.77 (20.27-27.42)	57.26±4.84 (49.34-69.13)	0.00
		72	32.83±2.78 (28.19-39.62)	72.00±7.18 (60.57-90.40)	0.00
	M	48	19.43±1.48 (16.72-22.65)	50.66±4.00 (44.03-60.33)	0.00
		72	26.03±1.75 (22.94-29.99)	56.45±4.30 (49.30-66.77)	0.01
F. officinalis	A	48	42.07±5.42 (33.86-57.63)	99.00±15.01 (77.01-143.6)	0.00
		72	53.16±7.73 (41.85-76.78)	112.77±18.81 (85.84-171.42)	0.012
	M	48	38.52±4.66 (31.32-51.50)	93.16±13.37 (73.33-131.97)	0.00
		72	60.79±9.69 (46.96-91.87)	122.58±22.06 (91.60-194.46)	0.056
P. hysterophorus	A	48	39.97±3.51 (34.25-48.76)	78.88±10.11 (66.06-99.87)	0.003
		72	52.06±5.87 (43.01-68.17)	97.36±12.61 (78.29-132.62)	0.059
	M	48	37.19±3.27 (31.82-45.36)	76.99±7.96 (64.41-97.61)	0.008
		72	49.85±5.49 (41.34-64.75)	95.02±12.06 (76.69-128.47)	0.016

(A: Aqueous, M: Methanolic, IP: Incubation Period)

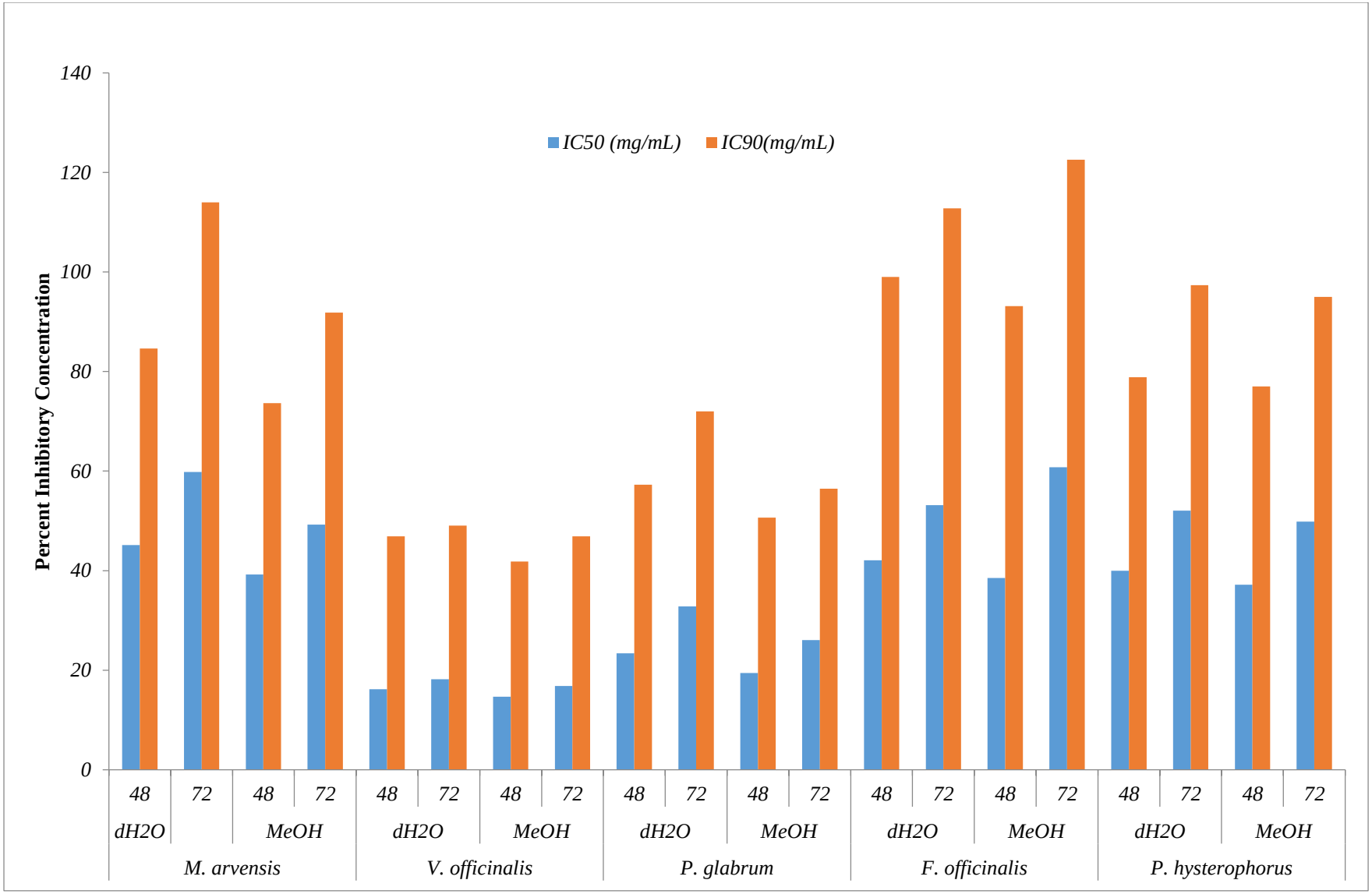


Figure 3.7: Graphical representation of IC_{50} & IC_{90} concentrations of the experimented plants

3.11. *In-vivo* Anticoccidial Activity

The anticoccidial activity of *in-vitro* active herbs were investigated in the *Eimeria* infected Ross-308 chicks, treated with different concentrations of *P. glabrum* and *V. officinalis.*

3.11. Birds Performance

3.11.1. Mean Weight Gain and Feed Conversion Ratio

The two basic parameters of MWG and FCR of all experimental groups were calculated thrice at different ages i.e. 21st, 28th, and 35th day age with the interval of 7 days. The summary of the average weight gain showed the medium concentration (10 mg/ml) of *V. officinalis* and a high dose (15 mg/ml) of *P. glabrum* were found effective against *E. tenella* oocysts sporulation and the individuals were found with enough mean weight gain. Adding to it, the MWG of the chicks as a result of the effective concentration "E2"of *V. officinalis* was 290±30.3, 313±22.8, and 621±6.9 gm MWG. Whereas, group E6 treated with *P. glabrum* (15 mg/ml) was noticed with 250±16, 292±11 and 513±10.5 gm MWG at day 21, 28 and 35 *resp*ectively, the results showed direct relation between MWG and time after drug administration i.e. with the passage of time the MWG of the chick's increases. In the present *in-vivo* study, the detail analysis of MWG of different experimented groups confirms the effectiveness of *V. officinalis* methanolic extract as compared to *P. glabrum* crude extract. The activity of both plant extracts were assured by comparing the MWG of the respective treated groups with SQX treated control group (C3) i.e. 259±17.6, 437±10.3, and 534±6.3 gm at the respective age, which is almost near to the MWG of E2 and E6. In short, the booster effect of methanolic crude extracts of both plants resulted in maximum weight gain with growing age, without harming the chicks (Table 3.7).

3.11.2. Oocysts Count and Diarrhea Grading

The oocyst count of each group was counted under microscope using the McMaster chamber on daily basis till the end of the experiment. In all infected groups, the number of oocysts was decreased after oral treatment with methanolic crude extract of *V. officinalis* and *P. glabrum to the chicks.* The uninfected group "C1" remained negative for *E. tenella* oocysts, while the fecal sample of group C2 was found with a huge number of oocysts i.e. 21,850 OPG, C3 showed 5250 OPG at age of 18 days and raised to 10,300

OPG at 23 days age and after treatment with SQX, it dropped to 3100 OPG at the age of 35 days. The group (E2) treated with *V. officinalis* (10 mg/ml) was diagnosed with 6950 OPG at the age of 17 days and rises to a peak level of 10,900 OPG at day 25 age, but when the respective methanolic extract came into action, the OPG dropped to 3950 oocysts. Similarly, the group E6 was initially reported with 5150 OPG at 18 day age and gradually raised to 12,750 OPG at 27 day age and after treatment with *P. glabrum* (15 mg/ml), the OPG dropped to 5700 oocyst sat 35 day age. In short, the methanolic crude extract of both plants has an inhibitory role in the sporulation of *E. tenella* oocysts but *V. officinalis* was found more potent with low number of OPG at the end of trial (Fig. 3.8).

Table 3.7: The mean weight gain (gm) and feed conversion ratio of broilers groups at age day 21 and 28 & 35

Treatment		**Mean weight gain (gm)**			**Feed Conversion Ratio**		
	Groups	**7th DPI**	**7th DPT**	**14th DPT**	**7th DPI**	**7th DPT**	**14th DPT**
Control	C1	435 ± 18^{a}	525 ± 10^{a}	700 ± 23^{a}	1.1^{f}	1.27^{f}	1.29^{f}
	C2	242 ± 15^{b}	185 ± 9.6^{e}	143 ± 1.5^{g}	2.14^{cd}	2.59^{a}	3.07^{a}
	C3	259 ± 17.6^{b}	437 ± 10.3^{b}	534 ± 6.3^{b}	1.87^{e}	1.50^{e}	1.87^{d}
V. officinalis	E1	270 ± 9.5^{b}	316 ± 24.7^{cd}	293 ± 67.9^{e}	1.98^{de}	1.72^{de}	2.29^{c}
	E2	290 ± 30.3^{b}	313 ± 22.8^{c}	621 ± 6.9^{bc}	2.67^{ab}	1.95^{cd}	1.95^{d}
	E3	283 ± 15.6^{b}	283 ± 15.6^{b}	493 ± 84^{cd}	1.99^{de}	1.57^{e}	1.69^{e}
P. glabrum	E4	270 ± 27.5^{b}	270 ± 27.5^{b}	278 ± 25^{f}	2.79^{a}	2.22^{b}	2.79^{b}
	E5	264 ± 1.6^{b}	264 ± 1.6^{b}	439 ± 65.2^{e}	2.39^{bc}	2.07^{bc}	2.00^{d}
	E6	250 ± 16^{b}	250 ± 16^{b}	513 ± 10.5^{d}	2.09^{de}	1.88^{cd}	1.71^{e}

Bloody diarrhea appeared within 7 days in all the *E. tenella* infected chicks' groups after oral infection. The appearance of diarrhea varied in different groups and even reached the severe level, but after the treatment on day 21 age with the respective concentrations and the severity of coccidiosis decreased due to its healing potential i.e. E2 and E3 treated with (medium and high concentration) *V. officinalis* recovered at 6th day of treatment, SQX treated group recovered at 7th day & *P. glabrum* treated groups took 9 days to recover from

diarrhea after treatment and ultimately bloody diarrhea slowly disappeared with the healing potential of methanolic extracts of *V. officinalis* and *P. glabrum* (Fig. 3.9).

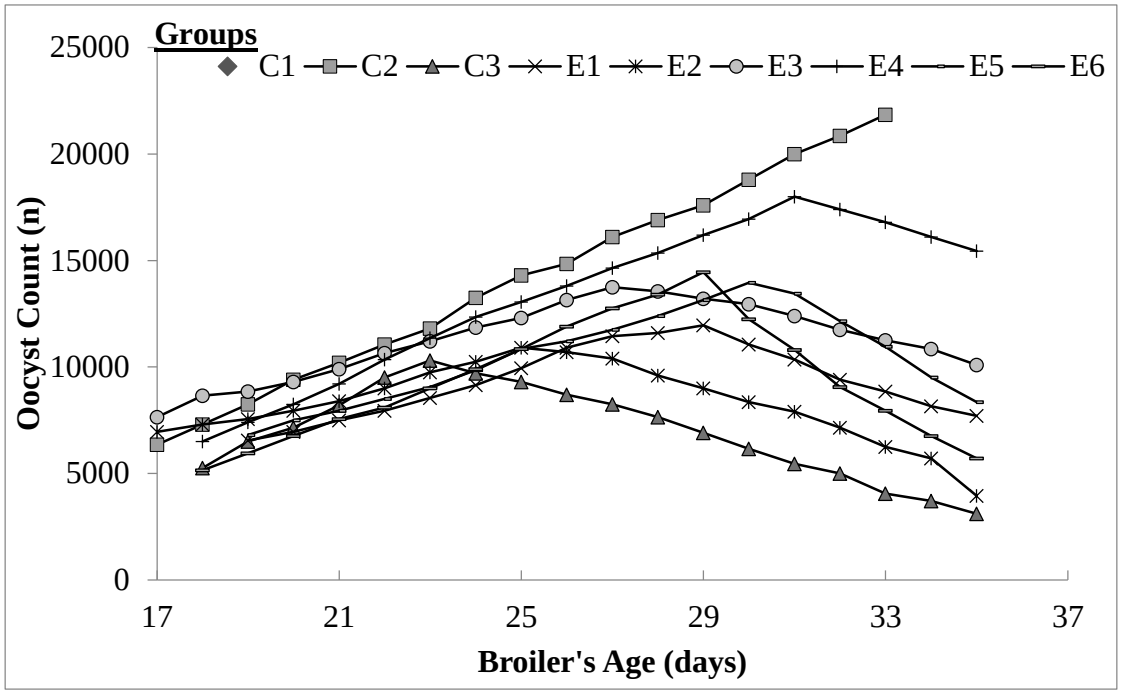


Figure 3.8: The oocyst count (OPG) of *E. tenella* diagnosed in various broiler groups

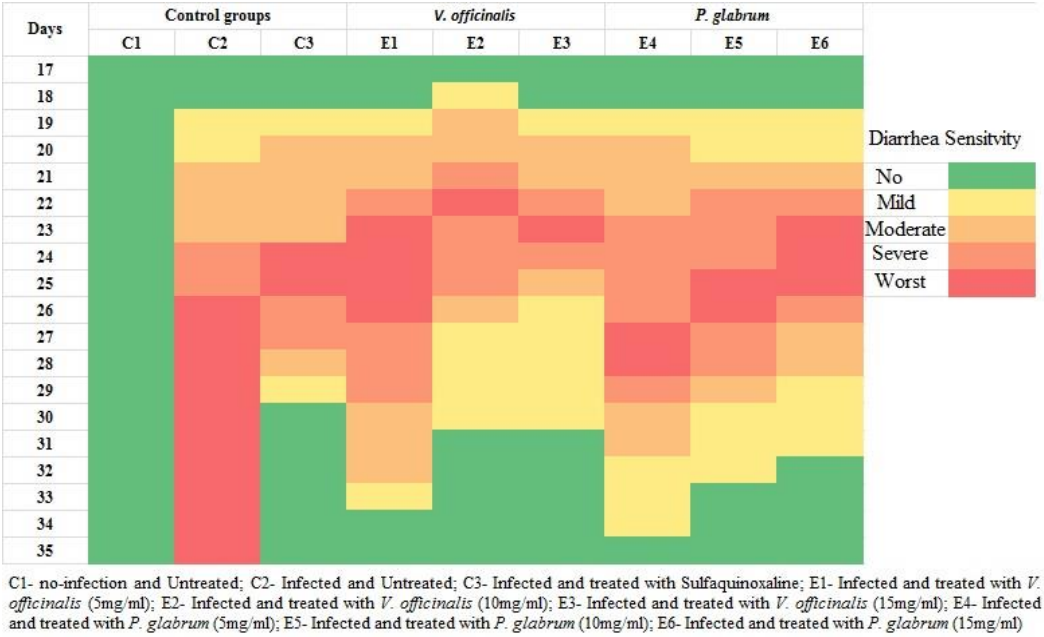


Figure 3.9: The heat map showing severity level of bloody diarrhea post infection and treatment of with respective plant extract

3.11.3.1. Biochemical Study

The biochemistry of all groups of chicks was examined by determining the quantity of serum protein, carbohydrate, ALT, and AST at the intervals of 7 days at 21st, 28th, and 35th day. The quantity of protein was 3.17±0.03, 3.22±0.03, and 3.21±0.02 g/l (Fig. 3.10a) and level of carbohydrates in the uninfected control group (C1) was 308±.007, 292±0.01 and 302.4±0.01 mg/ml at day 21, 28 and 35 age respectively, remains normal throughout the trial(Fig. 3.10b).

In contrast, the infected groups were found to have lower level of these macromolecules initially but after the treatment with the methanolic crude extracts of *V. officinalis* and *P. glabrum,* some groups showed a continuous increase in protein level i.e. E2 treated with *V. officinalis* (10 mg/ml) has 0.97±0.01, 1.96±0.02 and 3.06±0.02 g/l protein contents, E3 treated with *V. officinalis* (15 mg/ml) has 0.98±0.03, 2.11±0.01 and 3.85±0.04 g/l and group E6 treated with *P. glabrum* (15 mg/ml) was recorded with increasing level of protein i.e. 1.05±0.02, 2.06±0.1 and 3.90±0.04 g/l over time. Similarly, the level of carbohydrates of *V. officinalis* and *P. glabrum* treated groups were significant and similar to that of the uninfected group i.e. groups E2 has 272±0.01 mg/ml and E6 had 278±0.1 mg/ml at the age of 35 days respectively.

Similarly, the amount of ALT and AST was lowered in the uninfected and higher in the infected groups. The level of both enzymes decreased in the extract-treated groups, whereas the untreated group (C2) showed a continuous increase in mean ALT and AST levels. The ALT level of group E2 treated with *V. officinalis* (10 mg/ml) crude methanolic extract was 165±0.08, 124±0.07, and 107±0.03 U/L and the group E6 treated with *P. glabrum* (15 mg/ml) crude methanolic extract was found 165±0.3, 84±0.05, and 80±0.05 U/L at the age of day 21, 28 and 35 respectively (Fig. 3.10c).

The AST level of group E2 treated with *V. officinalis* (10 mg/ml) crude methanolic extract was 97.54±0.03, 87.99±0.02 and 31.14±0.01 U/L and E6 treated with methanolic crude extract of *P. glabrum* (15 mg/ml) was found 81.42±0.02, 44.81±0.03 and 31.85±0.01 U/L at the age of day 21, 28 and 35 respectively (Fig. 3.10d).

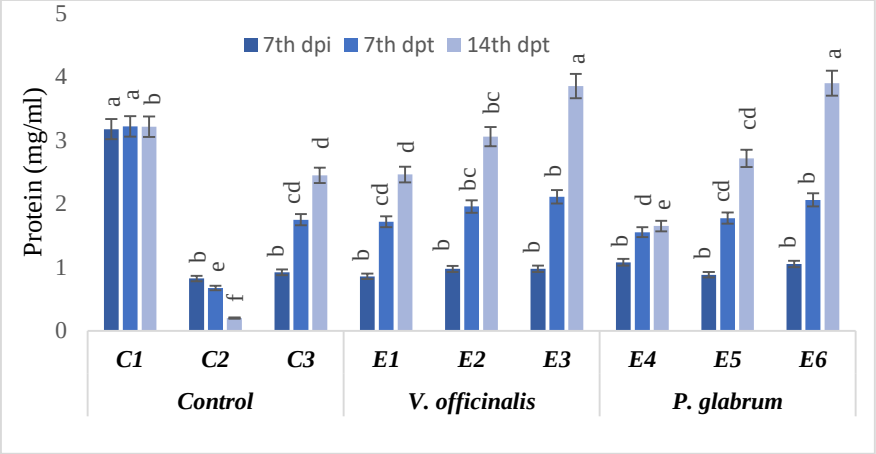


Figure 3.10(a): The protein, level of various broiler groups at age day 21, 28 and 35. (DPI: day post infection, DPT: day post treatment

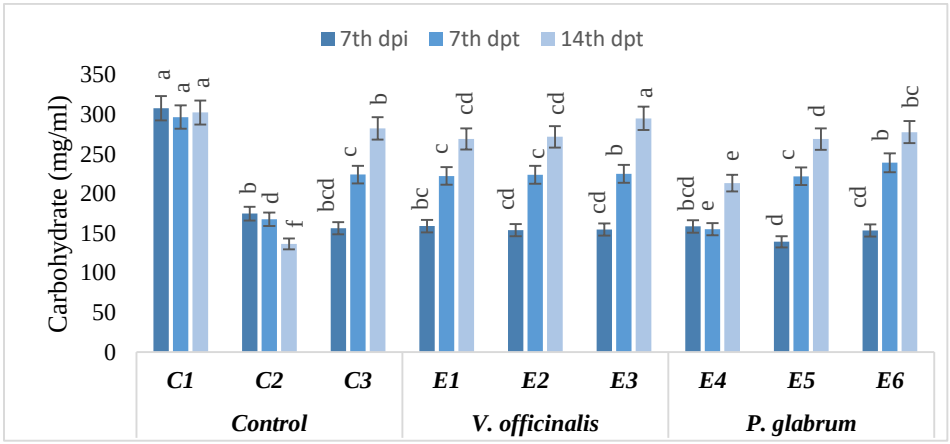


Figure 3.10(b): The carbohydrates level of various broiler groups at age day 21, 28 and 35. (DPI: day post infection, DPT: day post treatment

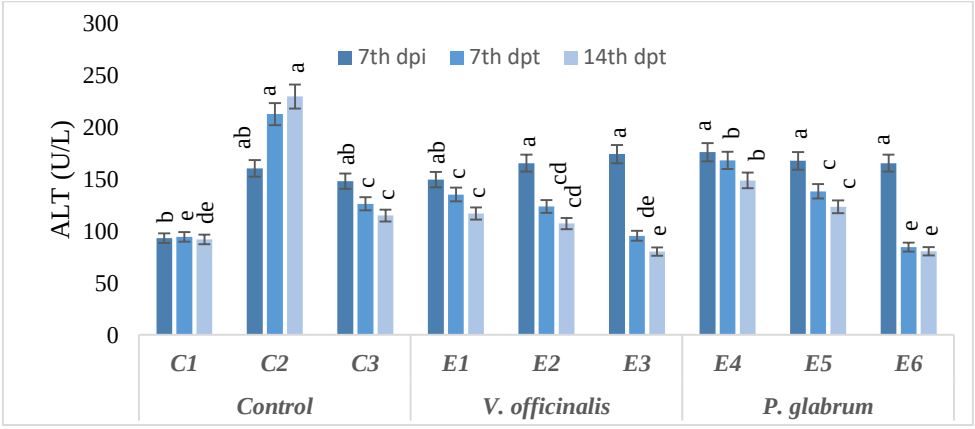


Figure 3.10(c): The ALT level of various broiler groups at age day 21, 28 and 35. (DPI: day post infection, DPT: day post treatment

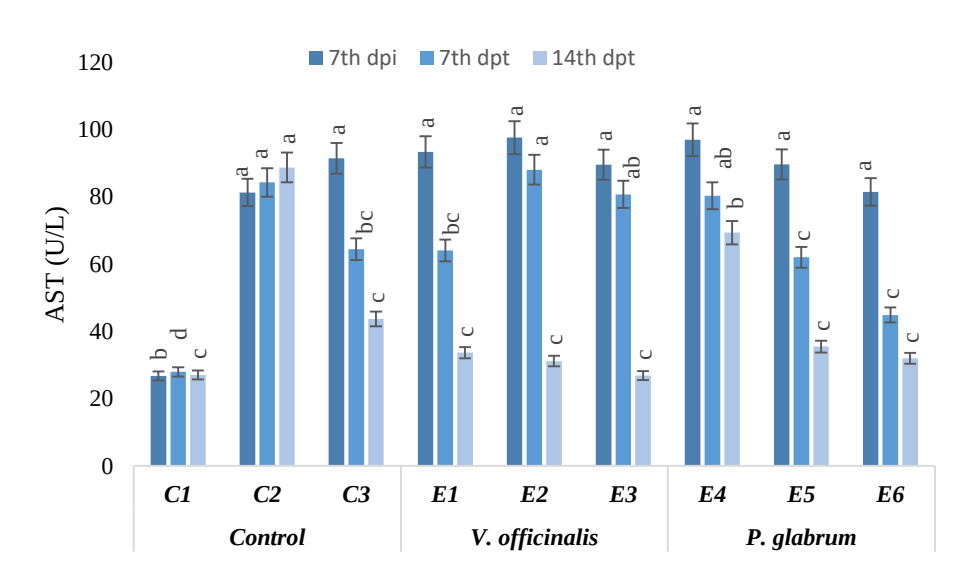


Figure 3.10(d): The AST level of various broiler groups at age day 21, 28 and 35. (DPI: day post infection, DPT: day post treatment

3.11.3.2. Hematological Study

The effect of methanolic plant extracts of *V. officinalis* and *P. glabrum* on the blood composition of *all* chicks groups was investigated using different hematological parameters (Hb, RBCs, WBCs, Platelets, Lymphocytes, Monocytes, and Granulocytes). The mean values of different hematological parameters of the groups E2 and E6 were found in the normal range just like the uninfected and untreated (C1) and SQX treated group (C3), with $P < 0.05$, Hb level, RBCs, WBCs, platelets and monocytes count of groups E2 and E6 showed an increase with increase in age, similar to the normal uninfected chicks.

In contrast, the level of lymphocytes and granulocytes drops with the passing age, similar patterns were noticed in C3 group. The hematological outcome of the studied groups showed a positive impact of the respective concentrations on the methanolic extracts (Table 3.8).

Table 3.8: The hematological profile of different broiler groups at age day 21, 28 and 35

Hematology Parameters		**Control groups**			***V. officinalis***			***P. glabrum***		
		C1	**C2**	**C3**	**E1**	**E2**	**E3**	**E4**	**E5**	E6
Hb (g/dl)	1	8.7 ± 0.7^{a}	7.6 ± 0.3^{b}	7.5 ± 0.5^{b}	7.8 ± 0.2^{ab}	7.7 ± 0.1^{ab}	7.8 ± 0.3^{ab}	7.7 ± 0.4^{ab}	7.9 ± 0.4^{ab}	7.4 ± 0.2^{b}
	2	10.6 ± 0.4^{a}	7.8 ± 0.9^{d}	9.2 ± 0.2^{bc}	7.9 ± 0.3^{d}	9.6 ± 0.5^{ab}	8.1 ± 0.3^{cd}	7.8 ± 0.5^{d}	8.0 ± 0.4^{cd}	9.6 ± 0.4^{ab}
	3	13.1 ± 0.2^{a}	7.4 ± 0.2^{e}	12.1 ± 0.6^{b}	7.7 ± 0.1^{de}	10.8 ± 0.3^{c}	8.6 ± 0.1^{d}	7.5 ± 0.2^{e}	8.1 ± 0.2^{de}	10.1 ± 0.4^{c}
RBC ($\times10^{6}$/µl)	1	1.8 ± 0.07^{a}	1.62 ± 0.05^{abc}	1.6 ± 0.06^{abc}	1.7 ± 0.14^{ab}	1.6 ± 0.1^{abc}	1.5 ± 0.06^{bc}	1.4 ± 0.10^{c}	1.6 ± 0.02^{bc}	1.59 ± 0.03^{abc}
	2	2.2 ± 0.15^{a}	1.57 ± 0.08^{b}	1.7 ± 0.10^{b}	1.3 ± 0.28^{b}	1.8 ± 0.14^{b}	1.4 ± 0.20^{b}	1.5 ± 0.08^{b}	1.7 ± 0.05^{b}	1.66 ± 0.06^{b}
	3	2.9 ± 0.02^{a}	1.50 ± 0.02^{c}	2.4 ± 0.40^{ab}	1.7 ± 0.26^{bc}	2.3 ± 0.4^{ab}	1.9 ± 0.08^{bc}	1.4 ± 0.30^{c}	1.8 ± 0.06^{bc}	2.03 ± 0.06^{bc}
WBC ($\times10^{3}$/µl)	1	8.0 ± 28^{d}	8.9 ± 36^{a}	8.9 ± 18^{a}	8.9 ± 78^{a}	8.3 ± 1.0^{b}	8.9 ± 14^{a}	8.1 ± 15^{d}	8.2 ± 16^{c}	8.3 ± 8.0^{bc}
	2	9.1 ± 42^{a}	8.2 ± 9.0^{c}	9.0 ± 28^{a}	8.1 ± 49^{d}	9.1 ± 15^{a}	8.2 ± 48^{cd}	8.5 ± 42^{b}	8.6 ± 62^{b}	9.0 ± 51^{a}
	3	1.0 ± 9.0^{a}	7.7 ± 27^{i}	10 ± 39^{c}	7.9 ± 25^{h}	10 ± 17^{b}	9.0 ± 26^{e}	8.1 ± 29^{g}	8.8 ± 28^{f}	9.8 ± 10^{d}
Platelets ($\times10^{5}$/µl)	1	4.4 ± 1.1^{e}	4.7 ± 1.8^{b}	4.7 ± 3.3^{bc}	4.8 ± 06^{a}	4.8 ± 0.9^{a}	4.7 ± 1.4^{bc}	4.8 ± 0.9^{a}	4.6 ± 1.9^{c}	4.5 ± 0.3^{d}
	2	4.4 ± 3.5^{e}	4.6 ± 0.8^{d}	4.6 ± 4.1^{a}	4.7 ± 0.7^{c}	4.8 ± 0.6^{a}	4.6 ± 3.0^{d}	4.7 ± 0.6^{bc}	47 ± 0.2^{ab}	4.6 ± 0.5^{cd}
	3	4.6 ± 2.8^{ef}	4.5 ± 0.6^{g}	5.3 ± 4.4^{a}	4.6 ± 1.0^{f}	4.9 ± 0.8^{b}	4.8 ± 0.9^{d}	4.6 ± 0.9^{e}	4.8 ± 0.5^{c}	4.9 ± 0.6^{c}
Lymphocytes (%)	1	64.9 ± 1.4^{d}	77.5 ± 0.7^{a}	77 ± 0.3^{ab}	75.5 ± 0.5^{bc}	77 ± 0.5^{abc}	77 ± 0.9^{ab}	75 ± 0.2^{c}	76.7 ± 0.4^{abc}	77.3 ± 0.5^{a}
	2	65.6 ± 0.93^{f}	86.0 ± 0.7^{a}	73 ± 0.7^{e}	79.8 ± 0.6^{c}	74 ± 0.4^{de}	83 ± 0.6^{b}	79 ± 0.9^{c}	78.6 ± 0.7^{c}	75.7 ± 0.5^{d}
	3	66.2 ± 1.1^{f}	91.5 ± 1.1^{a}	72 ± 1.1^{e}	83.6 ± 1.2^{bc}	72 ± 0.1^{e}	80 ± 0.9^{d}	85 ± 0.8^{b}	82.3 ± 0.7^{cd}	73.8 ± 0.3^{e}
Monocytes (%)	1	15.8 ± 0.4^{c}	22.0 ± 1.5^{ab}	22.8 ± 0.5^{ab}	20.8 ± 0.4^{b}	21.0 ± 0.6^{b}	21.2 ± 0.8^{b}	23.5 ± 0.6^{a}	22.7 ± 0.6^{ab}	22.0 ± 0.9^{ab}
	2	15.7 ± 0.8^{d}	18.0 ± 0.4^{c}	23.0 ± 0.4^{a}	18.9 ± 0.4^{c}	16.6 ± 0.4^{a}	16.6 ± 0.4^{d}	20.7 ± 0.4^{b}	18.9 ± 0.3^{c}	22.7 ± 0.5^{a}
	3	15.6 ± 0.7^{c}	15.0 ± 0.6^{c}	27.6 ± 0.3^{a}	14.5 ± 0.5^{c}	19.6 ± 0.9^{a}	19.6 ± 0.8^{b}	15.8 ± 0.8^{c}	18.2 ± 0.3^{b}	25.9 ± 0.9^{a}
Granulocytes (%)	1	16.0 ± 0.4^{a}	18.0 ± 0.3^{a}	16.7 ± 0.5^{b}	16.9 ± 0.4^{ab}	16 ± 0.5^{b}	16.9 ± 0.6^{ab}	17.4 ± 0.5^{ab}	17.9 ± 0.5^{a}	17.0 ± 0.4^{ab}
	2	16.9 ± 0.8^{a}	20.0 ± 0.7^{a}	13.1 ± 0.2^{d}	19.0 ± 0.8^{a}	14 ± 0.3^{cd}	19.0 ± 0.5^{a}	19.8 ± 0.4^{a}	19.3 ± 0.3^{a}	15.0 ± 0.4^{c}
	3	17.9 ± 0.8^{a}	21.2 ± 1.5^{a}	11.1 ± 0.2^{d}	22.0 ± 0.7^{a}	11.9 ± 0.2^{cd}	16.3 ± 0.5^{b}	22.9 ± 0.3^{a}	22.1 ± 0.3^{a}	13.7 ± 0.4^{c}

1: 7^{th} DPI, 2: 7^{th} DPT, 3: 14^{th} DPT

3.11.4. Histology

A comprehensive histo-pathological examination of all groups was performed. The ceacal, liver and kidney tissues were stained to observe the changes. The remarkable changes in the histomicrographs of the said tissues were noticed, which illustrates the recovery of individuals of different groups treated with *V. officinalis* (Fig. 3.11b) and *P. glabrum* (Fig. 3.11c) in comparison with the untreated and *E. tenella* infected group (Fig. 3.11a). The histological micrographs (Fig. 3.11a) of the control C1 group showed normal morphology as it remained uninfected, whereas the morphology of C2's liver is altered having compromised sinusoidal space, and the distribution of hepatocytes was changed as compared to C1, while the observation of kidney tissues demonstrates the shrunken glomerulus, degenerative collecting ducts, vacuole formation and increased bowman space, all these observations ensure the presence of parasitism. The protective groups i.e. C3 which is treated with SQX showed a recovery of sinusoidal spaces in liver tissues and lowering of spaces observed between hepatocytes. The glomerulus and tubules morphology in the kidney tissues also showed recovery as compared to the control C1 group (C: ceca, L: liver, and K: kidney). As for the experimental groups concerned which were treated with various dilutions of methanolic extracts of *V. officinalis* and *P. glabrum*, the histological study of the *V. officinalis* treated tissues was present in figure (3.11b), which showed that chicks of group E1, treated with lower concentration (5 mg/ml) of the extract, was not recovered by forming vacuole in kidney tissues, the appearance of sinusoidal spaces in liver and degeneration of cecum tissues (Fig. 3.11b). The individuals of groups E2 and E3, which has improved FCR and maximum weight gain by treatment with *V. officinalis* methanolic extract (10 & 15 mg/ml) respectively (Table 3.7), the reason is that the histological observations of the respective group illustrate recovery from coccidiosis in the form of no vacuoles in kidney tissues and no such apparent degeneration in liver and cecum tissues. The histomicrographs of the various tissues (Fig. 3.11c) of group E4 individuals has shown no recovery in all three types of tissues, after treatment with 5 mg/ml methanolic extract of *P. glabrum*. Whereas the kidney and liver tissues recovered by treatment with 10 and 15 mg/ml, appeared as no degeneration in tissue structure, and no vacuole formation with exception of a small amount of disruption in cecum and kidney tissues of group E6.

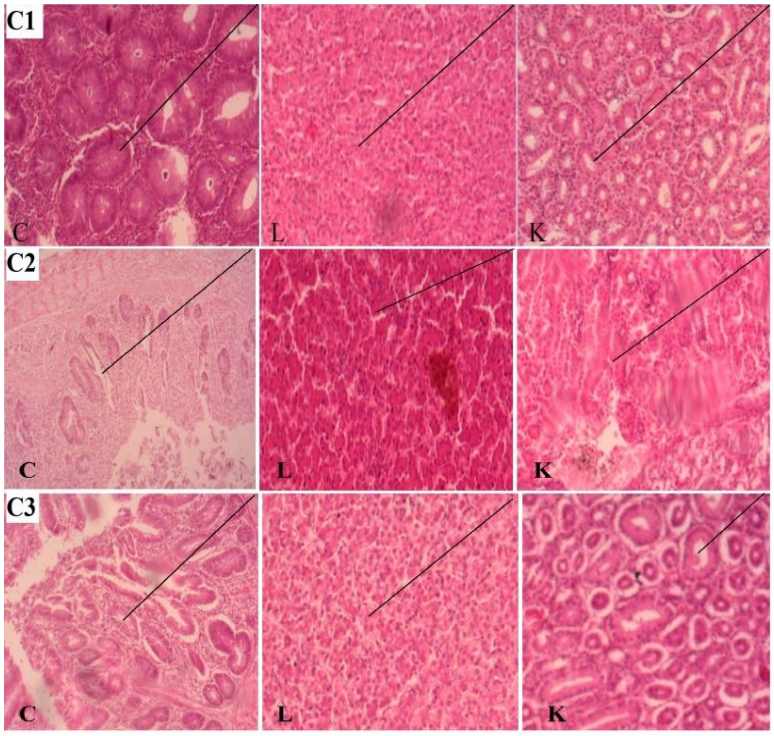


Figure 3.11(a): Photomicrograph of the histological study of the control groups (C: ceca, L: liver and K: kidney)

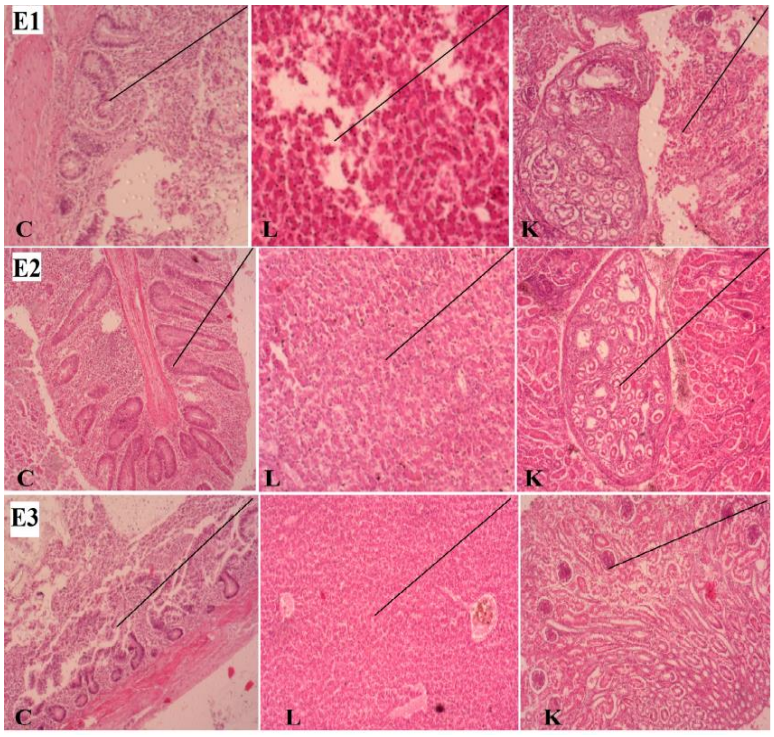


Figure 3.11(b): Photomicrograph of the histological study of the *V. officinalis* (C: ceca, L: liver and K: kidney)

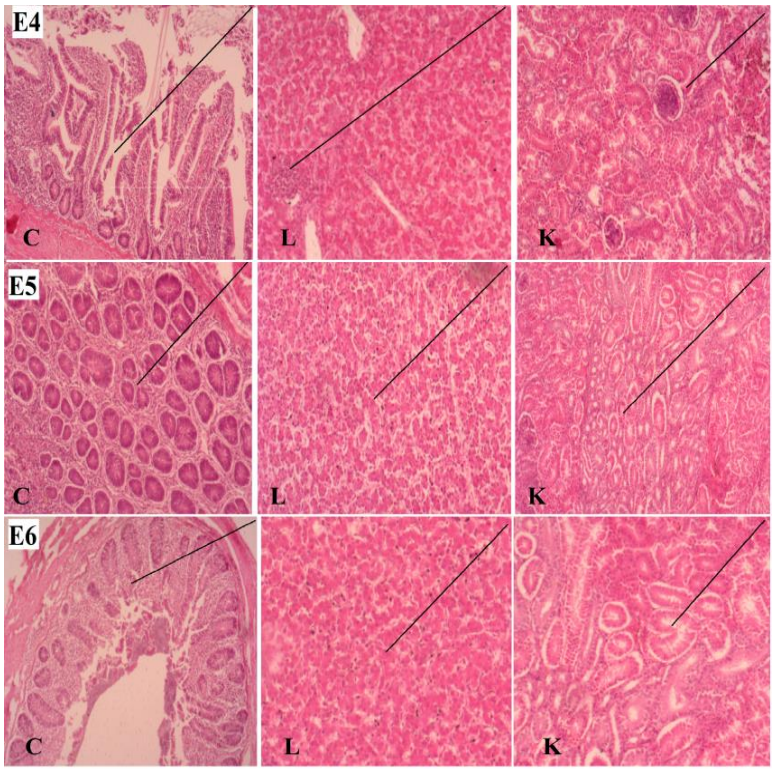


Figure 3.11(c): Photomicrograph of the histological study of the *P. glabrum* (C: ceca, L: liver and K: kidney)

3.12. Molecular Modeling

All 22 ligands isolated from methanolic extract of *V. officinalis* were docked with SAM synthetase. The binding affinities between of different ligands with target protein showed different score values. The lower will be the binding score; the higher will the binding affinity. The binding score values of ligands greater than −5.0 kcal/mol were excluded. Among all protein/receptor–ligand pairs, only four ligands showed the lowest binding energy ranging from −5.0 to -6.4 kcal/mol (Table 3.9). The hydrophobic interactions in 2D structures generated via LigPlot+ (Fig: 3.12a-d), showed the involvement of relatively varied number of interacting amino acid residues across the different ligand–receptor complexes. These interactions enhance the binding affinity and biological activity of the complex molecules and help in stabilizing the biochemical environments of target-drug complexes. The hydrophobic interaction of ligands with amino acid residues of SAM synthetase was demonstrated in table 3.9. Among all four

ligands, Strychane, 1-acetyl-20a-hydroxy-16-methylene demonstrated the best linkage with lowest binding score of -6.4 Kcal/mol (Fig: 3.12a).

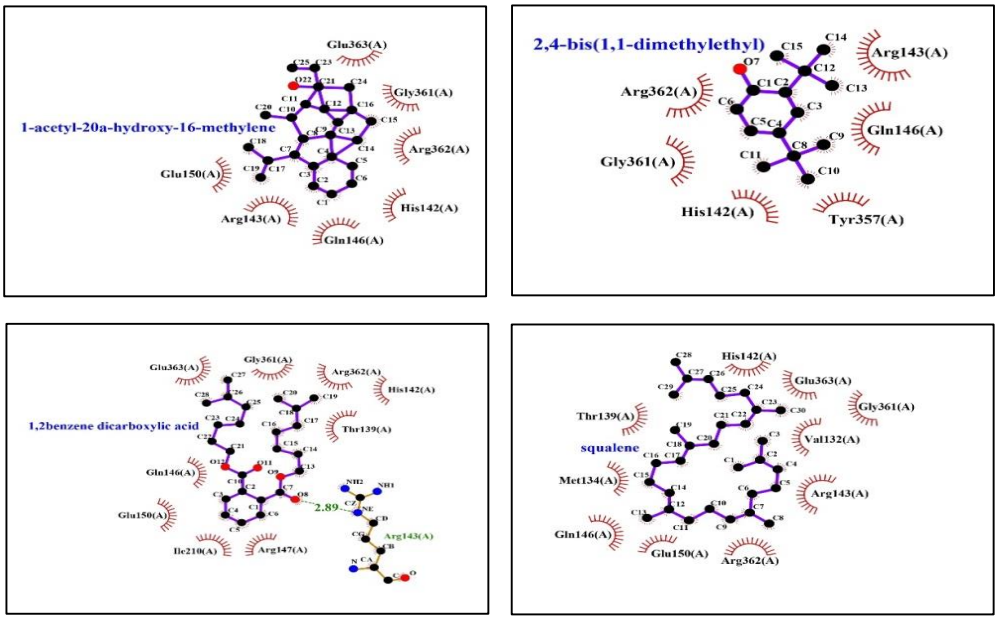


Figure 3.12: Graphical presentation of ligands with S. adenosyl methionine synthetase. (a) Strychane, 1-acetyl-20a-hydroxy-16-methylene (b) Phenol, 2,4-bis(1,1-dimethyl ethyl), (c) 1,2 benzene dicarboxylic acid, di-isooctyl ester, (d) Squalene

Table 3.9: Binding score, interactive residues and number of total residues of Ligand and S adenosyl methionine synthetase interaction

S no.	Ligand	Binding score (kcal/mol)	Interactive residues	Total Residues
1	Strychane, 1-acetyl-20a-hydroxy-16-methylene	-6.4	ARG143, GLU150, GLN146, GLU363, ARG362, GLY361, HIS142	7
2	Phenol, 2,4-bis(1,1-dimethyl ethyl)	-5.6	ARG362, TYR357, HIS142, ARG143, GLY361, GLN146	6
3	1,2 benzene dicarboxylic acid, diisooctyl ester	-5.2	GLU363, ARG362, GLY361, HIS142, THR139, ARG143, GLN146, ILE210, GLU150, ARG147	10
4	Squalene	-5.0	GLY361, GLU363, VAL132, MET134, THR139, HIS142, ARG362, GLN146, ARG143, GLU150	10

DISCUSSION

Eimeria species are deadly harmful to the poultry industry and lead to millions of dollars loss annually. To overcome this issue veterinary sector conducted many trials, some of them worked but most leads to making the parasite more resistant. The current study is conducted to check the effect of natural, economic, and easily available products or flora, which recommends that a higher rate of SPI were recorded at a higher concentration of methanolic extract of *V. officinalis* with IC_{50} 0.14 mg/mL against *E. tenella* at 48 hours incubation. Further, this plant was found rich in ingredients as shown in the qualitative phytochemical analysis. *P. glabrum* also showed more than 50% activity at both medium and higher concentrations.

The constituents derived from *V. officinalis* have an impressive effect on the prevention of cancer in a human colon cell line with IC_{50} <20 mg/ml (Encalada *et al*., 2015). In humans, it helps in the regulation of lactation and menstrual cycle (Hernandez *et al*., 2000), and is also used in curing rheumatism and joint pain (Li *et al*., 2003). In edema, it showed effective results in comparison with marketed ointment peroxicam (Calvo, 2006). The oil derived from *V. officinalis* can cause cell death in the case of chronic lymphocytic leukemia (Martino *et al*., 2011). The number of medicinally important herbs with 70 to 100% efficacy against *E. tenella*, which concludes that *Sophora flavescens* to cure the life-threatening avian infection coccidiosis (Youn and Noh, 2001), *Punica granatum*, commonly known Pomegranate is effective against *Eimeria* spp. by lowering its population up to 50% in the infected flock (Dkhil, 2013).

Artemisinin derived from *Artemisia sieberi* is capable to decrease the quantity of oocysts in chicks infected with *E. tenella* and *E. acervulina* (Arab *et al*., 2006). Methanolic extract of *V. officinalis* extract has shown round about 30% activity against fungus species *Rhizopus stolonifer* and *Penicillium expansum* (Casanova *et al*., 2008). It showed culicidal activity with LC_{50} of 38 ppm (Pavela *et al*., 2009). The ethanolic content of *V. officinalis* stem is effective against *Staphylococcus aureus* (Ahmad *et al*., 2012). The commercially available drug apacox was tested against *E. tenella* with better results like lowering of diarrhea and good feed conversion rate (Christaki *et al*., 2004). SOD is the enzymes that can help in the conversion of superoxide anion to hydrogen peroxide (Sheng

et al., 2014), and the peroxide species like H_2O_2 are found to be effective against coccidian oocysts by deactivating or arresting it from further development (Lee and Lee, 2007), this statement provides the evidence that the plant extract or drug having more quantity of SOD will effective against parasite population, and in current study *V. officinalis* has a high level of SOD than other plants (Fig. 3.6) and that's why it has shown much inhibition against *E. tenella* (Fig. 3.10a,b).

GSH commonly known as glutathione is the reducing form of glutathione, it acts as a neutralizer in a cell by neutralizing oxidants molecules, and in turn, it detoxifies the harmful effect of various compounds, it has shown in the study (Li *et al*., 2010) that S-nitroso glutathione, actually a derivative of glutathione has an inhibitory effect on *Eimeria* spp. and can interrupt the sporulation process of the parasite, which may keep the *Eimeria* oocysts in arrest stage. So it is concluded from figure (3.6), that a high level of glutathione will have a more inhibitory effect and vice versa. The asymptomatic coccidiosis in chicks interrupts many anabolic activities, which ultimately lead to lesser protein production and as a result, it disturbs the performance of chicks (Mathews *et al*., 2000).

The protein content in the diet or drug can have a positive impact on the live performance of chicks (Lee *et al*., 2011), which in turn makes it healthy in the form of improved immunity. In the current study, the *V. officinalis* extract has a sufficient amount of protein contents which can up-regulate the chicks performance as compared to *P. glabrum* and *M. arvensis.* The results demonstrate the higher inhibition activity of *V. officinalis* and comparative of all three plant extractives showed a minute difference between methanolic and aqueous extracts, which means that methanol extractives have some extra ingredients which enhance their inhibition rate against *Eimeria* oocysts. As for the *in-vivo* trial concerned, several medicinal herbs was reported to control coccidiosis from poultry; some common plants used against different *Eimeria* spp. i.e., *Sophora flavescens* with 70% anticoccidial efficacy (Youn and Noh, 2001), *Punica granatum* commonly known as Pomegranate showed 50% effectiveness (Dkhil, 2013). The inhibitory activity of *Azadirachta indica, Nicotiana tobaccum, Trachyspermum ammi*, and *Calotrpois procera* has good results against sporulating oocysts in comparison with amprolium (Zaman *et al*., 2012). *Artemisia sieberi* (artimisinin) *is* capable to decrease the *E. tenella* and *E. acervulina* oocysts quantity (Arab *et al*., 2006).

