Ethnopharmacological Uses of Medicinal Plants among the Communities of Murree Hills with Special Emphasis on their Selected Biological Activities

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In

Plant Sciences

(Plant Systematics and Biodiversity)

By

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HINA GUL

ABSTRACT

Ethnopharmacology is a cross-cultural exploration of the utilization of plant based medicines by human population. The current study was aimed to evaluate the indigenous knowledge of medicinal plants of Murree hills of Pakistan, because insufficient ethnobotanical data of this area is available in the literature. The present work is based on systematic account of ethnopharmacological uses, phytochemical screening and biological activity of important medicinal plants of Murree Hills. During this study, 131 plant species belonging to 104 genera and 64 families were recorded. The ethnopharmacological information was obtained from 84 community consultants (50 males and 34 females). A quantitative index was used to assess relative use values, whicg were found to be high for *Myrsine africana* L. (0.9), *Dodonaea viscosa* (L). Jacq. (0.9), *Debregeasia salicifolia* (D.Don) Rendle (0.88), *Bauhinia variegata* L. (0.88), *Rumex hastatus* D.Don (0.87), *Vitex negundo* L. (0.83) and *Indigofera heterantha* Wall. ex Brandis (0.75). The dominating functional group was O-H stretch, Carboxylic acids followed by O-H stretch, H-bonded alcohols and phenols. The plants showed a significant quantity of fatty acids (Oleic acid) as well as ascorbic acid, while some uncommon compounds were found in these plants extracts. Those previously unreported were ricinoleic acid ($C_{18}H_{34}O_3$) in *D. viscosa* flowers, Ar-tumerone ($C_{15}H_{20}O$), tumerone ($C_{15}H_{22}O$) and curlone ($C_{15}H_{22}O$) in *R. hastatus* leaves. Of the plants evaluated, *R. hastatus* leaves showed the highest anticancer activity (87.89±2.86%), followed by *V. negundo* leaves 85.9±2.11%. Hepato-protective activity of plant extracts was measured by evaluating blood liver markers, antioxidant enzymes, direct bilirubins and total proteins. *V. negundo* and *M. africana* leaves chloroform leaf extracts showed curative effects against abnormalities at the concentration of 300 mg/kg. Histopathological slides showed that cellular abnormalities induced by carbon tetrachloride were rectified by *D. salicifolia* and *M. africana* leaves extracts. The above findings revealed that flavonoids (quercetin, rutin and p- coumeric acid), phenols, essential and non-essential fatty acids present in plant extracts possess activities *in vitro* and *in vivo*, thus supporting ethnomedicinal uses of the above plants. Therefore further isolation, characterization and pharmacological analysis of novel medicinal plant constituents should be carried out to extract new and potent compounds.

CHAPTER 1

INTRODUCTION

1.1 Medicinal plants and primary health care: A global perspective

Traditional herbal therapies are considered as the most valuable methods for curing the common diseases of human population in different parts of the world. People from different continents of the world such as Asia, Latin America and Africa trust on method of treatment by using herbal medicines against different infections. The ethno-botanical knowledge has started from ancient times and ethnic people transferred these important information from generation to generation through cultural practices. Although plants have numerous uses in human society but the most significant is remedial use for human's existence and it is also source of health management. Subsequently, medicinal plants have been in practice of all cultures of human being from past to present scenario to cure not only human but also livestock infirmities. Traditional medicinal plants signify leading human use with respect to species number of natural world (Ahmad *et al.*, 2016).

According to WHO reports 80 percent of rural people from different part of world depend on the herbal medicines to fulfill their needs of primary health care. The methods to use traditional medicinal plants to control diseases are equally diverse as per different cultures. Plant's world has the full potential for the discovery of new drugs and it has many more hidden secrets which are needed to be explored. These resources have been originated from local plants with their knowledge which is affordable and simple in use. A traditional medicinal plant not only helps to fulfill the gaps of demand and supply in modern pharmaceuticals but it also extends primary health care substitutes for the future generations with no side effects (WHO, 2011:Meragiaw *et al.*, 2016). Herbal plants, either in the form of raw, processed, single or combined or were recommended as herbal medicines / drugs in TCM (Traditional Chinese Medicine) for curing of selected types of diseases. Most importantly it derived from either nature sources or from China and some other countries those were using herbs or their extracts from thousands of years.

Although, plant extracts are natural and safe products but required some precautions in their uses however, in past there was no monitoring of safety measures. Recently, a lot of herbal medicines are acknowledged by those countries which have not been familiar to herbal medicines in their past history (Xia *et al.*, 2017).The main sources of traditional medicines in Africa are tree species and more than 65percent of documented medicinal plants are shrubs or trees. Among African traditional medicines woody plants share greater part in contrast to herbs due to their availability and usage. Now, by the wide examination of plants we found anti-inflammatory, antioxidant, antimicrobial and anthelmintic activities in parts of plant which are beneficial for human health. African plants have great economical and valuable formulation of herbal medicines and natural products those are useful for human health (Van Wyk, 2011).

The microbial infections have been considered the most important cause of mortality specifically in children among medieval societies. Ailments such as tuberculosis, typhus, typhoid, cholera, pneumonia, dysentery and diphtheria were prevailed in medieval societies of British Isles followed by ethno linguistic Celts including Briton in 5th century AD. After 9th century AD, Celtic tribes turn out to be limited towards west (wales &Cornwall) and north (Scotland and Western Isles). Chronological archives and herbal documents involving in traditional Celtic drug, directed an extensive broad plants pharmacopeia for handling microbial infections. Since 13th century AD, text represented 800 remedies and most of native species. It also expressed past verbal data and local apothecary. This information was generally used by hereditary learnt physicians of Western Isles such as Beatons in Islay and Mull, Macleans in Skye, O'Conachers in Argyll. Medical families of that time persistently practiced traditional Celtic medicines till modern times in Western Isles (Wagner *et al* ., 2017).

Herbs contain active ingredients in the form of herbal materials, preparations or products which are known as herbal medicines, botanical medicines and phytomedicines. People primarily rely on crude botanical material for medicinal requirements in all regions especially in Africa and Asia. Previous records on the practice of herbal plants dated back to 5,000 years

ago in Sumerians, 60,000 years ago in Iran and 8000 years ago in China. Over the past few years, by the advent of western medicines, herbal drugs have been challenged due to the lack of scientific evidence. Due to less curative modern therapies followed by high microbial resistance and side effects, there is a revival of the use of herbal medicines (Pan *et al.*, 2014).

1.2 Ethno-pharmacological documentation of medicinal plants across the world

Traditional healers have great knowledge of the herbal plants and their usage against different diseases. Herbal preparations are widely use in the form of powder or tablets to overcome the harmful effects of different diseases such as vomiting, diarrhea, diuresis and genital ulcer. There is a need to enhancing the awareness in traditional healers regarding correct handling of patients and properly cite symptoms of particular disease. In Africa, *Gymnosporia senegalensis* (Lam.) Loes., *Leucosidea sericea* Eckl. & Zeyh., *Kigelia africana* (Lam.)Benth., *Achyranthes aspera* L., *Asparagus racemosus* Willd., *Parinari curatellifolia* Planch. ex Benth., *Oncoba spinosa* Forssk., *Garcinia livingstonei* T.Anderson, *Acacia nilotica* (L.) Willd. ex Delile, *Mimo pigra* L., *Hibiscus vitifolius* L. and *Combretum collinum* Fresen. have been in use to tackle the different diseases like infections, diarrhea, dysentery and inflammation in powder form or decoction or infusion (Chinsembu *et al.*, 2015: Mafole *et al.*, 2017).

Achyranthes bidentata Blume and *Achyranthes aspera* L. are used in Asia, specifically in India and China these plants were documented in pharmacopeia (Japanese, Chinese and Korean) and they were used for the nourishment of liver, kidney and strengthen bones. They act as carminative, laxative, stomachic, and also have the anti-helminthic activity for cattle and have the ability to remove urolith followed by expelling of placenta in goat. Both plants are also well admired in various traditional systems Unani, Sidha, Ayurveda and Homeopathy

(He *et al.*, 2017). *Polygonum multiflorum* Thunb. is documented in the Chinese pharmacopeia and possesses medicinal properties such as strengthen sinew, bones, liver and kidney, recharge essence and blood; darken the beard, hair, delaying the aging process and tonic. It is also experimentally proven that to lessen the toxicity in liver produced (hepatotoxicity) by carbon tetra chloride and cure toxic hepatitis, fatty liver ailments via oral administration of P. *multiflorum* by reducing lipid per oxidation as well as inflammation. This plant is rich in bioactive compounds including anthraquinones, glucoside possessing antioxidants, antiapoptosis and lipid regulation properties (Xia *et al.*, 2017). *Marsdenia tenacissima* (Roxb.) Moon is a Chinese therapeutic herb, its dried stems alone or in combination traditionally used to control the adverse effects of cancer because it have diversity of important therapeutic compounds that is also proved clinically(Kenneth K.W. Teoh *et al.*, 2016).

Waltheria indica L. has been used in many countries for curing cough, asthma, pain, sore throat, fever, inflammation and cancer. It is used for inflammation related ailments worldwide for instances cancer and rheumatism (Mexico), Asthma (Hawaii), conjunctivitis (Medagascar) and ingivitis (Brazil). It is rich in flavonoids (Quercetin and epicatechin) and various other compounds which have the antifungal and antitrypanosomal activities (Monteillier *et al.*, 2017).

1.3 Medicinal plants and an overview of their biological activities

Medicinal plants are well-known for the presence of biological active compounds and their natural products for instances phenols and flavonoids. There are many herbal derived formulates which expresses the beneficial properties on man health. In the outlook of amazing usefulness followed by less adversarial possessions of medicinal plants along with its significant bioactive compounds, this aid an important therapy for the management of several human diseases such as cardiovascular and neurodegenerative diseases (Nabavi *et al.*, 2016). Study of traditional medicines turn out to be burning subject in developing as well as developed countries for their fewer side effects. There is an emerging interest towards

unveiling the secrecies of herbal remedies from plant compositional screening to the clinical trials and resulted in the discovery of recognized drugs. There is a new perception towards the findings of curative products are plants. There is an estimate that 65 – 80percentof population relies on active properties of medicinal plants. To tackle the mortality of diseases plenty of interests are being evoked to the discovery of antioxidants from natural products such as leafy vegetables and fruits which are considered to have great potential. Crude extracts or pure compounds of plants showed the antioxidants as well as oxidant scavenging properties (Karakas *et al.*, 2016: Asase and Yohonu, 2016).

Cancer is considered as the main reasons of deaths in the world. Cancer cells are unable to perform functions as normal cells and cancerous cells divide continuously than normal cells. According to an estimate of WHO 84 million people has been died due to cancer and to minimize its threat a lot of struggles have been done to decrease the risk of cancer. Chemotherapy is found to be a significant option in the modern treatments and some anticancer drugs are also used for the treatment of some solid tumors, leukemia and lymphoma. But chemotherapy results in adverse effects on human body such as nausea and failure in bone marrow etc. and because of these side effects there is a shift from synthetic medicines to nature based active medicines for human cancer treatments (Elyasi *et al.*, 2016). Liver diseases are the serious ailments in human health and modern medicines have not proved to be an effective remedy and cause serious complications on the liver. The interferon, penicillamine, corticosteroids and colchicines treatment have not been much effective and sometimes left injurious side effects on liver. Due to lack of reliability in allopathic medicines herbs can play pivotal role for curing the liver disorders. There should be a focus to unveil alternative approaches and methods for curing hepatic diseases. Therefore, medicinal plants and its preparations are found to be useful for the treatment of hepatic diseases among traditional medicines (Toori *et al.*, 2015).

Infections such as Hepatitis B and C are the alarming causes of liver diseases. These infections can be responsible for many liver cancers and the most adverse is hepatocellular carcinoma. Five commonly occurred cancer in the world and two in Southeast Asia are found to be liver cancer. There are no epidemiological figures available in the Cambodia regarding

various liver disorders. Though it is estimated that hepatocellular carcinoma is ranked first in all different types of cancers in men (Chassagne *et al.,* 2017).

Patients infected with various diseases depend upon herbalists as well as herbal drugs and traditional use of these medicines root back to the old civilization. Presently, these medicines are largely used all over the world. The evidence of medicinal properties of plants supports the traditional uses and novel discovery of drugs (Ahmad *et al.,* 2015). Plant contains biologically active metabolites in the different parts and scientists collect various plant parts from the plants followed by extract preparation and are evaluated for searching novel chemotherapeutics for curing cancer, microbial and viral infections. Cytotoxic plant screening is the initial step to isolate plant active compounds (Nemati *et al.,* 2013: Desai *et al.*, 2014). So the medicinal plants attract the local people for curing various diseases. These potent compounds make plant kingdom diverse field for study (Ahmad *et al.*, 2011). From the previous three decades, global problem of insufficient disease control arises by the repetitively usage of commercial drugs which develops the resistance against antimicrobial compounds or drugs. It forced the researchers to find antimicrobial substances from plants. Plants and plant associated drugs has been clinically reported as chemotherapeutic and antimicrobial agents (Sathiamoorthy *et al.,* 2007: Hussain *et al.*, 2016). By the haphazardly use of synthetic antimicrobial drugs for curing infectious diseases, MDR (Multiple drug resistance) has been developed. Additionally, the use of antibiotics resulted in multiple adverse side effects on the health of consumer includes allergic diseases, immune suppression and hypersensitivity. These problems compel the researchers to realize the need to explore new antimicrobial agents. The alarming condition of antibiotic resistance to bacteria turned out in a need of effective therapeutic materials. Hence, it is important to produce alternative antimicrobial based drugs from the medicinal plants for curing infectious ailments (Ramamurthy *et al.,* 2013: To et *al.*, 2017).

The consumption of food rich in flavonoids, phenols and different antioxidant compounds helps to overcome the ailments. The medicinal plants, fruits and vegetables are rich in natural antioxidants such as tocopherol, flavonoids, phenols, carotenoids, ascorbic acid, tannins and diterpenes. These phytochemicals exhibit strong scavenging capacities for

humans. Free radicals such as hydrogen peroxide and singlet oxygen molecules affect human body cells by causing the pathogenesis of alzheimer, atherosclerosis, diabetes, cancer and rheumatoid arthritis. The plant compounds showed antioxidant and antibacterial activities for humans to cure these infectious diseases. The medicinal plants become very reliable and economical source for the cytotoxic, antitumor, anti-hemolytic and hepato-protective activities. These activities proposed idea for pharmaceutical companies to develop new drug for human (Letsyo *et al.*, 2017: Russo *et al.*, 2017).

1.4 Phytochemical screening of medicinal plants

Plant derived Phytochemical defined the medicinal properties of plants. They are basically non-nutritive compounds, and classify into qualitative (carbohydrates, proteins, amino acids and chlorophyll) and quantitative metabolites (phenolic, flavonoids, alkaloids, steroids, saponins and tannins). Plant extracts also contains essential oils which have various volatile chemical compounds with diversified functional groups. Among plant compounds polyphenols are the most essential group which comprises on flavonoids (quercetin),and it have anti-hepatotoxic, anti-platelets, antioxidants and cytotoxic activities (Guardia *et al.*, 2001:Savithramma *et al.*, 2012: Upadhyay, 2015: Li *et al.*, 2017). Plants donate dynamic role in diet with multiple uses by adding flavors, preservation and pharmacological properties which are contributed by phytochemical compounds (phenols and flavonoids). Plants derived secondary metabolites impart some biological activities i.e. anti-oxidative, anticancer, anti-allergic, hypoglycemic and antibiotic (Dib *et al.*, 2017).

1.4.1 Phenolic compounds

1.4.1.1 Tannins

Tannins are called secondary metabolites, the important members of polyphenolic compounds and it is found in leaves, fruits, roots, bark and wood. It forms complexes along with carbohydrates, proteins, vitamins, minerals and alkaloids due to containing high molecular weights 500-3000. It has useful effects upon metabolism of protein in ruminants followed by decrease in dietary protein degradation and accelerating amino acids absorption in small intestine. Tannins plays significant role in pharmaceutical, food and leather industries. On the basis of chemical structures with properties, tannins are classified into condensed and hydrolysable tannins. Condensed tannins contain flavonoid units associated with carbon-carbon bonds while hydrolysable tannins are found to be in small quantity in plants instead of condensed tannins. Tannins have been found in flowering and non-flowering plants (Hassanpour *et al.,* 2011).

1.4.1.2 Phenols and Flavonoids

Largest group of phytochemicals are Phenols and its derivatives, they act as an antioxidants which possess scavenging potential against reactive oxygen species (ROS) and free radicals, and broadly found in plants and their products (Hossain and shah, 2015). Phenols contain wide range of biological activities i.e. antioxidant, anti-carcinogenic, antibacterial, anti-parasitic, antimicrobial, antiviral and anti-mutagenic. It can modify the gene expressions, useful against cancers and cardiovascular diseases (John *et al.,* 2014).Phenolic compounds are commonly present in crude extracts of herbs, cereals, fruits, vegetables and some other plant based materials. They play an important role in food industry as antioxidants with health benefits (Baba *et al.*, 2015).

Likewise, plant parts also contain many flavonoids present in free-state or glycosides. It acts as antioxidants because of their polyphenolic structure. Oxidizing molecules such as reactive oxygen species, superoxide, hydroxyl radicals and free radicals are effectively suppressed by flavonoids to inhibit diseases (Nirmal *et al.*, 2014). They possessed pharmacological activities such as antimicrobial, antiulcer, anticancer, anti-arthritic,

antiangiogenic, mitochondrial adhesion inhibition and protein kinase inhibition. Flavonoids showed protection for heart diseases by acting as heart stimulant, different types of cancers, aging associated cell degeneration and cure hepatotoxins by anti-inflammatory effects (Xu *et al.*, 2012).

1.4.1.3 Alkaloid

Alkaloid is an Arabic word and its meaning is "a/-qali, it is a nitrogenous compound normally obtains from those plants which have wide spread flowers (Kutchan, 1995). Alkaloids are the diverse class of phytochemicals; it has nitrogenous atoms either in quaternary or tertiary bonds and has alkaline properties. It exists among living organisms and consists of groups of different structures types, biological activities and biosynthetic pathways. It extracts from different sources includes microorganisms, animals and plants. In many medicines and drugs alkaloid has been used as a significant component (Itokawa *et al.*, 1997: Roberts and Wink, 1998). Pyrrolizidine alkaloids, caffeine, theobromine and theophylline are the examples of alkaloid; being an important class of secondary metabolites it played additional role in food products i.e. coffee, cocoa, mate, tea, chocolate and soft drinks (Ashihara and Crozier, 1999: Hartmann and Ober, 2000).

1.4.2 Saponins

Saponins are the important group of secondary metabolites and it found in plant species in large amounts. Plant's saponins are the basically members of triterpenes and steroid glycosides that have the active bio-components, biological and pharmacological properties (Dubois and Wagner, 2000: Szakiel *et al.*, 2011). Furthermore, it possesses the food based properties, pharmaceutical properties, hemolytic and fish toxic properties because of amphiphilic character (Dubois, 2000).

1.4.3 Antioxidant potential of plants

Reactive oxygen species (ROS) interact with bio-molecules i.e. carbohydrates, protein, lipids and DNA converts the cis fatty acids into trans fatty acids that causes the several chronic diseases such as heart diseases, cancer, aging and chronic inflammations (Yasir *et al.*,2016). Plant contains natural antioxidants to scavenge free radicals by protecting cells from oxidative damage. Structures containing conjugated rings followed by hydroxyl groups make antioxidant potential or free radical scavenging potential by the process of hydrogenation or make complex to oxidizing species. Plants and plant based materials are rich in antioxidants. The antioxidants which are produced from natural sources (plants) have been found to be most effective than synthetic ones. Among antioxidants, great attention has been given to the flavonoids and phenolic compounds. There are so many compounds reported which inhibits or slow down the process of oxidation but plants have the great potential to cure the oxidative stress which causes the diseases (Cancer, diabetes, cardio vascular diseases, arthritis, AIDS and Alzheimer's diseases). Literature has been confirmed that the more consumption of fruits and vegetables decreases the risks of degenerative diseases (Ruch *et al.,* 1989; Babu *et al.,* 2001: Stankovic, 2011).

Quality and stability of food can be enhanced with the use of natural antioxidants; to reduce free radicals in the biological system, provide health benefits and can be used as nutaceuticals. Butylated hydroxyl anisole and butylated hydroxyl toluene are the synthetic antioxidants and their use has been reduced because of their injurious effects. Hence, there is a need to explore new sources of natural antioxidants (Seal, 2012).The presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases for example; ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders. Medicinal plants are therefore being investigated for their antioxidant properties, and their demand for natural antioxidants and food preservatives is increasing (Baba and Malik, 2015).

1.5 Diversity of Medicinal plants in Pakistan

Pakistan has inimitable biodiversity and subtropical climate. Approximately 6000 higher plant species are present in Pakistan. Nearly, 720 floral species have the medicinal value and out of these, 500 species have been investigated to have bio-active constituents. Plants diversity followed by variations among plant people interaction have been influenced by wild plants selection for the sake of food and some cultural uses. Ethno-botanical investigation aims to document the indigenous information linked with the plant species (Abbas *et al.*, 2016). About 66 % people in Pakistan lived in rural regions and out of it, majority of the population is poor and unable to meet their health needs due to unaffordable drugs. In remote areas people use herbal plants as a drug in different ways against various diseases. The pharmaceuticals prepared drugs which have the active constituents from plant materials and used to control different diseases. The discovery of active plants and their elucidation guide us to progress in alternative and complementary medicines for the treatment of various diseases. Some specific plants have been explored from the different areas of Pakistan like *Vincetoxicum arnottianum* (Wight) Wight use against different diseases such as inflammation, diarrheal, diabetic and spasmodic, it also have the antibiotic and antioxidant activities, clinically reported from Kashmir, Hazara, Shinkiari, Swat and Kaghan (Mahmood *et al.*, 2013).

Berberis orthobotrys Bien. ex Aitch. is an important shrub used to treat stomach disorders, uterine tumor, kidney stones, wounds, blood purification, gastrointestinal diseases, ulcers, jaundice followed by animal model (cardiac depressant and antihypertensive activity) and it has been reported from Astor, Gilgit, Baltistan, Skardu and Chitral. *Onosma hispida* is a medicinal herb and it has present in different areas such as Baluchistan, Chitral, Hazara, Swat and Gilgit, and used against typhoid fever, pneumonia, ulcers, kidney stones, hair problems, rheumatism leads to antibacterial activity and it also has the variety of phytochemicals. *Caccinia macranthera* (Banks & Sol.) Brand. is found from Balochistan and used to cure the liver disorders, dermal infection and cough followed by anticancer activity in vivo study. But very little efforts have been made to explore the medicinal properties of plant species of Pakistan (Tariq *et al.*, 2016: Engel *et al.*, 2016).

1.6 Medicinal plants of Murree hills

In Pakistan medicinal plants from many areas has been reported for their herbal uses but there is a still need to explore more medicinal plants and their uses against different diseases (Aziz *et al.,* 2016). Murree is considered as the important part of Himalayan region due to the presence of vast biodiversity of medicinal plants. Local people still use the traditional phytotherapy and visit to "hakeem" or "herbalists" for their primary health solutions. These herbalists have great knowledge of symptoms of different diseases which appears on human body and they use local plants to treat the diseases (Abbasi *et al.,* 2013).

In few studies researchers try to explore this area but there is no study documented regarding current work which focuses on the important biological studies of these plants. This study includes ethno-botanical information based ethno-pharmacological studies i.e. hepatoprotective, anticancer, antituberculosis, anti-hemolytic activity, cytotoxic, antitumor, antioxidant, antibacterial activities and quantification of active compounds by using HPLC, GCMS and FTIR techniques.

Plate.1. Medicinal plants: panoramic view at Murree hills

Plate.2. Floral diversity of plants in Murree hills

1.7 Background justification of Present project

The ineffectiveness and side effects of modern medicines against different diseases shifts the trend from modern to traditional medicines. Modern medicines are the fast heal ailments but they left toxic effects on different organs of human body and these medicines are out of reach of lay man because of their prices. Currently, numerous studies have been conducted for the discovery of new and novel medicinal plants. Medicinal or herbal plants are the burning topics due to the low cost, easy availability and reservoir of biological active compounds which exert pharmacological activities (Langrand *et al.*, 2014). The most dynamic secondary metabolites in medicinal plants are the Phenolic compounds which include phenols, flavonoids, saponins followed by alkaloids, triterpenes, monoterpenes, coumarins, vitamins and minerals. Phenols and flavonoids are present in medicinal plants and play an important role in the prevention of different diseases. These compounds possess antioxidant activities and in plants they scavenge oxidants such as reactive oxygen species and peroxidases, and this property make plant defensive system strong. Those plants help to prevent against different diseases including cancer, liver and heart diseases (Chiu *et al.*, 2002: He *et al.*, 2015).

Oxidants produced by the process of oxidation in human body are basically the toxicant which damages the normal functioning of different organs such as liver and kidney, and leads to cancer. Medicinal plants contains rich amounts of oxidant scavenging properties that's why these plants are used to remove toxicants from liver, kidney, brain and other organs of animal models. Toxicity of liver or liver infections (Hepatitis) resulted in elevated levels of liver enzymes such as ALT, ALP and AST, and use of herbal or medicinal plants has been proved to reduce these levels (Wu *et al.*, 2012: Lei *et al.*, 2015).

The plants or plant compounds have been used against cytotoxicity and the cytotoxic effects of required plants can be evaluated on the brine shrimps as an initial step before

examination of cancer cell lines. Expensive and painful treatment of cancer people starts to use and depend on traditional medicines for its control. Now days a lot of plants and compounds isolated from plants have been used against different cancer cell line in in-vitro studies. Tuberculosis imparts very adverse effects on human body and can cause death. So, researchers are focusing on the natural resources to cure tuberculosis and bacterial infections. Plants show pharmacological and biological activities due to the existence of bioactive compounds in their parts which require being quantified or characterized. So, some techniques have been used to explore different bioactive compounds such as GC-MS (Gas chromatography-Mass spectrometry), HPLC (High performance liquid chromatography) and FTIR (Fourier Transform Infrared Spectroscopy). GC-MS is used to analyze the compounds and fatty acid profile which exhibits the beneficial effects to human health, and also useful for the production of bio-fuel. HPLC is basically high performance liquid chromatography and it uses to analyze the chemical compounds including quercetin and many other phenolic compounds quantitatively. Quercetin compound is a type of flavonoid which has the properties of antioxidant. FTIR is an important technique known to use for detecting the functional groups of chemical compounds present in the plant extracts.

Murree hills are diversified area which has the number of medicinally important plants. These medicinal plants are in used to cure human as well as animal's diseases and also use for food, feed and timber. A systematic study was conducted on the medicinally important plants of Murree hills by conducting ethno botanical surveys. After the detailed study of area which includes the collection of ethno-pharmacological data regarding different diseases summed up in to 7 plants which are in use dominatingly by the local communities. These plants have been further scrutinized for the different biological activities.

1.7.1 An overview of targeted Medicinal plants

1.7.1.1 *Debregeasia salicifolia* (D.Don) Rendle

Debregeasia salicifolia is a small shrub and belongs to family Urticaceae. It contains numerous compounds (oleanolic acid, ursolic acid, triterpenes, lupeol, tormentic acid,

pomolic aid and stigmasterol) with medicinal characteristics such as antifungal and antimicrobial properties against skin diseases (Nisa *et al.*, 2011: Zulqarnain *et al.*, 2015).

1.7.1.2 *Indigofera heterantha* Wall. ex Brandis

Indigofera heterantha commonly known as *Indigofera Himalayan* is a member of family Fabaceae and it is a deciduous shrub. This genus possesses abundant of phytochemicals like triterpenes, flavonoids and steroids. It records of bioactivities are existed such as phytotoxicity (Uddin *et al.*, 2011: Rahman *et al.*, 2014).

1.7.1.3 *Rumex hastatus* D.Don

Rumex hastatus is commonly recognized as khatimal and it belongs to family Polygonaceae native to northern Pakistan. Whole plant of rumex has been reported to be used as drug. This plant acts as tonic, laxative and alterative by healing rheumatism, piles, lungs bleeding, skin diseases and bilious complaints. It use against various diseases in the form of different formulations. There is an evidence to use rumex for the treatment of AIDS, blood pressure and various diseases. Different compounds have been isolated from it especially phenolic compounds such as hastatusides (A/B), rumexoside, rutin, nepodin, resveratrol and orientaloside (Sahreen *et al.*, 2013: Ahmad *et al.*, 2015).

1.7.1.4 *Myrsine africana* L.

Myrsine africana L. belongs to family Primulaceae and this consists of 35 genera and 1000 species. It includes mesophytic trees, shrubs, lianas and woody plants along with berry fruits. It is traditionally used in tea, carminative, spices, appetizer and flavoring agent. Fruits and aerial parts of this plant were utilized as anthelmintic against anti-tumor, phytotoxic,

diarrhea, purgative, toothache, rheumatism, relieving hemorrhage, anti-helminthic, haemaglutination and pulmonary tuberculosis (Ahmad *et al.*, 2011; Abbhi *et al.*, 2011).

1.7.1.5 *Bauhinia variegata* L.

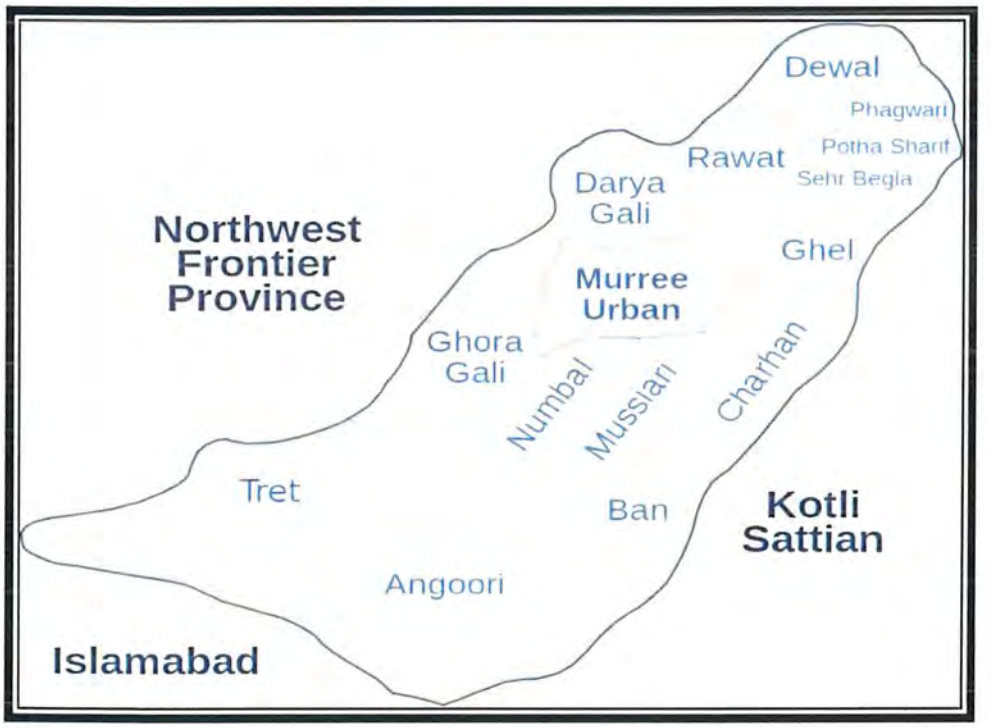
Bauhinia variegata belongs to family Fabaceae or Leguminosae and commonly called as cow's hoof and cow's paw because of their leaves shape. They are commonly present in tropical countries and mainly in Asia. It is commonly used in conventional medicines for the remedial purposes against various ailments such as inflammatory diseases, diabetes, pain and infections. This plant was investigated in vivo and in vitro studies that proved that it possess biological properties. It is examined for phytochemicals screening and compounds isolation, and resulted in alkaloids, terpenes, steroids, quinones, aromatic compounds and lactones (Mali *et al.*, 2007: Filho, 2009).

1.7.1.6 *Vitex negundo* L.

Vitex negundo L. belongs to family Lamiaceae known as Banna in common and is a small shrub. This plant is widely present in Asia and Africa. Its fresh leaves posses to have anti-inflammatory, anti-itching, antihistamine, antiarthritic, antimicrobial, antifungal, anti-genotoxic and hepatoprotective properties. It is also blessed with wide range of secondary metabolites especially polyphenolic compounds including flavonoids, phenols, alkaloids, terpenoids, flavone and iridoid glycosides and these polyphenols act as antioxidants leads to prevent many chronic diseases (Dharmasiri *et al.*, 2003:Kumar *et al.*, 2010: Chandramu *et al.*, 2003:Sathiamoorthy*et al.*, 2007).

1.7.1.7 *Dodonaea viscosa* (L.) Jacq.

Dodonaea viscosa L. belongs to family Sapindaceae and it exists as a shrub or seldom small tree. It is commonly known as vilayati Mehndi and widely present in south East Asia. It is used as remedial medicine in different part of the world. It was traditionally used for the treatment of malaria, ulcer, diarrhea, sore throat, cold, itching, gum relief, tooth pain, soreness, fractures, rheumatism, gout and snakebite. Antibacterial, anti-insecticidal, anti-ulcer, anti-inflammatory and wound healing activities of this plant are proved experimentally (Jawahar *et al.*, 2004: Ramamurthy *et al.*, 2013).



Map.1 Geographic location of Murree hills in Pakistan

1.8 OBJECTIVES

The objectives are following as

1. To document the traditionally important medicinal plants for the management of various diseases.
2. To characterize the plants for the phytochemical screening by using HPLC, GCMS and FTIR.
3. To explore the bioactivities of selected medicinal plants by assessing *In vitro* antioxidant, antibacterial, anti-tuberculosis, anti-hemolytic and antitumor (potato disc assay) activity.
4. To examine the plant extracts against cytotoxicity assay (Cancer cell line and brine shrimp assay).
5. To investigate the *in vivo* hepatoprotective activities of plant extracts in mice.

CHAPTER 2

MATERIALS AND METHODS

2.1 Collection of plant materials

Samples of various parts of eight different plants were collected from Murree hills areas during March and April 2015. The samples were collected in fine plastic bags duly labeled with name, date and areas of collection. The samples were shade dried followed by sun and oven drying for overnight at 60° C. The dried samples were ground by using electrical grinder, sieve (80 meshes) and saved in the fine plastic bags for further uses.
The Plants samples were collected with the help of local informant and the samples were properly identified by expert taxonomist on the basis of previous literature, herbarium specimens and by different sites (Plant lists, flora of Pakistan). Cleaned specimens were subjected to drying followed by pressing, poisoning and mounting of plant species and then submitted to herbarium of Pakistan (ISL) for the future record.

Collection and utilization of the plants were on the basis of ethnobotanical knowledge gained by local healers. Study deals with the sustainable use of plant resources and highlighted the safer use of medicinal plants. Furthermore current study was conducted following the rules formulated for conventional biological diversity for the plants of this region.

2.2 Ethno botanical data documentation

Total 84 informants were interviewed during study, using method of semi structure questionnaires, meetings and group discussions about the medicinal plants those are being used by these peoples in their local areas were conducted. Out of 84 informants 50 were males and 34 were females. Their ages were ranged from 21 to 77 years. The medium of interview was Urdu and local language and during interviews information regarding local name, parts of plant used, herbal recipes and ethno-botanical uses of plants were recorded.

2.3 Data analysis

This ethno botanical data is subjected to various quantitative and qualitative parameters.

2.3.1 Qualitative analysis

The qualitative analysis (Part used, Life forms, Dominating families, Route of application, Route of administration and Recipe types) were calculated by the method of Ahmed, 2016.

2.3.2 Quantitative analysis

2.3.2.1 Used value (UV)

To assess the importance of the medicinal plants which have highest use against different ailments (Butt *et al.*, 2015).

UV = No of ailments/ No of informants

2.3.2.2 Relative frequency citation (RFC)

It was calculated to intricate the knowledge of traditional use.

RFC= FC/N (0<RFC<0)

Where RFC stands for relative frequency citation while FC is frequency citation (Species citation by number of informants) and N stands for total informant numbers (Ahmad *et al.*, 2016).

2.3.2.3 Informant consensus factor

It represents that how much plants has been used for the treatment of different diseases with in an area. The range of ICF values should be lies (0.0 to 1.0). Greater ICF is

information consistency and represents large of people that has been used enormous number of plants (Pandikumar *et al.*, 2011).

$ICF = n_{ur} - n_i / n_{ur} - 1$

2.4 Preparation of plant powder

Plant samples (*Myrsine africana* Leaves and Fruits, *Dodonea viscosa* Flowers, *Bauhinia variegata* Flower, *Debregeasia salicifolia* Leaves, *Vitex negundo* Leaves, *Rumex hastatus* Leaves, *Indigofera heterantha* Leaves) were collected from the different parts of Murree. Take the desired parts of the plant sample followed by shade drying. Grind it to a fine powder and sieved. After sieving place the sample in heating oven at 37°c to remove moisture for complete drying. After complete drying the powdered material was ready for the further analysis.

2.6 Preparation of Plant Extracts

Total 500 grams of samples were dissolved in methanol, ethanol chloroform and n-hexane. They were extracted by using Soxhlet apparatus and rotary evaporator techniques followed by shaking for overnight. Filtration of all the extracts was done so that the residue gets separated from the desired extracts. The percentage yields of extracts were calculated and dried extracts were stored in air tight vials for further processes.

2.7 Chemicals required

Methanol, ethanol, chloroform, DPPH, ABTS, Hydrogen peroxide, EDTA, Formalin, Xylene, Heamotoxylin, Eosin, KH_2PO_4 buffer, ALT Alanine aminotransferase, AST Aspartate aminotransferase, ALP alkaline phosphatase and bilirubin were purchased. All the solvents were of analytical grade. All chemicals and reagents used in this experiment were a grade and purchase from local dealers of Sigma aldrich and Merck.

Plate 3 & 4: Collection of medicinal plants

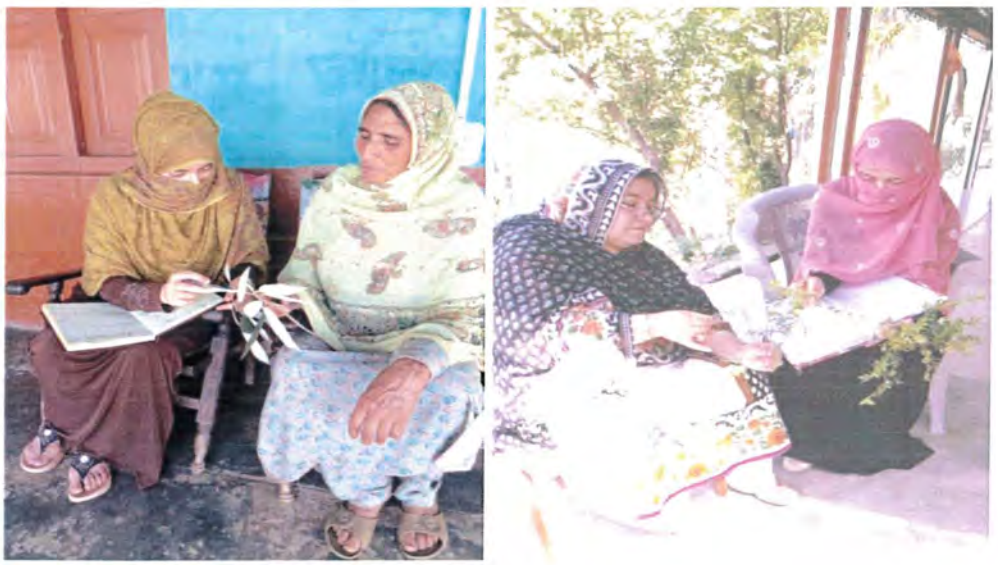


Plate 5 & 6: Documentation of traditional knowledge from local people

Plate 7: *Bauhinia variegata* L.

Plate 8: *Dodonaea viscosa* (L). Jacq.

Plate 9: *Myrsine africana* L. leaves

Plate 10: *Myrsine africana* L. fruits

Plate 11: *Indigofera heterantha* Wall.ex Brandis

Plate 12: *Rumex hastatus* D.Don

Plate 13: *Debregeasia salicifolia* (D.Don) Rendle

Plate 14: *Vitex negundo* L.

2.8 Qualitative and Quantitative analysis of Phytochemicals

Qualitative and quantitative estimation of flavonoids, phenols alkaloids, tannins, saponins, glycosides and steroids were carried out by the specific methods described by (Harborne, 1998, Siddiqui *et al.*, 2009: AOAC, 2003, Marinova *et al.*, 2005: Abbasi *et al.*, 2015).

2.9 Antioxidant Activity of Plants

2.9.1 DPPH Scavenging Activity

This method is assessed by using the modified protocol (Moon and Shibamoto, 2009). Different concentrations ranges from 20 to 100 µg/ml of plant sample along with 2ml of DPPH solution and left the mixture for 30 minutes in darkness. The reading was taken at 517nm. The activity was determined by the following formula

DPPH % = $[A^{\wedge} - A^{H}/A^{\wedge}]*100$

$A^{\wedge}$ is the reaction mixture absorbance except plant extract.

A^{H} is the reaction mixture absorbance having plant extract.

Ascorbic acid and Gallic acid were used as a Standard or Positive control. IC 50 was measured by linear regression analysis and defined as the 50% inhibition concentration of the extract.

2.9.2 Iron Chelating Assay

The method used is described by the (Dinis *et al.*, 1994). The plant extracts ranged from 20 to100 µg/ml were used for the chelating of Fe 2+. Then add 1ml of 2mM ferrous sulphate and with the addition of 0.25mM Ferrozine (1ml) reaction was initiated. After shaking, left the mixture for10 minutes and at 517 nm absorbance was recorded.

Chelating rate % = $[A^{\wedge} - A^{H}/A^{\wedge}]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

Ascorbic acid and Gallic acid were used as a Standard.