In general, the *Eimeria* spp. affected hosts has lowered MWG as compared to the healthy one, due to the disturbed nurturing or metabolism (Orengo *et al*., 2012), Similarly the current study showed the lowered protein and carbohydrates contents of the infected chicks, the probable reason for this decline is the disruption of the epithelial lining of the intestine, which is unable to assimilate sufficient amount of nutrients and hence leads to weight loss. Further, this statement also supports the recovery of chicks treated with *V. officinalis* and *P. glabrum*, which is reflected in the form of good MWG, high quantity of proteins and carbohydrates, and histomicrographs of the ceacal part of the respective groups (Morris and Gasser, 2006). The disturbed assimilation process leads to imbalanced hematology and biochemical profile in different infected groups at the 7th day post-infection. The phenomenon of weight loss or stunted growth was controlled by conducting the bioactivity of ethanolic crude extracts of *V. officinalis* and *P. glabrum* against *E. tenella* due to its medicinal importance as reported in earlier research. Further, the naturally derived compounds can augment the immunity of the host (Freier *et al*., 2003).

The significant weight gain and other parameters of C1 broilers elucidate the sterility of the environment provided to the chicks (Orengo *et al*., 2012). The heat map of phyto-compounds (Fig. 3.2) showed the richness of both plants (*V. officinalis* and *P. glabrum*), whereas the GC report of methanolic extract of *V. officinalis* showed the presence of bioactive phyto-components therefore the Freier and coworkers (2003) states that, naturally derived compounds boost the immune response to parasitic infection. The effective concentrations of *V. officinalis* (10 mg/ml) and *P. glabrum* (15 mg/ml) results in good weight gain of chicks' just next to the SQX treated control group, especially in group E2 and E6, similar findings were also reported by Kaingu and colleagues in 2017, in which maximum weight was gained by chicks after treatment with *Aloe secundiflora* extract, his findings supports the phenomenon of slow growth by group C2, which is remained infected and untreated. The FCR of groups E1, E4, and E5 was recorded above 2.00 and C2 was a poor FCR of 3.07, whereas the groups E2 and E6 and controls "C1 and C3" has FCR values in the normal range with normal hematology (Table 3.8) and biochemical profile (Fig. 10a). Similarly, Naidoo and coworkers (2008) reported the activity *Tulbaghia violacea*, *Vitis vinifera*, and *Artemisia afra extracts* and toltrazuril treated chicks have good FCR as compared to infected chicks. The methanolic crude extracts of *V. officinalis* and *P.*

glabrum exhibit antioxidant activity (Casanova *et al*., 2008; Raja and Ramya, 2017), which supports the reduction in oocysts shedding in the effective groups. The natural products usually don't harm a living being with some exceptions, as El Banna, (2016) said that *Moringa olifera* doesn't lead to any toxic effect. Similarly, the chicks under trial don't bear any negative pressure of *V. officinalis* and *P. glabrum* methanolic extracts except for the higher concentration (15 mg/ml) of *V. officinalis*, which yields better FCR but negatively affected the blood and serum profile severely, the exact reason of which is unclear but may be possible due to overdose.

The derivatives of *V. officinalis* showed anticancer activities with LC_{50} < 20 mg/ml in a human cell line (Encalada *et al*., 2015). It also assists in the regulation of human lactation and menstrual cycle (Hernandez *et al*., 2000), curing rheumatism and joint pain (Li *et al*., 2003). In comparison marketed ointment piroxicam, *V. officinalis* is effective against edema (Calvo, 2006). The oil derivatives of *V. officinalis* have an apoptotic effect in chronic lymphocytic leukemia (Martino *et al*., 2011). The methanolic extract of *V. officinalis* has shown 30% antifungal activity against *Rhizopus stolonifer* and *Penicillium expansum* (Casanova *et al*., 2006), while the culicidal activity with LC_{50} of 38 ppm (Pavela, 2009). The methanolic crude extract of *V. officinalis* has antibacterial activity against *Staphylococcus aureus* (Sahile *et al*., 2105). The purpose of selecting *P. glabrum* against avian coccidiosis is that it exhibits some qualities and different activities have been done with it against different diseases and pathogens as elaborated above, in addition, the member of the same genus "*Polygonum aviculare"* was found effective against coccidiosis (Youn and Noh, 2001).

The level of AST in chicks rises with coccidiosis infection (El-Maksoud *et al*., 2014), same is the case with the current experiment which showed elevation of AST in the infected groups earlier but later on, the healing potential of *V. officinalis* and *P. glabrum* decreased its level. In the current study, the results of increasing the level of protein with decreased ALT and AST profile in the recovered groups were comparable with the findings of Cowieson (2020). The thiamine receptors help in carbohydrate synthesis which enhances *Eimeria* oocysts sporulation, the synthetic drug amprolium as its antagonist blocks the thiamine receptor and inhibit sporulation process (Ali and Abdelhalim, 2020). Prinzo and collegues (1999) reported the flavonoids as antagonist to

thiamine. Considering the blockage of thiamine receptors, the flavonoids may block the thiamine receptor like amprolium due to its antagonistic behavior and act as anticoccidial agent. Further, the effectiveness of the studied herbal extracts was supported by the results of different hematological, biochemical parameters, and histomorphology. Additionally, the clinical signs like oocyst abundance or bloody diarrhea, the common indicators of coccidiosis, was also used to evaluate the potency of *V. officinalis* and *P. glabrum* that either any change in those parameters occurs with the treatment of the respective concentrations of the studied herbs.

The phyto-components of methanolic crude extract of *V. officinalis* exhibits some biological activities which enhance its importance, some of the properties are sited in this work. The anti-microbial activity organic compounds like tetradecane (Idris *et al*., 2019), hexadecane (Padma *et al*., 2019), 6-octadecenoic acid methyl ester (Adegoke *et al*., 2019), linoleic acid ethyl ester (Huang *et al*., 2010), hexacosane (Banakar and Jayaraj, 2018) were reported. The decane (Idris *et al*., 2019), and phenol, 2,4-bis(1,1-dimethylethyl)- (Devi *et al*., 2021) were found active against fungus. Dodecane (Padma *et al*., 2019), eicosane (Idris *et al*., 2019), and heptacosane (Khatua *et al*., 2016) exhibits antibacterial property. The hexadecane (Padma *et al*., 2019), hexadecanoic acid, methyl ester (Sermakkani and Thangapandian, 2012), and squalene, 1-Monolinoleoyl glycerol trimethylsilylether (Banakar and Jayaraj, 2018) were reported as antioxidant agents. The anti-cancerous activity of squalene, 1-monolinoleoyl glycerol trimethylsilyl ether was also noticed by Banakar and Jayaraj in 2018. Ecosane has cytotoxic activity (Idris *et al*., 2019), whereas tetratetracontane is cyto-protective in nature (Amudha *et al*., 2018). The compounds like octacosane (Rajkumar and Jebanesan, 2004), hexadecanoic acid, methyl ester (Sermakkani and Thangapandian, 2012) have best insecticidal activity. The sulfurous acid, cyclohexyl methyloctadecyl ester has synergistic behavior by acting as catalyst or enhancing the rate of reaction (Rukaiyat *et al*., 2015). The octadecane is used for the treatment of phobic disorders (Kumaresan *et al*., 2015). 1, 2 benzene dicarboxylic acid, diisooctyl ester is used in the synthesis of various perfumes and cosmatics (Hema *et al*., 2011).

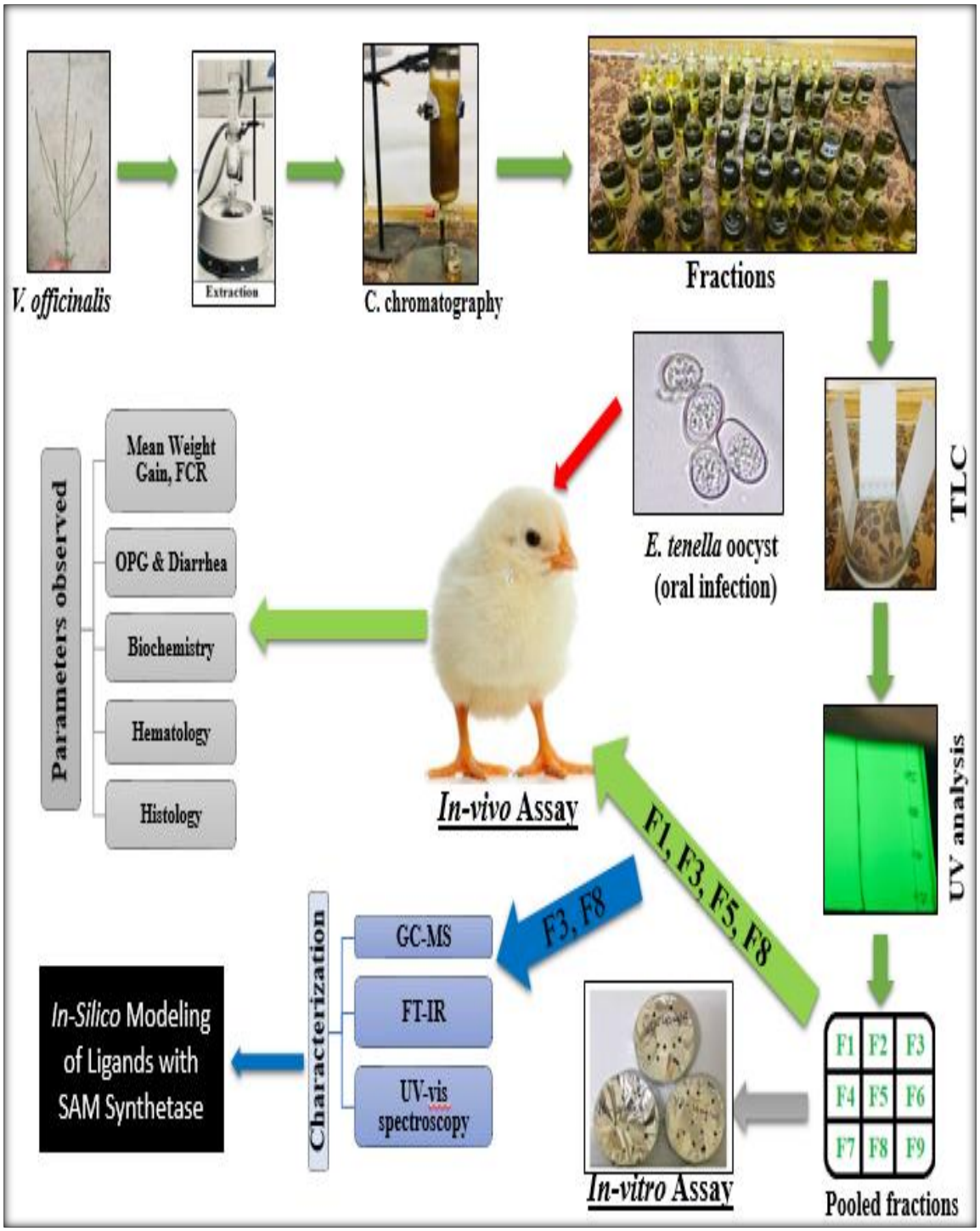
CONCLUSION

In the current study, it is concluded that the aqueous and methanolic crude extracts of *V. officinalis* and *P. glabrum* showed significant activity against *E. tenella* sporulating oocysts in terms of low IC_{50} as compared to *M. arvensis, F. officinalis and P. hysterophorous* (*in-vitro*). Similarly, the groups treated with methanolic crude extracts of *V. officinalis* (*in-vivo*) results in enough weight gain and good FCR with recovered hematological and biochemical profile, bearing no negative impact. The molecular docking of phyto-components of *V. officinalis* suggests: 1,2benzene dicarboxylic acid, diisooctyl ester exhibits anticoccidial potential. More extensive investigation of *V. officinalis* should be carried out, especially bio-guided fractionation to find the most effective fraction against the avian coccidiosis and further the chemical characterization of compound structures.

Chapter: 04

Anticoccidial activity of *V. officinalis'* fractions and prediction of available natural compounds as inhibitors of *Eimeria tenella* through molecular docking

GRAPHICAL ABSTRACT



ABSTRACT

In the current study, the chromatography based fractions of *Verbena officinalis* was used against *Eimeria tenella*. The water and methanol based extracts of various fractions were applied in-vitro for assessment of sporulation inhibition assay and probit analysis against *E. tenella* oocysts in comparison with control groups (Potassium dichromate and Sulfaquinoxaline) in triplicate. The most active fractions with low IC_{50} were subjected to *in-vivo* anticoccidial activity in the 15 groups of 14-day-old *E. tenella*-infected chicks and treated post-infection. The parameters like weight gain, fecal oocyst count, diarrheal discharge, biochemical and hematology contents and histology were studied at post infection and post treatment intervals and analyze using tukey (ANOVA) test. The active fractions were characterized by Fourier transmission & infrared and Ultra-Violet Visible spectroscopy using Origin software and Gas Chromatography-Mass Spectroscopy; and each ligand identified in the active fraction was docked (ChemDraw, and Autodock Vina) to S-adenosyl methionine synthetase *in-silico*, to find out the best inhibitory compound with the lowest binding energy.

Initially, all the fractions (F1-F9) were tested in the sporulation inhibition assay, with F1, F3, F5 and F8 exhibiting maximal (> 70%) inhibitory activity at 40 mg/ml concentration and lowest IC_{50} of 16.83, 8.59, 10.65, and 10.32 mg/ml, respectively. The *in-vivo* anticoccidial activity of active fractions was conducted in the *E. tenella*-infected chicks, resulting in the significant mean weight gain of 885.00±7.08 gm and 814.57±14.46 gm incase of 15 mg/ml of F3 and F8 at 14^{th} post-treatment, respectively, with less than 1 FCR value. Both the groups showed lowest oocyst number (6.5×10^3, 7×10^3/gm) at the end of experiment, with recovered diarrhea, normal hematology, histology, and biochemical profiles and has significant difference ($P < 0.05$) to uninfected groups as indicated by Tukey statistics. The functional groups and purity of active fractions was characterized with UV-Vis and FT-IR spectroscopy, GC-MS, reveals the presence of seven compounds in F3 and twenty in F8. Docking of all the ligands with S-adenosyl methionine synthetase, resulted in the best binding with a-sitosterol, 1,2 benzenedicarboxylic acid, mono(2 -ethylhexyl) ester, and 3,9-epoxypregnane-11,14,18-triol-20-one, 16-cyano-3-methoxy-, 11-acetate with binding energies of -6.2, -6.1, and -5.3 respectively, suggests their best binding compatibility with the target protein and can be used as an inhibitory substance against the avian coccidiosis to alter the methylation of DNA, which will interfere with the gene expression of parasite.

MATERIALS AND METHODS

4.1. Plant Collection

The aerial parts (Fig. 4.1) of *V. officinalis* were harvested from the fields of the northern region of district Mardan, Pakistan (34.3410° N, 72.2897° E) during the summer season and stored in paper bags to be safe from humidity, which is a possible reason of fungal infection. The allergic reactions were avoided by using preventive measures (Iqbal *et al*., 2022).

Figure 4.1: Aerial parts of *V. officinalis* collected anticoccidial activity

4.1.2. Preparation of Crude Extract

The aerial parts of *V. officinalis* were washed with tap water to remove the dust particles and shade dried in a humidity-free place at room temperature (25 to 37°C). The dried parts were grinded and sieved to obtain fine powder by passing through mesh (0.5 mm pore size) (Iqbal *et al*., 2022). The crude extract was prepared from 30 g of powder in 300 ml of absolute methanol at 40-60^{o}C in the Soxhelt apparatus (Shanghai Heqi, China) (Fig. 4.2). After filtration (Whatman no. 1), the solvent was removed with a vacuum rotary evaporator (R-300, Rotavapor) at 50^{o}C (Shaheen *et al*., 2020).

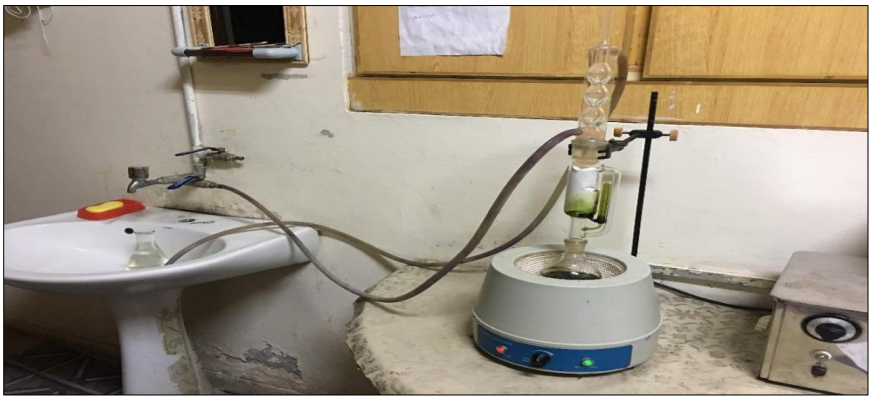


Figure 4.2: *V. officinalis* extract preparation in Soxhelt apparatus

4.1.3. Column Chromatography and TLC

The fractions were prepared from the crude extract of *V. officinalis* in a series of solvents (Sigma Aldrich) such as n-hexane, ethyl acetate, chloroform, and methanol, depending on the polarity. The glass column (14 × 2 inches) was mounted on a steel stand 3 inches above the ground (Fig. 4.3a) and plugged with a cotton swab at the outlet next to the stopper to prevent sand and silica release. The autoclaved sand was poured at the bottom and filled with 125 gm of dry fine silica gel (0.063-0.200 mm) up to 8 inches. The slurry (crude extract and silica) was poured up to 3 inches. The silica was settled down by pouring 300 ml of absolute n-hexane, out of which 100 ml of n-hexane was collected in a beaker, and the rest was absorbed onto the silica with the appearance of a clear band along the length of the glass column.

The solvents were poured using a step gradient method through the column in combinations of n-hexane-ethyl acetate, ethyl acetate-chloroform, and chloroform-methanol in pre-defined ratios (80:20, 60:40, 40:60, and 20:80) and harvested in 10 ml glass bottles. The same nature fractions were pooled on the basis of thin-layer chromatography (Fig. 3b) using silica-coated paper as the stationary phase, and the same solvents were chosen for the mobile phase. After TLC, the plates were examined under UV light to find out the exact path traveled by each fraction (Fig. 3c). The rf value of each fraction was calculated, and the fractions were pooled. The solvent from each pooled fraction was evaporated and stored at 4°C for further use (Kaur *et al*., 2014; Iqbal *et al*., 2022).

Figure 4.3(a): Fraction isolation by column chromatography, (b) Fractions obtained. (c): Thin layer chromatography of *V. officinalis*' fractions

4.1.4. Phytochemical Characterization

The active fractions of *V. officinalis* were characterized by UV-Vis, FT-IR spectroscopy, and GC-MS. The proximate analysis of active fractions was investigated by UV-visible spectroscopy. The fractions were centrifuged at 3000 rpm for 15 min and filtered through a syringe filter (0.45 µm). The samples were diluted to obtain fine spectra by scanning in the wavelength range of 200 to 800 nm using a spectrophotometer (Shimadzu), and characteristic peaks were observed (Ansir *et al*., 2020). The functional groups in the fractions were observed in the wavelength range of 400 to 4000 cm^{-1} using FT-IR spectroscopy. The samples were prepared by mixing 2 mg of solute with 100 mg potassium bromide (KBr) and a ~3 mm salt disc loaded into a FT-IR spectrometer (Bruker Platinum ATR) and scanned for confirmation within the above mentioned range (Hemavathy *et al.,* 2019).

The inert composition of the active fractions was analyzed with the GC-MS (Thermo Scientific DSQ; Version: 2.0.7 1). The GC system was provided with an auto-sampler, an auto-injector, selective detector and a silica capillary column measuring 0.25 mm × 30 m × 0.25 µm. 1 µL of the sample was injected at a temperature of 250^{o}C. The GC system was started at 100^{o}C for 0.5 minutes and then ramped to 280^{o}C in 3 minutes using helium gas as a carrier (1 ml/min). The mass spectral scan range was 50-350 m/z at 70 eV. A comparison of retention times and mass spectra was used to identify specific compounds conserved in Mainlib and Replib, and peaks were visualized on chromatograms (Ashraf *et al*., 2020).

4.2. *In-vitro* Assay

4.2.1. Purification *E. tenella* Oocysts

The ceca *E. tenella*-infected chicks were acquired from a poultry research institute in 2.5% $K_2Cr_2O_7$ and pierced to harvest the excreta and filtered (cloth mesh) to obtain an oocyst suspension. The suspension was centrifuged at 3000 rpm for 10 min, and the oocysts settled down. The supernatant was discarded and kept in the refrigerator for further use (Shah *et al*., 2023).

4.2.1.1. Sporulation Inhibition Assay

To examine the oocysticidal activity of various fractions (F1-F9), a sporulation inhibition assay was performed on unsporulated *E. tenella* oocysts *in-vitro*. The experiment was performed in Petri dishes in triplicate and provided with an equal amount of oocyst suspension, and each was treated with a respective concentration (2.5, 5, 10, 20, and 40 mg/ml) of various *V. officinalis* fractions along with sulfaquinoxaline-positive control and $K_2Cr_2O_7$-negative control and incubated at room temperature (28°C), provided oxygen for 72 h, and observed at 10X magnification under the microscope using a neubauer chamber (Wajiha and Qureshi, 2021).

$$Percent\ sporulation(sp)\ inhibition = \frac{Sp\ \%\ of\ control - Sp\ \%\ of\ extract}{Sp\ \%\ of\ control} \times 100$$

4.3. *In-vivo* Anticoccidial Assay

4.3.1. Experimental Animals

Two hundred and twenty-five-day-old chicks (Ross 308) were purchased from a local hatchery and housed in an animal house (Department of Zoology, Quaid-i-Azam University, Islamabad). All the chicks were kept together for the first two weeks and fed with a coccidiostat free starter ratio and water *ad libitum*. The floor was lined with sawdust bedding, and the litter was replaced after a week to avoid humidity that could promote fungal growth. The temperature of the room was well maintained by ventilation to avoid asphyxia. All the chicks were supplemented with vitamins and vaccinated against infectious bursal disease (IBD) on day 3 and Newcastle disease (ND) on day 5 (Wajiha and Qureshi, 2021).

$$Amount\ of\ feed\ required\ per\ day = \frac{No\ of\ Chicks\ \times 4.5\ \times Age\ in\ days}{1000}$$

4.3.3. Infectious Dose Inoculation

On day 14, chicks were divided into 15 groups, with 15 birds per group having a triplicate of 5, i.e., the control groups (C1, C2, C3) and experimental or *in-vitro* active groups (F1, F3, F5, and F8). All groups received an infectious dose (microtitre) of *E. tenella* except C1 (Shah *et al*., 2023).

4.3.3. Preparation and Administration of Treatments

The stock solution (10,000 ppm) of each fraction was prepared in deionized water and diluted using the diluted formula "$C_1V_1=C_2V_2$" into working concentrations of 5, 10, and 15 mg/ml, and orally administered at day 21 to the respective *E. tenella*-infected group labeled as F1L, F1M, F1H, F3L, F3M, F3H, F5L, F5M, F5H, F8L, F8M, and F8H for evaluation of the *in-vivo* anticoccidial effect (Sidiropoulou *et al*., 2020). Three groups were kept as controls: C1 (uninfected-untreated), C2 (infected-untreated) and C3 (infected-treated with sulfaquinoxaline).

4.3.4. Evaluation of *In-vivo* Anticoccidial Activity

The *in-vivo* anticoccidial activity of different fractions of *V. officinalis* was evaluated by analyzing health parameters that may vary with any sort of infection and therapeutic measure. These parameters include weight gain, stool examination, hematological, biochemical, and histopathological profiles (Sidiropoulou *et al*., 2020).

4.3.4.1. Physical Parameters

The infected chicks from different groups showed numerous clinical signs. The most common signs include bloody diarrhea, weight loss or poor weight gain, ruffled feathers, and examination of a stool sample under the microscope revealed the presence of oocysts. For this purpose, the body weight gain, feed conversion rate, degree of diarrhea, and oocysts per gram were recorded daily (Desalegn and Ahmad, 2020).

4.3.4.2. Sample Collection

The hematology, biochemistry, and histology of the experimental groups were studied by collecting the blood, serum, cecal, and liver tissues at different time intervals of the experiment (Adamu *et al*., 2013). The serum of all the control and treated groups of chicks was examined for protein, carbohydrate, AST, and ALT thrice during trial, i.e. 7^{th} DPI, 7^{th} and 14^{th} DPT, using biochemistry analyzer (Matenu MTN-659D) at specified wavelengths. The protein and carbohydrate contents were estimated using the lowery protein estimation and phenol-sulfuric acid method, respectively (Ashraf *et al*., 2020). The hematological parameters (hemoglobin, RBC, WBC, platelets, granulocytes, lymphocytes and monocytes) were examined on at 14^{th} DPI using a hematology analyzer (Mindray

BC2800) (Adamu *et al*., 2013). The cecum and liver histopathological examination of all experimental groups was conducted to examine the effect of various fractions of *Verbena officinalis* and sulfaquinoxaline on their anatomy on the 14th DPT using the standard protocol (David *et al*., 2022).

4.4. Homology Modeling and Molecular Docking

4.4.1. Selection and Preparation of Target protein

The S-Adenosylmethionine synthetase was selected to control avian coccidiosis, which is essential for the *Eimeria* spp. division (Shah *et al*., 2023). The 3D crystal structure of SAM synthetase was acquired from the Protein Drug Bank with ID 1FUG. All the attached ligands and water molecules found within the protein structure were removed by PyMOL software 2.1.0 and AutoDock (Schrodinger, 2015). Polar hydrogen molecules and Kollman united atom charges were assigned to them, converted into the PDB format, and finally saved in the PDBQT file format (Trott and Olson, 2010).

4.4.2. *In-silico* Preparation of Ligand Molecule

The compounds present in the *V. officinalis* fractions identified by GC-MS (Tables 4.7a & b) were docked with SAM synthetase. The 3D structures of all 27 compounds were built with Chemdraw (11), saved in .sdf format, then converted to PDB format via PyMOL software, minimized by calculating Gasteiger charges, and finally converted to PDBQT file format with AutoDock Tools (Trott and Olson, 2010).

4.4.4. Molecular Docking Simulation

The protein and ligands were accessed via AutoDock (4.2) Tools, and suitable grid maps were calculated for protein–ligand combinations. The center grid parameters were set to 26.536, 17.719, and 58.288 for the x-, y-, and z-axes and maintained at 26 degrees between the x, y, and z axes with a spacing of 1.00A and centered on the active sites. A configuration file consisting of the grid box properties has been created and saved in a .txt file format. The command line was inserted into the command prompt application using AutoDock Vina (1.5.6) to generate the output score, which suggests the best fit model from the different conformations generated for each ligand (Trott and Olson, 2010). The amino acid residues found in the binding site were visualized using LigPlot+ software, and their

hydrogen bonding and hydrophobic interactions with each ligand are summarized in table 4.7c (Laskowski and Swindells, 2011).

4.5. Statistical analysis

The significant difference in the percent SPI data was calculated using the Tukey test ($P < 0.05$), and IC_{50} for each fraction of *V. officinalis* was calculated using Probit analysis (Minitab: V-19) (Monzote *et al*., 2014). The parameters of the *in-vivo* anticoccidial assay were statistically analyzed by one-way ANOVA "Tukey test" (Statistix V-9) to check for HSD (Iqbal *et al*., 2022). The data from UV-Vis and FT-IR spectroscopy was analyzed using Origin 8.5 (Ansir *et al*., 2020).

RESULTS

The aqueous extract of *V officinalis* was processed using column chromatography. After the whole process, more than 90 isolates were acquired in glass bottles, which were pooled into 9 fractions using paper chromatography and subjected to *in-vitro*, *in-vivo* and *in-silico* anticoccidial studies.

4.6 Sporulation Inhibition Assay

These fractions were tested for sporulation inhibition against *E. tenella* oocysts *in-vitro* in the form of aqueous and methanolic extracts. The methanolic extract has somewhat more inhibitory activity than aqueous. Among all the 9 fractions, only four fractions have shown more than 70% inhibitory effect against sporulating oocysts, i.e. the 40 mg/ml of aqueous extracts of F1, F3, F5 and F8, has 68.69±1.81%, 74.12±1.07%, 82.27±0.51% and 79.01±1.29% activity respectively, after 48 hours, which is slightly decreased after 72 hours of incubation i.e. 67.66±2.07%, 71.85±0.69%, 77.69±2.17, and 77.79±1.28% respectively.

Among the effective fractions, F5 was found more potent and has a high rate of sporulation inhibition. Similarly, the 20 mg/ml of all the above mentioned fractions has more than 50% activity against the sporulating oocysts. Further, both the control groups have shown remarkable activity i.e. maximum inhibitory activity was recorded in the SQX-treated groups and negligible activity was shown by $K_2Cr_2O_7$. The microscopic observations of the same groups after 72 hr of incubation period were almost similar with slight differences, such a minute increase in percent sporulation was observed which is elaborated in table 4.1a in detail. The clear trend observed in all fraction assay is that the rate of percent sporulation inhibition is decreased with the decrease the concentration of the both aqueous and methanolic extracts. In addition, the lower concentrations of the effective fractions have shown more activity than the higher concentrations of the ineffective fractions.

Table 4.1(a): Percent sporulation inhibition of *E. tenella* oocysts by using various fractions of *V. officinalis*

F	E	IP	Concentrations (mg/ml) of *V. officinalis*' fractions					Control groups	
			40	20	10	5	2.5	$K_2Cr_2O_7$	SQX
F 1	A	48	68.69±1.81^{b}	57.75±3.79^{de}	44.29±4.34^{gh}	32.82±1.52^{ij}	27.63±1.63^{jk}	2.3±1.22^{l}	98.04±0.19^{a}
		72	67.66±2.07^{bc}	58.69±1.99^{de}	43.57±6.11^{gh}	32.08±0.19^{ij}	24.47±2.72^{k}	2.93±2.39^{l}	97.63±1.03^{a}
	M	48	72.59±2.31^{b}	60.47±2.10^{cd}	52.95±1.24^{ef}	37.72±1.25^{hi}	30.39±1.98^{jk}	3.33±1.75^{l}	98.05±0.25^{a}
		72	70.43±2.28^{b}	57.92±2.96^{de}	50.12±1.48^{ij}	34.69±1.94^{ij}	28.59±1.29^{jk}	3.49±0.41^{l}	96.53±0.91^{a}
F 2	A	48	47.92±1.74^{b}	36.56±1.04^{de}	23.54±0.28^{gh}	21.26±0.97^{ghi}	15.56±1.47^{jkl}	2.06±0.88^{m}	97.66±0.05^{a}
		72	41.78±2.50^{cd}	32.17±1.95^{ef}	20.16±1.86^{ghij}	18.17±0.57^{hij}	11.99±1.20^{kl}	4.03±1.19^{m}	98.22±1.56^{a}
	M	48	47.06±3.83^{bc}	38.28±2.05^{d}	29.63±1.55^{f}	23.76±1.66^{g}	16.11±2.22^{ijkl}	2.73±1.76^{m}	97.42±1.80^{a}
		72	37.62±2.03^{de}	30.13±2.24^{f}	22.87±2.46^{gh}	16.52±2.50^{ijk}	10.53±0.62^{l}	4.2±0.46^{m}	96.73±0.93^{a}
F 3	A	48	74.12±1.07^{b}	59.45±1.62^{cd}	55.08±0.82^{de}	47.09±1.82^{gh}	38.93±1.32^{jk}	3.37±0.97^{l}	96.77±0.81^{a}
		72	71.85±0.69^{b}	56.98±2.68^{de}	53.89±1.22^{ef}	45.05±2.21^{ghi}	36.14±1.21^{k}	2.99±1.55^{l}	98.42±1.37^{a}
	M	48	74.95±1.24^{b}	63.52±1.07^{c}	56.90±0.89^{de}	49.81±0.45^{fg}	42.97±2.51^{hij}	2.61±0.^{47l}	98.28±0.69^{a}
		72	73.13±1.03^{b}	59.88±3.26^{cd}	55.05±0.66^{de}	47.62±0.89^{gh}	40.61±2.59^{ijk}	2.61±1.^{72l}	97.27±0.63^{a}
F 4	A	48	37.92±1.68^{bc}	25.30±1.61^{fgh}	17.72±2.14^{ijk}	13.10±2.68^{klm}	9.85±0.96^{lmn}	2.35±1.17^{o}	95.68±0.21^{a}
		72	32.86±4.04^{cde}	20.46±3.66^{ghij}	14.78±0.87^{jkl}	11.05±0.96^{lm}	6.74±0.49^{mno}	2.99±2.04^{o}	97.76±0.66^{a}
	M	48	37.92±1.59^{b}	25.30±3.88^{def}	17.72±2.65^{ghi}	13.10±2.77^{hijk}	9.84±1.69^{jkl}	2.35±0.50^{o}	95.68±0.29^{a}
		72	35.73±2.14^{bcd}	26.77±3.45^{efg}	19.87±2.73^{hij}	15.39±0.83^{jkl}	4.47±0.87^{no}	2.95±1.02^{o}	97.18±0.88^{a}
F 5	A	48	82.27±0.51^{ab}	72.77±0.62^{bcd}	61.21±2.05^{cdefg}	49.09±0.75^{efgh}	42.24±1.05^{gh}	2.06±0.56^{l}	95.2±2.29^{a}
		72	77.69±2.17^{ab}	70.69±1.74^{bcd}	57.69±1.37^{cdefg}	46.69±1.63^{efgh}	41.57±1.71^{gh}	3.12±0.53^{l}	97.69±0.27^{a}
	M	48	82.27±0.54^{ab}	72.92±0.87^{bcd}	60.46±0.69^{cdefg}	52.53±2.21^{defg}	43.72±1.18^{fgh}	2.0±0.36^{l}	97.87±0.55^{a}
		72	65.23±23.9^{bcde}	63.96±12.84^{bcdef}	58.53±2.80^{cdefg}	48.70±5.00^{efgh}	30.14±19.2^{h}	1.64±0.46^{l}	97.13±0.32^{a}
F 6	A	48	39.85±1.62^{bc}	34.55±2.51^{cde}	27.63±1.73^{efgh}	20.55±0.84^{ijkl}	13.81±3.59^{lmn}	2.48±0.86^{p}	96.93±1.36^{a}
		72	32.42±3.98^{def}	25.28±1.415^{ghi}	23.52±2.09^{hijk}	15.07±1.19^{lm}	7.88±2.62^{nop}	2.60±0.96^{p}	98.20±1.58^{a}
	M	48	43.07±2.14^{bcd}	36.88±1.85^{bcd}	30.61±2.32^{defg}	24.15±1.85^{ghij}	18.45±3.98^{jkl}	3.27±2.16^{p}	98.42±0.44^{a}
		72	33.95±4.16^{defg}	30.98±0.58^{defg}	26.21±1.47^{fghi}	16.99±1.38^{klm}	10.72±2.87^{mno}	4.29±2.57^{p}	97.04±0.96^{a}
F 7	A	48	39.14±2.33^{bc}	31.64±2.57^{def}	24.99±2.83^{fghi}	21.53±2.65^{ghij}	16.19±2.61^{jklm}	4.34±2.73^{op}	96.20±1.65^{a}
		72	33.61±1.95^{cde}	25.52±2.92^{fgh}	17.82±2.31^{ijkl}	13.54±1.51^{klmn}	9.78±2.47^{mnop}	4.58±2.84^{op}	97.84±2.33^{a}
	M	48	43.36±1.74^{b}	35.92±2.17^{bcd}	29.98±0.64^{def}	26.30±0.89^{fgh}	18.86±3.09^{ijk}	2.91±1.99^{p}	96.48±3.49^{a}
		72	36.04±3.76^{bcd}	27.11±2.38^{efg}	20.09±1.04^{ghijk}	15.40±3.54^{jklm}	10.43±1.28^{lmno}	6.72±0.41^{nop}	96.90±0.57^{a}

F 8	A	48	79.01±1.29^{b}	67.95±0.89^{cd}	55.67±1.46^{e}	44.57±0.84^{f}	36.42±1.28^{g}	1.72±0.54^{h}	95.42±0.62^{a}
		72	77.79±1.28^{b}	66.71±0.74^{cd}	54.77±0.68^{e}	42.87±1.76^{f}	34.27±1.45^{g}	2.41±0.39^{h}	98.97±0.89^{a}
	M	48	79.95±1.58^{b}	71.58±2.84^{c}	56.96±0.89^{e}	48.08±1.43^{f}	35.99±1.13^{g}	2.82±1.59^{h}	98.15±0.57^{a}
		72	78.72±3.63^{b}	65.75±3.39^{d}	54.37±1.82^{e}	44.99±2.59^{f}	33.47±3.62^{g}	2.58±0.64^{h}	96.99±0.13^{a}
F 9	A	48	46.07±1.99^{bc}	39.67±0.96^{de}	32.58±1.10^{fg}	26.51±1.16^{hij}	20.21±0.49^{k}	5.14±0.49^{o}	95.71±1.2^{a}
		72	38.69±0.62^{de}	32.04±0.63^{fgh}	24.81±1.31^{ijk}	21.34±1.22^{jk}	14.27±3.89^{lm}	3.19±1.46^{o}	97.79±1.43^{a}
	M	48	48.83±2.08^{b}	42.59±2.66^{d}	35.04±2.51^{f}	27.25±0.98^{hi}	19.08±3.55^{l}	3.78±1.34^{o}	98.61±0.22^{a}
		72	40.89±1.60^{cd}	32.36±3.68^{fg}	27.48±3.68^{ghi}	20.65±1.77^{k}	12.27±1.32^{mn}	7.27±1.59^{no}	95.40±0.11^{a}

F: Fraction, E: Extract solvent, IP: Incubation period, SQX: Sulfaquinoxaline

Table 4.1(b): Inhibitory concentration (IC_{50}) of aqueous (A) and methanolic (M) *V. officinalis* fractions against *E. tenella* oocysts

F	Solvent	IP (hrs)	Mean SPI (%)	IC_{50} (mg/ml) (UCL-LCL)	IC_{90} (mg/ml) (UCL-LCL)
F 1	A	48	18.83±1.24	18.83(16.47-21.41)	65.04(57.3-75.9)
		72	19.69±1.22	19.69(17.38-22.24)	64.44(57-74.80)
	M	48	14.24±1.21	14.24(11.80-16.60)	61.06(53.68-71.55)
		72	16.83±1.21	16.83(14.27-19.30)	63.15(55.61-73.82)
F 2	A	48	39.11±2.68	39.11(33.86-44.40)	91.77(79.01-110.8)
		72	45.91±3.48	45.91(39.09-52.72)	101.08(86.07-124.14)
	M	48	39.74±3.18	39.74(33.55-46.01)	101.02(84.93-126.6)
		72	50.75±4.39	50.75(42.14-59.35)	111.52(93.19-141.13)
F 3	A	48	9.37±1.62	9.37(6.19-12.55)	67.70(57.67-83.29)
		72	11.83±1.55	11.83(8.80-14.86)	79.44(59.98-86.69)
	M	48	5.66±1.89	5.66(1.95-9,38)	66.33(56.15-85.51)
		72	8.59±1.77	8.59(5.12-12.06)	70.9(59.77-88.74)
F 4	A	48	50.91±3.88	50.91(43.30-58.51)	103.92(88.64-127.29)
		72	56.61±4.56	56.61(47.67-65.55)	109.59(92.94-135.48)
	M	48	50.22±4.69	50.22(41.02-59.43)	116.57(96.08-150.96)
		72	51.20±3.92	51.20(43.52-58.88)	104.16(88.78-127.73)
F 5	A	48	4.33±1.44	4.33(1.49-7.16)	48.13(42.44-56.08)
		72	6.03±1.51	6.03(3.07-8.98)	54.83(47.86-64.95)
	M	48	3.13±1.58	3.13(0.04-6.23)	48.86(43.91-57.38)
		72	10.65±1.88	10.65(6.96-14.33)	80.35(66.51-103.7)
F 6	A	48	49.03±4.75	49.03(39.72-58.35)	118.48(96.9-155.48)
		72	60.77±6.45	60.77(48.14-73.40)	130.13(105.42-173.64)
	M	48	45.59±4.66	45.59(36.46-54.72)	120.89(97.59-162.39)
		72	59.32±6.82	59.32(44.39-80.99)	136.17(108.39-187.61)
F 7	A	48	52.84±5.35	52.84(42.36-63.33)	123.55(100.51-163.64)
		72	57.10±5.22	57.10(46.87-67.34)	117.99(98.4-150.8)
	M	48	46.62±5.03	46.62(36.77-56.47)	125.59(100.37-171.84)
		72	54.79±5.03	54.79(44.94-64.64)	117.25(97.24-150.25)
F 8	A	48	9.05±1.21	9.05(6.68-11.41)	51.85(45.97-60.01)
		72	10.56±1.17	10.56(8.26-12.85)	53.58(47.52-61.99)
	M	48	7.52±1.25	7.52(5.08-9.97)	49.89(44.27-57.68)
		72	10.32±1.15	10.32(8.06-12.58)	52.449(46.60-60.50)
F 9	A	48	41.78±4.16	41.78(33.63-49.94)	117.17(94.76-156.92)
		72	53.21±5.69	53.21(42.05-64.37)	127.85(102.89-172.65)
	M	48	36.81±3.16	36.81(30.62-43.01)	104.07(86.21-103.70)
		72	48.39±4.43	48.39(39.69-57.11)	114.31(94.41-147.52)

The IC_{50} for the effective aqueous concentrations of F1, F3, F5 and F8 was 18.83, 11.83, 4.33 and 9.05 mg/ml respectively, after 48 hours of incubation period, which showed the potency of the respective fractions of *V. officinalis* as compared to the rest (Table 4.1b). Similarly, the IC_{90} for the same fractions is recorded as 65.04, 67.70, 48.13 and 51.85 mg/ml respectively after the same incubation period. As for the methanolic extractives of

these fraction concerned, F1, F3, F5 and F8 showed 50% inhibition in the sporulating oocysts19.69, 5.66, 3.13 and 7.52 mg/ml respectively, and 90% sporulation inhibition of the same effective fraction with 61.06, 66.33, 48.86 and 49.89 mg/ml after 48 hours of incubation.

4.7. Anticoccidial Potential of Active Fractions (*In-vivo*)

The anti-coccidial activity of certain *in-vitro* active fractions of *V. officinalis* was evaluated in *E. tenella* infected broiler Ross 308 chicks. The chicks were initially infected with parasitic microtitre. After an infection, each group was treated with it designated treatment. The effectiveness of these treatments against infection was assessed by keeping a daily based record of diarrheal discharge, oocyst count, mean weight gain and feed consumption. Additionally, the serum biochemistry, hematology and histology profile were studied at the end of the experimental trail.

4.7.1 Mean Weight Gain

The effectiveness of the *in-vitro*-active fractions of *Verbena officinalis* in different ratios of certain organic and inorganic solvents against the *E. tenella* oocysts were tested against the same protozoan *in-vivo* in the chick model (Broiler: Ross-308). To overcome the environmental and other relevant factors, three types of control groups were simultaneously run with the experimental groups, including uninfected and untreated C1 group, the infected C2 group and infected plus sulfa drug treated C3 group. The group C3 showed recovering result in terms of weight gain (856.29 gm), which is quite similar to the MWG of C1 (858.71 gm) at the end of experiment as the tukey analysis indicated. Discussing the effect of various fractions of *V. officinalis*, the fractions F3 and F8 with active composition against coccidiosis in terms of MWG as compared to the rest of the two groups as shown in table 4.2. While observing the peaks shown by higher concentrations of F3 (885 gm) and F8 (814.57 gm) at the closing, it makes the results more clear and understandable (Fig. 4.4).

In addition, F3 is considered as more potent against coccidial infection due to its efficacy in case of medium concentration. Furthermore, the maximal feed consumption of 0.62 and 0.81 FCR was observed in the groups treated with high concentrations of fractions F3 and F8, in contrast to the infected group, which was above 2.00, suggesting low feed

consumption. Whereas, the fractions F1 and F5 also had low feed consumption due to severe intestinal infection with 1.04 and 1.51 FCR values (Table 4.3).

Table 4.2: Mean weight gain of the *E. tenella* infected broiler chicks' treated with various fractions of *V. officinalis*

Groups	**Concentrations**	**Mean Weight Gain (gm)**		
		7th DPI	**7th DPT**	**14th DPT**
Controls	C1	299.29±4.16^{a}	652.57±18.11	858.71±11.61^{a}
	C2	247.86±7.19^{defg}	298.57±42.89	451.00±27.17^{i}
	C3	248.43±15.17^{abcd}	645.14±1.78^{a}	856.29±3.79^{a}
F1	H	260.86±7.43^{abcd}	369.43±35.52^{cd}	687.57±51.95^{f}
	M	263.14±6.46^{cdef}	307.14±14.43^{ef}	559.86±15.88^{h}
	L	243.43±10.06^{fgh}	262.71±6.13^{g}	472.57±22.86^{i}
F3	H	290.14±6.18^{ab}	486.29±6.42^{b}	885.00±7.08^{b}
	M	256.57±6.38^{bcde}	428.83±11.67^{c}	854.86±30.19^{c}
	L	294.14±12.41^{abc}	341.00±13.33^{c}	752.14±9.26^{e}
F5	H	223.00±5.72^{gh}	336.86±5.16^{ef}	404.71±4.38^{i}
	M	237.71±2.06^{efgh}	275.71±12.22^{fg}	421.14±14.83^{i}
	L	225.86±7.96^{h}	270.86±2.025^{g}	412.86±9.29^{i}
F8	H	273.71±11.65^{abcd}	373.86±15.93^{c}	814.57±14.46^{fg}
	M	276.29±8.78^{abcd}	274.29±9.48^{efg}	700.43±11.29^{g}
	L	262.29±6.61^{bcd}	322.57±2.92^{de}	627.43±8.06^{g}

Superscript of all treated and untreated groups denotes tukey test variables were different, showed significant difference. C1: Normal, C2: Infected, C3: Sulfaquinoxaline treated, H: High (15 mg/ml), M: Medium (10 mg/ml), L: Low (5 mg/ml)

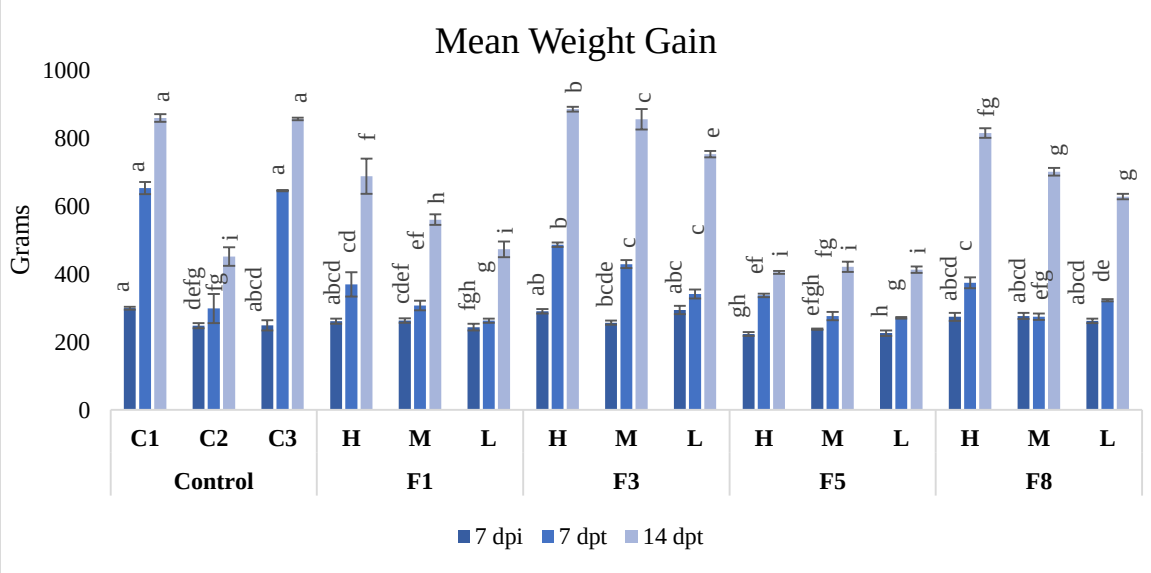


Figure 4.4: Mean weight gain of the *E. tenella* infected broiler chicks' treated with various fractions of *V. officinalis*

4.7.2. Feed Conversion Ratio

To assess the metabolic activity, the feed consumption and feed conversion ratio of all the experimented groups of chicks were calculated. Initially, first week post-infection (3 week age) the feed consumption of all infected groups were same, lower than that of the non-infected group (5062.5 gm) with significant FCR value. In the 4th week, the treated groups acted differently, i.e. the feed consumption of sulfa treated groups (5512.5 gm; 0.41) rises as the individuals of uninfected normal control (6300.0 gm; 0.36) with lower most FCR value, Similarly the infected groups treated with F3H fraction has also responded in well manner with good feed consumption and FCR (5512.5gm; 0.46), showing it efficient reabsorption of nutrients. In the end of the experiment, the feed consumption calculated showed good feed consumption in the case of F3H (6048 gm; 0.62) had been followed by F3M (5040 gm; 0.75) and F8H (5040 gm; 0.81).The groups treated with fraction F1H-L(4032, 3154.5, 3154.5 gm with 1.04, 1.63 and 1.92 FCR respectively) and F5H-L (4032, 3564.0 and 3573 gm feed consumption with poorest FCR of 1.51, 2.24 and 2.36 respectively), indicating that all the groups treated with F1 and F5 has consumed insufficient amount of feed with poor FCR (Table 4.3).