2.9.3 Hydroxyl Radical Scavenging Assay

Plants extracts ranges from 20 to100 µg/ml were investigated by the deoxyribose method (Nagai *et al.*, 2005). Add Sodium phosphate buffer (0.2M) of 7 pH followed by 2-deoxyribose (10Mm), FeSO 4 -EDTA (10Mm), H 2 O 2 (10mM), 525µl of H 2 O. Then add the mixture of TCA (2.8%) and TBA (1%) in Na OH. Absorbance was observed at 520nm. Ascorbic acid and Gallic acids are used as Positive control.

Scavenging activity = $[1- A^H /A^A]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

2.9.4 ABTS (2, 2- azinobis [3- ethylbenzothiazoline-6- sulfonate]) Radical Cation Decolorisation Assay

The method used is described by (Ashafa *et al.* 2010: Dehghan and Khoshkam, (2012). The mixture of 3mM (final concentration) ABTS (2, 2- azinobis [3- ethylbenzothiazoline-6-sulfonate]) with water was oxidized by adding potassium persulfate that is 2.5Mm. Place this mixture in dark for 12 hours. After diluting this ABTS + solution with distilled water absorbance should be 2.51±0.05.The plant extracts ranged from 20 to100 µg/ml. Absorbance was measured at 734nm. Ascorbic acid and Gallic acids were used as Standard.

Percent Scavenging potential = $[A^A - A^H /A^A]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

2.9.5 Reducing Power Assay

FRAP (Ferric ion reducing power) value was measured (Hazra *et al.*, 2008: Adedapo *et al.*, 2009). Different concentration of plant samples ranged from 20 to100 µg/ml was taken

with 0.2M phosphate buffer and potassium ferricyanide (0.1%). Incubate the mixture in water bath till 20 minutes. By adding trichloroacetic acid (10%) the above reaction was ended. The above layer of this solution was added in distilled water (2ml) followed by ferric chloride (0.01%) and incubated for 20 minutes. The readings of blank and samples were observed at 700nm. Ascorbic acid and Gallic acid were used as positive control. The results were expressed as GAE (mg/g of compound extracted).

2.9.6 Hydrogen Peroxide Scavenging Activity ($H_2 O_2$)

This activity was described as the method (Aiyegoro and Okoh, 2010). Add $H_2 O_2$ solution 4Mm (preparation inphosphate buffer) indifferent plant concentrations followed by incubation for 10 minutes. The absorbance was observed at 230nm.

Scavenging activity % = $[A^{\wedge} - A^{H} / A^{\wedge}]*100$

$A^{\wedge}$ is the reaction mixture absorbance except plant extract.

A^{H} is the reaction mixture absorbance having plant extract.

2.9.7 Superoxide Assay

The assay was used with some modified procedure (Beauchamp and Fridovich, 1971). Add 50mM Phosphate buffer, Riboflavin, 20Mm PMS, 0.5Mm NBT in various concentrations of plant samples and incubate for 20 minutes. The absorbance was recorded at 560nm.The positive control was Ascorbic acid.

Scavenging percentage = $[1- A^{H} / A^{\wedge}]*100$

$A^{\wedge}$ is the reaction mixture absorbance except plant extract.

A^{H} is the reaction mixture absorbance having plant extract.

2.10 Analysis of Flavonoids with High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography analysis of plant extracts were performed by using Shimadzu HPLC system (Tokyo, Japan) equipped with C18 column (250 mm × 4.5 mm, 5 m) gradient pump and UV/Visible detector. Crude extract (10 mg) was used with the help of HPLC grade methanol to prepare tested sample. The compounds were eluted by using gradient elution of mobile phases (Acetonitrile and 0.1% phosphoric acid; 36:64), and the injection volume for all samples was 10µl. Flavonoids were monitored at 280 nm and 285 nm at a flow rate of 1 ml/min with 20 min retention time. The quercetin was used as standard and all determinations were performed in triplicates.

2.11 Molecular docking

Molecular docking studies were conducted using GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1. The interaction of quercetin glycosides (ligand) with the crystal structure of human estrogen protein receptor (PDBID= 3ERD) was retrieved from the Protein Data Bank (PDB). The view of docking results and analysis of their surface with graphical representations were performed using Discovery studio visualizer (Ahmad *et al.*, 2016).

2.12 GC-MS

2.12.1 Analysis with Gas chromatography and Mass Spectrometry Analysis

GC-MS QP2010 model (Shimadzu®) using capillary column RTx- 5MS (cross bond 5% diphenyl – 95% dimethylpolysiloxane) with 30m x 0.25mm x 0.25 µm film thickness in the following conditions. Split less inject mode at 250 °C using helium as carrier gas. Column flow is 1.2 ml/min at a constant linear velocity mode. The column oven temperature program started 150 °C (hold 1 min) then programmed at 4 °C / min to 150 °C (hold 10 min). The temperature of injector was 275 °C carrier gas was N2 (1.0 mL/min), 0.2 µl injection volume and split ratio was 50:1. The mass spectra was operated electron ionization (70 eV) in Selected Ion Monitoring (SIM) mode. Ions were chosen (*m/z*) and various peaks were highlighted and compounds were identified from spectral data base of NIST library as suggested earlier by (Daferera *et al.*, 2000: Upadhyay, 2015).

2.12.2 Identification of compounds

Interpretation of mass spectrum GC-MS was conducted using database of National Institute Standard and Technology (NIST). The name, molecular weight and structure of the components of the test materials were determined. The percentage amount of each component was calculated by comparing it average area to total area. The spectrum of unknown components was compared with version 2005, software, and Turbo mass 5.2. Purpose was to find out individual compound or group of compounds which may demonstrate its current commercial and traditional uses.

2.13 FT-IR analysis of Plant Samples

The plant extracts were processed to infrared spectrum analysis by FT-IR (Fourier transform Infrared) spectroscopy shimadzu machine, IR affinity 1, Japan. The loaded samples were initially ground by KBr (1:100 w/w) followed by scan range (400-4000cm) with $4cm^{-1}$ resolution. Samples were subjected for structural characterization and are used to identify functional groups which are chemical bonds types (Zargar *et al.*, 2014).

2.14 Determination of antibacterial activities of extracts

2.14.1 Microorganism Tested

The following bacteria *Echerichia coli* (ATCC15224), *klebsiella pneumonia* (MTCC618), *Salmonella gallinarum*, *Staphylococcus aureus* (ATCC 6538), *Microcrocus lotus*, *Enterobacter auregens*, *Bacillus brevis*, *Pseudomonas aeruginosa* were tested for antimicrobial activity by Well diffusion method. Inoculum of microbes was made in Lauria-Broth gL- 1 and kept it in shaking incubator for 24hours (contained 10 8 cfu/ml) at 37 °C.

2.14.2 Well diffusion Method

Antimicrobial activity was done by well diffusion method (Ettebong and Nwafor, 2009) with some modifications. Media contained Lauria-Bertini (LB) agar and autoclaved the agar (121 °C) for 15 min. After cooling poured it in the Petri plates in laminar flow hood. Add 30 µl of inoculums in all the plates for inoculation. Then the disk is impregnated into the plates with sterilized forceps after dipping in the plant extracts (50 µl). Incubation of plates was done (37 °C) for 24 hours. The inhibition zones were observed after 24 hours and presented in millimeter.

2.14.3 Minimum Inhibitory Concentration (MIC)

MIC (Minimum inhibitory concentration) of the plant samples was examined by tube dilution method in Lauria-Bertini broth media. Each dilution (3.125-50 mg/mL) was inoculatedwith 5 x 10 6 of the bacterial strain culture incubated for 24 h at 37°C. The standard drug (Gentamicine) was used as positive control at same concentration. The absorbance of the suspension was observed at 420 nm along with the blank. The MIC was measured as lowest concentration of plant extract that inhibits the test cultures with no observable growth (Hemandez-Hernandez et al., 2017).

2.15 Anti-tuberculosis activity

The two drug resistant strains of *Mycobacterium tuberculosis* bg 206 and bg 1972, while a sensitive strain H37Rv were obtained from National TB Reference Laboratory, National TB Control Program Chak-Shahzad, Islamabad.Lowenstein-Jensen (LJ) medium was used in this experiment for the growth of isolates. Plant extract was incorporated in to culture medium and set for incubation at 37°C and for 40 days. Readings were taken in triplicate manner. Control was also taken and susceptibility of MDR strains was done against standard rifampicin. MIC is the lowest extract concentration and it was determined by using 96 well micro titer plates (Gemechu *et al.*, 2013: Ishikawa *et al.*, 2017).

2.16 Antihemolytic activity

The method reported by (Alinezhad *et al.*, 2013) with some modification was used to determine anti-hemolytic activates of plant extracts. The Methanol plant extracts were assessed against Human erythrocytes and absorbance was measured at 640 nm by using UV- Spectrophotometer and described as

Antihemolytic activity %= Control- Sample/Control*100.

2.17 Antitumor activity

Potato disc assay was adopted as described by (Hussain *et al.*, 2007), and results were described as Percentage inhibition (%) = 100- Number of tumors per sample/ Number of tumors in control*100.

2.18. Cytotoxicity Assay

2.18.1 Assessment of cytotoxicity (Brine shrimp assay)

Brine shrimps cytotoxicity assay was carried out to evaluate the cytotoxic effect of plant extracts by using the method reported by (McGaw *et al.*, 2007) with minor modifications and artificial sea water was used for the hatching of brine shrimp eggs.

2.18.2 Determination of cytotoxicity of extracts by using Sulforhodamine B (SRB) assay

The anti-proliferative SRB assay was performed to assess growth inhibition, this is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye. Finally unbound SRB was removed quickly by washing the wells five times with 1 % acetic acid and then air dried. Total 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker and optical density was recorded on ELISA reader at 515 nm as reported by (Skehan *et al.*, 1990).

2.19 In vivo Study (Hepato protective assay)

2.19.1 Selection and Purchase of Animals

Total 270 albino mice of either sex (body weight 55.2±2.5g) were purchased from the National institute for health, Islamabad. Guide lines of ethics committee about handling of experimental animals were followed. The animals were provided standard animals house conditions, fed with commercial mice chow (Feed Mills, Islamabad) and allowed water *ad libitum*. Animals were provided feed on different time duration but allowed free access to water. All animals were carefully monitored and maintained in standard house condition.

2.19.2 Acute oral toxicity study

Acute toxicity study was conducted to selected suitable doses of plant extracts for animals as earlier described by (Li *et al.*, 2017).

2.19.3 Experimental design

Animals were divided randomly in 10 groups of 5 animals in each group.

Group I: Normal control group, animals of this group was given normal feed up to 21 days.
Group II: Olive oil group, animals were provided 1 mL of olive oil with their feed upto 21 days.
Group III: Animals of this group were given only 1 mL/ kg b.w of CCl4 intraperitoneal for 21 days.
Group IV: Animals of this group were administrated 100 mg/kg b.w of methanolic flower extract after induction of CCl4.
Group V: Animals of this group were given 200 mg/kg b.w of methanolic extract after induction of CCl4.
Group VI: Animals of this group were provided 300 mg/kg b.w of methanolic extract after induction of CCl4.
Group VII: Animals of this group were given 100 mg/kg b.w of chloroform extract after induction of CCl4.

Group VIII: Animals of this group were administrated 200 mg/kg b.w of chloroform extract after induction of CCl4.

Group IX: Animals of this group were provided 300 mg/kg b.w of chloroform extract after induction of CCl4.

Group X: Animals of this group were administrated 100 mg/kg b.w of Silymarine after induction of CCl4 for 14 days. Doses of flower extracts were administrated to animals by gavages.

Animals from all groups were slaughtered on 21 days. Blood was obtained from the heart of mice and serum was separated by centrifugation at 3000 rpm for 10 minutes. After that both blood and serum were stored in freezer at -20°C before analysis. The body weight of animals were recorded, various organs of animals were taken out and washed properly, and stored in freezer till analysis.

2.19.4 Analysis of Blood samples

The biochemical activities of serum (ALT, AST, ALP and Bilirubin) were analyzed by using AMS diagnostic kits (Italy). RBC, WBC and platelets in blood sample were measured by using the method reported by (Dacie and Lewis, 1991).Whereas level of anti-oxidant enzymes (CAT, SOD, GPx) and total protein were measured by method proposed by (Aebi, 1984), (Misra and Fridovich, 1972), (Flohe and Gunzler, 1984) and (Lowry *et al.*, 1951).

2.19.5 Histopathological Study

The livers, kidney and spleen of mice were removed carefully after slaughtering and preserved in 10 % formalin in order to save them from damage. Dehydration of small part of tested specimen was done in alcohol ascending series lead to fix paraffin section. The tissue specimen was passed into two xylene changes followed to embed into molten paraffin. Sections of paraffin were cut down in 5µm thickness by using microtome and tissues were mounted on slides. For the purpose of histopathological studies tissue sections were stained by means of Ehrlich's hematoxylin with eosin counter stained as reported earlier by (Yakubu *et al.*, 2007: Giribabu *et al.*, 2017).

2.20 Statistical Analysis

Mean, standard error, probability and Pearson coefficient correlation were calculated by using statistical software prism pad 7.

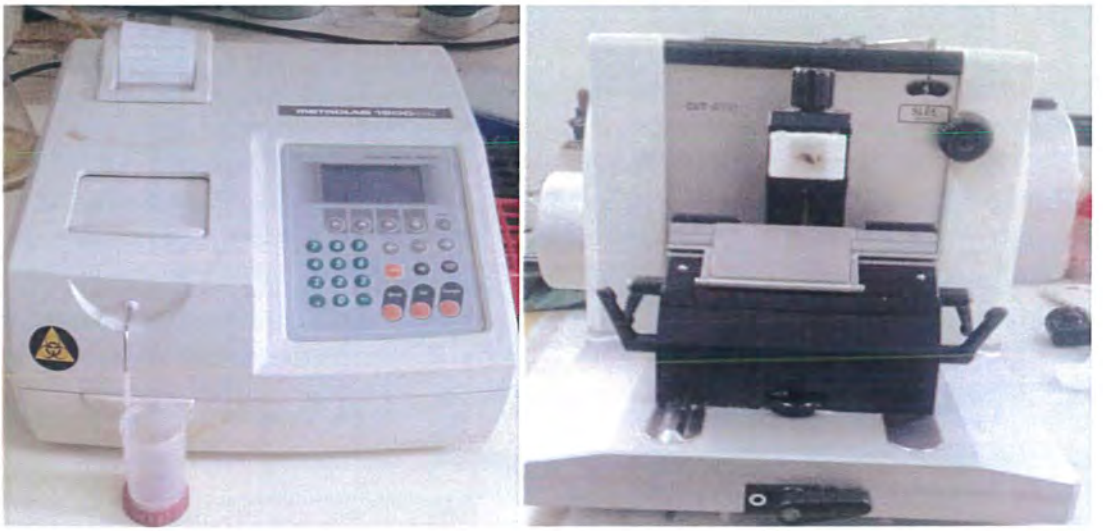


Plate 15 & 16: Serum analysis and preparation of histopathological specimen slides

Plate 17 & 18: Microscopic examination of slides and GC-MS analysis of phytochemicals

Plate 19 & 20: Preparation of microbial media and treatment during in vivo study

Plate 21& 22: Oral administration of plant extract and dissection of mice

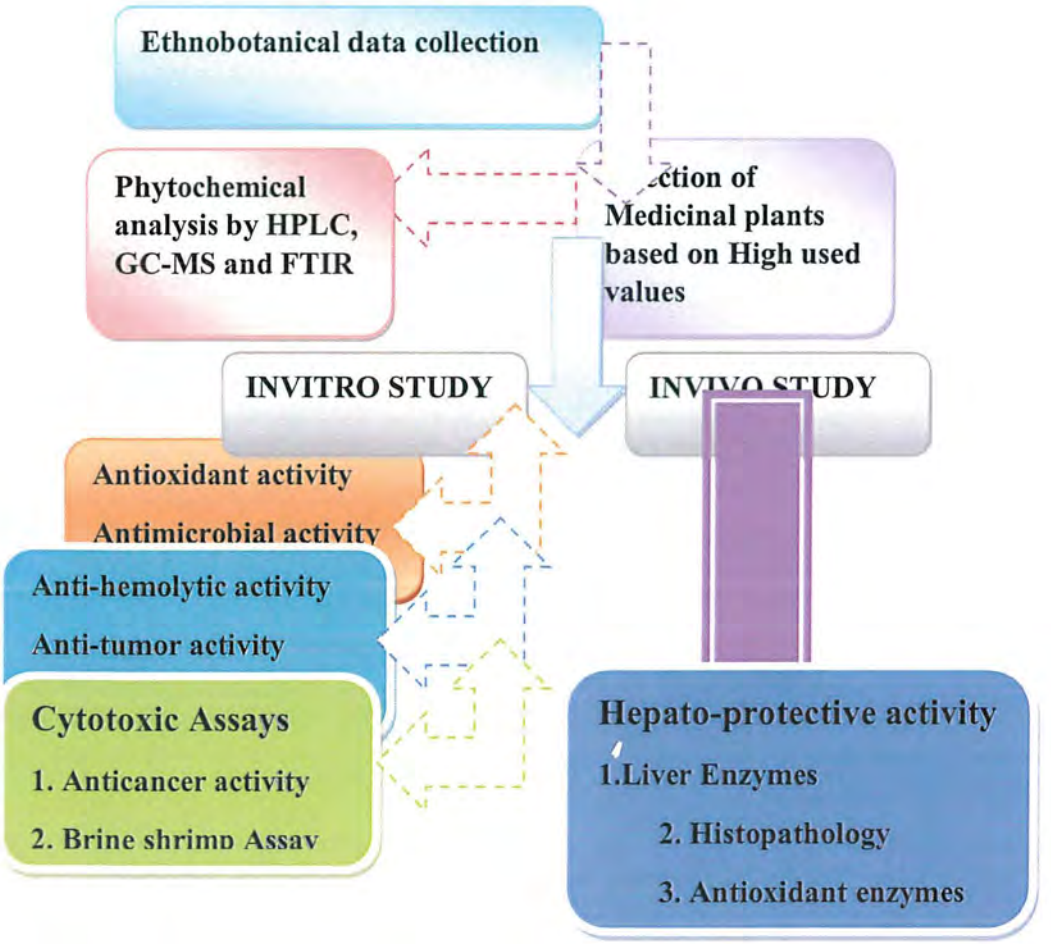


Plate23: Diagrammatic view of Ethnobotanical and pharmacological techniques used

ANNEXURE 1

ETHNOPHARMACOLOGICAL DATA COLLECTION QUESTIONNAIRE

Informant demographic data:

Gender of Informant: ----------

Age of Informants: ----------

Informant Education: ---------- Date: ----------

Ethno botanical information of Medicinal plants:

1) Plant botanical name: ----------
2) Local name: ----------
3) Voucher number: ----------
4) Locality: ----------
5) Flowering season:----------
6) Part of Plant used:----------
7) Method of administration:----------

Open ended questions

1. **Which plant have you used very frequent for the medicinal purposes?**

   ---

2. **For which disease do you use the plants?**

   ---

3. **How do you utilize the plants?**

ANNEXURE 2

Medicinal plants traditional uses based Questionnaire

1) Plant botanical name: ----------
2) Plant synonym: ----------
3) Local name: ----------
4) Voucher number: ----------
5) Locality: ----------
6) Plant flowering period: ----------
7) Collectors(Male/Female/Children):----------
8) Plant Collection technique: ----------
9) Conservation status: ----------
10) Medicinal uses:----------
11) Part of Plant used:----------
12) Fresh/ Dried form Use: ----------
13) Use (Powder/decoction/Infusion/Paste):----------
14) Utilization (External/Internal): ----------
15) Traditional recipe:----------
16) Duration of plant consumption----------
17) Approximate dose----------
18) Relief after consumption----------
19) Useful or not useful----------
20) Recommend or not----------
21) Other uses----------
22) Knowledge----------

Remarks

Botanical name of plant ----------

Family name of plant----------

Identified by ----------

Signature of Researcher----------

CHAPTER 3

RESULTS

SECTION 1

ETHNOMEDICINAL FINDINGS

Medicinal plants are various valuable traditional medicines; those are playing an important role for the curing of various diseases of people from both developed and developing countries especially inhabitants residing in the rural areas. These medicines heal up the infectious person with lower cost medicines as compared to costly modern medicines. In the current study attempt has been made to collect information's regarding the ethno-botanical and ethno-pharmacological uses of medicinal plants.

3.1 Qualitative analysis

3.1.1 Ethno-botanical data documentation

The plants were excessively collected from wild habitat however few of them were from the cultivated habitats. The medicinal plants documented in this study were 70% wild and 30% cultivated. Herbs have been reported 41% followed by shrubs (31 %) and trees (28%). The herbs were among the frequent used habitats followed by shrubs and trees.

3.1.2 Taxonomic diversity

During this study 131 species, 104 genus and 64 families were recorded. Highly reported family found in this study was Asteraceae (19%) with 10 species followed by Moraceae (11%) with 8 species, Rosaceae, Lamiaceae and Amaranthaceae (10%) with 5 species and Acanthaceae, Brassicaceae, Convolvulaceae, Euphorbiaceae and Solanaceae (8%) with 4 species (Fig. 1). Whereas Malvaceae, Mimosaceae, Oleaceae, Polygonaceae, Rhamnaceae, Sapindaceae and Verbenaceae each was found with 3 species. Adiantaceae, chenopodiaceae, Caesalpinaceae, and Flacourticaea were reported with 2 species. Aizoaceae, Asclepiadaceae, Commelinaceae, Celasteraceae, Fagaceae and Fumariaceae were reported with 1 species.

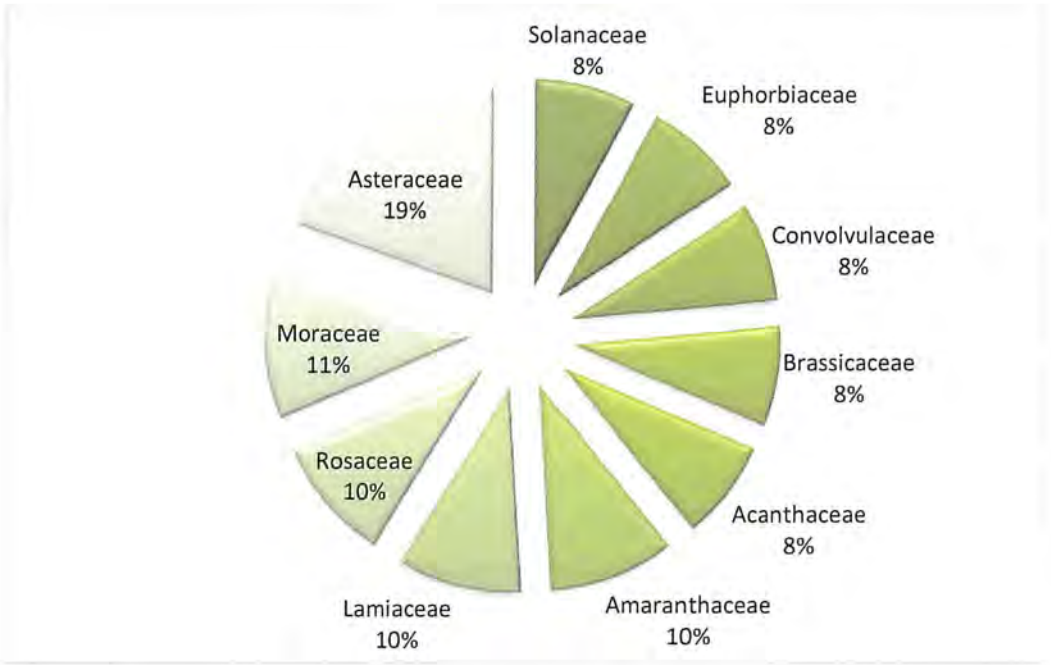


Fig.1.Percentage (%) of various Families used in study area

3.1.3 Plant Parts

The parts of plants commonly utilized for medicines are Leaves (76), Whole plant (39), Fruit (29), Branches (24), Seeds (14), Bark (7), Wood (7), Flowers (6), Gum (5), Shoots (3), Stem (2), Berries (1), Legume (1), Bud (1), Lumentum (1) and Pulp (1). The majority of plant remedy found was decoctions (56), powder (38), paste (20), extract (14) and juice (5).

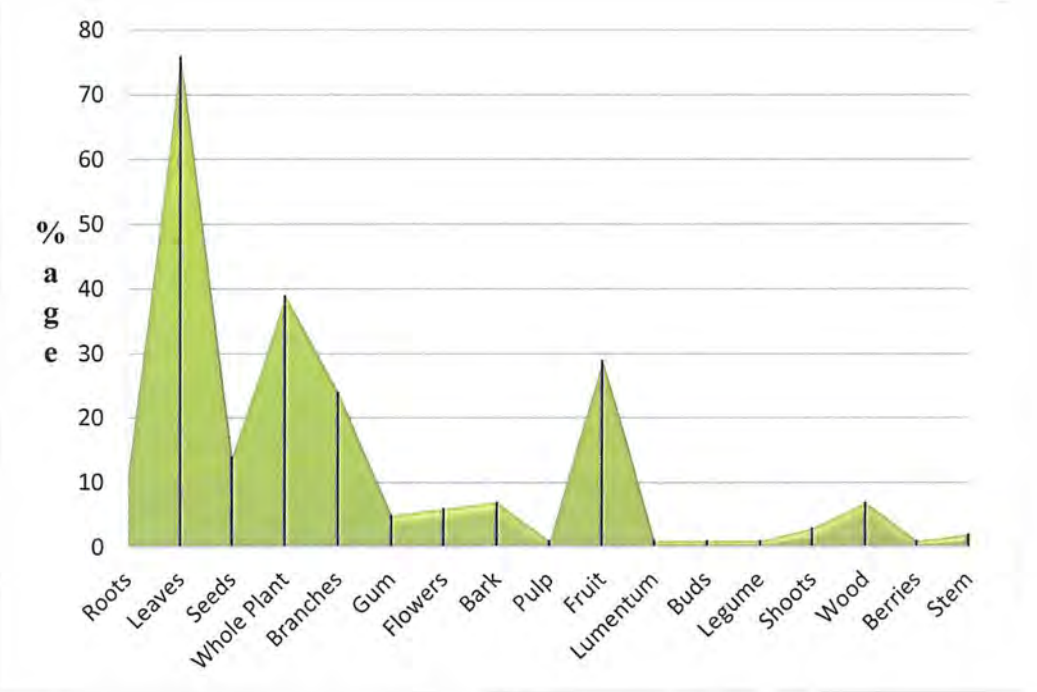


Fig.2.Parts of plants frequently used in local medicines

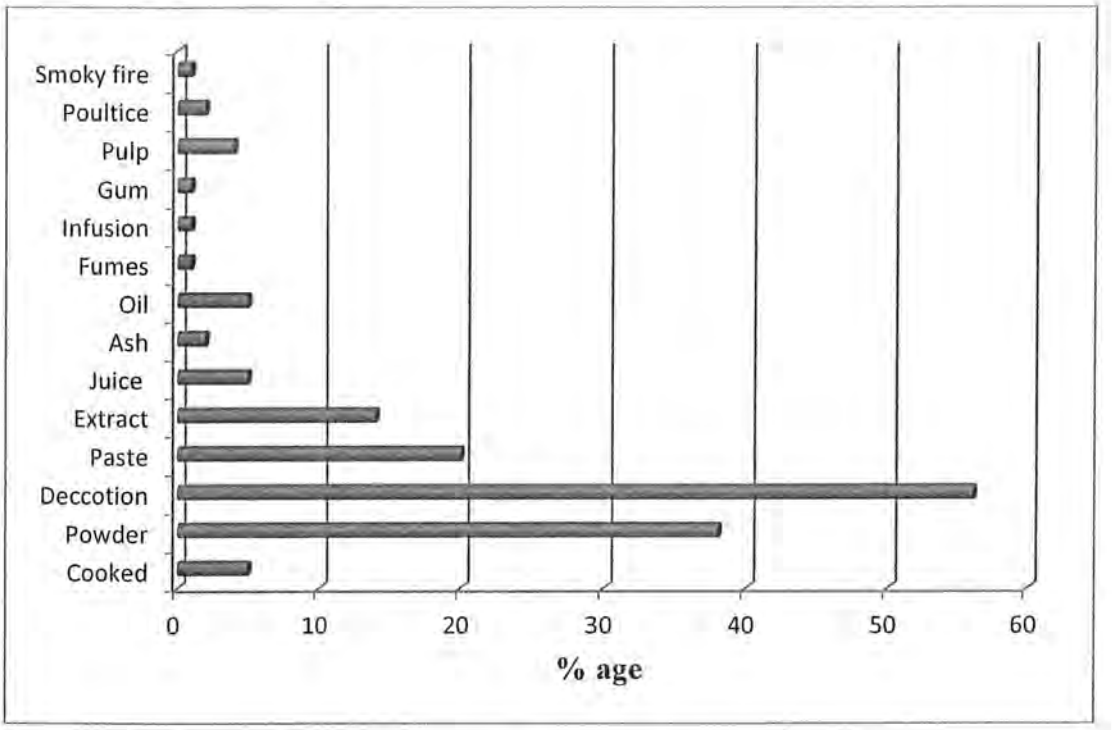


Fig.3.Methods used for remedy preparation

3.1.4 Informant's profile

Total informants involved in the ethno-botanical study were found to be 84. Out of 84 informants, 50 males and 34 females were reported. The age of total informants was between 17 to 70 years whereas female informants found to have more knowledge in early age as compared to male especially between 17-20 years.

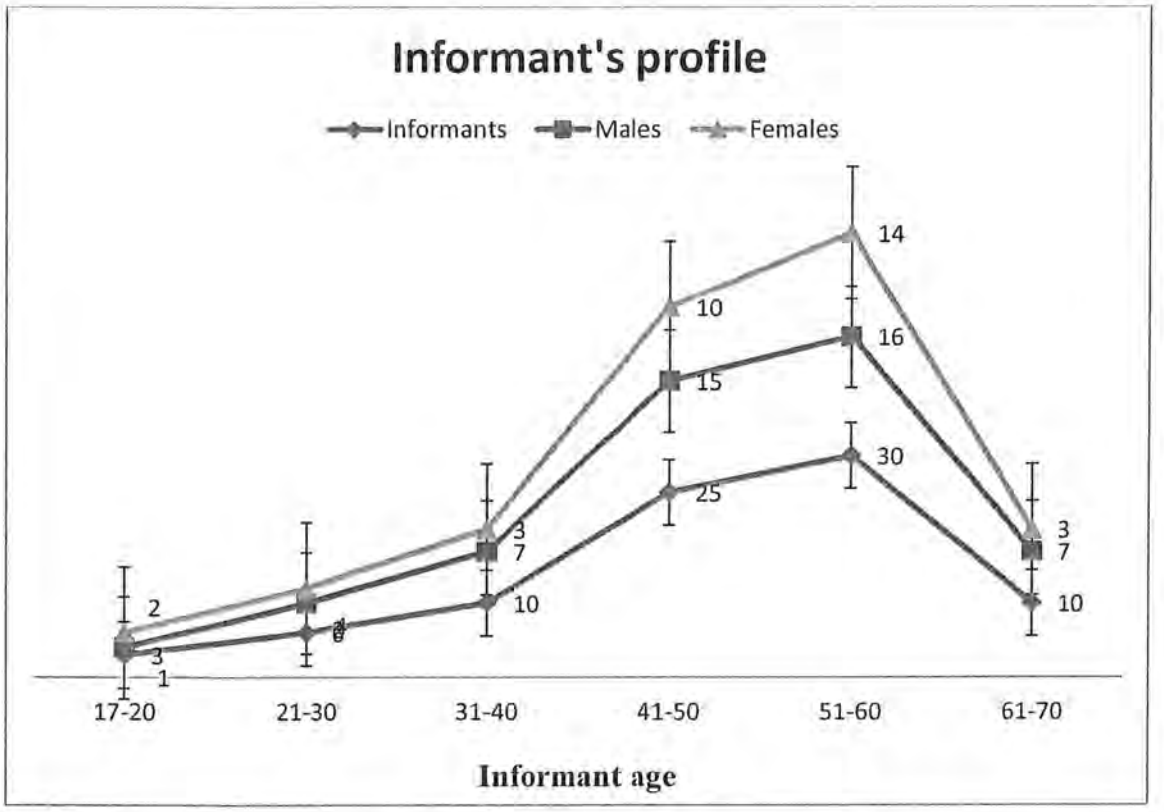


Fig.4.The profile of informants conducted in the study area

3.2 Quantitative analysis

3.2.1 Medicinal uses (ICF)

Informants were knowledgeable regarding ethno-medicinal uses against disease categories. The dominating plants were used for gastrointestinal problems (64) followed by dermatological disorder (39), glandular disorder (33), Muscular-skeletal disorder (21), respiratory disorder (20) (Fig.5).

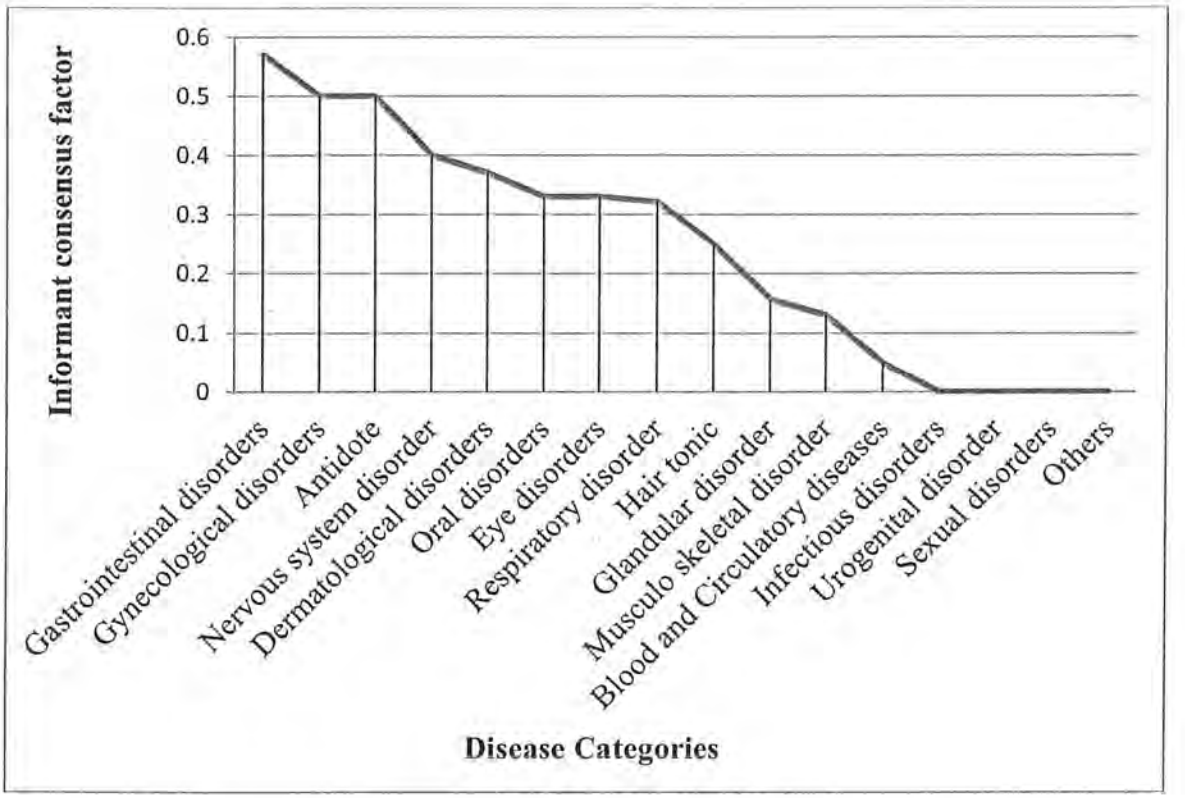


Fig.5.Management of diseases with medicinal plants in the study area

3.2.2 Most cited plants (UV)

Myrsine africana and *Dodoneae viscosa* were the commonly used plants having higher used value (0.90) and have been used for the management of many diseases (Fig.6.). *Debregeasia salicifolia* and *Bauhinia variegata* has been widely used by local people against different ailments with having used value (0.88).

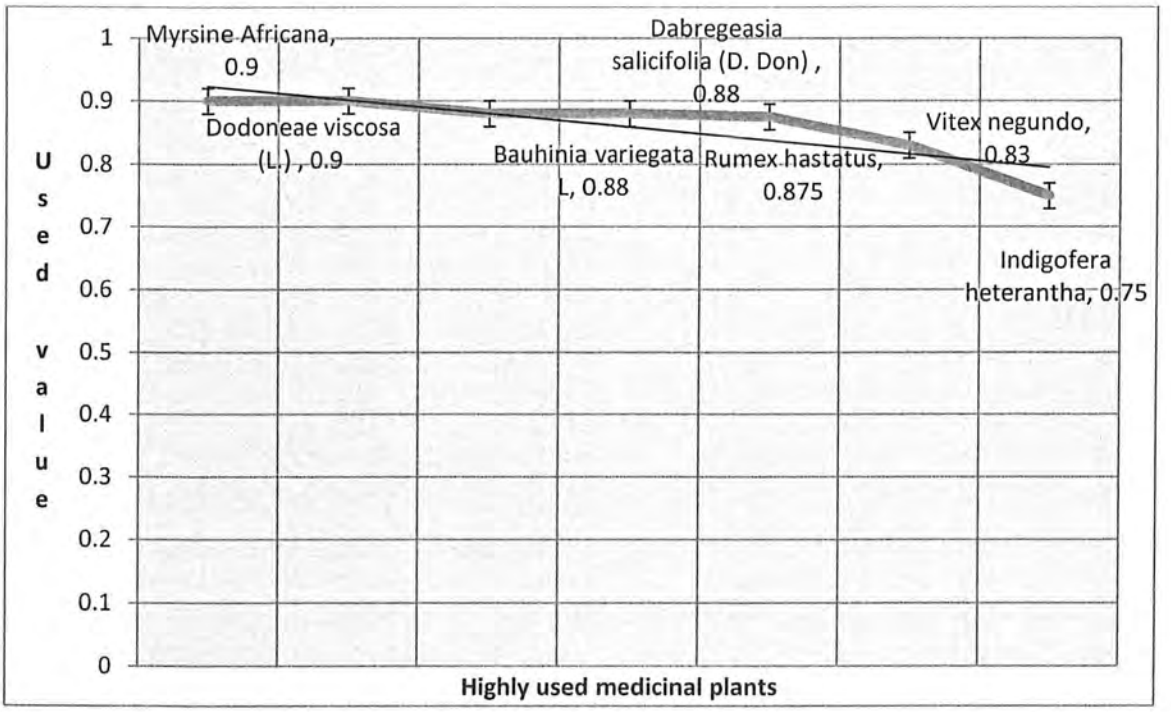


Fig.6.The higher use values of selected medicinal plants

Table.1. Ethnopharmacological documentation of Medicinal plants

Botanical name	Local Name	Family	Habitat	Life form	Part used	Mode of utilization	Diseases	USES	UV
Achyranthes aspera L. var. aspera	Puth kanda	Amaranthaceae	H	W	R/ Lv	Pw	Tooth ache, Digestive problem, Bloody Diarrhea	3	0.6
Acacia catechu (Linn.f.) Willd.	Kikar	Mimosaceae	T	W	Br/G		Tooth cleaner, Tonic	2	0.14
Acacia modesta Wall.	Phulahi, Reenn	Mimosaceae	T	W/Cu	G	G	Jointpain, Backache, Diabetes, Leucorrhea,	4	0.23
Acacia nilotica(L.) Willd. ex Del.	Jangli kikar, Babool	Mimosaceae	T	W	Fl, Lu, B, G, R	Pw	Sexual diseases, Leucorrhea, Tooth problems, Arthritis, Back pain	5	0.41
Adhatoda zelyanica Medic.	Bhaikar	Acanthaceae	Sb	W	Wh	D, A, P	Blood purifier, Cough, Diabetes, Hepatitis, Respiratory disorders, Sweating smell, Pimples and pustules	7	0.36
Adiantum capillus-veneris L.	Hansraal	Adiantaceae	Sb	W/Cu	Lv	D	Cough, Bronchitis	2	0.33
Adiantum incisum Forssk	Pakhi	Adiantaceae	Sb	W/Cu	Wh	P	Heat stroke, Hepatitis, Liver disorders	3	0.6
Aesculus indica (Wall. ex Camb.) Hook.	Bankhor	Hippocastinaceae	T	W	S	Pw	Digestive problem, Acidity	2	0.66
Aloe barbadensis Mill.	Ghee gawar, Kanwaar gandal	Liliaceae	H	W	Lv, Pl	Pl	Diabetes, Backache, Piles, Ulcers, Acnes, Increase milk production in cattles	5	0.26
Aloe vera (L.) Burm.f.	Kanwar gandal	Aloaceae	H	W/Cu	Wh	Pl	Skin tumors, Skin diseases, Hair fall	3	0.21
Alternanthera pungens Kunth	Lundri	Amaranthaceae	H	W	Wh	Pw	Hepatitis A	1	0.2
Ajuga bracteosa Wall. ex Benth.	Kauri Booti	Lamiaceae	H	W	Lv	D	Hepatoprotective, Stomach relieving, Anxiety, Diarrhea	4	0.16
Amaranthus ovalifolius L.	Choleri	Amaranthaceae	H	W	Lv	D	Constipation, Soothing effect	2	0.33

Amaranthus virdis L.	Cholai	Amaranthaceae	H	W	Lv	D	Antiseptic, Constipation, Soothing effect, Scorpion bite, Snake bite	5	0.23
Artemisia dubia Wall. ex Besser		Asteraceae	Sb	W	Wh	D	Malaria, Anthelminthic	2	0.66
Artemisia japonica Thunb.		Asteraceae	Sb	W	Lv	D	Digestive problems, Hypertension	2	0.66
Artemisia roxburghiana Wall. ex Besser		Asteraceae	Sb	W	Wh	Pw	Brain Tonic, Liver uses, Epilepsy	3	0.6
Artemisia scoparia Waldst. & Kit	Chahoo	Asteraceae	Sb	W/Cu	Lv	D	Skin diseases, Malaria	2	0.5
Asparagus adscendense	Sumbloo, Sufaid Musli	Asparagaceae	H	W	R	Pw	Vomiting, Motion	2	0.66
Asphodelus tenuifolius Cav.	Piazi	Liliaceae	H	W	Wh	C	Arteriosclerosis, Antiseptic	2	0.33
Barleria cristata L.	Kal pouth	Acanthaceae	Sb	W	Wh	D	Antipyretic, Fever, Respiratory tract diseases	3	0.21
Bauhinia variegata L.	Kachnar, Kuliarh	Caesalpinaceae	T	W/Cu	Bd, Fl, Br	Pw/D	Digestive problems, Brain Tonic, Liver problems, Laxative, Antidiabetic, Heart diseases, Abdominal cramp, Bloating	8	0.88
Berberis lycium Royle	Sumbal	Berberidaceae	Sb	W/Cu	R	D	Wound healing, Bone fractures	2	0.15
Bergenia ciliata (Haw.) Sternb.	Bhat-Phay	Saxifragaceae	H	W	R	D	Cardiovascular diseases, Diabetes, Skin diseases	3	1.5
Bergenia stracheyi (Hook.f. & Thomson) Engl.	Bhat-Phay	Saxifragaceae	H	W	R	D	Digestive track ulcer, Healing wounds	2	0.28
Bombax ceiba L.	Sanbal	Bombacaceae	T	W/Cu	B	D	Healing wounds	1	0.33
Broussonetia papyrifera (L.) L'Hér. ex Vent.	Jangli Toot	Moraceae	T	W	Wh	Pw	Fuel Wd	1	
Calotropis procera (Aiton) W.T.Aiton	Ak, Gul e madaar	Asclepiadaceae	Sb	W	Wh	D	Pimples and pustules, TB, Blood pressure, Jointpain, Body heat, Wound healing	6	0.12

Carrisa opaca Stapf ex Haines	Garanda	Apocynaceae	Sb	W/Cu	F,Lv, R	D,J, Pw	Hepatitis A, Respiratory diseases, Liver diseases, Diabetes, Purify Blood, Blood thinner	2	0.11
Cassia fistula L.	Amaltas	Caesalpinaceae	T	W	F	Pl	Constipation	1	0.1
Cannabis sativa L.	Bhang	Cannabinaceae	Sb	W	Lv, S	D	Antiseptic, Epilepsy, Diarrhea, Antipyretic, Sedative	5	0.71
Cardiospermum halicacabum L.	Sat-barga	Sapindaceae	H	W	Wh	D	Heart problems	1	0.5
Cedrus deodara (Roxb. ex D.Don) G.Don	Diar, Deodar	Pinaceae	T	W/Cu	St	D	Stimulate sexual desires	1	0.12
Chenopodium album L.	Bathu	Chenopodiacea e	H	W	R, Br, Lv, S	P, O	Jaundice, Hepatoprotective, Urinary tract problems, Soothing effect, Worm killer	5	0.31
Chenopodium ambrisoides (L.) Mosyakin & Clemants	Chandan Bathu	Chenopodiacea e	H	W	Wh, S	D, P	Back and Jointmassage, Cough, motions, Diuretic, Stomach tonic, Swelling	6	0.35
Cotinus coggygria Scop.	Bhann, Phann.	Anacardiaceae	Sb	W	Br,Lv		Fuel, Fodder	2	
Cichorium intybus L.	Kaasni	Asteraceae	Sb	W	Wh	D, P	Liver diseases, Blood purifyer, Blood thinner	3	0.6
Cissampelos pareira L.	Pla jarrhi	Menispermacea e	H	W	Lv	E	Diarrhea, Digestive problems	2	0.66
Commelina benghalensis L.	Kana Keerai	Commelinaceae	H	W	Wh, Lv	D	Ulcer, Lesion, pimples	3	0.15
Convolvulus arvensis L.	Lehli	Convolvulaceae	H	W	Wh, Sh	E	Soothing effect, Remove worms, Skin diseases	3	0.42
Coronopus didymus (L.)Smith	Thandi Booti	Brassicaceae	H	W	Wh	Pw	Purgative effect, Insecticidal	2	0.66
Cupressus sempervirens L.	Saru	Cupressaceae	T	W	S	Pw	Piles	1	0.5
Cynodon dactylon (L.)Pers.	Khabbal ghaas	Poaceae	H	W/Cu	Wh	Po	Outer injury	1	0.1
Cynoglossum denticulatum	Pitrus	Boraginaceae	H	W	R	Pw	Intestinal worms, Colic pains, Purify Blood,	4	0.66

A.DC.	(KaSbmiri)						Pimples and pustules		
Dalbergia sissoo Roxb. ex DC.	SbiSbum, Taali	Papilionaceae	T	W/Cu	Wd, Lv	Po, D	Pimples and pustules, Hair fall, Fuel, Timber purpose	4	0.125
Dodonaea viscosa (L.) Jacq.	Sanatha	Sapindaceae	Sb	W	Lv	D, Pw, E	Respiratory ailments, Itching, pustules, Hepatic problems, Cough, body Relaxant, Throat sores, Asthma, Lungs problems	9	0.9
Dryopteris ramosa (C. Hope) C	Pakha, Pakhi	Dryopteridacea e	Sb	W	Lv	C	Stomach problems	1	0.1
Dabregeasia salicifolia (D.Don) Rendle	Sindwaar	Urticaceae	Sb	W	Lv, Br, F	E	Fodder, Extreme Diarrhea, Stomach problems, Hepatitis, throat problems, Vomiting, Dysentery, Arthritis, Joint pain	8	0.88
Dicliptera roxburghiana var. bupleuroides (Nees) C.B.Clarke	Andhoosi	Acanthaceae	Sb	W	Lv, Fl, Wh	Pw	Body Tonic, Heart strokes in Buffaloes	2	0.4
Diosypros lotus L.	Amlok	Ebenaceae	T	W/Cu	F	D	Stomach ailments	1	0.12
Duchesnea indica f. japonica (Kitam.) M.MizuSb.		Rosaceae	H	W/Cu	Wh, F	P	Diarrhea, Fodder	1	0.5
Echinops echinatus Roxb.	Hand, Barhong	Asteraceae	H	W	Lv	D	Swelling, Fodder	1	0.14
Emblica officianalis Gaertn.	Amla	Euphorbiaceae	T	W/Cu	F, Lv, Wd	D	Digestive system, Cooling effect, Liver diseases, Goat grazing, Fuel	3	0.15
Eruca sativa var. vesicaria (L.) Coss.	Tara Meera	Brassicaceae	H	W/Cu	Lv, Wh, Sh	O	Skin diseases, Constipation, Digestive ulcers, Fodder	3	0.12
Eucalyptus cammoldulensis Dehnh.	Safaida	Myrtaceae	T	W/Cu	Lv	Fu	Fever, Flu, Charge phone battery	3	0.3
Euphorbia prostrata Aiton	Thandi booti,	Euphorbiaceae	H	W	Wh	Pw, D, P,	Fever, Abdomen problems, Purify Blood,	6	0.21

	Makhni booti, Hazaar dani						Liver diseases, Skin diseases, Buffaloes fever,		
Ficus carica L.	Anjeer	Moraceae	T	W/Cu	F	Pw	Laxative, Piles, constipation	3	0.12
Ficus glomerata Blanco	Phagwarh	Moraceae	T	W/Cu	Lv, F, Br	C	Intestinal problems, Constipation, Fuel	3	0.13
Ficus variegata Blume	Tussi	Moraceae	T	W/Cu	F, Lv, Br	D	Digestive problems, Constipation, cooling effect, Fuel Wood	4	0.11
Flacourtia indica (Burm.f.) Merr.	Kokoh	Flacourtiaceae	Sb/T	W	F, Lv, Br	D	Diabetes, Fodder, Fuel Wood	3	0.14
Fumaria indica (Hausskn.) Pugsley	Sbahtra papra	Fumariaceae	H	W	Wh	J,Pw	Cough, Hepatic problems, Purify Blood, Soothing, Purgative effect	5	0.25
Geranium rotundifolium L.	Saanchal/ Gul bakhSb	Geraniaceae	H	W	Wh	D	Constipation	1	0.12
Grewia optiva J.R.Drumm. ex Burret	Tamman, Dhamman	Tiliaceae	T	W	Lv, Br, B		Quick discharge afterbirth in cattle, induce animals puberty, Making ropes, Fodder	4	
Hedera nepalensis K.Koch	Baleri, Albhambar	Araliaceae	H	W	Lv, Br	Pw	Diabetes	1	0.2
Indigofera heterantha Wall. ex Brandis	Kathi	Fabaceae	Sb	W	Lv, Fl, Wh	Pw, D	Liver problems, Respiratory problems, Bloating, Abdomen pain, Stomach problems, Inflammation, Skin diseases, Pustules	8	0.75
Ipomoea crassipes var. crassipes	MuSbki	Convolvulaceae	H	W	Wh	P	Sores	1	0.33
Ipomoea pentaphylla Jacq.	Kaan Kati	Convolvulaceae	H	W	S	E	Liver problems, Abdomen pain, Inflammation	3	0.25
Ipomoea purpurea (L.) Roth	Aerh	Convolvulaceae	H	W	Lv, Wh	J	Hair lices, Fodder	2	0.2
Jasminum officinale L.	Chambeli	Oleaceae	Sb	W/Cu	Lv,	J	Scabies, Allergic problem	2	0.125
Juglans regia L.	Akhore,	Juglandiaceae	T	W/Cu	S, F, Lv,	D/O	Cardiac problems, mouth tonic, clean teeth,	6	0.12

	Akhrot				B, Wd		Mouth ulcers, Furniture, Fuel.		
Justicia adhatoda L.	Baikkarh	Acanthaceae	Sb	W	Lv	Pw, J, SF	Diabetes, Scabies, Bloating, Pimples, Fuel, Drive away the insects from cattle	6	0.14
Lantana indica Roxb.	Soi	Verbenaceae	Sb	W	Lv	D	Digestion problems	1	0.33
Lepidium sativum L.	Halyan	Brassicaceae	H	W	S	E	Cleaning eyes	1	0.12
Lonicera quinquelocularis Hardw.	Phutuk	Caprifoliaceae	Sb	W	Lv	E	Cataract, Improve vision, Fodder for goats	3	0.6
Mallotus philipensis (Lam.) Müll.Arg.	Kamila	Euphorbiaceae	T	W	F, Lv, Br	D	Bloody diarrhea, Washing scrub, Fodder, Fuel.	3	0.17
Malva parviflora L.	Sonchal	Malvaceae	H	W	Wh	D	Respiratory ailments, Antipyretic	2	0.4
Malvastrum coromandelianum (L.) Garcke	Damhni, Phabchi	Malvaceae	H	W	Wh , Lv, Fl, S	P, Pw	Body pain, Perspiration, Diabetes, Leprosy	4	0.26
Maytenus royleana (Wall. ex M .A. Lawson) Cufod.	Patakhi / Sumbal (Hindkoh	Celasteraceae	T	W	B, Lv	P	Bone fracture	1	0.16
Medicago polymorpha L.	Maeserhi, Maina	Fabaceae	H	W	Lv, Br, Wh	Pw	Constipation, Digestive problems, Fodder	3	0.15
Melia azadirachta L.	Dharek	Meliaceae	T	W/Cu	Lv	E	Scabies, Pustules, Pimples, Bloating, Fodder, Fuel	6	0.22
Mentha arvensis L.	Kala Poodina	Lamiaceae	H	W/Cu	Lv	Pw	Carminative, Cooling effect, Digestive stimulant.	3	0.6
Mentha longifolia var. asiatica (Boriss.) Rech.f.	Sufaid Poodina	Lamiaceae	H	W/Cu	Lv, F	D/ Pw	Vomiting, Digestive problems, Cholera.	3	0.6
Micromeria biflora (Buch-Ham.ex D. Don) Benth.	Persia o waSban	Lamiaceae	H	W/Cu	Wh	D	Respiratory disorders, Cold	2	0.16

Morus alba L.	Sufaid Sbehtoot	Moraceae	T	W/Cu	F, Lv, Wd		Digestive stimulant, Purgative effect, Constipation, Fodder, Furniture, Fuel	6	0.3
Morus nigra L.	Kala Sbehtoot	Moraceae	T	W/Cu	F,Lv, Wd		Digestive stimulant, Digestive problems, Fodder, Furniture, Fuel.	5	0.19
Myrsine africana L.	Khokhal	Myrsinaceae	Sb	W/Cu	F, Lv, Br	Pw	Intestinal worms, Constipation, Hepatic problems, Fodder, Fuel, abdominal cramps, Skin infections, Stomach problems, body tonic, jaundice, Diarrhea	9	0.9
Nerium indicum var. splendens Poit.	Ganeera, Kaner	Apocynaceae	Sb	W/Cu	Br	E	Clean tooth, Worms	2	0.11
Olea ferruginea (Aiton) Steud.	Kahu	Oleaceae	T	W/Cu	Lv	D	Flue, Skin diseases, Mouth ulcers, Stomach burning, Roof thatching, Fodder, Fuel	7	0.3
Olea glandulifera Wallich	Barh-koh	Oleaceae	T	W/Cu	Lv, Br		Fodder, Fuel	2	
Otostegia limbata Bentham	Chita jand	Lamiaceae	Sb	W	Lv,	D	Mouth ulcers, Skin diseases, Browsed by goats	3	0.6
Oxalis corniculata L.	Khati Booti	Oxalidaceae	H	W	Wh, Lv	D	Skin disorders, Fever, Diarrhea, Snake bite, Liver diseases, Eyesight	6	0.4
Parthenium hysterophorus L.	Chatak chandni	Asteraceae	Sb	W	Wh	D	Flue, Diarrhea, Stomach disorders	3	0.5
Pinus roxburghii Sarg.	Cheer	Pinaceae	T	W/Cu	G, Sh, S	Pw	Backache, Measles	2	0.11
Plantago lanceolata L.	Batti, Chamchi patra	Plantaginaceae	H	W/Cu	Lv	Pw	Stomach burning	1	0.25
Punica granatum L.	Darruni	Punicaceae	Sb	W/Cu	S, F, Br	E	Digestive stimulant, Carminative, Cooling effect, Diarrhea in humans and cattle, Fuel	5	0.25
Pyrus paSbia var. obtusata Cardot	Batangi	Rosaceae	T	W/Cu	F, Lv, Br	Pw	Diarrhea, Fodder, Fuel	3	0.42

Quercus incana	Rein, Sbah-baloot	Fagaceae	T	W/Cu	B, Br	D	Joint pain, Cooling effect,Roof thatching, Fuel.	4	0.57
Ricinus communis L.	Arand, Hernoli	Euphorbiaceae	Sb	W/Cu	S, Lv, Br, Wh	O, P	Purgative, Laxative, Digestive problems, Rheumatic joints, Fuel	5	0.156
Rosa brunonii Rydb.	Jangli Gulab	Rosaceae	Sb	W/Cu	Fl	P	Scabies, Digestive problems, Heart problems	3	0.21
Rubus ellipticus Sm.	Aakha	Rosaceae	Sb	W/Cu	F, Lv	D	Stomach burning, Browsed by goats	2	0.16
Rubus Ficosus L.	Aakha	Rosaceae	Sb	W/Cu	F, Lv	D	Stomach burning, Browsed by goats	2	0.1
Rumex dentatis L.	Khoe, Jangli Palak	Polygonaceae	H	W	Lv, Wh	E,D	Antiseptic, Wound healing, Skin problems, Fodder	4	0.16
Rumex hastatus D.Don	Chukki, Khatimmer	Polygonaceae	H	W	Lv, R	Pw, D	Skin diseases, Jaundice, Liver diseases, Brain tonic, Pustules, Skin infections, Body relaxant	7	0.875
Rumex nepelansis Spreng.	Khoe, Jangli Palak	Polygonaceae	H	W	Lv	E	Antiseptic, Wounds healing, Skin problems	3	0.2
Salix babylonica L.	Beiss	Salicaceae	T	W	R, Wd, Br	Pw	Cooling effects, Furniture, Fuel	3	0.42
Sapindus mukorossi Gaertn.	Retha	Sapindaceae	T	W/Cu	F, Br	P	Healthy and silky hairs, Fuel.	2	0.1
Saussuria heteromala (D. Don) Handel-Mazzetti	Kali Zeer	Asteraceae	H	W/Cu	S	Pw	Scabies, Pimples, Fodder	3	0.2
Sida cordata (Burm.f.) Borss.Waalk.	Tuman	Malvaceae	Sb	W/Cu	Wh	Pw	Goat Fodder at delivery	1	
Sisymbrium irio L.	Khoob Kalan	Brassicaceae	H	W/Cu	S	O	Swelling of body parts	1	0.2
Solanum nigrum L.	Mako, Kach maach	Solanaceae	H/Sb	W/Cu	Lv, F, Fl	Pw	Abdominal swellings, Stomach-ache, Cardiac problems, Respiratory ailments, Body pain	5	0.15
Solanum surattense Burm.f.	Kandiari/Chho	Solanaceae	H	W/Cu	Fl, F, Lv,	D, P	Respiratory problems, Body aches,	5	0.14

	ti Mahokari				Berries		Toothache, Wound healing, Skin diseases		
Solanum violaceum Ortega	Kach-maach	Solanaceae	H	W/Cu	Lv	C	Cardiac problems	1	0.11
Sonchus arvensis L.	Dodh Bhatal	Asteraceae	H	W	St, Lv	D	Purgative effect, Body pain, Tuberculosis	3	0.21
Sonchus asper (L.) Hill	Dudda	Asteraceae	H	W	Lv, Wh, R	D, P	Fodder, Cough, Diabetes, Skin problems, Heal wounds	5	0.22
Spermadictyon suaveolens Roxb.	Phisanni	Rubiaceae	Sb	W	Wh		Fodder	1	
Swertia chirayita (Roxb.) H.Karst.	Charaita	Gentianaceae	H	W/Cu	Lv	Pw, P	Malaria Fever	1	0.5
Swertia ciliata (D. Don ex G. Don) B.L. Burtt	Charaita	Gentianaceae	H	W/Cu	Lv	P	Malaria Fever	1	0.5
Syzygium cumini (L.) Skeels	Jaman	Myrtaceae	T	W/Cu	F, S, Lv, Wd	D,Pw	Cardiac problems, Diabetes, Fodder purpose, Fuel, Furniture	5	0.29
Trianthema portulacastrum L.	Itsit	Aizoaceae	H	W	Lv, Wh	E, A	Healing wound, Rheumatism, Pyretic, Hepatic problems, Body swelling	5	0.5
Tribulus terrestris L.	Bhakrha	Zygophyllaceae	H	W	Wh, F	Pw	Menstrual flow, Relieve constipation, Abortion, Renal problems	4	0.26
Trichodesma indicum (L.) Sm.	Hundusi, Gao-zaban	Boraginaceae	Sb	W	Lv	P	Diarrhea, Dysentery	2	0.66
Verbena officinalis L.	Chooroon	Verbenaceae	H	W	Lv	P	Rheumatism, Joint pain	2	0.22
Viola canescens Wall.	BanafSba	Violaceae	Sb	W/Cu	Lv	I	Cough, Cold, Respiratory problems	3	0.2
Vitex negundo L.	Marvan, Banna	Verbenaceae	Sb	W	Lv, Br	D	Skin diseases, Insect pests, Fuel, Fertility, Pustules, Irritation, Chicken pox, Bacterial infection, Brain tonic, Brain relaxant, Skin sores, Body relaxant	10	0.83
Withania somnifera (L.)	Asgand	Solanaceae	Sb	W/Cu	R	D, P	Stomach ulcers, Wounds, Arthritis,	6	0.33

Dunal							Respiratory diseases, Renal problems, Labour pain		
Wdfordia Ficosa (L.) Kurz	Dhaawi, Taavi	Vitaceae	H	W	Fl,Lv, Br	Pw	Abortion, Menstrual flow, Fodder, Fuel	4	0.6
Xylosma longifolium Clos	Batti	Flacourtiaceae		W	Br		Fuel, Support purposes	2	
Zanthoxylum alatum Roxburgh	Timber, Timmer	Rutaceae	Sb/T	W/Cu	F, Lv, Br	Es	Carminative, Stomach-ache, Tooth cleaning, Walking sticks	5	0.62
Zizyphus mauritiana Lam	Bair, Beri	Rhamnaceae	T	W/Cu	Lv , F	D	Digestive stimulant, hair fall, Blood heat reduces, Browsed by goats	4	0.16
Zizyphus nummularia (Burm.f.) Wight & Arn.	Jangli bairi	Rhamnaceae	T	W/Cu	R, Lv	D	Diabetes, Pyretic, Fever, Fodder	4	0.23
Zizyphus oxyphylla	Amnui	Rhamnaceae	T	W/Cu	R, F	D	Pustules, Diabetes, Jaundice, Browsed by goats	4	0.66

H=Herb, Sb=Shrub, T=Tree, Wild=W, Cultivated=Cu, Fl= Flower, Lv=Leaves, L=Lumentum, Wh= Whole plant, Sh=Shoot, R=Root, G=gum, B=Bark, S=Seed, St=Stem, Br=Branches, Be=Berries, Wd=Wood D=Decoction, P=Paste, A=Ash, Pl=Pulp, C=Cooked, Bd=Buds, Fruits=F, Pw=Powder, J=Juice, O=Oil, E=Extract, Po=Poultice, Fumes=Fu, Smoky Fire=SF, Infusion=I

SECTION 2

PHYTOCHEMICAL SCREENING

OF

MEDICINAL PLANTS

3.2.1 Extraction of plant extracts

Medicinal plants are rich source of various variety of biological active compounds that leads to the discovery of pharmacological activities and finally to the drug development. Therefore, this study focused on qualitative and quantitative investigation of secondary metabolites having important pharmacological activities. Therefore in the present experiment seven plants (*Myrsine Africana* leaves (ML) and fruits (MF), *Debregeasia salicifolia* Leaves (DS), *Bauhinia variegata* flower (BV), *Dodneae viscosa* flower (DV), *Indigofera heterantha* Leaves (IH) and *Rumex hastatus* Leaves (RH)) were initially extracted with different solvents as given below. In this study extraction yield (%) of all methanol extracts were found higher than other solvents used. Methanol is a polar solvent and able to dissolves maximum of polar compounds. Extraction depends on procedure of extraction, size of particles, time duration and type of solvents used. On the basis of yield obtained, further analysis was carried out.

Table 2. Crude extraction % of plants by using various solvents

Plant and part name	Methanol	Chloroform	N-hexane	Ethanol
Debregeasia salicifolia leaves	11.4±0.7%	7.9±1.3%	5.2±0.37%	6.56±0.21%
Indigofera heterantha leaves	12.7±1.1%	10.1±2.5%	9.6 ±1.8%	7.41±1.3%
Myrsine africana leaves	13.8±0.9%	9.2±1.42%	6.1±0.69%	10.43±0.82%
Myrsine africana fruit	9.8±0.34%	5.22±0.7%	5.17 ±0.91%	8.40±1.9%
Dodonaea viscosa flower	7.06±0.52%	6.91±1.09%	4.22 ±0.4%	5.08±0.74%
Bauhinia variegata flower	7.3±1.5%	5.76±1.4%	3.9±0.83%	6.011±0.50%
Vitex negundo leaves	13.2±2.3%	8.94 ±0.81%	8.37±1.5%	5.07±0.09%
Rumex hastatus leaves	12.9±1.09%	7.54±0.26%	7.24 ±0.72%	9.011±2.25%

3.2.2 Phytochemical Analysis

The current study focused on qualitative and quantitative estimation of secondary metabolites. The curative effect of these plants were might be due to these biological metabolites Alkaloids, phenols, flavonoids, saponins, anthraquinone, cardiac glycosides, terpenoids are presented in table 3.

Myrsine africana leaves have higher quantity of phenols 362±9 mg/g of dry weight followed by 358.4±5 350±1.7 in *Myrsine africana* fruit and *Vitex negundo. Indigofera heterantha* have higher alkaloids concentrations (28.3±1.57 mg/g dry weight) (Table 3). The higher flavonoids contents were found in *Vitex negundo* 120±2.9 mg/g which is slightly higher than other extracts tested.

Phytochemicals	*Debregeasia salicifolia* Leaves	*Indigofera heterantha* Leaves	*Myrsine africana* Leaves	*Myrsine africana* Fruit	*Dodonaea viscosa* Flower	*Bauhinia variegata* Flower	*Vitex negundo* Leaves	*Rumex hastatus* Leaves
Flavonoids	+	+	+	+	+	-	+	+
Alkaloids	+	+	-	+	-	+	+	+
Anthraquinones	-	+	+	+	+	+	-	+
Coumarins	+	-	-	-	+	-	+	+
Tannins	+	+	+	+	-	+	+	-
Cardiac glycosides	+	-	-	+	+	-	+	+
Saponins	+	+	+	+	+	+	+	+
Terpenoids	-	+	+	-	-	+	+	+
Reducing sugars	+	+	+	+	+	+	+	+
Steroids	-	+	-	+	-	+	+	-

Table.3: Qualitative estimation of phytochemical constituents from Medicinal plants

-= not present += present

Table.4. Quantitative analysis of phytochemicals from Medicinal plants

Plant name and part used	Total flavonoid contents	Total Phenolic contents	Tannins	Alkaloids	Saponins
Debregeasia salicifolia **Leaves**	75.1±2.8[a]	224.9±8[a]	26±1.1[a]	13.3±4.2[a]	11.25±3.1[a]
Indigofera heterantha **Leaves**	119± 4[a]	136±5.5[a]	29±2.9[a]	28.3±1.57[a]	8.9±0.9[a]
Myrsine africana **Leaves**	90±3.4[a]	362±9[a]	13±0.73[a]	22.5±2.8[a]	8.75±1.5[a]
Myrsine africana **Fruit**	88.1±2[a]	358.4±5[a]	25±2.2[a]	17.9±2.14[a]	7.9±1.83[a]
Dodonaea viscosa **Flower**	98±7[a]	174±4[a]	17±4.7[a]	20.4±3.2[a]	7.29±1.36[a]
Bauhinia variegata **Flower**	63±5.2[a]	220±9.1[a]	12±0.83[a]	19.5±2.82[a]	4.1±0.83[a]
Vitex negundo **Leaves**	120±2.9[a]	350±1.7[a]	20±2.5[a]	20.8±1.6[a]	7.91±1.22[a]
Rumex hastatus **Leaves**	74±1.78[a]	127±6.4[a]	23±3.7[a]	26.2±4.78[a]	7.29±0.99[a]

[a]= $p<0.01$, [a]= $p<0.05$ and measured in mg/g dry weight.

3.2.3 Antioxidant assays of plant extracts

Methanol extracts of plants were assessed against different antioxidant assays. The DPPH scavenging assay is used to evaluate the sample ability as hydrogen atoms donor while changing DPPH radical into DPPH-H (reduced form). In the FRAP, Ferric reduces to ferrous ion due to the presence of antioxidants. FRAP deals sample antioxidants as reductants during redox associated colorimetric reaction (Prasad *et al.*, 2010).

Debregeasia salicifolia Methanol showed highest IC50 (37.4 ± 1 and 37.9 ± 4 µg/ml) against hydroxyl radical assay and DPPH assay following *Indigofera heterantha* Methanol which showed higher activity (IC50 47.01 ± 2 and 48.9 ± 1.9 µg/ml) against iron chelating and DPPH assay. Ascorbic acid shows lower value of IC50 (14.5 ± 0.84 and 15.7 ± 3 µg/ml) against hydroxyl radical assay and DPPH assay (Table 4). According to results significant IC 50 values of *Myrsine africana* fruit and leaves against Hydrogen peroxide, DDPH, ABTS and FRAP were (14.7±2, 58.6±6.1, 85±9.1, 344.6±7 µg/ml) and (18.8±4,30.5±1.6, 160.2±1.7, 220.5±11 µg/ml) extracts indicates good antioxidant potential as compared to all other extracts. *Dodonaea viscosa* has higher antioxidant activity comparing to others as 1.37±0.4 µg/ml and 9±0.56 µg/ml against hydrogen peroxide and hydroxyl radical assay. *Vitex negundo* and *Rumex hastatus* have significant antioxidant activities as 100.9±5µg/ml and 122.3±7.1 µg/ml against FRAP followed by 92±8 µg/ml and 87±5.3 µg/ml against DPPH assay. It was observed that *Bauhinia variegata* has produced significantly higher antioxidant activities. *Bauhinia variegata* has high hydroxyl radical scavenging assay 12.6±3 µg/ml followed by Iron chelating assay 18±3.1 and DPPH scavenging activity 28.1±1.3 µg/ml. The correlation of TPC and TFC with antioxidants was given in (Table 5 to 9).

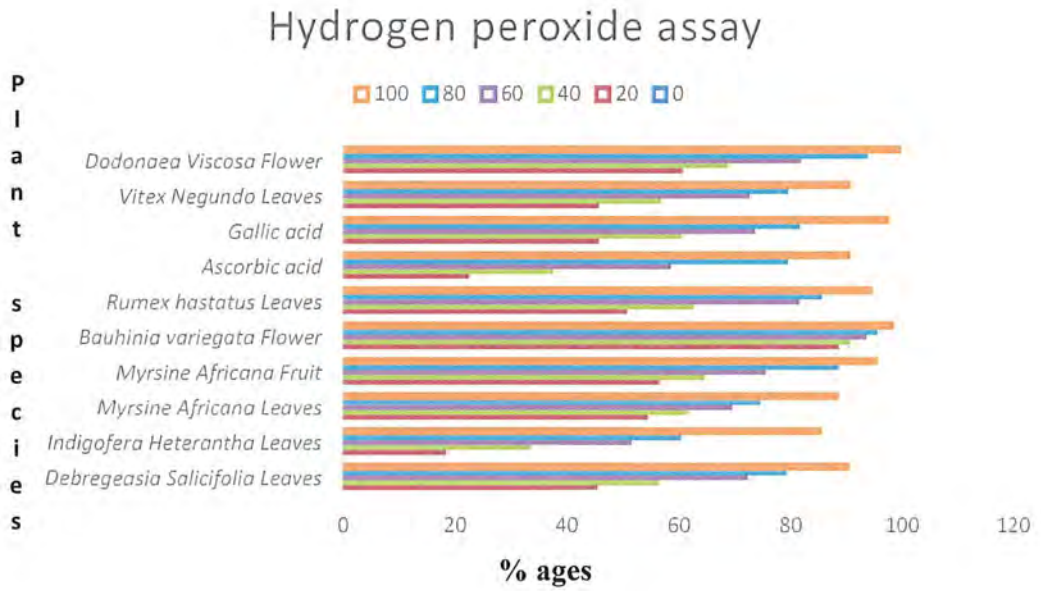


Fig.1. Scavenging capacity of medicinal plants, P<0.05

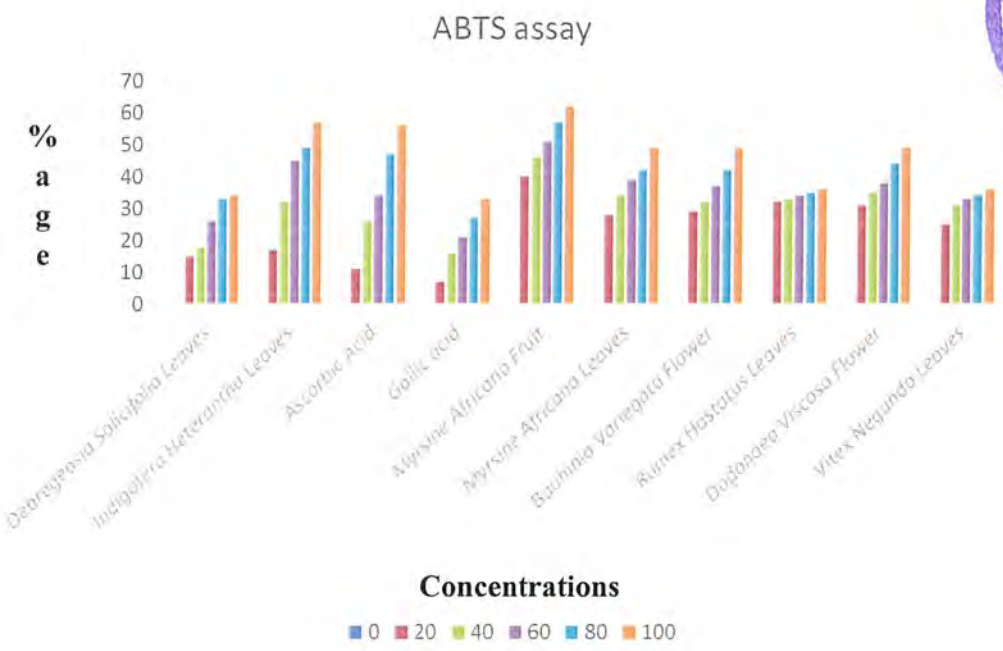


Fig.2. ABTS scavenging activity of selected medicinal plants extracts

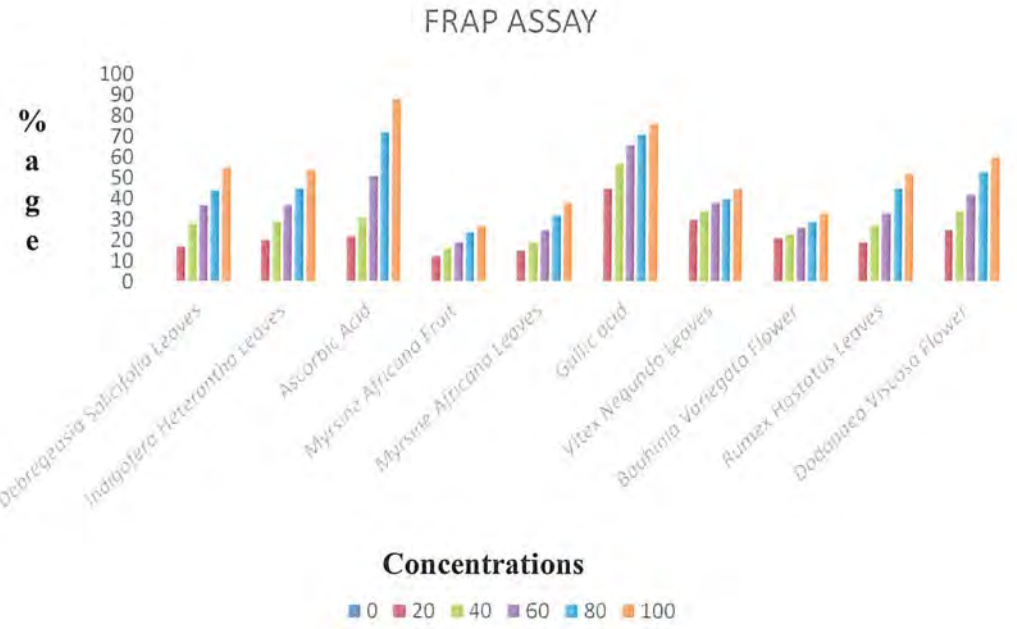


Fig.3.Ferric reducing power assay for plant extracts where p<0.05

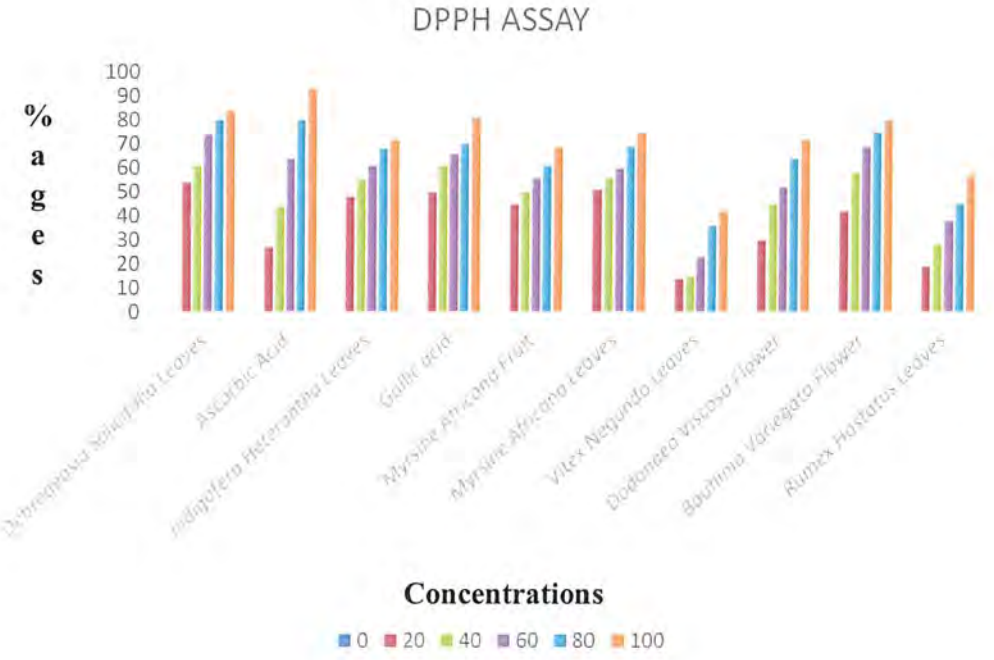


Fig.4.DPPH activity against various plant extracts

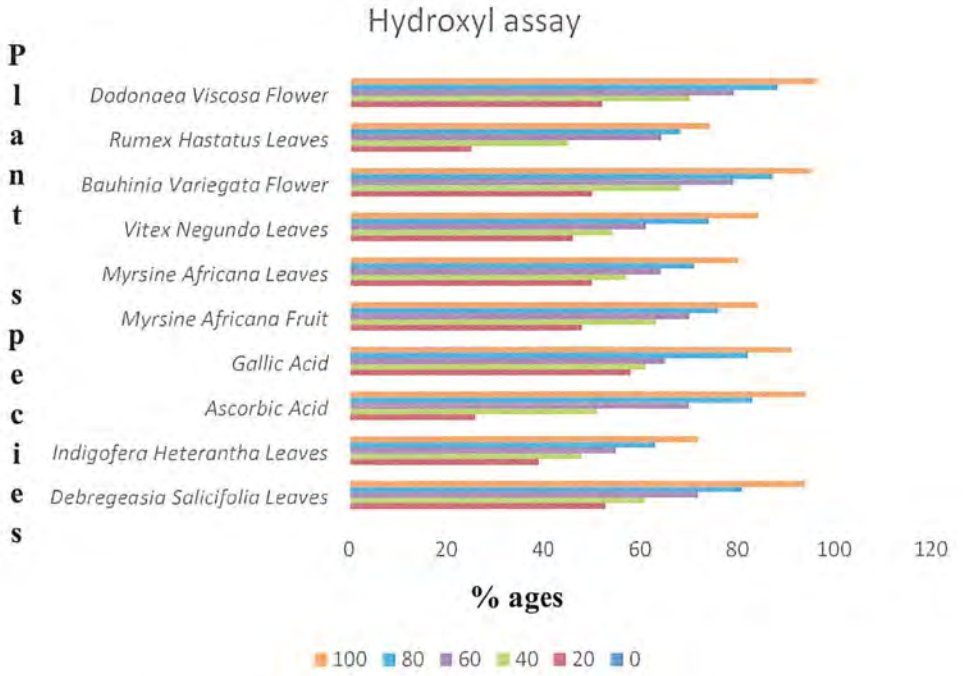


Fig.5.Hydroxyl power assay against plant extracts p<0.05

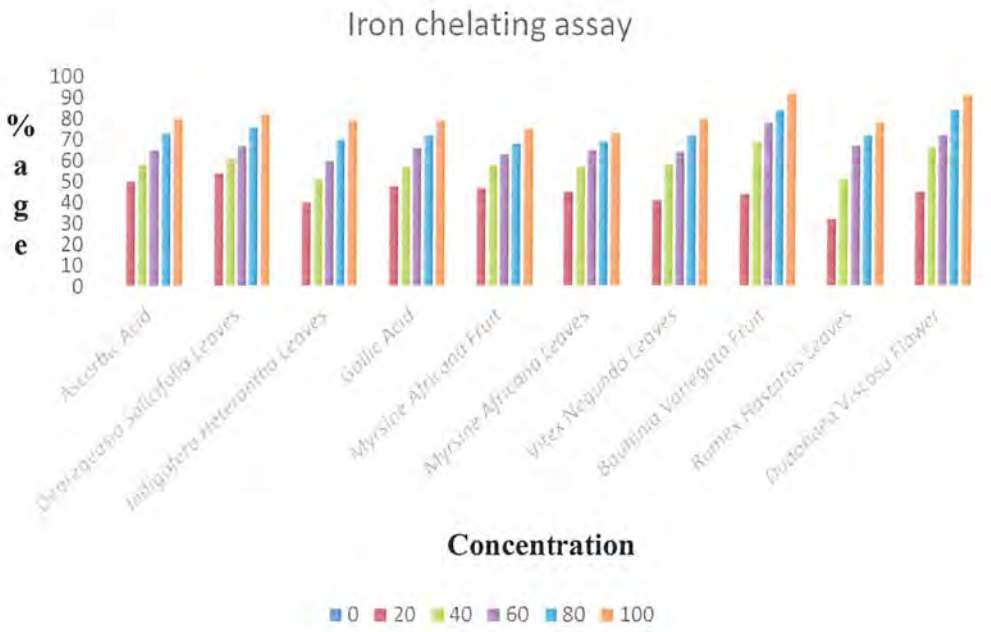


Fig.6.Plants extracts showing potential against iron chelating assay p<0.05

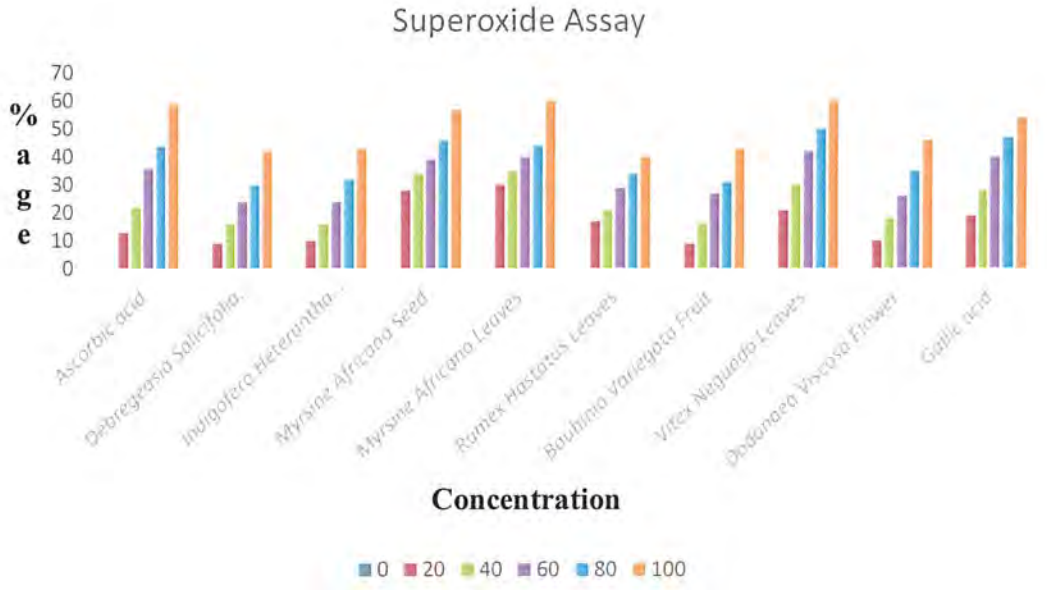


Fig.7. Superoxide potential of different plants extracts p<0.05

Table.5. IC_{50} Values of various plant extracts of medicinal plant extracts

	ABTS radical cation decolorisation Assay	Reducing power Assay	DPPH free radical scavenging Assay	Iron chelating Assay	Hydrogen peroxide ASSAY	Hydroxyl radical scavenging Assay	Superoxide Assay
Bauhinia variegata	108.8±15	152±11	28.1±1.3	18±3.1	235.2±3	12.6±3	119.7±2
Myrsine africana **Fruit**	85±9.1	344.6±7	58.6±6.1	35.9±2	14.7±2	26±2.2	131.3±1.7
Dodonaea viscosa	107.1±11.4	75.59±4	54.95±2.1	20.7±1.3	1.37±0.4	9±0.56	111.6±2.1
Indigofera heterantha	79.6±5	88±2.2	48.9±1.9	47.01±2	61±3	56.11±3	120.6±4
Debregeasia salicifolia	137.5±6.2	88±4.7	37.9±4	40.3±2	41.3±2	37.4±1	124.4±7.3
Myrsine africana Leaves	160.2±1.7	220.5±11	30.5±1.6	38±4	18.8±4	32.5±0.8	125.6±6.2
Vitex negundo	205.6±12.4	100.9±5	122.3±7.1	31.7±2.1	27.67±1.3	31.25±2.3	79.1±2
Rumex hastatus	232±10	92±8	87±5.3	42.3±5	17.8±2.6	51.4±1.3	133.8±5.5
Ascorbic acid	119±7.9	25.7±2	15.7±3	29.2±.7	16.8±2.1	14.5±0.84	116.6±2.8
Gallic acid	229±15	39.2±1	24.7±2	34.8±2	13.1±1	16.2±1	134.2±5.6