Table 4.3: Feed consumption and FCR of the *E. tenella* infected broiler chicks' treated with various fractions of *V. officinalis*

Groups	**Concentrations**	**Feed Consumption (gm) and Feed Conversion Ratio**		
		7 DPI	**7 DPT**	**14 DPT**
Controls	C1	5062.5 – 0.32	6300.0 – 0.36	7056.0 – 0.52
	C2	4797.0 – 0.38	3978.0 – 0.85	2421.0 – 2.46
	C3	4716.0 – 0.38	5512.5 – 0.41	6048.0 – 0.57
F1	H	3879.0 – 0.40	3937.5 – 0.69	4032.0 – 1.04
	M	3964.5 – 0.39	3937.5 – 0.83	3154.5 – 1.63
	L	3964.5 – 0.43	3937.5 – 0.94	3154.5 – 1.92
F3	H	4567.5 – 0.35	5512.5 – 0.46	6048.0 – 0.62
	M	4374.0 – 0.38	4725.0 – 0.58	5040.0 – 0.75
	L	3892.5 – 0.37	3573.0 – 0.75	3024.0 – 1.29
F5	H	4126.5 – 0.43	3937.5 – 0.79	4032.0 – 1.51
	M	3807.0 – 0.43	3937.5 – 0.87	3564.0 – 2.24
	L	3739.5 – 0.45	3937.5 – 0.94	3573.0 – 2.36
F8	H	4410.0 – 0.37	4828.5 – 0.61	5040.0 – 0.81
	M	4333.5 – 0.37	4599.0 – 0.73	4032.0 – 1.19
	L	4063.5 – 0.39	3690.0 – 0.80	3024.0 – 1.50

C1: Normal, C2: Infected, C3: Sulfaquinoxaline treated, H: High (15 mg/ml), M: Medium (10 mg/ml), L: Low (5 mg/ml)

4.6.3. Oocysts Count (OPG)

To assess the effect of fractions of *V. officinalis* against the *E. tenella* infected groups, the oocysts count was taken under observation both post infection and post treatment. Initially the broiler groups were infected at the 14th day of age. As a result, some

of the groups (9) become infected at day 19 and others (5) release oocysts in feces on day 20. The numbers in all infected groups began to rise, but after a week, i.e. day 21 infected groups were treated with their designated concentration of *V. officinalis* fractions.

Till the 27th day, the oocyst numbers were rising reflecting no effect of any kind of treatment, even in the control group treated with drug but on 28th day the decline was observed in some groups and the rest was showing a similar pattern just like the untreated control group. The blue spectrum lines in the graph (Fig. 4.5) reflect the groups of chicks recovered from *E. tenella* infection, which correspond to the right side of the secondary y-axis, whereas red lines showed the infected and unrecovered groups as the treatment become non effective, corresponding to the primary y-axis.

The individuals of the control group (C2) released a maximum number (22.2×10^3 OPG) of oocysts in feces, reflecting a severe type of infection in all groups. In contrast, the group (C3) treated with sulfa drug primarily released, raising the number of oocysts, but as the drug becomes effective, the oocysts number falls to the lowest level (5.0×10^3 OPG), whereas C1, the uninfective group, showed no single oocyst in feces.

In terms of oocysts shedding, a declining number was observed in two fractions of *V. officinalis*, i.e. F3 and F8. The infected group treated with higher concentration of fraction 3 was reported with 4.35×10^3 OPG initially at day 19 age, rising to highest value of 12.25×10^3 OPG at 27th day but as the concerned amount of *V. officinalis* fraction begin to act the oocysts number declines and at the end of experiment 6.55×10^3 oocyst per gram were recorded finally and the second most effective fraction was the higher concentration of fraction 8, which was noticed with 4.35×10^3 OPG at day 20 age, the oocyst number was continuously raised till 27 day age up to maximum amount of 13.7×10^3 OPG and then the treatment become effective in terms of lowering down the amount of oocyst in the respective group and finally ends on 7.05×10^3 OPG at 35 day age.

The same declining oocyst rate were also observed in both medium and lower concentrations of fractions 3 and 8 of *V. officinalis*, whereas fractions F1 (18.75×10^3) and F5 (19×10^3) does not react against the rising oocysts number in the infected groups, having no therapeutic effect.

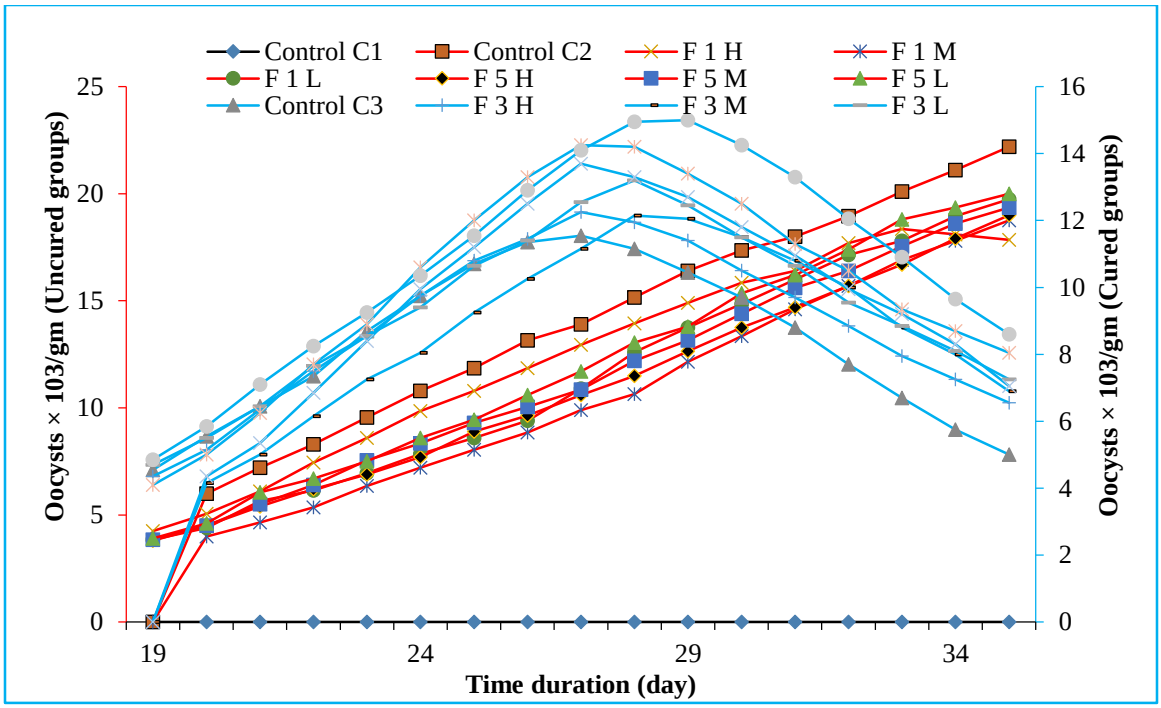


Figure 4.5: Treatment dependent reduction of oocysts shedding in response to *V. officinalis* fractions

4.7.4. Diarrheal record of infected and treated chicks

The diarrhea grades of the experimental groups were observed post-treatment with different concentrations of the *in-vitro* fractions of *V. officinalis.* The presence of diarrhea is graded into 4 levels on the basis of severity, i.e. 1 to 4; the colors in the heat map reflect diarrheal severity of each group from the day of infection to the end of the experiment. The normal control group (C1) was found to have no diarrheal fluid in the fecal material as it was not infected with *E. tenella*. The control C2 was found to have severe diarrhea as it was not treated with any kind of drug or supplement; whereas C3 had mild diarrhea for two weeks after having an infection as it was treated with sulfaquinoxaline as a standard for the newly tested fractions of *V. officinalis* extract.

In this regard, the fractions F3 and F8 have satisfactory results in terms of getting rid of diarrhea caused by coccidiosis, though both groups took a little bit more time, i.e., at 33 days, the F3H-treated group has normalized the diarrhea, whereas the F8H-treated group showed no diarrheal fluid at day 34. Further, the medium concentration of the fraction F3 has also shown curing results from diarrhea. The groups treated with F1's concentration

have experienced severe infection and end with a moderate level, whereas the F5-treated groups have the longest severe infection period and end with mild type infection at the termination of the experimental trail (Fig. 4.6).

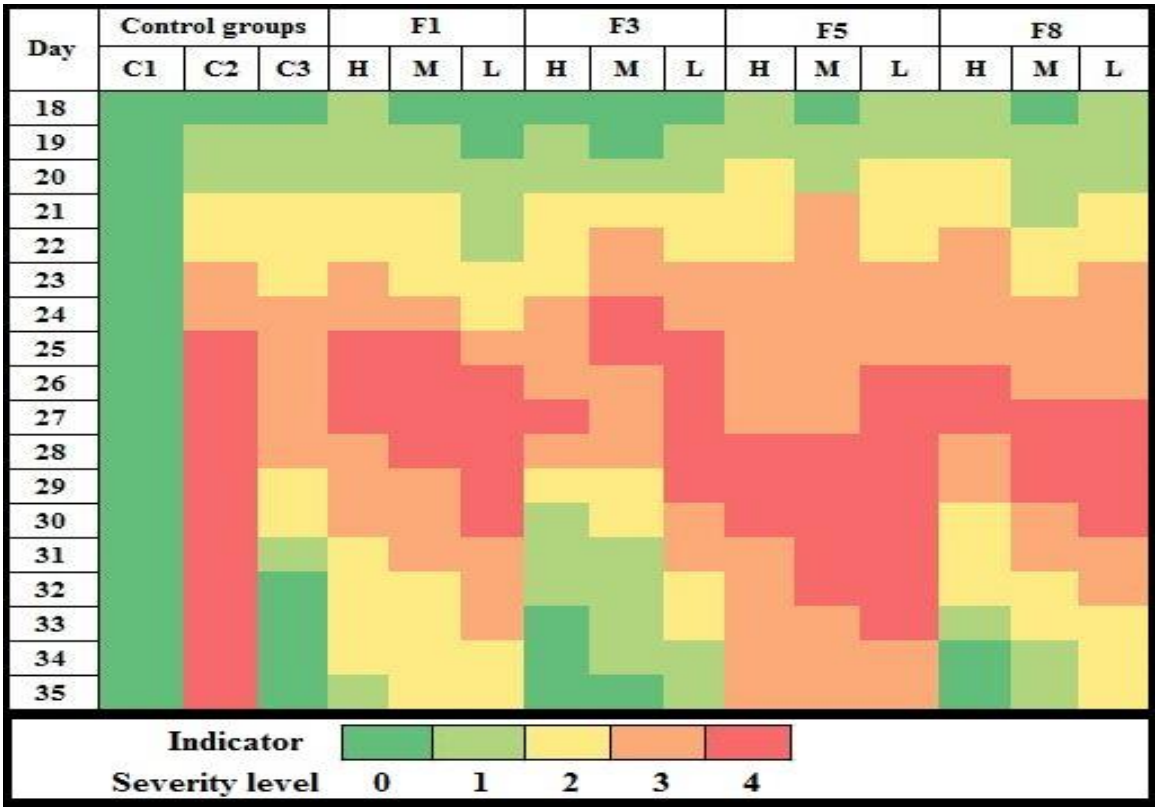


Figure 4.6: Heat map showing severity of bloody diarrhea post infection and post treatment with respective fraction

4.7.4.1 Biochemistry

To assess the effectiveness of *Eimeria* infection and treatment with *V. officinalis* fractions on the biochemical profile of experimental groups of broiler chicks, they were observed thrice in the experimental period, i.e., pre-treatment & post-infection (7^{th} DPI / 21 day age) and post-treatment (7^{th} & 14^{th} DPT / 28 & 35 day age). Four common parameters like protein, carbohydrate, ALT, and AST were selected for observation to find out the negative or positive impact of all the experimental fractions of *V. officinalis* on the health of broiler chicks. The biochemical changes occur in blood composition in response to parasitic infection and drug treatment; in short, these changes act as health indicators. The level of protein and carbohydrates generally falls with the occurrence of infection as

the organism is unable to utilize the food in a good way. Whenever the cell experiences injury, parasitic infection, or poison infusion, it releases ALT and AST into bloodstream, and as a result, the level of both contents raises, signaling the presence of infection or liver injury. In the current experiment, the *E. tenella*-infected chicks were treated with different concentrations of four fractions of *V. officinalis* for the evaluation of its anti coccidial activity. The anticoccidial activity of these fractions was analyzed by estimating the different biochemical contents of blood serum protein and carbohydrates to test the reabsorption capacity of the intestinal epithelium of chicks in all the drug- and fraction-treated groups as compared to the infected group, whereas ALT and AST were measured to observe the liver-recovery action of these fractions.

Initially, at 7th DPI, the protein and carbohydrates (Fig. 4.7a & b; Table 4.4a & b) levels of all experimental groups were below normal except for C1, which remained uninfected, and a gradual increase was observed in both contents of each group, showing how birds in each group respond to their respective treatments. As for the liver function concerned, the ALT and AST were initially high except for the uninfected control, but with the passage of time, the liver responded well to the *Eimeria* parasite, and the level of both liver contents declined to normal according to the treatment.

The infected group was found to have a low level of protein (0.25±0.008^{g} mg/ml) and carbohydrate (135.02±12.95^{g} mg/ml) and a high amount of AST (93.41±7.90^{ab} U/L) and ALT (222.32±2.18^{a} U/L) (Fig. 4.7c & d; Table 4.4c & d). In contrast, the normal and drug-treated control groups have just opposite amounts of these parameters. The observation of all the experimented groups showed the potential of fraction F3 against coccidiosis with a high level of protein content (2.04±0.013^{cd}) just like the sulfa-treated group (2.89±0.037^{b}) followed by fraction F8H with 2.22±0.007^{c} mg/ml protein. Similar results were observed in the F3H (242.81±17.81^{c} mg/ml) treated group for carbohydrate content. Which showed that F3-treated groups have enhanced reabsorption activity of macronutrients compared to the rest of the groups. As for as the AST and ALT was concerned, the amounts of both contents in blood serum were found satisfactory in a group treated with the F3H fraction of *V. officinalis*, i.e., 42.78±2.47^{f} U/L and 119.31±0.79^{hi} U/L, respectively, showed the decline of parasitic infection.

Table 4.4a: Protein estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

Groups	Concentrations	Protein Estimation (mg/ml)		
		7 DPI	7 DPT	14 DPT
Controls	C1	3.51±0.009^{a}	3.68±0.005^{a}	3.74±0.011^{a}
	C2	0.82±0.020^{b}	0.92±0.005^{fg}	0.25±0.008^{g}
	C3	0.83±0.025^{b}	1.62±0.010^{b}	2.89±0.037^{b}
F1	H	0.81±0.009^{b}	0.74±0.005g^{h}	0.69±0.006^{ef}
	M	0.62±0.005^{b}	0.67±0.009^{h}	0.59±0.009^{f}
	L	0.79±0.010^{b}	0.65±0.008^{h}	0.56±0.006^{fg}
F3	H	1.01±0.006^{b}	1.60±0.021^{bc}	2.04±0.013^{cd}
	M	0.89±0.033^{b}	1.34±0.011^{cd}	1.89±0.010^{cd}
	L	0.77±0.023^{b}	1.18±0.006^{def}	1.83±0.024^{d}
F5	H	0.88±0.018^{b}	0.87±0.013^{gh}	0.95±0.007^{e}
	M	0.78±0.007^{b}	0.74±0.002^{gh}	0.88±0.005^{ef}
	L	0.78±0.019^{b}	0.68±0.008^{h}	0.75±0.006^{ef}
F8	H	0.62±0.044^{b}	1.54±0.013^{bc}	2.22±0.007^{c}
	M	0.57±0.013^{b}	1.22±0.012^{de}	1.96±0.009^{cd}
	L	0.57±0.044^{b}	0.98±0.019^{efg}	1.82±0.008^{d}

Superscript of all treated and untreated groups denotes tukey test variables were different, showed significant differences with $P \leq 0.05$. C1: Normal, C2: Infected, C3: Sulfaquinoxaline treated, H: High (15 mg/ml), M: Medium (10 mg/ml), L: Low (5 mg/ml)

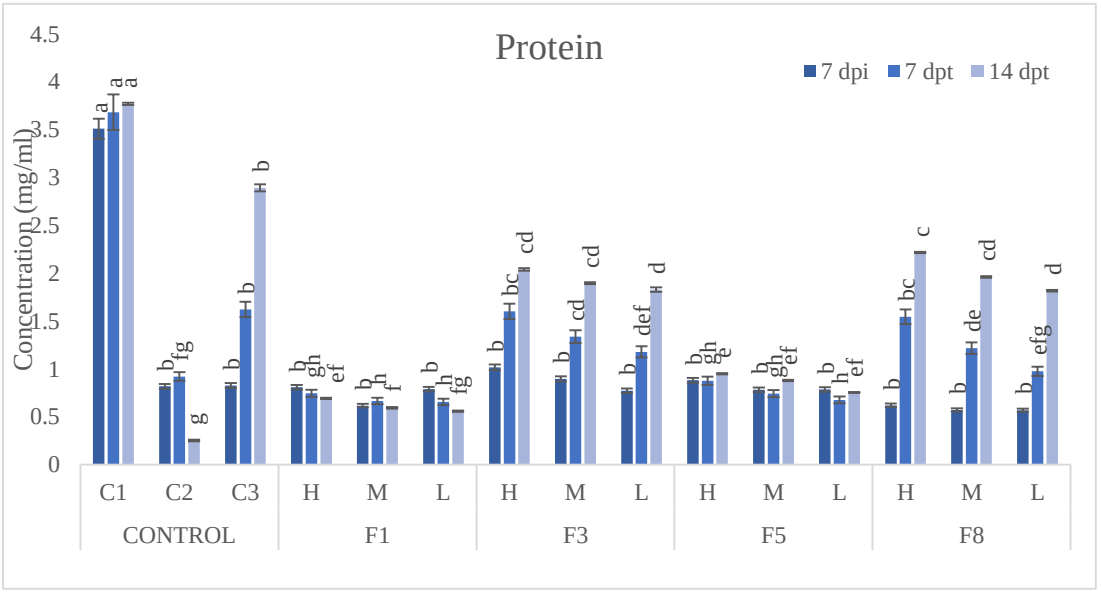


Figure 4.7a: Protein estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

Table 4.4b: Carbohydrate estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

Groups	Concentrations	Carbohydrate Estimation (mg/ml)		
		7 DPI	7 DPT	14 DPT
Controls	C1	322.17±23.90^{a}	316.25±18.18^{a}	319.48±16.94^{a}
	C2	157.44±23.09^{b}	152.17±12.96^{de}	135.02±12.95^{g}
	C3	156.47±13.99^{b}	227.44±14.80^{b}	289.91±23.90^{b}
F1	H	140.50±20.89^{b}	148.19±13.02^{de}	145.93±12.76^{fg}
	M	142.76±14.20^{b}	148.03±13.45^{de}	145.02±12.95^{fg}
	L	144.43±15.16^{b}	146.79±14.97^{de}	144.43±13.52^{fg}
F3	H	149.32±15.98^{b}	164.05±14.07^{c}	242.81±17.81^{c}
	M	143.24±14.02^{b}	156.15±12.99^{cd}	182.65±19.15^{d}
	L	143.78±14.13^{b}	147.70±14.47d^{e}	166.89±15.05^{de}
F5	H	146.79±15.46^{b}	143.40±13.60^{e}	147.81±15.66^{fg}
	M	148.24±13.63^{b}	143.35±12.63^{e}	147.49±13.47^{fg}
	L	150.98±15.18^{b}	143.03±13.56^{e}	147.44±13.68^{fg}
F8	H	150.72±14.55^{b}	151.58±14.62^{de}	154.10±14.04^{de}
	M	152.17±14.31^{b}	150.88±15.67^{de}	152.49±15.88^{de}
	L	152.65±17.96^{b}	149.43±14.22^{de}	150.50±14.78^{de}

Superscript of all treated and untreated groups denotes tukey test variables were different, showed significant differences with P ≤ 0.05. C1: Normal, C2: Infected, C3: Sulfaquinoxaline treated, H: High (15 mg/ml), M: Medium (10 mg/ml), L: Low (5 mg/ml)

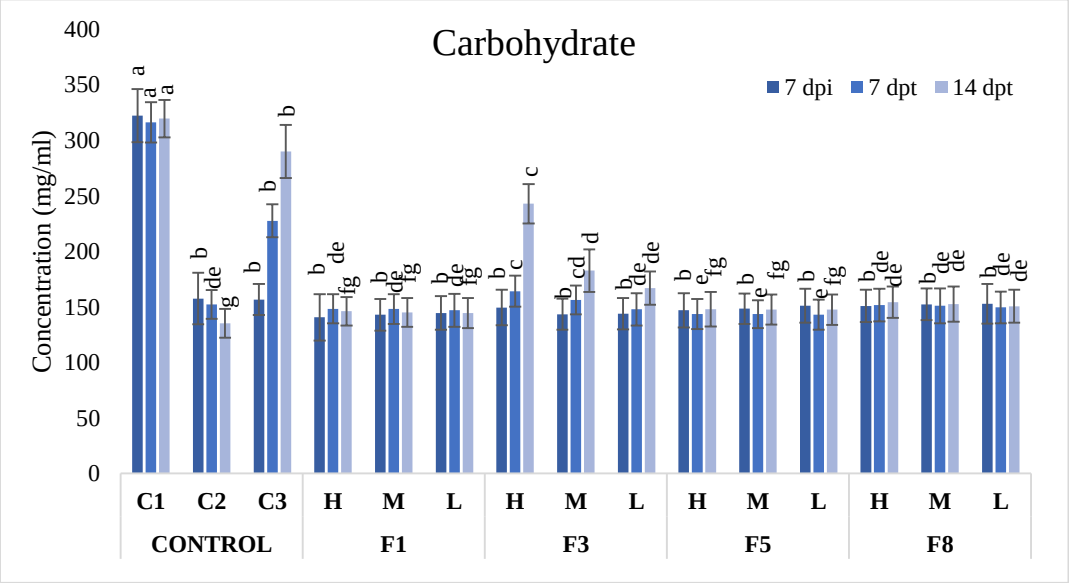


Figure 4.7b: Carbohydrate estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

Table 4.4c: ALT estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

Groups	Concentrations	Alanine aminotransferase (U/L)		
		7 DPI	7 DPT	14 DPT
Controls	C1	91.26±1.39^{c}	94.23±5.17^{e}	91.89±3.28^{j}
	C2	139.21±2.14^{ab}	170.06±2.66^{a}	222.32±2.18^{a}
	C3	143.81±1.21^{a}	122.05±1.86^{d}	113.89±1.51^{i}
F1	H	136.48±1.44^{ab}	141.66±1.25^{b}	143.64±1.52^{b}
	M	141.08±1.27^{ab}	138.11±1.28^{b}	144.57±1.50^{b}
	L	140.84±4.49^{ab}	141.54±1.67^{b}	144.51±1.08^{b}
F3	H	142.65±0.89^{ab}	125.95±0.50^{d}	119.31±0.79^{hi}
	M	140.49±0.87^{ab}	129.67±0.81^{cd}	125.31±0.87^{g}
	L	139.79±0.54^{ab}	136.30±1.88^{bc}	129.03±0.99f^{g}
F5	H	141.25±3.59^{ab}	138.05±2.45^{b}	134.44±1.11^{def}
	M	139.56±1.19^{ab}	138.75±2.45^{b}	137.88±0.36^{bc}
	L	143.23±1.09^{ab}	143.23±1.09^{b}	142.71±0.93^{bc}
F8	H	141.66±1.25^{ab}	138.52±1.81^{b}	124.26±.186^{gh}
	M	138.11±1.28^{ab}	139.04±0.79^{b}	131.24±1.37^{ef}
	L	141.54±1.67^{ab}	137.29±0.70^{bc}	135.55±0.87^{de}

C1: Normal, C2: Infected, C3: Sulfaquinoxaline treated, H: High (15 mg/ml), M: Medium (10 mg/ml), L: Low (5 mg/ml)

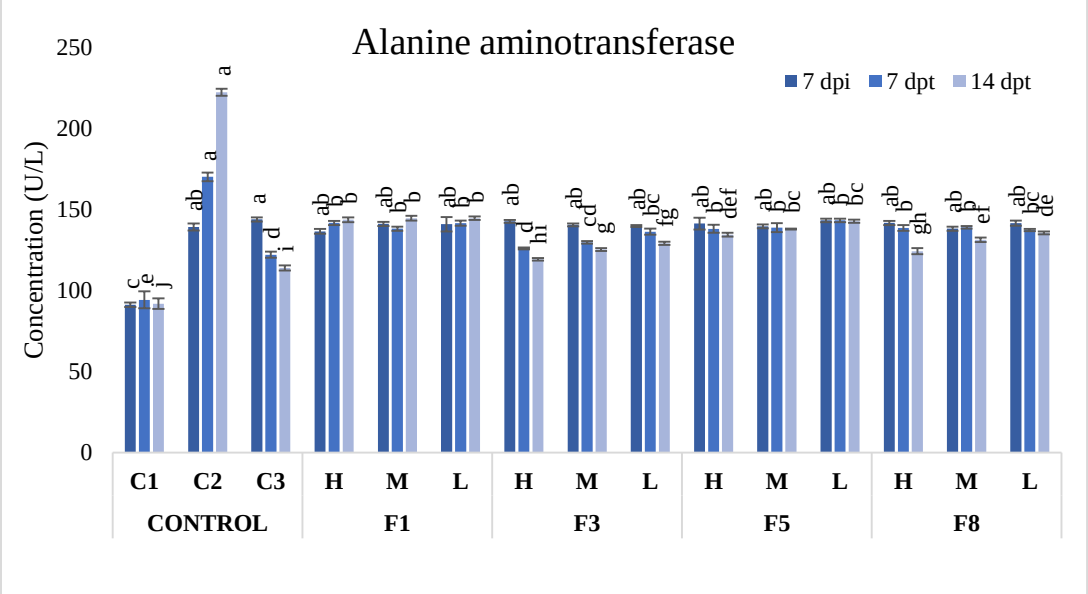


Figure 4.7c: ALT estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

Table 4.4d: AST estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

Groups	Concentrations	Aspartate aminotransferase (U/L)		
		7 DPI	7 DPT	14 DPT
Controls	C1	$27.12\pm.171^{e}$	26.31 ± 1.66^{f}	25.20 ± 1.58^{g}
	C2	84.39 ± 2.12^{cd}	83.52 ± 3.05^{cd}	93.41 ± 7.90^{ab}
	C3	94.05 ± 3.79^{abc}	63.38 ± 3.57^{e}	41.32 ± 5.03^{f}
F1	H	88.81 ± 3.33^{bcd}	86.78 ± 2.47^{bcd}	85.32 ± 1.78^{bcd}
	M	93.76 ± 3.79^{abc}	91.32 ± 1.36^{abc}	93.29 ± 2.22^{ab}
	L	93.12 ± 1.86^{abc}	99.76 ± 2.49^{a}	101.85 ± 2.07^{a}
F3	H	100.16 ± 2.81^{a}	67.86 ± 1.93^{e}	42.78 ± 2.47^{f}
	M	90.56 ± 1.93^{abcd}	81.18 ± 1.08^{d}	56.80 ± 2.99^{e}
	L	96.90 ± 1.59^{ab}	91.49 ± 1.31^{abc}	77.87 ± 2.43^{cd}
F5	H	95.97 ± 2.87^{ab}	86.14 ± 2.29^{bcd}	61.34 ± 1.51^{e}
	M	99.93 ± 5.04^{a}	92.36 ± 2.15^{abc}	75.60 ± 1.54^{d}
	L	94.87 ± 2.92^{abc}	93.64 ± 0.36^{ab}	87.94 ± 1.09^{bc}
F8	H	79.85 ± 4.73^{d}	59.59 ± 4.61^{e}	75.72 ± 4.78^{d}
	M	87.88 ± 1.01^{bcd}	80.14 ± 3.23^{d}	89.34 ± 1.65^{bc}
	L	97.54 ± 1.94^{ab}	87.82 ± 2.33^{bcd}	92.01 ± 0.51^{ab}

Superscript of all treated and untreated groups denotes tukey test variables were different, showed significant differences with $P \leq 0.05$. C1: Normal, C2: Infected, C3: Sulfaquinoxaline treated, H: High (15 mg/ml), M: Medium (10 mg/ml), L: Low (5 mg/ml)

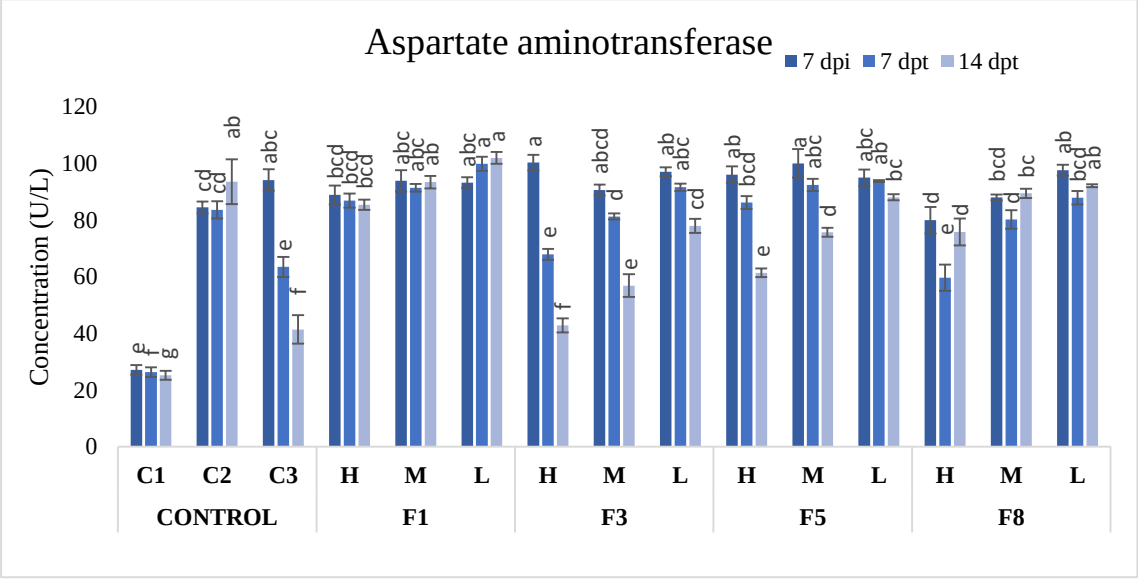


Figure 4.7d: AST estimation of the *E. tenella* infected broiler chicks post treatment with *V. officinalis* fractions

4.7.4.2 Hematology

The complete blood count of various experimental groups of *E. tenella*-infected broiler chicks was analyzed after treatment with various concentrations of *V. officinalis'* active fractions (*in-vitro*) in different ratios of organic and inorganic solvents in comparison with normal uninfected chicks and the sulfa-treated group as a control. Observing the different blood contents of the studied groups, it seems clear that the experiment was conducted in good laboratory conditions because the sulfa treated group has recovered action against the coccidiosis in broilers in terms of hemoglobin, RBC, WBC, platelets, lymphocytes, monocytes and granulocytes because its level falls near the normal control group, in contrast to the chicks of the untreated control.

The hemoglobin contents of the infected groups showed an increase in treatment with higher concentrations of fraction F3 (11.96 ± 0.09^{c} g/dL), followed by F8 (10.64 ± 0.11^{d} g/dL), respectively, whereas the untreated group has the lowest level of about 6.84 ± 0.22^{i} g/dL, as indicated by tukey analysis. In the same way, the RBCs level rises with an increase in concentration of fraction F3 (H: 1.88 ± 0.014^{bb}, M: 1.77 ± 0.021^{bc} and L: $1.75\pm0.021^{de}\times10^{6}$/µL), followed by F1 (H: $1.75\pm0.026d^{e}\times10^{6}$/µL) and F8 (H: 1.73 ± 0.094^{cd} $\times10^{6}$/µL) treated groups, whereas drug treated control has $1.96\pm0.169^{b}\times10^{6}$ RBCs/µL. Just like RBC, a similar pattern is adopted by WBC level in response to the treatment fraction F3 (H: 9.98 ± 44.58^{c}, M: 8.77 ± 44.47^{d}, L: $8.53\pm32.51^{fg}\times10^{3}$/µL) followed by F8 (H: $8.62\pm34.43^{ef}\times10^{3}$/µL) and F1 (H: $8.67\pm56.75^{de}\times10^{3}$/µL) treatment. The platelets observed in the infected and untreated control were too low ($382\pm5.6^{f}\times10^{3}$/µL) from the normal at 14th DPT. whereas, the fractions-treated groups have shown better recovery from the rest of the groups. In this aspect, group F3 had a normalizing level of $447\pm3.0^{b}\times10^{3}$, followed by the F8-treated group (H: $434\pm2.7^{c}\times10^{3}$ Platelets/µL).

The immune system responded to the infection in such a way that the level of immune cells rose to a high level to cope with the infection in a good manner, and the level of lymphocytes, monocytes, and granulocytes recorded in the infected group (C2) were 97.03 ± 0.29^{a}%, 33.78 ± 0.39^{a}%, and 30.86 ± 0.44^{a}%, respectively. But in the case of treatment with the sulfa drug (Group-C3), the level of these contents falls, indicating the recovery of the control group. Similarly, fraction F3 has the best recovery results with 65.94±0.34% lymphocytes, 16.83±0.22% monocytes, and 16.10±0.48% granulocytes, followed by the

infected group treated with fraction F8 with 67.13±0.30% lymphocytes, 18.04±0.17% monocytes, and 18.12±0.19% granulocytes (Table 4.5).

Table 4.5: Estimation of blood parameters of various experimented broiler groups treated with different fractions of *V. officinalis*

G	C	Hb (g/dL)	RBC ($\times10^6$/µL)	WBC ($\times10^3$/µL)	Plt (10^3/µL)	L-cytes (%)	M-cytes (%)	G-cytes (%)
Control	C1	13.59±0.34^{a}	2.17±0.306^{a}	10.43±44.55^{a}	471±5.9^{a}	61.15±2.08^{i}	15.88±0.48^{i}	14.73±0.52^{j}
	C2	6.84±0.22^{i}	1.62±0.172^{g}	7.26±75.96^{k}	382±5.6^{f}	97.03±0.29^{a}	33.78±0.39^{a}	30.86±0.44^{a}
	C3	12.65±0.45^{b}	1.96±0.169^{b}	10.23±36.47^{b}	452±4.0^{b}	64.21±0.57^{h}	16.38±0.47^{ij}	15.66±0.48^{ij}
F1	H	8.79±0.09^{ef}	1.75±0.026d^{e}	8.67±56.75^{de}	409±2.5^{e}	65.29±0.39^{gh}	23.78±0.33^{d}	23.40±0.49^{e}
	M	8.09±0.07^{g}	1.71±0.040^{def}	8.29±30.75^{h}	390±3.1^{f}	65.88±0.15^{fgh}	25.38±0.57^{c}	27.49±0.65^{c}
	L	7.17±0.03^{hi}	1.58±0.035^{ef}	8.10±24.00^{i}	387±2.0^{f}	67.53±0.20^{ef}	28.56±0.59^{b}	28.97±0.31^{bc}
F3	H	11.96±0.09^{c}	1.88±0.014^{bb}	9.98±44.58^{c}	447±3.0^{b}	65.94±0.34^{fgh}	16.83±0.22^{hi}	16.10±0.48^{ij}
	M	10.94±0.09^{d}	1.77±0.021^{bc}	8.77±44.47^{d}	429±3.5^{cd}	67.17±0.37^{efg}	18.43±0.41^{fg}	17.62±0.53^{hi}
	L	8.27±0.18^{fg}	1.75±0.021^{de}	8.53±32.51^{fg}	423±2.5^{d}	68.94±0.25^{e}	19.78±0.44^{ef}	19.59±0.63^{efg}
F5	H	8.88±0.06^{ef}	1.60±0.080^{ef}	8.18±45.18^{hi}	391±2.5^{f}	72.18±0.62^{cd}	27.42±0.48^{b}	25.86±0.56^{d}
	M	7.41±0.49^{hi}	1.71±0.080^{ef}	7.91±43.55^{j}	389±1.5^{f}	73.08±0.24^{c}	28.73±0.51^{b}	28.16±0.76^{c}
	L	7.25±0.06^{hi}	1.59±0.075^{f}	7.79±38.68^{j}	384±3.0^{f}	81.07±0.62^{b}	33.14±0.59^{a}	30.29±0.44^{ab}
F8	H	10.64±0.11^{d}	1.73±0.094^{cd}	8.62±34.43^{ef}	434±2.7^{c}	67.13±0.30^{efg}	18.04±0.17^{gh}	18.12±0.19^{gh}
	M	9.14±0.22^{e}	1.58±0.092^{de}	8.42±34.66^{g}	426±4.2^{cd}	70.99±0.29^{d}	19.97±0.64^{e}	19.55±0.67^{fg}
	L	7.67±0.11^{gh}	1.53±0.071^{ef}	8.17±44.55^{hi}	421±2.5^{d}	73.13±0.13^{c}	20.15±0.28^{e}	20.34±0.53^{f}

Superscript of all treated and untreated groups denotes tukey test variables were different, showed significant differences with $P \leq 0.05$. C1: Normal, C2: Infected, C3: Sulfaquinoxaline treated, H: High (15 mg/ml), M: Medium (10 mg/ml), L: Low (5 mg/ml) (Plt Platelets; L-cytes: Leucocytes; M-cytes: Monocytes; G-cytes: Granulocytes)

4.7.5. Histology of Experimental Groups Post-treatment

The histological profile of experimental chicks' groups was studied to examine the impact of *E. tenella* infection and the treatment effect of *in-vitro* active fractions of *V. officinalis* in *Eimeria*-challenged broiler chicks. For this purpose, the histology of three essential organs like the cecum, liver, and kidney of both the normal uninfected chicks was compared with fractions and sulfaquinoxaline treated groups at the termination of the experimental trail, i.e., day 35. The histological observation of the intestinal epithelium was done after *E. tenella* infection and treatment of various groups of broiler chicks to investigate the effectiveness of different fractions of *V. officinalis.* For this purpose, the flock of broiler chicks was divided into certain groups by infecting all the groups by day 14 except one (normal control group-C1). Each of the infected groups was treated with a specific drug (C3- Sulfaquinoxaline) and various concentrations of fractions F1, F3, F5, and F8 at the age of 21 days and was fed according to the protocol. Again one of the

infected groups was left untreated (C2) for comparison with the normal and treated groups. One member from each group was sacrificed for histological observation of liver and intestinal tissues to investigate the effectiveness of the said fractions of *V. officinalis*.

The histological observation of group C1 revealed the normal position of the intestinal tissues of the ceacal part; further, this group was found to have a meaningful mean weight gain and feed conversion ratio. The group C2 (control) was left untreated with any kind of drug and remained infected throughout the experimentation period. The histological observation of this group (C2) demonstrates the degenerated intestinal epithelial cells (Fig. 4.8a), which definitely had disturbed nutrient reabsorption as reflected in low weight gain (Table 4.2) and low quantities of protein and carbohydrates (Table 4.4a-c). The control group (C3) was treated with sulfaquinoxaline for comparative analysis of newly synthesized factions of *V. officinalis*.

The histological study of C3 revealed a little bit of disturbance in intestinal epithelial tissues, but the overall position is satisfactory with recovered cells after sulfaquinoxaline treatment and has good MWG and FCR records as well. Coming towards the *E. tenella*-infected groups treated with numerous concentrations of the *V. officinalis* fractions, the groups treated with high and medium concentration of F3 fraction and high concentration of F8 fraction, demonstrated normal or recovered intestinal tissue in histological observation. The said fractions were effective against the coccidial infection; that's why the tissues were recovered to their normal position as in the C3 control group. Further, these groups have a good feed conversion ratio with a normal biochemical and hematology profile through the study. As far as the rest of the fractions like F1 and F5 were concerned, they don't respond well to the induced parasitic infection, and the intestinal histology demonstrates the degenerated tissues with cavities inside. That's why the intestinal epithelial cells of these groups do not perform their function properly, leading to the malnourishment of chicks. In addition, the poor biochemical (Table 4.4a-c), hematology profile (Table 4.5) and minimum weight gain in these groups provide evidence of an unrecovered condition.

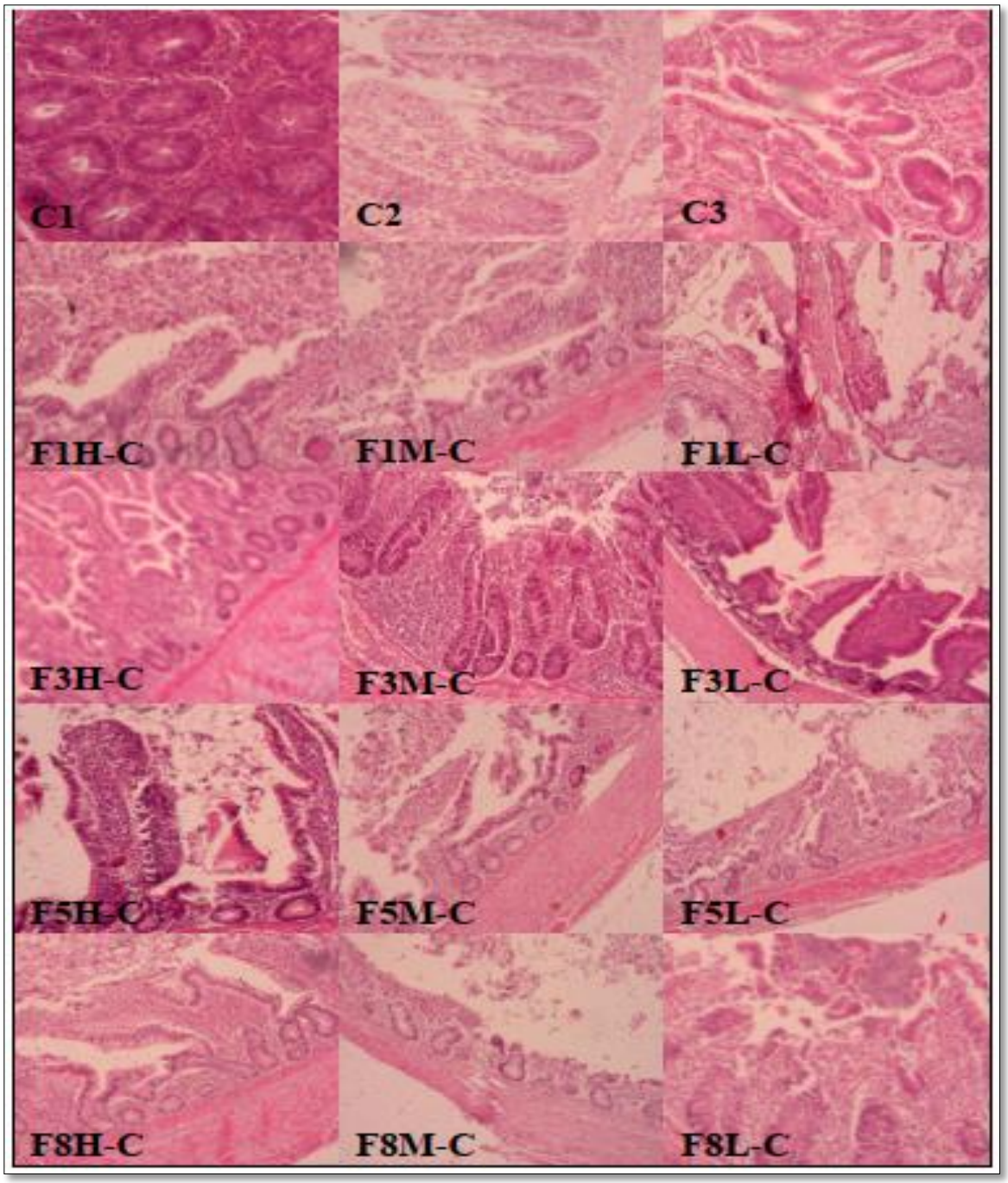


Figure 4.8a: Histology micrographs of cecal section of *Eimeria* infected chicks, treated with different concentrations of *V. officinalis in-vitro* active fractions. (C: Ceca, C1: Normal, C2: Infected, C3: Infected & SQX treated, F1, F3, F5 and F8 are active fractions of *V. officinalis*, H: High concentration, M: Medium concentration, L: Low concentration)

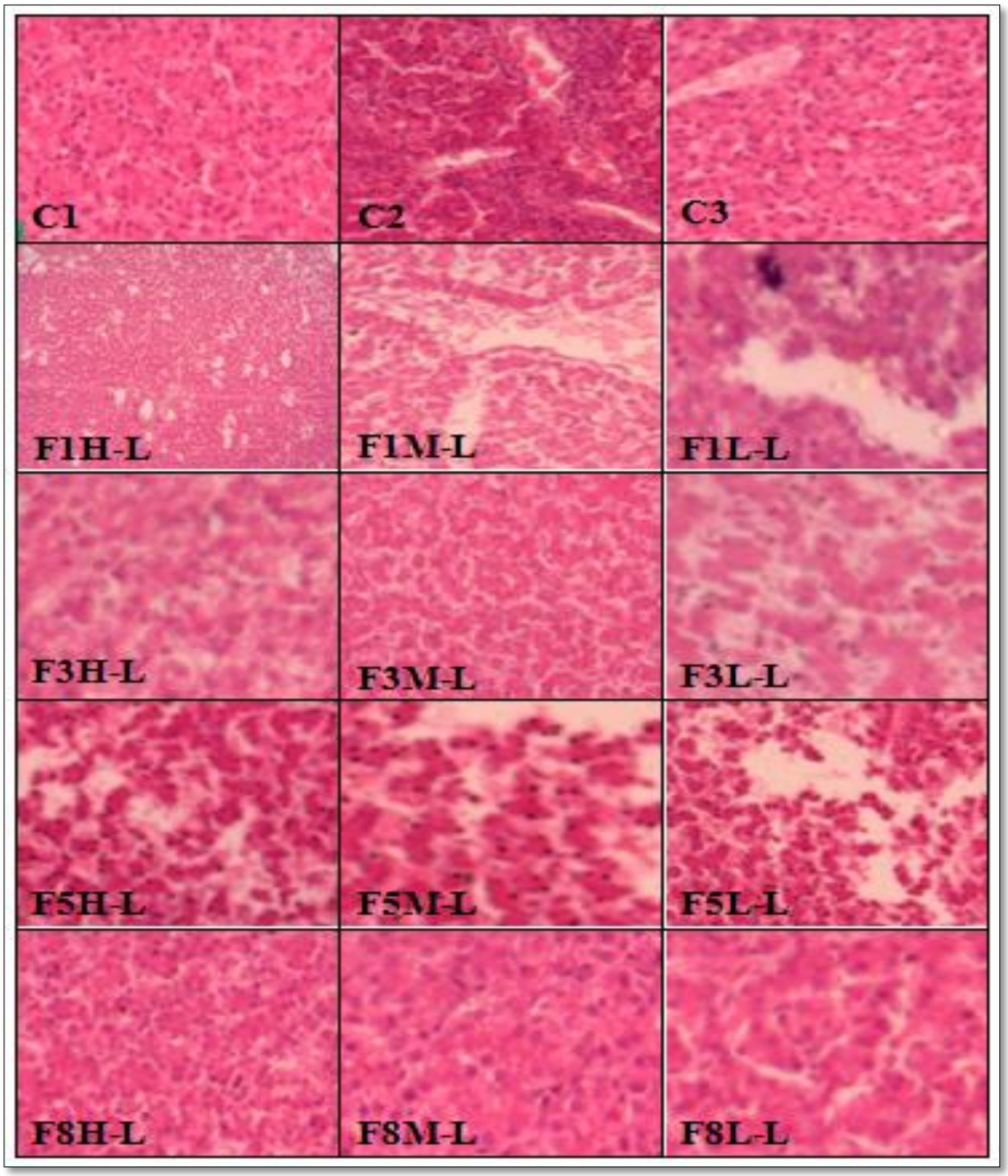


Figure 4.8(b): Histology micrographs of liver section of *Eimeria* infected chicks, treated with different concentrations of *V. officinalis in-vitro* active fractions. (C: Ceca, C1: Normal, C2: Infected, C3: Infected & SQX treated, F1, F3, F5 and F8 are active fractions of *V. officinalis*, H: High concentration, M: Medium concentration, L: Low concentration)

4.8. Column Chromatography

The prior work and potency of *V. officinalis* in different aspects regarding therapeutic activities were mentioned earlier in this study. Considering the therapeutic history of *V. officinalis*, its fractionation was conducted using column chromatography, resulting in 90 fractions of 10 ml each. These fractions were further pooled up by thin layer chromatography, yielding 9 fractions, i.e., F1 to F9. The fraction (F1) showed no band due to n-hexane.

Table 4.6: Pooling of various fractions of *V. officinalis* based on Rf values

S. No	Fractions	Solvents ratio	Rf Values
F 1	1-13	Pure nh & nh: E A (80:20)	No spot observed
F 2	14-25	nh: E A (60:40,40:60, 20:80) & Pure EA	0.405
F 3	26-29	Pure EA	0.327
F 4	30-43	EA : C (80:20, 60:40, 40:60)	0.389
F 5	44-59	EA : C (20:80), Pure C	0.358
F 6	60-65	C:M (80:20, 60:40)	0.429
F 7	66-74	C:M (40:60, 20:80)	0.417
F 8	75-82	C:M (20:80) & Pure M	0.309
F 9	83-90	Pure M	0.439

nh-nhexane, EA- Ethyl Acetate, C- chloroform, M- Methanol

4.9. Characterization

4.9.1. UV-Vis Spectroscopy

UV-Vis spectroscopy is a technique that showed the interaction of light with matter in the UV-Vis region, and upon the absorption of light, the electrons excite from the valence band to the excitatory band. The UV-Vis spectroscopic study of active fractions of *V. officinalis* showed the presence of hetero atoms like sulfur, nitrogen, and oxygen with unsaturated alkenes in the UV region below 400 nm wavelength with π-π^* transition, whereas in the visible region of 400-800 nm wavelength, it showed the presence of carbonyls and chromophores with n-π^* transition (Fig. 4.9).

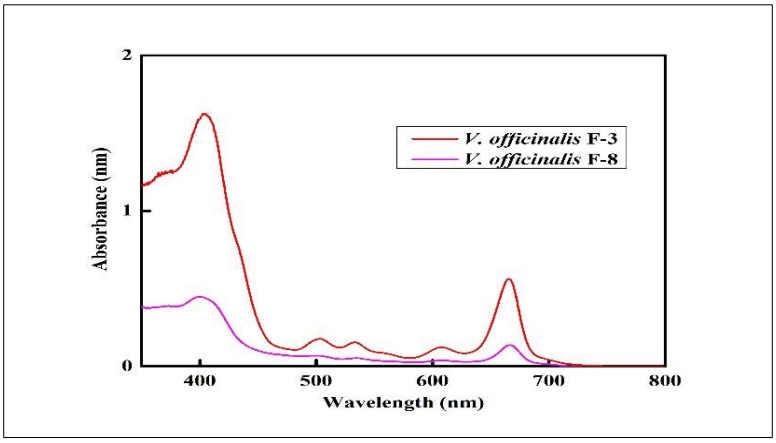


Figure 4.9: UV-Vis spectroscopy of F3 (red) and F8 (pink) fractions of *V. officinalis*

4.9.2. FT-IR spectroscopy

The peaks 3334 of Vo-3, and 3339 of Vo-8 fall in the range of 3550-3000 cm^{-1} wavenumber, showed the presence of alcoholic and phenolic functionalities. The second peak with the highest wavenumber observed was 2973.98 cm^{-1} and 2974.32 cm^{-1} in the FT-IR of the Vo-3 and Vo-8 fractions of *V. officinalis*, respectively, which indicates the presence of alkanes. The appearance of 1648.80 and 1654.05 cm^{-1} showed the availability of amines and oximes functional groups in both the anticoccidial active fractions. Both fractions have shown peaks at 1380 cm^{-1} wavenumber, which indicate the presence of florides, whereas the peak at 1044 cm^{-1} falls in the range with C–F stretching. Whereas below 600cm^{-1} wavenumber C-X (C–Cl, C–I, C-Br) exist, as indicated in figures 4.10a and b (Pavia *et al*., 2003).

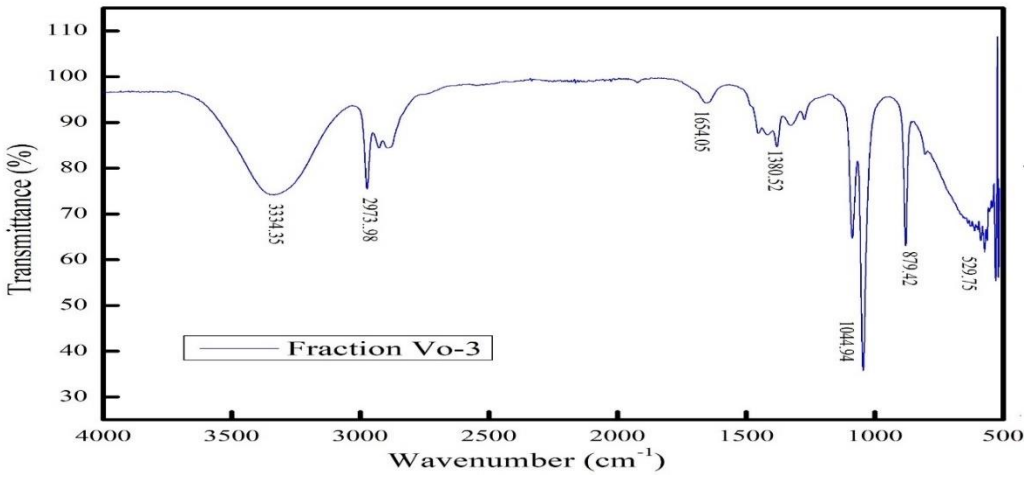


Figure 4.10(a): FT-IR spectra of active fraction Vo-3 of *V. officinalis*

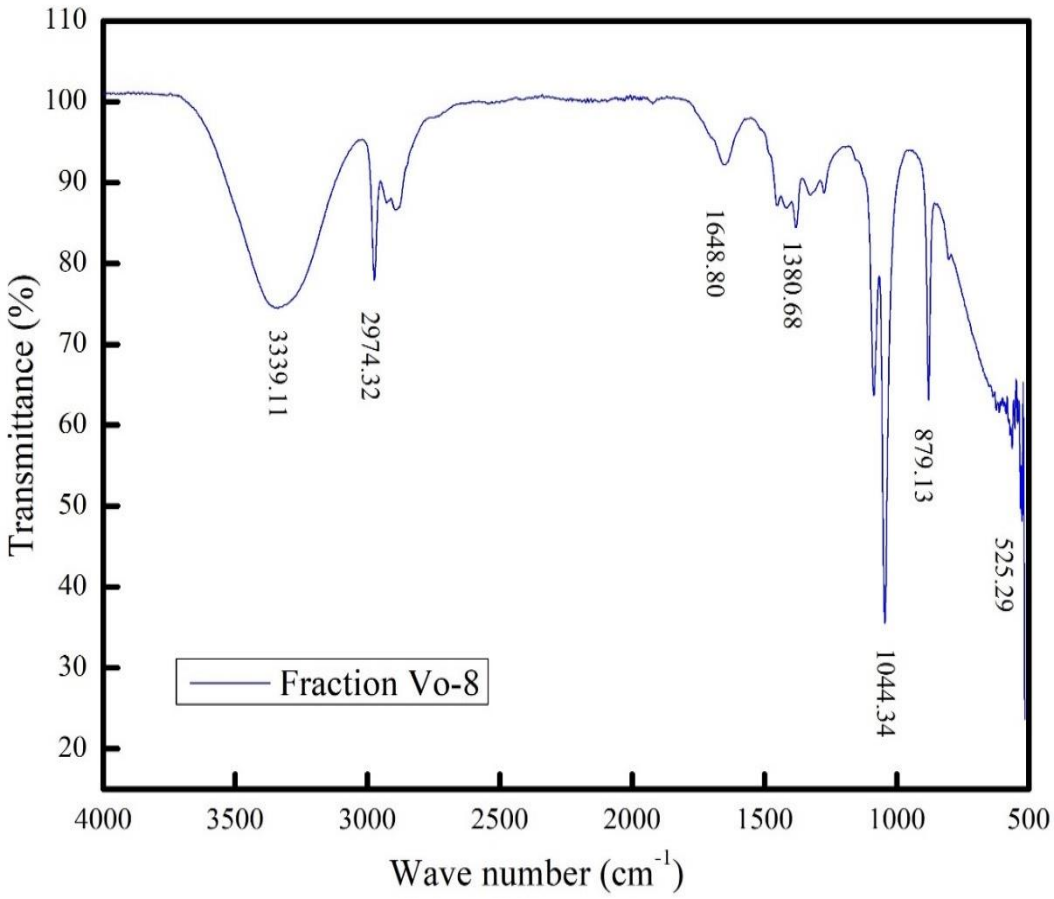


Figure 4.10(b): FT-IR spectra of active fraction Vo-8 of *V. officinalis*

4.9.3. GC-MS

Among the four fractions of *V. officinalis*, Vo-3 (solvent: ethyl acetate) and Vo-8 (solvent: chloroform & methanol) had good anticoccidial activity in the *Eimeria*-infected chicks, as indicated by the mean weight gain, diarrheal recovery, and normalization of biochemical and hematology profiles. So both of the fractions were characterized through GC-MS to find out their inert composition for *in-silico* studies against the target protein. Both fractions were run for 31 minutes, and various compounds were identified through online compound libraries, i.e., replib and mainlib, which showed the presence of 7 compounds in Vo-3 (Fig. 4.11a) and 20 compounds in Vo-8 (Fig. 4.11b). All these compounds were subjected to docking against SAM synthetase.

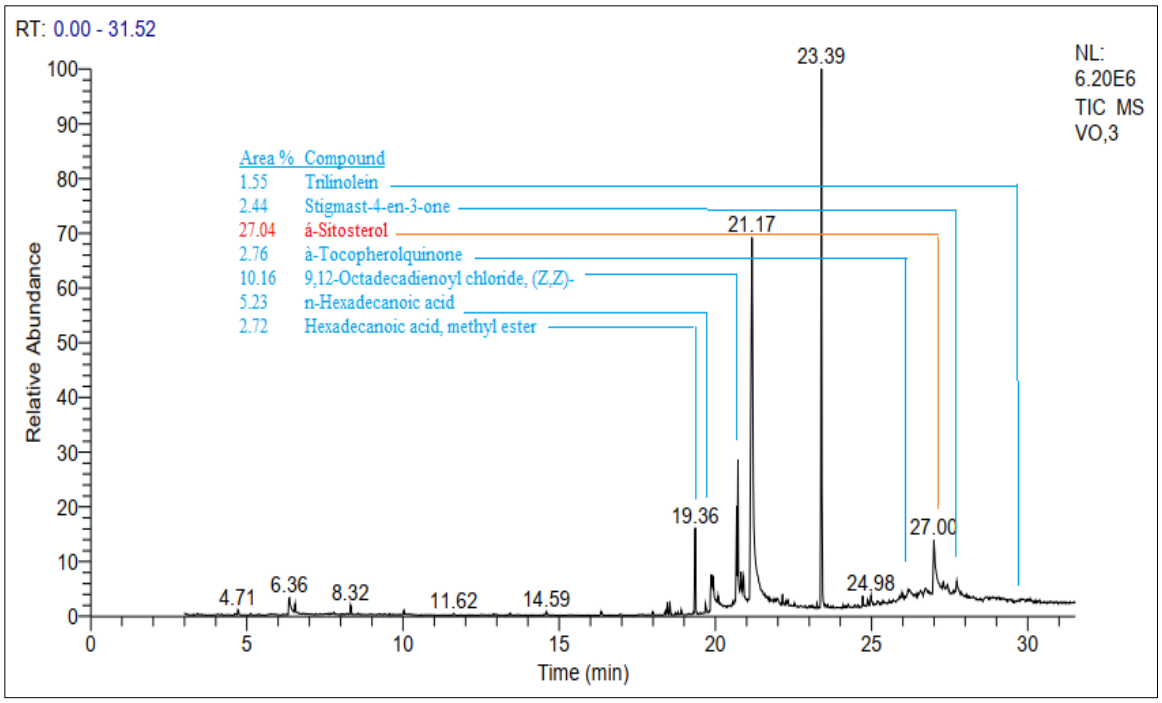


Figure 11a: Chromatogram of active fraction (Vo-3) of *V. officinalis* extracted in ethyl acetate

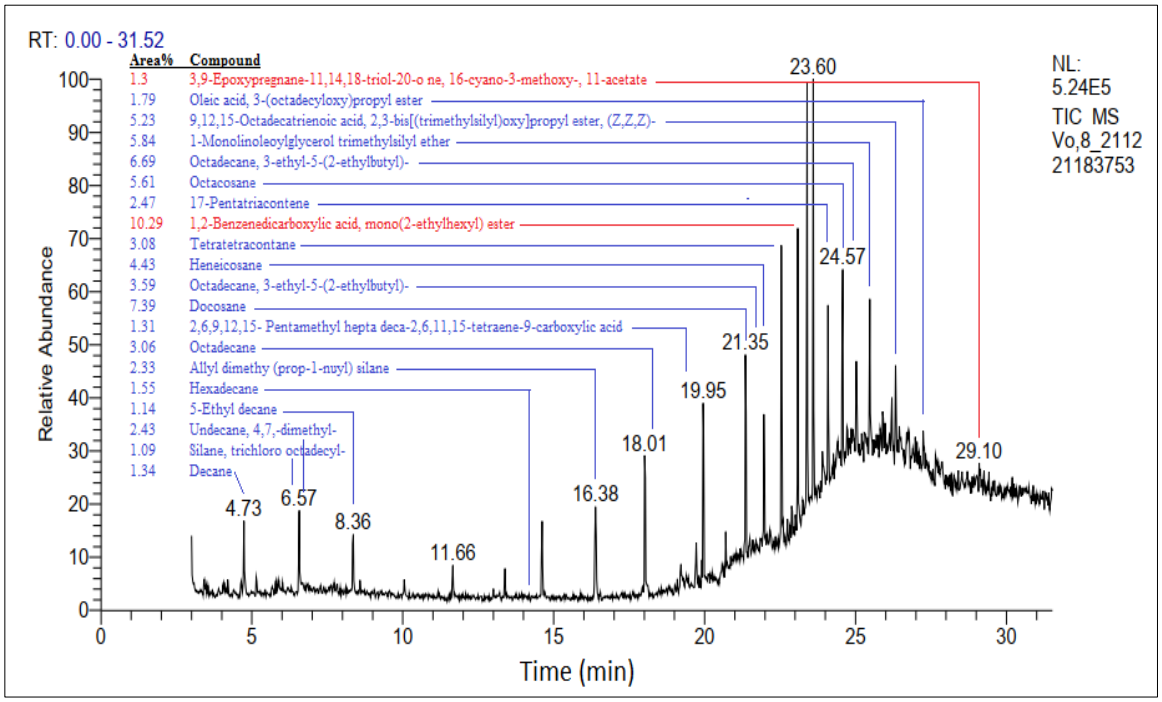


Figure 4.11b: Chromatogram of active fraction (Vo-8) of *V. officinalis* extracted in chloroform: methanol (20:80) & Pure methanol

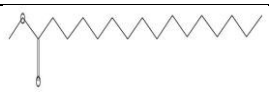
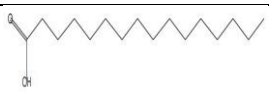
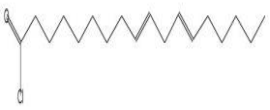
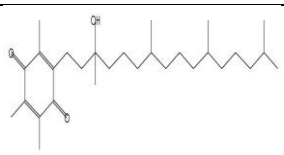
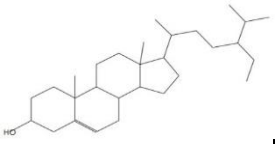
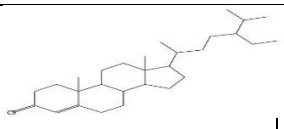
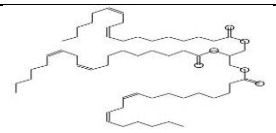
4.10 Molecular docking

All 27 ligands identified in the fractions of *V. officinalis* were docked with S-Adenosylmethionine synthetase. The binding affinities between different ligands and target protein showed different score values (Table 4.7a & b). The lower the binding score, the higher the binding affinity.

The 2D structural model generated via LigPlot+, the hydrophobic interaction analyzed showed the involvement of a relatively varied number of interacting amino acid residues across the different ligand–receptor complexes. Hydrophobic interactions enhance the binding affinity and biological activity of the complex molecules and help in stabilizing the biochemical environments of target-drug complexes.

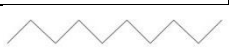
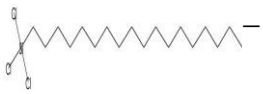
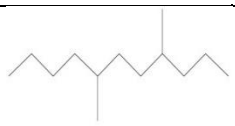
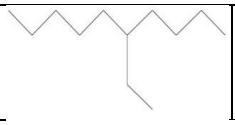
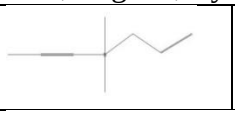
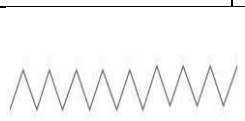
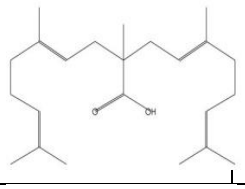
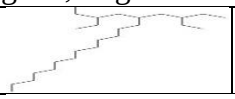
The hydrophobic interactions of protein with each ligand molecule, along with binding scores, were summarized in Table 4.7c. Among all ligands of fraction Vo-3, 4 compounds showed interaction with SAM synthetase, but a-sitosterol showed the lowest binding score (-6.2) and was considered the best inhibiting molecule, and its interaction with the S-Adenosylmethionine synthetase enzyme is shown in figure 4.12a. Whereas among the 20 ligands of the Vo-8 fraction only nine showed interaction with the target molecule: 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and 3,9-Epoxy pregnane-11 ,14,18-triol-20-one,16-cyano-3-methoxy-, 11-acetate with binding scores of -6.1 and -5.3 (Fig. 4.12b & c). All ligand-protein complexes with hydrogen bonding residues, interactive atoms, and bond length was given in Table 4.7c.

Table 4.7a: Binding score of different (Vo-3) ligand-protein complex along with hydrophobic interacting amino acid residues as demonstrated in ligPlot+

S no.	Ligands name	RT	Area %	MF	MW	Structure	BS	TR
1	Hexadecanoic acid, methyl ester	19.36	2.72	$C_{17}H_{34}O_2$	270		-4.4	10
	IR: Arg147, Glu150, Gln146, Arg143, Tyr357, Gly361, Phe360, Gly358, Arg362, Ile210							
2	n-Hexadecanoic acid	19.91	5.23	$C_{16}H_{32}O_2$	256		-4.6	7
	IR: Arg362, Ile210, Gln146, His142, Arg143, Arg147, Gly361							
3	9,12-Octadeca dienoyl chloride, (Z,Z)-	20.72	10.16	$C_{18}H_{31}ClO$	298		-4.7	8
	IR: Glu150, Arg362, Gly361, Thr139, Arg143, Gln146, Ile210, Arg147							
4	à-Tocopherol quinone	26.22	2.76	$C_{29}H_{50}O_3$	446		–	–
5	a-Sitosterol	27.04	23.13	$C_{29}H_{50}O$	414		-6.2	8
	IR: Glu363, Glu150, Arg362, Arg143, Gly358, Gly361, Gln146, Asp131							
6	Stigmast-4-en-3-one	27.73	2.44	$C_{29}H_{48}O$	412		–	–
7	Trilinolein	29.03	1.55	$C_{57}H_{98}O_6$	878		–	–

RT: Retention time, MW: Molecular weight, MF: Molecular formula, BS: Binding Score, TR: Total residue, IR: Interactive residue

Table 4.7b: Binding score of different (Vo-8) ligand-protein complex along with hydrophobic interacting amino acid residues as demonstrated in ligPlot+

S no.	Ligand	RT	Area %	MF	MW	Structure	BS	TR
1	Decane	4.73	1.34	$C_{10}H_{22}$	142		-4.1	15
	IR: Phe360, Tyr251, Phe262, Phe123, Thr250, Arg256, Ala275, Gly258, Met122, Leu121, Asp271, Leu121, Gly259, Gly260, His257							
2	Silane, trichloro octadecyl-	5.87	1.09	$C_{18}H_{37}Cl_3Si$	386		–	–
3	Undecane, 4,7-dimethyl-	6.59	2.43	$C_{13}H_{28}$	184		-4.6	6
	IR: Arg143, Gln146, His142, Gly361, Thr139, Ile210							
4	5-Ethyldecane	8.36	1.14	$C_{12}H_{26}$	170		–	–
5	Hexadecane	14.61	1.55	$C_{16}H_{34}$	226		-4.5	9
	IR: His142, Gln146, Arg362, Gly361, Thr139, Ile210, Glu150, Arg143, Tyr357							
6	Allyldimethyl(prop-1-ynyl)silane	16.38	2.33	$C_8H_{14}Si$	138		–	–
7	Octadecane	18.03	3.06	$C_{18}H_{38}$	254		-4.4	9
	IR: Gln146, His142, Ile210 Glu150, Arg362, Arg147, Tyr357, Arg143, Gly361							
8	2,6,9,12,16-Pentamethylheptadeca-2,6,1 1,15-tetraene-9-carboxylic acid	19.24	1.31	$C_{23}H_{38}O_2$	346		–	–
9	Docosane	21.35	7.39	$C_{22}H_{46}$	310		-3.9	8
	IR: Gln146, His142, Glu150, Arg147, Ile210, Gly361, Arg362, Arg143							
10	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	21.97	3.59	$C_{26}H_{54}$	366		–	–
11	Heneicosane	22.54	4.43	$C_{21}H_{44}$	296		–	–
12	Tetratetracontane	23.09	3.08	$C_{44}H_{90}$	618		–	–

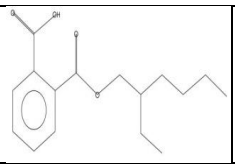
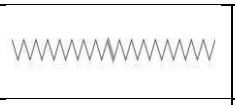
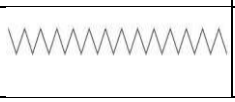
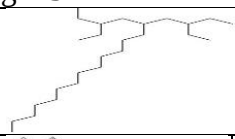
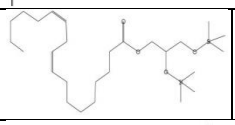
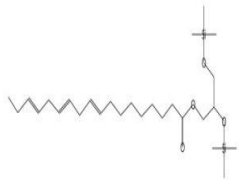
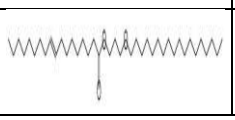
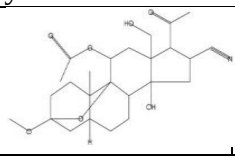
13	1,2-Benzene dicarboxylic acid, mono(2-ethylhexyl) ester	23.39	10.29	$C_{24}H_{38}O_4$	390		-6.1	6
	IR: Thr139, His142,Gly361, Arg147, Glu150, Ile210							
14	17-Pentatria contene	24.08	2.47	$C_{35}H_{70}$	490		–	–
15	Octacosane	24.57	5.61	$C_{28}H_{58}$	394		-3.9	7
	IR: Gln146, Ile210, Arg362, His142, Thr139, Arg147, Arg143							
16	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	25.02	6.69	$C_{26}H_{54}$	366		–	–
17	1-Monolino leoylglycerol trimethylsilyl ether	25.90	5.84	$C_{27}H_{54}O_4Si_2$	498		–	–
18	9,12,15-Octa decatrienoic acid, 2,3-bis [(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	26.73	5.23	$C_{27}H_{52}O_4Si_2$	496		–	–
19	Oleic acid, 3-(octa decyloxy)propyl ester	27.26	1.79	$C_{39}H_{76}O_3$	592		-4.6	7
	IR: Arg362, Gln146, Glu363, Gly361, Ile210, Arg143, Gly358							
20	3,9-Epoxy pregnane-11 ,14,18-triol-20-one,16-cyano-3-methoxy-, 11-acetate	29.10	1.30	$C_{25}H_{35}NO_7$	461		-5.3	7
	IR: Thr139, Gly361, Arg362, Asp131, Glu363, Ile210, Glu150							

Table 4.7c: Different ligand molecules with hydrogen binding residues along with bond length and interactive atoms

S no.	Ligand Name	Hydrogen Binding residues	Bond Length	Interactive Atoms
1.	a-Sitosterol	His142	3.02	ND1—O
2.	1,2-benzenedicarboxylic acid	Arg362, Gln146, Arg143	3.05 3.06 2.91	NE—O3 OE1—O3 NE—O4
3.	3,9-epoxypregnane-11,14,18-triol-20-one, 16-cyano-3-methoxy-, 11-acetate	Arg143	3.05	NE—O5

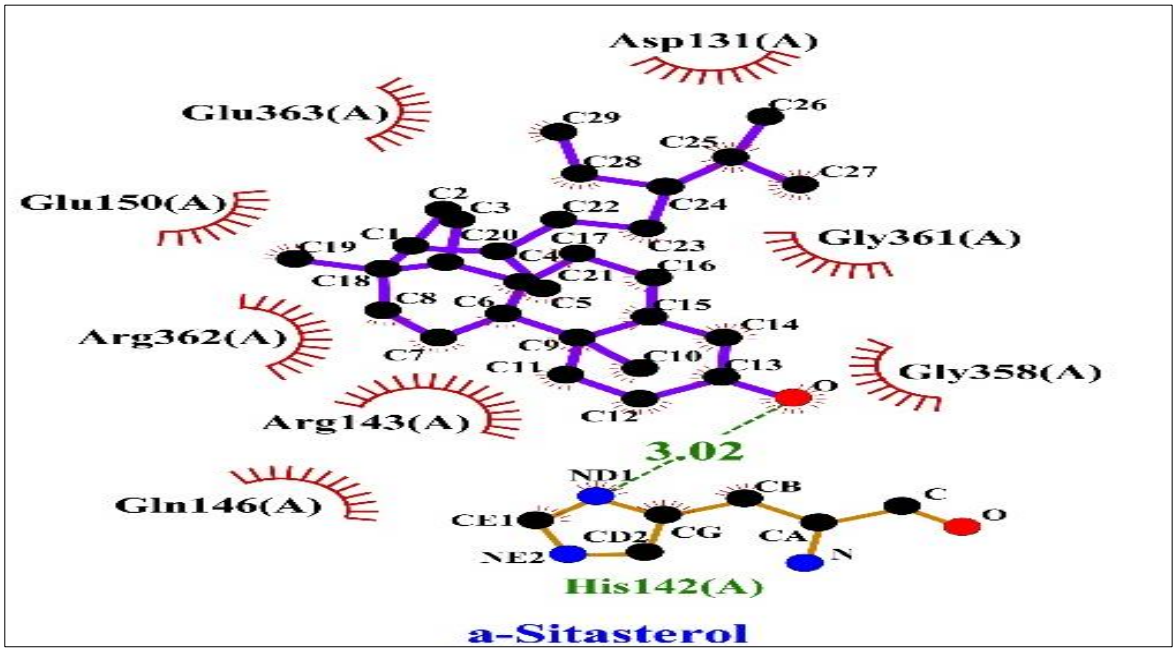


Figure 4.12a: 2D structure of a-sitosterol, showing interaction with SAM synthetase with 3.02A H-bond length

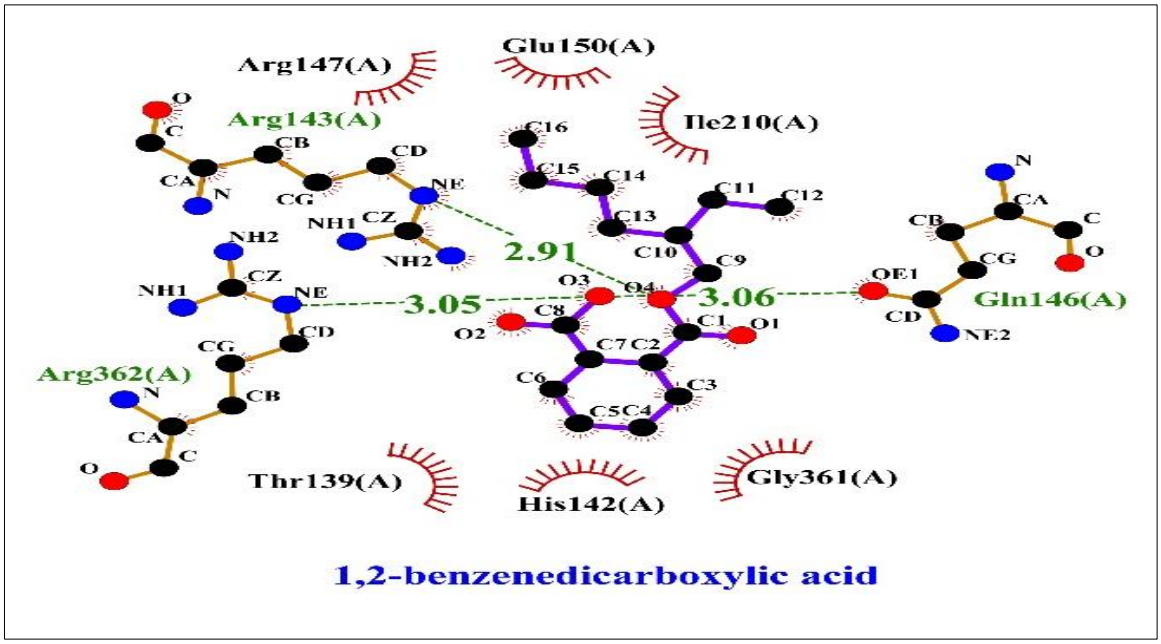


Figure 4.12b: 2D structure of 1, 2 benzenedicarboxylic acid, showing interaction with SAM synthetase with 3.06, 3.05 and 2.91A H-bond length

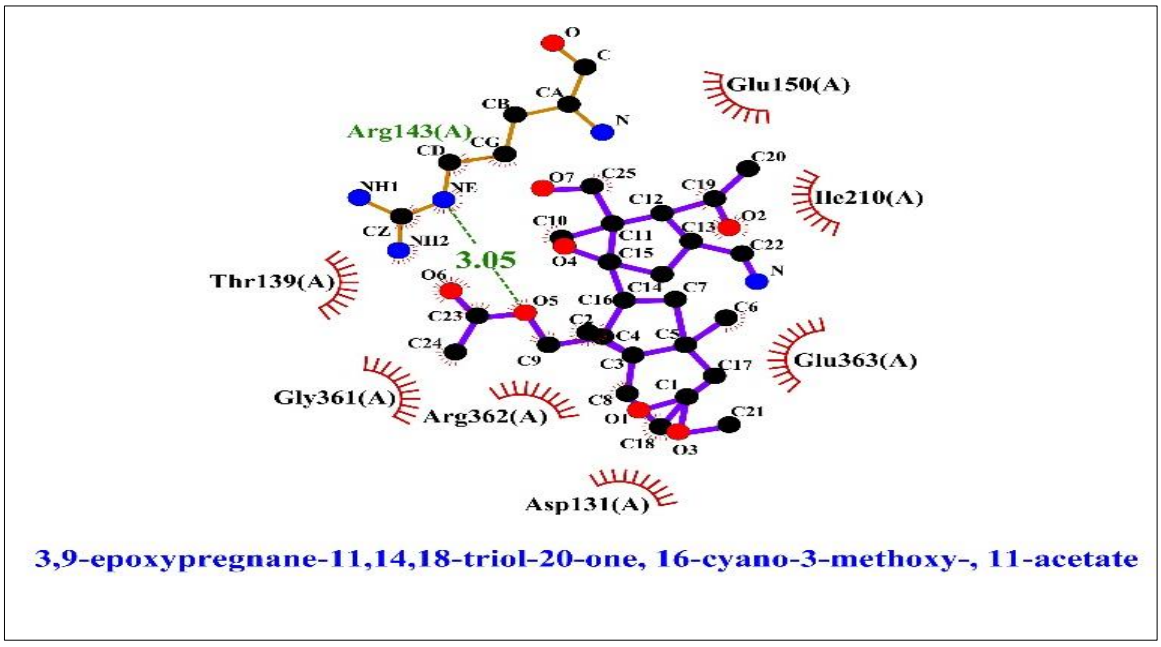


Figure 4.12c: 2D structure of 3, 9-epoxypregnane-11, 14, 18-triol-20-one, 16-cyano-3-methoxy, 11-acetate, showing interaction with SAM synthetase with 3.06, 3.05 and 2.91A H-bond length

DISCUSSION

The crude form of *Verbena officinalis* was found active against *E. tenella* both in sporulation inhibition and anticoccidial activity, therefore, it has been processed further through the advanced chemistry technique of column and thin layer chromatography to obtain its fine fractions in various compounds based on the principle of polarity. The purpose of this research is to find out the whole composition of anticoccidial active ingredients in *V. officinalis*, which was simulated computationally after *in-vitro* and *in-vivo* trials.

In the first phase, all nine fractions of *V. officinalis* were subjected to an *in-vitro* sporulation inhibition assay, which concluded the effectiveness of some fractions with more than 70% SPI. This was confirmed by the 50% inhibition against melanoma with a minute amount (2.85 µg/ml) by n-henxane, ethyl acetate, and chloroform fractions of *V. officinalis* (Nisar *et al*., 2022). The methanol and chloroform lead fractions of *V. officinalis* are reported to have anti-inflamatory activity with more than 50% inhibition, with verbenalin and ursolic acid as its major components, respectively (Deepak and Handa, 2000).

The *in-vivo* anti-coccidial efficacy of various fractions that were found active during the induction of the sporulation inhibition assay against the sporulating *E. tenella* oocysts. The feed consumed by different birds was assessed in terms of average weight gain at different time intervals. The ethanolic extract of *Allium sativum* showed the best performance against coccidiosis, proving the effectiveness of naturally available products like flavonoids (Indrasanti *et al*., 2017). The growth performance of the *Eimeria*-infected broilers was evaluated post-treatment with *Carica papaya* leaves, resulting in a significant change in the average mass of the birds (Banjoko *et al*., 2020). The coccidial infection leads to less feed consumption due to reduced hunger, which ultimately results in a lesser weight of chicks (El-Ghany, 2020).

The FCR values of the F3 and F8 fractions of *V. officinalis* treated groups was quite meaningful as compared to the untreated birds and have better results than those of *Artemisia splendens* in Ross-308 (Abdullah and Al-Barwary, 2020). In the larger image, fraction Vo-3 showed a better result than fraction Vo-8 in terms of good MWG, feed

consumption, and FCR. Though both fractions was of the same herb, the only difference is the extraction solvents used during the process of column chromatography, i.e., fraction F3 was extracted in pure ethyl acetate whereas F8 was a combination of chloroform and methanol, which means that the components extracted in ethyl acetate was more favorable or enhance the digestion chemistry of the birds during the coccidial attack.

The diarrheal profile of the treated birds showed that ethyl acetate extract is providing healing in a better way. That's why the same groups were reported to have lowered OPG and the best nutrient assimilation through the intestinal mucosa. The diarrhea of the experimented groups was observed on a daily basis post-infection and post-treatment to evaluate the effectiveness of certain fractions of *V. officinalis* extract. Whenever the chick experiences the coccidial infection, the *Eimeria* reside in the intestinal epithelial tissues for its lifecycle, and eventually it damages the hosting tissues, which become ruptured and drain with fecal material in the form of bloody diarrhea (Nahed *et al*., 2022). Initially, all infected groups were reported with the absence of diarrhea, which means that the infection was not so mature that it could cause tissue hemorrhages and result in diarrhea (Qaid *et al*., 2021). For instance, the data of all groups was presented in the form of a heat map on the basis of case severity. It is evidenced that natural flora like *Pulsatilla koreana*, *Sophora flavescens*, and *Sinomenium acutum* have anti-diarrheal ability against *E. tenella* due to their inert composition during coccidiosis in poultry (Nahed *et al*., 2022). The severity of infection varied among all the infected groups, but the common thing observed was the incidence of bloody diarrhea, which is a sign of parasitic infection implantation (Al-Shaibani *et al*., 2020).

The presence of oocysts in the excretory discharge is a clear indication of the clinical form of coccidiosis, which can be focused at 400X magnification (Abebe and Gugsa, 2018). The omni-survival of oocysts in harsh environments and hosts for long period of time can complicate the elimination of *Eimeria* spp., it can be managed within the host but can be altered in the environment due to its encysted form (Adhikari *et al*., 2008). As it can be managed within the host, a number of therapeutics can be applied against it and have satisfactory results with declining oocyst numbers, indicating the drug efficacy (Abbas *et al*., 2013), which supports the results of fractions F3 and F8 of *V. officinalis* regarding oocyst count in the fecal material of the treated groups. The herbal

products restrict oocyst sporulation, and thus the parasitic load on the host tends to decline, and chicks showed improved feed consumption and a significant feed conversion ratio, which saves the resources spent on poultry practice (Nahed *et al*., 2022). The F3H fraction of *V. officinalis* was observed to have the lowest oocyst density at the end of the trail, just like *Allium sativum*, as compared to the untreated or ineffective treatments, because all experimental groups were highly infectious a week ago (Al-Shaibani *et al*., 2020).

As for the biochemistry of the studied groups of treated chicks challenged with *Eimeria*, fractions with ethyl acetate and chloroform-methanol combinations play a vital role in managing the induced parasitic infection. The fractions F1 and F5 do not have a significant effect on liver function, and that's why the ALT and AST concentrations were continuously dropping in the groups treated with their respective fractions. This kind of result was also found in the study on *Artemesia splendens*, showed that the integral composition of these fractions doesn't have the potency to alter liver function against the parasitic infection in the same host used in the current study. Despite treatment, the serum protein and carbohydrates of the same groups were recorded as being too low, which concludes the malabsorption of essential nutrients during the assimilation process (Abdullah and Al-Barwary, 2020), whereas the F3 and F8 fractions perform well just as well as the sulfaquinoxaline in all biochemical aspects. The protein contents of the coccidial-infected chicks did not change significantly with certain commercial drugs, but in the current experiment, the extract-treated groups, especially F3 and F8, showed significant improvement (Lei *et al*., 2022).

The infected chicks experienced a low protein condition as compared to the healthy ones, indicating a decline in serum protein due to the *E. tenella* infection (Mondal *et al*., 2011). Hashemnia and coworkers report a decreasing level of serum protein content with infection, indicating the loss of intestinal epithelial protein. The parasitic infection, whenever it leads to disruption of the tissue, leads to the loss of biomolecules like protein and carbohydrates. That's why the attack of *Eimeria* disrupts the intestinal epithelium, which excretes feces in the form of bloody diarrhea (Patra *et al*., 2010). The reason for the decline in protein level is its conversion into urea due to stress (Hrzenjak *et al*., 2021). Usually, the coccidial infected liver leads to enhanced serum ALT and AST quantities (El-Shazly *et al*., 2020), whereas the cocci-free host showed low-quantity serum biochemical

parameters (Biu *et al*., 2021). Similarly, the effective treatment dose of probiotics lowered the AST and ALT levels, indicating the curing capacity of the operative drug (Arczewska *et al*., 2022). The methanol extract of *Glycyrrhiza glabrais* proved to be liver-protective by normalizing the level of liver secretions in *Eimeria* infected chicks (Hussain *et al*., 2022).

The blood profile was observed during the experimental trail on *Eimeria*-infected chicks treated with different extracts (with different solvents) of *V. officinalis* for the assessment of their good and bad impact on the chick's hematology. The hemoglobin concentration of *Aloe barbadensis, Tinaspora cordifolia, Bambusa arundinacea, Emblica officinalis, Ferulafoetida regal,* and *Tamarindus indica*-treated coccidial-induced chicks supports the hemoglobin concentration produced after treatment with a higher concentration of F3 and F8 fractions of *V. officinalis*. Further, *V. officinalis* is proved to be more efficient because the amount administered in the current research was quite low as compared to the plant product mentioned earlier (Moryani *et al*., 2021), proving it as an erythropoiesis-stimulating agent because the rest of the blood contents were also improved with this treatment. The Hb content of the chicks treated with all fractions of *V. officinalis* has a better quantity than that of uninfected chicks, and crude ethanol extracts of *Salvadora persica, Zingeber officinale,* and *Curcuma longa* treated coccidial infected chicks have a higher RBC level than the said extracts (Aljedaie and Al-Malki, 2020).

Similarly, *V. officinalis*-treated broilers yield an enormous amount of hemoglobin as compared to vitamin E-Selenium conjugate with a short period of time against *E. tenella* (Singh *et al*., 2013). Generally, the number of defensive components like lymphocytes and monocytes decreases in recovering situations. The fall in lymphocytes was observed in the birds treated with effective fractions of *V. officinalis*, just like dexamethasone-treated birds (Crouch *et al*., 2022). The immune cells like WBC, monocytes, and lymphocytes of the *Eimeria*-infected broiler chicks was following similar patterns of recovery with *Vernonia amygdalina* treatment in the experimented groups as active fractions 3 and 8 of *V. officinalis* (Banjoko *et al*., 2019). The ethanolic garlic extract plays a vital role in normalizing the platelet amount in *Eimeria*-infected organisms, showed the same recovery from infection as *V. officinalis* treatment, whereas a low platelet number was found in the untreated organism (Indrasanti *et al*., 2017). The blood components like RBC's and hemoglobin of the *Eimeria*-infected hubbird breed stabilized by serving probiotics, which

evidences the better efficacy of the natural product against coccidiosis (AbdAllah *et al.*, 2013). The treatment of *Carica papaya* leaf extract has shown better results in increasing body weight with a good FCR and hematology profile in the *Eimeria*-infected host (Banjoko *et al*., 2020).

The sections of cecal and liver tissues of the experimental groups treated with various fractions of *V. officinalis* extracted in different solvents were studied. The purpose of using different extraction solvents is to evaluate the differently extracted components from the same herb, which is reflected in healing the tissues of ethyl acetate and chloroform-methanol-derived fractions, i.e., F3 and F8 groups. The *Eimeria* lead infection causes damage to the intestinal epithelium, as the histology of the infected chicks reflects. This phenomenon eventually causes malabsorption of essential nutrients, which causes low weight gain (Chadwick *et al*., 2020).

The histological observation revealed tissue damage in the intestinal epithelium due to the pathogenesis of *E. tenella*, whereas a curative agent like *V. officinalis* inhibits parasitic infections and recovers the tissues (Olabode *et al*., 2020). The histopathological observations of the untreated birds' group revealed necrosis of epithelial tissue, and similar types of damage were found in ineffective groups (Ayana *et al.,* 2017). The parasitic infection immensely disrupts the tissues, leading to the appearance of large crypts in the intestinal epithelium (Abebe and Gugsa, 2018), and the enterocytes seem to be ruptured with the infection, whereas in cases of effective treatment dosage, they remain intact in a healthy condition (Jenkins *et al*., 2017). The improved histological appearance evidenced the effective treatment of *Phyllanthus emblica* by preventing *E. tenella* infection in birds (Sharma *et al*., 2021).

As the crude extract of *V. officinalis* effectively cures avian coccidiosis, it indicates the presence of effective components in the herbal product, such as coconut oil, against *E. tenella* (Hafeez *et al*., 2020). During coccidiosis, the increase in dimensions of the intestinal parts observed means that the anatomical structure is disturbed by such a parasitic infection (Abudabos *et al*., 2018). The anticoccidial potential of the *in-vivo* active fractions of *V. officinalis* is due to the existence of potent biomolecules in its composition. In the current study, we reported its active components through the GC-MS characterization technique and active functional groups with the means of FT-IR and UV-Vis spectroscopy, but also

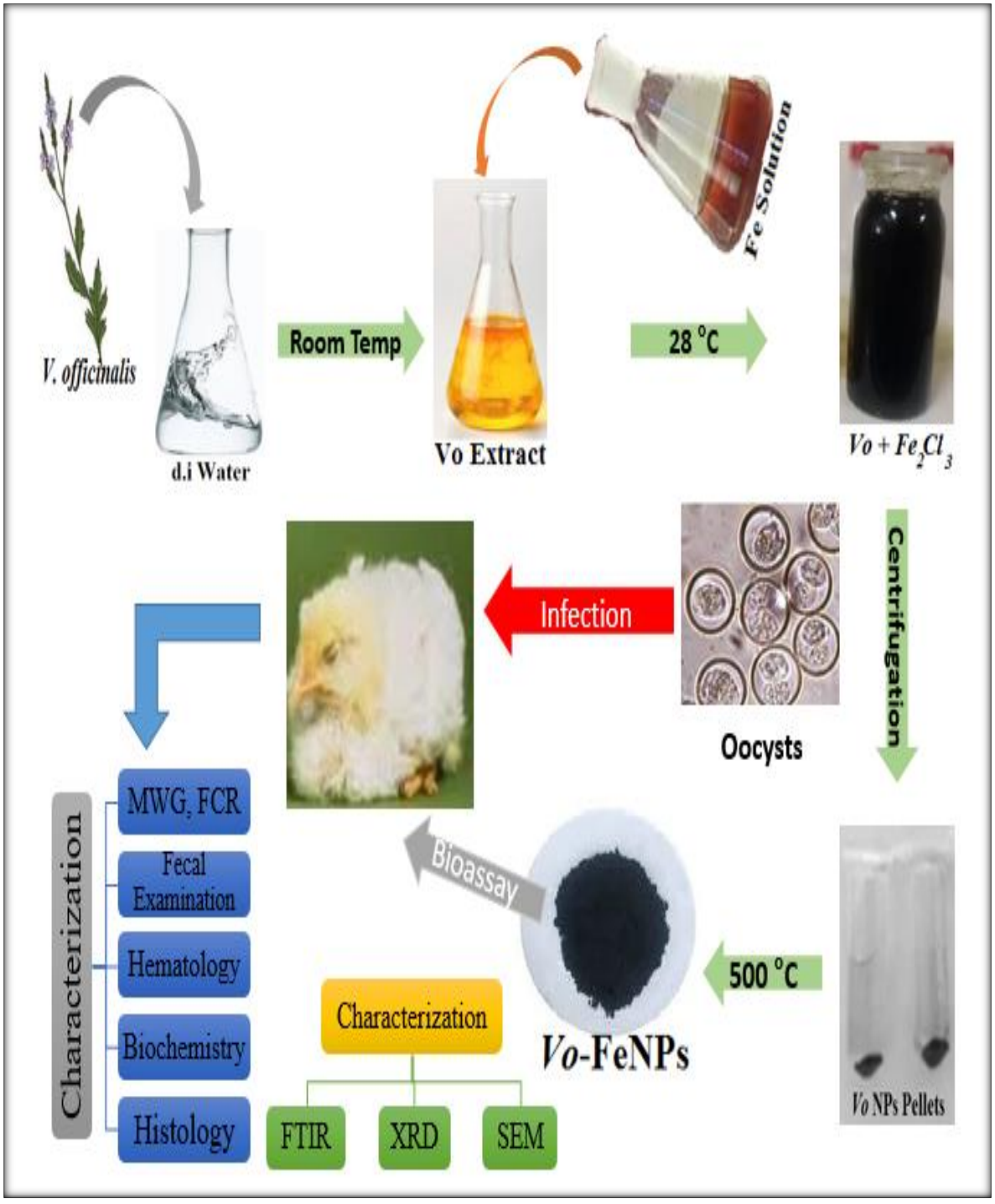
the composition of the same plant belonging to different locations or latitudes with slight changes was documented earlier as represented in the form of a chromatogram (Shams *et al*., 2003). The flavonoid content increases the antioxidant capacity of *V. officinalis*, and such free radicals play a key role in managing the parasitic load in cases of coccidial treatment (Rehecho *et al*., 2011; Abbas *et al*., 2013). The presence of triterpenoids is reported in the chloroform fraction (Kaur *et al*., 2014), flavonoids like Apigenin-7-*O*-glucoside in the aerial parts of *V. officinalis* (Hritcu and Cioanca, 2016), and the enrichment of the ethyl acetate fraction of *V. officinalis* with flavonoids demonstrates its activeness against mental disorders like common anxiety and almost has a similar result as the standard market drugs (Kumar *et al*., 2017). Previously, the antitumor potential of *V. officinalis* was successfully tested with satisfactory results (Kuo *et al*., 2020). The essential oil extracted from *V. officinalis* is enriched with citral, playing an apoptotic role in leukemia by triggering the required biochemical pathways (Ganjewala *et al*., 2012). *V. officinalis* exhibits antioxidant activity with the lowest IC_{50} of about 15 mg/ml (Tammar *et al*., 2021). The essential oils of *V. officinalis* were reported to have the presence of numerous compounds belonging to diverse groups but was highly enriched with aldehydes (De Almeida *et al*., 2010). The vigorous composition of *V. officinalis* is reflected in its bioactivities as tested against certain diseases, ranging from antibacterial activity to lethal dilemmas like cancer (Gharachorloo and Amouheidari, 2016; Nasir *et al*., 2022).