Table.6.Correlation of TPC and TFC with antioxidant assays of Medicinal plants

Assays	*Bauhinia variegata* Total Phenolic contents mg/g	*Bauhinia variegata* Total Flavonoids contents mg/g	*Debregeasia Salicifolia* Total Phenolic contents mg/g	*Debregeasia Salicifolia* Total Flavonoids contents mg/g
Hydrogen peroxide assay IC 50	0.9851	0.972	0.9553	0.9417
ABTS radical scavenging activity IC 50	0.9797	0.9549	0.9966	0.8436
FRAP radical assay IC 50	0.9797	0.9616	0.936	0.9809
DPPH Radical scavenging assay IC 50	0.8724	0.9442	0.9736	0.8823
Hydroxyl assay IC 50	0.9008	0.9716	0.9417	0.9679
Iron chelating Assay IC 50	0.8236	0.9392	0.9718	0.9536
Superoxide assay IC 50	0.993	0.9708	0.9137	0.9807

Table.7. Correlation of TPC and TFC with antioxidant assays of medicinal plants

Assays	*IndigoferaHeterantha* Total Phenolic contents mg/g	*Indigofera Heterantha* Total Flavonoids contents mg/g	*Rumex hastatus* Total Phenolic contents mg/g	*Rumex hastatus* Total Flavonoids contents mg/g
Hydrogen peroxide assay IC 50	0.9653	0.9035	0.8662	0.7649
ABTS radical scavenging activity IC 50	0.9843	0.8338	0.9714	0.8974
FRAP radical assay IC 50	0.9988	0.8082	0.9829	0.9065
DPPH Radical scavenging assay IC 50	0.9956	0.7309	0.9706	0.916
Hydroxyl assay IC 50	0.998	0.8185	0.7909	0.6709
Iron chelating Assay IC 50	0.9998	0.7825	0.8134	0.6962
Superoxide assay IC 50	0.9845	0.869	0.9596	0.8842

Table.8. Correlation of TPC and TFC with antioxidant assays of medicinal plants

	Myrsine africana Leaves	*Myrsine Africana Leaves*	*Myrsine africana Fruit*	*Myrsine africana Fruit*
Assays	**Total Phenolic contents mg/g**	**Total Flavonoids contents mg/g**	**Total Phenolic contents mg/g**	**Total Flavonoids contents mg/g**
Hydrogen peroxide assay IC 50	0.9671	0.9908	0.9761	0.9759
ABTS radical scavenging activity IC 50	0.986	0.9829	0.9942	0.9906
FRAP radical assay IC 50	0.9823	0.9722	0.99	0.9793
DPPH Radical scavenging assay IC 50	0.9821	0.9461	0.9827	0.962
Hydroxyl assay IC 50	0.9928	0.9829	0.973	0.9894
Iron chelating Assay IC 50	0.9395	0.8885	0.9841	0.9902
Superoxide assay IC 50	0.9069	0.95	0.9754	0.9341

Table.9. Correlation between TPC and TFC with antioxidants of Medicinal plants

Assays	*Vitex negundo* Total Phenolic contents mg/g	*Vitex negundo* Total Flavonoids contents mg/g	*Dodoneae viscosa* Total Phenolic contents mg/g	*Dodoneae Viscose* Total Flavonoids contents mg/g
Hydrogen peroxide assay IC 50	0.9876	0.918	0.9461	0.9376
ABTS radical scavenging activity IC 50	0.8501	0.8471	0.971	0.933
FRAP radical assay IC 50	0.992	0.969	0.9706	0.9655
DPPH Radical scavenging assay IC 50	0.9326	0.8488	0.9744	0.988
Hydroxyl assay IC 50	0.9722	0.939	0.9467	0.9797
Iron chelating Assay IC 50	0.9295	0.9214	0.9491	0.9773
Superoxide assay IC 50	0.994	0.9351	0.9872	0.9738

Correlation between TPC and TFC with antioxidants and values are significantly different $p \leq 0.05$

3.2.4 High performance liquid chromatography

HPLC was used to estimate composition analysis of selected plants. It is used to determine percentage of quercetin in different plant extracts. Quercetin standard were taken ranged from 1.5 to 25µg/ml and regression equation was Y=28917x+40502, R^2=0.998. Plant extracts showed different results as presented in table.9.

Myrsine africana leaves chloroform extract found to have higher quercetin content 66.98586µg/ml at 12.5 retention time with an area of 1977532cm. *Dodonaea viscosa* methanol extract contained significant amount of quercetin 59.49186 µg/ml at an area of 1760828cm and at retention time of 12.5.*Debregeasia salicifolia* methanol extract also found to have high quantity of quercetin 42.20455 µg/ml at 12.5 retention time and an area of 1260931cm. The relation between peak areas and concentration was linear found in the range of 1.5 to 25ug/ml. The quercetin coefficient correlation was found to be R^2=0.998 and Y=28917x+40502, exhibiting outstanding correlation of peak area and concentration of plant drugs. Chloroform extracts of *Bahinia variegata*, *Dodonaea viscosa*, *Indigofera heterantha* and *Vitex negundo* showed no quercetin at 12.5 retention time.

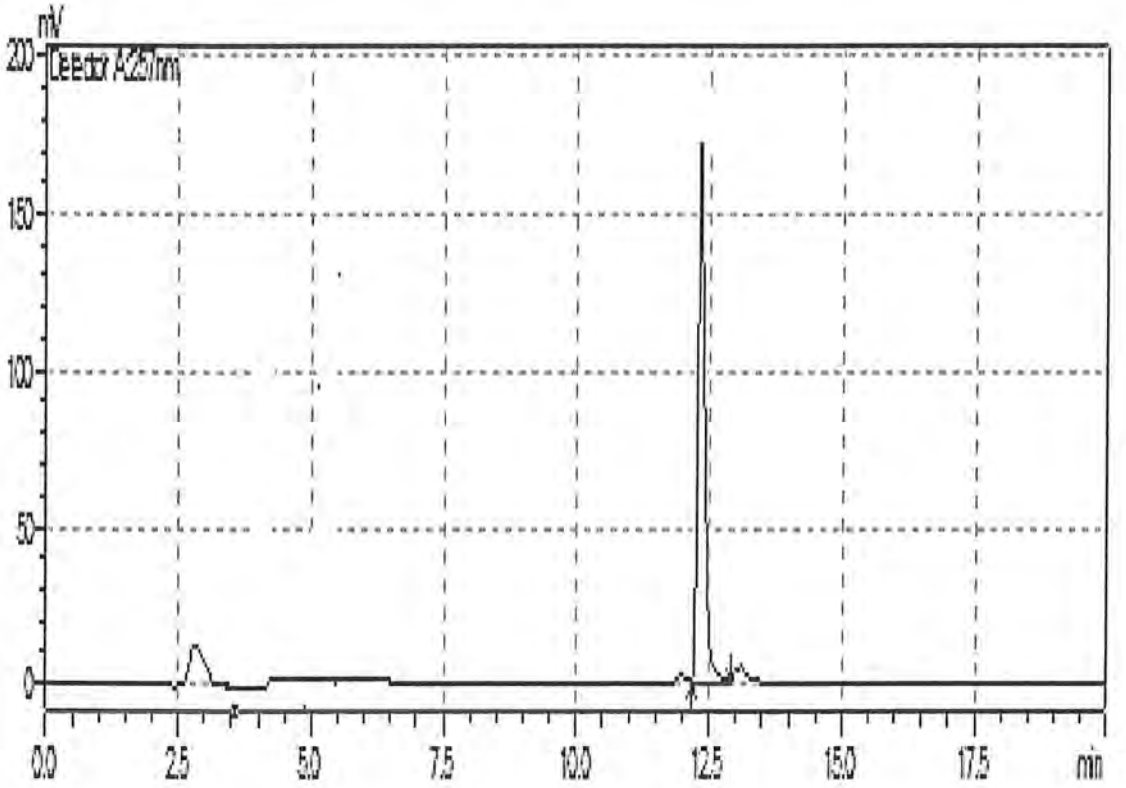


Fig.8. HPLC chromatography of Quercetin compound at 12.5 retention time

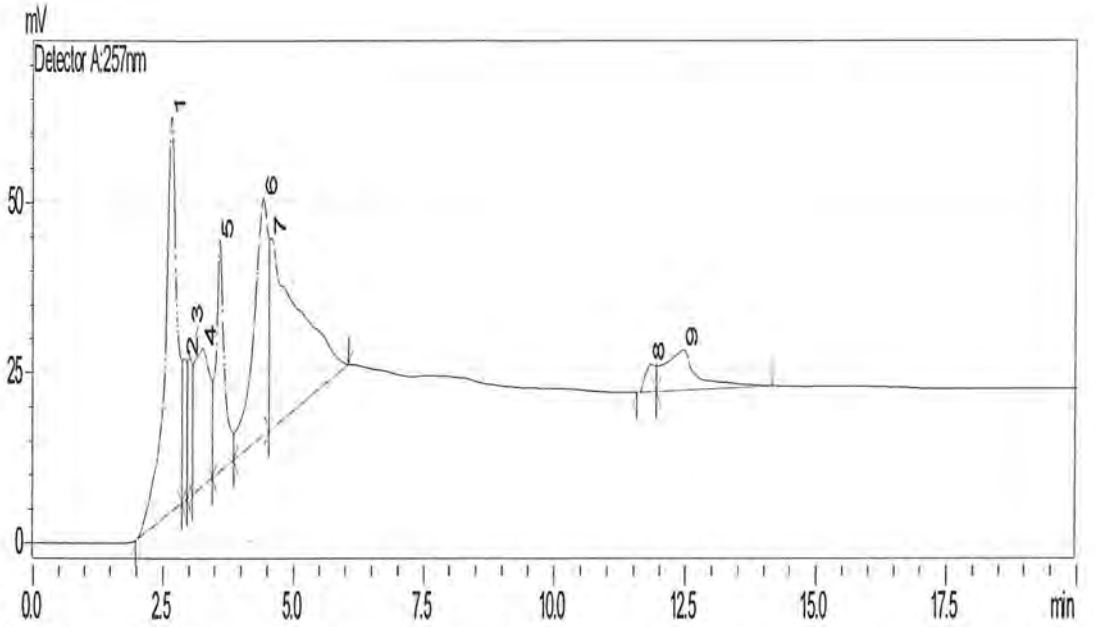


Fig.9.Various compounds of methanol extract of *Myrsine africana* Leaves

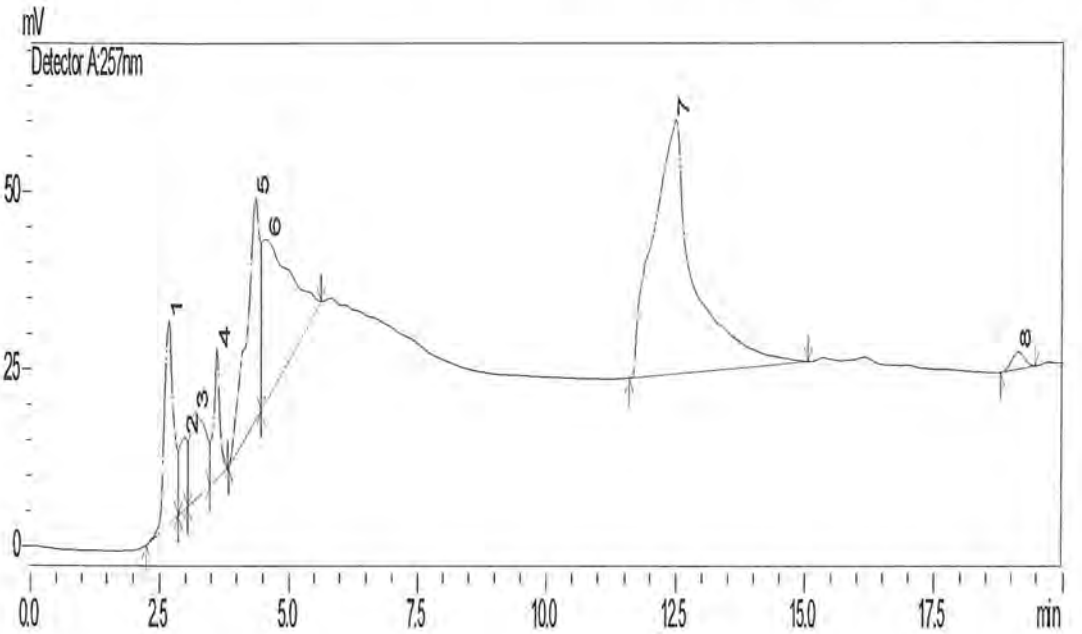


Fig.10. HPLC chromatogram of *Myrsine africana* Leaves Chloroform extract

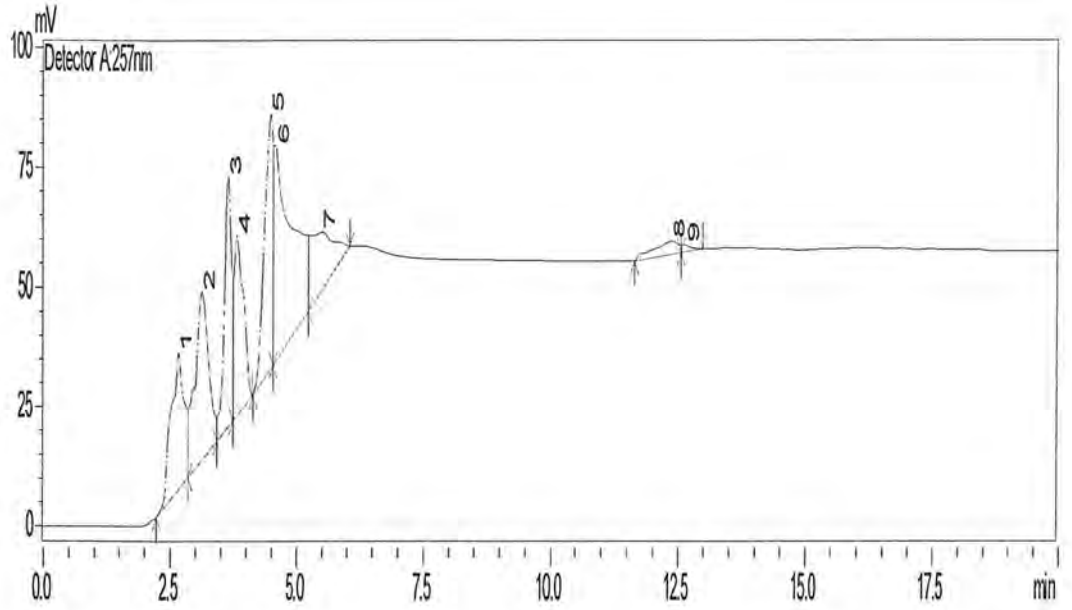


Fig.11. Analysis of quercetin from methanol extract of *Myrsine africana* Fruit

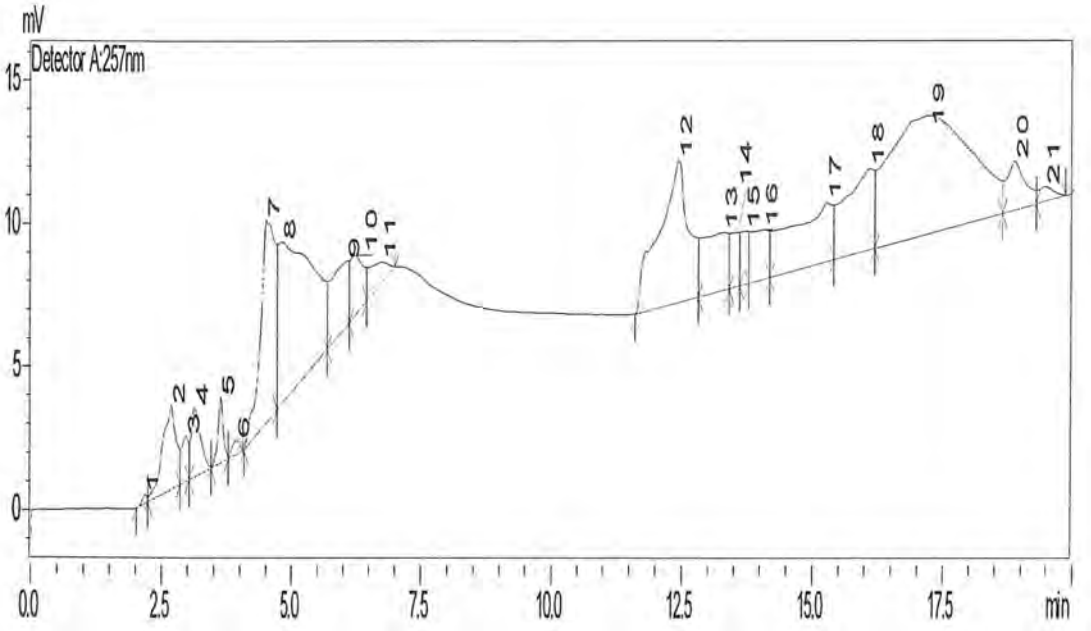


Fig.12.Quercetin estimation from chloroform extract of *Myrsine africana* Fruit

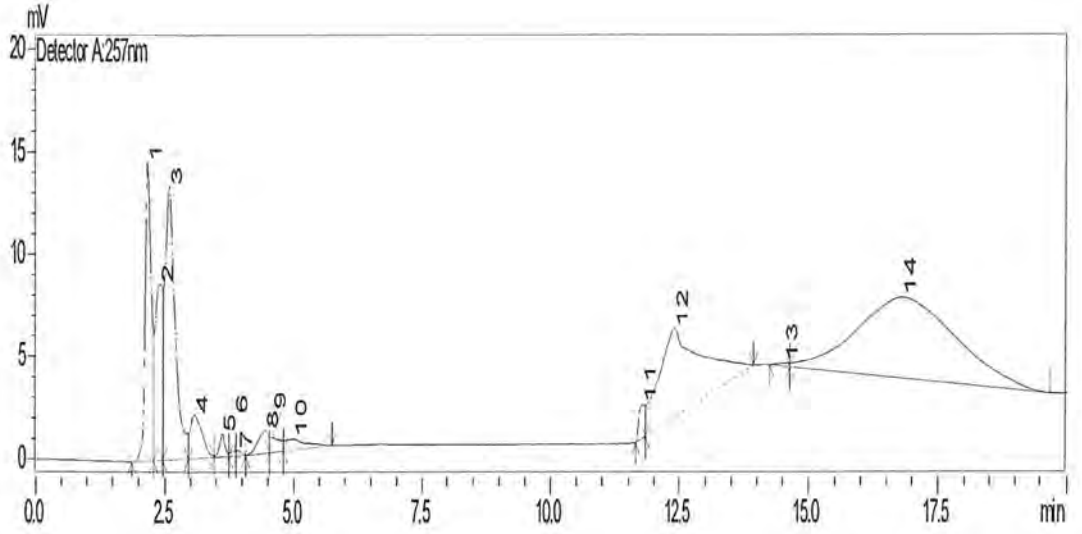


Figure.13. Analysis of quercetin from methanol extracts of *B. variegata*

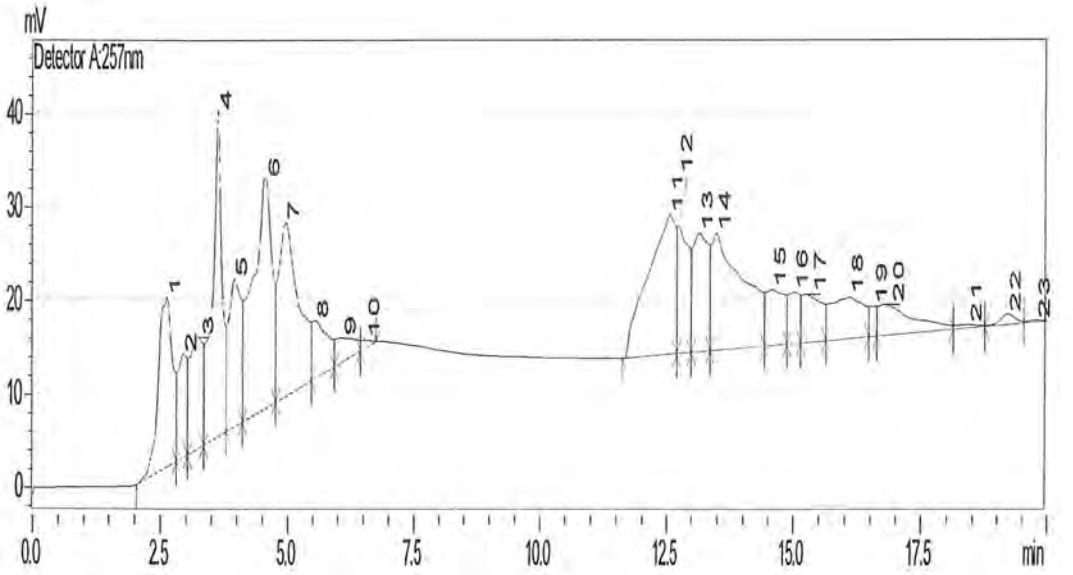


Figure.14. Analysis of quercetin from chloroform extracts of *B. variegata*

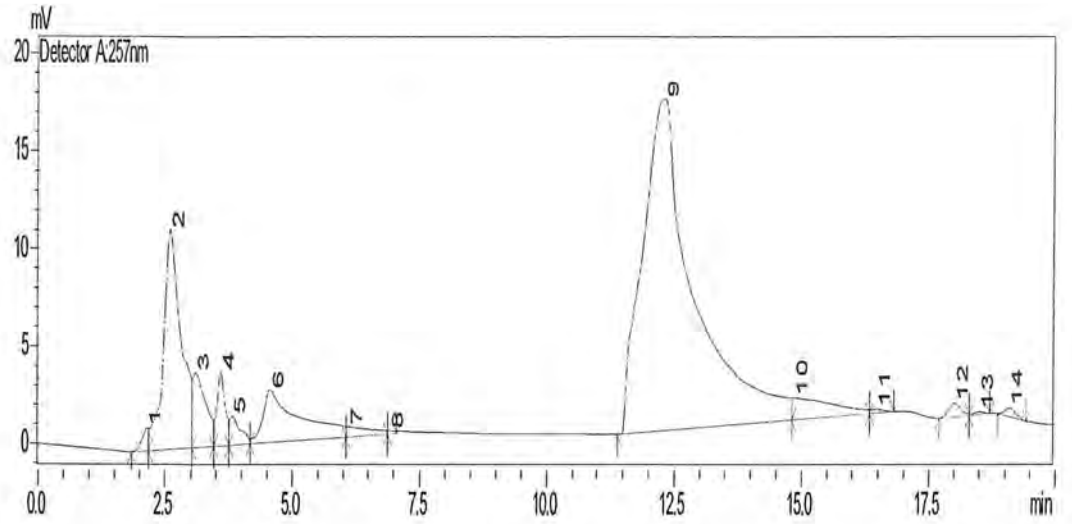


Fig.15. Quercetin estimation from Methanol extract of *Indigofera heterantha*

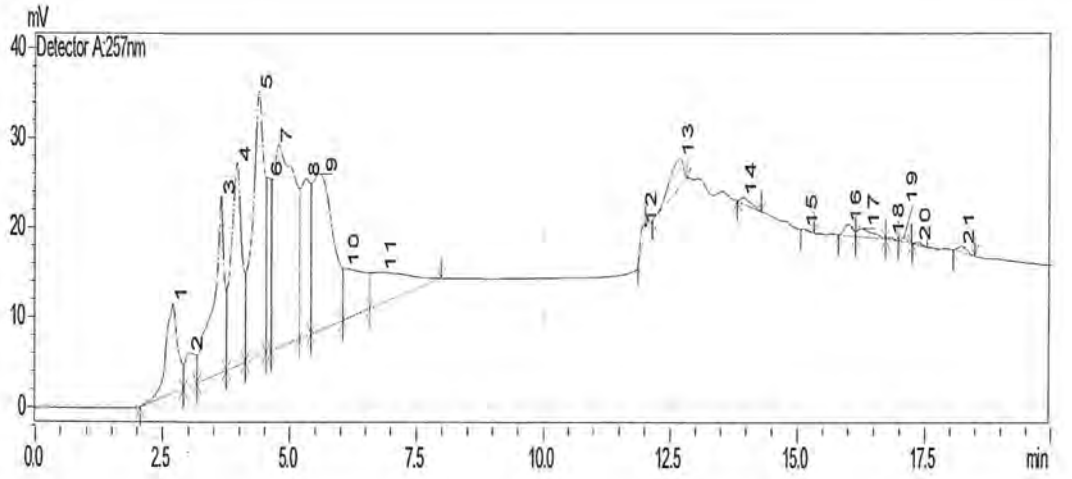


Fig.16.Quercetin estimation from Chloroform extract of *Indigofera heterantha*

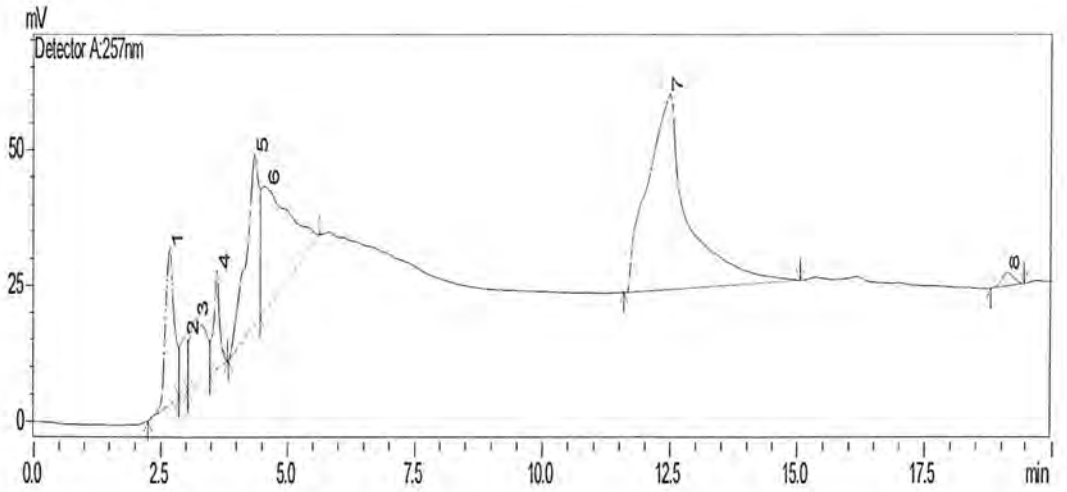


Fig.17. HPLC chromatogram of Methanol extract of *Debregeasia salicifolia*

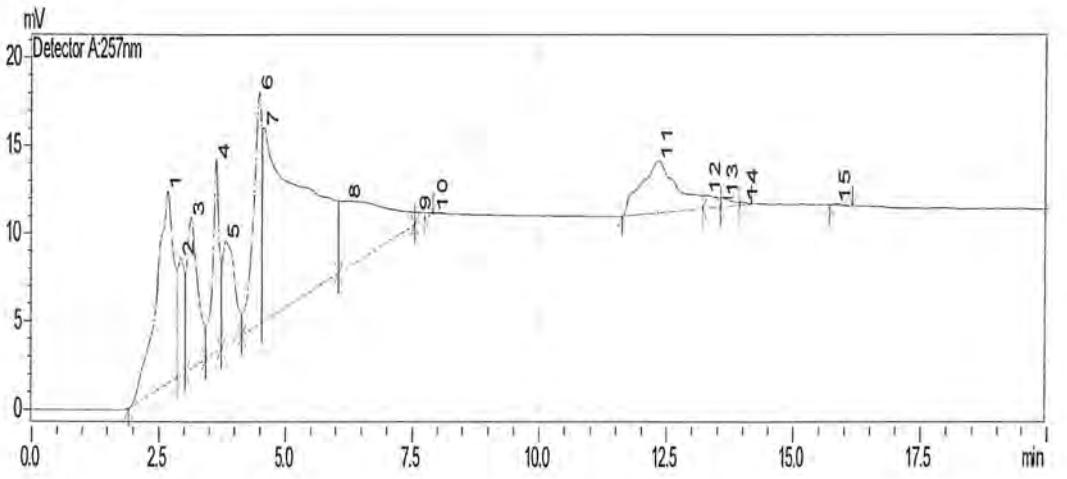


Fig.18.HPLC quantification of Chloroform extracts of *Debregeasia salicifolia*

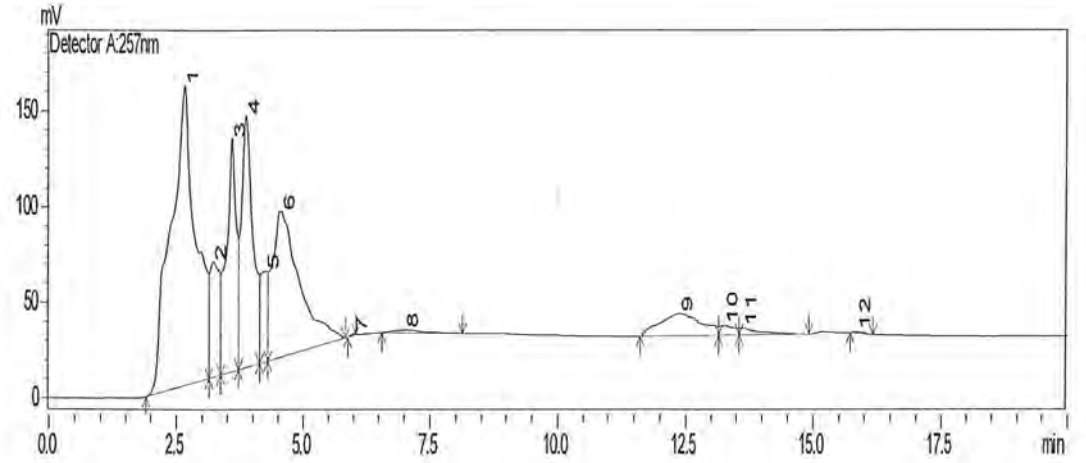


Fig.19.Quantification of quercetin from Methanol extract of *Dodonaea viscosa*

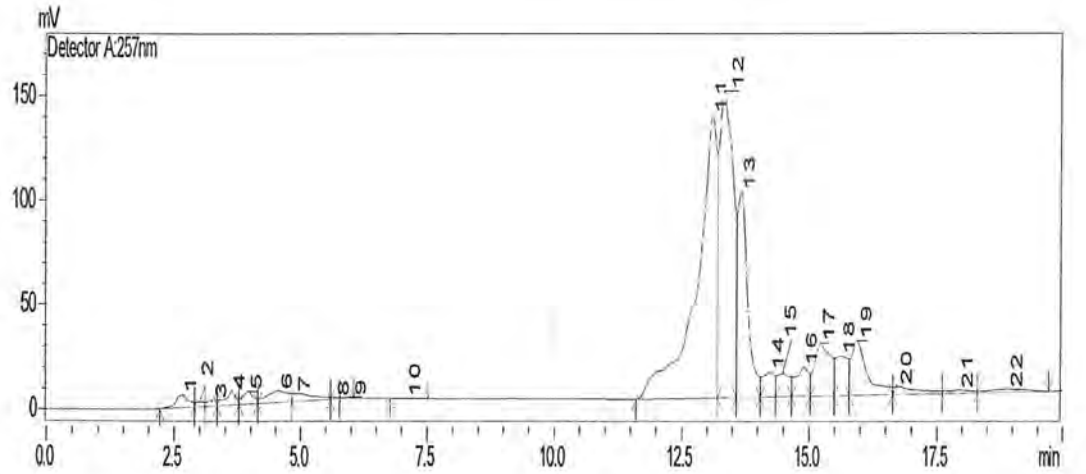


Fig.20.Estimation of quercetin from Chloroform extract of *Dodonaea viscosa*

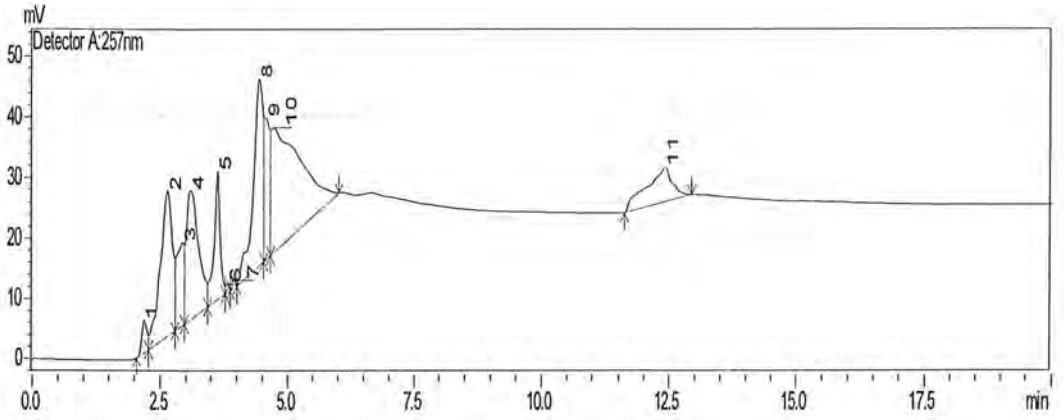


Fig.21. Analysis of quercetin from chloroform extracts *Rumex hastatus*

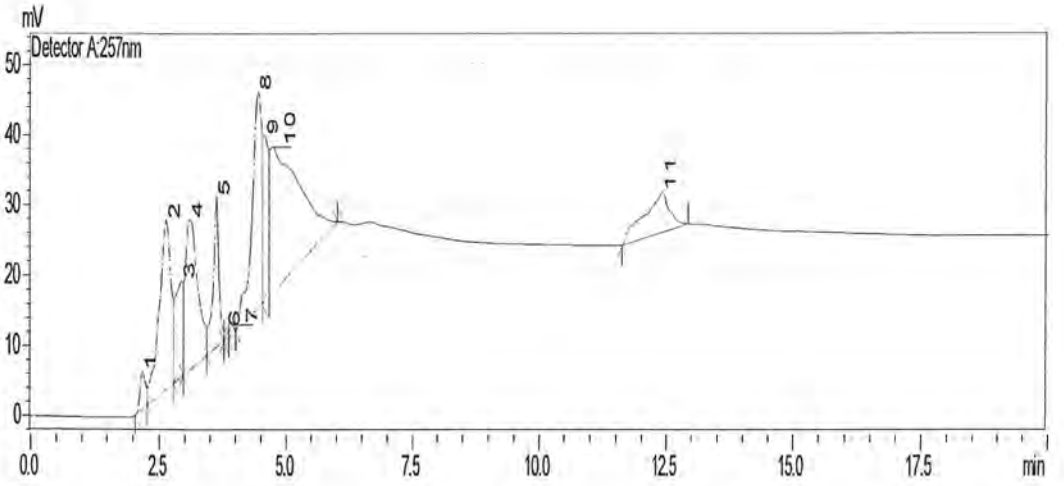


Fig.22.Quercetin analysis from Methanol extract of *Rumex hastatus*

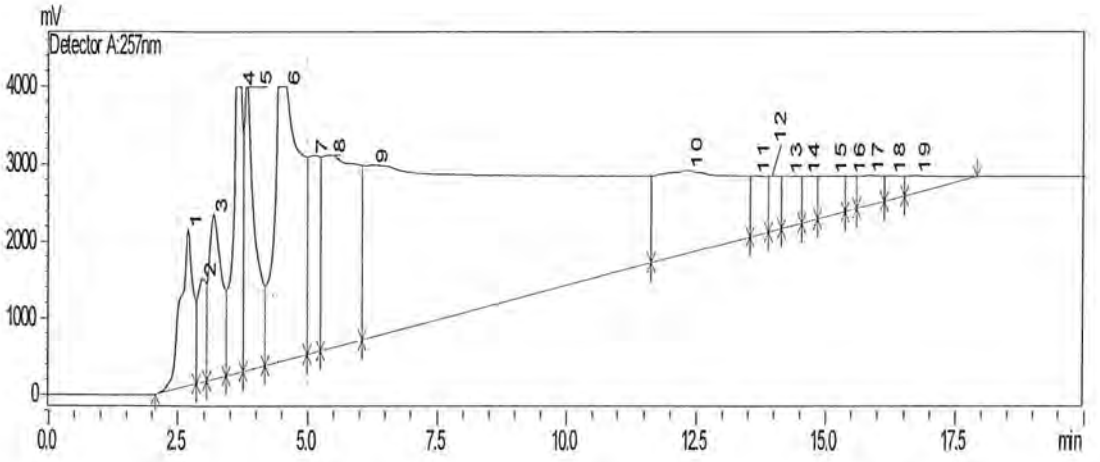


Figure.23. HPLC analysis of chloroform extract of *Vitex negundo* leaves

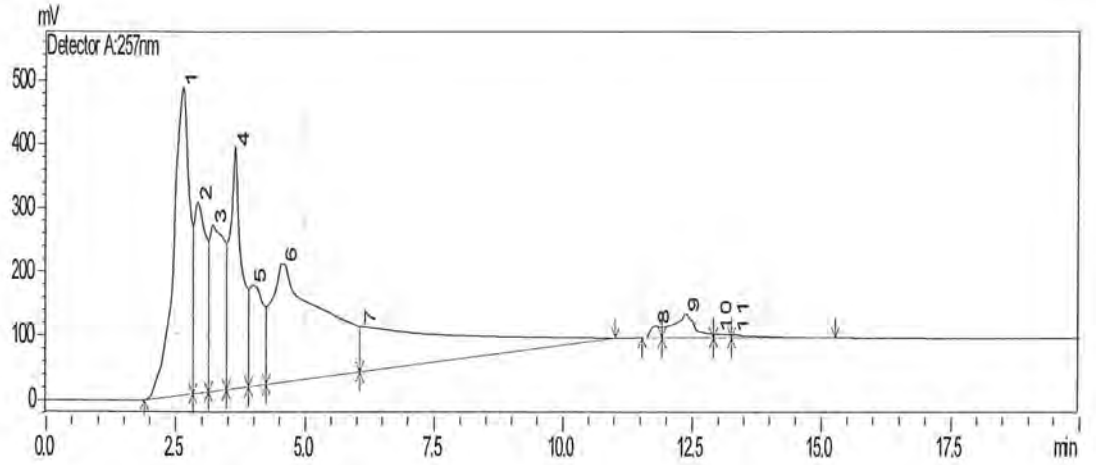


Figure.24. HPLC analysis of *Vitex negundo* leaves methanol extract

Table.10. Quercetin estimation of medicinal plant samples

Plant name	Area	Concentration µg/ml	RT
Myrsine africana leaves methanol	258749	7.54736	12.5
Myrsine africana leaves Chloroform	1977532	66.98586	12.5
Myrsine africana fruit Chloroform	193642	5.295847	12.5
Myrsine africana fruits Methanol	97632	1.975654	12.5
Bauhinia variegata Methanol	248178	7.181796	12.5
Bauhinia variegata Chloroform	0	0	12.5
Debregeasia salicifolia Leaves Methanol	1260931	42.20455	12.5
Debregeasia salicifolia Leaves chloroform	146963	3.681606	12.5
Dodonaea viscosa Methanol	1760828	59.49186	12.5
Dodonaea viscosa Chloroform	0	0	12.5
Indigofera heterantha Methanol	1178348	39.34869	12.5
Indigofera heterantha chloroform	0	0	12.5
Rumex hastatus chloroform	130959	3.12816	12.5
Rumex hastatus Methanol	209776	5.853788	12.5
Vitex negundo Methanol	1095575	36.48625	12.5
Vitex negundo Chloroform	0	0	12.5

3.2.4.1 Molecualr docking of Quercetin

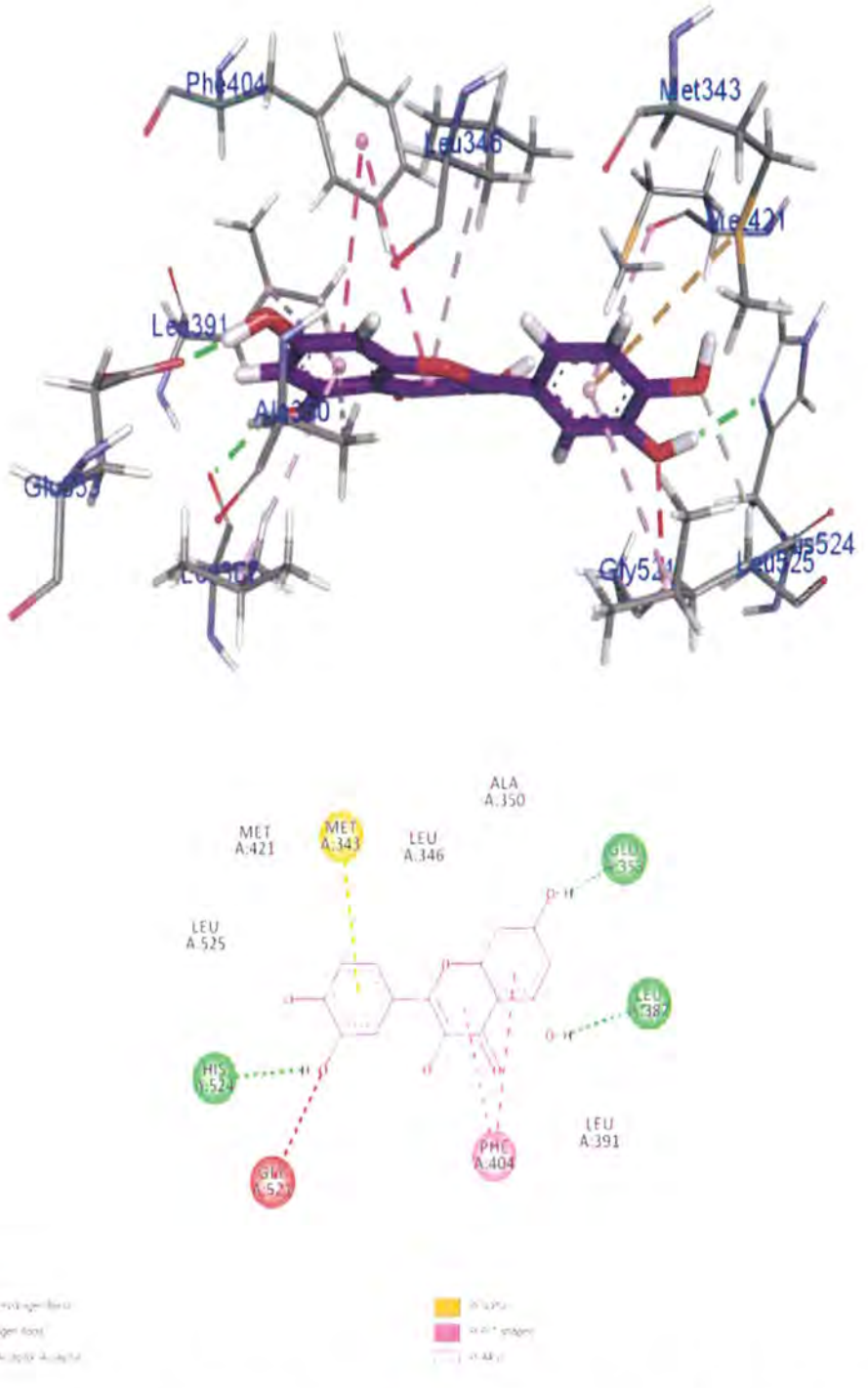


Fig.25.Crystallographic structures of protein receptor (A) after interaction with quercetin glycosides (ligand) (B) during Molecular docking process.