CONCLUSION

The *V. officinalis*-derived fractions were tested for sporulation inhibition assays. Fractions F1, F3, F5, and F8 were found to have maximum sporulation inhibition (lowest IC_{50} (*in-vitro*), The *in-vivo* anticoccidial of the active fraction concluded that the ethyl acetate-based (Vo-3) and chloroform-methanol (Vo-8) fractions of *V. officinalis* extract showed significant activity against *E. tenella*-infected chicks, with the best mean weight gain and recovering hematology and biochemical profile. The molecular docking of phyto-components of Vo-3 suggests a-Sitosterol (binding energy = -6.2) and 1,2 benzenedicarboxylic acid, mono(2-ethylhexyl) Ester (binding energy = -6.1), 3,9-Epoxypregnane-11,14,18-triol-20-one, 16-cyano-3-methoxy-, 11-acetate (binding energy = -5.3).

Chapter: 05
Ecofriendly Phyto-synthesis of Iron Oxide Nanoparticles of *Verbena officinalis*; Characterization and Bio-activity

GRAPHICAL ABSTRACT



ABSTRACT

The modern day community uses alternative ways to cope with deadly pathogenic diseases; in this aspect, green synthesized nanoparticles gained importance due to its targeted delivery mode and nanosize. The iron oxide nanoparticles of *Verbena officinalis* were green synthesized using an aqueous extract. The confirmation, purity, nanosize, and crystalline structure were found by X-ray diffraction, Fourier transmission infrared spectroscopy, and scanning electron microscopy. The antiprotozoal iron nanoparticles of *V. officinalis* were found in *E. tenella*-infected chicks to make them applicable in the life race. The newly synthesized iron oxide nanoparticle of *V. officinalis* was 22.35 nm in size with a crystalline structure. The SEM confirms that round, spherical shape of iron nanoparticles clustered together, plays an important role in bioactivities; further, the FT-IR spectra revealed various significant peaks, which evidence the stability of nanoparticles.

To investigate the anticoccidial effect of these nanoparticles against *Eimeria*-infected chicks on growth performance and several other parameters in comparison with sulfaquinoxaline, which revealed significant weight gain in iron nanoparticle treated groups with maximum weight gain (801±2.01 gm) after treatment with 15 mg/ml dosage with lowest feed conversion ratio (1.07) like uninfected birds ($P < 0.05$), leaving negligible wastage. The lowest oocysts number (0.53×10^3/g) and cured from diarrhea at the 9th day of infection with no lesion in the treated group (T3) evidenced the recovery from infection with declined casualties.

The hematological profile of T3 groups showed normal hemoglobin, RBC, WBC, platelets, monocytes, lymphocytes, and granulocytes, similar to uninfected control (C3), whereas carbohydrate, protein, and ALT was normalized at 14th day post-treatment. In the histopathological profile of the cecum and hepatic tissues, all groups showed no lesion or damage as in the infected and untreated control group (C2), showed significant recovery in the treated groups. The means of all studied parameters were analyzed statistically using ANOVA (tukey test) to confirm the significant difference ($P < 0.05$) between the treated and untreated groups.

MATERIALS AND METHODS

5.1.1 Biosynthesis of Iron Oxide Nanoparticles

The aerial parts of *V. officinalis*, leaves in particular were collected in the month of August from the locality of district Mardan, Pakistan (34.3410° N, 72.2897° E**)**. The floral parts of the respective herb processed by washing with distilled water to remove dust particles, dried in shade for two weeks in germ free environment and crushed into a fine powder (Shah *et al*., 2017).

The 15 gm fine plant powder was mixed with 100 ml of deionized water, sonicated for 30 minutes using a magnetic stirrer. The solution was filtered thrice with whatman no.1 filter paper. The 6 gm precursor salt ($Fe_2Cl_3.6H_2O$) was added to 100 ml of filtered plant extract and stirred for 2-3 hours and subjected to cool for overnight. The solution was centrifuged for 10 minutes at 10,000 rpm to acquire pallet and remove the supernatant. The pallet was washed thrice with deionized and dried for 2 hours in a furnace at 500°C. The iron oxide nanoparticles of *Verbena officinalis* were produced as a dried powder and characterized with biological and physiochemical activities (Shah *et al*., 2017).

5.1.2. Characterization of Iron Oxide nanoparticles

The green synthesized iron oxide nanoparticles of *V. officinalis* were characterized by confirming its shape and size using XRD, FT-IR and SEM analysis (Behera *et al*., 2012).

5.1.2.1 XRD Analysis

The crystalline structure and iron oxide forms were studied using X-ray powder diffraction. It is also used to determine the purity and presence. Diffraction peaks were identified at different angles during the XRD study (Molina *et al*., 2019).

5.1.2.2 FT-IR Spectroscopy

An IR spectrophotometer was used for the FT-IR analysis. Infrared light is used to scan test materials and examine their chemical qualities. In the absorption mode, the IR spectra was recorded in the middle infrared region of 400-4000 cm^{-1} with a resolution of 4 cm^{-1} (Abdel-Raouf *et al*., 2019).

5.1.2.3 Scanning Electron Microscopy

SEM was used to establish the presence of *Vo*-Fe NP. It produces crystal-clear, high-resolution photographs of the material's size and surface texture. To ensure the synthesis of Fe NPs, SEM was performed at a different resolution (Valsalam *et al*., 2019).

5.1.3 Sample Collection and Storage

Coccidiosis-infected chicks' guts were collected in a 2.5% potassium dichromate solution and identified at Peshawar Veterinary Research Institute. To ensure successful sporulation, a small amount of air was allowed in the sample solution. The material was transported to the laboratory for processing (Eassa *et al*., 2019).

5.1.3.1 Isolation of Oocysts

The intestines were pierced and the cecum contents and debris were removed with spatula and immersed overnight in 2.5% potassium dichromate solution. The suspension was poured into a petri dish after being filtered through the two folds of the muslin sheet with fine sieve. Then, the suspension was kept expose to environmental oxygen for sporulation. After sporulation, the sample was centrifuged for 3 min at 1500 rpm. The centrifugation at same conditions was repeated. The supernatant was removed again, and the top layer was piped out and mixed with water overnight. The oocysts suspension was resuspended in a 2.5% potassium dichromate solution and incubated at 30°C for 24 to 72 hours. The sporulated oocysts were then deposited in 2.5% potassium dichromate solution and stored in a refrigerator at 4°C. The number of sporulated oocysts per inoculum was adjusted to 20,000-30,000 (Eassa *et al*., 2019).

5.1.4 Chicks Housing

A total of 90 day-old chicks were purchased from a hatchery in Rawalpindi. For research purposes, they were kept at the Primate House at QAU for 5 weeks. The cages were thoroughly cleaned and disinfected before the chicks were placed. On the first day, the room temperature was kept constant at 32°C, then dropped to 22°C on day 21. The chicks were given sawdust as a bedding material. A 24-hour artificial light source was provided. Chicks were given coccidiostates and antibiotic-free diet throughout the

experiment. The diet was provided in the meshed form. The chicks were transferred to stainless steel cages (Williams, 2006).

5.1.5 Experimental Infection

The fecal litter was collected to confirm coccidiosis before inoculation of the infection. The stored oocysts were taken out of refrigerator and centrifuged at 1500 rpm for 10 min to remove potassium dichromate, then washed 3-4 times with distilled water. On day 14, each bird was inoculated with 2 ml suspension containing approximately 10×10^3 oocysts/ml of *Eimeria* species orally. The C2 group received no infection (Kaingu *et al*., 2017).

5.1.6 Experimental Groups

On day 21, oral gavage was used to administer manufactured dosages of various quantities to the three experimental groups T1, T2, and T3. Sulfaquinoxaline was administered to C3 at a rate of 2 ml per bird. Because the oxidant stress generated by the coccidian parasite lasts about 5 days, the doses were administered for a total of 5 days (Akhtar *et al*., 2012).

The 6 experimental groups were as follows:

1. C1: Control group (Uninfected, Untreated).
2. C2: Control group (Infected, Untreated).
3. C3: Control group (Infected, Treated with Sulfha drug).
4. T1: Treated with 5 mg/ml Fe NPS of *V. officinalis.*
5. T2: Treated with 10 mg/ml Fe NPS of *V. officinalis.*
6. T3: Treated with 15 mg/ml Fe NPS of *V. officinalis.*

5.1.7 Experimental Parameters

The following parameters were used to determine the effectiveness of the drug. Here are the details: Increase in body weight, feed intake, feed conversion ratio, bloody diarrhea, oocyst count, lesion score, survival rate, death rate, and hematological analysis,

histology, and biochemical analysis were used to determine survival and mortality (Youn and Noh, 2001).

5.1.7.1 Body Weight Gain

The weights of all the birds were calculated separately using the scale on the 14th day before infection and were considered as initial weight, followed by 21th, 28th, and 35st days weight. The formula was used to calculate the average weight gain (Wajiha and Qureshi, 2021).

$$\mathrm{MWG} = \mathrm{MFW} - \mathrm{MIW} + \mathrm{WDB}$$

5.1.7.2 Feed Intake

Feed intake was calculated as the feed left at night was subtracted from the feed provided in morning.

5.1.7.3 Group Feed Conversion Ratio

The group feed conversion ratio was estimated by using the formula (Naidoo *et al*., 2008).

$$FCR = \frac{FCG}{WSB - WDB}$$

Whereas FCR: Feed conversion ratio, FCG: Feed consumed per group, WSB: Weight gain of surviving birds, WDB: Weight gain of dead birds

5.1.7.4 Oocysts count

The birds began to shed oocysts in their feces on the 5th of infection. The number of oocysts per gram of feces was calculated from day 7 to the 13 post-infection. The McMaster counting technique was used to count the fecal samples from each group. The feces (3 ml) was mixed with tap water (42 ml) and left overnight. The suspension was filtered through double folded cheesecloth next day, then both chambers of the McMaster slide was filled with filtrate and left to stand for 3 min before being examined under the microscope. The number of oocysts per gram in the fecal sample was counted using the formula below (Zhang *et al*., 2013).

$$\mathrm{X}/0.15 \times 45 \times 10 \times 1/3 = \mathrm{X}1000$$

Whereas X= average oocyst counts in one chamber, 0.15=volume under the ruled area of McMaster slide, 45= total volume of suspension, 10=1/10 dilution factor, 1/3= correction factor.

5.1.7.5 Lesion Score

The color of the epithelium, fluid accumulation, and the general appearance of the intestine were studied after day 8 post-inoculation using a formula, and five ranks were assigned based pre mentioned characters (Raman *et al*., 2011). The cecum mucosa and submucosa were scored using a microscopic lesion grading system using the adopted method. The lesions were graded as: 0 = No parasite/lesion, 1 = Parasite/lesion in one field, 2 = Parasite or lesion in two fields, 3 = Parasite/lesion in three fields, 4 = Parasite/lesion in four fields (Yang *et al*., 2019).

5.1.7.6 Bloody Diarrhea

Bloody diarrhea was examined from the 4^{th} to 7^{th} day post infection. It was categorized into four categories, ranging from 0 (-) to 3 (+++). A normal status was zero, 25% was considered as grade 1, 26-50% was considered as grade 2, 51-75% was considered as grade 3, and more than 75% was considered as grade 4 with hemorrhagic stool in the total fecal sample over 24 hours (Patra *et al*., 2010).

5.1.7.7 Survival Rate

The survival rate was estimated by dividing the number of surviving chicks by the number of initial chicks (Du and Hu, 2004).

$$S\,\% = \frac{N - DB}{N} \times 100$$

Whereas; S%: Survival percentage, N: Total number of birds, DB: Total number of dead birds

5.1.7.8 Mortality Rate

The death rate of birds was calculated using the formula stated by (Naidoo *et al*., 2008).

$$M\,\% = \frac{DB}{IN} \times 100$$

Whereas M%: Percent mortality, DB: Total number of dead chicks in a cage, IN: Initial number of birds in a cage

5.1.7.9 Hematology

The blood samples were taken on the last day of the experiment to estimate the hemoglobin concentration and red blood cells count. The blood samples were collected in EDTA tubes to avoid coagulation (Wakenel, 2010).

5.1.7.10 Histological Parameters

Following the whole histological procedure, birds from each group were slaughtered and the cecum, liver and kidney tissues were extracted in a 10% formalin solution, subjected several grades of alcohol, and finally embedded in paraffin wax. It was sectioned and chopped into thin cubes, the slides were prepared and examined under the microscope (Deepa *et al*., 2020).

5.1.7.11 Biochemical Analysis

All the blood samples were placed into gel tubes and clot activator tubes and centrifuged, to extract serum. The sera from various samples was extracted, placed into Eppendorf tubes using micro pipette, and stored at -20 ˚C for future biochemical analysis (Adamu *et al*., 2013). Carbohydrates, proteins, AST/GOT at 30 ˚C, and ALT/GPT at 30 ˚C were among the assays performed.

5.1.8 Statistics

The anticoccidial activity of iron oxide nanoparticales of *V. officinalis* was statistically analyzed by finding the mean and standard deviation of the results. The parameters such as mean weight gain, feed conversion ration, oocysts count, hematology and biochemistry of *in-vivo* anticoccidial assay were statistically analyzed by one way ANOVA "Tukey test" (Statistix V-9) to check HSD under the condition of $P < 0.05$ (Iqbal *et al*., 2022). The data of UV-Vis and FT-IR spectroscopy was analyzed using Origin 8.5 (Ansir *et al*., 2020).

RESULTS

5.2.1 Physical Qualities

The synthesis of iron oxide nanoparticles was initially observed by the change in color after the addition of iron chloride solution to the aqueous crude extract of *V. officinalis*, as shown in figure 5.1.

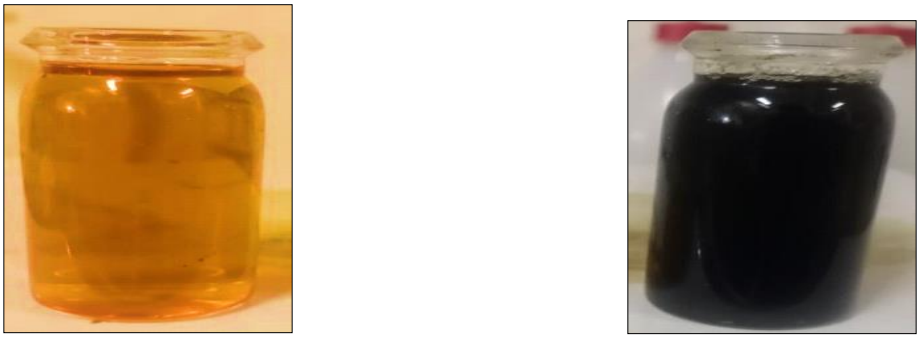


Figure 5.1: Color change indicating green synthesis of Fe NPs from the crude extract of *Verbena officinalis*

5.2.2 Morphological identification of sporulated *Eimeria tenella* oocysts

Sporulated oocysts were examined microscopically prior to infection. The sporulated oocysts of *Eimeria tenella* have an oval shape and are distinguished by the number of sporocysts, i.e., each sporulated oocyst contain two sporocysts (Fig: 5.2).

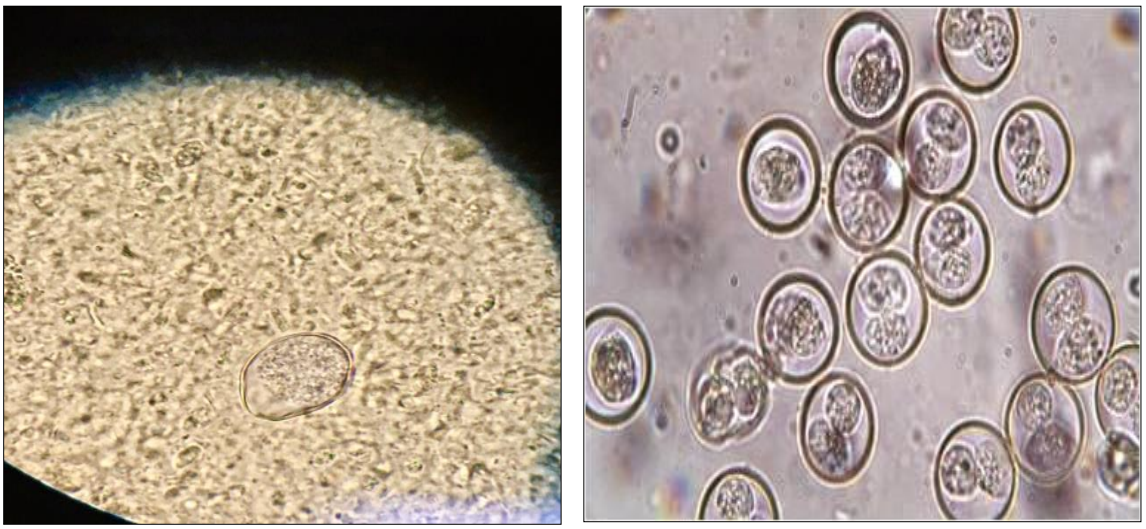


Figure 5.2: Sporulated and unsporulated oocysts of *Eimeria tenella*

5.2.3 XRD Analysis

Using XRD analysis, the synthesis of iron oxide nanoparticles was confirmed. The formation of the sharp peaks shows that the crystalline structure of the FeO nanoparticle has been formed (Fig. 5.3). The size was approximately 22.35 nm based on XRD examination. Strong diffraction peaks with 2θ were formed at 24˚ = 012, 33˚ = 104, 35˚ = 110, 40˚ = 113, 49˚ = 024, 54˚ = 116, 57˚ = 018, 62˚ = 214, and 63˚ = 300.

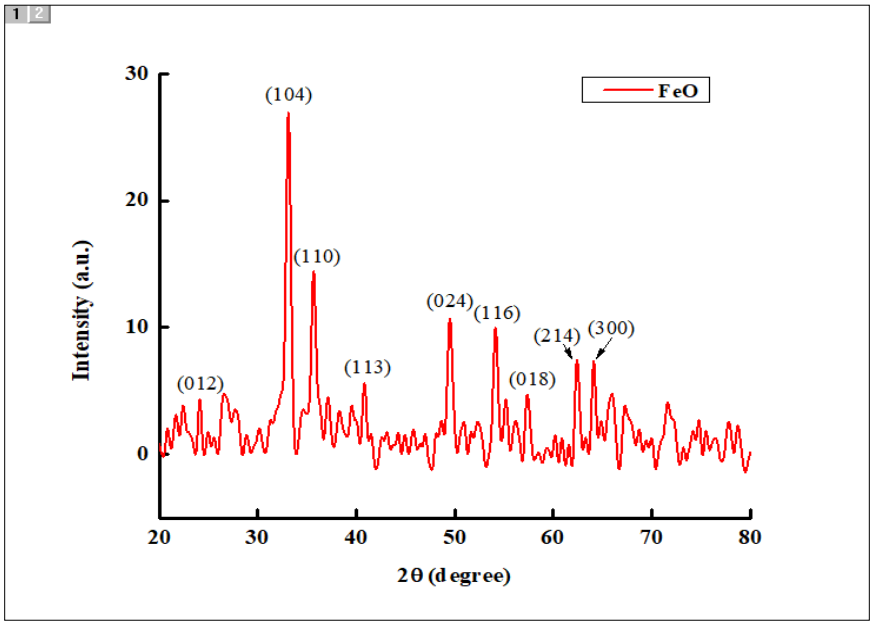


Figure 5.3: XRD analysis of synthesized Fe NPs of *Verbena officinalis*

5.2.4 FT-IR Spectroscopy

FT-IR analysis were performed to evaluate and identify potential biomolecules in the plant extract that might be responsible for the formation and stabilization of Fe NPs. Peaks in the FT-IR analysis were formed between 500 and 2500 cm^{-1} (Fig. 5.4). A magnetite band was observed between 500 and 640 cm^{-1}. A maghemite band was observed between 580 and 620 cm^{-1}. A hematite band was observed between 520 and 570 cm^{-1}. Therefore, it can be estimated that iron oxide nanoparticles were formed and strongly interacted with the compounds in the leaf extract. Absorbance was observed on the y-axis, and wavenumber was observed on the x-axis.

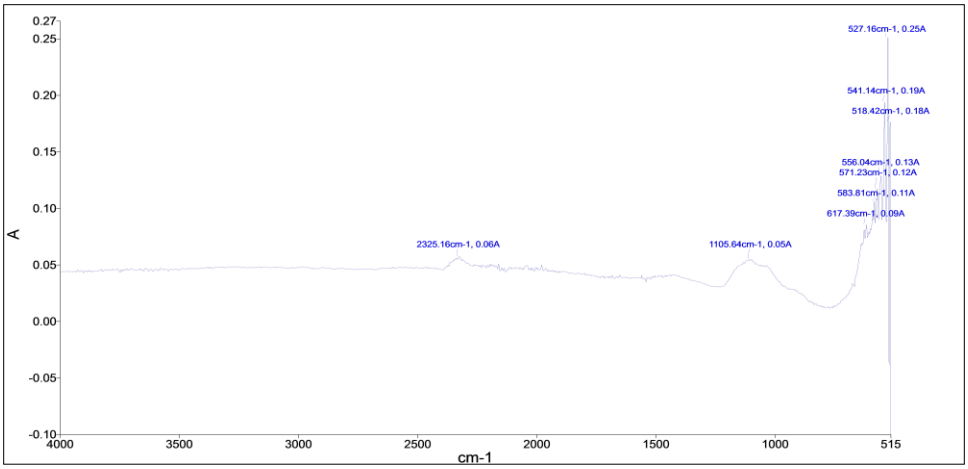


Figure 5.4: FT-IR analysis of synthesized Fe NPs of *Verbena officinalis*

5.2.5 Scanning Electron Microscopy

Scanning electron microscopy was used to determine the shape and size of the nanoparticles. Images taken at 20 kV with different resolutions, showed that iron oxide nanoparticles have a spherical shape (Fig. 5.5).

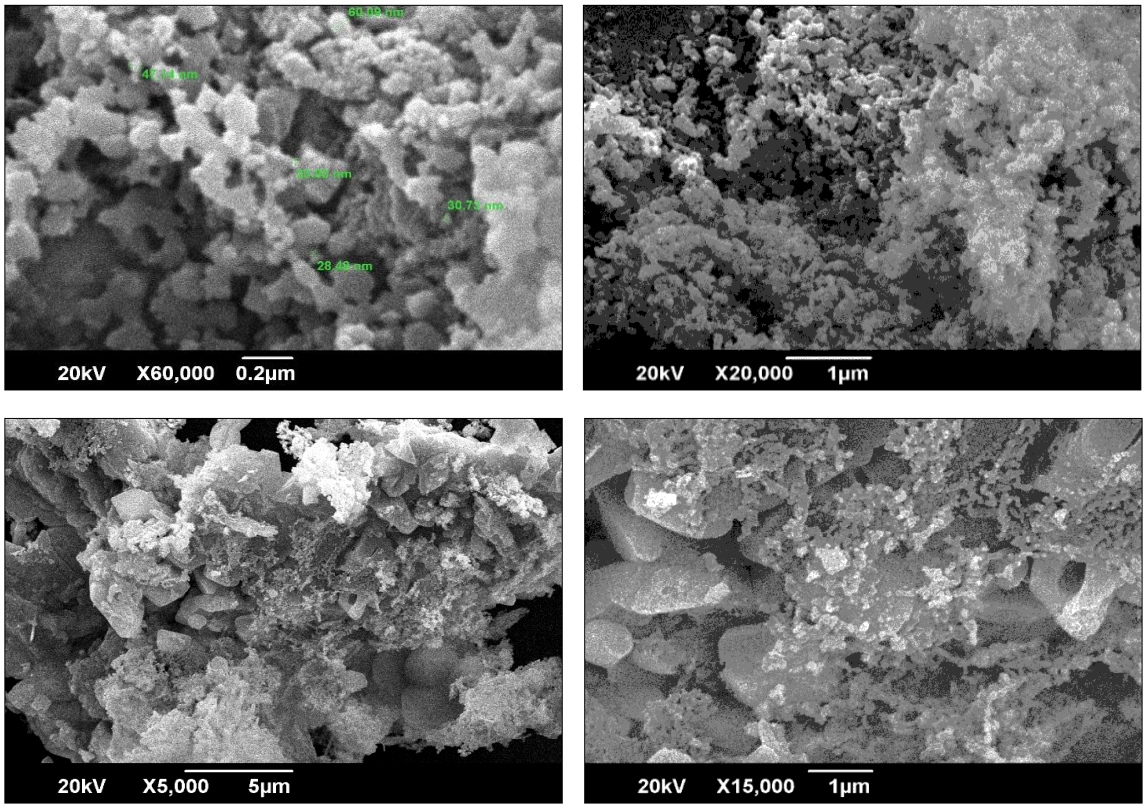


Figure 5.5: SEM images from synthesized Fe NPs of *Verbena officinalis*

5.3. *In-vivo* Anticoccidial Activity

Birds that had been orally injected with *Eimeria* sp. showed signs of coccidiosis on day 5 after infection, including reduced body weight gain, weakness, immobility, ruffled feathers, and bloody diarrhea. These clinical sings peaked on the 7th and 8th day post-infection and subsequently began to subside until the end of the experiment. Various concentrations of commercial and synthetic drugs were given to different groups, and these groups showed fewer signs than the other groups. None of the treated groups showed any mortality.

5.3.1 Effect of *Verbena officinalis* Fe-NP on broiler chicks

All birds were randomly divided into 6 groups on day 14, and each group was orally dosed with 10,000 sporulated oocysts, except for the uninfected and unmedicated control groups. The findings demonstrate that the mean weight of the birds treated with different doses was significantly increased compared to the infected but untreated group. Reduced weight gain in the birds after illness may be due to the malabsorption resulted from infection. However, chick feed consumption and weight gain were positively increased after treatment. Among the different groups, the uninfected, non-medicated, and the drug treated control group showed maximum weight gain, with no significant difference. The maximum weight gain in the control group (uninfected, unmedicated) was 877±3.51 gm (Fig. 5.6). Compared to all other groups, the infected but unmedicated group showed a maximum reduction in weight gain of 305±4.04 gm. Among the different groups, no significant difference ($P < 0.001$) was found between groups treated with 5, 10, and 15% of *Verbena officinalis* nanoparticles, among which sulfaquinoxaline showed a maximum weight gain of 825±2.51 gm. The feed consumption ratio (FCR) of all groups was recorded from the 1st to the 3rd week after infection. The highest FCR was measured in the infected group, untreated having of FCR 1.82±0.02 as compared with the group that was given sulphonamides, which showed an FCR of about 1.09±0.05, and that was non-infected and non-medicated, showed an FCR 1.10±0.05, Both was lower as compared with the C2 (infected and untreated) group (Table 5.1).

Table 5.1**:** Effect of different concentrations of *V. officinalis* Fe NPs and commercial drugs on the mean weight gain (g) means±S.D of *Eimeria* infected birds

	Time	C1	C2	C3	T1	T2	T3
MWG (gm)	7 DPI	353±8.086^{a}	201±3.51^{b}	212±3.21^{b}	207±2.64^{b}	204±1.52^{b}	205±5.68^{b}
	7 DPT	578±6.02^{a}	249±2.51^{e}	525±2.64^{b}	475±3.51^{d}	487±5.56^{cd}	493±4.01^{c}
	14 DPT	877±9.89^{a}	305±14.3^{f}	825±11.3^{b}	674±13.5^{e}	708±6.01^{d}	801±18.3^{c}
FCR	7 DPI	1.71±0.02^{b}	2.55±0.11^{a}	2.61±0.03^{a}	2.62±0.04^{a}	2.60±0.03^{a}	2.53±0.06^{a}
	7 DPT	1.43±0.01^{c}	2.17±0.02^{a}	1.34±0.05^{d}	1.24±0.05^{e}	1.35±0.01^{d}	1.52±0.01^{b}
	14 DPT	1.10±0.05^{b}	1.82±0.02^{a}	1.09±0.05^{b}	1.03±0.05^{c}	1.02±0.01^{c}	1.07±0.02^{b}

Superscript of all treated and untreated groups denotes tukey test variables was different, showed significant differences with P ≤ 0.05.

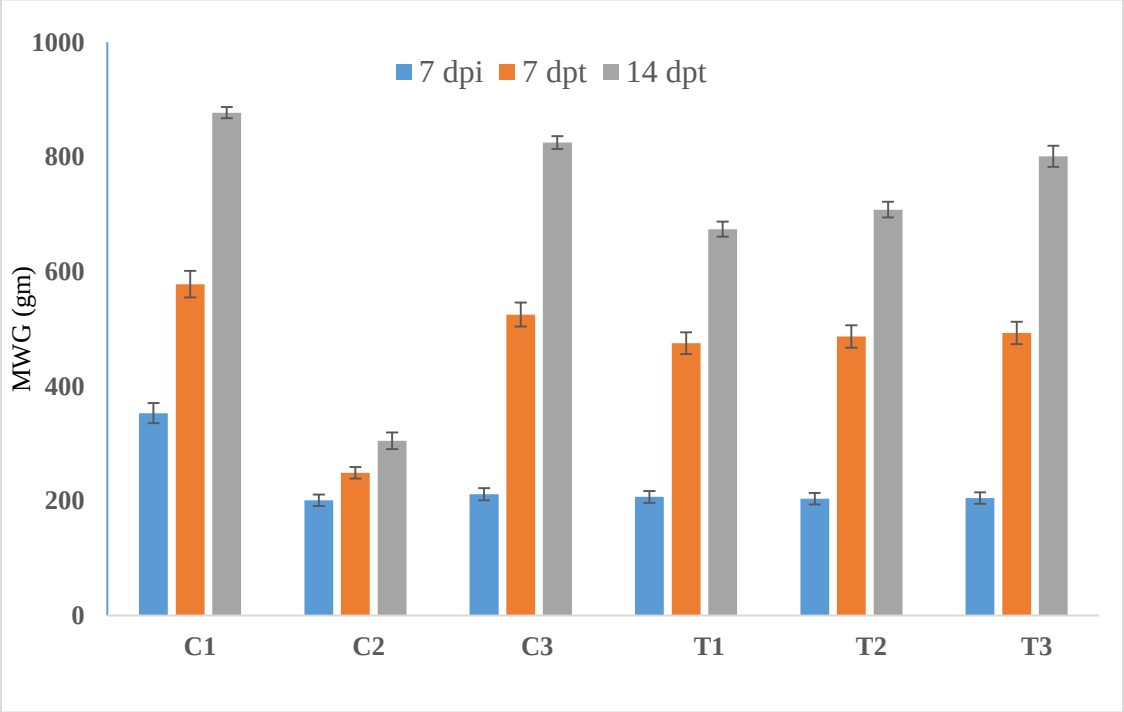


Figure 5.6: Mean weight gain (mean±S.D) of all experimental groups at 7 DPI, 7 & 14 DPT

5.3.2 Feed Consumption

The food intake of all studied groups was calculated from the 1st to the 3rd week after infection. The uninfected, untreated group (C1) showed a maximum average feed consumption compared to the rest of the groups, i.e., 975±5.56 gm. The feed consumption of the infected-untreated group was the lowest, i.e., 562±3.78 gm. In the supplemented group, the groups treated with 10% and 15% Fe NPs of *V. officinalis* showed feed consumption of 726±1.52 gm and 862±1.52 gm, similar to feed + consumption of the group treated with commercial drug, i.e., 910±5.00 gm (Table 5.2; Fig. 5.7).

Table 5.2**:** Effect of different concentration of *V. officinalis* Fe NPs and commercial drug on feed consumption (mean ± S.D) of *Eimeria* infected birds

Time	C1	C2	C3	T1	T2	T3
7 DPI	611 ± 9.50^{a}	533 ± 7.50^{cd}	555 ± 3.05^{b}	543 ± 2.51^{bc}	533 ± 2.51^{cd}	522 ± 1.00^{d}
7 DPT	836 ± 5.13^{a}	494 ± 2.51^{f}	709 ± 5.56^{c}	593 ± 3.05^{e}	663 ± 2.00^{d}	751 ± 1.52^{b}
14 DPT	975 ± 5.56^{a}	562 ± 3.78^{f}	910 ± 5.00^{b}	655 ± 3.21^{e}	726 ± 1.52^{d}	862 ± 1.52^{c}

Superscript of all treated and untreated groups denotes tukey test variables was different, showed significant differences with $P \leq 0.05$.

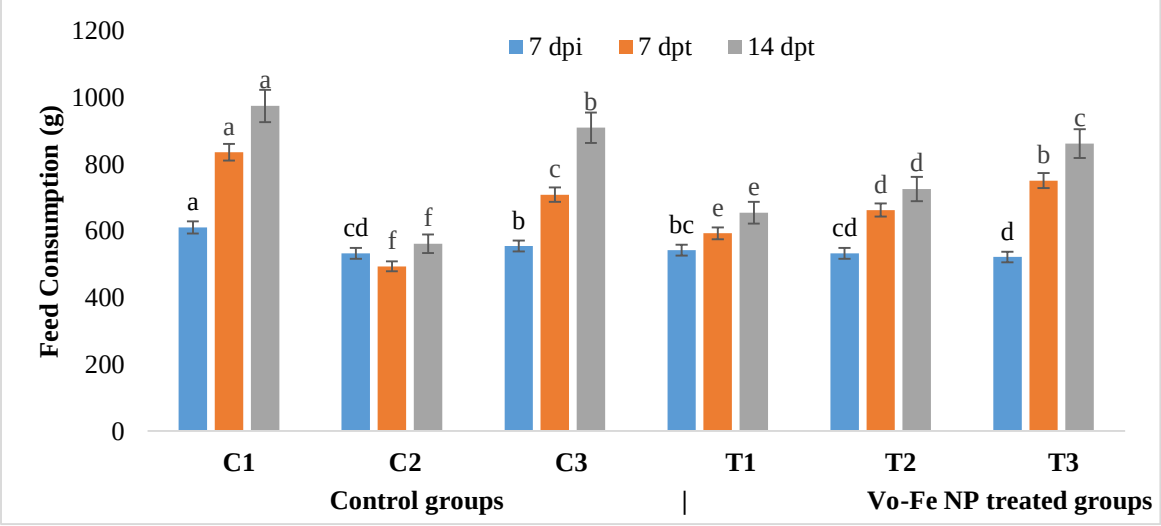


Figure 5.7: Effect of different concentrations of *V. officinalis* Fe NPs and commercial drugs on the Feed consumption birds infected with *Eimeria tenella*.

5.3.3 Oocyst Count and Diarrhea

From day 6 to 10 days after infection, stool samples were collected from each group and oocysts counted. On days 7 and 8, the highest number of oocysts was calculated using the McMaster technique. No oocysts were observed in the infected and untreated groups (Table 5.3). The highest number of oocysts was calculated for the infected-untreated group, i.e., 37.05±4.07. After treatment, no or reduced oocysts were observed in the treated groups, as all groups showed a reduction and complete disappearance of oocysts in the stool samples after treatment (Fig. 5.8).

Table 5.3**:** Oocyst count ($\times 10^3$/ gram feces) of *Eimeria* infected bird groups, treated with sulfaquinoxaline and various doses of synthesized Fe NPs

Time (day)	C1	C2	C3	T1	T2	T3
6th	0.00	3.33±0.57^{a}	3.66±0.57^{a}	3.00±1.00^{a}	3.43±0.51^{a}	3.73±0.64^{a}
7th	0.00	10.43±0.51^{a}	6.76±0.68^{b}	7.23±0.32^{b}	6.16±0.15^{b}	7.06±0.11^{b}
8th	0.00	12.30±0.26^{a}	4.50±0.20^{d}	5.66±0.15^{b}	5.33±0.15^{bc}	5.10±0.10^{c}
9th	0.00	7.66±0.57^{a}	2.16±0.15^{c}	2.66±0.15^{bc}	3.30±0.10^{b}	2.23±0.05^{c}
10th	0.00	3.30±0.10^{a}	0.43±0.05^{c}	1.60±0.10^{b}	1.40±0.10^{b}	0.53±0.15^{c}
Total	0.00	37.05±4.07^{a}	17.51±2.39^{c}	20.15±2.33^{b}	19.62±1.86^{c}	18.65±2.52^{c}

Superscript of all treated and untreated groups denotes tukey test variables was different, showed significant differences with $P \leq 0.05$.

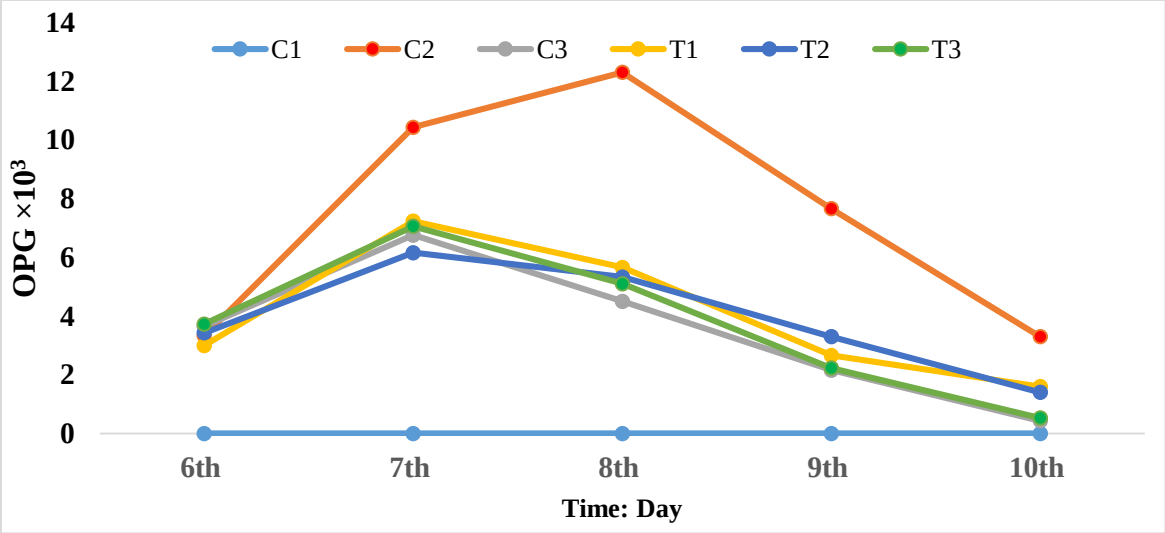


Figure 5.8: Oocyst count per gram of feces (mean±S.D) of infected groups from 6th to 10th day post-infection

The mortality was mainly observed in all groups except the uninfected-untreated group. The highest mortality rate was recorded in the infected-untreated group. After treatment, all the groups had no deaths. No mortality was observed in the normal control and all treated group post treatment. Bloody diarrhea was observed from day 6 to day 10 after infection. The uninfected-untreated group had no diarrhea discharge, whereas the infected-untreated group had a high level. All the groups showed a reduction in their bloody diarrhea after being given treatment (Table 5.4).

Table 5.4: Comparative bloody diarrhea, lesion score, survival, and mortality rate of *Eimeria* infected birds, treated with *V. officinalis* Fe NPs

Groups	**Bloody Diarrhea (Day)**					**Lesion Score**	**Survival %**	**Mortality %**
	6^{th}	7^{th}	8^{th}	9^{th}	10^{th}			
C1	-	-	-	-	-		100	0.00
C2	-	+	+++	++	+	+3	60	40
C3	-	+	++	+	-	0	100	0.00
T1	-	+	++	+	-	+2	100	0.00
T2	-	+	++	+	-	+1	100	0.00
T3	-	+	+	-	-	0	100	0.00

Superscript of all treated and untreated groups denotes tukey test variables was different, showed significant differences with $P \leq 0.05$.

5.3.4 Hematological Analysis

The red blood cell (RBC) count was also different in all the experimental groups, showed a significant difference compared to the control group. The RBC count in the infected and untreated group 'C2' is significantly different compared to the normal control 'C1' ($P > 0.01$). The normal control group 'C1' showed a significant difference in hemoglobin from the C2 group but very little difference from the sulfaquinoxaline -treated 'C3' group. All the groups treated with iron oxide nanoparticles showed highly non-significant results compared to the C1 group. Post-inoculation values showed highly significant differences in platelets between 'C1' and all other infected groups. Post-treatment values indicate a rapid recovery of platelets in the blood plasma of birds.

The white blood cell (WBC) count in the 'C1' group was significantly different ($P > 0.001$) compared to the 'C2 and C3' groups. The WBCs in the T3 group were not significantly different compared to the T2 group, but both groups showed a relatively significant difference ($P > 0.001$) to the T1 group (Table 5.5; Fig. 5.9a).

The highest non-significant differences were found in groups C3, T2, and T3 compared to the normal control group 'C1'. Post-infection and post-treatment of birds, the control group 'C1' showed a highly significant difference ($P > 0.001$) in lymphocytes compared to all other groups. All the infected groups have relatively large numbers of lymphocytes. After treatment, the lymphocyte count begins to approach the normal range. The groups 'C3, T2, and T3' showed relatively non-significant differences compared to the normal control group 'C1' after treatment. When the birds were infected with *Eimeria*, the number of monocytes began to increase. After one week of treatment, all groups had decreased lymphocyte counts, which eventually returned to normal after 2 weeks of treatment. The results showed a significant difference in lymphocyte counts in the 'C2' group compared to all other groups. The groups 'C3, T2, and T3' showed non-significant results as compared to the C1 group. The increased granulocyte count was observed in the infected group. After treatment, the granulocyte count was reduced in all except the C2 group, which was infected but did not took medication. The results showed that there was no significant difference between groups 'C3, T2, and T3' but a relatively significant difference in the T1 group and a highly significant difference in result with the C2 group (Fig. 5.9b).

Table 5.5: Hematological parameters (means ± S.D) of different groups at 14th day post-treatment with iron oxide nanoparticles of *V. officinalis*

Parameter	**C1**	**C2**	**C3**	**T1**	**T2**	**T3**
Hb (g/dl)	12.12±0.02^{a}	6.53±0.04^{e}	12.14±0.0^{a}	9.24±0.06^{d}	10.33±0.03^{c}	11.62±0.02^{b}
RBC (×10^{6}/µl)	4.22±0.01^{a}	1.54±0.03^{d}	4.26±0.06^{a}	3.46±0.04^{c}	3.82±0.02^{b}	4.16±0.04^{a}
WBC (×10^{3}/µl)	7901±1.00^{f}	12466±1.00^{a}	8035±1.00^{d}	8733±1.0^{b}	8471±1.00^{c}	7949±0.51^{e}
Platelets (×10^{3}/µl)	432±1.52^{c}	335±0.37^{d}	455±0.07^{b}	455±0.26^{b}	458±0.11^{a}	457±1.00^{ab}
Lymphocytes (%)	74.27±0.06^{c}	94.17±0.15^{a}	72.3±0.09^{e}	80.12±0.2^{b}	73.5±0.14^{d}	70.22±0.09^{f}
Monocytes (%)	15.43±0.11^{e}	34.35±0.15^{a}	15.32±0.01^{e}	18.47±0.7^{b}	17.43±0.08^{c}	16.26±0.07^{d}
Granulocytes (%)	15.28±0.04^{e}	29.18±0.11^{a}	15.21±0.10^{e}	24.3±0.10^{b}	20.25±0.15^{c}	17.28±0.11^{d}

Superscript of all treated and untreated groups denotes tukey test variables were different, showed significant differences with $P \leq 0.05$.

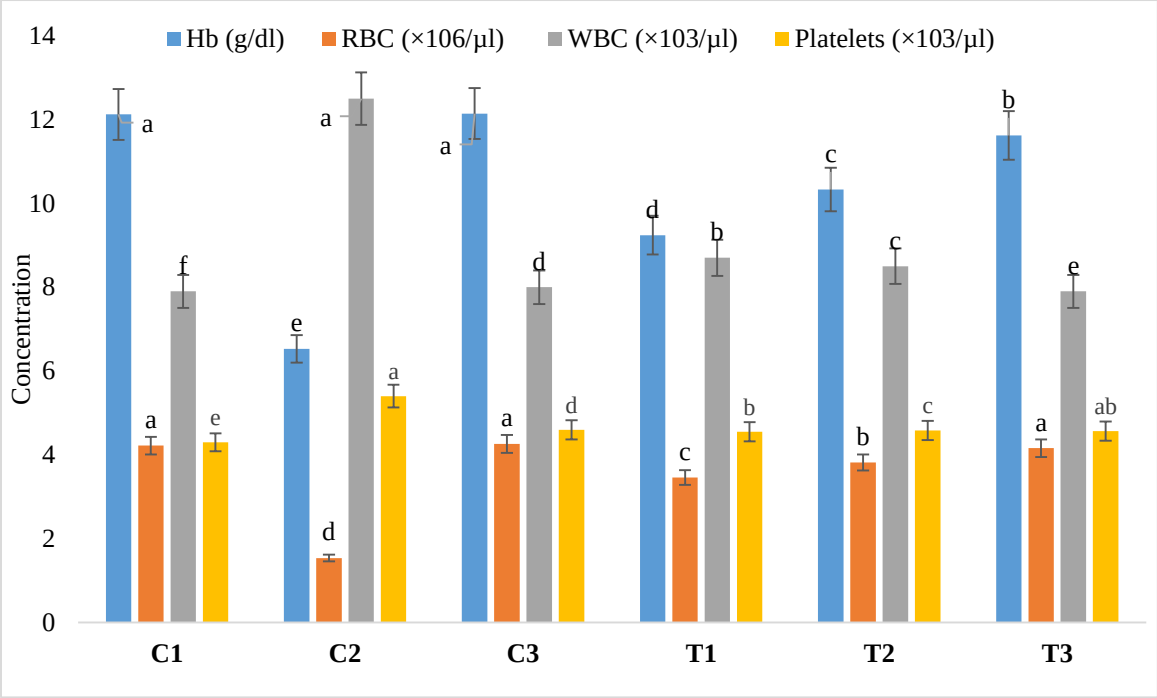


Figure 5.9a: Hematological parameters (Hb, RBC, WBC and Platelets) of different groups at 14th day post-treatment

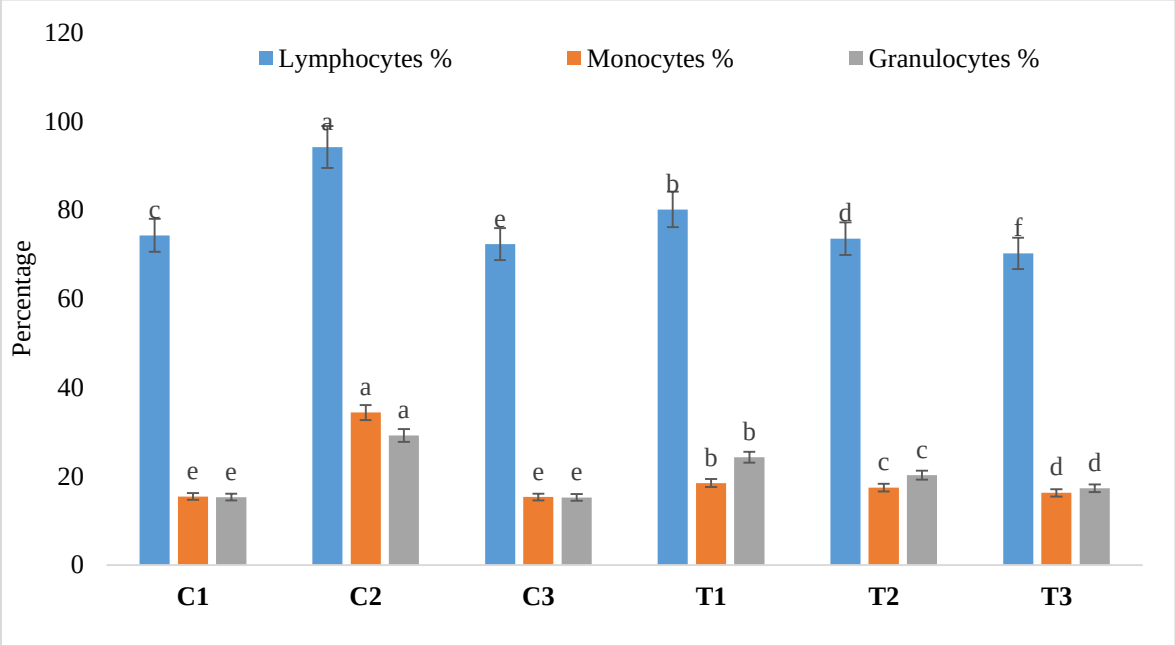


Figure 5.9b: Hematological parameters (Lymphocytes, Monocytes and Granulocytes) of different groups at 14th day post-treatment

5.3.5 Biochemical Analysis

The serum was separated from the blood to measure the carbohydrate, protein (Fig. 5.10a & b), and AST and ALT levels (Fig. 5.10b & c). AST levels showed a significant increase immediately after the infection. All the groups receiving infection showed significant differences in AST levels compared to normal group 'C1'. Post-treatment AST values showed a significant difference between the C1 and C2 groups. No significant difference was found between the C1 and C3 groups, but there is a significant difference in the T1 group. The serum glucose level showed that after infection, the serum glucose level was significantly decreased in all infected groups compared to the normal group. One week after treatment, the birds' glucose level was elevated and approached the normal range. The results showed highly non-significant differences between the C3 and C1 groups as well as with the T2 group. The results showed non-significant outcome between the C3, T2, and T3 groups and highly significant results for the C2 group as compared to groups C1 and C3 (Table 5.6).

The protein level begins to drop when birds become infected. All the infected birds showed significant differences compared to the normal control group C1. After treating birds, all infected birds showed a significant difference from the normal control group 'C1', i.e., protein content of treated birds was raised and approaching its normal range. The protein level in the infected but untreated group significantly differs from the normal group. There is no significant difference in the protein content between the untreated and treated groups.

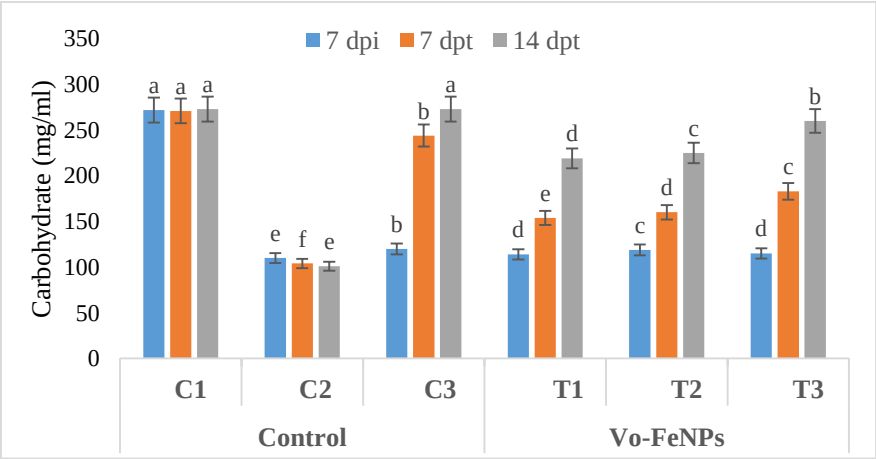


Figure 5.10a: **C**arbohydrate levels in the blood of coccidian-free and medicated groups at 7 DPI, 7 and 14 DPT

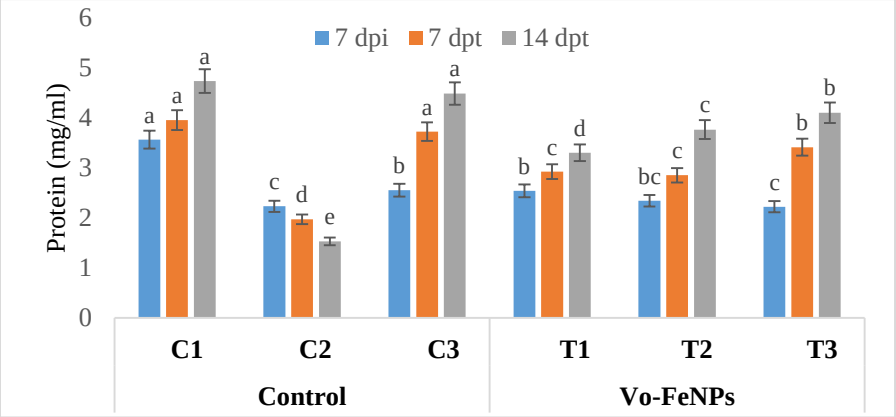


Figure 5.10b: Protein levels in the blood of coccidian-free and medicated groups at 7 DPI, 7 and 14 DPT

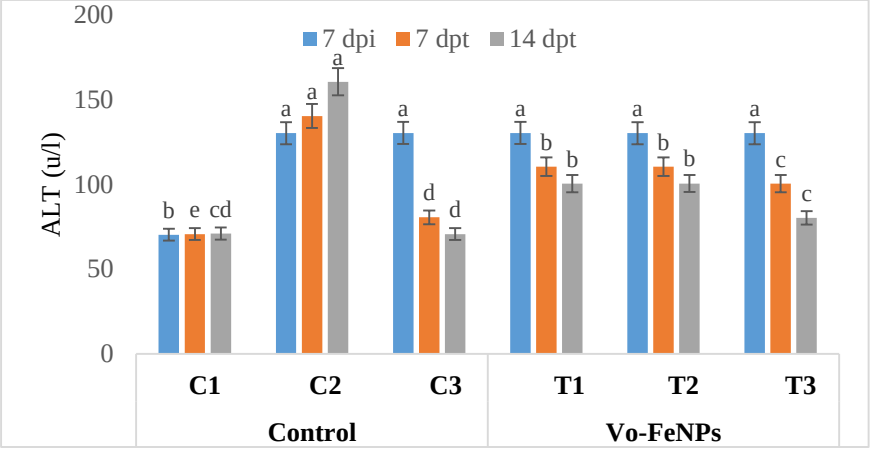


Figure 5.10c: ALT levels at 30°C of all experimental groups at 7 DPI, 7 and 14 DPT

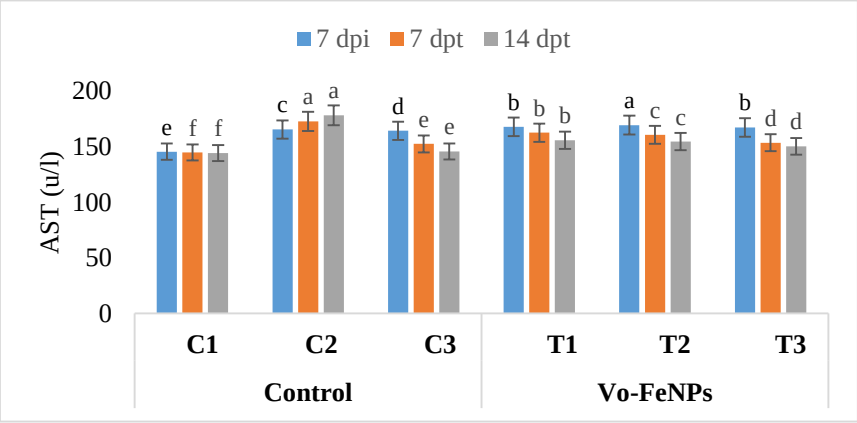


Figure 5.10d: AST levels at 30°C of all experimental groups at 7 DPI, 7 and 14 DPT

Table 5.6: Serum Biochemistry of coccidian-free and medicated (*V. officinalis*, FeNP) groups at 7 DPI, 7 and 14 DPT

Time		C1	C2	C3	T1	T2	T3
Carbohydrate (mg/ml)	1	272±0.25^{a}	110±0.67^{e}	120±0.10^{b}	114±0.16^{d}	119±0.09^{c}	115±0.05^{d}
	2	271±0.09^{a}	104±0.11^{f}	244±1.23^{b}	154±0.18^{e}	160±0.05^{d}	183±0.17^{c}
	3	273±0.16^{a}	101±0.09^{e}	273±1.51^{a}	219±0.13^{d}	225±0.14^{c}	260±0.08^{b}
Protein (mg/ml)	1	3.56±0.04^{a}	2.23±0.05^{c}	2.55±0.02^{b}	2.54±0.06^{b}	2.34±0.17^{bc}	2.22±0.08^{c}
	2	3.95±0.02^{a}	1.97±0.03^{d}	3.72±0.01^{a}	2.92±0.01^{c}	2.85±0.09^{c}	3.41±0.17^{b}
	3	4.73±0.03^{a}	1.53±0.03^{e}	4.48±0.04^{a}	3.30±0.21^{d}	3.76±0.18^{c}	4.14±0.07^{b}
ALT (U/L)	1	70.28±0.07^{b}	130.17±0.02^{a}	130.26±0.09^{a}	130.29±0.04^{a}	130.24±0.09^{a}	130.23±0.10^{a}
	2	70.64±0.11^{e}	140.34±0.21^{a}	80.49±0.19^{d}	110.43±0.02^{b}	110.40±0.05^{b}	100.41±0.17^{c}
	3	70.94±0.02^{cd}	160.53±0.15^{a}	70.64±0.15^{d}	100.41±0.08^{b}	100.48±0.14^{b}	80.21±0.06^{c}
AST (U/L)	1	145.4±0.25^{e}	165.3±0.25^{c}	164.2±0.58^{d}	167.6±0.15^{b}	169.24±0.09^{a}	167.2±0.10^{b}
	2	144.6±0.10^{f}	172.7±0.58^{a}	152.4±0.20^{e}	162.4±0.18^{b}	160.4±0.05^{c}	153.4±0.17^{d}
	3	144.2±0.10^{f}	178.0±0.49^{a}	145.6±0.15^{e}	155.5±0.16^{b}	154.4±0.14^{c}	150.2±0.06^{d}

Superscript of all treated and untreated groups denotes tukey test variables were different, showed significant differences with $P \leq 0.05$. (1: 7 DPI, 2: 7 DPT, 3: 14 DPT)

5.3.5 Histological Analysis

A histological examination of the cecal and liver tissues was performed. The results differed between the infected and FeNP-treated groups. The unembellished oocysts and damage to the intestinal epithelial lining were seen in the infected group, whereas improvement and mild clinical signs were seen in the treated groups (Fig. 5.11a). Infected but untreated birds had more severe hepatic congestion and liver lesions, whereas treated groups had milder signs (Fig. 5.11b).

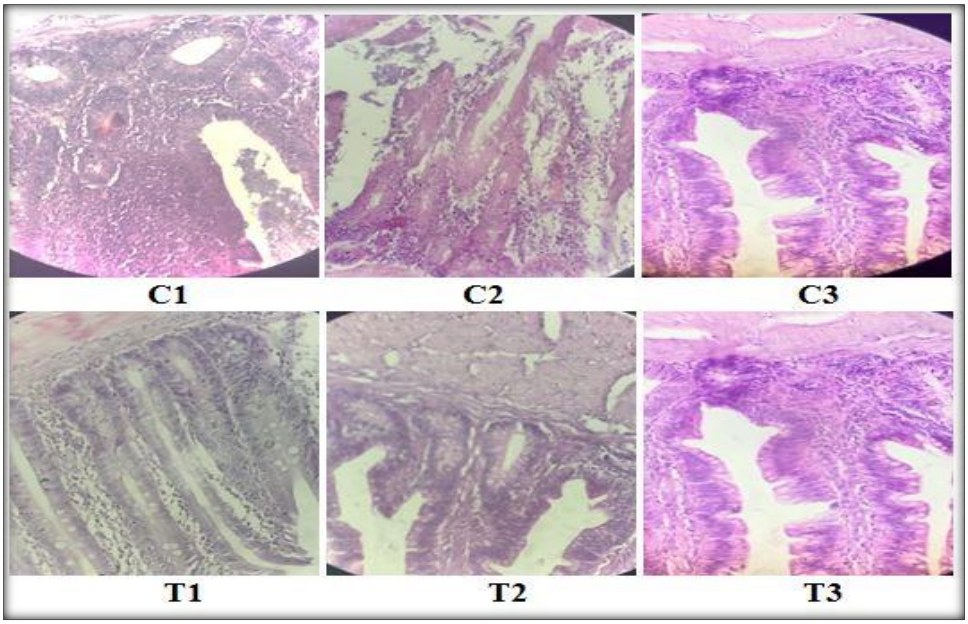


Figure 5.11a: Histopathological examination of cecal tissues, 14 day post-treatment; (C1) uninfected untreated group, (C2) infected, untreated, (C3) infected and treated with SQX, and (T1, T2 and T3) refers to groups treated with 5,10 and 15% Fe NP of *V. Officinalis*

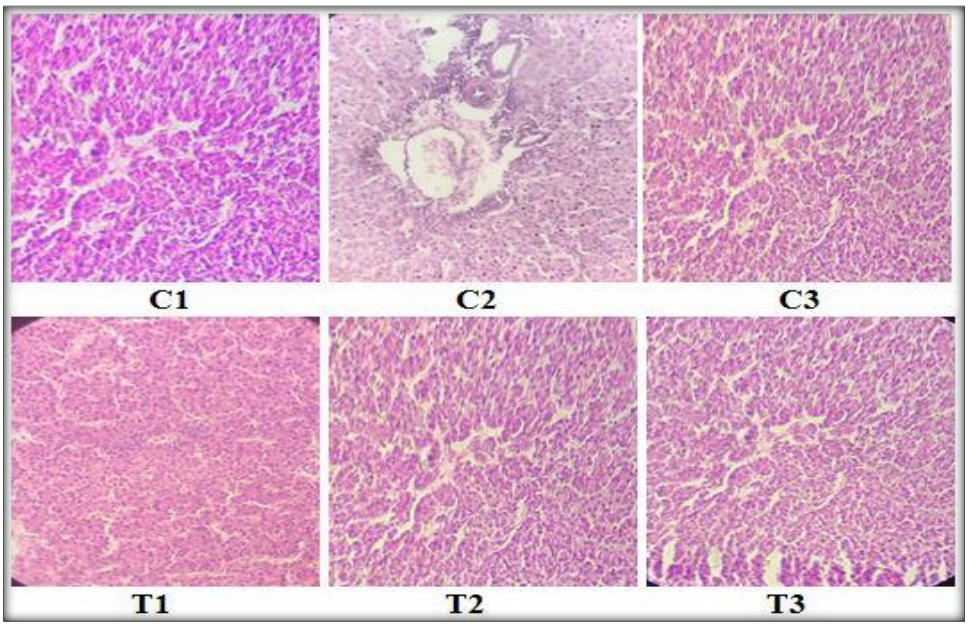


Figure 5.11b: Histopathological examination of hepatic tissues, 14 day post-treatment; (C1) uninfected untreated group, (C2) infected, untreated, (C3) infected and treated with SULFAQUINOXALINE, and (T1, T2 and T3) refers to groups treated with 5,10 and 15% Fe NP of *V. Officinalis*

DISCUSSION

Coccidiosis is one of the most harmful and self-limiting gastrointestinal infections, caused by *Eimeria* that enters primarily through the cecal villi, causing a severe but sometimes fatal infection (Hernandez *et al*., 2019). The coccidial-causing protozoan primarily resides in the intestines of most domestic and wild avian species (Wang *et al*., 2021). Seven different *Eimeria* species can infect chicks, depending on their location in the gut; the most common and lethal species is *E. tenella* (Pop *et al*., 2019). The major causes of cecal coccidiosis include decreased weight gain, increased bloody diarrhea, reduced feed intake, and even death (Muhammad *et al*., 2019). The clinical manifestations of the disease appear when sufficient numbers of coccidian parasites were present to initiate infection. In addition, widespread drug resistance, a high parasite prevalence, and environmental persistence contribute to subclinical infections, with significant negative impacts on productivity and food security (Acharya and Acharya, 2017).

The infection by coccidian parasites can cause significant economic losses to poultry businesses. The main protozoan parasite, *E. tenella,* invades the intestinal tract, causing extreme morbidity and high mortality. This causes severe damage to the intestinal tract. The young chicks (3 - 18 weeks) was very susceptible to coccidiosis. Clinical coccidiosis is primarily caused by the virulence of *Eimeria* species and results primarily from inappropriate management practices, chick age, and relative oocysts consumption (Abdisa *et al*., 2019). Over the past 60 years, several anticoccidial agents have been introduced to treat this disease, but resistance to all of these drugs has emerged, making coccidiosis control techniques much more puzzling and complex (Wallach, 2010). By eliminating some resistance mechanisms, improving bioavailability, and amplifying the therapeutic targets, nanoparticles may be a potential tool to reduce the risk of drug resistance to conventional therapies.

Elements like iron and silica have also been utilized due to their anti-microbial qualities, and some polysaccharides, such as chitosan, was effective as drug delivery systems (Muraleedharan and Chhabra, 2018). Therefore, the current study is based on a green synthesis of *Verbena officinalis* iron oxide nanoparticles prepared in various aqueous concentrations and used to treat coccidiosis in broiler chicks *in-vivo*. Chhabra and

colleagues (2014) reviewed herbal remedies and their internal mechanisms of action for coccidiosis.

Verbena officinalis (Verbenaceae), commonly known as vervain, simpler's joy, turkey grass, wild verbena, and verveine officinale, is a well-known medicinal herb. It is widely distributed in natural habitats in Europe, America, North and Central Africa, Asia, and Australia (Kubica *et al*., 2020). It is generally accepted that the mineral constituents of this herb, especially the macrominerals and trace elements, play important roles in the activation of enzymatic systems and the metabolism of biomolecules due to its antioxidant action (Rehecho *et al.*, 2011). The purpose of this study is to evaluate the *in-vivo* efficacy of green synthesized iron oxide nanoparticles against experimentally induced coccidiosis in broiler chicks. Iron oxide nanoparticles were synthesized using distilled water and ($FeCl_3.6H_2O$) ferric chloride hexahydrate (Balamurugan *et al*., 2014).

The characterization of iron oxide nanoparticles was performed to confirm their synthesis and purification. Iridoids, phenylpropanoids, flavonoids, luteolin, and terpenoids make up most of the active compounds in *V. officinalis* (Rehecho *et al*., 2011). XRD analysis was used for confirmation. XRD studies revealed an average-size nanoparticle of 22.35 nm. Strong diffraction peaks with 2θ values matching the crystal planes of 24˚=012, 33˚=104, 35˚=110, 40˚=113, 49˚=024, 54˚=116, 57˚=018, 62˚=214, and 63˚=300 correspond to the synthesis of Fe_2O_3 nanoparticles. The results showed the spinal phase structure of magnetite, showed similarity to magnetite nanoparticles (Pan *et al*., 2020). The analyzed diffraction peaks matched well with the standard magnetite XRD pattern using JCPDS numbers. A broad peak was obtained due to the nano-size effect of the synthesized particles (Yew *et al*., 2016).

The morphology, texture, and composition of iron NPs were determined using SEM. Iron oxide nanoparticles were found to be spherical (Devatha *et al*., 2016). Scanning electron micrographs play a very important role in the morphology determination of iron oxide nanoparticles, since the biological role of nanoparticles is highly dependent on their size and shape (Laurent *et al*., 2008).

The FT-IR spectroscopy of synthesized iron oxide nanoparticles was performed to analyze and evaluate biomolecules bound to the iron oxide nanoparticles (Gottimukkala *et*

al., 2017). The FT-IR revealed several peaks in the range of 500-2500 cm^{-1}. Maghemite bands were observed between 580 and 620 cm^{-1} and magnetite bands were observed between 500 and 640 cm^{-1}. A hematite band is observed at 520-570 cm^{-1} (Demirezen *et al*., 2019). An asymmetric band around 1105 cm^{-1} indicates the presence of C=C stretching in polyphenols. From these findings, the formation of iron oxide NPs can be estimated and interact strongly with the compounds of leaf extract (Kouhbanani *et al*., 2019).

The assay takes into account many parameters related to coccidiosis, i.e., feed consumption, mean weight gain, and feed conversion ratio, which were observed and recorded for 3 weeks post-infection. The observation showed that the infected but treated birds (FeNP treatment) gained significantly less weight than the uninfected birds, as Zaman and colleagues (2012) revealed in their study.

The reduced weight gain in some groups may be due to the low feed intake of these birds. The results also showed that weight gain in uninfected and unmedicated birds, infected birds treated with drugs, and medium and high doses of iron oxide nanoparticles is significantly higher than the reports of Zaman (2012). It also showed a significant increase compared to the group (Ali *et al.,* 2019). The highest average weight gain was observed in chicks of different groups (C1, C3, and T3), while the lowest average weight gain was observed in chicks of group C2 (Habibi *et al*., 2016).

The findings of the current study was analogous to those of Gogoi and co-researchers (2019), who found that the body weight of the medicated group was not significantly different from that of the uninfected control group. The T2 and T3 groups had the highest feed intake and were comparable to control groups (C1 and C3). In this context, team research team of Gogoi (2019) reported some general findings. Moreover, the positive control (C2), had the lowest feed intake compared to the other groups, which may be the reason why the C2 group had the lowest body weight gain. The T3 group had the lowest FCR, as did the negative and drug-treated controls. The highest FCR in the negative control group was determined by Chand and colleagues (2016); they also showed a decreased FCR in a group like the negative control group. Similar results for favorable feed conversion ratios in the medicated groups were shown by (Muthamilselvan *et al*., 2016).

The 14 day-old chicks were infected. After one week, they started shedding oocysts in their feces, which were collected and observed for two weeks. Fewer oocysts were detected in all infected and treatment groups. The group administered with 15% *V. officinalis* Fe NPs produced similar oocysts to the drug-treated group. The number of oocysts was lower in the treated group compared to the control group. The oocyst count in this study showed similarity to the study of Parent and colleagues (2018). The findings of Jang and coauthors (2007) on the consistency of the C2 group showed the maximum number of oocysts on the 7^{th} and 8^{th} days after infection. A progressive decrease in oocysts was observed from day 8 post-infection, similar to Kim's study (2007). In addition, a reduction in fecal oocysts has also been reported (Chauke and Siebrits, 2012). No oocysts were observed in the group infected within the first 6 days, just like the findings of Moines (2014).

Bloody diarrhea was observed in all groups with severe diarrheal discharge except the uninfected one, whereas the treated birds showed a decreasing level of diarrheal discharge and cured with the passage of time (Ishaq *et al*., 2022). The symptomatic bloody diarrhea was observed on the 4^{th} day of infection in various groups. Severe bloody diarrhea was observed in the C2 group, whereas mild diarrhea was observed in the T1 group. The treated groups (C3, T2, and T3) showed mild to no bloody diarrhea (Rizwan *et al*., 2022), which indicates the efficacy of medium and high doses of synthesized herbal drugs against coccidiosis. Group C2 had the highest lesion score (+3), while Group T1 had the lowest (+2) score. While groups C1, C3, and T3 had no or low lesion scores, whereas group T2 had a grade +1 lesion score, the lower lesion score in cases where an effective dose saved birds from any casualties was associated with reduced oocysts shedding (Almeida *et al*., 2014). All treatment groups had the best survival rate, with no mortality. The sulfaquinoxaline-treated group had no mortality and a 100% survival rate, as did the groups treated with various doses of Fe NPs, similar to the findings of Vijayalingam *et al*., (2019), who found that treated birds had higher survival rates.

Based on the serum biochemical analysis, the AST and Alt levels were significantly elevated in *E. tenella*-infected chicks compared to the negative control. The findings of the current study was similar to those of Cowieson and colleagues (2020), who stated that raised levels of AST and ALT were due to hyperactive metabolic tissues; these elevated

levels appear to be caused by tissue damage. After treatment with various doses of Fe NPs, both enzymes start to decline, except for the infected, untreated group. Adamu (2013) revealed elevated AST and ALT levels in cases of *E. tenella* infection. The high AST level may be caused by cecal inflammation, damage to the cells lining the intestine, and blood loss, leading to tissue damage and elevated AST activity.

Similar results obtained from Biu *et al.,* (2021) showed elevated AST and ALT and were significantly reduced after treatment with the aqueous extracts. Elevated AST and ALT indicated damage in hepatocytes and intestinal mucosal cells. The results was similar to those of Melkamu *et al*., (2018), who also presented the same findings. The extensive destruction in colon tissues raised the serum ALT activity with the dramatic drop in glucose after infection and a rise significantly to normal levels after treatment. The current results were consistent with those of Langerudi *et al*., (2022), indicating hypoglycemia with infection.

Histopathological observation showed changes in the livers of infected and treated chicks. The hepatic congestion was evident in the mild and high-dose groups, mild hepatic congestion was noted in the low-dose and untreated groups. No lesions were observed in sulfaquinoxaline-treated chicks. The liver observations of treated chicks revealed similarity to the findings of Mikail (2019). Histopathological analysis of the cecum revealed epithelial necrosis, cystic abnormalities, and parasitic growth in the submucosa. The oocysts were noticed in both infected and sulphonamide-treated birds, but no lesions were observed after treatment. The cecum of birds also showed severe tissue degeneration caused by the parasite. The most damaged intestines was found in untreated infected birds. As the infection spreads throughout the chick's body, the epithelium begins to shed, resulting in the malabsorbtion of nutrients.

The results were consistent with Olabode's study (2020), which reported similar histological results where low or no lesions were observed in treated groups. In contrast, significant lesions in the cecum and liver were observed in unmedicated groups, the lesions include swelling, thrombosed distension, tissue debris, and a thickened cecal wall that causes edema and the formation of scar tissue. Manafi (2011) states the lesion minimalization happened as a result of effective treatment. In addition, histopathological studies showed that chicks treated with 10 and 15 mg/ml of Fe-NPs had a lesser impact on

Eimeria-affected tissue with no hemorrhages and less-damaged intestinal mucosa (Moines *et al*., 2014).

The decreased hemoglobin and the RBC count were observed, indicating disruption of the intestinal epithelium and mucosal lining, similar to the findings of Akhter (2021). Since lymphocytes and WBCs were the first line of defense against parasitic infection, they also showed a similar decline in PCV count. Once the infection was treated, the number of WBCs began to rise and approached normal levels.

Comparing to the standard data range, the total number of erythrocytes in the infected case was lower than in healthy chicks (Eze *et al*., 2022). According to Messai and Redouane (2022), the most common abnormalities found in chicks were a decrease in RBC count and hemoglobin. A reduced RBC count is caused by both viral diseases and blood loss in the gastrointestinal tract. The infected chicks showed increased lymphocytes, monocytes, and granulocytes compared to healthy chicks, this increased number can be caused by inflammation in ceca (Umar *et al*., 2022).

CONCLUSION

In the current study, a green synthesis of iron oxide nanoparticles from *Verbena officinalis* was performed, and their anticoccidial effect was determined in broiler chicks along with sulfaquinoxaline against coccidiosis. This recent study demonstrated that iron oxide NPs synthesized from *Verbena officinalis* was effective against experimentally induced coccidiosis in broiler chicks, showed a dose-dependent effect.

GENERAL CONCLUSION

- The coccidiosis prevalence data showed a decline from 2017 to 2019.
- It was also shown that coccidiosis infection has a direct relationship with temperature and an inverse relationship with humidity.
- The age-wise data showed that *Eimeria* infection occurs in adult chicks.
- The percent adoption of the management parameters concluded that the use of vaccines, disinfectants, special shoes, fresh bedding, clean feeders, and feeding formula showed significant results ($P < 0.05$) with the least number of cases.
- Among the five plant extracts, *V. officinalis* and *P. glabrum* were observed to have maximum SPI (76 and 65%), respectively, and the best anticoccidial activity with maximum weight gain, low oocyst count, diarrhea, and a normal biochemical, hematological, and histological profile.
- The components of *V. officinalis* were identified with GC-MS, docked against S-Adenosyl methionine synthase, suggesting its best binding with strychane, 1-acetyl-20a-hydroxy-16-methylene with the lowest binding enegery (-6.4).
- The SPI of all fractions of the *V. officinalis* showed that F1, F3, F5, & F8 have a maximum SPI of 70, 73, 65 and 78% respectively.
- The anticoccidial activity of active fractions was investigated and it was suggested that (EA/F3 and C:M/F8) fraction treatment resulted in maximum mean weight gain, low oocyst count, and low diarrhea in chicks with a normal biochemical, hematological, and histological profile.
- The most active fractions were characterized with GC-MS, and all the identified compounds were docked against SAM synthase, suggesting that a-sitosterol (EA) and 1,2 benzenedicarboxylic acid, mono(2-ethylhexyl) ester (C:M) have the best binding with the lowest binding energy of -6.2 and -6.1, respectively.
- The *in-vivo* assay of Fe NPs of *V. officinalis* suggested its anticoccidial activity against *Eimeria* infected chicks with good mean weight gain and feed conversion ratio, the lowest oocyst count and a normal biochemical, hematological, and histological profile.

REFERENCES

Abbas, A., Abbas, R. Z., Khan, M. K., Raza, M. A., Mahmood, M. S., Saleemi, M. K., Hussain, T., Khan, J. A. & Sindhu, Z. U. D. (2019). Anticoccidial effects of *Trachyspermum ammi* (Ajwain) in broiler chickens. *Pakistan Veterinary Journal*, *39*(2), 301-304.

Abbas, A., Iqbal, Z., Abbas, R. Z., Khan, M. K., & Khan, J. A. (2015). *In-vitro* anticoccidial potential of *Saccharum officinarum* extract against *Eimeria* oocysts. *Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants*, *14*(6), 456-461.

Abbas, A., Iqbal, Z., Abbas, R. Z., Khan, M. K., & Khan, J. A. (2017). Immunomodulatory activity of *Pinus radiata* extract against coccidiosis in broiler chicken. *Pakistan Veterinary Journal*, *37*(2), 145-149.

Abbas, A., Iqbal, Z., Abbas, R. Z., Khan, M. K., Khan, J. A., Hussain, K., Mahmood, M. S. & Rizwan, H. M. (2017). Immunomodulatory effects of *Camellia sinensis* against coccidiosis in chickens. *Journal of Animal & Plant Sciences*, *27*(2), 415-421.

Abbas, G., Khan, S. H., Hassan, M., Mahmood, S., Naz, S., & Gilani, S. S. (2015). Incidence of poultry diseases in different seasons in Khushab district, Pakistan. *Journal of Advanced Veterinary and Animal Research*, *2*(2), 141-145.

Abbas, R. Z., Abbas, A., Raza, M. A., Khan, M. K., Saleemi, M. K., & Saeed, Z. (2019). *In-vitro* anticoccidial activity of *Trachyspermum ammi* (Ajwain) extract on oocysts of *Eimeria* species of chicken. *Advancements in Life Sciences*, *7*(1), 44-47.

Abbas, R. Z., Colwell, D. D., & Gilleard, J. (2012). Botanicals: an alternative approach for the control of avian coccidiosis. *World's Poultry Science Journal*, *68*(2), 203-215.

Abbas, R. Z., Iqbal, Z., Khan, M. N., Zafar, M. A., & Zia, M. A. (2010). Anticoccidial activity of *Curcuma longa* L. in broilers. *Brazilian Archives of Biology and Technology*, *53*(1), 63-67.

Abbas, R., Iqbal, Z., Blake, D., Khan, M., & Saleemi, M. (2011). Anticoccidial drug resistance in fowl coccidia: the state of play revisited. *Worlds Poultry Science Journal, 67*(2), 337-350.

Abbas, R., Iqbal, Z., Mansoor, M., Sindhu, Z., Zia, M. A., & Khan, J. A. (2013). Role of natural antioxidants for the control of coccidiosis in poultry. *Pakistan Veterinary Journal, 33*(4), 401-407.

AbdAlla, A. M., Abd-Alla, O. A. b., Emad, W. G., Walid, A., & Fatma, F. M. 2013. Efficacy of probiotic on intestinal coccidiosis in infected broilers: hematological, biochemical and pathological. *Kafrelsheikh Veterinary Medical Journal, 11*(2), 175-202.

Abdel-Raouf, N., Al-Enazi, N. M., Ibraheem, I. B. M., Alharbi, R. M., & Alkhulaifi, M. M. (2019). Biosynthesis of silver nanoparticles by using of the marine brown alga *Padina pavonia* and their characterization. *Saudi Journal of Biological Sciences, 26*(6), 1207-1215.

Abdisa, T., Hasen, R., Tagesu, T., Regea, G., & Tadese, G. (2019). Poultry coccidiosis and its prevention, control. *Journal of Veterinary and Animal Research, 2*(1), 103-108.

Abdulhamid, A., Fakai, I. M., & Ogwihi, O. J. (2017). Phytochemical screening, chromatographic studies and antibacterial activity of *Carica papaya* leaves extracts. *International Journal of Medicinal Plant Natural Production, 3*(1), 11-15.

Abdulhamid, A., Onyenonachi, I. C., & Ismail, A. M. (2017). Phytochemical screening, chromatographic studies and antibacterial activity of *Eclipta alba* L. leaves extracts. *Indo Global Journal of Pharmaceutical Sciences, 7*(2), 143-147.

Abdullah, L. N., & Al-Barwary, L. T. O. (2020). Effect of *Artemisia splendens* powder and extract on broiler chicken's performance, lymphoid organ weight, gut morphology and serum biochemicals during coccidiosis challenge. *The Iraqi Journal of Agricultural Science, 51*(2), 611-618.

Abebe, E., & Gugsa, G. (2018). A review on poultry coccidiosis. *Abyssinia Journal of Science and Technology, 3*(1), 1-12.

Abudabos, A. M., Alyemni, A. H., Hussein, E. O., & Al-Ghadi, M. A. Q. (2018). Anticoccidial effect of some natural products in experimentally induced *Eimeria* spp. infection on carcass quality, intestinal lesion and ileal histology in broilers. *Journal of Animal and Plant Science*, *28*(1), 73-79.

Acharya, K. P., & Acharya, N. (2017). Alternatives to fight against coccidiosis: A review. *Nepalese Veterinary Journal*, *34*, 152-167.

Adamu, M., Boonkaewwan, C., Gongruttananun, N., & Vongpakorn, M. (2013). Hematological, biochemical and histopathological changes caused by coccidiosis in chickens. *Agriculture and Natural Resources*, *47*(2), 238-246.

Adediran, O. A., Uwalaka, E. C., & Kolapo, T. U. (2014). Antihelminthic and anticoccidial effects of *Vernonia amygdalina* in goats. *Journal of Advanced Veterinary Research, 4*(7), 610-615.

Adegoke, A. S., Jerry, O. V., & Ademola, O. G. (2019). GC-MS analysis of phytochemical constituents in methanol extract of wood bark from *Durio zibethinus* Murr. *International Journal of Medicinal Plants and Natural Production*, *5*(3), 1-11.

Adhikari, A., Gupta, R., & Pant, G. R. (2008). Prevalence and identification of coccidian parasite (*Eimeria* species) in layer chicken of Ratnanagar municipality, Chitwan district. *Nepal Journal of National History, 23,* 45-50.

Affian, E. A. S., Clarisse, K. O., Witabouna, K. M. A., & Elie, E. M. (2017). Anti-coccidian effect of almonds of *Azadirachta indica* (Meliaceae) against *Eimeria* spp. of the dwarf goat. *International Journal of Agronomy and Agricultural Research, 10*(1), 52-59.

Agelet, A., & Valles, J. (2011). Studies on pharmaceutical ethnobotany in the region of Pallars (Pyrenees, Catalonia, Iberian Peninsula). Part I. General results and new or very rare medicinal plants. *Journal of Ethnopharmacology, 1-77*(1), 57-70.

Ahad, S., Tanveer, S., Malik, T. A., & Nawchoo, I. A. (2018). Anticoccidial activity of fruit peel of *Punica granatum* L. *Microbial pathogenesis, 116,* 78-83.

Ahad, S., Tanveer, S., Nawchoo, I. A., & Malik, T. A. (2017). Anticoccidial activity of *Artemisia vestita* (Anthemideae, Asteraceae)-a traditional herb growing in the Western Himalayas, Kashmir, India. *Microbial pathogenesis*, *104*, 289-295.

Ahaotu, E. O., De los Ríos, P., Ibe, L. C., & Singh, R. R. (2019). Climate change in poultry production system—A review. *Acta Scientific Agriculture*, *3*(9), 113-117.

Ahmadov, E. I., Sha, T., Hasanova, J. V., & Namazova, A. A. (2016). Effects of herbal plants on ducks and quail infected with *Eimeria* species. *Journal of Entomology and Zoology Studies*, *4*, 1150-1152.

Ahmadov, E.I., Hasanova, J.V., Mammadova, F.Z., & Topchiyeva Sh, A. (2019). Effects of the *Artemisia absinthium L*. extracts on *Eimeria tenella* oocysts *in-vitro*. *Acta Scientific Microbiology*, *2*(9), 135-140.

Ahmed, D., Chaudhary, M. A., Raza, A., Waheed, A., Khan, S. R., & Ikram, M. (2012). Comparative study of antibacterial activity and mineral contents of various parts of *Verbena officinalis* Linn. *Asian Journal of Chemistry*, *24*(1), 68.

Akerreta, S., Cavero, R. Y., & Calvo, M. I. (2007). First comprehensive contribution to medical ethnobotany of Western Pyrenees. *Journal of Ethnobiology and Ethnomedicine*, *3*(1), 1-3.

Akhtar, M., Tariq, A. F., Awais, M. M., Iqbal, Z., Muhammad, F., Shahid, M., & Hiszczynska, S. E. (2012). Studies on wheat bran Arabinoxylan for its immunostimulatory and protective effects against avian coccidiosis. *Carbohydrate Polymers*, *1*, 90(1), 333-339.

Akhter, M. J., Aziz, F. B., Hasan, M. M., Islam, R., Parvez, M. M. M., Sarkar, S., & Meher, M. M. (2021). Comparative effect of papaya (*Carica papaya*) leaves extract and Toltrazuril on growth performance, hematological parameter, and protozoal load in Sonali chickens infected by mixed *Eimeria* spp. *Journal of Advanced Veterinary and Animal Research*, *8*(1), 91-100.

Akintunde, O. K., & Adeoti, A.I. (2014). Assessment of factors affecting the level of poultry disease management in Southwest, Nigeria. *Trends in Agricultural economics*, *7*, 41-56.

Akram, M. Z., Zaman, M. A., Jalal, H., Yousaf, S., Khan, A. Y., Farooq, M. Z., Rehaman, T., Sakandar, A., Qamar, M. F., & Bowman. D. D. (2018). Prevalence of Gastrointestinal Parasites of Captive Birds in Punjab, Pakistan. *Pakistan Veterinary Journal*, *39*(1), 132-134.

Alam, M. Z., Dey, A. R., Parvin, S., Akter, S., & Rony, S. A. (2021). ITS1-PCR based identification of chicken *Eimeria* species in poultry litter from Mymensingh district, Bangladesh. *Journal of Advanced Veterinary and Animal Research*, *8*(3), 489-493.

Ali, H., Naqvi, F., & Tariq, N. (2014). Prevalence of coccidiosis and its association with risk factors in poultry of Quetta, Pakistan. *Asian Journal of Applied Sciences*, *2*(4), 554-558.

Ali, M., Chand, N., Khan, R. U., Naz, S., & Gul, S. (2019). Anticoccidial effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) against experimentally induced coccidiosis in broiler chickens. *Journal of Applied Animal Research*, *47*(1), 79-84.

Ali, S., & Abdelhalim, A. (2002). Removal of amprolium from water by roots and seeds ash of *Salvadora persica*. *Journal of Taibah University for Science*, *1*, 14(1), 1604-1612.

Aljedaie, M. M., & Al-Malki, E. S. (2020). Anticoccidial activities of *Salvadora persica* (arak), *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) extracts on the control of chicken coccidiosis. *Journal of King Saud University - Science*, *32*, 2810–2817.

Aljedaie, M. M., Alam, P., & Al-Malki, E. S. (2021). Prediction of novel natural inhibitors of avian coccidia (*Eimeria tenella*) through molecular docking. *Tropical Journal of Pharmaceutical Research*, *20*(7), 1441-1445.

Allen, P. C., & Fetterer, R. (2002). Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clinical Microbiology Reviews*, *15*(1), 58-65.

Allen, P. C., Danforth, H. D., & Augustine, P. C. (1998). Dietary modulation of avian coccidiosis. *International Journal for Parasitology*, *28*(7), 1131-1140.

Almeida, G. F., Thamsborg, S. M., Madeira, A. M., Ferreira, J. F., Magalhaes, P. M., Dematte Filho, L. C., & Hermansen, J. E. (2014). The effects of combining *Artemisia annua* and *Curcuma longa* ethanolic extracts in broilers challenged with infective oocysts of *Eimeria acervulina* and *E. maxima*. *Parasitology*, *141*(3), 347-355.

Al-Mathal, E. M. (2010). Efficacy of *Commiphora molmol* against hepatic coccidiosis (*Eimeria stiedae*) in the domestic rabbit. *Journal of Food, Agriculture and Environment*, *8*(3/4), 1072-1080.

Alnassan, A. A., Thabet, A., Daugschies, A., & Bangoura, B. (2015). *In-vitro* efficacy of allicin on chicken *Eimeria tenella* sporozoites. *Parasitology research*, *114*(10), 3913-3915.

Al-Shaibani, I., Al-Khadher, A., & AlHibah, A. (2020). Anticoccidial activity of *Allium sativum* and *Punica granatum* against experimentally induced *Eimeria tenella* infection in broiler chickens. *Asian Journal of Research in Animal and Veterinary Sciences*, *5*(4), 20-29.

Amin, Y., Aslam, A., Anwar, K., Pervez, & Ali, Z. (2014). Seasonal prevalence of eimeriosis in broiler chicken. *Advancements in Life Sciences*, *1*(3), 160-164.

Amudha, P., Jayalakshmi, M., Pushpabharathi, N., & Vanitha, V. (2018). Identification of bioactive components in *Enhalus acoroides* seagrass extract by gas chromatography-mass spectrometry. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(10), 313-315.

Anosa, G. N., & Okoro, O. J. (2011). Anticoccidial activity of the methanolic extract of *Musa paradisiaca* root in chickens. *Tropical Animal Health and Production*, *43*(1), 245-248.

Ansir, R., Shah, S. M., Ullah, N., & Hussain, M. N. (2020). Performance of pyrocatechol violet and carminic acid sensitized ZnO/CdS nanostructured photoactive materials for dye sensitized solar cell. *Solid-State Electronics*, *172*, 107886.

Aouadi, M., Sebai, E., Saratsis, A., Kantzoura, V., Saratsi, K., Msaada, K., Sotiraki, S., & Akkari, H. (2021). Essential oil of *Rosmarinus officinalis* induces *in-vitro* anthelmintic and anticoccidial effects against *Haemonchus contortus* and *Eimeria* spp. in small ruminants. *Veterinarny Medicina*, *66*(4), 146-155.

Arab, H. A., Rahbari, S., Rassouli, A., Moslemi, M. H., & Khosravirad, F. (2006). Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens. *Tropical Animal Health and Production*, *38*(6), 497-503.

Arczewska-Włosek, A., Swiątkiewicz, S., Ognik, K., & Jozefiak, D. (2022). Effects of a dietary multi-strain probiotic and vaccination with a live anticoccidial vaccine on growth performance and haematological, biochemical and redox status indicators of broiler chickens. *Animals*, *12*(24), 3489.

Arlette, N. T., Nadia, N. A. C., Jeanette, Y., Gertrude, M.T., Josue, W. P., & Mbida, M. (2019). Anticoccidial effects of *Ageratum conyzoides* (Asteraceae) and *Vernonia amygdalina* (Asteraceae) leaves extracts on broiler chickens. *South Asian Journal of Parasitology*, *2(1), 1-10.*

Ashraf, A., Qureshi, N. A., Shaheen, N., Iqbal, A., Fatima, H., Afzal, M., Alhewairini, S. S., & Qureshi, M. Z. (2020). Termiticidal and protozocidal potentials of eight tropical plant extracts evaluated against *Odontotermes obesus* Rambur (Blattodea; Termitidae) and *Heterotermes indicola* Wasmann (Blattodea; Rhinotermitidae). *Polish Journal of Environmental Studies*, *29*(5), 3493-3507.

Attia, Y. A., Rahman, M. T., Hossain, M. J., Basiouni, S., Khafaga, A. F., Shehata, A. A., & Hafez, H. M. (2022). Poultry production and sustainability in developing countries under the COVID-19 crisis: Lessons learned. *Animals*, *12*(5), 644-655.

Attree, E., Sanchez-Arsuaga, G., Jones, M., Xia, D., Marugan-Hernandez, V., Blake, D., & Tomley, F. (2021). Controlling the causative agents of coccidiosis in domestic chickens; an eye on the past and considerations for the future. *CABI Agriculture and Bioscience*, *2*(1), 1-16.

Auta, I. K., Samaila, S. J., & Solomon, B. (2021). Prevalence Of Coccidiosis Among Poultry Birds Slaughtered At Selected Markets In Kaduna Metropolis, Kaduna. *International Research Journal of Applied Sciences, Engineering and Technology*, *7*(8), 1-9.

Awais, M. M., Akhtar, M., Iqbal, Z., Muhammad, F., & Anwar, M. I. (2012). Seasonal prevalence of coccidiosis in industrial broiler chickens in Faisalabad, Punjab, Pakistan. *Tropical Animal Health Production*, *44*, 323-328.

Ayana, D., Mohammed, A., Kelemwork, E., Ashenafi, H., & Waktole, H. (2017). Study on poultry coccidiosis in different production systems in and around Bishoftu, Oromia region, Ethiopia. *Advances in Biological Research*, *1*(4), 190-197.

Ayaz, M. M., Akhter, M., Hayat, C. S., Hafeez, M.A., & Haq, A. (2003). Prevalence of coccsidiosis in broiler chickens in Faisalabad, Pakistan. *Pakistan Veterinary Journal*, 23(1), 29-37.

Babitha, S., David, B., & Otilia, J. F. (2012). Investigation on antioxidant and hepatoprotective activity of ethanolic leaf extract of *Polygonum glabrum* Wild on carbon tetrachloride-induced hepatotoxicity in rats. *Spatula DD*, *2*(4), 199-205.

Bachaya, H. A., Raza, M. A., Khan, M. N., Iqbal, Z., Abbas, R. Z., Murtaza, S., & Badar, N. (2012). Predominance and detection of different *Eimeria* species causing coccidiosis in layer chickens. *The Journal of Animal & Plant Sciences*, *22*(3), 597-600.

Bachaya, H. A., Abbas, R. Z., Raza, M. A., Iqbal, Z., Rehman, T. U., Baber, W., & Hussain, R. (2015). Existence of coccidiosis and associated risk factors in broiler chickens in Southern Punjab, Pakistan. *Pakistan Veterinary Journal*, *35*(1), 81-84.

Balamurugan, M., Saravanan, S., & Soga, T. (2014). Synthesis of iron oxide nanoparticles by using *Eucalyptus globulus* plant extract. *e-Journal of Surface Science and Nanotechnology*, *12*, 363-367.

Balicka-Ramisz, A., Laurans, L., Batko, M., & Ramisz, A. (2021). The influence of coccidiostatic Baycox on the course of coccidiosis in broiler chicken. *Russian parasitological journal*, *15*(2), 95-100.

Banakar, P., & Jayaraj, M. (2018). GC-MS analysis of bioactive compounds from ethanolic leaf extract of *Waltheria indica* Linn. and their pharmacological activities. *International Journal of Pharmaceutical Science Research*, *9*(5), 2005-10.

Banjoko, O. J., Adebayo, I. A., Osho, I. B., Olumide, M. D., Fagbiye, O. O. A., Ajayi, O. A., & Akinboye, O. E. (2020). Evaluation of varying levels of *Carica papaya* leaf meal on growth, carcass, hematological parameters and its use as anticoccidial for broiler chicken. *Nigerian Journal of Animal Science*, *22*(3), 229-241.

Banjoko, O. J., IA, A., Osho, I. B., & Olumide, M. D. (2019). Evaluation of varying levels of *Vernonia amygdalina* leaf meal on growth, hematological parameters and as anticoccidial. *International Journal of Livestock Production*, *10*(8), 192-197.

Bawm, S., Win, S. Y., Soe, N. C., Thaw, Y. N., Hmoon, M. M., Htun, L. L., Nakao, R., & Katakura, K. (2021). Microscopic and molecular detection of *Eimeria maxima* and *Eimeria praecox* naturally infected in free-range village chickens of Myanmar. *Acta Parasitologica*, *66*, 1074-1078.

Begum, B., Tajbar, S., Khan, B., & Rafiq, L. (2021). Identification of relationships between climate indices and precipitation fluctuation in Peshawar City-Pakistan. *Journal of Research in Environmental Earth Sciences*, *10*, 264-278.

Behera, S. S., Patra, J. K., Pramanik, K., Panda, N., & Thatoi, H. (2012). Characterization and evaluation of antibacterial activities of chemically synthesized iron oxide nanoparticles. *World Journal of Nano Science and Engineering*, *2*, 196-200.

Beretta, G., & Facino, R. M. (2010). Recent advances in the assessment of the antioxidant capacity of pharmaceutical drugs: from *in-vitro* to *in-vivo* evidence. *Analytical and Bioanalytical Chemistry*, *398*(1), 67-75.

Bhati, D., & Jain, S. (2016). Micro nutrient composition of unconventional wild fruits and vegetables. *The Bioscan. Save Nature To Survive*, *11*(4), 2405-2409.

Bindari, Y. R., Kheravii, S. K., Morton, C. L., Wu, S. B., Walkden-Brown, S. W., & Gerber, P. F. (2021). Molecular detection of *Eimeria* species and *Clostridium perfringens* in poultry dust and pooled excreta of commercial broiler chicken flocks differing in productive performance. *Veterinary Parasitology*, *291*, 109361.