Three dimensional (3 D) Molecular docking of quercetin indicated its important interaction with others active molecules (Fig.17). Aligand of quercetin glycosides was prepared and positioned at the binding site. The active sites of ligand conformation in estrogen protein receptor are based on its polar interaction. The docking results indicated binding of the ligand with active sites of the receptor at Gly 521, His 524, Leu 387and Glu 353. The pi pi T-shaped bond was found in Phe 404 whereas the dihydroxyphenyl group with Met 343 represents pi-sulphur binding. The GOLD fitness score was 57.415.A protein cavity becomes active after interaction with a ligand (Morris *et al.*, 2009) and may produce further activation or inhibition of protein (enzyme) activity.

3.2.5 FT-IR analysis of Medicinal plants

Fourier transform infrared spectroscopy gives the (IR) spectrum of the samples. It is used to collects higher resolution data and identifies functional groups along with the covalent bond illustration (Griffiths and de Hasseth, 2007). It gives absorption spectrum that shows wavelength and identifies functional groups of the sample. Plant extracts possess different functional groups as given in tables (10-17). The functional groups were measured by comparing with chart at different wavelengths. FTIR spectra of methanol extract of *Myrsine africana* fruit showed higher peak on the wavelength of 2925.76nm indicating O-H stretch, Carboxylic acids. At the higher wavelength 3401.25nm of spectra corresponds to O-H stretch, H-bonded alcohols and phenols. Methanolic leaves extracts of *M. africana* showed highest peak on 2924.45nm corresponds to C-H stretch, Alkanes. It showed significant peak on the wavelength of 3379.73 indicating O-H stretch, H-bonded corresponds to alcohols and phenols. BV showed highest peak at 1030.65 indicating C-O stretch, Alcohols, Carboxylic acids, esters, ethers. Peak 1 showed O-H stretch, H- bond corresponds to Alcohols and Phenols at 3338.46nm. Similarly, *Dodonaea visocosa* also showed highest peak (2926.68nm) corresponding O-H stretch, Carboxylic acids followed by O-H stretch, H-bonded signifying Alcohols, phenols at 3399.20nm. Peaks of *Vitex negundo*, *Rumex hastatus*, *Indigofera heterantha* and *Debregeasia salicifolia* showed significant amount of O-H stretch, H-bonded indicating alcohols, phenols and O-H stretch corresponds to Carboxylic acids. Carboxylic acid expressed acidity and act as preservative in food products manufacturing.

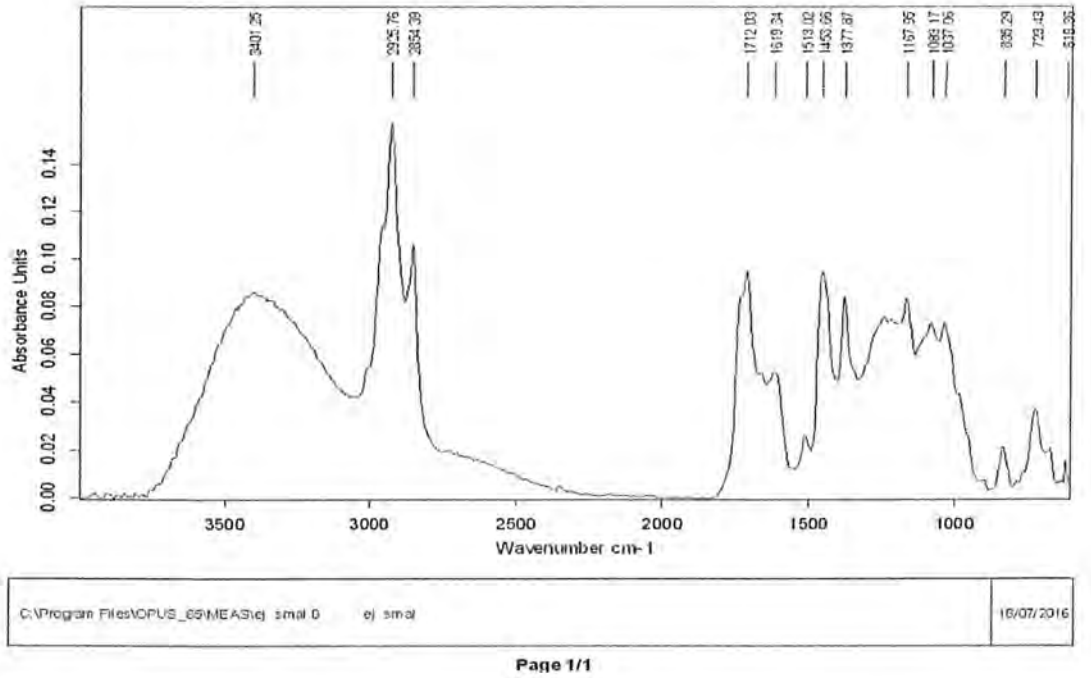


Fig.26.FTIR spectra of *Myrsine Africana* fruits showing various functional groups

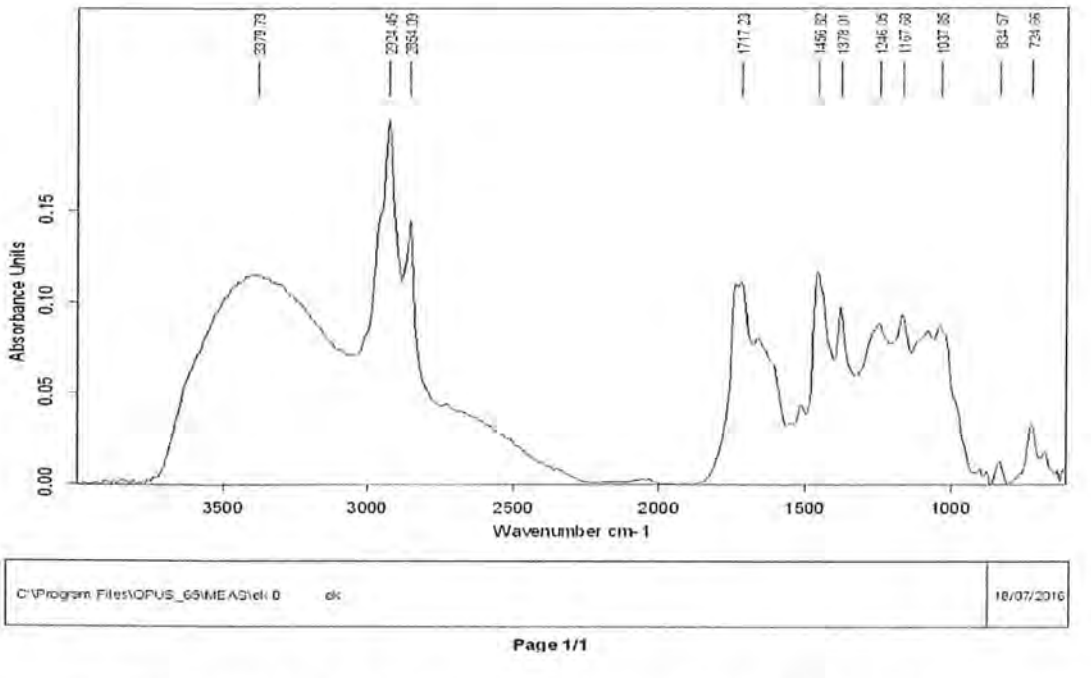


Fig.27. FTIR spectra of various functional group from *Myrsine Africana* leaves

Table.11. Fourier infrared spectrometry analysis of *Myrsine Africana* fruits

Peaks	Wavelength	Bond	Functional group
1	3401.25	O-H stretch, H-bonded	Alcohols, phenols
2	2925.76	O-H stretch	Carboxylic acids
3	2854.39	C-H stretch	Alkanes
4	1712.03	C=O stretch	Carbonyl (general)
5	1619.34	N-H bend	1° amines
6	1513.02	N-O asymmetric stretch	Nitro compounds
7	1453.66	C-H bend	Alkanes
9	1167.95	C-O stretch	Alcohols, carboxylic acids, esters, ethers
10	1083.17	C-N stretch	Aliphatic amines
11	1037.06	C-N stretch	Aliphatic amines
12	835.29	C-Cl stretch	Alkyl halides
13	723.43	C-H rock	Alkanes
14	618.36	C-Br stretch	Alkyl halides

Table.12. FT-IR analysis *Myrsine africana* leaves

Peaks	Wavelength	Bond	Functional group
1	3379.73	O-H stretch, H-bonded	Alcohols, phenols
2	2924.45	C-H stretch	Alkanes
3	2854.09	C-H stretch	Alkanes
4	1717.23	C=O stretch	Carbonyls (general)
5	1456.82	C-H bend	Alkanes
7	1246.05	C-N stretch	Aliphatic amines
8	1167.68	C-H wag (-CH_2X)	Alkyl halides
9	1037.85	C-N stretch	Aliphatic amines
10	834.57	C-Cl stretch	Alkyl halides
11	724.66	C-H rock	Alkanes

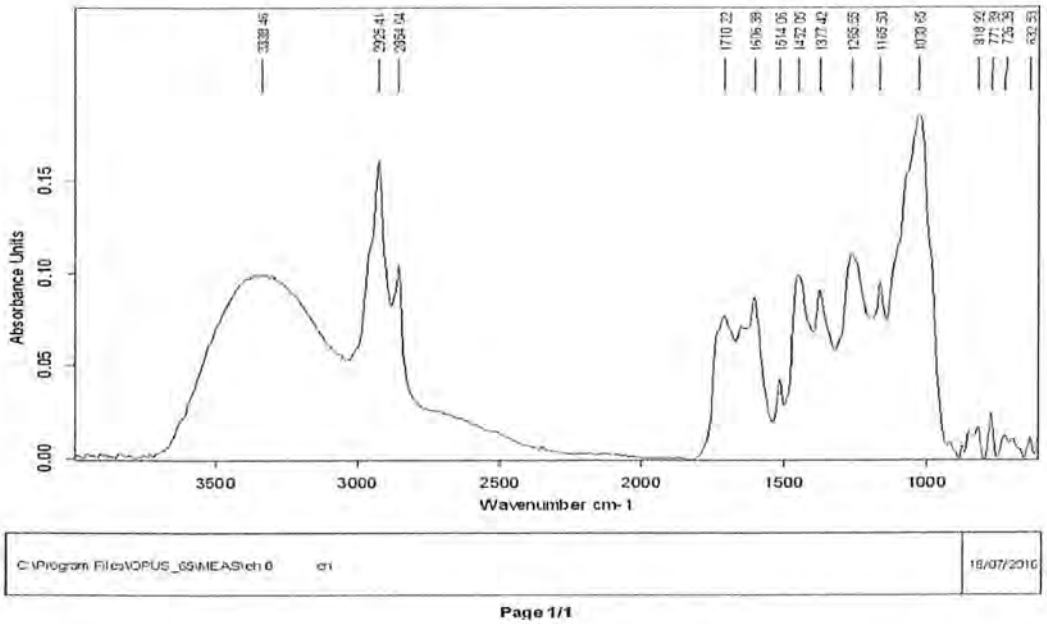


Fig.28.Fourier infrared spectrometry analysis of *Bauhinia variegata*

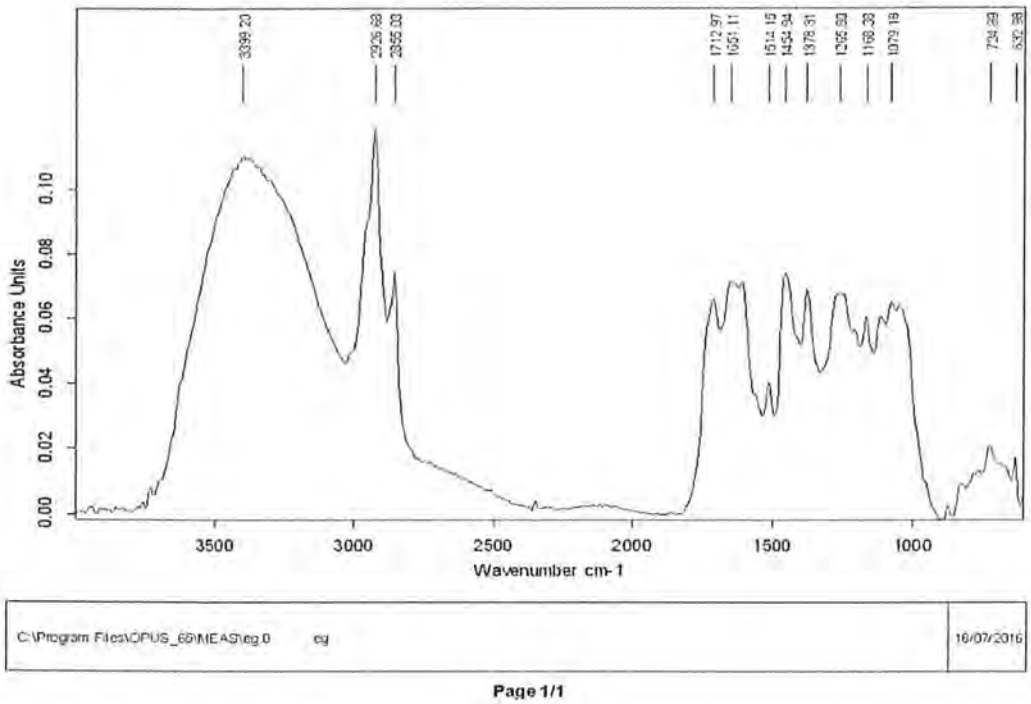


Fig.29.Fourier infrared spectrometry analysis of *Dodonaea viscosa*

Table.13. Fourier infrared spectrometry analysis of *Bauhinia variegata* flowers

Peaks	Wavelength	Bond	Functional group
1	3338.46	O-H stretch, H- bond	Alcohols, Phenols
2	2925.41	C-H stretch	Alkanes
3	2854.64	C-H stretch	Alkanes
4	1710.22	C=O Stretch	Carbonyls, carboxylic acids
5	1606.38	N-H bend	1° amines
6	1514.06	N-O asymmetric stretch	Nitro compounds
7	1452.05	C-C stretch, C-H bend	Aromatic, alkanes
9	1265.55	C-H wag (-CH_2X)	Alkyl halides
10	1165.50	C-N stretch	Aliphatic amines
11	1030.65	C-O stretch	Alcohols, Carboxylic acids, esters, ethers
12	818.92	C-Cl stretch	Alkyl halides
13	771.39	C-Cl stretch	Alkyl halides
14	726.28	C-Cl stretch	Alkyl halides
15	632.53	C-Br stretch	Alkyl halides

Table.14. FT-IR analysis of *Dodonaea viscosa* flowers

Peaks	Wavelength	Bond	Functional group
1	3399.20	O-H stretch, H-bonded	Alcohols, phenols
2	2926.68	O-H stretch	Carboxylic acids
3	2855.03	C-H stretch	Alkanes
4	1712.97	C=O stretch	Carbonyl (general)
5	1651.11	-C=C- stretch	Alkenes
6	1514.15	N-O asymmetric stretch	Nitro compounds
7	1454.94	C-H Bend	Alkanes
9	1265.80	C-H wag (-CH_2X)	Alkyl halides
10	1168.38	C-N stretch	Aliphatic amines
11	1079.18	C-N stretch	Aliphatic amines
12	724.89	C-H rock	Alkanes
13	632.98	C-Br stretch	Alkyl halides

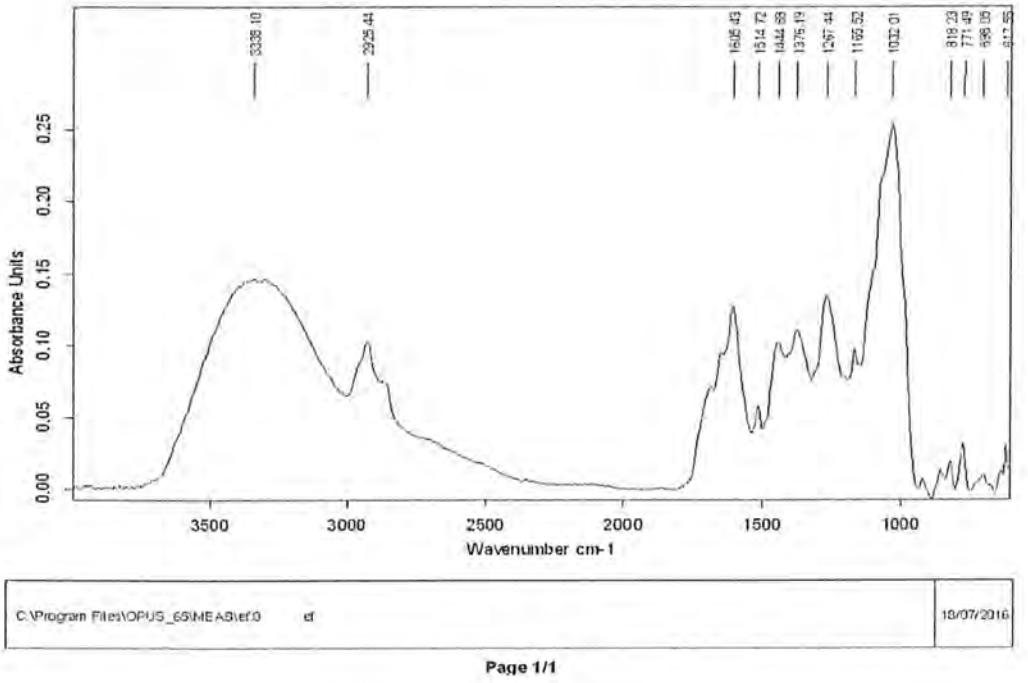


Fig.30. FT-IR spectra showing functional groups of *Vitex negundo*

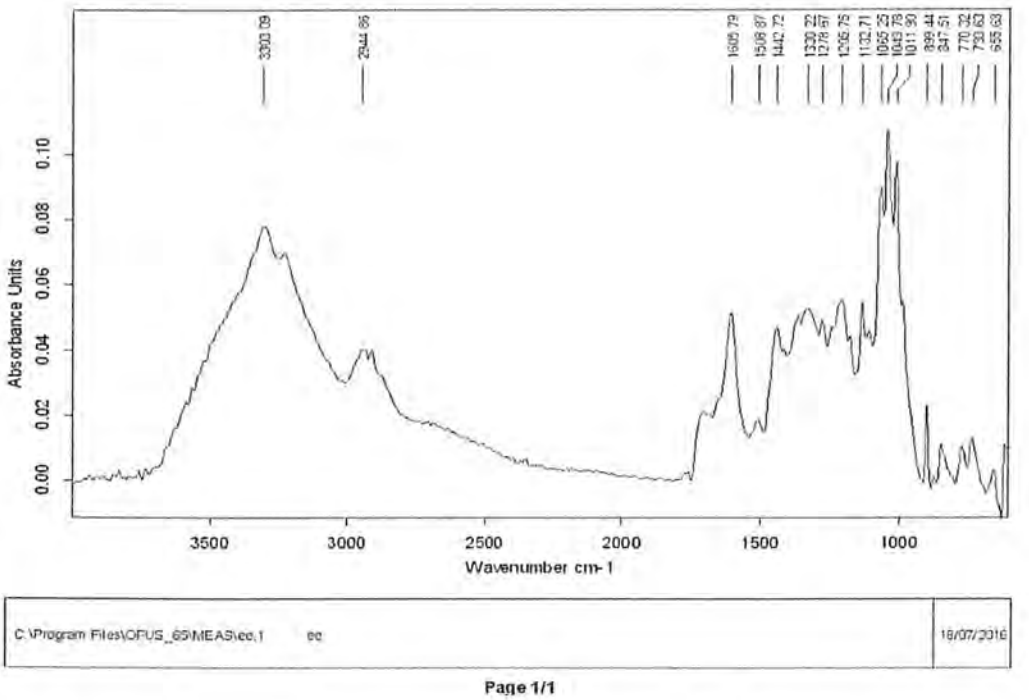


Fig.31. FT-IR spectra of various functional groups from *Rumex hastatus*

Table.15. FTIR analysis of *Vitex negundo* Leaves

Peaks	Wavelength	Bond	Functional group
1	3338.10	O-H stretch, H-bonded	Alcohols, phenols
2	2925.44	O-H stretch	Carboxylic acids
3	1605.43	N-H bend	1° amines
4	1514.72	N-O asymmetric stretch	Nitro compounds
5	1444.88	C-C stretch (in-ring)	Aromatics
7	1267.44	CN- Stretch	Aromatic amines
8	1165.52	C-O stretch	Alcohols, carboxylic acids, esters, ethers
9	1032.01	C-N stretch	Aliphatic amines
10	818.23	C-Cl stretch	Alkyl halides
11	771.49	C-Cl stretch	Alkyl halides
12	698.05	-C=C-H: C-H bend	Alkynes
13	617.55	C-Br stretch	Alkyl halides

Table.16. FTIR analysis of *Rumex hastatus* leaves

Peaks	Wavelength	Bond	Functional group
1	3303.09	O-H stretch, H-bonded	Alcohols, phenols
2	2944.86	O-H stretch	Carboxylic acids
3	1605.79	N-H bend	1° amines
4	1508.87	N-O asymmetric stretch	Nitro compounds
5	1442.72	C-C stretch (in-ring)	Aromatics
6	1330.22	N-O symmetric stretch	Nitro compounds
7	1278.67	CN- Stretch	Aromatic amines
8	1205.75	C-N stretch	Aliphatic amines
9	1132.71	C-N stretch	Aliphatic amines
10	1065.25	C-N stretch	Aliphatic amines
11	1043.78	C-N stretch	Aliphatic amines
12	1011.90	C-N stretch	Aliphatic amines
13	899.44	C-H "oop"	Aromatics
14	847.51	C-Cl stretch	Alkyl halides
15	770.32	C-Cl stretch	Alkyl halides
16	733.63	C-Cl stretch	Alkyl halides
17	655.63	C-Br stretch	Alkyl halides

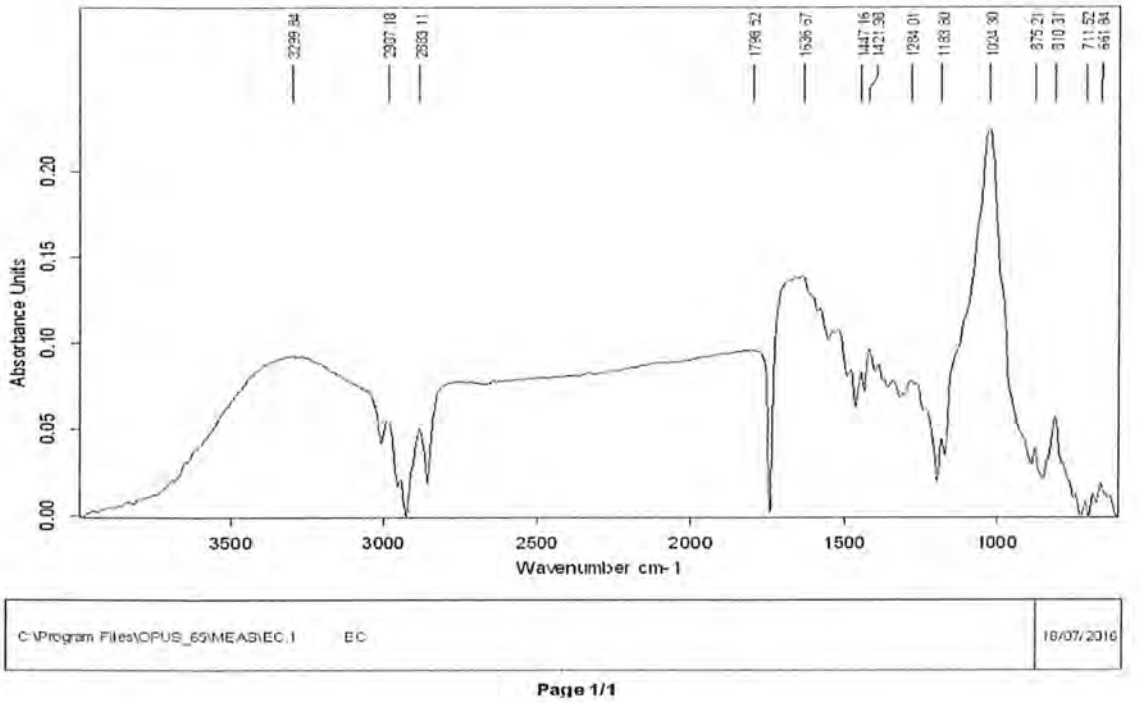


Fig.32. FTIR spectra of *Indigofera heterantha* leaves

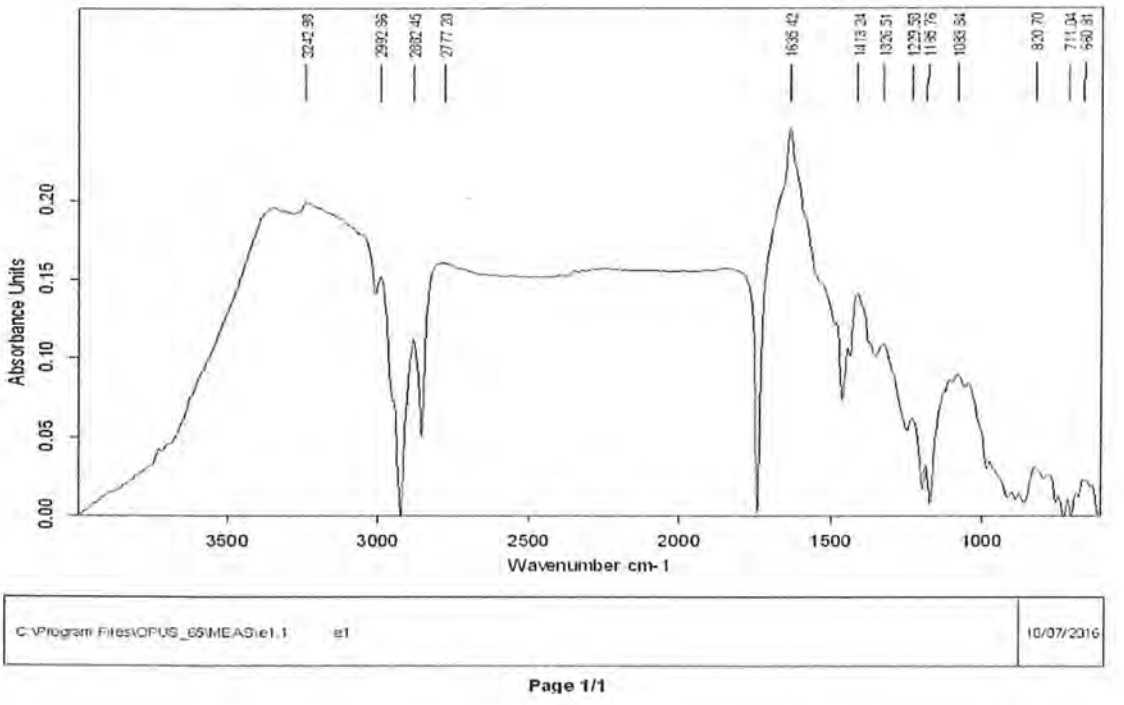


Fig.33. FTIR spectra of *Debregeasia salicifolia* leaves

Table.17. FT-IR analysis of *Indigofera heterantha* leaves

Peaks	Wavelength	Bond	Functional group
1	3299.84	O-H stretch, H-bonded	Alcohols, phenols
2	2987.18	O-H stretch	Carboxylic acids
3	2883.11	C-H stretch	Alkanes
5	1636.67	N-H bend	1^{o} amines
6	1447.16	C-C stretch (in-ring)	Aromatics
7	1421.98	C-C stretch (in-ring)	Aromatics
8	1284.01	C-H wag ($-CH_2X$)	Alkyl halides
9	1183.80	C-N stretch	Aliphatic amines
10	1024.30	C-O stretch	Alcohols, carboxylic acids, esters, ethers
11	875.21	C-H "oop"	Aromatics
12	810.31	C-Cl stretch	Alkyl halides
13	711.52	C-H rock	Alkanes
14	661.84	C-Br stretch	Alkyl halides

Table.18. FTIR analysis of *Debregeasia salicifolia* leaves

Peaks	Wavelength	Bond	Functional group
1	3242.98	O-H stretch, H-bonded	Alcohols, phenols
2	2992.86	C-H stretch	Alkanes
3	2882.45	C-H stretch	Alkanes
4	2777.20	H-C=O:C-H stretch	Aldehydes
5	1635.42	N-H bend	1° amines
6	1413.24	C-C stretch (in-ring)	Aromatics
7	1326.51	N-O symmetric stretch	Nitro compounds
8	1229.58	C-N stretch	Aliphatic amines
9	1185.76	C-N stretch	Aliphatic amines
10	1083.84	C-N stretch	Aliphatic amines
11	820.70	C-Cl stretch	Alkyl halides
12	711.04	C-Cl stretch	Alkyl halides
13	660.81	C-Br stretch	Alkyl halides

3.2.6 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) Analysis

Volatile organic compounds like fatty acids present in the plant extracts were analyzed by using GC-MS. Results were obtained in the form of chromatograms by comparing chromatogram, peak areas (%) of compounds were calculated and other information were obtained from NIST library data base by matching these compounds with standard. According to results higher quantity of ascorbic acid and stearic acids were present in plant extracts. *Myrsine africana* fruit contained significant quantity of oleic acid ($C_{18}H_{34}O_2$) (36.86%), glycidol stearate ($C_{21}H_{40}O_3$) (1.82%) and cyclohexane carboxylic acid ($C_{19}H_{32}O_2$) (0.69%) which were not previously reported. Higher compound found in *Myrsine Africana* leaves was octadecenoic acid ($C_{18}H_{34}O_2$) (24.36%) followed by some important compounds palmitoyl chloride ($C_{16}H_{31}C_{10}$) (3.02%), phosphaheptacos ($C_{44}H_{84}NO_8$)(2.14%) and Heptacosyl heptafluorobutyrate ($C_{31}H_{55}F_7O_2$) (3.60%).

The results revealed presence of 10 organic compounds in *Bauhinia variegata* and among that 4 compounds were dominating as 1-(+)-Ascorbic acid 2, 6-dihexadecanoate (Peak 3) (35.092 %), formula $C_{38}H_{68}O_8$, docosanoic anhydride ($C_{44}H_{86}O_3$) (1.580%), sebacic acid ($C_{23}H_{40}O_4$) (0.616%) and hexadecenoic acid ($C_{36}H_{70}O_2$) (0.271%). Oleic ($C_{18}H_{34}O_2$)(18.93%), octadecenoic acid ($C_{18}H_{34}O_2$) (10.41%) and fumaric acid ($C_{23}H_{40}O_4$) (1.68%) were frequently found in *Vitex negundo*.

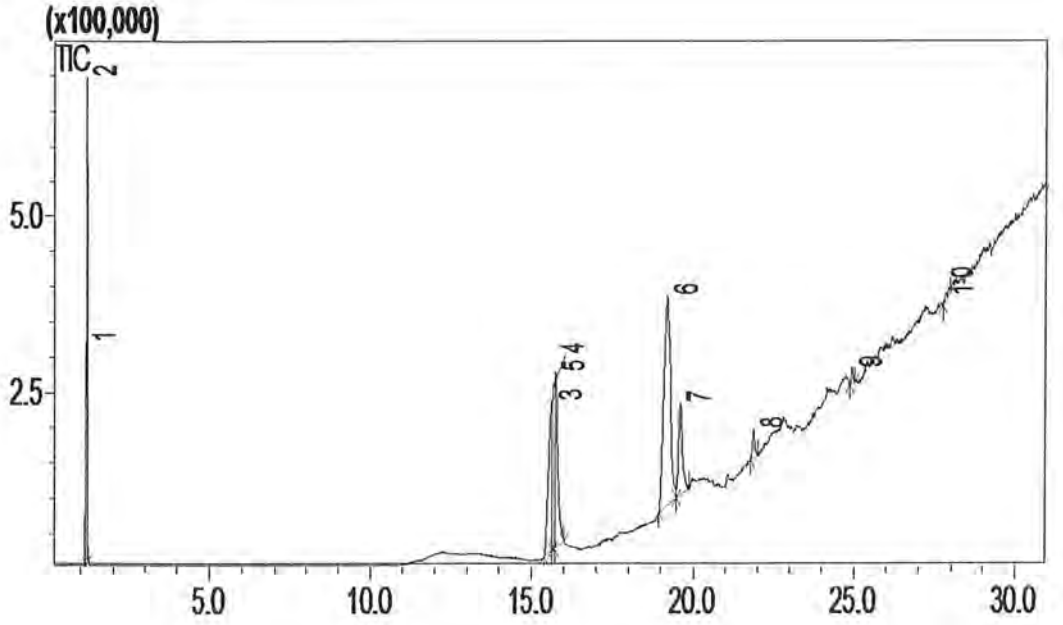


Fig.34.GC chromatogram of *Myrsine africana* Fruit

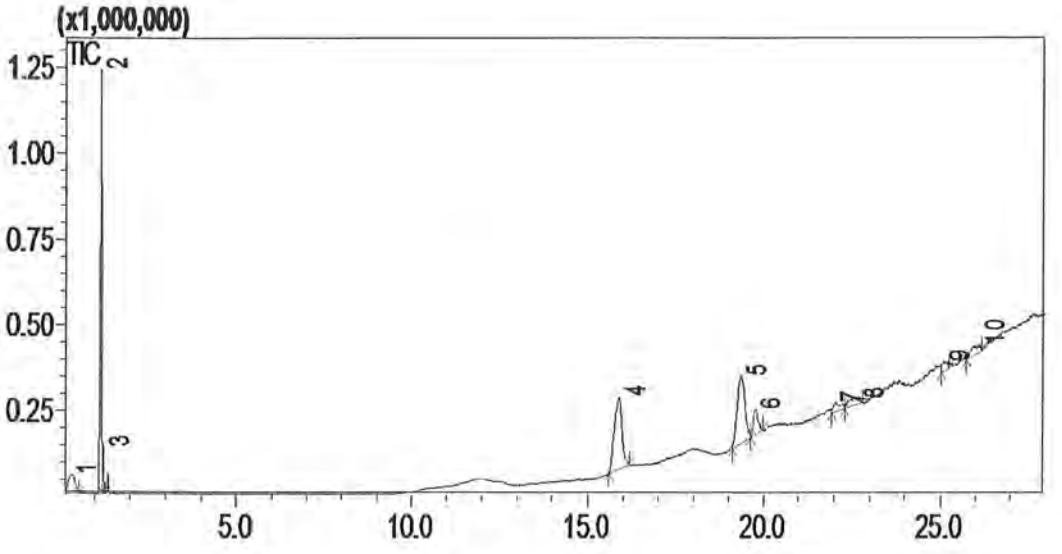


Fig.35.GC chromatogram of *Myrsine africana* Leaves

Table.19. GCMS analysis of *Myrsine africana* fruit

Peaks	Compounds name	Formula	Molecular weight	Area %	Retention time
1	Nickel tetracarbonyl	C_4NiO_4	170	3.86	1.136
2	Propanone	C_3H_6O	58	9.98	1.178
3	Ascorbic acid	$C_{38}H_{68}O_8$	652	13.78	15.620
4	Ascorbic acid	$C_{38}H_{68}O_8$	652	8.43	15.675
5	Ascorbic acid	$C_{38}H_{68}O_8$	652	14.58	15.732
6	Oleic acid	$C_{18}H_{34}O_2$	282	36.86	19.182
7	Stearic acid	$C_{18}H_{36}O_2$	284	9.12	19.590
8	Glycidol stearate	$C_{21}H_{40}O_3$	340	1.82	21.881
9	Cyclohexanecarboxylic acid	$C_{19}H_{32}O_2$	292	0.69	24.933
10	Hexacontane	$C_{60}H_{122}$	842	0.88	27.775

Table.20. GCMS analysis of *Myrsine africana* leaves

Peaks	Compounds name	Formula	Molecular weight	Area%	Retentin time
1	Heptacosanol	$C_{27}H_{56}O$	396	6.07	0.321
2	Ethanol	C_2H_6O	46	25.95	1.167
3	Isobutyl alcohol	$C_4H_{10}O$	74	1.73	1.247
4	Ascorbic acid	$C_{38}H_{68}O_8$	652	24.26	15.889
5	Octadecenoic acid	$C_{18}H_{34}O_2$	282	24.36	19.352
6	Stearic acid	$C_{18}H_{36}O_2$	284	5.59	19.760
7	Palmitoyl chloride	$C_{16}H_{31}C_{10}$	274	3.02	22.025
8	Hexacontane	$C_{60}H_{122}$	842	3.28	22.596
9	Phosphaheptacos	$C_{44}H_{84}NO_8$	785	2.14	25.087
10	HeptacosylHeptafluorobutyrate	$C_{31}H_{55}F_7O_2$	592	3.60	26.106