Biswas, N. N., Saha, S., & Ali, M. K. (2014). Antioxidant, antimicrobial, cytotoxic and analgesic activities of ethanolic extract of *Mentha arvensis* L. *Asian Pacific Journal Tropical Biomedicine*, *4*(10), 792-797.

Biu, A. A., Yusuf, S. D., & Rabo, J. S. (2021). Use of neem (*Azadirachta indica*) aqueous extract as a treatment for poultry coccidiosis in Borno State, Nigeria. *African Scientist*, *7*(3), 147-153.

Blake, D. P. (2015). *Eimeria* genomics: Where are we now and where are we going? *Veterinary Parasitology*, *212*(1-2), 68-74.

Blake, D. P., & Tomley, F. M. (2014). Securing poultry production from the ever-present *Eimeria* challenge. *Trends in Parasitology*, *30*(1):12–9.

Bo, R., Dai, X., Huang, J., Wei, S., Liu, M., & Li, J. (2020). Evaluation of optimum conditions for decoquinate nanoliposomes and their anticoccidial efficacy against diclazuril-resistant *Eimeria tenella* infections in broilers. *Veterinary Parasitology*, *283*, 109186.

Boudriche, L., Safaei, Z., Ramasamy, D., Sillanpaa, M., & Boudjemaa, A. (2019). Sulfaquinoxaline oxidation by UV-C activated sodium persulfate: Degradation kinetics and toxicological evaluation. *Water Environment Research*, *91*(11), 1412-1419.

Bozkurt, M., Aysul, N., Kucukyilmaz, K., Aypak, S., Ege, G., Catli, A. U., Aksit, H., Coven, F., Seyrek, K., & Cinar, M. (2014). Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers. *Poultry Science*, *93*(2), 389-399.

Bozkurt, M., Giannenas, I., Kucukyilmaz, K., Christaki, E., & Florou-Paneri, P. (2013). An update on approaches to controlling coccidia in poultry using botanical extracts. *British Poultry Science*, *54*(6), 713-727.

Buragohain, B., Phukan, A., Dutta, T. C., Barua, C. C., & Phukan, S. C. (2013). Prevalence of coccidiosis in kids in and around Guwahati. *Journal of Veterinary Parasitology*, *27*(1), 25-28.

Burrell, A., Tomley, F. M., Vaughan, S., & Marugan-Hernandez, V. (2020). Life cycle stages, specific organelles and invasion mechanisms of *Eimeria* species. *Parasitology*, *147*(3), 263-278.

Bussiere, F. I., Niepceron, A., Sausset, A., Esnault, E., Silvestre, A., Walker, R.A., Smith, N.C., Quere, P. and Laurent, F., (2018). Establishment of an *in-vitro* chicken epithelial cell line model to investigate *Eimeria tenella* gamete development. *Parasites & Vectors*, *11*, 1-8.

Cakic, M., Glisic, S., Cvetkovic, D., Cvetinov, M., Stanojevic, L., Danilovic, B. & Cakic, K. (2018). Green synthesis, characterization and antimicrobial activity of silver nanoparticles produced from *Fumaria officinalis* L. Plant Extract. *Colloid Journal, 80*(6), 803-813.

Calvo, M. I. (2006). Anti-inflammatory and analgesic activity of the topical preparation of *Verbena officinalis* L. *Journal of Ethnopharmacology*, *107*, 380-382.

Campbell, T., & Ellis, C. K. (2008). (Third edition). Avian and exotic animal hematology and cytology. Black well publishing. *Journal of Exotic Pet Medicine*, *17*(1), 60–61.

Carrisosa, M., Jin, S., McCrea, B. A., Macklin, K. S., Dormitorio, T., & Hauck, R. (2021). Prevalence of select intestinal parasites in Alabama backyard poultry flocks. *Animals*, *11*(4), 939.

Casanova, E., Garcia, J. M. M., & Calvo, M. I. (2008). Antioxidant and antifungal activity of *Verbena officinalis* L. leaves. *Plant Foods for Human Nutrition*, *63*, 93–97.

Castaneda, R. E. Q., & Gonzalez, E. D. (2015). Control of avian coccidiosis: Future and present natural alternatives. *BioMed Research International*, *2015*, 2-11.

Cedric, Y., Payne, V.K., Nadia, N.A.C., Kodjio, N., Kollins, E., Megwi, L., & Kuiate, J.R. (2018). *In-vitro* antioxidant and anticoccidial studies of *Pentaclethra macrophylla* extracts against *Eimeria magna, Eimeria flavescens, Eimeria intestinalis, Eimeria stiedae* oocysts and sporozoites of rabbits. *Journal of Advances in Parasitology*, *5*(3), 38-48.

Cervantes, H. M., McDougald, L. R., & Jenkins, M. C. (2020). Coccidiosis. *Diseases of poultry*, *2*, 1193-217.

Chadwick, E., Rahimi, S., Grimes, J., Pitts, J., & Beckstead, R. (2020). Sodium bisulfate feed additive aids broilers in growth and intestinal health during a coccidiosis challenge. *Poultry Science*, *99*(11), 5324-5330.

Chan, C. H., Yusoff, R., Ngoh, G. C., & Kung, F. W. L. (2011). Microwave-assisted extractions of active ingredients from plants. *Journal of Chromatography A*, *1218*(37), 6213-6225.

Chand, N., Faheem, H., Khan, R. U., Qureshi, M. S., Alhidary, I. A., & Abudabos, A. M. (2016). Anticoccidial effect of mananoligosacharide against experimentally induced coccidiosis in broiler. *Environmental Science and Pollution Research*, *23*(14), 14414-14421.

Chang, C. L. T., Yang, C. Y., Muthamilselvan, T., & Yang, W. C. (2016). Field trial of medicinal plant, *Bidens pilosa*, against eimeriosis in broilers. *Scientific Reports*, *6*(1), 1-7.

Chapman H. D., Jeffers, T. K., & Williams R. B. (2010). Forty years of monensin for the control of coccidiosis in poultry. *Poultry Science*, *89*, 1788–1801.

Chapman, H. D. (2000). Practical use of vaccines for the control of coccidiosis in the chicken. *World's Poultry Science Journal*, *56*(1), 7-20.

Chapman, H. D. (2014). Milestones in avian coccidiosis research: a review. *Poultry science*, *93*(3), 501-511.

Chapman, H. D., Cherry, T. E., Danforth, H. D., Richards, G., Shirley, M. W., & Williams, R. B. (2002). Sustainable coccidiosis control in poultry production: the role of live vaccines. *International Journal for Parasitology*, *32*(5), 617-629.

Chauke, N., & Siebrits, F. K. (2012). Evaluation of silver nanoparticles as a possible coccidiostat in broiler production. *South African Journal of Animal Science*, *42*(5), 493-497.

Chen, W., Ma, C., Li, G., Jia, Z., Yang, X., Pan, X., & Ma, D. (2021). Specific EtMIC3-binding peptides inhibit *Eimeria tenella* sporozoites entry into host cells. *Veterinary research*, *52*(1), 1-11.

Chhabra, M. B., Pathak, K. M. L., & Muraleedharan, K. (2014). Medicinal plants as an alternative for control of livestock parasites. 2. Protozoa. *Indian Journal of Animal Sciences*, *84*, 599-608.

Christaki, E., Florou-Paneri, P., Giannenas, I., Papazahariadou, M., Botsoglou, N. A., & Spais, A. B. (2004). Effect of a mixture of herbal extracts on broiler chickens infected with *Eimeria tenella*. *Animal Research*, *53*(2), 137-144.

Chookyinox, L. U., Stella, U., & Sandy, O. (2009). Coccidiosis. Backyard poultry information centre. Php BB 2004-2009/ backyard.

Clark, E. L., Tomley, F. M., & Blake, D. P. (2017). Are *Eimeria* genetically diverse, and does it matter?. *Trends in Parasitology*, *33*(3), 231-241.

Conway, D. P., & McKenzie, M. E. (2007). Poultry coccidiosis: diagnostic and testing procedures. John Wiley & Sons, Blackwell publishing, Ames, Iowa, 3rd edition, 10–76.

Coutinho, H. D. M., Costa, J. G. M., Lima, E. O., Falcao, V. S., Silva., & Siqueira J. P. (2009). Potentiating effect of *Mentha arvensis* and chlorpromazine in the resistance to aminoglycosides of aethicillin – resistant *Staphylococcus aureus*. *In-Vivo*, *23*, 287-290.

Cowieson, A. J., Livingston, M. L., Nogal, B., Hoang, V., Crespo, R., & Livingston, K. A. (2020). Effect of coccidial challenge and vaccination on the performance, veterinary

postmortem scores, and blood biochemistry of broiler chickens. *Poultry Science*, *99*(8), 3831-3840.

Crouch, E. E., Reinoso-Perez, M. T., Vanderstichel, R. V., Dhondt, K. V., Dhondt, A. A., Cruz Otero, J. D., Piech, t., & Forzan, M. J. (2022). The effect of dexamethasone on hematologic profiles, hemosporidian infection, and splenic histology in house finches (*Haemorhous mexicanus*). *The Journal of Wildlife Diseases*, *58*(3), 512-523.

Dakpogan, H. B., Mensah, S., Attindehou, S., Chysostome, C., Aboh, A., Naciri, M., Salifou, S., & Mensah, G. A. (2018). Anticoccidial activity of *Carica papaya* and *Vernonia amygdalina* extract. *International Journal of Biological and Chemical Sciences*, *12*(5), 2101-2108.

Dalloul, R. A., & Lillehoj, H. S. (2006). Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Review of Vaccines*, *5*(1), 143-163.

Das, M. (2021). Diversity of *Eimeria* species in backyard poultry of subtropical hilly region of Meghalaya, India. *Journal of Entomology and Zoology Studies*, *9*(2), 360-365.

Datta, A., Patra, C., Bharadwaj, H., Kaur, S., Dimri, N. & Khajuria, R. (2017). Green synthesis of zinc oxide nanoparticles using *Parthenium hysterophorus* leaf extract and evaluation of their antibacterial properties. *Journal of Biotechnology and Biomaterial*, *7*(3), 271-276.

David, M., Ain, Q. U., Jahan, S., Ahmad, M., Shah, Q., Afsar, T., Shafique, H., Alanezi, T.N., Almajwal, A., Ijaz, M.U. & Razak, S. (2022). Determination of possible contraceptive potential of methanolic leaf extract of *Mentha longifolia* L. in adult male rats: a biochemical and histological study. *Toxicology Research*, *11*(6), 951-961.

De Almeida, L. F. R., Frei, F., Mancini, E., De Martino, L., & De Feo, V. (2010). Phytotoxic activities of Mediterranean essential oils. *Molecules*, *15*(6), 4309-4323.

De Andrade, J. P., Pigni, N. B., Torras-Claveria, L., Berkov, S., Codina, C., Viladomat, F. & Bastida, J. (2012). Bioactive alkaloid extracts from Narcissus broussonetii: Mass spectral studies. *Journal of pharmaceutical Biomedicine. Anal*, *70*, 13-25.

De Assis, D. C. S., da Silva, G. R., Lanza, I. P., Ribeiro, A. Cd. S. R., Lana, A. M. Q., Lara, L. J. C., de Figueiredo, T. C., & Cancado, S. D. V. (2016). Evaluation of the presence and levels of enrofloxacin, ciprofloxacin, sulfaquinoxaline and oxytetracycline in broiler chickens after drug administration. *PLoS ONE*, *11*(11), e0166402.

Debata, B., Patnaik, P., & Mishra, A. (2020). COVID-19 pandemic! It's impact on people, economy, and environment. *Journal of Public Affairs*, *20*(4), e2372.

Deepak, M., & Handa, S. S. (2000). Antiinflammatory activity and chemical composition of extracts of *Verbena officinalis*. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *14*(6), 463-465.

Demirezen, D. A., Yıldız, Y. S., Yılmaz, S., & Yılmaz, D. D. (2019). Green synthesis and characterization of iron oxide nanoparticles using *Ficus carica* (Common Fig) dried fruit extract. *Journal of Bioscience and Bioengineering*, *127*(2), 241-245.

Desalegn, A. Y., & Ahmed, M. R. (2020). Anticoccidial activity of *Aloe debrana* and *Aloe pulcherrima* leaf gel against Eimeria oocysts. *Journal of Parasitology Research*, *8524973*, 1-10.

Devatha, C. P., Thalla, A. K., & Katte, S. Y. (2016). Green synthesis of iron nanoparticles using different leaf extracts for treatment of domestic waste water. *Journal of Cleaner Production*, *139*, 1425-1435.

Devi, T. S., Vijay, K., Vidhyavathi, R. M., Kumar, P., Govarthanan, M., & Kavitha, T. (2021). Antifungal activity and molecular docking of phenol, 2, 4-bis (1, 1-dimethylethyl) produced by plant growth-promoting actinobacterium *Kutzneria sp.* strain TSII from mangrove sediments. *Archives of Microbiology*, *203*, 4051-4064.

Dkhil, M. A., Abdel-Baki, A. S., Wunderlich, F., Sies, H., & Al-Quraishy, S. (2011). Anticoccidial and antiinflammatory activity of garlic in murine *Eimeria papillata* infections. *Veterinary Parasitology*, *175*(1-2), 66-72.

Dowling, L. (1992). Ionophore toxicity in chickens: a review of pathology and diagnosis. *Avian Pathology*, *21*(3), 355-368.

Dragan, L., Gyorke, A., Ferreira, J. F., Pop, I. A., Dunca, I., Dragan, M., Mircean, V., Dan, I. & Cozma, V. (2014). Effects of *Artemisia annua* and *Foeniculum vulgare* on chickens highly infected with *Eimeria tenella* (Phylum Apicomplexa). *Acta Veterinaria Scandinavica*, *56*(1), 1-7.

Drouin, P., & Toux, J. Y. (2000). Decontamination of free-range poultry housing. *Sciences & Techniques Avicoles*, 39-49.

Du, A., & Hu, S. (2004). Effects of a herbal complex against *Eimeria tenella* infection in chickens. *Journal of Veterinary Medicine, Series B*, *51*(4), 194-197.

Dubey, J. P. (2019). Coccidiosis in Livestock, Poultry, Companion Animals, and Humans. (Boca Raton, Florida: CRC Press).

Dubey, R., Harrison, B., Dangoudoubiyam, S., Bandini, G., Cheng, K., Kosber, A., Agop-Nersesian, C., Howe, D.K., Samuelson, J., Ferguson, D.J., & Gubbels, M. J. (2017). Differential roles for inner membrane complex proteins across *Toxoplasma gondii* and *Sarcocystis neurona* development. *Msphere*, *2*(5), e00409-17.

Dubremetz, J. F., Reguet, N. G., Conseil, V., & Fourmaux, M. N. (1998). Invited review apical organelles and host-cell invasion by apicomplexa. *International Journal for Parasitology*, *28*(7), 1007-1013.

Eassa, A., El Ayis, A. A., & Muna, A. K. M. (2019). Isolation and characterization of *Eimeria* species in poultry using conventional methods and coccimorph program in khartoum state. 2019; 8(2): 54-67.

Egner, U., Kratzschmar, J., Kreft, B., Pohlenz, H. D., & Schneider, M. (2005). The target discovery process. *Chembiochem*, *6*(3), 468-479.

El Banna, H. A., Atef, M., & Nabil, G. (2016). Anti-coccidial activity of *Moringa oleifera* plant. *Animal Veterinary Sciences*, *4*(2), 19-25.

El-Ghany, W. A. A. (2020). Coccidiosis: a parasitic disease of significant importance in rabbits. *World's Veterinary Journal*, *10*(4), 499-507.

El-Khtam, A. O., Abd El Latif, A., & El-Hewaity, M. H. (2014). Efficacy of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) on *Eimeria* species in broilers. *International Journal of Basic and Applied Sciences*, *3*(3), 349.

El-Maddawy, Z. K., El-Sawy, A. E. S. F., Ashoura, N. R., Aboelenin, S. M., Soliman, M. M., Ellakany, H. F., & El-Shall, N. A. (2022). Use of Zinc Oxide Nanoparticles as Anticoccidial Agents in Broiler Chickens along with Its Impact on Growth Performance, Antioxidant Status and Hematobiochemical Profile. *Life*, *12*(1), 74.

El-Maksoud, A., Afaf, H. A., Abdel-Magid, D., & El-Badry, M. A. (2014). Biochemical effect of coccidia infestation in laying hen. *Benha Veterinary Medicine Journal*, *26*(1), 127-133.

Elmusharaf, M. A., & Beynen, A. C. (2007). Coccidiosis in poultry with emphasis on alternative anticoccidial treatments. *Alternative Anticoccidial Treatment of Broiler Chickens*, 9(1), 9-44.

Elshafie, H. S., Mancini, E., Camele, I., De Martino, L., & De Feo, V. (2015). *In-vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. *Industrial Crops and Products*, *66*, 11-15.

El-Shazly, K. A., El-Latif, A. A., Abdo, W., El-Morsey, A., El-Aziz, M. I. A., & El-Mogazy, H. (2020). The anticoccidial activity of the fluoroquinolone lomefloxacin against experimental *Eimeria tenella* infection in broiler chickens. *Parasitology Research*, *119*, 1955-1968.

Elson, H. A. (2010). Poultry housing and husbandry. *British Poultry Science*, *51*(1), 23-34.

Encalada, M. A., Rehecho, S., Ansorena, D., Astiasar, I., Cavero, R. Y. & Calvo, M.I. (2015). Antiproliferative effect of phenylethanoid glycosides from *Verbena officinalis* L. on colon cancer cell lines. *LWT. Food Science and Technology*, *63*, 1016-1022.

Eze, C. P., Obi, C. F., Idika, I. K., & Nwosu, C. O. (2022). Evaluation of the anti-coccidial effect of aqueous *Sacoglottis gabonensis* (Magnoliophyta, Humiriaceae) stem bark extract in broilers experimentally infected with mixed *Eimeria* species. *Spanish Journal of Agricultural Research*, *20*(1), e0503.

Fadunsin, S. D. O., & Ademola, I. O. (2014). Anticoccidial effects of *Morinda lucida* acetone extracts on broiler chickens naturally infected with *Eimeria* species. *Pharmaceutical Biology*, *52*(3), 330-334.

Fadunsin, S. D. O., Ganiyu, I. A., Rabiu, M., Hussain, K., Sanda, I. M., Musa, S. A., Uwabujo, P.I. & Furo, N. A. (2019). Gastrointestinal parasites of different avian species in Ilorin, North Central Nigeria. *Journal of Advanced Veterinary and Animal Research*, *6*(1), 108.

Fadunsin, S. D. O., Rabiu, M., Hussain, K., Sanda, I. M., Ganiyu, I. A. (2020). Epidemiological studies of *Eimeria* species of cattle in Ilorin, North-Central Nigeria. *Annals of Parasitology*, *66*(3), 373–384.

Fadunsin, S. D. O., Uwabujo, P. I., Sanda, I. M., Hussain, K., Ganiyu, I. A., Rabiu, M., & Balogun, R. B. (2019). Cross-sectional study of *Eimeria* species of poultry in Kwara State, North-Central Nigeria. *Journal of Parasitic Diseases*, *43*(1), 87-95.

FAO. (2011). World Livestock 2011, Livestock in Food Security. Rome: Food and Agriculture Organization of the United Nations (FAO).

Farrell, D. (2013). The role of poultry in human nutrition. *Poultry Development Review. Rome: Food and Agriculture Organization*, *1*, 2-9.

Fatima, S., Akhtar, M. F., Ashraf, K. M., Sharif, A., Saleem, A., Akhtar, B., Peerzada, S., Shabbir, M., Ali, S., & Ashraf, W. (2019). Antioxidant and alpha amylase inhibitory activities of *Fumaria officinalis* and its antidiabetic potential against alloxan induced diabetes. *Cell Molecular Biology*, *65*(2), 50-57.

Fatoba, A. J., & Adeleke, M. A. (2018). Diagnosis and control of chicken coccidiosis: a recent update. *Journal of Parasitic Diseases*, *42*, 483-493.

Fatoba, A. J., & Adeleke, M. A. (2020). Transgenic *Eimeria* parasite: A potential control strategy for chicken coccidiosis. *Acta Tropica*, *205*, 105417, 1-6.

Fazal, H., Ahmad, N., Ullah, I., Inayat, H., Khan, L. & Abbasi B. H. (2011). Antibacterial Potential in *Parthenium hysterophorus, Steviar rebaudiana* and *Ginkgob biloba. Pakistan Journal of Botany*, *43*(2), 1307-1313.

Fonseca, A. P., Alcala-Canto, Y., Salem, A. Z., & Alberti-Navarro, A. B. (2016). Anticoccidial efficacy of naringenin and a grapefruit peel extract in growing lambs naturally-infected with *Eimeria* spp. *Veterinary Parasitology*, *232*, 58-65.

Fontana, I., Tullo, E., Butterworth, A., & Guarino, M. (2015). An innovative approach to predict the growth in intensive poultry farming. *Computers and Electronics in Agriculture*, *119*, 178-183.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, *66*(5), 365-378.

Gadelhaq, S. M., Arafa, W. M., & Abolhadid, S. M. (2018). *In-vitro* activity of natural and chemical products on sporulation of *Eimeria* species oocysts of chickens. *Veterinary Parasitology*, *251*, 12-16.

Ganjewala, D., Gupta, A. K., & Muhury, R. (2012). An update on bioactive potential of a monoterpene aldehyde citral. *Journal of Biologically Active Products from Nature*, *2*(4), 186-199.

Gazoni, F. L., Adorno, F. C., Matte, F., Alves, A. J., Campagnoni, I. D. P., Urbano, T., Zampar, A., Boiago, M. M., & da Silva, A. S. (2020). Correlation between intestinal health and coccidiosis prevalence in broilers in Brazilian agroindustries. *Parasitology International*, *76*, 102027.

Geng, T., Lei, Z., Ye, C., Shen, B., Fang, R., Hu, M., & Zhao, J. (2020). Prevalence of Eimeria parasites and sulfachloropyrazine sodium resistance in chicken farms in the Hubei and Henan provinces. *Research Square*, *1*, 1-18.

Genova, B. M. D., & Knoll, L. J. (2021). Comparisons of the sexual cycles for the coccidian parasites *Eimeria*. *Molecular Basis of Stage Conversion in Apicomplexan Parasites*, *10*, 1-54.

Gerhold, R. W. 2015. Overview of coccidiosis in poultry. Merck Manual—Veterinary Manual. 10th ed. Merck & Co., Inc., Kenilworth, NJ, USA. Accessed Oct. 2018.

Gharachorloo, M., & Amouheidari, M. (2016). Chemical composition, antibacterial and antioxidant activities of the essential oil isolated from *Verbena officinalis*. *Journal of Food Biosciences and Technology*, *6*(1), 33-40.

Girard, Y. A., Johnson, C. K., Fritz, H. M., Shapiro, K., Packham, A. E., Melli, A. C., Carlson-Bremer, D., Gulland, F. M., Rejmanek, D. & Conrad, P. A. (2016). Detection and characterization of diverse coccidian protozoa shed by California sea lions. *International Journal for Parasitology: Parasites and Wildlife*, *5*(1), 5-16.

Gogoi, C., Sarma, J., Barua, C. C., Tamuly, S., Upadhyaya, T. N., Islam, S., Sonowal, J., Borthakur, U., Banerjee, D. K., & Barkathullah, N. (2019). Evaluation of nano-curcumin on experimentally induced coccidiosis in broiler chicks. *International Journal of Conservation Science*, *7*(3), 4514-4520.

Gottimukkala, K. S. V., Harika, R. P., & Zamare, D. (2017). Green synthesis of iron nanoparticles using green tea leaves extract. *Journal of Nanomedicine & Biotherapeutic Discovery*, *7*, 151-155

Graat, E. A. M., Van der Kooij, E., Frankena, K., Henken, A. M., Smeets, J. F. M., & Hekerman, M. T. J. (1998). Quantifying risk factors of coccidiosis in broilers using on-farm data based on a veterinary practice. *Preventive Veterinary Medicine*, *33*(1-4), 297-308.

Grube, M., Muter, O., Strikauska, S., Gavare, M. & Limane, B. (2008). Application of FT-IR spectroscopy for control of the medium composition during the biodegradation of nitro aromatic compounds. *Journal of Industrial Microbiology and Biotechnology*, *35*(11), 1545-1549.

Guarrera, P. M., Forti, G., & Marignoli, S. (2005). Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy). *Journal of Ethnopharmacology. 15, 96*(3), 429-44.

Gueye, F. (2005), Gender aspects in family poultry management systems in developing countries. In: 22nd World's Poultry Congress, 8-13 Jan 2004, Istanbul (Turkey). *World's Poultry Science Journal, 61*, 39-46.

Gunasekaran, S. (2003). UV-VIS spectroscopic analysis of blood serum. *Asian Journal of Microbiology Biotechnology and Environmental Sciences, 5*(4), 581-582.

Gupta, P. C, Sharma, N. & Rao, C.V. (2012). A review on ethnobotany, phytochemistry and pharmacology of *Fumaria indica* (Fumitory). *Asian Pacific Journal of Tropical Biomedicine, 2*(8), 665-669.

Gussem, M. D. (2007). Coccidiosis in poultry: review on diagnosis, control, prevention and interaction with overall gut health. In *Proceedings of the 16th European Symposium on Poultry Nutrition, Series B-2610,* 253-261.

Gyorke, A., Pop, L., & Cozma, V. (2013). Prevalence and distribution of *Eimeria* species in broiler chicken farms of different capacities. *Parasite, 20,* (50), 20-8.

Habibi, H., Firouzi, S., Nili, H., Razavi, M., Asadi, S. L., & Daneshi, S. (2016). Anticoccidial effects of herbal extracts on *Eimeria tenella* infection in broiler chickens: *In-vitro* and *in vivo* study. *Journal of Parasitic Diseases, 40*(2), 401-407.

Hadipour, M. M., Olyaie, A., Naderi, M., Azad, F., & Nekouie, O. (2021). Investigation of the occurrence of *Eimeria* species in native chickens of Shiraz, Iran. *Advance Journal of Microbiology Research, 15*(1), 1-23.

Hady, M. M., & Zaki, M. M. (2012). Efficacy of Some Herbal Feed Additives on Performance and Control of Cecal Coccidiosis in Broilers. *APCBEE Procedia, 4*, 163-168.

Hafeez, A., Ullah, Z., Khan, R. U., Ullah, Q., & Naz, S. (2020). Effect of diet supplemented with coconut essential oil on performance and villus histomorphology in broiler exposed to avian coccidiosis. *Tropical Animal Health and Production, 52*, 2499-2504.

Hafez, H. M. (2008). Poultry coccidiosis: Prevention and control approaches. *Archive Fur Geflugelkunde*, *72*(1), 2-7.

Hamid, P. H., Kristianingrum, Y. P., Wardhana, A. H., Prastowo, S., & Silva, L. M. R. D. (2018). Chicken coccidiosis in Central Java, Indonesia: A recent update. *Veterinary medicine international, 2018*, 1-7.

Han, H. Y., Zhu, S. H., Jiang, L. L., Li, Y., Dong, H., Zhao, Q. P., Kong, C. L., & Huang, B. (2013). Molecular characterization and analysis of a novel calcium-dependent protein kinase from *Eimeria tenella*. *Parasitology*, *140*(6), 746-755.

Haq, I. U., Pasha, T. N., & Khalique, A. (2011). Comparative efficacy of herbal and allopathy drugs against coccidiosis in poultry. *Italian Journal of Animal Science*, *10*(1), 3.

Harper, J. F., & Harmon, A. C. (2005). Plants, symbiosis and parasites: a calcium signaling connection. *Nature Reviews Molecular Cell Biology, 6*, 555-566.

Hector, M., Cervantes, L. R. M., & Mark, C. J. (2020) Coccidiosis. In: Diseases of Poultry, 14 edn. John Wiley & Sons Inc., USA, 1193-217.

Hema, R., Kumaravel, S., & Alagusundaram, K. (2011). GC/MS determination of bioactive components of Murraya koenigii. *Journal of American Science, 7*(1), 80-83.

Hemavathy, A., Shanthi, P., Sowndharya, C., Thiripura, S. S., & Priyadharshni, K. (2019). Extraction and isolation of bioactive compounds from a therapeutic medicinal plant-*Wrightia tinctoria* (Roxb.) R. Br, *International Journal of Pharmacognosy Phytochemical Research*, *11*(3), 199-204.

Hernandez, C. S., Gomez del, C., Campo, J. A, Castro. L. T., Mendoza-Martinez, G. D., Gloria-Trujillo, A. (2019). Evaluation of a feed plant additive for coccidiosis control in broilers herbals for coccidiosis control. *Brazilian Journal of Poultry Science*, *21*(1), 1-6.

Hernandez, N. E., Tereschuk, M. L., & Abdala, L. R. (2000). Antimicrobial activity of flavonoids in medicinal plants from Tafı del Valle (Tucuman, Argentina). *Journal of Ethnopharmacology. 1, 73*(1-2), 317-322.

Hernandez, V. M., Jeremiah, G., Martins, K, A., Burrell, A., Vaughan, S., Xia, D., Randle, N., & Tomley, F. (2020). The growth of *Eimeria tenella*: characterization and application of quantitative methods to assess sporozoite invasion and endogenous development in cell culture. *Frontiers in Cellular and Infection Microbiology*, *10*, 579833, 1-13.

Hritcu, L., & Cioanca, O. (2016). Prevalence of use of herbal medicines and complementary and alternative medicine in Europe. *Herbal Medicine in Depression: Traditional Medicine to Innovative Drug Delivery*, *1*, 135-181.

Hrzenjak, N. M., Zadravec, M., Svara, T., & Racnik, J. (2021). Hepatic coccidiosis in two pet rabbits. *Journal of Exotic Pet Medicine*, *36*, 53-56.

Huang, C. B., George, B., & Ebersole, J. L. (2010). Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Archives of Oral Biology*, *55*(8), 555-560.

Huang, Y., Ruan, X., Li, L., & Zeng, M. (2017). Prevalence of *Eimeria* species in domestic chickens in Anhui province, China. *Journal of Parasitic Diseases*, *41*(4), 1014-1019.

Hussain, J., Rabbani, I., Aslam, S., & Ahmad, H. A. (2015). An overview of poultry industry in Pakistan. *World's Poultry Science Journal*, *71*(4), 689-700.

Hussain, K., Alsayeqh, A. F., Abbas, A., Abbas, R. Z., Rehman, A., Waqar, Z. A. I. B., Rehman, T.U., & Mahmood, M. S. (2022). Potential of *Glycyrrhiza glabra* (Licorice) extract an alternative biochemical and therapeutic agent against coccidiosis in broiler chickens. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *28*(5), 585-591.

Idris, O. A., Wintola, O. A., & Afolayan, A. J. (2019). Comparison of the proximate composition, vitamins (ascorbic acid, α-tocopherol and retinol), anti-nutrients (phytate and oxalate) and the GC-MS analysis of the essential oil of the root and leaf of *Rumex crispus* L. *Plants*, *8*(3), 51.

Ikura, M. (1996). Calcium binding and conformational response in EF-hand proteins. *Trends in Biochemical Sciences, 21*(1), 14-17.

Indrasanti, D., Indradji, M., Hastuti, S., Aprilliyani, E., Fatikha, F., & Rosyadi, K. A. (2017). The administration of garlic extract on *Eimeria stiedai* oocysts and the hematological profile of the coccidia infected rabbits. *Media Peternakan*, *40*(3), 158-164.

Iouknane, N. D., Benbarek, H., & Ayad, A. (2018). Prevalence and aetology of coccidiosis in broiler chickens in Bejaia province, Algeria. *Onderstepoort Journal of Veterinary Research*, *85*(1), 1-6.

Iqbal, A., Qureshi, N. A., Alhewairini, S. S., Shaheen, N., Hamid, A., Qureshi, M.Z. (2022). Biocidal action, characterization, and molecular docking of *Mentha piperita* (Lamiaceae) leaves extract against *Culex quinquefasciatus* (Diptera: Culicidae) larvae. *PLoS One. 17*(7), e0270219.

Irvine, R. J. (2006). Parasites and the dynamics of wild mammal populations. *Animal Sciences*, *82*, 775–781.

Ishaq, A. N., Sani, D., Abdullahi, S. A., Jolayemi, K. O., Ebbo, A. A., Jatau, I. D., & Gadzama, I. M. (2022). Evaluation of anticoccidial activity of *Citrus aurantium* L. ethanolic leaf extract against experimental *Eimeria tenella* infection in broiler chickens *(Gallus gallus domesticus*). *Pharmacological Research-Modern Chinese Medicine*, *4*, 2-9.

Ishino, T., Orito, Y., Chinzei, Y., & Yuda, M. (2006). A calcium dependent protein kinase regulates *Plasmodium* ookinete access to the midgut epithelial cell. *Molecular Microbiology, 59*, 1175–1184.

Islam, S. K. M. A., Alauddin, M., Hassan, M. M., Khan, S. A., Alam, M. R., Hossain, M. B., Ahasan, A. S. M. L., Saifuddin, A. K. M., Sultana, S., Tun, H. M., Shaikat, A. H., Debnath, N. C., & Hoque, M. A. (2012). Biochemical analysis on blood and crop contents of household chickens along with their production and health status in Bangladesh. *Pakistan Veterinary Journal*, *32*, 575-578.

Jabrani, S. A., Omidyar, G. R., & Makhmalbaf, R. M. (2019). Predominance of *Eimeria* species in searching local chickens of Shiraz, Iran. *International Journal of Biochemistry and Biotechnology*, *8*(5), 1-4.

Jack, A. A., Adewumi, M. K., Adegbeye, M. J., Ekanem, D. E., Salem, A. Z., & Faniyi, T. O. (2020). Growth-promoting effect of water-washed neem (*Azadirachta indica* A. Juss) fruit inclusion in West African dwarf rams. *Tropical Animal Health and Production*, *52*(6), 3467-3474.

Jain, P. K., Soni, A., Jain, P., & Bhawsar, J. (2016). Phytochemical analysis of *Mentha spicata* plant extract using UV-VIS, FTIR and GC/MS technique. *Journal of Chemical and Pharmaceutical Research*, *8*(2), 1-6.

Jamil, M., Mansoor, M., Khan, M. K., Amanullah, Khan, A., Haq, R., & Anwar, F. (2016). Prevalence of coccidiosis in broiler chickens in District Dera Ismail Khan, Pakistan. *The Journal of Zoology Studies*, *3*(3), 41-45.

Jang, S. I., Jun, M. H., Lillehoj, H. S., Dalloul, R. A., Kong, I. K., Kim, S., & Min, W. (2007). Anticoccidial effect of green tea-based diets against *Eimeria maxima*. *Veterinary Parasitology*, *144*(1-2), 172-175.

Jansen, M. S., Nyangiwe, N., Yawa, M., Dastile, M., Mabhece, V., Muchenje, V., & Mpendulo, T. C. (2020). Prevalence and seasonal variation of gastrointestinal nematodes and coccidia infecting ovine grazing on communal rangelands in the Eastern Cape, South Africa. *Parasitology Research*, *119*(12), 4169-4175.

Jenkins, M. C., Dubey, J. P., Miska, K., & Fetterer, R. (2017). Differences in fecundity of *Eimeria maxima* strains exhibiting different levels of pathogenicity in its avian host. *Veterinary Parasitology*, *236*, 1-6.

Jitviriyanon, S., Phanthong, P., Lomarat, P., Bunyapraphatsara, N., Porntrakulpipat, S., & Paraksa, N. (2016). *In-vitro* study of anti-coccidial activity of essential oils from indigenous plants against *Eimeria tenella*. *Veterinary Parasitology*, *228*, 96-102.

Jollow, D. J., Mitchell, J. R., Zampaglione, N., & Gillette, J. R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, *11*(3), 151-169.

Kaingu, F., Liu, D., Wang, L., Tao, J., Waihenya, R., & Kutima, H. (2017). Anticoccidial effects of *Aloe secundiflora* leaf extract against *Eimeria tenella* in broiler chicken. *Tropical Animal Health and Production*, *49*(4), 823-238.

Kanthal, L. K., Dey, A., Satyavathi, K., & Bhojaraju, P. (2014). GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. *Pharmacogonosy Research*, *6*, 58-61.

Karpagasundari, C., & Kulothungan, S. (2014). Analysis of bioactive compounds in *Physalis minima* leaves using GC MS, HPLC, UV-VIS and FTIR techniques. *Joiurnal of Pharmacognosy and Phytochemistry*, *3*(4), 196-201.

Kashani, H., Seyed-Hosseini, E., Nikzad, H., & Aarabi, M. H. (2012). Pharmacological properties of medicinal herbs by focus on secondary metabolites. *Journal of Life Science*, *9*(1), 509-520.

Kaur, J., Kumar, D., Madaan, R., & Kumar, S. (2014). Estimation of isolated triterpenoid-ursolic acid in *Verbena officinalis* L. aerial parts using TLC densitometry. *Journal of Pharmaceutical Technology, Research and Management*, *2*(2), 121-135.

Kellenberger, E., Rodrigo, J., Muller, P., & Rognan, D. (2004). Comparative evaluation of eight docking tools for docking and virtual screening accuracy. *Proteins: Structure, Function, and Bioinformatics*, *57*(2), 225-242.

Khan, M. J. (2019). Epidimiological studies of different eimerian species and their identifcation in commercial poultry of southern Punjab, Pakistan, *Frontiers in Nanoscience and Nanotechnology*, *5*, 1-4.

Khan, M. Q., Irshad, H., Anjum, R., Jahangir, M., & Nasir, U. (2006). Eimeriosis in poultry of Rawalpindi/Islamabad area. *Pakistan Veterinary Journal*, *26*(2), 85-87.

Khater, H. F., Ziam, H., Abbas, A., Abbas, R. Z., Raza, M. A., Hussain, K., & Selim, A. (2020). Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. *Agrobiology Records*, *1*, 11-25.

Khatua, S., Pandey, A., & Biswas, S. J. (2016). Phytochemical evaluation and antimicrobial properties of *Trichosanthes dioica* root extract. *Journal of Pharmacognosy and Phytochemistry*, *5*(5), 410-413.

Khawaja, J. Z., Naeem, K., Ahmad, Z., & Ahmad, S. (2005). Surveillance of Avian influenza virus in wild birds in area adjacent to epicenter of an outbreak in federal capital territory of Pakistan. *International Journal of Poultry Science*, *4*, 39-43.

Kiani, R., Rasadi, M., & Mohammadian, M. N. (2007). Sources and routes of introduction of *Eimeria* oocysts into broiler chick's houses. *International Journal of Poultry Science*, *6*(12), 925-927.

Kieschnick, H., Wakefield, T., Narducci, C. A., & Beckers, C. (2001). *Toxoplasma gondii* attachment to host cells is regulated by a calmodulin-like domain protein kinase. *Journal of Biological Chemistry*, *276*(15), 12369-12377.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C. Y. & Kim, Y. K. Lee, Y. S., Jeong, D. H., & Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, *3*(1), 95-101.

Kleyn, F. J., & Ciacciariello, M. (2021). Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies?. *World's Poultry Science Journal*, *77*(2), 267-278.

Kohli, R. K., & Daizy, R. (1994). *Parthenium hysterophorus* -a review. Research Bulletin of the Panjab University, *Science*, *44*(1/4), 105-149.

Kostadinovic, L., & Levic, J. (2018). Effects of phytoadditives in poultry and pigs diseases. *Journal of Agronomy, Technology and Engineering Management. 1*(1), 1-7.

Kostadinovic, L., Popovic, S., Pelic, D. L., Cabarkapa, I., Duragic, O., & Levic, J. (2019). Medicinal plants as natural alternative to coccidial synthetic drugs in broiler chicken production. *Journal of Agronomy, Technology and Engineering Management*, *2*(5), 325-334.

Kou, W. Z., Yang, J., Yang, Q. H., Wang, Y., Wang, Z. F., Xu, S. L., & Liu, J. (2013). Study on *in-vivo* anti-tumor activity of *Verbena officinalis* extract. *African Journal of Traditional, Complementary and Alternative Medicines*, *10*(3), 512-517.

Kouhbanani, M. A. J., Beheshtkhoo, N., Taghizadeh, S., Amani, A. M., & Alimardani, V. (2019). One-step green synthesis and characterization of iron oxide nanoparticles using aqueous leaf extract of *Teucrium polium* and their catalytic application in dye degradation. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *10*(1), 015007.

Krishnakumari, V., & Nagaraj, R. (2012). Binding of peptides corresponding to the carboxy- terminal region of human-β-defensins-1–3 with model membranes investigated by isothermal titration calorimetry. *Biochimicaet Biophysica Acta (BBA)-Biomembranes*, *1818*(5), 1386-1394.

Krishnavignesh, L., Mahalakshmipriya, A., & Ramesh, M. (2013). *In-vitro* analysis of phytochemical screening and antimicrobial activity of *Parthenium hysterophorus* L. against pathogenic microorganisms. *Asian Journal of Pharmaceutical and Clinical Research*, *6*(5), 41-44.

Kubica, P., Szopa, A., Dominiak, J., Luczkiewicz, M., & Ekiert, H. (2020). *Verbena officinalis* (Common Vervain) – a review on the investigations of this medicinally important plant species. *Planta Medica*, *86*(17), 1241-1257.

Kumar, A., Shukla, R., Singh, P., Singh, A. K., & Dubey, N. K. (2009). Use of essential oil from *Mentha arvensis* L. to control storage moulds and insects in stored chickpea. *Journal of Science, Food and Agriculture*, *89*, 2643–2649.

Kumar, M., Dahiya, S. P., & Ratwan, P. (2021). Backyard poultry farming in India: A tool for nutritional security and women empowerment. *Biological Rhythm Research*, *52*(10), 1476-1491.

Kumar, P., Madaan, R., & Sidhu, S. (2017). Antianxiety activity of fractions and isolated compounds of *Verbena officinalis* aerial parts. *International Journal of Pharmaceutical Sciences and Drug Research*, *9*(2), 79-82.

Kumar, S., Chashoo, G., Saxena, A. K., & Pandey, A. K. (2013). *Parthenium hysterophorus:* A probable source of anticancer, antioxidant and anti-hiv agents. *BioMed Research International*, 1-11.

Kumar, S., Pandey, S., & Pandey, A. K. (2014). *In-vitro* antibacterial, antioxidant, and cytotoxic activities of *Parthenium hysterophorus* and characterization of extracts by LC-MS analysis. *BioMed Research International*, *4*, 495154.

Kumar, S., Singh, A. P., Nair, G., Batra, S., Seth, A., Wahab, N., & Warikoo, R. (2011). Impact of *Parthenium hysterophorus* leaf extracts on the fecundity, fertility and behavioral response of *Aedes aegypti* L. *Parasitology Research, 108*, 853–859.

Kumaresan, S., Senthilkumar, V., Stephen, A., & Balakumar, B. S. (2015). GC-MS analysis and pass-assisted prediction of biological activity spectra of extract of *Phomopsis sp.* isolated from *Andrographis paniculata*. *World Journal of Pharmaceutical Research*, *4*(1), 1035-1053.

Kundalic, B. S., Fialova, S., Dobes, C., Olzant, S., Tekelova, D., Grancai, D., Reznicek, G., & Saukel, J. (2009). Multivariate numerical taxonomy of *Mentha* species, hybrids, varieties and cultivars. *Scientia Pharmaceutica*, *77*(4), 851-876.

Kuo, T. T., Chang, H. Y., Chen, T. Y., Liu, B. C., Chen, H. Y., Hsiung, Y. C., Hsia, S. M., Chang, C. J., & Huang, T. C. (2020). *Melissa officinalis* extract induces apoptosis and inhibits migration in human colorectal cancer cells. *ACS Omega*, *5*(49), 31792-31800.

Kushwaha, V. B., & Maurya, S. (2012). Biological utilities of *Parthenium hysterophorus. Journal of Applied and Natural Science*, *4*(1), 137-143.

Lahlou, R. A., Bounechada, M., Mohammedi, A., Silva, L. R., & Alves, G. (2021). Dietary use of *Rosmarinus officinalis* and *Thymus vulgaris* as anticoccidial alternatives in poultry. *Animal Feed Science and Technology*, *273*, 114826.

Lai, L., Bumstead, J., Liu, Y., Garnett, J., Campanero-Rhodes, M. A., Blake, D. P., Palma, A. S., Chai, W., Ferguson, D.J., Simpson, P., Feizi, T., Tomley, F.M., & Matthews, S. (2011). The role of sialyl glycan recognition in host tissue tropism of the avian parasite *Eimeria tenella*. *PLoS Pathogens*, *7*, e1002296.

Lakkanatinaporn, P., & Matayatsuk, C. (2004). Simultaneous HPLC method for determination of sodium trimethoprim phenyl propanol disulphonate and sodium sulfaquinoxaline in veterinary drugs. *Songklanakarin Journal of Science and Technology*, *26*(6), 849-854.

Lan, L. H., Sun, B. B., Zuo, B. X. Z., Chen, X. Q., & Du, A. F. (2017). Prevalence and drug resistance of avian *Eimeria* species in broiler chicken farms of Zhejiang province, China. *Poultry Science*, *96*(7), 2104-2109.

Lan, L., Zuo, B., Ding, H., Huang, Y., Chen, X., & Du, A. (2016). Anticoccidial evaluation of a traditional Chinese medicine—*Brucea javanica*—in broilers. *Poultry Science*, *95*(4), 811-818.

Langerudi, M. T., Youssefi, M. R., & Tabari, M. A. (2022). Ameliorative effect of *Psidium guajava* essential oil supplemented feed on chicken experimental coccidiosis. *Tropical Animal Health and Production*, *54*(2), 1-9.

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling*, *51*(10), 2778-2786.

Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., & Muller, R. N. (2008). Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chemical Reviews*, *108*(6), 2064-2110.

Lavinas, F. C., Macedo, E. H. B., Sa, G. B., Amaral, A. C. F., Silva, J. R., Azevedo, M. M., & Rodrigues, I. A. (2019). Brazilian stingless bee propolis and geopropolis: promising sources of biologically active compounds. *Revista Brasileira de Farmacognosia*, *29*(3), 389-399.

Lawal, J. R., Jajere, S. M., Ibrahim, U. I., Geidam, Y. A., Gulani, I. A., Musa, G., & Ibekwe, B. U. (2016). Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria. *Veterinary World*, *9*, 653–659.

Lee, J. J., Kim, D. H., Lim, J. J., Kim, D. G., Min, W., Kim, G. S., Lee, H. J., Rhee, M. H., Park, H., Kim, S. M., Chang, H. H., & Kim, S. (2012). Anticoccidial effect of supplemental dietary *Galla rhois* against infection with *Eimeria tenella* in chickens. *Avian Pathology*, *41*(4), 403-407.

Lei, T., Wu, D., Song, Z., Ren, Y., Yu, Q., Qi, C., Xiao, P., & Gong, J. (2022). Research Note: Effects of different anticoccidial regimens on the growth performance, hematological parameters, immune response, and intestinal coccidial lesion scores of yellow-feathered broilers. *Poultry Science*, *101*, 102019.

Levine, N. D. (1985). Veterinary Protozoology. Iowa State University Press, Ames, Iowa, USA.

Londonkar, R. L., & Poddar, P.V. (2009). Studies on activity of various extracts of *Mentha arvensis* Linn against drug induced gastric ulcer in mammals**.** *World Journal Gastrointestinal Oncology*, *15*, 1(1), 82–88.

Lopez, A. M., Munoz, M. C., Molina, J. M., Hermosilla, C., Taubert, A., Zarate, R., Hildebrandt, I., McNaughton-Smith, G., Eiroa, J. L., & Ruiz, A. (2019). Anticoccidial efficacy of Canary rue (*Ruta pinnata*) extracts against the caprine apicomplexan *Eimeria ninakohlyakimovae*. *Journal of Animal Science*, *97*(1), 101-110.

Lourido, S., Shuman, J., Zhang, C., Shokat, K. M., Hui, R. & Sibley, L. D. (2010). Calcium-dependent protein kinase 1 is an essential regulator of exocytosis in Toxoplasma. *Nature, London, 465*, 359-362.

Lozano, J., Salinero, A. P., Anaya, A., Lux-Hoppe, E., Gomes, L., Oliveira, M., Silva, A. P., Rebelo, M. T., & de Carvalho, L. M. (2021). Long-term survey of *Eimeria* spp. prevalence and faecal shedding in a traditional Portuguese free-range chicken farm. *Revista Portuguesa de Zootecnia*, *6*(1), 9-16.

Lu, Z. J., & Markham, G.D. (2002). Enzymatic Properties of *S*-Adenosylmethionine Synthetase from the Archaeon *Methanococcus jannaschii. The Journal of Biological Chemistry*, *277*(19), 16624-16631.

Maheswari, P., & Revathi, K. 2017. Antimicrobial effects of 1-monocaprylin and 1-monocaproin through *in-vitro* growth inhibition and molecular mocking studies. *Journal of Biochemistry and Biotechnology*, *13*(3), 123-131.