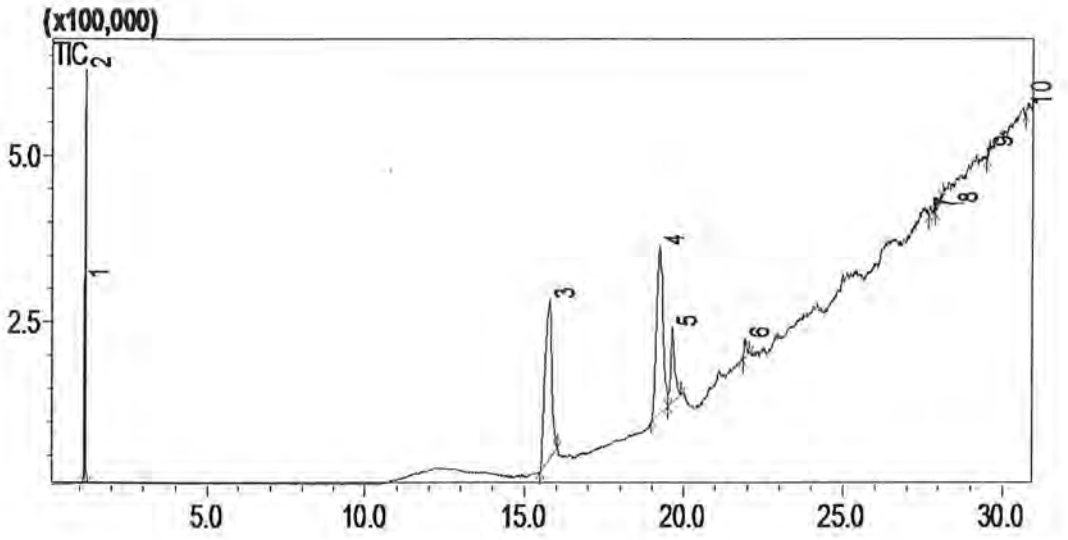


Fig.36.GCMS Chromatogram of *Bauhinia variegata* flowers

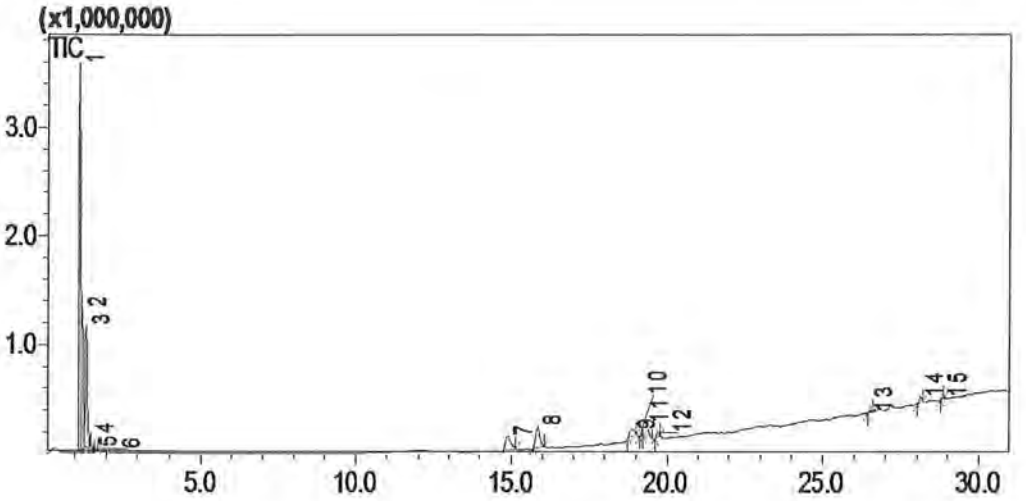


Fig.37.GCMS Chromatogram of *Dodonaea viscosa* flowers

Table.21. GCMS analysis of *Bauhinia variegata* flowers

Peaks	Compounds name	Formula	Molecular weight	Area (%)	Retention time
1	Nickel tetracarbonyl	C_4NiO_4	170	4.213	1.136
2	Propanone	C_3H_6O	58	10.321	1.178
3	Ascorbic acid	$C_{38}H_{68}O_8$	652	35.092	15.776
4	Dihydroxy propylelaidate	$C_{21}H_{40}O_4$	356	35.740	19.215
5	Stearic acid	$C_{18}H_{36}O_2$	284	10.314	19.626
6	Docosanoic anhydride	$C_{44}H_{86}O_3$	662	1.580	21.907
7	Sebacic acid	$C_{23}H_{40}O_4$	380	0.616	27.710
8	Hexacontane	$C_{60}H_{122}$	842	0.710	27.940
9	Hexadecenoic acid	$C_{36}H_{70}O_2$	534	0.271	29.510
10	Tetrapentacontane	$C_{54}H_{108}Br_2$	914	1.140	30.750

Table.22. GCMS of *Dodonaea viscosa* flowers compounds

Peaks	Compounds name	Formula	Molecular weight	Area %	Retention time
1	Ethyl fluoride	C_2H_5F	48	44.66	1.132
2	Isobutyl alcohol	$C_4H_{10}O$	74	16.36	1.218
3	Isopentyl alcohol	$C_5H_{12}O$	88	16.78	1.350
4	Furanone	$C_5H_8O_2$	100	1.58	1.488
5	Dimethyl sulfoxoniumformylmethylide	$C_4H_8O_2S$	120	0.34	1.612
6	Isopentyl alcohol	$C_7H_{14}O_2$	130	0.30	1.660
7	Ascorbic acid	$C_{38}H_{68}O_8$	652	4.56	14.900
8	Ascorbic acid	$C_{38}H_{68}O_8$	652	5.56	15.846
9	Octadecenoic acid	$C_{18}H_{34}O_2$	282	2.11	18.858
10	Ricinoleic acid	$C_{18}H_{34}O_3$	298	1.02	19.205
11	Octadecenoic acid	$C_{18}H_{34}O_2$	282	4.30	19.278
12	Stearic acid	$C_{18}H_{36}O_2$	284	0.74	19.697
13	Carboxylic acid	$C_{23}H_{32}O_4$	372	0.40	26.460
14	Cyclopentanone	$C_{15}H_{20}O$	216	1.08	28.112
15	Cedrol	$C_{15}H_{26}O$	222	0.21	28.862

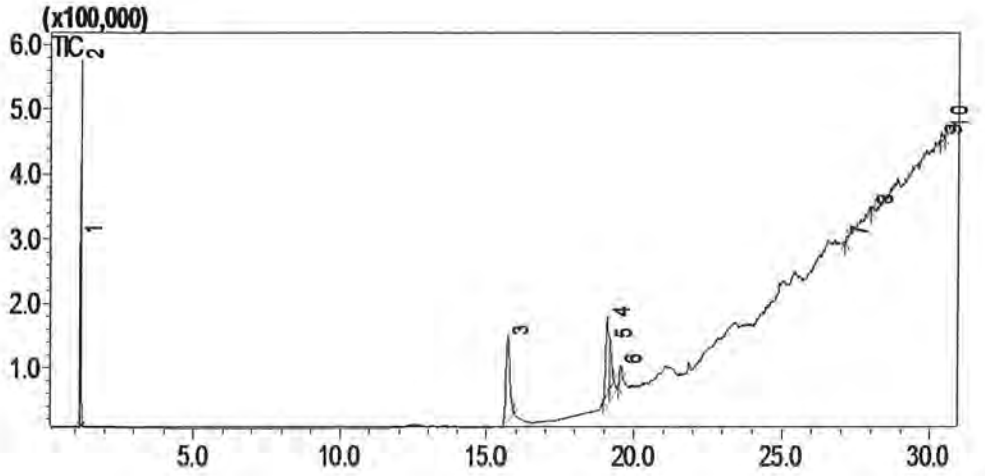


Fig.38. GCMS quantification of *Vitex negundo* leaves

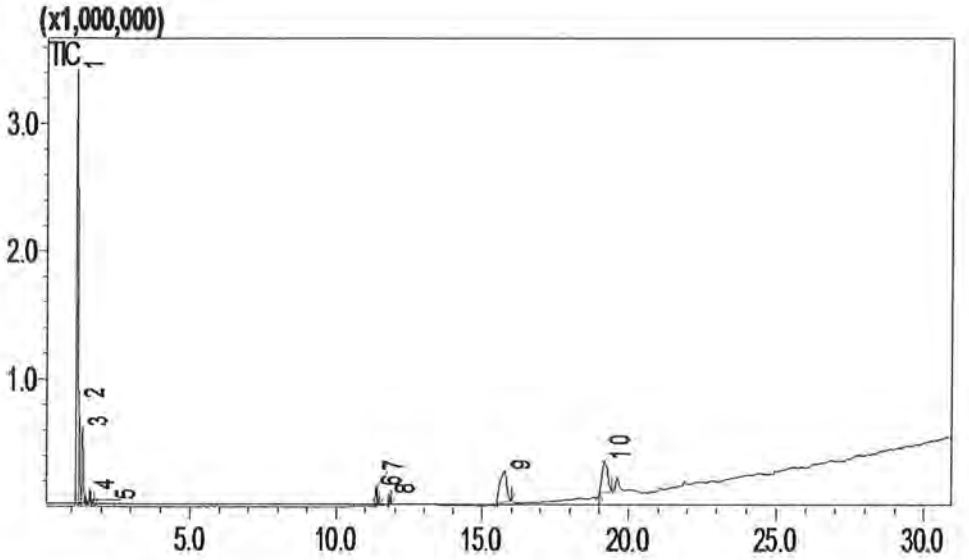


Fig.39. GCMS quantification of *Rumex hastatus* leaves

Table.23. GCMS analysis of *Vitex negundo* leaves

Peaks	Compounds name	Formula	Molecular weight	Area %	Retention time
1	Nickel tetracarbonyl	C_4NiO_4	170	10.49	1.135
2	Propanone	C_3H_6O	58	22.76	1.177
3	Ascorbic acid	$C_{38}H_{68}O_8$	652	29.43	15.688
4	Oleic acid	$C_{18}H_{34}O_2$	282	18.93	19.098
5	Octadecenoic acid	$C_{18}H_{34}O_2$	282	10.41	19.185
6	Stearic acid	$C_{18}H_{36}O_2$	284	3.18	19.536
7	Di methyl Phosphino methyl	$C_{11}H_{27}P_3$	252	1.16	27.155
8	n-Butyl-2 methyl – trans-decahydr	$C_{14}H_{27}NO$	225	1.10	28.005
9	Fumaric acid	$C_{23}H_{40}O_4$	380	1.68	30.335
10	Fumaric acid	$C_{26}H_{46}O_4$	422	0.86	30.524

Table.24. Chemical composition of *Rumex hastatus* leaves by GCMS

Peaks	Compounds name	Formula	Molecular weight	Area	Retention time
1	Pentanone	$C_6H_{12}O_2$	116	51.30	1.146
2	delta Valerola	$C_5H_8O_2$	100	8.93	1.217
3	Isopentyl alcohol	$C_5H_{12}O$	88	8.22	1.343
4	Dimethylsulfoxoniumformylmethylide	$C_4H_8O_2S$	120	1.59	1.587
5	P-Dime	C_8H_{10}	106	0.58	1.700
6	Ar-tumerone	$C_{15}H_{20}O$	216	1.37	11.359
7	Tumerone	$C_{15}H_{22}O$	218	1.56	11.414
8	Curlone	$C_{15}H_{22}O$	218	0.76	11.815
9	Ascorbic acid	$C_{38}H_{68}O_8$	652	13.10	15.778
10	Octadecenoic acid	$C_{18}H_{34}O_2$	282	12.59	19.180

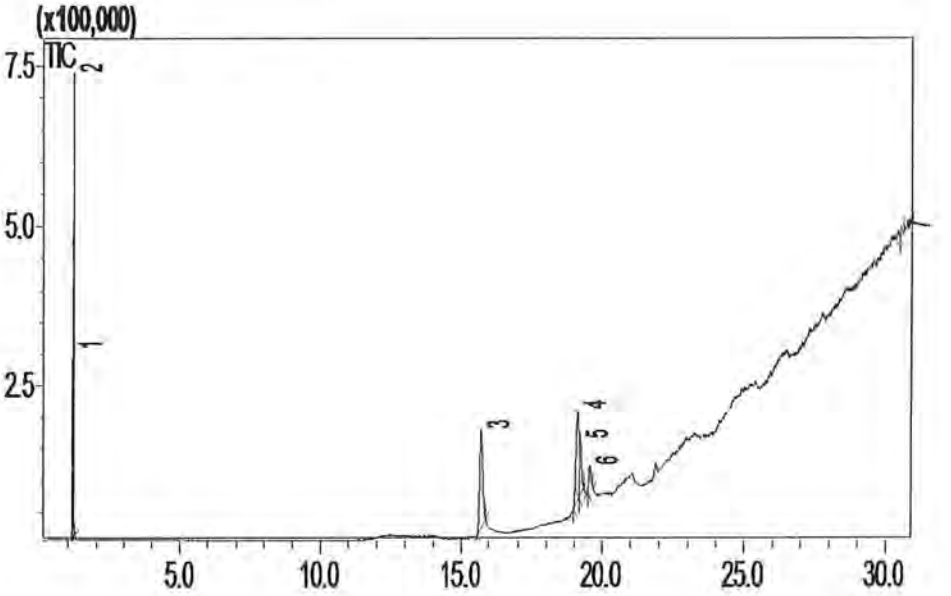


Fig.40. Gas chromatography mass spectrometry chromatogram of *Indigofera heterantha* leaves

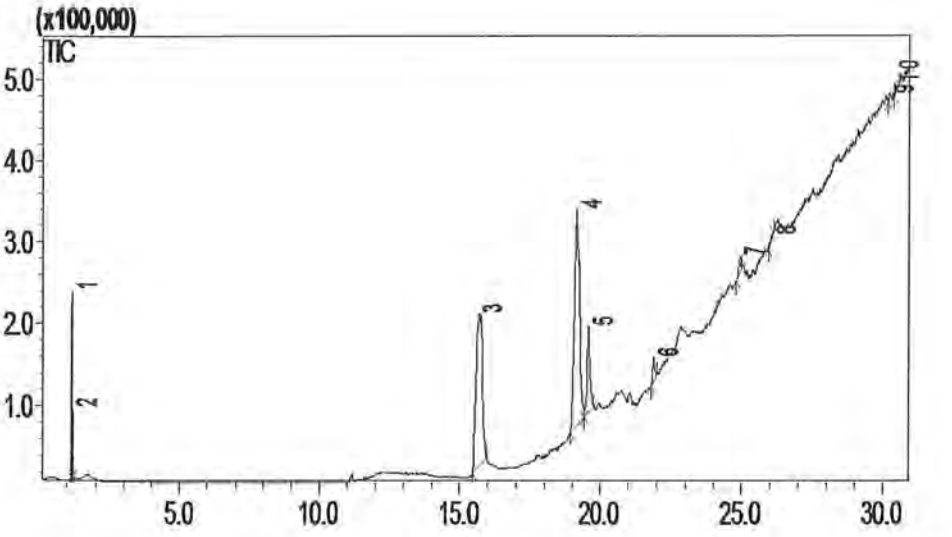


Fig.41. Gas chromatography mass spectrometry chromatogram of *Debregeasia salicifolia* leaves

Table.25. Chemical composition of *Indigofera heterantha* by GCMS analysis

Peaks	Compounds name	Formula	Molecular weight	Area %	Retention time
1	Propanone	C_3H_6O	58	9.00	1.136
2	Ascorbic acid	$C_{38}H_{68}O_8$	652	24.52	1.178
3	Ascorbic acid	$C_{38}H_{68}O_8$	652	28.60	15.697
4	Octadecenoic acid	$C_{18}H_{34}O_2$	282	20.60	19.119
5	Dihydroxy propyl elaidate	$C_{21}H_{40}O_4$	356	11.42	19.215
6	Stearic acid	$C_{18}H_{36}O_2$	284	4.69	19.558
7	Fumaric acid	$C_{25}H_{44}O_4$	408	1.17	30.545

Table.26. Chemical compositional analysis of *Debregeasia salicifolia*

Peaks	Compounds name	Formula	Molecular weight	Area%	Retention time
1	Nickel tetracarbonyl	C_4NiO_4	170	3.93	1.136
2	Ethanol	C_2H_6O	46	1.32	1.165
3	Ascorbic acid	$C_{38}H_{68}O_8$	652	37.57	15.644
4	Oleic acid	$C_{18}H_{34}O_2$	282	42.44	19.138
5	Stearic acid	$C_{18}H_{36}O_2$	284	9.63	19.565
6	Glycidol stearate	$C_{21}H_{40}O_3$	340	2.03	21.869
7	Propanetriyl ester	$C_{57}H_{104}O_6$	884	1.35	24.925
8	Sebacic acid	$C_{23}H_{40}O_4$	380	0.59	26.035
9	Methyltntnacontane	$C_{34}H_{70}$	478	0.55	30.185

SECTION 3

BIOLOGICAL ACTIVITIES OF MEDICINAL PLANTS

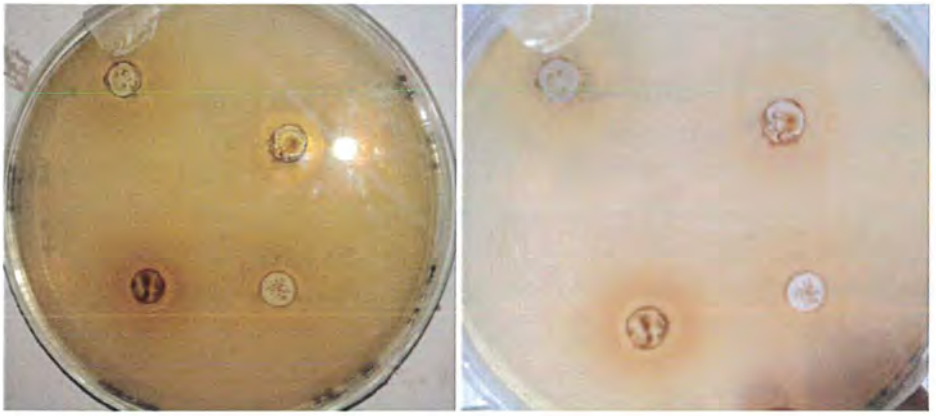
3.3.1 Antimicrobial activity of Medicinal plants

Extracts of seven different plants were analyzed against different bacterial strains and tested plants showed good activity against *Staphylococcus aureus* and *Escherichia coli.* Plants extracts were assessed against different gram positive and negative bacterial strains. Different fractions of plants were evaluated and results are in Table 27 and 28.

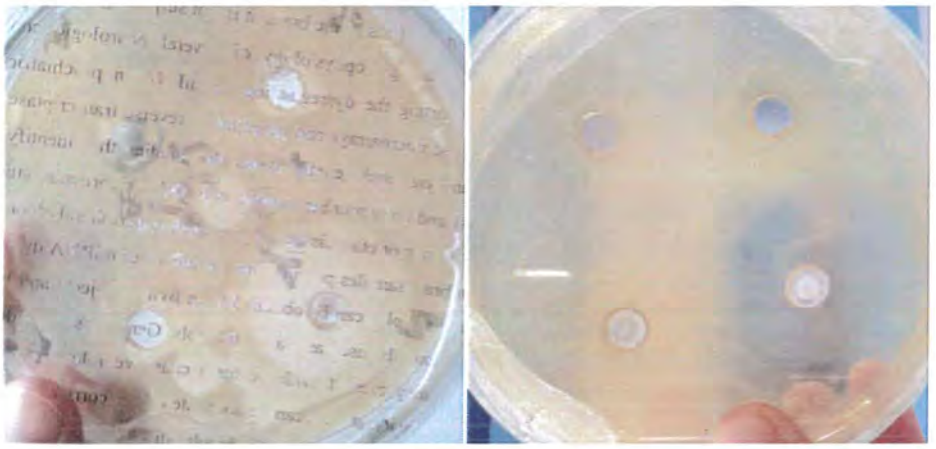
Myrsine africana leaves methanol was found to have higher activity against *E. Coli* 25±1.5mm zone followed by acetone extract 22±1.56mm zone against *S. aureus*. *Myrsine africana* fruit methanol extract showed highest activity against *S.aureus*22±6mm zone. Methanol, acetonitrile and Ethyl acetate extracts of *Rumex hastatus* showed higher zones against *S.aureus*21±3, 22±0.6mm and 23±1.2mm. *Indigofera heterantha n*-hexane and methanol extracts showed highest activities against *S.aureus*25±1mm zone *E. coli* 23±2mm zone. Methanol extract of *Bauhinia variegata* showed highest zones 23±1.7mm against *E.coli,* 21±2mm against *S.aureus* followed by methanol extract which showed 19±4mm against *P. aeruginosa*. Methanol and ethyl acetate extracts of *Debregeasia salicifolia* showed highest activity 22±2.1mm against *E. coli* and 20±2.7mm against *S.aureus.* Among all plant extracts acetonitrile extract of *Vitex negundo* showed highest activity 27±5mm against *E. coli* followed by *n*-hexane extract 22±4mm zone, acetonitrile 20±2mm zone and methanol 20±2mm against *S.aureus.*

Table.3. MIC of Medicinal plants

Extracts	*Staphylococcus aureus*	*Echerichia Coli*
***Myrsine africana* Leaves Methnol**	6.25mg/ml	12.5mg/ml
***Myrsine africana* Leaves Acetone**	3.125mg/ml	3.125mg/ml
***Myrsine africana* Leaves N-hexane**	6.25mg/ml	12.5mg/ml
***Myrsine africana* Fruits Nhexane**	6.25mg/ml	12.5mg/ml
***Myrsine africana* Fruits Methanol**	3.125mg/ml	3.125mg/ml
***Myrsine africana* Fruits Ethyl acetate**	-	-
***Myrsine africana* Fruits Acetonitryl**	-	6.25mg/ml
***Bauhinia variegata* Flower Methanol**	25mg/ml	12.5mg/ml
***Rumex hastatus* Leaves Acetonityrl**	-	3.125mg/ml
***Rumex hastatus* Leaves Methanol**	6.25mg/ml	12.5mg/ml
***Rumex hastatus* Leaves Ethyl acetate**	25mg/ml	12.5mg/ml
***Dodonaea viscose* Flower Acetonityrl**	25mg/ml	25mg/ml
***Dodonaea viscosa* Flower N-hexane**	12.5mg/ml	-
***Indigofera* heterantha Methanol**	3.125mg/ml	6.25mg/ml
***Indigofera* heterantha n-hexane**	-	6.25mg/ml
***Indigofera* heterantha Acetone**	12.5mg/ml	12.5mg/ml
***Debregeasia salicifolia* Leaves Methanol**	12.5mg/ml	6.25mg/ml
***Debregeasia salicifolia* Leaves Ethyl acetate**	3.125mg/ml	12.5mg/ml
***Vitex negundo* Leaves Acetonityrl**	-	12.5mg/ml
***Vitex negundo* Leaves Methanol**	3.125mg/ml	3.125mg/ml
***Vitex negundo* Leaves n-hexane**	12.5mg/ml	25mg/ml



Myrsine africana Leaves *Dodonaea viscosa* flowers



Vitex negundo leaves Streptomycin

Fig.1. Plants showed different zones against bacteria's

3.3.2 Anti-tuberculosis assay

Anti-tuberculosis activity of plants extracts was tested against three strains bg 1972, bg 206 and H37Rv. Plant extracts were evaluated against three tuberculosis strains bg 1972, H37Rv and bg 206. Various concentrations of 5 mg, 10 mg and 50 mg / ml were used, and by

increasing the concentration percentage inhibition of tuberculosis was increased. Plant extracts showed higher inhibition against H37Rv strains. Plants extracts showed lower resistance against strain bg 206 as compared to other strains of tuberculosis. *Debregeasia salicifolia* showed more antituberculosis activity and leads to higher minimum inhibitory concentration by *Debregeasia salicifolia* 3.12 µg / ml against H37Rv strain.

Rifampicin was the standard drug showed minimum inhibitory concentration at 0.125 against H37Rv strain. Percentage inhibition was measured and results were given in the Table 30. Minimum inhibitory concentrations of above three strains were tested against plants extracts and showed great resistant to tuberculosis strain for their growth given in the Table 31.

Tabl.4. Anti tuberculosis activity of Medicinal plants

Extracts	Isolates	Mean CFU on media				Percentage Inhibition		
***M. africana* Leaves**		Control	5mg/mL	10mg/mL	50mg/Ml	5mg/mL	10mg/mL	50mg/mL
	bg 1972	130	71	40	0	46	69	100
	H37Rv	140	20	0	0	86	100	100
	bg 206	150	42	15	0	73	90	100
***M. africana* Fruit**	bg 1972	130	65	50	0	50	62	100
	H37Rv	140	35	0	0	79	100	100
	bg 206	150	45	20	0	70	87	100
***D. Salicifolia* Leaves**	bg 1972	130	58	31	0	55	76	100
	H37Rv	140	19	0	0	86	100	100
	bg 206	150	35	5	0	77	95	100
	bg 1972	130	74	51	0	43	61	100
***B. variegata* Flowers**	H37Rv	140	36	2	0	74	99	100
	bg 206	150	49	23	0	67	85	100
***D. viscosa*Flower**	bg 1972	130	80	55	0	38	58	100
	H37Rv	140	40	4	0	71	97	100
	bg 206	150	53	26	0	65	83	100
***I. heterantha* Leaves**	bg 1972	130	75	53	0	42	59	100
	H37Rv	140	37	2	0	74	99	100

	bg 206	150	50	24	0	67	84	100
***R. hastatus* Leaves**	bg 1972	130	81	56	0	38	49	100
	H37Rv	140	43	5	0	69	96	100
	bg 206	150	56	28	0	63	81	100
***V. negundo* Leaves**	bg 1972	130	60	42	0	54	68	100
	H37Rv	140	22	0	0	84	100	100
	bg 206	150	40	10	0	73	93	100

Percentage Inhibition = Cc – Ct/Cc x100 Cc = No of colony in the control media slope, Ct = No colony in the Test media slope

Table.5. Minimum inhibitory concentration of Medicinal plants

Plant names	**Strains**		
	H37Rv	bg 1972	bg 206
DS	3.12	12.5	6.25
ML	6.25	25	12.5
VN	12.5	25	12.5
MF	12.5	25	12.5
BV	12.5	50	25
IH	12.5	100	25
DV	25	100	25
RH	25	100	50
Rif	0.125	0.5	0.25

3.3.3 Cytotoxicity assay of plant extracts

Cytotoxicity assays consist of two assessments such as brine shrimp and cancer cell line assay. Plants extract's showed toxicity which kills cancer cells to grow.

3.3.3.1 Brine shrimp

Plants extract's showed toxicity against shrimps and toxicity increases with increased concentrations of plants extract. Plants showed moderate to good activity against shrimps as given in Fig. 50. Plant showed cytotoxicity against brine shrimp as followed *Bauhinia variegata*, *Indigofera heterantha*, *Rumex hastatus*, *Debregeasia salicifolia*, *Vitex negundo*, *Myrsine africana* leaves, *Myrsine africana* fruit and *Dodonaea viscosa*. LD_{50} is 50% lethal concentration of plants extracts and higher LD_{50} is 10 found in *Bauhinia variegata* and positive control K_2CrO_7. Toxicity produced by these plants extracts found to be active against brine shrimp and tumors in Fig. 51.

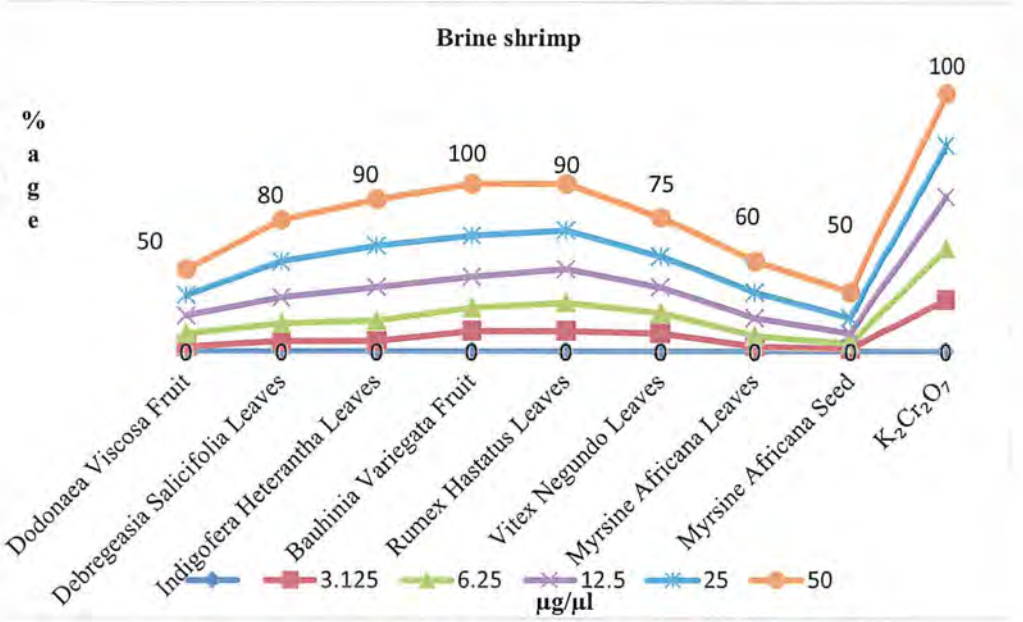


Figure.50.It illustrated that results are expressed in %

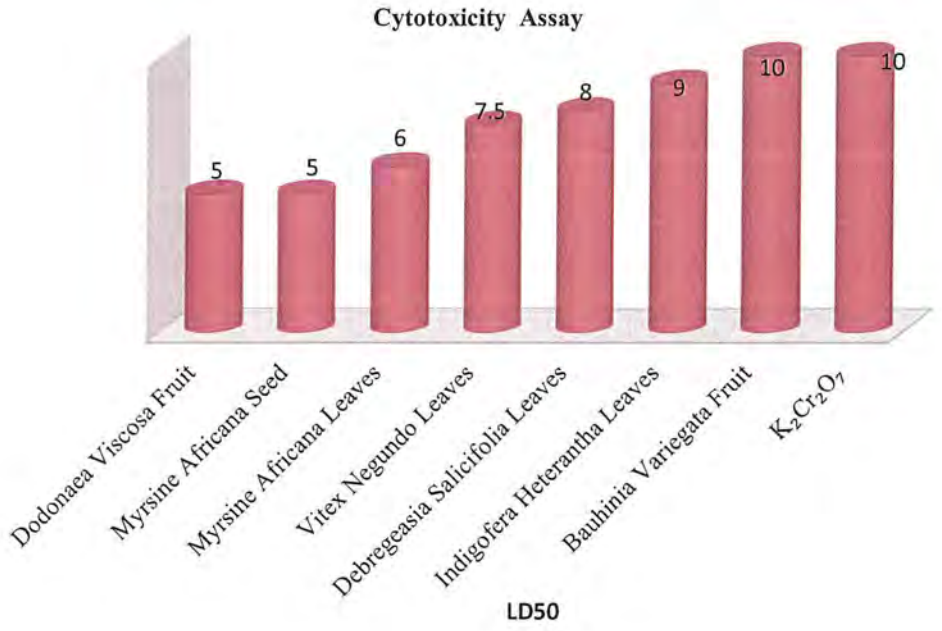


Fig.51. Showing different plant extracts lethality

3.3.3.2 Anti-proliferative assay (Cancer cell line)

Plants extracts were screened against human cell line HepG2 (Liver cancer cell line). Plating density was 10000 cells per well exposed for 24 hours in Fig. 52.The results of in vitro cytotoxicity using SRB assay are presented herein based on the recommendation of the National Cancer Institute (NCI, USA) that 30 µg/mL is the upper IC_{50} limit considered promising for purification and further utilization of plant based natural products (Newman and Cragg, 2012).

Plants extracts were examined against human liver carcinoma cell line to check their cytotoxicity. The images that showed more viability expressed the fewer defenses to cancer cells. As the cytotoxicity increases viability % of cell became lower. The higher anticancer activity was shown by RH DMSO extract 87.89 % inhibition which means 12.11±2.86 % cancer cells were viable. *Vitex negundo* methanol showed 14.1±2.11% viability and 85.9% inhibition which proved it stronger anticancer potential plant extracts. Methanol extract of *Debregeasia salicifolia* showed 64.55±3.19 % viability and this showed that plant extracts possess lower activity 36.45±2.1%. *Indigofera heterantha* methanol extract possessed 74.92± 3.78 % viability and (26.08±0.87%) inhibition against liver carcinoma cell line. Results indicates that 68.61 % viability that means 32.39 % of anticancer activities of methanol flower extracts of *Bauhinia variegata* which may be considered useful for accidence where synthetic drugs might have lower or no action due to some other complication in organisms.

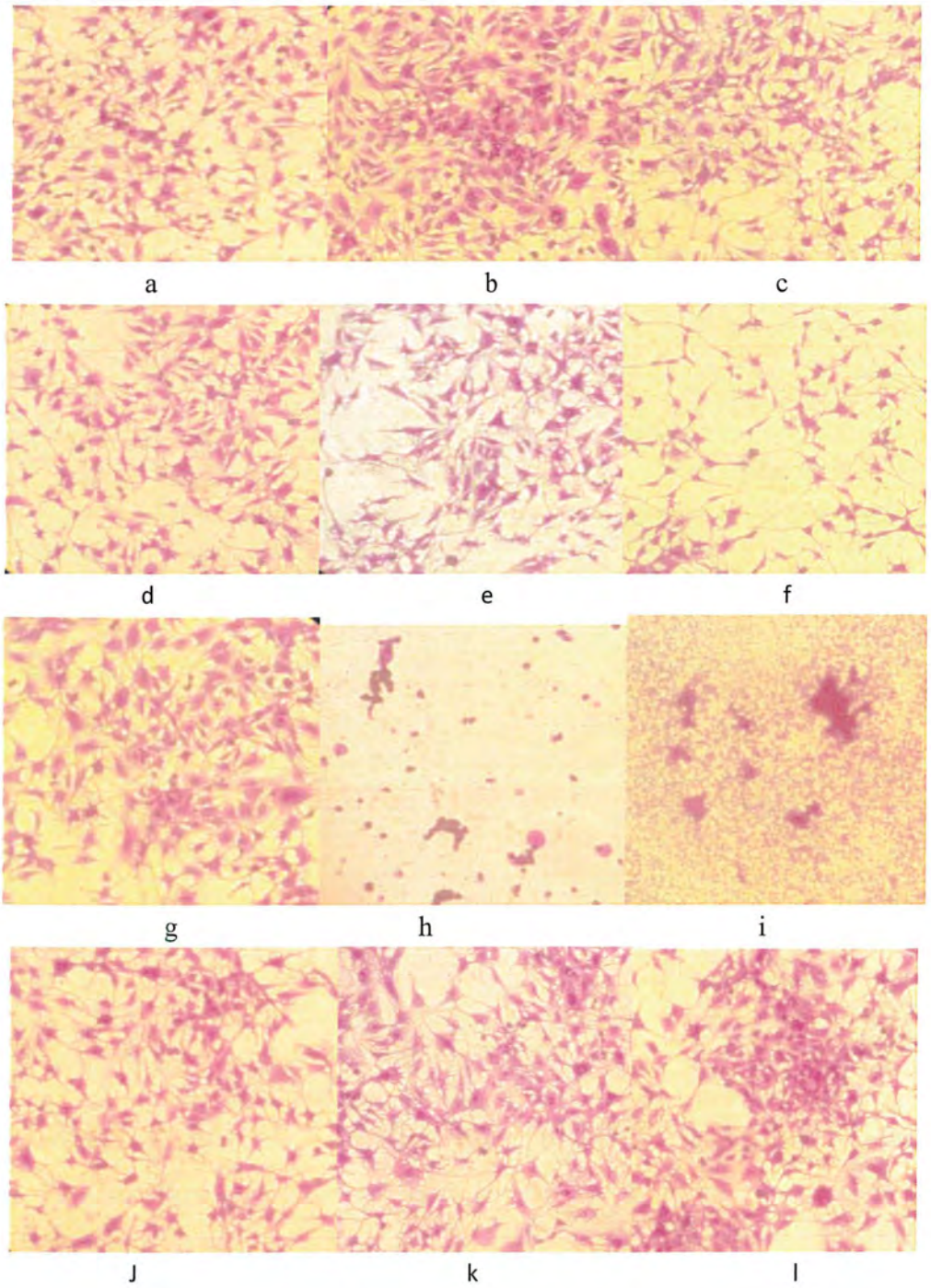


Fig.52. a: Untreated cancer cells. Fig. b, c, d, e &g. Less activity of plants as cancer cells viability was more. Figure h and f. Good to moderate activity against cancer cells and less viability.

Table.32. Anticancer activity of Medicinal plants

Sr. No.	Extract/Compound	% Viability	Anticancer activity %	SD
1	ML n-Hexane	-	-	-
2	DV Methanol	-	-	-
3	DV DMSO	-	-	-
4	DV Acetone	-	-	-
5	BV Methanol	68.61	31.39	2.7
6	BV DMSO	-	-	-
7	BV Acetone	-	-	-
8	ML Methanol	77.68	22.32	2.5
9	ML DMSO	75.59	24.41	2.4
10	ML Acetone	-	-	-
11	MF Methanol	76.43	23.57	2.73
12	MF DMSO	-	-	-
13	MF Acetone	78.41	21.59	4.01
14	DS Methanol	64.55	35.45	3.19
15	DS DMSO	93.46	6.54	2.8
16	DS Acetone	-	-	-
17	RH Methanol	33.7	66.3	2.49
18	RH DMSO	12.11	87.89	2.86
19	RH Acetone	-	-	-
20	IH Methanol	74.92	25.08	3.78
21	IH DMSO	-	-	-
22	IH Acetonitrile	73.8	26.2	3.14
23	VN Methanol	14.1	85.9	2.11
24	VN DMSO	-	-	-
25	VN Acetone	-	-	-

3.3.4 Potato disc assay of Medicinal plants

Plants extracts were analyzed against tumor which was induced by *Agrobacterium tumefacien* with different fractions. Plant extracts shows good defense as shown in Table 33. Potato disc assay of these plants extracts were assessed with different fractions. 10, 100 and 1000µg/ml concentrations were analyzed in the four fractions (Methanol, Ethanol, chloroform and n-hexane).

Table.33. Antitumor activity of Medicinal plants

Plant name	Chloroform	N-hexane	Methanol	Ethanol
Debregeasia salicifolia Leaves	56±4.8	33±0.9	90±2.8	80±6
Indigofera heterantha Leaves	79±3.2	81±2.5	85±3	32±2
Myrsine africana Leaves	88±9.5	33.4±1.3	44.5±1.7	86.5±5
Myrsine africana Fruits	83.4±7.1	82.4±7	64.5±4	30±2
Vitex negundo Leaves	38±2	85±3.6	34.5±2	80±9
Dodonea viscosa flower	81.7±5	31±2.7	80±6	77.6±5
Bauhinia variegata Flower	82.8±10	77±9	29.1±2	48±2
Rumex hastatus Leaves	54.7±3	72.9±4	38.2±1	79.3±1.6

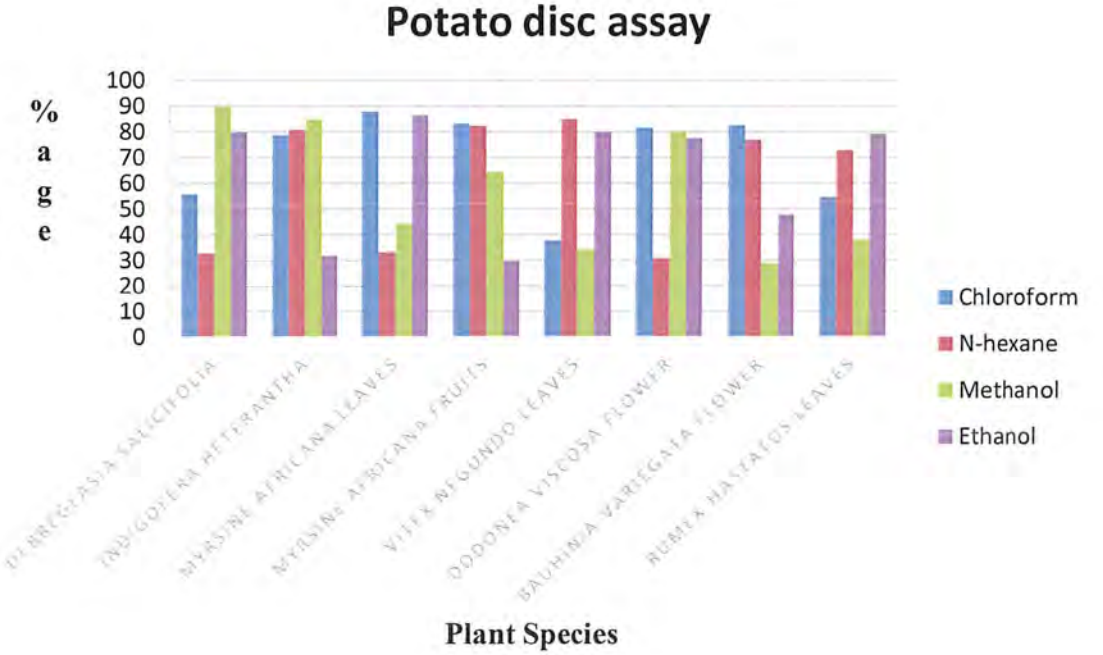


Fig.53. Medicinal plants showing potential against tumor

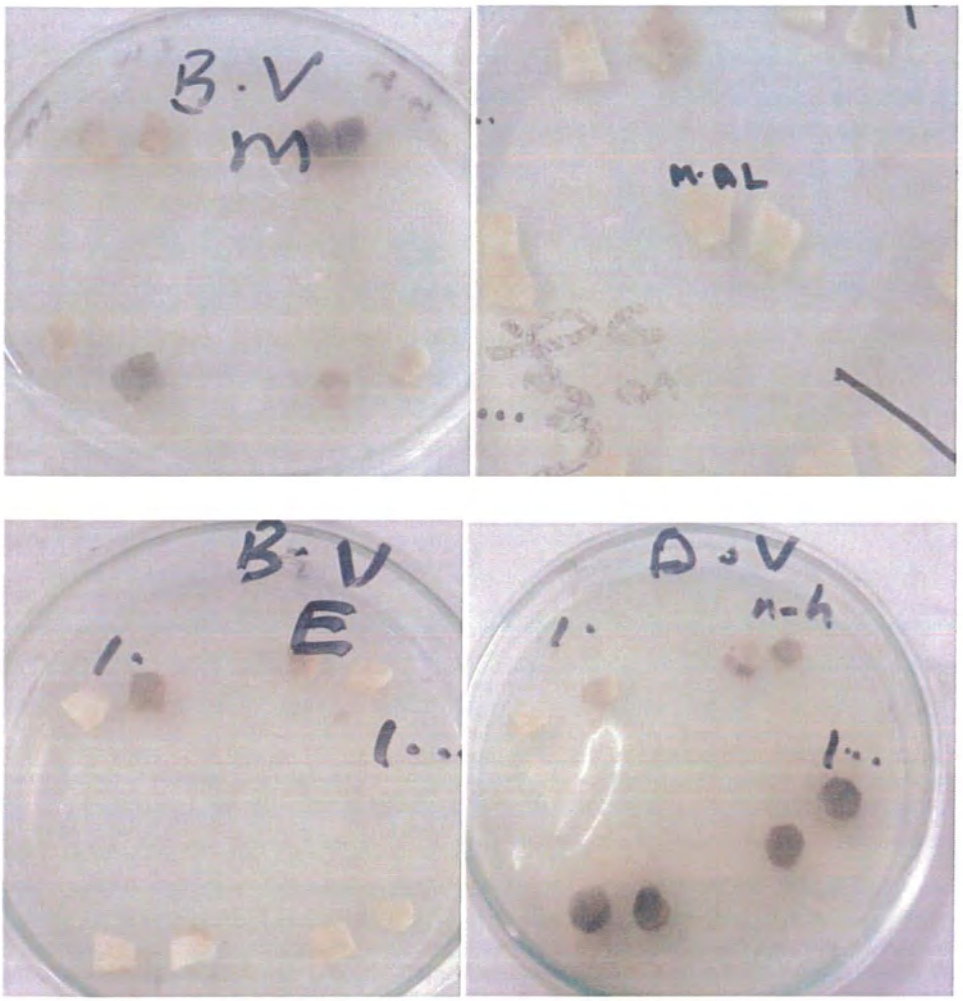


Fig.54. Antitumor activity of various extracts of medicinal plants

3.3.5 Anti-hemolytic activity (%)

Different plants extracts were analyzed by inducing hemolysis in human blood. Percentage of resistance was increased with increasing concentration as given in the table 34 and figure 55. Methanolic and other extracts of samples showed good to moderate activity and $p<0.05$. The higher percentages of samples in all extracts are shown *Vitex negundo* (70±9%), *Dodonaea viscosa* (67±1.6%), RH (61±3.6%), *Bauhinia variegata* (76±1.6%), *Indigofera heterantha* (77±3%),DS(82±5%), *Myrsine africana* fruit (60±3.8%), and *Myrsine africana* leaves (62±0.5%).