Makkar, M. K., Sharma, S., & Kaur, H. (2018). Evaluation of *Mentha arvensis* essential oil and its major constituents for fungitoxicity. *Journal of Food Science & Technology*, 55, 3840-3844.

Malik, T. A., Kamili, A.N., Chishti, M.Z., Tanveer, S., Ahad, S., & Johri, R. K. (2016). Synergistic approach for treatment of chicken coccidiosis using berberine - A plant natural product. *Microbial Pathogenesis*, *93*, 56-62.

Malik, U., Das, S., & Barik, A. (2018). Biology of *Galerucella placida* Baly (Coleoptera: Chrysomelidae) on the rice-field weed *Polygonum orientale* L. (Polygonaceae). *In Proceedings of the Zoological Society*, *71*(3), 257-264.

Manafi, M., Mohan, K., & Ali, M. N. (2011). Effect of ochratoxin A on coccidiosis-challenged broiler chicks. *World Mycotoxin Journal*, *4*(2), 177-181.

Mancinelli, C., Alice, Mattioli, S., Twining, C., Bosco, A.D., Donoghue, A. M., Arsi, K., Angelucci, E., Chiattelli, D., & Castellini, C. (1969). Poultry meat and eggs as an alternative source of n-3 long-chain polyunsaturated fatty acids for human nutrition. *Nutrients.* 14(9), 1-29.

Manh, K. D., & Tuyet, O. T. (2020). Larvicidal and repellent activity of *Mentha arvensis* L. essential oil against *Aedes aegypti*. *Insects*, *11*(3), 198.

Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L., & Poli, A. (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. *Food & nutrition research*, *59*(1), 27606, 1-11.

Mathew, M., Nair, C., Shenoy, T., & Varghese, J. (2011). Preventive and curative effects of *Acalypha indica* on acetaminophen-induced hepatotoxicity. *International Journal of Green Pharmacy*, *5*(1), 49.

McDougald, L. R., Hofacre, C., Mathis, G., Fuller, L., Hargrove, J. L., Greenspan, P., & Hartle, D. K. (2008). Enhancement of resistance to coccidiosis and necrotic enteritis in broiler chickens by dietary muscadine pomace. *Avian Diseases*, *52*(4), 646-651.

Melkamu, S., Chane, M., & Asrat, M. (2018). Biochemical changes caused by *Eimeria* spp in broiler chickens. *Journal of Animal Research, 8*(5), 899.

Memon, F. U., Yang, Y., Soliman, A. M., Lv, F., Rajput, N., Zhang, G., Baig, M. B., Wang, Y., & Si, H. (2021). Dietary supplementation with *Piper sarmentosum* extract on gut health of chickens infected with *Eimeria tenella*. *Tropical Animal Health and Production*, *53*(5), 1-10.

Mesa, C., Gomez-Osorio, L. M., Lopez-Osorio, S., Williams, S. M., & Chaparro-Gutierrez, J. J. (2021). Survey of coccidia on commercial broiler farms in Colombia: Frequency of *Eimeria* species, anticoccidial sensitivity, and histopathology. *Poultry Science*, *100*(8), 101239.

Messai, A., & Redouane-Salah, S. (2022). Dietary use of *Artemisia herba alba* Asso as a potential coccidiostat against cæcal coccidiosis: haematological parameter variations. *Tropical Animal Health and Production*, *54*(1), 1-7.

Metwaly, M. S., Dkhil, M. A., Al-Quraishy, S., & Al Omar, S. Y. (2015). Protective effects of palm pollen aqueous extract against *Eimeria papillata* induced intestinal damage in mice. *Pakistan Journal of Zoology*, *47*(4), 971-979.

Mikail, H. G., Abubakar, I. A., Mohammed, B. R., Yusuf, M., Hussain, G., & Fatihu, M. Y. (2019). Effects of methanolic leaf extract of *Lannea schimperi* on some organ

histopathology in experimentally induced coccidiosis in broiler chickens. *Journal of Veterinary Science and Animal Husbandry*, *6*(6), 602.

Million, D., Nigatu, L., Bekeko, Z., Legesse, H. (2021). "Distribution and Impact of Parthenium (*Parthenium hysterophorus L*.) on weed species diversity in maize fields in western Gojjam Zone, Amhara National Regional State, Ethiopia. *International Journal of Agronomy*, 5514528, 1-10.

Mohammed, B. R., & Sunday, O. S. (2015). An overview of the prevalence of avian coccidiosis in poultry production and its economic importance in Nigeria. *Veterinary Research International*, *3*(3), 35-45.

Mohammed, M. T., Sidi, A. A., Saleh, H., Aliyu, M. H., Ibrahim, R., & Sabo, Y. H. (2021). Prevalence of intestinal parasites *in Gallus domesticus* (local chicken) slaughtered at Abu Baker Musa Market Soba Kaduna State. *Infection*, *45*, 30.

Mohsin, M., Abbas, R. Z., Yin, G., Sindhu, Z. U. D., Abbas, A., Huang, Z., Aleem, M. T., Saeed, Z., Afzal, M. Z., Ejaz, A., & Shoaib, M. (2021). Probiotics as therapeutic, antioxidant and immunomodulatory agents against poultry coccidiosis. *World's Poultry Science Journal*, *77*(2), 331-345.

Moine, E., Sabourin, C. D., Grockiego, F. D., Silpa, L., Gorgette, O., Barale, J. C., & Gueiffier, C. E. (2015). A small-molecule cell-based screen led to the identification of biphenylimidazoazines with highly potent and broad-spectrum anti-apicomplexan activity. *European Journal of Medicinal Chemistry*, *89*, 386-400.

Molan, A. L., Liu, Z., & De, S. (2009). Effect of pine bark (*Pinus radiata*) extracts on sporulation of coccidian oocysts. *Folia Parasitologica*, *56*(1), 1-5.

Molina, C., Kaialy, W., & Nokhodchi, A. (2019). The crucial role of leucine concentration on spray dried mannitol-leucine as a single carrier to enhance the aerosolization performance of Albuterol sulfate. *Journal of Drug Delivery Science and Technology*, *49*, 97-106.

Mondal, D. K., Chattopadhyay, S., Batabyal, S., Bera, A. K., & Bhattacharya, D. (2011). Plasma biochemical indices at various stages of infection with a field isolate of *Eimeria tenella* in broiler chicken. *Veterinary World*, *4*(9), 404.

Monzote, L., Garcia, M., Pastor, J., Gil, L., Scull, R., Maes, L., & Gille, L. (2014). Essential oil from *Chenopodium ambrosioides* and main components: activity against *Leishmania*, their mitochondria and other microorganisms. *Experimental Parasitology*, *136*, 20-26.

Moreno, S. N., & Docampo, R. (2003). Calcium regulation in protozoan parasites. *Current Opinion in Microbiology*, *6*(4), 359-364.

Morgoglione, M. E., Bosco, A., Maurelli, M. P., Alves, L. C., Saralli, G., Bruni, G., Cringoli, G., & Rinaldi, L. (2020). A 10-Year Surveillance of *Eimeria* spp. in Cattle and Buffaloes in a Mediterranean Area. *Frontiers in Veterinary Science*, *7*, 410.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785-2791.

Moryani, A. A., Rajput, N., Naeem, M., Shah, A. H., & Jahejo, A. R. (2021). Screening of the Herbs and Evaluation of their Combined Effects on the Health and Immunity of Coccidiosis Challenged Broiler Chickens, *Pakistan Veterinary Journal*, *41*(2), 228-234.

Mottet, A., & Tempio, G. (2017). Global poultry production: Current state and future outlook and challenges. *World's Poultry Science Journal*, *73*(2), 245-256.

Muhammad, K., Khan, S. R., Kalhoro, N. H., Ullah, S., Khan, M. S., Malik, M. I., Zaman, A. A., Khan, T. A., Rehman, A., & Naimatullah, S. A. R. (2019). Efficiency of Diaveridine and Sulphaquinoxaline with and without vitamin A and K to treat the broiler caecal coccidiosis. *Pure and Applied Biology (PAB)*, *8*(2), 1592-1599.

Munir, A. A. (2002). A Taxonomic Revision of the Genus *Verbena* L. (Verbenaceae) In Australia. *Journal of the Adelaide Botanic Gardens*, *18*(1), 21-103.

Mura, C., & McAnany, C. E. (2014). An introduction to biomolecular simulations and docking. *Molecular Simulation*, *40*(10-11), 732-764.

Muraleedharan, K., & Chhabra, M. B. (2018). Nanotechnology Applications and Potential in Parasitology: An Overview. *Veterinary Immunology & Biotechnology*, *1*(1), 25.

Mustafa M. Y., & Ali, S. S. (2005). Prevalence Of infectious diseases in local and fayoumi breeds of rural poultry (*Gallus domesticus*). *Punjab University Journal of Zoology*, *20*(2), 177-180.

Muthamilselvan, T., Kuo, T. F., Wu, Y. C., & Yang, W. C. (2016). Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. *Evidence-based Complementary and Alternative Medicine*, *2016*, 2-19.

Nahed, A., Abd El-Hack, M. E., Albaqami, N. M., Khafaga, A. F., Taha, A. E., Swelum, A. A., El-Saadony, M. T., Salem, H. M., El-Tahan, A. M., Abu Qamar, S. F., El-Tarabily, K. A., & Elbestawy, A. R. (2022). Phytochemical control of poultry coccidiosis: a review. *Poultry Science*, *101*(1), 101542.

Naidoo, R., Balmford, A., Costanza, R., Fisher, B., Green, R. E., Lehner, B., & Ricketts, T. H. (2008). Global mapping of ecosystem services and conservation priorities. *Proceedings of the National Academy of Sciences*, *105*(28), 9495-9500.

Naidoo, V., McGaw, L. J., Bisschop, S. P. R., Duncan, N., & Eloff, J. N. (2008). The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. *Veterinary Parasitology*, *153*(3-4), 214-219.

Naseem, Z., Hanif, M. A., Zahid, M., Tahir, S., Anjum, F., & Bhatti, H. N. (2022). Ultrasound-assisted deep eutectic solvent–based extraction of phytochemicals from *Mentha arvensis*: optimization using Box-Behnken design. *Biomass Conversion and Biorefinery*, *12*(1), 35-45.

Nayak, V., Singh, K. R., Verma, R., Pandey, M. D., Singh, J., & Singh, R. P. (2022). Recent advancements of biogenic iron nanoparticles in cancer theranostics. *Materials Letters*, *313*, 131769.

Nidaullah, H., Durrani, F. R., Ahmad, S., Jan, I. U., & Gul, S. (2010). Aqueous extract from different medicinal plants as anticoccidial, growth promotive, and immunostimulant in broilers. *Journal of Agricultural and Biological Science*, *5*(1), 53-59.

Nisar, R., Adhikary, S., Ahmad, S., & Alam, M. A. (2022). *In vitro* antimelanoma properties of *Verbena officinalis* fractions. *Molecules*, *27*(19), 6329.

Noack, S., Chapman, H. D., & Selzer, P. M. (2019). Anticoccidial drugs of the livestock industry. *Parasitology Research*, *118*(7), 2009-2026.

Nonkookhetkhong, T., & Chalalai, T. (2021). Lesion scores, oocysts output, hematological and histopathological changes of the 7 days life cycle of *Eimeria tenella* in Broilers. *Journal of Parasitic Diseases*, *45*(1), 1-13.

Nweze, N. E., & Obiwulu, I. S. (2009). Anticoccidial effects of *Ageratum conyzoides. Journal of Ethnopharmacology*, *122*, 6–9.

Nwonuma, C. O., Adelani-Akande, T.A., Osemwegie, O.O., Olaniran, A. F., & Adeyemo, T. A. 2019. Comparative study of the *in vitro* phytochemicals and antimicrobial potential of six medicinal plants. *F1000 Research*, *8*, 81.

Nwosu, C. O., Yahayah, K., & Igbokwe, I. O. (2011). Toxicity and anticoccidial efficacy of some plants used in the traditional treatment of avian coccidiosis in semi arid northeastern Nigeria. *Research Journal of Parasitology*, *6*(1), 18-30.

Odden, A., Stuen, S., Enemark, H. L., Robertson, L. J., Molina, J. M., & Ruiz, A. (2019). Preliminary studies on *in-vitro* methods for the evaluation of anticoccidial efficacy/resistance in ruminants. *Experimental parasitology*, *201*, 34-41.

Ojimelukwe, A. E., Emedhem, D. E., Agu, G. O., Nduka, F. O., & Abah, A. E. (2018). Populations of *Eimeria tenella* express resistance to commonly used anticoccidial drugs in

southern Nigeria. *International Journal of Veterinary Science and Medicine*, *6*(2), 192-200.

Olabode, V. B., Gunya, D. Y., Alsea, U. M., Choji, T. P. P., & Barde, I. J. (2020). Histopathological lesions of coccidiosis natural infestation in chickens. *Asian Journal of Research Animal and Veterinary Science*, *5*(2), 41-45.

Orengo, J., Buendia, A. J., Ruiz-Ibanez, M. R., Madrid, J., Del Rio, L., Catala-Gregori, P.,Gracia, V., & Hernandez, F. (2012). Evaluating the efficacy of cinnamaldehyde and *Echinacea purpurea* plant extract in broilers against *Eimeria acervulina*. *Veterinary Parasitology*, *185*(2-4), 158-163.

Osorio, S. L., Chaparro-Gutierrez, J. J., & Gomez-Osorio, L. M. (2020). Overview of poultry *Eimeria* life cycle and host-parasite interactions. *Frontiers in Veterinary Science*, *7*, 384.

Osorio, S. L., Villar, D., Failing, K., Taubert, A., Hermosilla, C., & Chaparro-Gutierrez, J. J. (2020). Epidemiological survey and risk factor analysis on *Eimeria* infections in calves and young cattle up to 1 year old in Colombia. *Parasitology Research*, *119*(1), 255-266.

Owings, W. J., Reynolds, D. L., Hasiak, R. J., & Ferket, P. R. (1990). Influence of dietary supplementation with *Streptococcus faecium* M-74 on broiler body weight, feed conversion, carcass characteristics, and intestinal microbial colonization. *Poultry Science*, *69*(8), 1257-1264.

Padma, M., Ganesan, S., Jayaseelan, T., Azhagumadhavan, S., Sasikala, P., Senthilkumar, S., & Mani, P. (2019). Phytochemical screening and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L). *Journal of drug delivery and therapeutics*, *9*(1), 85-89.

Palani, R., Karunakaran, D., Rajesh, V., Mathivanan, K., Jayaraman, P. (2014). Analysis of antioxidant, antimicrobial activity and phytochemical potential of *Cleistanthus collinus Roxb*., *Polygonum glabrum* Wild. and *Melia azedarch* Linn. *Asian Journal of Medicine and Pharmaceutical Science*, *2*(2), 149-59.

Paltinean, R., Mocan, A., Vlase, L., Gheldiu, A., Crisan, G., Ielciu, I., Vostinaru, O. & Crisan, O. (2017). Evaluation of polyphenolic content, antioxidant and diuretic activities of six *Fumaria* species. *Molecules, 22*, 639.

Pan, Z., Lin, Y., Sarkar, B., Owens, G., & Chen, Z. (2020). Green synthesis of iron nanoparticles using red peanut skin extract: Synthesis mechanism, characterization and effect of conditions on chromium removal. *Journal of Colloid and Interface Science, 558*, 106-114.

Parent, E., Fernandez, D., & Boulianne, M. (2018). The use of a live non-attenuated coccidiosis vaccine modifies *Eimeria* spp. excretion in commercial antibiotic-free broiler chicken flocks compared to conventional shuttle anticoccidial programs. *Poultry Science, 97*(8), 2740-2744.

Patra, G., Ali, M. A., Chanu, K. V., Jonathan, L., Joy, L. K., Prava, M., Ravindran, R., Das, G., & Devi, L. I. (2010). PCR based diagnosis of *Eimeria tenella* infection in broiler chicken. *International Journal of Poultry Science*, *9*, 813-818.

Pavela, R. (2009). Larvicidal effects of some Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Parasitology Research*. *105*(3), 887-92.

Pawestri, W., Nuraini, D. M., & Andityas, M. (2020). The estimation of economic losses due to coccidiosis in broiler chickens in Central Java, Indonesia. In *IOP Conference Series: Earth and Environmental Science, 411*(1), 012030.

Pereira, J., Guedes, D. S., Leite, E., & Souza, D. (2018). Investigation of damage to *Escherichia coli, Listeria monocytogenes* and *Salmonella Enteritidis* exposed to *Mentha arvensis* L. and *M. piperita* L. essential oils in pineapple and mango juice by flow cytometry. *Food Microbiology, 76*, 564-571.

Pop, L. M., Varga, E., Coroian, M., Nedișan, M. E., Mircean, V., Dumitrache, M. O., Farczadi, L., Fulop, I., Croitoru, M.D., Fazakas, M. & Gyorke, A. (2019). Efficacy of a commercial herbal formula in chicken experimental coccidiosis. *Parasites & Vectors, 12*(1), 1-9.

Price, K. R. (2012). Use of live vaccines for coccidiosis control in replacement layer pullets. *Journal of Applied Poultry Research*, *21*(3), 679-692.

Prinzo, Z. W. (1999). Thiamine deficiency and its prevention and control in major emergencies. *WHO*, 51-52.

Qaid, M. M., Al-Mufarrej, S. I., Azzam, M. M., & Al-Garadi, M. A. (2021). Anticoccidial effectivity of a traditional medicinal plant, *Cinnamomum verum*, in broiler chickens infected with *Eimeria tenella*. *Poultry Science*, *100*(3), 100902.

Qaid, M. M., Al-Mufarrej, S. I., Azzam, M. M., Al-Garadi, M. A., Albaadani, H. H., Alhidary, I. A., & Aljumaah, R. S. (2021). Anti-coccidial effect of *Rumex nervosus* leaf powder on broiler chickens infected with *Eimeria tenella* oocyst. *Animals*, *11*(1), 167.

Rahman, H. U., Rehman, T. U., Ali, A., Shah, S. A. A., & Ismail, M. (2015). *In-vitro* anti-leishmanial activity of *Polygonum glabrum* stem extract on *Leishmania tropica* (KWH23) Strain. *Journal of Advances Biology and Biotechnology*, *3*, 23-28.

Raja, S., Ramya, I. (2017). A comprehensive review on *Polygonum glabrum*. *International Journal of Phytomedicine*, *8*(4), 457-67.

Rajkumar, S., & Jebanesan, A. (2004). Mosquitocidal activities of octacosane from *Moschosma polystachyum* Linn. (Lamiaceae). *Journal of ethnopharmacology*, *90*(1), 87-89.

Ramadan, A., El-Sooud, K. A., & El-Bahy, M. M. (1997). Anticoccidial efficacy of toltrazuril and halofuginone against *Eimeria tenella* infection in broiler chickens in Egypt. *Research in Veterinary Science*, *62*(2), 175-178.

Raman, D., Sobolik-Delmaire, T., & Richmond, A. (2011). Chemokines in health and disease. *Experimental Cell Research*, *317*(5), 575-589.

Ramimoghadam, D., Bagheri, S., & Abd Hamid, S. B. (2014). Progress in electrochemical synthesis of magnetic iron oxide nanoparticles. *Journal of Magnetism and Magnetic Materials*, *368*, 207-229.

Rarey, M., Kramer, B., Lengauer, T., & Klebe, G. (1996). A fast flexible docking method using an incremental construction algorithm. *Journal of Molecular Biology*, *261*(3), 470-489.

Rashid, M., Akbar, H., Bakhsh, A., Rashid, M. I., Hassan, M. A., Ullah, R., Hussain, T., Manzoor, S., & Yin, H. (2019). Assessing the prevalence and economic significance of coccidiosis individually and in combination with concurrent infections in Pakistani commercial poultry farms. *Poultry Science*, *98*(3), 1167-1175.

Rehecho, S., Hidalgo, O., de Cirano, M. G. I., Navarro, I., Astiasaran, I., Ansorena, D., Cavero, R. Y., & Calvo, M. I. (2011). Chemical composition, mineral content and antioxidant activity of *Verbena officinalis* L. *LWT-Food Science and Technology*, *44*(4), 875-882.

Reid, A. J. Y., Blake, D. P., Ansari, H. R., Billington, K., Browne, H. P., Bryant, J., Dunn, M., Hung, S.S., Kawahara, F., Miranda-Saavedra, D. & Pain, A. (2014). Genomic analysis of the causative agents of coccidiosis in domestic chickens. *Genome Research*, *24*(10), 1676-1685.

Reperant, J. M., Thomas-Henaff, M., Benoit, C., Le Bihannic, P., & Eterradossi, N. (2021). The impact of maturity on the ability of *Eimeria acervulina* and *Eimeria meleagrimitis* oocysts to sporulate. *Parasite*, *28*, 1-7.

Reytor, E., Perez-Miguelsanz, J., Alvarez, L., Perez-Sala, D., & Pajares, M.A. (2009). Conformational signals in the C-terminal domain of methionine adenosyltransferase I/III determine its nucleocytoplasmic distribution. *Faderation of American Society for Experimental Biology Journal*, *23*(10), 3347-3360.

Rizwan, H. M., Khan, M. K., Mughal, M. A. S., Abbas, Z., Abbas, R. Z., Sajid, M. S., & Nadeem, M. (2022). A new insight in immunomodulatory impact of botanicals in treating avian coccidiosis. *Journal of Parasitic Diseases*, *46*(4), 1164-1175.

Roghini, R., & Vijayalakshmi, K. (2018). Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. *International Journal of Pharmaceutical Science and Research*, *1,9*(11), 4859-64.

Ruff, M. D. (1999). Important parasites in poultry production systems. *Veterinary Parasitology*, *84*(3-4), 337-347.

Ruggiero, M. A., Gordon, D. P., Orrell, T. M., Bailly, N., Bourgoin, T., Brusca, R. C., Cavalier-Smith, T., Guiry, M. D., & Kirk, P. M. (2015). A higher level classification of all living organisms. *PLoS ONE*, *10*: e0119248.

Rukaiyat, M., Garba, S., & Labaran, S. (2015). Antimicrobial activities of hexacosane isolated from *Sanseveria liberica* (Gerome and Labroy) plant. *Adv Med Plant Res*, *3*(3), 120-125.

Safari, M. R., Azizi, O., Heidary, S. S., Kheiripour, N. & Ravan, A. P. (2018). Antiglycation and antioxidant activity of four Iranian medical plant extracts. *Journal of Pharmacopuncture*, *21*(2), 82-89.

Sahar, M., Gadelhaq,, Waleed, M., Arafa., Shawky, M., & Abolhadid. (2018). *In vitro* activity of natural and chemical products on sporulation of Eimeria species oocysts of chickens. *Veterinary Parasitology*, *251*, 12–16.

Sahile, S., Habtamu, M., & Chandrodyam, S. (2015). Anti-bacterial activity of *Verbena officinalis* (Linn.) crude extract against some human pathogens. *International Journal Pure & Applied Biosciences*, *3*(6), 34-41.

Said, M. S., Chinchansure, A. A., Nawale, L., Durge, A., Wadhwani, A., Kulkarni, S. S., Sarkar, D., Joshi, S.P. (2015). A new butenolide cinnamate and other biological active chemical constituents from *Polygonum glabrum*. *Natural Product Research*, *29*(22), 2080-6.

Saif, S., Tahir, A., & Chen, Y. (2016). Green synthesis of iron nanoparticles and their environmental applications and implications. *Nanomaterials*, *6*(11), 209.

Santos, K. K. A., Matias, E. F. F., Souza, C. E. S., Tintino, S. R., Braga, M. F. B. M., Guedes, G. M. M., Nogueira, L. F. B., Morais, E. C., Costa, J. G. M., Menezes, I. R. A. & Coutinho, H. D. M. (2012). Anti-*Candida* activity of *Mentha arvensis* and *Turnera ulmifolia*. *Journal of Medicinal Food*, *15*(3), 322-324.

Sarala, V., Radhakrishnan, M., & Balagurunathan, R. (2011). Inhibitory activity of terpenoid from the medicinal plant *Andrographis paniculata* against biofouling bacteria. *International Journal of ChemTech Research*, *3*(3), 1225-1231.

Sarker, D. K. (2005). Engineering of nanoemulsions for drug delivery. *Current drug delivery*, *2*(4), 297-310.

Sato, H., Nitanai, A., Kurosawa, T., & Oikawa, S. (2004). Anticoccidial efficacy of medium-chain triglycerides (MCT) in calves. *Journal of veterinary medical science*, *66*(12), 1583-1585.

Scanes, C. G. (2007). The global importance of poultry. *Poultry science*, *86*(6), 1057.

Scartazzini, L., Tosati, J. V., Cortez, D.H.C., Rossi, M.J., Flôres, S.H., Hubinger, M.D., Di Luccio, M. & Monteiro, A.R., (2019). Gelatin edible coatings with mint essential oil (*Mentha arvensis*): film characterization and antifungal properties. *Journal of Food Science & Technology*, *56*, 4045-4056.

Schrodinger, L. L. C. (2015). The PyMOL molecular graphics system, 6.6. *Schrodinger LLC*, *1*, 7.

Schwarz, R. S., Jenkins, M. C., Klopp, S., & Miska, K. B. (2009). Genomic analysis of *Eimeria* spp. populations in relation to performance levels of broiler chicken farms in Arkansas and North Carolina. *Journal of Parasitology*, *95*(4), 871-880.

Senapati, S., Ahmad, A., Khan, M. I., Sastry, M., & Kumar, R. (2005). Extracellular biosynthesis of bimetallic Au–Ag alloy nanoparticles. *Small*, *1*(5), 517-520.

Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z., & Ercisli S. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pakistan Journal of Pharmaceutical Science, 22*(1), 102-106.

Sermakkani, M., & Thangapandian, V. (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(2), 90-94.

Shah, S. A. A., Ashraf, A., & Qureshi, N. A. (2017). Morphological characterization and biological control of *Alitropus typus* (Isopoda: Aegidae). *Entomological Research*, *47*(6), 352-365.

Shah, S. A. A., Qureshi, N. A., Qureshi, M. Z., Alhewairini, S. S., Saleem, A., & Zeb, A. (2023). Characterization and bioactivities of *M. arvensis*, *V. officinalis* and *P. glabrum*: In-silico modeling of *V. officinalis* as a potential drug source. *Saudi Journal of Biological Sciences*, *30*(6), 103646.

Shaheen, N., Qureshi, N. A., Ashraf, A., Hamid, A., Iqbal, A., & Fatima, H. (2020). *In vitro* anti-leishmanial activity of *Prunus armeniaca* fractions on *Leishmania tropica* and molecular docking studies. *Journal of Photochemistry and Photobiology B: Biology*, *213*, 112077.

Shahraki, F., Shariati-Sharifi, F., Nabavi, R., & Jamshidian, A. (2018). Coccidiosis in Sistan: the prevalence of *Eimeria* species in native chicken and its histopathological changes. *Comparative Clinical Pathology*, *27*(6), 1537-1543.

Shamim, A., Hassan, M., Yousaf, A., Iqbal, M. F., Zafar, M. A., Siddique, R. M., & Abubakar, M. (2015). Occurrence and identification of *Eimeria* species in broiler rearing under traditional system. *Journal of Animal Science and Technology*, *57*(1), 1-4.

Shams, A. M., Mosadegh, M., & Shafaati, A. (2003). Volatile constituents from the aerial parts of *Verbena officinalis* L. (Vervain). *Iranian Journal of Pharmaceutical Research*, *2*, 39-42.

Shamsardakani, M. R., Mosaddegh, M., & Shafaati, A. (2010). Volatile constituents from the aerial part of *Verbena officinalis* L. (Vervain). *Iranian Journal of Pharmaceutical Research*, (1), 39-42.

Sharef, A. Y., Aziz, F. M., & Adham A. N. (2020). The protective effect of *Fumaria officinalis* against the testicular toxicity of fluoxetine in rat. *Zanco Journal of Medical Sciences*, *24*(1), 117-131.

Sharma, P., & Chitnis, C. E. (2013). Key molecular events during host cell invasion by apicomplexan pathogens. *Current Opinion in Microbiology*, *16*(4), 432-437.

Sharma, S., Iqbal, A., Azmi, S., Mushtaq, I., Wani, Z. A., & Ahmad, S. (2015). Prevalence of poultry coccidiosis in Jammu region of Jammu & Kashmir State. *Journal of Parasitic Diseases*, *39*(1), 85-89.

Sharma, S., Rasal, V. P., Patil, P. A., & Joshi, R. K. (2018). *Mentha arvensis* essential oil suppressed airway changes induced by histamine and ovalbumin in experimental animals. *Natural Product Research*, *32*(4), 468-472.

Sharma, U, R., Surendra, V., Goli, D. (2014). Evaluation of Analgesic activity of Ethanolic extracts of *Fumaria officinalis* Linn. in experimental animals. *Journal of Fundamental Pharmacy Research*, 2(1), 49-56.

Sharma, U. N. S., Fernando, D. D., Wijesundara, K. K., Manawadu, A., Pathirana, I., & Rajapakse, R. J. (2021). Anticoccidial effects of *Phyllanthus emblica* (Indian gooseberry) extracts: Potential for controlling avian coccidiosis. *Veterinary Parasitology: Regional Studies and Reports*, *25*, 100592.

Sharma, U. R., Goli, D., & Surendra. (2014). Evaluation of neuropharmacological activity of *Fumaria officinalis Linn.* by study of anticonvulsant activity on experimental animals. *Journal of Fundamental Pharmaceutical Research*, *2*(2), 22-29.

Shetshak, M. A., Suleiman, M. M., Jatau, I. D., Ameh, M. P., & Akefe, I. O. (2021). Anticoccidial efficacy of *Garcinia kola* (Heckel H.) against experimental *Eimeria tenella* infection in chicks. *Journal of Parasitic Diseases*, *45*(4), 1034-1048.

Shirzad, M. R., Seifi, S., Gheisari, H. R., Hachesoo, B. A., Habibi, H., & Bujmehrani, H. (2011). Prevalence and risk factors for subclinical coccidiosis in broiler chicken farms in Mazandaran province, Iran. *Tropical Animal Health and Production*, *43*(8), 1601-1604.

Sidiropoulou, E., Skoufos, I., Marugan-Hernandez, V., Giannenas, I., Bonos, E., Aguiar-Martins, K., Lazari, D., Blake, D.P. and Tzora, A. (2020). *In-vitro* anticoccidial study of oregano and garlic essential oils and effects on growth performance, fecal oocyst output, and intestinal microbiota *in-vivo*. *Frontiers in Veterinary Science*, *7*, 420.

Silva, L. M. R., Carrau, T., Vila-ViCosa, M. J. M., Musella, V., Rinaldi, L., Failing, K., Cortes, H. C. E., Taubert, A., & Hermosilla C. (2020). All authors have read and approved the manuscript. Analysis of potential risk factors of caprine coccidiosis. *Veterinary Parasitology: Regional Studies and Reports*, *22*, 100458.

Silva, M. A. D., Pessotti, B. M. D. S., Zanini, S. F., Colnago, G. L., Rodrigues, M. R. A., Nunes, L. D. C., & Martins, I. V. F. (2009). Intestinal mucosa structure of broiler chickens infected experimentally with *Eimeria tenella* and treated with essential oil of oregano. *Ciencia Rural*, *39*, 1471-1477.

Silva, R. E., Sausset, A., Bussiere, F. I., Laurent, F., Lacroix-Lamande, S., & Silvestre, A. (2021). Genome-wide expression patterns of rhoptry kinases during the *Eimeria tenella* life-cycle. *Microorganisms*, *9*(8), 1621.

Singh, H. P., Batish, D. R., Setia, N., & Kohli, R. K. (2005). Herbicidal activity of volatile oils from *Eucalyptus citriodora* against *Parthenium hysterophorus. Annals of Applied Biology, 146*, 89–94.

Singh, J., Petter, R. C., Baillie, T. A., & Whitty, A. (2011). The resurgence of covalent drugs. *Nature reviews Drug discovery*, *10*(4), 307-317.

Singh, M., Kaur, P., Singla, L. D., Kashyap, N., & Bal, M. S. (2021). Assessment of risk factors associated with prevalence of gastrointestinal parasites in poultry of central plain zone of Punjab, India. *Veterinary World*, *14*(4), 972.

Singh, V. S., Palod, J., Vatsya, S., & Shukla, S. K. (2013). Effect of herbal vitamin E-selenium with organic chromium on growth, hematological and parasitological parameters of broilers chicken during mixed *Eimeria* infection. *Veterinary Research International*, *1*(1), 25-30.

Snafi, A., & Esmail, A. 2020. Constituents and pharmacology of *Fumaria officinalis*- A review. *IOSR Journal of Pharmacy*, *10*(1), 17-25.

Sohail, M., Zainab, B., Sajid, M., Ullah, H., & Shoaib, M. (2019). Prevalence of Coccidiosis in Commercial Broiler Chicken in District Abbottabad Khyberpakhtunkhwa, Pakistan. *EC Veterinary Science*, *4*(7), 482-487

Song, X., Li, Y., Chen, S., Jia, R., Huang, Y., Zou, Y., Li, L., Zhao, X., & Yin, Z. (2020). Anticoccidial effect of herbal powder "Shi Ying Zi" in chickens infected with *Eimeria tenella*. *Animals*, *10*(9), 1484.

Sorour, S. S., Asa, S. A., Elhawary, N. M., Ghazy, E. W., Abd El Latif, A., El-Abasy, M. A., & Khalifa, H. O. (2018). Anticoccidial and hepatoprotective effects of artemisinin liquid extract, cinnamon essential oil and clove essential oil against *Eimeria stiedae* infection in rabbits. *Tropical Biomedicine*, *35*(4), 926-943.

Soulsby, E. J. L. (1986). Helminths, Arthropods and Protozoa of domesticated animals. 7th edn., Bailliere, Tindall, London, 807.

Sticher, O. (2008). Natural product isolation. *Natural Product Reports*, 25, 517-554.

Sultana, R., Hussain, S. A., Maqbool, A., Chaudry S. I., & Hussain, S. (2009). Epidemiology of eimeriosis in broiler and layer flocks in and around Lahore, Pakistan. *Punjab University Journal of Zoology*, *24*(1-2), 81-86.

Sundar, S. B., Harikrishnan, T. J., Latha, B. R., Chandra, G. S., & Kumar, T. M. S. A. (2017). Anticoccidial drug resistance in chicken coccidiosis and promising solutions: A review. *Journal of Entomology and Zoology Studies*, *5*(4), 1526-1529.

Tadesse, T., & Teshome, L. (2018). A study on copro-epidemiology of poultry coccidiosis in and around Jimma Town, Oromia Regional State, Ethiopia. *Journal of Veterinary Medical Research*, *5*(11), 1167.

Tammar, S., Salem, N., AidiWannes, W., Limam, H., Bourgou, S., Fares, N., Dakhlaoui, S., Hammami, M., Khammassi, S., Re, G.D., & Hessini K. (2021). Chemometric profiling and bioactivity of verbena (*Aloysia citrodora*) methanolic extract from four localities in Tunisia. *Foods*, *10*(12), 2912.

Tamrat, H., Mekonnen, N., Ferede, Y., Cassini, R., & Belayneh, N. (2020). Epidemiological study on calf diarrhea and coccidiosis in dairy farms in Bahir Dar, North West Ethiopia. *Irish Veterinary Journal*, *73*(1), 1-8.

Tang, B., Bi, W., Tian, M., & Row, K. H. (2012). Application of ionic liquid for extraction and separation of bioactive compounds from plants. *Journal of Chromatography B*, *904*, 1-21.

Tartaj, P., Morales, M. P., Gonzalez-Carreno, T., Verdaguer, S. V., & Serna, C. J. (2005). Advances in magnetic nanoparticles for biotechnology applications. *Journal of Magnetism and Magnetic Materials*, *290*, 28-34.

Tawab, H. A., Abdel-Baki, A. S., El-Mallah, A. M., Al-Quraishy, S., & Abdel-Haleem, H. M. (2020). *In-vivo* and *in-vitro* anticoccidial efficacy of *Astragalus membranaceus* against *Eimeria papillata* infection. *Journal of King Saud University-Science*, *32*(3), 2269-2275.

Tewari, A. K., & Maharana, B. (2011). Control of poultry coccidiosis: Changing trends. *Journal of Parasitic Diseases*, *35*(1), 10-17.

Thagfan, F. A., Dkhil, M. A., & Al-Quraishy, S. (2017). *In-vivo* anticoccidial activity of *Salvadora persica* root extracts. *Pakistan Journal of Zoology*, *49*(1), 53-57.

Thawkar, B. S., Amol, G., Jawarkar, Kalamkar, P. V., Pawar, K. P., & Kale, M. K. (2016). Phytochemical and pharmacological review of *Mentha arvensis*. *International Journal of Green Pharmacy*, *10*(2), 71.

Trott, O., & Olson, A. J. (2010). AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455-461.

Uddin, M. A., Ayaz, M., Aggoune, E. H., & Sajjad, M. (2014). Wireless sensor network: A complete solution for poultry farming. *2nd International Symposium on Telecommunication Technologies,* IEEE, 321-325.

Udo, E. J., & Abba, A. M. (2018). Comparative Study of *in-vitro* anticoccidial efficacy of *Allium sativum* and *Carica papaya*. *Journal of Zoology Research*, *2*(2), 10-14.

Ullah, H., Ali, R. H., & Javed, H. (2020). Prevalence of Coccidiosis in Broiler Chickens in Lahore, Pakistan. *Journal of Poultry Science and Technology, 8*(2), 26-28.

Ullah, N., Shah, S. M., Hussain. H., Ansir, R., & Hussain, M. N. (2020). Pyrocatechol violet sensitized Ho-TiO2/ZnO nanostructured material: as photoanode for dye sensitized solar cells. *Materials Research Express, 7*(3), 035003.

Umar, F. S., Usman, S. G., Muhammed, M. S., Enam, S. J., Babashani, M., & Adamu, S. (2022). Comparison of hematological changes associated with coccidiosis in commercial layer chickens at different production stages in Zaria, Nigeria. *Sokoto Journal of Veterinary Sciences*, *20*(5), 181-186.

Urbano, V. R., Maniero, M. G., Guimaraes, J. R., Del Valle, L. J., & Perez-Moya, M. (2021). Sulfaquinoxaline Oxidation and Toxicity Reduction by Photo-Fenton Process.*International Journal of Environmental Research and Public Health*, *18*(3), 1005.

Usman, J. G., Gadzama, U. N., Kwaghe, A. V., & Madziga, H. A. (2011). Anticoccidial resistance in poultry: a review. *New York Science Journal*, *4*(8), 102-109.

Valgas, C., Souza, S. M. D., Smania, E. F., & Smania Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, *38*(2), 369-380.

Valsalam, S., Agastian, P., Arasu, M. V., Al-Dhabi, N. A., Ghilan, A. K. M., Kaviyarasu, K., & Arokiyaraj, S. (2019). Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of *Tropaeolum majus L*. and its enhanced *in-vitro* antibacterial, antifungal, antioxidant and anticancer properties. *Journal of Photochemistry and Photobiology B: Biology*, *191*, 65-74.

Vermeulen, A. N., Schaap, D. C., & Schetters, T. P. (2001). Control of coccidiosis in chickens by vaccination. *Veterinary Parasitology*, *100*(1-2), 13-20.

Vijayalingam, T. A., Rajesh, N. V., & Ilavarasan, S. (2019). A report on intestinal coccidiosis in a Kairali Desi chicken farm in Ramanathapuram district. *Journal of Entmological and Zoological Studies*, *7*, 965-968.

Waheed, K. A., Arif-Ullah K, Touqeer A. 2016. Anticonvulsant, anxiolytic, andsedative activities of *Verbena officinalis*. *Frontiers in Pharmacology*, *7*, 499.

Wajiha, & Qureshi, N. A. (2021). *In-vitro* Anticoccidial, antioxidant activities and biochemical screening of methanolic and aqueous leaves extracts of selected plants. *Pakistan Veterinary Journal*, *41*(1), 53-63.

Walaa, I. M., Nahla, H.S., & Eman, M. A. (2018). Prevalence of *Eimeria* species among sheep and goats in Suez Governorate, Egypt. *International Journal of Veterinary Science and Medicine*, *6*:65-72.

Wall, M. E., Wani, M. C., Brown, D. M., Fullas, F., Olwald, J. B., Josephson, F. F. & Kinghorn, A. D. (1996). Effect of tannins on screening of plant extracts for enzyme inhibitory activity and techniques for their removal. *Phytomedicine*, *3*(3), 281-285.

Wallach, M. (2010). Role of antibody in immunity and control of chicken coccidiosis. *Trends in Parasitology*, *26*(8), 382-387.

Wang, D., Zhou, L., Li, W., Zhou, H., & Hou, G. (2018). Anticoccidial effects of areca nut (*Areca catechu* L.) extract on broiler chicks experimentally infected with *Eimeria tenella*. *Experimental parasitology*, *184*, 16-21.

Wang, Y., Zhang, K., Chen, Y., Li, X., & Zhang, L. (2021). Cryptosporidium and cryptosporidiosis in wild birds: A One Health perspective. *Parasitology Research, 120,* 3035-3044.

Wiedmer, S., Kurth, T., Buder, U., Bleischwitz, S., Entzeroth, R., & Kurth, M. (2020). Correlative light and electron microscopy of wall formation in *Eimeria nieschulzi*. *Parasitology Research, 119*(8), 2667-2678.

Wiedosari, E., & Wardhana, A. H. (2018). Anticoccidial activity of artemisinin and extract of *Artemesia annua* leaves in chicken infected by *Eimeria tenella*. *Journal of Animal and Veterinary Science*, *22*(4), 196-204.

Williams, M. G., Simon, O. R., West, M. E. (2006). The past and present use of plants for medicines. *West Indian Medical Journal, 55*(4), 217.

Williams, R. B., Marshall, R. N., Pages, M., Dardi, M., & del Cacho, E. (2009). Pathogenesis of *Eimeria praecox* in chickens: virulence of field strains compared with laboratory strains of *E. praecox* and *Eimeria acervulina*. *Avian Pathology*, *38*(5), 359-366.

Willis, G. M., & Baker, D. H. (1981). Performance of healthy and *Eimeria acervulina*-lnfected chicks as influenced by anticoccidial drugs and source of dietary protein. *Poultry Science*, *60*(10), 2284-2288.

Wondimu, A., Mesfin, E., & Bayu, Y. (2019). Prevalence of poultry coccidiosis and associated risk factors in intensive farming system of Gondar Town, Ethiopia. *Veterinary medicine international*, *2019*, 1-6.

Wu, H., Hang, M., & Yang, Z. (2019). Repellent activity screening of 12 essential oils against *Aedes albopictus* Skuse: Repellent liquid preparation of *Mentha arvensis* and *Litsea cubeba* oils and bioassay on hand skin. *Industrial Crops and Products*, *128*, 464-470.

Yakhchali, M., & Fakhri, M. (2017). Prevalence of *Eimeria* species in free-range chickens of villages of Khoy suburbs, Iran. *Veterinary Researches & Biological Products*, *30*(1), 206-211.

Yan, X., Han, W., Liu, X., & Suo, X. (2021). Exogenous nitric oxide stimulates early egress of *Eimeria tenella* sporozoites from primary chicken kidney cells *in-vitro*. *Parasite*, *28*, 11.

Yang, W. C., Yang, C. Y., Liang, Y. C., Yang, C. W., Li, W. Q., Chung, C. Y., & Chang, C. L. T. (2019). Anti-coccidial properties and mechanisms of an edible herb, *Bidens pilosa*, and its active compounds for coccidiosis. *Scientific Reports*, *9*(1), 1-11.

Yew, Y. P., Shameli, K., Miyake, M., Kuwano, N., Bt Ahmad Khairudin, N. B., Bt Mohamad, S. E., & Lee, K. X. (2016). Green synthesis of magnetite (Fe_3O_4) nanoparticles using seaweed (*Kappaphycus alvarezii*) extract. *Nanoscale Research Letters*, *11*(1), 1-7.

Youn, H. J., & Noh, J. W. (2011). Screening of the anticoccidial effects of herb extracts against *Eimeria tenella*. *Veterinary Parasitology*, *96*, 257–263.

Yousaf, A., Shahnawaz, R., Jamil, T., & Mushtaq, A. (2018). Prevalence of coccidiosis in different broiler poultry farms in Potohar region (distract Rawalpindi) of Punjab-Pakistan. *Journal of Dairy, Veterinary & Animal Research, 7*(3), 87-90.

Yunus, A. W, Nasir, M. K., Farooq, U., & Bohm, J. (2008). Prevalence of poultry diseases in district Chakwal and their interaction with mycotoxicosis: 1. affects of age and flock size. *The Journal of Animal and Plant Sciences*, 18, 107-113.

Yunus, A. W., Nasir, M. K., Aziz, T., & Bohm, J. (2009). Prevalence of poultry diseases in district chakwal and their interaction with mycotoxicosis: 2. Effects of season and feed. *The Journal of Animal and Plant Sciences*, *19*, 1-5.

Zaman, M. A., Iqbal, Z., Abbas, R. Z., & Khan, M. N. (2012). Anticoccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. *Parasitology*, *139*(2), 237-243.

Zhang, D. F., Sun, B. B., Yue, Y. Y., Zhou, Q. J., & Du, A. F. (2012). Anticoccidial activity of traditional Chinese herbal *Dichroa febrifuga* Lour. extract against *Eimeria tenella* infection in chickens. *Parasitology research*, *111*(6), 2229-2233.

Zhang, J. J., Wang, L. X., Ruan, W. K., & An, J. (2013). Investigation into the prevalence of coccidiosis and maduramycin drug resistance in chickens in China. *Veterinary Parasitology*, *191*(1-2), 29-34.

Zhang, K., Li, X., Na, C., Abbas, A., Abbas, R. Z., & Zaman, M. A. (2020). Anticoccidial effects of *Camellia sinensis* (green tea) extract and its effect on blood and serum chemistry of broiler chickens. *Pakistan Veterinary Journal*, *40*, 77-80.

Zoubiri, S., & Baaliouamer, A. (2011). Larvicidal activity of two algerian verbenaceae essential oils against *Culex pipiens*. *Veterinary Parasitology*, *181*(2-4), 370-373.

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Original article

Characterization and bioactivities of *M. arvensis, V. officinalis and P. glabrum*: In-silico modeling of *V. officinalis* as a potential drug source

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ABSTRACT

In current study the pharmaceutically active herbs was used against coccidiosis, caused by a protozoan: *Eimeria*, lead to $ 3 billion loss annually. The aqueous and methanolic extracts of whole plants were applied *in-vitro* to assess sporulation inhibition (spi) assay and calculated the inhibitory concentration (IC_{50}). For *in-vivo* study 9 groups of 14 day old broiler chicks were infected with *Eimeria tenella* and three groups were treated different concentrations of methanolic extracts of *Verbena officinalis* and *Polygonum glabrum* post infection. The mean weight gain, oocyst count, diarrhea, biochemical tests, hematology, and histopathology of all groups were analyzed. The herbs were characterized by antioxidant assay, phyto-chemical screening, Fourier transmission and infrared (FT-IR), Ultra Violet-visible (UV–Vis) spectroscopy and Gas chromatography and mass spectroscopy (GC–MS). The GC–MS identified phyto-compounds of *V. officinalis* were docked with S-Adenosyl methionine (SAM) synthetase. The *in-vitro* study revealed that *V. officinalis* and *P. glabrum* have minimum IC_{50} of 0.14 and 12 mg/ml respectively. The *in-vivo* experiment showed that *V. officinalis* had significantly high anticoccidial potential with significant hematological profile like drug treated controls. The histology of treated chicks also showed recovery in the studied tissues. The antioxidant assay showed that *V. officinalis* have 4.19U/mg Superoxide dismutase (SOD) and 33.96 μM/mg Glutathione (GSH) quantities. The chemical characterization confirmed the presence of large number of organic compounds, however Flavonoids found only in *V. officinalis*, which suggests the anticoccidial potential of *V. officinalis* because flavonoids as antagonist of thiamine (Prinzo, 1999), because it promotes the carbohydrate synthesis required. Strychane, 1-acetyl-20a-hydroxy-16-methylene has best binding of with target protein with lowest binding score (-6.4 Kcal/mol), suggests its anticoccidial potential in poultry.

Abbreviations: SOD, Superoxide dismutase; GSH, Glutathione; spi, sporulation inhibition; sp, sporulation; MWG, Mean weight gain; MIW, Mean initial weight; MFW, Mean final weight; WDB, weight of dead birds; opg, oocysts per gram; FCR, feed consumption ratio; SQX, sulfaquinoxaline; K2Cr2O7, Potassium dichromate; AST, Aspartate aminnotransferase; ALT, Alanine transaminase; SAM, S-Adenosyl methionine; IC, inhibitory concentration; wbc, white blood cell; rbc, red blood cell; Hb, hemoglobin; FT-IR, Fourier transmission and Infrared spectroscopy; UV-Vis, Ultra voilet Visible; GC-MS, Gas chromatography and mass spectroscopy.

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