Table.34. Anti-hemolytic assay of selected plants

	Myrsine africana Leaves	*Myrsine africana* Fruits	*Debregeasia salicifolia Leaves*	*Indigofera heterantha Leaves*	*Bauhinia variegata Fruit*	*Rumex hastatus Leaves*	*Dodonaeav iscosa Flower*	*Vitex negundo Leaves*
20	17±0.62	15±3.7	13±2.13	8±0.99	16±2	11±3	8±2.3	18±3
40	29±1.4	33±1.2	31±1.6	20±3.5	39±4	21±2.7	19±2	35±2.8
60	43±1.9	45±1.1	48±4	39±6.2	47±2.8	30±4	28±3.4	43±4
80	56±2	53±2.5	65±7.2	60±9	60±3.1	47±1	44±6	52±2.3
100	62±0.5	60±3.8	82±5	77±3	76±1.6	61±3.6	67±1.6	70±9

Values are taken in triplicates manner along standard deviations

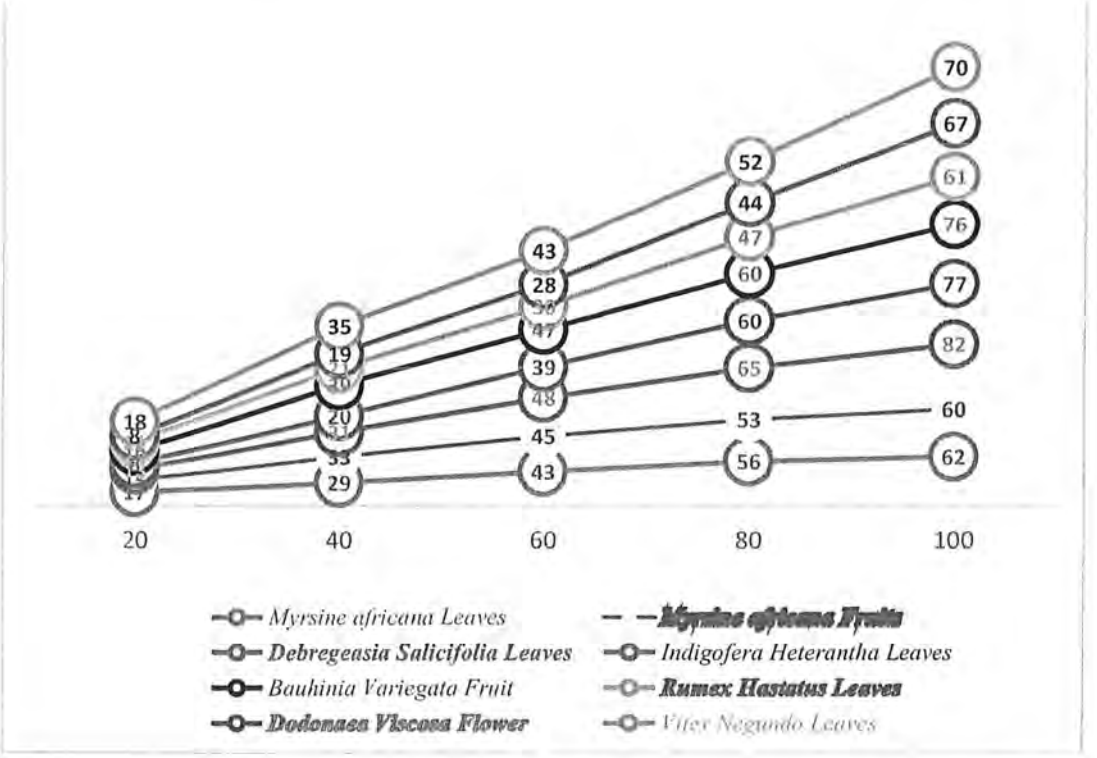


Fig.55. Responses of medicinal plants against hemolysis

Table.1. Antimicrobial activity of plants extracts

	Microcrocus lotus					*Enterobacterauregens*					*Bacillus brevis*					*Pseudomonas aeruginosa*				
Plant extract	AN	A	M	N.H	E.A	AN	A	M	N.H	E.A	AN	A	M	N.H	E.A	AN	A	M	N.H	E.A
Bauhinia variegata Fruit	-	-	13±3.5	7±1	14±3	-	15±2	10±2.4	9±0.6	-	6±1	7±2.9	11±3	11±1.9	17±3	-	-	19±4	-	-
Rumex hastatus Leaves	11±3	16±1.2	11±1.1	11±3	12±4	10±2	16±2.3	-	-	-	6±1	7±2	18±1	6±2	6±2	17±4	10±2	-	-	8±1.3
Myrsine africana Leaves	5±2.7	10±3.8	-	-	-	2±.089	-	8±0.38	7±0.65	-	-	6±3	-	7±2	7±0.97	10±4	12±2.5	10±1.1	-	-
Dodonaea viscosa Flower	7±2	14±1.4	7±2	9±1	10±2	-	10±2	14±2	16±1.5	-	10±2.5	6±2	11±3.1	-	7±2	6±2.4	10±4	6±1	8±2	-
Indigoferahetetrantha Leaves	11±0.7	-	3±0.7	-	6±0.2	8±0.6	14±3	8±0.5	7±0.1	8±1	-	11±0.2	5±0.8	8±2	9±1	15±3	7±2	9±2	10±2.3	8±2.6
Debregeasiasalicifolia Leaves	14±3	12±1.4	8±1	-	15±4	16±5.6	15±4	14±2	8±2	-	17±3	9±2	10±2.5	12±4.1	15±3	12±5	-	12±2	17±5	10±3
Vitex negundo Leaves	17±4	14±2	12±4.3	16±2	15±5	-	-	16±2	12±3.2	5±2	10±2.7	7±3	7±2.5	13±3	10±2	10±3	9±2	10±4	12±2.7	13±5
Myrsine africana Fruit	5±2.7	10±1.8	-	9±1	-	8±2.2	-	-	10±3	9±1.9	-	3±1.2	9±1	7±2.2	6±2	6±2.3	10±1.7	7±2.4	-	-
Streptomycin	25±4	20±6	-	19±2.5	-	18±5.3	-	-	20±0.93	19±3.6	16±5.2	18±3.9	16±1.6	19±7	12±4	22±5	-	-	19±3.4	18±5

Table.2. Antimicrobial activity of different plants

Plant extract	*Microcrocus lotus*					*Enterobacterauregens*					*Bacillus brevis*					*Pseudomonas aeruginosa*				
	AN	A	M	N.H	E.A	AN	A	M	N.H	E.A	AN	A	M	N.H	E.A	AN	A	M	N.H	E.A
Bauhinia variegata Fruit	-	-	13 ±3 .5	7±1	14± 3	-	15 ±2	10 ±2 .4	9±0.6	-	6±1	7±2. 9	11±3	11±1. 9	17± 3	-	-	19±4	-	-
Rumex hastatus Leaves	11 ±3	1 6 ± 1. 2	11 ±1 .1	11± 3	12± 4	10±2	16 ±2. 3	-	-	-	6±1	7±2	18±1	6±2	6±2	17±4	10 ±2	-	-	8±1. 3
Myrsine africana Leaves	5± 2.7	1 0 ± 3. 8	-	-	-	2±.08 9	-	8± 0. 38	7±0.6 5	-	-	6±3	-	7±2	7±0. 97	10±4	12 ±2. 5	10±1 .1	-	-
Dodonae aviscosa Flower	7± 2	1 4 ± 1. 4	7± 2	9±1	10± 2	-	10 ±2	14 ±2	16±1. 5	-	10±2 .5	6±2	11±3 .1	-	7±2	6±2. 4	10 ±4	6±1	8±2	-
Indigofer aheterant ha Leaves	11 ±0. 7	-	3± 0. 7	-	6±0. 2	8±0.6	14 ±3	8± 0. 5	7±0.1	8±1	-	11± 0.2	5±0. 8	8±2	9±1	15±3	7± 2	9±2	10±2 .3	8±2. 6
Debrege asiasalici folia Leaves	14 ±3	1 2 ± 1. 4	8± 1	-	15± 4	16±5. 6	15 ±4	14 ±2	8±2	-	17±3	9±2	10±2 .5	12±4. 1	15± 3	12±5	-	12±2	17±5	10± 3
Vitexneg	17	1	12	16±	15±	-	-	16	12±3.	5±2	10±2	7±3	7±2.	13±3	10±	10±3	9±	10±4	12±2	13±

undo Leaves	±4	4 ± 2	±4 .3	2	5			±2	2		.7		5		2		2		.7	5
Myrsinea fricana Fruit	5± 2.7	10 ± 1.8	-	9±1	-	8±2.2	-	-	10±3	9±1.9	-	3±1.2	9±1	7±2.2	6±2	6±2.3	10 ±1.7	7±2.4	-	-
Streptomycin	25 ±4	20 ± 6	-	19± 2.5	-	18±5.3	-	-	20±0.93	19±3.6	16±5.2	18± 3.9	16±1.6	19±7	12± 4	22±5	-	-	19±3.4	18± 5

Table.2. Antibacterial activity of plants and -=No activity

SECTION 4

IN VIVO STUDY OF MEDICINAL PLANTS

3.4 In vivo study of Medicinal plants

3.4.1 Protective effects of plant extracts

Weights of different groups of mice were recorded before and after administration of plant extracts, standard drugs and CCL_4. After due time the animals were slaughtered from all groups. Livers, kidneys and spleens were taken out and were examined. With the intake of plant extracts weight of mice and their organs (liver and kidney) were significantly increased and weight of spleen decreases to the normal weight by increasing the extract doses to 300mg/kg which remarkably showed significant results as observed in normal group.

Myrsine africana fruit chloroform (MFC) and *Debregeasia salicifolia* methanol (DSM) extract showed higher weight of liver 6.43±0.53 and 6.38±0.711g @ 300mg/kg. Liver weight of normal mice was found to have 5.60±1.21g and CCL_4 reduces weight of liver 3.96±1.18g.Liver of mice from silymarine group was found to have 6.02±0.749 which is less than chloroform and methanol extracts of *Myrsine africana* fruit followed by *Debregeasia salicifolia*. *Myrsine africana* leaves chloroform extract increased 0.55±0.02g the kidney weight when it was reduced to 0.34±0.12g by toxicity of CCL_4 and standard drug silymarine protected the kidney 0.5±0.17g which was less than plant extracts *Myrsine africana* Leaves (ML), *Debregeasia salicifolia* (DS), *Myrsine africana* fruit (MF), *Vitex negundo* (VN) and *Rumex hastatus* (RH). The normal weight of kidney was observed 0.48±0.1g (480mg).

When toxicity was induced weight of spleen increased by 3.34±0.5g from normal weight 2.080±0.19g and when plant extracts were given results in reduction of spleen weight was observed and higher reduction was noticed in *Vitex negundo* (VN) 300mg/kg methanol extract 2.05±0.25g. Average weight of mice were 40g in all groups and in normal group after 21 days there were 19±2.9% increased in weight was observed followed by olive oil group 20±3.10%, CCL_4 group 7.41±2.05% and silymarine group 22.27±0.09% increase. 300mg/kg chloroform extract of *Myrsine africana* fruit (MFC) and methanol extract of *Debregeasia salicifolia* (DSM) groups increased the body weight by 25.92±1.21% and 25.11±1.83% those

are highly active extracts found in this study. The lower increased in body weight was found in *Dodonaea viscosa* methanol (DVM) 15.52±1.75% which is less than standard drug, plant extracts might contain compounds those are active in other solvents or might be crude particles may not properly mixed with olive oil before oral administration to the mice.

Table.35.Methanol extract of various plants protective effect

Treatments	Weight of Liver (g)	Weight of Kidney (g)	Weight of Spleen (g)	Rise (%) in body weight
Normal	5.60±1.21	0.48±0.1	2.080±0.19	16±2.9
Olive oil Control	5.82±0.94	0.51±0.08	2.15±0.2	20±3.10
CCL_4	3.96±1.18	0.34±0.12	3.34±0.5	7.41±2.05
Silymarine+CCL_4	6.02±0.749	0.5±0.17	2.150±0.31	22.27±0.09
ML (100mg/kg)+CCL_4	4.27±0.62	0.39±0.05	2.99±0.7	10.66±0.71
ML (200mg/kg)+CCL_4	5.245±1.08	0.46±0.09	2.56±0.12	14.76±1.2
ML (300mg/kg)+CCL_4	6.17±0.90	0.53±0.14	2.13±0.79	21.83±1.05
MF (100mg/kg)+CCL_4	4.48±0.812	0.382±0.1	2.98±0.5	12.1±1.42
MF (200mg/kg)+CCL_4	5.323±0.29	0.45±0.03	2.62±0.8	15.20±1.29
MF (300mg/kg)+CCL_4	6.22±1.10	0.50±0.15	2.10±0.07	24.03±2.19
DS (100mg/kg)+CCL_4	4.37±0.40	0.40±0.02	3.06±0.4	12.8±2.01
DS (200mg/kg)+CCL_4	5.402±0.97	0.47±0.11	2.78±0.8	17.12±0.95
DS (300mg/kg)+CCL_4	6.38±0.711	0.52±0.02	2.45±0.26	25.11±1.83
VN (100mg/kg)+CCL_4	4.02±0.601	0.37±0.04	2.92±0.1	7.32±1.46
VN (200mg/kg)+CCL_4	4.701±0.809	0.425±0.13	2.42±0.7	15.60±0.95
VN (300mg/kg)+CCL_4	5.618±1.10	0.47±0.06	2.05±0.25	21.48±1.63
DV (100mg/kg)+CCL_4	4.016.±0.83	0.25±0.045	3.11±0.77	6.03±0.67
DV (200mg/kg)+CCL_4	4.67±0.71	0.33±0.01	2.85±0.3	10.060±1.04
DV (300mg/kg)+CCL_4	5.105±0.51	0.41±0.1	2.49±0.5	15.52±1.75
BV (100mg/kg)+CCL_4	4.31±0.84	0.35±0.02	3.09±0.9	8.13±1.13
BV (200mg/kg)+CCL_4	4.98±1.20	0.42±0.05	2.79±0.11	13.44±0.88
BV	5.49±0.912	0.50±0.08	2.35±0.2	19.04±1.31

(300mg/kg)+CCL_4 IH	4.27±0.67	0.28±0.04	2.98±0.36	8.52±0.815
(100mg/kg)+CCL_4 IH	4.75±0.90	0.35±0.1	2.61±0.5	11.6±1.29
(200mg/kg)+CCL_4 IH	5.04±0.53	0.44±0.13	2.38±0.4	18.92±2.11
(300mg/kg)+CCL_4 RH	4.12±0.80	0.335±0.08	2.86±0.5	8.43±1.20
(100mg/kg)+CCL_4 RH	4.65±1.01	0.38±0.1	2.53±0.9	12.5±0.91
(200mg/kg)+CCL_4 RH	5.21±0.70	0.49±0.02	2.20±0.4	17.83±1.50
(300mg/kg)+CCL_4				

Table.36.Chloroform extract of various plants protective effect

Treatments	Weight of Liver (g)	Weight of Kidney (g)	Weight of Spleen (g)	Rise (%) in body weight
Normal	5.60±1.21	0.48±0.05	2.080±0.1	16±2.9
Olive oil Control	5.82±0.94	0.51±0.02	2.15±0.5	20±3.10
CCL_4	3.96±1.18	0.34±0.08	3.34±0.2	7.41±2.05
Silymarine+CCL_4	6.02±0.749	0.5±0.1	2.150±0.16	22.27±0.09
ML (100mg/kg)+CCL_4	4.11±1.14	0.364±0.08	3.057±0.2	9.24±1.52
ML (200mg/kg)+CCL_4	5.036±0.97	0.451±0.03	2.72±0.1	13.82±0.97
ML (300mg/kg)+CCL_4	5.90±0.63	0.55±0.02	2.19±0.15	20.52±0.56
MF (100mg/kg)+CCL_4	4.53±0.74	0.37±0.05	3.08±0.6	11.5±0.93
MF (200mg/kg)+CCL_4	5.542±0.42	0.443±0.01	2.54±0.9	16.62±2.17
MF (300mg/kg)+CCL_4	6.43±0.53	0.49±0.1	2.12±0.5	25.92±1.21
DS (100mg/kg)+CCL_4	4.13±1.22	0.37±0.08	2.94±0.2	12.3±1.73
DS (200mg/kg)+CCL_4	5.088±0.65	0.42±0.2	2.53±0.08	16.43±0.72
DS (300mg/kg)+CCL_4	6.17±1.14	0.49±0.13	2.29±0.5	23.06±2.42
VN (100mg/kg)+CCL_4	3.85±0.32	0.39±0.05	2.97±0.11	7.11±0.93
VN (200mg/kg)+CCL_4	4.522±0.74	0.44±0.07	2.40±0.25	14.22±0.78
VN (300mg/kg)+CCL_4	5.27±0.83	0.51±0.1	2.12±0.6	20.55±2.18
DV (100mg/kg)+CCL_4	4.25.±0.92	0.28±0.03	3.06±0.8	7.22±0.92
DV (200mg/kg)+CCL_4	4.92±1.23	0.35±0.14	2.76±0.5	12.24±2.31
DV (300mg/kg)+CCL_4	5.56±0.92	0.46±0.02	2.3±0.33	16.94±2.08
BV (100mg/kg)+CCL_4	4.63±0.71	0.32±0.02	2.91±0.74	9.52±1.55
BV (200mg/kg)+CCL_4	5.09±0.82	0.38±0.05	2.67±0.1	15.62±1.54
BV (300mg/kg)+CCL_4	5.82±1.03	0.45±0.03	2.32±0.07	20.35±2.02
IH (100mg/kg)+CCL_4	4.03±0.92	0.25±0.08	2.85±0.35	7.81±0.773
IH (200mg/kg)+CCL_4	4.52±0.840	0.32±0.04	2.55±0.12	10.99±1.13
IH (300mg/kg)+CCL_4	5.02±0.72	0.38±0.1	2.30±0.05	17.13±1.58
RH (100mg/kg)+CCL_4	4.08±0.42	0.36±0.02	2.88±0.3	8.25±0.78
RH (200mg/kg)+CCL_4	4.47±0.94	0.41±0.2	2.59±0.81	11.3±1.41
RH (300mg/kg)+CCL_4	5.03±0.83	0.50±0.17	2.24±0.9	16.38±2.1

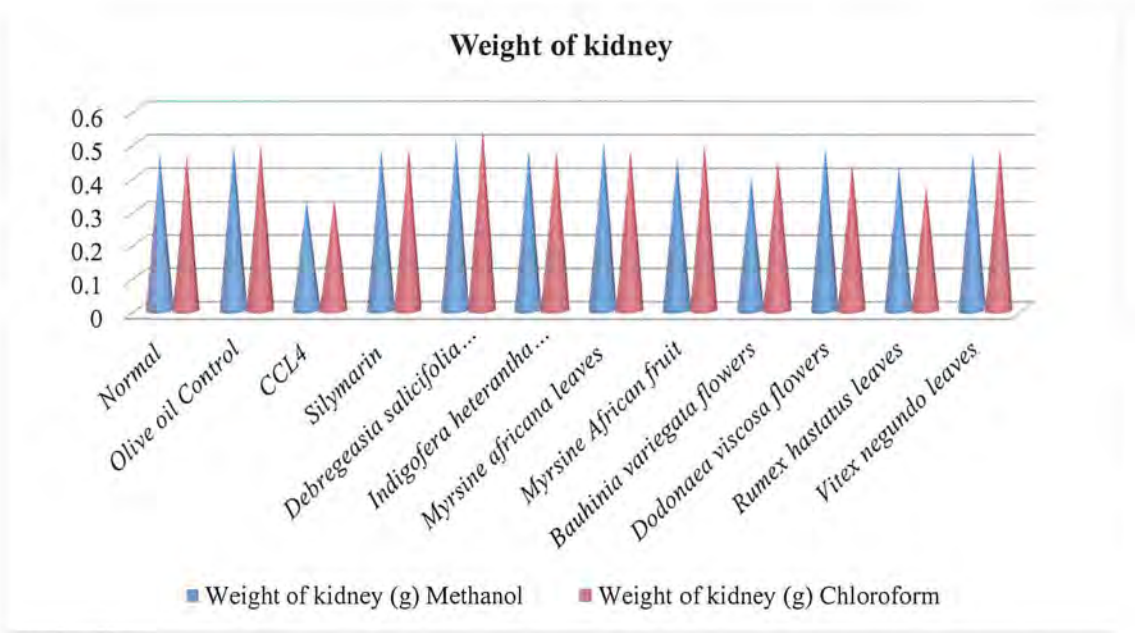


Figure.56.It represents the weight of kidney varying in different groups

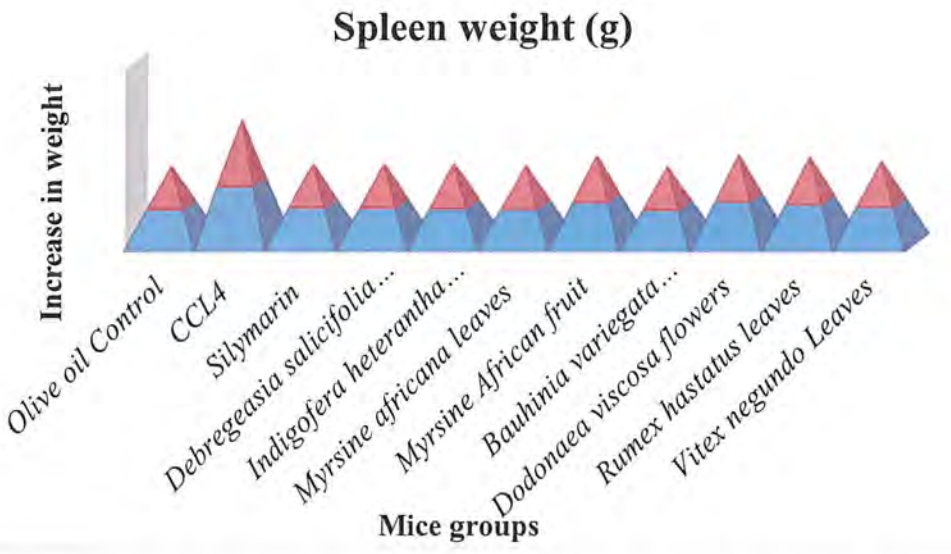


Figure57.The weight of Spleen in different group of animals

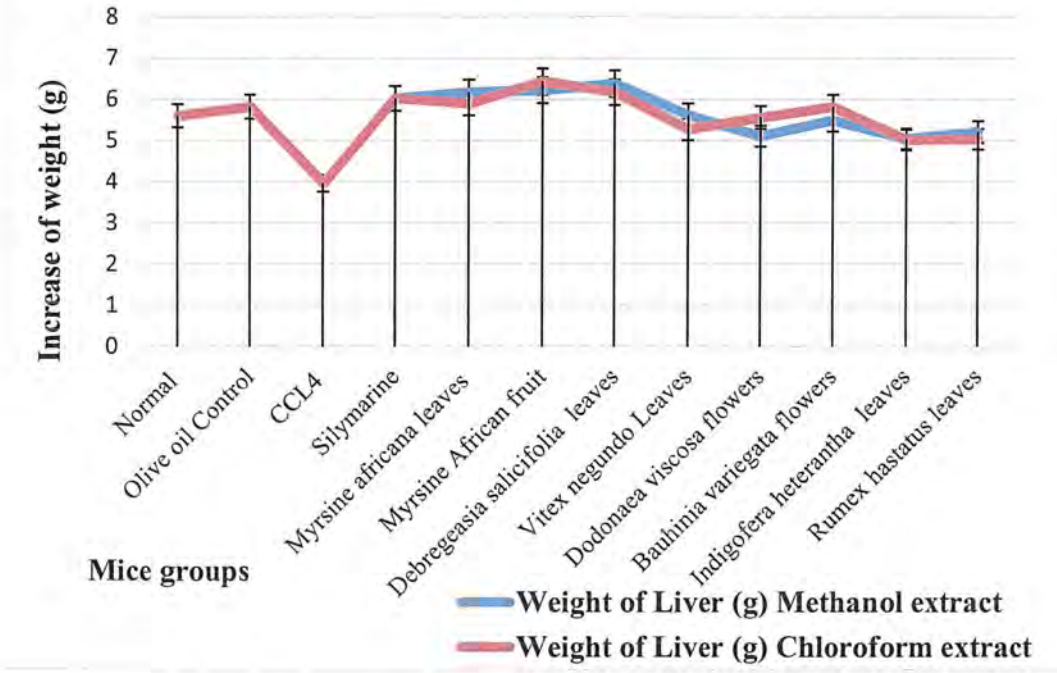


Figure.58.The weight of Liver in different group of mice

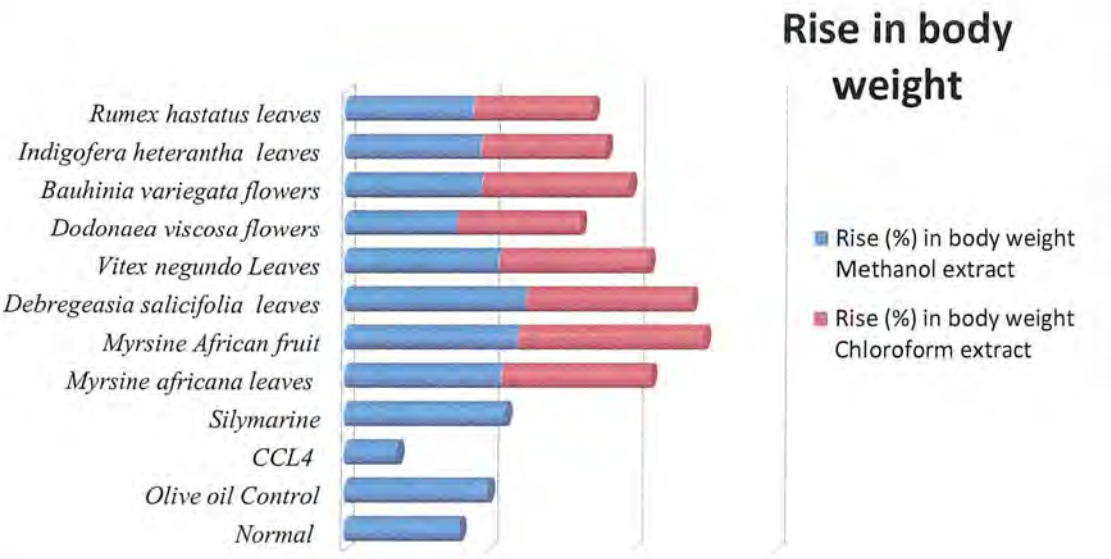


Figure.59. Increase in weight of animals from different groups

3.4.2 Hepato-protective activity

To replace synthetic medicines some active plants extracts were tested for their hepato-protective activity in albino mice. ALT (alanine aminotransaminase), AST (aspartate aminotransaminase), ALP (alkaline phosphatase) and direct bilirubin were assessed in the serum. Animals were divided into 6 groups including normal, CCL_4 (24 hours, 48 hours, 72 hours and 21 days), Silymarine Drug (positive control) and plant extracts (100mg, 200mg and 300mg). Carbon tetra chloride was induced through intraperitoneal technique. Plant extracts, positive control along with olive oil was given orally. Results showed that liver enzymes treated with CCL_4 after 24 hours have elevated enzymes level as compared to normal, olive, plant extracts and silymarine drug were observed. In carbon tetra chloride groups with increasing day's elevation in results were increased. CCL_4 increased enzymes levels (ALT, AST, ALP and direct bilirubin) in this biochemical studies (Zhang *et al.*, 2012: Dong *et al.*, 2013).

Both methanol and chloroform fraction of all the plants extracts showed protective effects and when there were less effect of these extracts enzymes levels increased. With increasing plant concentration the results were found to have more activities. Chloroform extract of *Vitex negundo* leaves 300mg/Kg; *Myrsine africana* leaves 300mg/kg, *Indigofera heterantha* leaves300mg/kg, *Dodonaea viscosa* flower 300mg/kg have more activity as compared to their methanol extract. Methanol extracts of *Myrsine africana* fruit, *Debregeasia salicifolia* leaves, *Rumex hastatus* leaves and *Bauhinia variegata* flowers showed higher activity than their chloroform extract.

Table.37.Analysis of blood serum for estimation of liver enzymes

	ALT	AST	ALP	Direct bilirubins
Normal control	38±0.83	80.2±4.1	110±9.2	0.2±0.001
Olive oil control	40±5.2	63±2	175±20	0.32±0.06
CCl_4 Control 24 hrs	74.5±7.7	90±11	208±5.1	1.0±0.2
CCl_4 Control 48 hrs	85±12	95±3.5	225±11	1.25±0.04
CCl_4 Control 72 hrs	89±14	109±9.2	247±2	1.7±0.45
CCl_4 Control 21days	120±9	127.8±8.6	294±15	1.91±0.08
Silymarine drug	66.4±7.2	76.1±12	185±31	0.53±0.09
MLM 100mg+ CCl4	65.5±8	97±16	190±23	0.80±0.5
MLM 200mg+ CCl4	60.7±8.4	85.4±20	181±45	0.68±0.9
MLM 300mg+ CCl4	46.8±14	70±8.8	172±29	0.63±1.1
MLC 100mg+ CCl4	85.5±13.1	180±29	221.5±35	0.82±0.77
MLC 200mg+ CCl4	80.8±5	155±21	172±12	0.6±0.68
MLC 300mg+ CCl4	43.5±23.5	86.5±7	121.6±6.1	0.418±0.34
MFM 100mg+ CCl4	66.5±18	100±7.2	265.6±40	1.0±0.14
MFM 200mg+ CCl4	66.1±4.8	98.15±14.2	203.4±13.2	0.81±0.28
MFM 300mg+ CCl4	62.5±3.8	85±15	190.8±11	0.72±0.31
MFC 100mg+ CCl4	72.8±11	108.2±19	345.7±42	0.9±0.067
MFC 200mg+ CCl4	68±6.5	101±5.6	298±37	0.85±0.43
MFC 300mg+ CCl4	66±7.1	95.7±9.9	269.4±29	0.71±0.041
DSM 100mg+ CCl4	73.4±5.4	108.5±13	331±45	0.84±0.42
DSM 200mg+ CCl4	62.2±8.9	96.1±8	234±7.2	0.79±0.56
DSM 300mg+ CCl4	59.6±11	75.7±7.2	183.9±58	0.7±0.09
DSC 100mg+ CCl4	75.5±9.0	126.7±10	332.5±35	0.94±0.04
DSC 200mg+ CCl4	72.2±14.3	95.5±13.4	253.3±30	0.89±0.1
DSC 300mg+ CCl4	51.5±0.99	93.7±6.5	204.7±7.7	0.81±0.09
RHM 100mg+ CCl4	79.9±8.2	133±27	247.3±28	1.7±0.21
RHM 200mg+ CCl4	72.7±4.8	123±5.1	228.8±17	0.88±0.02
RHM 300mg+ CCl4	67±3.1	98±24	111.8±2.9	0.62±0.31
RHC 100mg+ CCl4	90.4±5.2	139±12.1	321.6±48	1.1±0.12
RHC 200mg+ CCl4	80.8±2.1	129±8.9	275.8±16	0.89±0.04
RHC 300mg+ CCl4	72.6±5	95.5±5.5	154±13	0.85±0.06
IHM 100mg+ CCl4	84±1.2	110±8.2	405.6±55	1.8±0.52
IHM 200mg+ CCl4	81±4.5	109±2.7	377.7±21	1.1±0.35
IHM 300mg+ CCl4	78±2.8	108±3.0	351.5±17	0.9±0.12

IHC 100mg+ CCl4	74.5±6.1	108±2.8	253.6±9.8	1.1±0.05
IHC 200mg+ CCl4	71.2±12	96±7	292.2±14	1.0±0.02
IHC 300mg+ CCl4	71±5.2	60.5±5	283.2±10	0.94±0.06
DVM 100mg+ CCl4	62.5±8	104±20	264.8±11	0.7±0.08
DVM 200mg+ CCl4	61.8±4.5	88±11	236±14	0.6±0.01
DVM 300mg+ CCl4	59±2	76.5±6	197±9.8	0.56±0.02
DVC 100mg+ CCl4	61±5	96±7.1	357±12.4	1.1±0.2
DVC 200mg+ CCl4	72±3	84.3±5	283.6±3.2	0.98±0.05
DVC 300mg+ CCl4	53±6	74±4.3	229±11	0.81±0.07
BVM 100mg+ CCl4	70±9.4	84±7	319±5.7	0.9±0.08
BVM 200mg+ CCl4	57±6	79.5±4.1	300.8±12	0.75±0.05
BVM 300mg+ CCl4	56±3.35	63.5±2.5	238±9.1	0.7±0.02
BVC 100mg+ CCl4	74±5.1	110±12	337.7±15	1.1±0.009
BVC 200mg+ CCl4	72.2±2.3	89±8.1	295±13	1±0.03
BVC 300mg+ CCl4	64.5±3	81±10	275±20	0.8±0.01
VNM 100mg+ CCl4	80±6	90±15	374.5±29	1.1 0.06
VNM 200mg+ CCl4	69.3±2.4	88±11	269.2±19	1.0±0.02
VNM 300mg+ CCl4	62.5 1.9	83±2.9	255±16	0.85±0.35
VNC 100mg+ CCl4	64±5.2	95±1.6	263±22	1.3±0.03
VNC 200mg+ CCl4	61.8±4	86±4.1	252.7±43	0.95±0.31
VNC 300mg+ CCl4	27±3.9	76.5±9	209±20	0.9±0.07

3.4.3 Hematological studies

Hematological examination was done for the analysis of red blood cells, white blood cells and platelets. *Myrsine africana* leaves Methanol < *Debregeasia salicifolia* Chloroform < *Dodonaea viscosa* methanol were found to have greater RBCs values which are near to the normal control 4.80±0.06. The results were significant with $p<0.05$. For the analysis of white blood cells, *Dodonaea viscosa* methanol , *Debregeasia salicifolia* methanol, *Myrsine africana* leaves methanol and MF methanol showed the highest values equal to the results of olive oil group (5.50±0.47) and normal group (5.02±0.47) with $p<0.05$. *Debregeasia salicifolia* methanol, *Dodonaea viscosa* chloroform and *Dodonaea viscosa* methanol showed protective effects and results as compared to normal control (240±6.9) at $p<0.05$. While in present study it was observed that *Bauhinia Variegata* flower extracts has positive effects on hematological parameters that indicates its suitability of its uses against blood cells relevant disorders (Middleton,1984; Murray *et al.*,2000).

Table.38.Study of hematological parameters after treatment with plants extracts

Groups	RBCs	WBCs	Platelets
Normal group	4.80±0.06	5.02±0.47	240±6.9
Olive oil control	4.93±0.2	5.50±0.008	252±10.1
CCl_4 Control 24 hrs	3.05±0.081	3.68±0.03	170±5.03
CCl_4 Control 48 hrs	2.73±0.03	3.25±0.15	155.2±0.25
CCl_4 Control 72 hrs	2.40±0.009	2.913±0.092	140.01±0.17
CCl_4 Control 7 days	1.88±0.005	2.351±0.11	100.59±2.9
Silymarine drug	3.97±0.12	4.802±0.060	227.3±4.12
MLM 100mg+ CCl4	3.92±0.005	4.47±0.039	215.8±5.21
MLM 200mg+ CCl4	4.35±0.18	5.183±0.24	241.3±0.19
MLM 300mg+ CCl4	4.80±0.41	5.43±0.0088	250.8±3.76
MLC 100mg+ CCl4	3.52±0.007	4.25±0.23	208±0.29
MLC 200mg+ CCl4	3.802±0.014	4.5±0.17	223.05±0.081
MLC 300mg+ CCl4	4.40±0.02	4.905±0.3	241.9±0.53
MFM 100mg+ CCl4	3.78±0.1	4.202±0.0082	213.1±0.019
MFM 200mg+ CCl4	4.108±0.31	4.47±0.135	221.8±0.16
MFM 300mg+ CCl4	4.39±0.55	5.32±0.051	239.04±0.002
MFC 100mg+ CCl4	3.19±0.06	3.801±0.0071	180.21±0.05
MFC 200mg+ CCl4	3.55±0.072	4.04±0.020	200.16±0.0079
MFC 300mg+ CCl4	3.92±0.021	4.394±0.081	211.4±0.041
DSM 100mg+ CCl4	3.81±0.05	4.51±0.02	229.1±0.06
DSM 200mg+ CCl4	4.15±0.31	4.7±0.33	237.4±0.11
DSM 300mg+ CCl4	4.427±0.05	5.5±0.6	252.1±0.84
DSC 100mg+ CCl4	3.86±0.12	4.48±0.081	220.6±0.007
DSC 200mg+ CCl4	4.263±0.080	4.93±0.29	236.2±0.05
DSC 300mg+ CCl4	4.71±0.023	5.384±0.37	249.02±0.2
RHM 100mg+ CCl4	3.54±0.1	4.38±0.501	213.4±0.15
RHM 200mg+ CCl4	4.181±0.003	4.79±0.07	220.8±0.02

RHM 300mg+ CCl4	4.72±0.055	5.23±0.29	247.2±0.32
RHC 100mg+ CCl4	3.21±0.7	4.021±0.025	205.6±0.07
RHC 200mg+ CCl4	3.791±0.002	4.57±0.04	218.21±0.13
RHC 300mg+ CCl4	4.417±0.31	5.10±0.026	244.01±0.58
IHM 100mg+ CCl4	3.23±0.073	4.113±0.004	208.1±0.12
IHM 200mg+ CCl4	3.92±0.021	4.42±0.28	221.3±0.5
IHM 300mg+ CCl4	4.408±0.04	5.13±0.07	245±0.03
IHC 100mg+ CCl4	3.42±0.007	4.23±0.21	213.1±0.18
IHC 200mg+ CCl4	4.028±0.22	4.801±0.003	225.6±0.203
IHC 300mg+ CCl4	4.49±0.18	5.18±0.0013	248.1±0.11
DVM 100mg+ CCl4	3.7±0.016	4.67±0.04	240.3±0.15
DVM 200mg+ CCl4	4.181±0.004	5.002±0.12	247.09±0.21
DVM 300mg+ CCl4	4.62±0.21	5.56±0.01	256.6±0.82
DVC 100mg+ CCl4	3.612±0.093	4.205±0.83	234.1±0.26
DVC 200mg+ CCl4	4.05±0.02	4.84±0.131	248.3±0.03
DVC 300mg+ CCl4	4.43±0.1	5.291±0.20	254.20±0.19
BVM 100mg+ CCl4	3.521±0.07	4.089±0.31	232.9±0.0012
BVM 200mg+ CCl4	3.981±0.11	4.52±0.08	245.5±0.03
BVM 300mg+ CCl4	4.391±0.3	5.081±0.021	253.5±0.022
BVC 100mg+ CCl4	3.34±0.118	3.970±0.001	225.3±0.31
BVC 200mg+ CCl4	3.772±0.27	4.468±0.2	240.12±0.16
BVC 300mg+ CCl4	4.12±0.61	4.991±0.05	251.31±0.01
VNM 100mg+ CCl4	3.83±0.05	4.08±0.1	230.1±0.4
VNM 200mg+ CCl4	4.19±0.27	4.51±0.002	244.3±0.002
VNM 300mg+ CCl4	4.461±0.081	5.1±0.071	252±0.01
VNC 100mg+ CCl4	3.712±0.001	3.94±0.02	221.5±0.091
VNC 200mg+ CCl4	4.08±0.081	4.4218±0.001	240.6±0.15
VNC 300mg+ CCl4	4.412±0.02	5.067±0.21	249.9±0.001

3.4.4 Effects of plant extracts on antioxidant enzymes of liver

The effects of plant extracts on antioxidant enzymes and protein were tested in liver of mice. In antioxidants enzymes CAT, SOD, GPx and protein levels were measured from all the groups. In normal and olive oil control groups values of CAT (8.2 and 7.9 m mol / min / mg / protein), SOD (10.15 and 10.23U SOD / mg / protein), GPx (32.10 and 34.7 μ mol / min / mg / protein) and protein (3.15 and 3.2 μg / mg / tissue) were recorded statistically. When mice were slaughtered after 24 hours of CCL 4 dose the CAT, SOD, GPx and protein levels were found maximum (5.94 m mol /min / mg / protein, 6.89 m mol / min / mg / protein, 22.40U / mg / protein, 22.40 mol / min / mg /protein and 3.15 μg / mg / tissue, respectively) and after 21 days of CCL 4 dose toxicity level increased and enzymes activities and protein were reduced up to 4.75 m mol / min/ mg / protein for CAT, 5.75 U / mg / protein for SOD, 17.83 mol / min / mg / for GPx and 0.75μg / mg / tissue for protein (Table.39).

Positive control i.e. silymarine drug with both plant chloroform and methanol extracts exhibited significant results at $p<0.05$.CCL_4 reduces antioxidant enzymes (CAT, SOD and GPx) and protein which confirms the liver damage (Simeonova *et al.*, 2014) while these levels were regenerated after treatment with plant extracts. *Debregeasia salicifolia* methanol 300mg/Kg showed higher results 8.37±1.05 m mol / min/ mg / protein for CAT, 11.22±2 U / mg / protein for SOD, 35.02±1.4 mol / min / mg / for GPx and 3.26±0.8 μg / mg / tissue for protein. MLM 300mg also showed significantly good level of antioxidant enzymes 8.2±0.3 m mol / min/ mg / protein for CAT followed by 10.98±1.28 U / mg / protein for SOD,32±4.8 mol / min / mg / for GPx and 3.06±0.7μg / mg / tissue for protein.

Table.39.Effects of plant extracts on Antioxidant enzymes and total proteins

Groups	CAT (m mol /min/mg protein)	SOD (U SOD/mg protein)	GPx (μ mol/min/mg protein)	Protein (μg/mg tissue)
Normal control	8.2±2	10.15±1.4	32.10±3	3.15±0.4
Olive oil	7.9±0.5	10.23±2	34.7±4.5	3.2±0.25
CCL4 24 hours	5.94±1.3	6.89±0.7	22.40±3.7	1.2±0.11
CCL4 48 Hours	5.28±1.7	6.24±1	20.47±1.9	1.03±0.2
CCL4 72 Hours	5.03±0.43	5.98±1.4	18.16±2	0.98±0.3
CCL4 21 days	4.75±1.9	5.75±0.6	17.83±3	0.75±0.08
DSM 100mg + CCL4	7.70±1.2	8.34±2	27.9±1.6	1.96±0.5
DSM 200mg + CCL4	8.06±2.5	10.05±3.5	31.14±2.7	2.52±0.19
DSM 300mg + CCL4	8.37±1.05	11.22±2	35.02±1.4	3.26±0.8
DSC 100mg + CCL4	7.62±2.3	8.10±2.05	26.6±0.8	2.02±0.3
DSC 200mg + CCL4	7.88±1.8	9.86±1	30.47±1.5	2.67±0.85
DSC 300mg + CCL4	8.17±1.1	10.71±0.8	33.82±0.7	3.31±0.94
IHM 100mg + CCL4	7.44±1.9	8.06±0.25	26.07±1.9	1.82±0.08
IHM 200mg+ CCL4	7.76±0.4	9.79±2	29.65±0.2	2.37±0.65
IHM 300mg + CCL4	8.09±0.54	10.69±3.1	32.83±1.4	3.11±1.07
IHC 100mg + CCL4	7.61±2	8.28±2.5	27.15±2.6	1.99±0.2
IHC 200mg + CCL4	7.95±0.04	10.01±0.9	30.83±1.38	2.56±0.2
IHC 300mg + CCL4	8.29±1.2	11.19±1.4	34.64±2.6	3.29±1
BVM 100 mg+ CCl4	7.42 ±0.3	8.29±0.5	24.1±2.6	1.84±0.5
BVM 200 mg+ CCl4	7.91±0.7	9.95±0.6	28.72±2.8	2.29±0.7
BVM 300 mg + CCl4	8.15±0.8	11.03±0.8	32.51±3.6	3.10±0.8
BVC100 mg+ CCl4	7.05±0.6	8.04 ±0.7	22.4±2.1	1.76±0.6
BVC200 mg+ CCl4	7.61±0.4	9.52±0.8	26.25±2.5	2.35±0.3

BVC300 mg+ CCl4	7.99±0.6	10.78±1.5	30.63±3.1	2.92±0.2
DVM100 mg + CCl4	6.8±0.2	7.79±1	19.4±1.2	1.58±0.05
DVM200 mg + CCl4	7.3±0.8	8.58±2.3	23.0±3.5	2.1±0.4
DVM300 mg+ CCl4	7.7±1.03	9.7±0.5	28.4±2	2.6±0.08
DVC100 mg+CCl4	6.9±1.5	7.86±1.09	20.1±3	1.62±0.5
DVC200 mg+ CCl4	7.5±2	8.9±1.5	25.4±0.7	2.2±0.9
DVC300 mg+ CCl4	7.87±1	10.02±1	29.8±1	2.8±0.71
MLM100 mg+ CCl4	7.7±0.4	9.5±1.72	22.5±0.6	2.59±0.05
MLM200 mg+ CCl4	7.93±1.8	10.3±2	28.1±2.2	2.82±0.2
MLM300 mg+ CCl4	8.2±0.3	10.98±1.28	32±4.8	3.06±0.7
MLC100 mg+ CCl4	7.58±1	9.4±1.5	21.9±1.6	2.38±0.1
MLC200 mg+ CCl4	7.81±0.8	10.19±0.6	26.4±0.3	2.70±0.07
MLC300 mg+ CCl4	8.08±0.23	10.7±1	30.9±1	2.98±0.3
MFM100 mg+ CCl4	7.7±0.5	9.48±1	22.3±2	2.4±0.8
MFM200 mg+ CCl4	7.91±1.1	10.2±2.2	27.5±1.1	2.85±0.1
MFM300 mg+ CCl4	8.1±0.1	10.8±1.6	31.05±2.7	3.02±0.6
MFC100 mg+ CCl4	6.9±2	8.3±1.1	20.5±2	1.9±0.2
MFC 200 mg+ CCl4	7.4±1.7	9.06±2	24.8±3.9	2.2±0.6
MFC 300 mg+ CCl4	7.90±1.4	10.3±4.2	29.4±1	2.6±0.3
RHM100 mg+ CCl4	6.7±1	8.1±2	19.3±1.4	1.7±0.5
RHM200 mg+ CCl4	7.2±0.8	8.69±1.7	22.6±2.9	2.1±0.1
RHM300 mg+ CCl4	7.74±0.2	9.04±0.2	27.3±1.1	2.45±0.08
RHC100 mg+ CCl4	7.05±2	8.4±3	20.5±3	1.85±0.7
RHC200 mg+ CCl4	7.41±1.6	8.7±1.6	24.1±2.2	2.24±0.3
RHC300 mg+ CCl4	7.95±0.3	9.3±2	29.5±0.9	2.6±1
VNM100 mg+ CCl4	7.6±1	8.52±3.4	21±1.5	2.03±0.8
VNM200 mg+ CCl4	7.92±0.6	8.88±2	25.7±2	2.39±0.1
VNM300 mg+ CCl4	8.15±1.4	9.4±1.3	31.1±4	2.7±0.5
VNC100 mg+	7.52±0.2	8.42±0.3	24.1±3	2.08±0.6

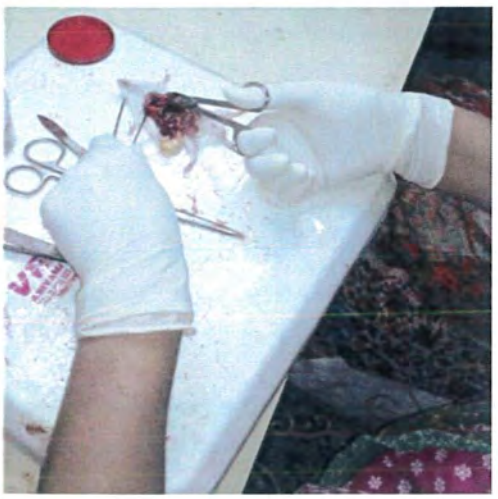
CCl4				
VNC200 mg+ CCl4	7.88±1	8.7±2	26.5±2.5	2.4±0.1
VNC300 mg+ CCl4	8.09±1.4	9.25±0.7	30.5±1.1	2.75±0.07
Silymarine + CCl4	8.11±1.2	11.62±0.6	33.7±1.5	3.22±0.8

3.4.5 Histopathological studies of animals

After dissection of mice organs were processed either for direct histopathology or preserved in formalin for the future uses. Animals treated with 100 and 200 mg/kg body weight slightly recovered the cellular organization, while plant extracts 300 mg/kg body weight has largely restored the structural integrity of tissues and proved its protective effects and recovery of damage tissues were comparable with standard drug (silymarine) (Fig.60). Similar results were also reported by Agbaje *et al.*, (2009). Figure "60a" was the view of dissection of mice followed by Figure "60b" showed the liver preserved in formalin. Figure "60c" and "60d" showed tumor on the organs which was resulted due to CCl_4 induction. While figures "61a", "61b", "61c", "61d," "61e", "61f", "61g" and "61h" showed histology of liver, kidney and spleen which showed necrosis and damage in the tissues in CCl_4 damaged slides and plants extracts showed no or less necrosis or damage by verifying protective effects on the organs. Silymarine drug also recovered the cellular organization of organs.

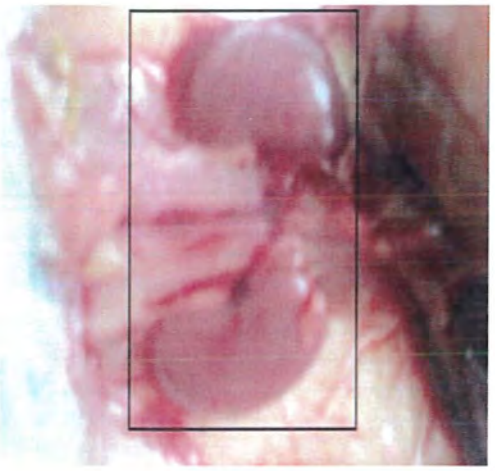
CHAPTER 4

DISCUSSION

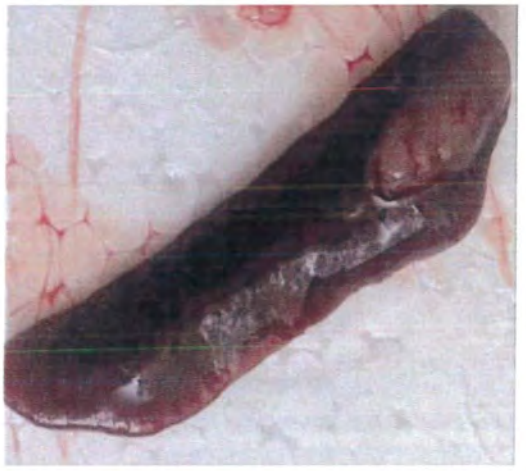


(a)

(b)



(c)



(d)

Fig.60. Dissection of mice and organs infected with tumors

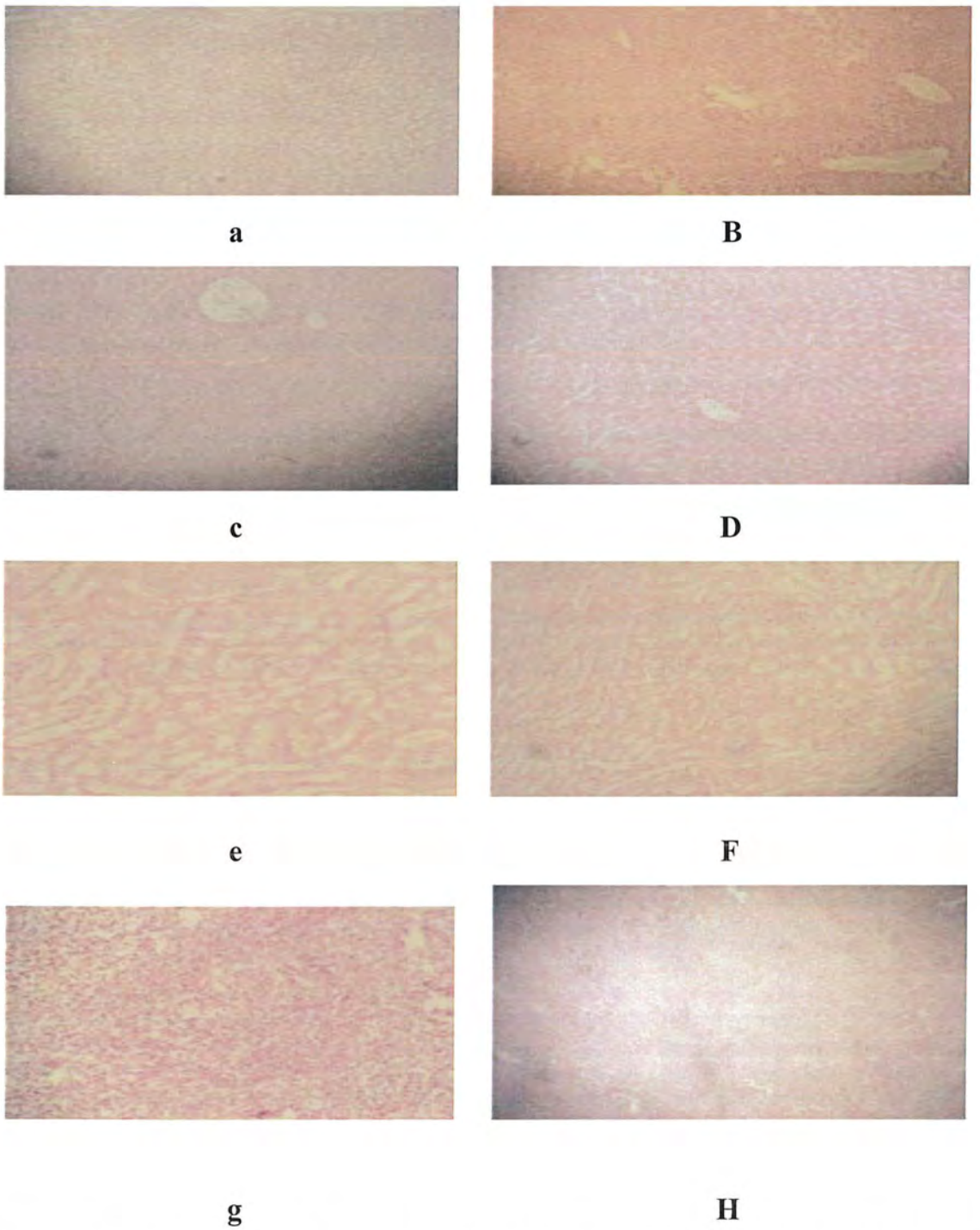


Figure.61. Histopathological slides of liver organ (a) Normal Liver (b) Damage Liver by CCl4 (c) Recover of tissue after treatment with methanol extract of *Vitex negundo* leaves (d) Recover of damaged liver tissue with standard drug Silymarine (e) Normal tissues of kidney before treatment (f) Treatment of damaged tissues of kidney with *Dodonaea viscosa* methanol extract (g) Normal tissues of spleen (h) Treatment of damaged tissues of spleen with chloroform extract of *Dodonaea viscosa.*

4.1 Discussion

The ethno-botanical and ethno-pharmacological study was investigated in the rich, cultural as well as traditional diversity of different localities in the Murree hills. The plants were excessively collected from wild habitat however few of them were from the cultivated habitats. Wild plants represented more in some studies as compared to cultivated (Sivasankari *et al.*, 2014). Herbs were dominating in some studies (Ahmad *et al.*, 2014: Hong *et al.*, 2015: Li and Xing, 2016). The herbs were among the frequent used habitats followed by shrubs and trees. It was reported earlier that herbs were commonly found everywhere with minimum side effects and biological active compounds can be easily extracted from herbs (Lulekal *et al.*, 2013: Yaseen *et al.*, 2015).

During this study 131 species, 104 genus and 64 families were recorded. Asteraceae was dominating with the highest species and it was found dominated in many studies with Euphorbiaceae and Rubiaceae (Abbasi *et al.*, 2011: Zheng *et al.*, 2013). The parts of plants commonly utilized for medicines are Leaves, Whole plant, Fruit, Branches, Seeds etc. These information correlated with the study previously reported (Kunwar *et al.*, 2006: Ahmad *et al.*, 2014) and the reason behind that leaves are the most chemically diversified part of plant. The majority of plant remedy found was decoctions, powder, paste, extract and juice. Plant preparations (decoctions) are mostly prepared with fresh materials formed by the boiling the plant material and it frequently found in some studies (Mahmood *et al.*, 2013: Liu *et al.*, 2013). It may be possible that active constituents are more active in fresh plants and highly soluble in water due to having polar property.

Males were more active than females regarding knowledge about medicinal as well as ethno-botanical uses. In many studies men were found more energetic for unveiling and conveying ethno-medicinal knowledge as compared to females (Ribeiro *et al.*, 2017). Females were reported to have more knowledge as compared to man in the early age. These results are corroborated with many studies reported earlier where females have more knowledge than men (Kainer and Duryea, 1992: Amorozo, 2002: Borba and Macedo, 2006). Informants between 51-60 years have more information about medicinal uses of plants as it was found earlier in some studies that elder age people knew and practice more traditional knowledge (Bitu *et al.*, 2015).Some respondents said that information's or traditional knowledge has been transmitted to them through oral and observation. According to some

informants, they have gathered knowledge from their parents and grandparents or older people and similar information available in literature (Li *et al.*, 2017).

Informants were knowledgeable regarding ethno-medicinal uses against disease categories. The highly treated diseases are gastrointestinal problems, dermatological disorder, glandular disorder, Muscular-skeletal disorder, respiratory disorder. The dominating ICF found in this study was gastrointestinal disorder (0.57), Gynecological disorder (0.5), Antidote (0.5), Nervous system disorder (0.4), Dermatological disorder (0.37), Oral disorder (0.33), eye disorder (0.33), Respiratory disorder (0.321), Hair tonic (0.25) and Glandular disorder (0.157). Similar results were as reported by other authors (Wang *et al.*, 2010: Lin, 2014). *Myrsine africana*, *Dodoneae viscosa, Debregeasia salicifolia* and *Bauhinia variegata* has been widely used by local people against different ailments with having high used value against various diseases and have sound recognition in the local community. *Cynodon dactylon*, *Dryopteris ramose*, *Calotropis procera*, *Cassia fistula*, *Justicia adhatoda* and *Ricinus communis* were the less commonly used by the local peoples with lower used values. This might be due to the less active constituents present in these plants or might be due to the presence of toxic compounds. The findings of present study were slightly similar with some other studies, similar plants have been less commonly used (Mahmood *et al.*, 2013: Kiyani *et al.*, 2015).

In this modern era plants still being used medicinally in different countries and their crude extracts are still sources of many potent and powerful drugs (Selvan and Velavan, 2015). Crude plant extracts consist of complex mixture of different chemicals having wide range of bioactivities (Gomathi *et al.*, 2008). The Chinese, Arabic,Unani and Ayurvedic medicines have been used for the betterment of human population from ancient times (Ibrahim *et al.*, 2012). Besides significance advances in modern medicine, plants still make an important contribution in the health care and about 25% of the pharmacological drugs were isolated from plants in the developed countries during last couple of years are being used against many human infections (Zengin *et al.*, 2011). In the quantitative analysis, total phenols, total flavonoids and tannins were estimated by comparing with standards (Ascorbic acid, quercetin and tannic acid). The discovery of the drugs from natural products are based on the investigation of biologically active constituents and their therapeutical screening (Pandey *et al.*, 2012). Among secondary metabolites phenolic compounds are considered the most important compounds and found to be present in more quantity as compared to other

compounds. Phenols were present in higher quantity as compared to other phytochemicals and quantified in the current study. Phenols impart various pharmacological properties and effective against different diseases e.g. anticancer activity, cardiovascular diseases, rheumatism (Azietaku *et al.*, 2017).

Alkaloids stored in storage organs and most of alkaloids have been stored in leaves extracts as compared to fruits and flowers extracts. There are various important chemical components of plants and alkaloids are believed to be one of them. They are commonly famous for growth termination, inhabitation and stimulation processes. They have been used against various human and animal diseases (Xu *et al.*, 2011: Rasulev *et al.*, 2014). After phenols, flavonoids are the second to be found in higher quantity as compared to other secondary metabolites. Flavonoids are well known parts of polyphenols are present in various plants and posses many actions e.g. antioxidant, anti-mutagenic, anti-inflammatory, antiviral, anti-allergic and anti-neoplastic (Khan *et al.*, 2012).

Plant extracts contained small quantity of tannins and saponins. Tannins have the potential of antibacterial, haemostatic, diuretic, insecticidal, antidote and stomach problems. Furthermore it protects microbiological degradation for dietary proteins (Hollman, 2001). Plant saponins possessed antimutagenic, anticancerous, hypocholesterolic, immunoadjuvent, antitoxic, antifungal, antiprotozoal, anti-inflammatory and antihaemolytic properties (Havsteen, 1983). Plant derived phytochemicals defined the medicinal properties of plants. They are basically non-nutritive and classified into qualitative (carbohydrates, proteins, amino acids and chlorophyll), and quantitative metabolites (phenolic, flavonoids, alkaloids, steroids, saponins and tannins). Plant extracts also contains essential oils which have various volatile chemical compounds with diversified functional groups. Among plant compounds polyphenols are the most essential group which comprises on flavonoids (quercetin) which have anti-hepatotoxic, anti-platelets, antioxidants and cytotoxic activities (Guardia *et al.*, 2001: Savithramma *et al.*, 2012: Upadhyay and Dixit, 2015).

Most of compounds which have been found in plant extracts have potent antioxidant properties. Plants which contain antioxidants play a vital role for human health. They protect organisms by oxidative damage and prevent various diseases such as cancer, heart diseases,

neurological diseases and diabetes (Shariatifar *et al.*, 2014). Due to several advantages plants extracts has been assessed for seven antioxidant assays such as Hydrogen peroxide assay, DPPH, ABTS, Hydroxyl assays, Reducing power, superoxide and Iron chelating assay. For antioxidants estimation single antioxidant method is not enough therefore it is an important to analyze different antioxidants assays (Dudonne *et al.*, 2009). IC_{50}value is the half inhibitory concentration that measures the substance of effectiveness while inhibiting a particular reaction. It represents the drug concentration needed for 50% inhibition. The inhibitor concentration increases by lowering the antagonist activity, which further results in higher IC 50 values. *Debregeasia salicifolia* Methanol and *Indigofera heterantha* Methanol has shown good antioxidants activities against all assays by possessing significant amount of phytochemicals and the plant *Debregeasia salicifolia* Methanol extract was found most effective. Higher antioxidant activities of plant extracts could be attributed with higher level of flavonoids and phenols obtained from fruit and leaves extracts. Results obtained in current study are comparable with results of antioxidant potential of plant extracts reported in literature by other authors including (Aberoumand and Deokule., 2008).

Results obtained in current study about antioxidants activities were more comparable with results reported by Rajani and Ashok (2009), when they worked on root and stem of *Bauhinia variegata*. Various studies revealed that *Bauhinia variegata* contains active constituents responsible for pharmacological and curative activities (Ali Esmail Al-Snafi, 2013) and possess significant amount of natural antioxidants (Pandey *et al.*, 2012). This can be attributed to the flavonoids and other phyto-constituents present in the plant extracts. The over production of ROS or inadequate antioxidant defense, there is always increase in ROS leading to oxidative stress. The antioxidant activity can be attributed to various mechanisms like prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reductive capacity, and radical scavenging activity (Gopinathan *et al.*, 2004; Kaur *et al.*, 2006).

Most of the antioxidants methods used for antioxidants assays showed strong correlations with phenolic contents. The strong relationship with phenols and antioxidants method revealed that the antoxidant activity is frequently contributed by phenols (Dudonne *et al.*, 2009). IC50 of plants were significantly correlated with total phenolic contents and total

flavonoid contents. Secondary metabolites such as flavonoids are the diverse group of natural compounds and are important antioxidants, while the phenols consists of the major compounds that imposed the properties of antioxidants as well as free radical scavenging activities. Flavonoids also impart pharmacological and biochemical effects such as hepato-protective, anti-cancerous and antioxidant activities (Miliauskas *et al.*, 2004: Alaklabi *et al.*, 2016: Hussein *et al.*, 2012).

High Performance Liquid Chromatography is an important analytical techniques used to quantify various plant based organic compounds those might be required for determination of bioactivities. It is used in the manufacturing of biological as well as pharmaceuticals goods (Gerber *et al.*, 2004).Quercetin is estimated from the different extracts of plants. Quercetin is a sub class of flavonoids, revealed pharmacological effects such as antioxidant, anti-arthritis, anti-inflammatory, anti-mutagenic and anti-hepatotoxic (Guardia *et al.*, 2001). Pytochemical screening of flavonoids contents in quantitative analysis signifies the importance of current findings. The plant extract which have higher value may be due to the active compounds that are properly dissolved in the HPLC grade methanol and the plants extracts those passed no activity might be due to inappropriate mixing of compounds. If the crude extracts did not properly dissolved or not shown any residue before injection would not show good activity.

Quercetin is basically a flavonol which is a class of flavonoid. It is widely distributed group of plant phenols and imparts colors in flowers. The most abundant flavonoid present in food is flavonol, responsible for taste, color, deterrence to oxidation of fat and protects vitamins and enzymes. Flavonoids are essential class of secondary metabolites and playing vital role for protection of human body against many infections. Flavonoids are capable of modulating the activity of enzymes, affect on the performance of human cells and possess anti hepatotoxic anti allergic, anti-inflammatory and antitumor activities (Canini *et al.* 2007: Tuberoso *et al.*, 2011). Furthermore flavonoids as a dietary component are providing health benefits due to their higher antioxidant ability, both *in vivo* and *in vitro* system (Cook and Samman, 1996). Flavonoids reduce health problems like cardiovascular diseases, atherosclerosis, Parkinson's disease, reduce blood pressure and blood cholesterol level (Kumar and Pandey, 2013).

Quercetin (C_{15} H_{10} O_7) having molecular weight of 302.235 g/mol, is polyphenolic flavonoids like other phenolic heterocyclic compounds. Quercetin have antioxidant, anti-inflammatory and anticancer activities (Marinova *et al.*, 2005; Newman and Gragg, 2012; Gopinathan *et al.*, 2014; Hollman *et al.*,1997). The binding of quercetin with the estrogen protein receptor showed an association with glycine, histidine, leucine,glutamine, phenylalanine and methionine indicating possible hydrophobic and hydrophilic sites for inhibition of the receptor (Li *et al.*, 2010). The molecular docking approach can be used to model interactions between small molecules (liqands) and protein at the atomic level. Quercetin inhibits the growth of several cancer cell lines and shows antiproliferative activity mediated by estradiol. It has a correlation with liver enzyme alkaline phosphatase (Christophe *et al.* 2004). Our results support the traditional use of all plant extracts and suggest that the flavonoids, including quercetin, are likely to contribute to its effects.

FTIR analysis confirmed presence of aromatic compounds like carboxylic acids, alcohols and phenols which verifies the presence of β- sitosterol, lupeol and friedelin groups (Zargar*et al.*, 2014). Phenols are the phytochemicals used in the manufacture of different dugs in pharmaceutical industries and used to identify crude dugs. These pharmaceuticals are the source of curative activities of plant extracts (Savithramma *et al.*, 2012).Phenolic compounds were found to have biological features by plummeting antioxidant stress. Hydroxyl group with unsaturated double bond make phenols significant antioxidants through their vulnerability to oxidation (Pantelic *et al.*, 2016).Chemical compounds in various plant extracts showed different spectra of IR absorption allow identifying the different samples. It depends on type of OH, CH and NH bonds in the plant materials. The features from FTIR spectrum combine with compositional and functional analysis of the desired compounds leads to indicate composition of plant extracts either new or unknown (Yang *et al.*, 2004).

Ricinoleic acid ($C_{18}H_{34}O_3$) (1.02%), carboxylic acid ($C_{23}H_{32}O_4$) (0.40%) and cedrol ($C_{15}H_{26}O$) (0.21) were quantified as some uncommon compounds in *Dodonaea viscosa*. Ricinoleic acid is unsaturated as well as hydroxylated fatty acids. Ricinoleic acid hinders bacteria, yeast, virus and mold and number of biological potential (Tunaru *et al.*, 2012). Oleic acid along with linoleic acid has been dominantly present unsaturated fatty acid. Fatty acids

(poly saturated and mono saturated) are essential for human nutrition and health, they help to reduce the blood pressure, decreases ulcers, oxidative damages and diabetes (Vassiliou *et al.*, 2009: Dong *et al.*, 2011: Silva *et al.*, 2014). Findings revealed some new compounds like Ar-tumerone ($C_{15}H_{20}O$), tumerone($C_{15}H_{22}O$) and curlone ($C_{15}H_{22}O$) were quantified in *Rumex hastatus*. Octadecenoic acid ($C_{18}H_{34}O_2$) (20.60%) and fumaric acid ($C_{23}H_{40}O_4$)(1.17%) were observed in *Indigofera heterantha*. Ar-tumerone is the sesquiterpenes, responsible for its pungent smell and taste and these compounds were found to have hypoglycemic activity (Ferreira *et al.*, 2013:Nishiyama *et al.*, 2005).

Sebacic ($C_{23}H_{40}O_4$) and Heptadecafluorononanoic($C_{27}H_{37}F_{17}O$) were significantly analyzed as newly reported compounds found in *Debregeasia salicifolia*. Ascorbic acid, phenols and poly phenols are the important antioxidants those found in fruits, vegetables, plants and inhibits the oxidation in plant extracts caused by free radicals (oxidants) and various diseases (Ghasemnezhad *et al.*, 2011: Pavlovic *et al.*, 2016). Fatty acids (poly saturated and mono saturated) are essential for human nutrition and health, they lower the blood pressure, reduces ulcers, oxidative damages and diabetes (Vassiliou *et al.*, 2009: Dong *et al.*, 2011: Silva *et al.*, 2014).

The Infectious diseases are the reason of mortality in humans and to control such infections pharmaceutical industries has been manufacturing different antibacterial drugs. Those antibiotics are becoming ineffective as resisted by the bacteria. Many plants are called medicinal as they have substances those are curing human diseases. Medicinal plants knowledge is occasionally known as curative reserve for local communities and pays occasionally to the health care (Betoni *et al.*, 2006). The plant extracts exhibited significant activities against different bacterial strains in different solvents. These plants extracts were active against different diseases and this led to the discovery of novel compounds. Some studies showed that the methanolic, *n*-hexane and chloroform extracts of the aerial parts of *Myrsine africana* and *B. variegata* showed good activity against *K. pneumonia* and *S. aureus*. Chloroform and aqueous extract of *Myrsine africana* showed no activity against *E. coli* (Ahmad *et al.*, 2011: Ali Esmail Al-Snafi, 2013).

Our findings regarding *Vitex negundo* were somewhat similar with the results found in some studies (Zargar *et al.*, 2011: Chandramu *et al.*, 2003).All other bacterial strains did

not exhibited significant zones of inhibition for some extracts. Some bacterial strains, Gram-negative were more resistant as compared to Gram-positive bacteria (Panda *et al.*, 2009). Streptomycin was the standard drug used in this assay. Minimum Inhibitory Concentration is the minimum quantity of an antimicrobial substances those stops the growth of bacteria after the incubation period. In diagnostic labs MIC was used to check resistance. It is commonly used as an instrument in the latest vitro studies for the research purpose (Andrews, 2001). Minimum inhibitory activities of plant extracts was assessed against two strains of bacteria and results obtained indicates lower level of MIC values for various plant extracts indicating their suitability as antimicrobial drugs.

The plants extract tested in this study showed stronger resistance against all tested strains of Mycobacterium tuberculosis and this might be due to presence of bioactive components in plant extracts with the methanol that are possibly antimycobacterial metabolites. Tuberculosis is responsible for many fatalities in the world and TB patients need to have extensive chemical treatment and ultimately it causes adverse effects to patient health. To reduce the uses of synthetic drugs resistance medicinal plants provide a great confidence as a potential source of bioactive antimycobacterial metabolites (Gemechu *et al.*, 2013). *Myrsine africana* leaves showed higher results against all strains as compared to *Myrsine africana* fruits. *Myrsine africana* has multiple roles starting from the flavoring agent, anti- rheumatic and tuberculosis effect to the inhibitory and larvacidal activities and possesses diversity of compounds such as benzoquinones, triterpenes, steroids, flavonoids, flavonol glycosides, Myrsinone A and B (Ahmad *et al.*, 2011).

Higher lethality against brine shrimp was shown by *Bauhinia variegata* as it may possess active constituents and plant extracts may have anticancerous activity because brine shrimps assay can be an initial step for anti cancerous activities (Nisar *et al.*, 2015). *Indigofera heterantha* possess higher cytotoxicity (90%) in comparison to this, some studies showed very less toxicity with the similar or different solvents (Arfan *et al.*, 2011). While lower lethality was found in *Dodonaea viscosa* and *Myrsine africana* fruit. *Myrsine africana* leaves were found to have 60% lethality. According to the results of cytotoxicity obtained for plants extracts during this study were lower than these reported earlier in the literature. Therefore leaves extracts of *Myrsine Africana* has shown suitable cytotoxicity might be due to presence of emodin and 2-hydroxychrysophanol (Ahmad *et al.*, 2011).

Plants have a long history of use in the treatment of cancer and the interest in nature as a source of potential chemotherapeutic agents continues (Schwatsmann *et al.*, 2002). The anti anticancer activity of plant extracts was proposing which indicates importance of flower extract of *Bauhinia variegata* that may be use against certain carcinogenicity of human or animals cells (Saha *et al.*, 2011; Acharaya *et al.*, 2010). In spite of comprehensive studies cancer becomes one of the primary reasons of mortality. Medicinal plants have been extensively used by the people for the quick efficacy and less side effects. Plants possess diversity of compounds that found to have anticancer activity (Lall *et al.*, 2015).In current senerio, cancer has been treated with synthetic drugs and procedure is known as chemotherapy, it might be effective but painful and sometime become resistance. So, the plants have been recommended to use against cancer cells as they possess biological active compounds (Alaklabi *et al.*, 2016).Plants were screened against liver carcinoma cell lines and found to possess less significant activity. It might be due to the absence of active constituents in the tested fraction, though the presence of biologically active compounds also depends on the solubility of solvents used (Juárez-Rojop *et al.*, 2014).

Methanol extract of *Debregeasia salicifolia* showed highest antitumor activity 90±2.8% and ethanol extract of *Debregeasia salicifolia* also strongly inhibit tumor to grow 80±6% while chloroform extract partially showed inhibition and n-hexane extract showed less inhibition. *Debregeasia salicifolia* showed higher activity in polar solvents that means significantly high quantity of active compounds was dissolved in polar solvents. Chloroform and ethanol extracts of *Myrsine africana* leaves showed 88±9.5% and86.5±5% antitumor activity. While other extracts showed less activity. Methanol extracts of *Indigofera heterantha* showed strong activity 85±3% and *n*-hexane extract also showed 81±2.5% defense against *Agrobacterium tumefacien.*

Chloroform extracts also has shown good inhibition which means *Indigofera heterantha* and *Myrsine africana* leaves are active with both polar and non polar solvents. Similarly *Vitex negundo n*-hexane and ethanol extracts showed strongest activity 85±3.6% and 80±9% followed by *Rumex hastatus* ethanol and *n*-hexane extracts showed good activity 79.3±1.6 % and 72.9±4% and same inhibition was showed by *Dodonaea viscosa*. They were found to be active in both polar and non polar solvents it can be due to the type of phyto-

constituents which are intended to be dissolved in particular solvent, nature of solvent and extraction procedure. *Myrsine africana* fruit and *Bauhinia variegata* showed high antitumor activity in non-polar solvents Chloroform (83.4±7.1%:82.8±10%) and n-hexane (82.4±7%:77±9%).It could be due to less polar or non polar compounds present in these plants extracts and found to be active not only against agrobacterium tumefacien strains but also against bacteria's, cancer cells and tuberculosis strains which justifies the significance of current results. As the concentration increases ultimately activity also increases. This study is relatively a quick, reasonable, nontoxic and statically consistent method for the selection of murine leukemia (antitumor activity) (Hussain *et al.*, 2007).The obtained values were significantly different $p<0.05$.

Hemolysis was induced in RBCs by H_2O_2 that is a toxicant and has oxidizing nature lead to degradation of cell membrane and finally hemoglobin discharged from cell. H_2O_2 arouse OH radical's production with Fe2+ mobilization by Ca2+ through Fenton reduction reaction. The above factors totally cause cell membrane disruption, indicating cell lysis (Kadali *et al.*, 2016). It was observed that H_2O_2 is toxicant that causes degradation in cell and also produces reactive oxidants. The above antioxidant studies of these plants support the results of anti-hemolytic activity by showing strong effects. This activity was not previously reported in any tested extracts. This activity demonstrated the capabilities of various extracts that stabilizes the membrane of human red blood cell, that is indication of extracts capability to avoid rupture and hemolysis in stress conditions induced by hypotonic solution.

The antioxidant activity of plant extracts might be due to polyphenolic flavonoids which carry oxidant scavenging activity. PLA2 (enzyme) acts on the membrane of red blood cells of human which is associated with phospholipids, liberates lysolecithin resulting hemolysis. The induction of oxidative hemolysis in human red blood cells (in vitro) was used to examine the free radical produce damage in biological membranes and inhibition of natural antioxidants. When red blood cells are exposed to injurious substances e.g. methyl salicylate and phenyl hydrazine causes membrane lysis along with hemoglobin's hemolysis and oxidation. The effects of hemolysis in hypotonic solution are associated with excessive fluid accumulation in cell resulting in membrane rupturing. RBC membrane injury makes the cell susceptible to secondary damage by free radical produced by lipid per-oxidation. This is frequently happening and causes bio-molecules breakdown leads to free radicals formation which increase cellular damage. Free radicals e.g. lipid peroxide and superoxide are

reportedly produced in conditions like stress hemolysis is due to destabilization of cell membrane (Kumarapppan *et al.*, 2011: Durairaj *et al.*, 2014).

CCL_4 reduced the mice body weight followed by liver and kidney weight while spleen weight was increased. Protective effect of different plants showed results near to normal and olive oil control. Liver size of CCL_4 mice was reduced as compared to normal mice. Olive oil itself showed protective effect on liver and weight increases. Methanol and chloroform plant extracts showed protection depending upon the solubility and nature of compounds. CCL_4 being a toxicant cause oxidative stress in the liver and these plants have varied amounts of secondary metabolites and antioxidants. Phenolics, tocopherols and oleic acids possess oxidant scavenging effect (Jia *et al.*, 2011)

Debregeasia salicifolia Leaves and *Myrsine africana* fruit showed high protective effects on liver and increases the weight of animals. Myrsine plant has significant bioactive compounds and fruit is very active against various diseases such as antitumor, antifertility, phytotoxic and haemagglunitation activity and verify it theraputant (Abbhi *et al.*, 2011). Results suggested that during in vivo study it was observed that plant extracts has recovered weight of animals without creating any behavior change and recovery was on dose depended manner. Similar results were reported by Gulfraz *et al.*, (2008), when they worked on other plant extracts.

Liver is an important organ of body and it can be damage by toxicants such as oxidants, virus and drugs. Toxicity is caused by carbon tetra chloride in albino mice, those started oxidation process and causes liver injury. However liver has inordinate ability to detoxify toxins up to some limits and fight against diseases. But liver diseases continue to have huge hazard to human health so far. Besides advancements in modern medicines, there has no effective drug available till now that protects liver and enhances its normal activities (Jia *et al.*, 2011: Dong *et al.*, 2011: Iqubal *et al.*, 2017). CCl_4 is a well known hepato-toxin and toxicity produced by it has been used in different experimental models for the investigation of Hepato-protective agents (Chen *et al.*, 2009: Liu *et al.*, 2011).

Curative effect of these plants might be due to biologically active compounds present in plant extracts. Flavonoids, triterpenoids, palmitate, ursolic and sitosterol play vital role for liver protection (Guardia *et al.*, 2001; Adewusi and Afolayan, 2010). However, administration of various doses especially 300 mg/kg body weight of methanolic *Bauhinia*

variegata flower extracts reduced the increase in the serum levels of three enzymes (ALT, AST and ALP) as well as other biochemical parameters those were elevated during CCl4 induction as reported earlier (Kumar *et al.*, 2009). *Bauhina variegata* bark was reported to have hepato-protective activity and its bark have tonic for liver. Flavonoids and glycosides were documented in different plant parts and known for hypoglycemic activity (Bodakhe and Alpana, 2007). *Vitex negundo* leaves found to have protection against liver and results were comparable with the findings of Tandon *et al.*, (2008). All the results were significant at the level of $p<0.01$.

It was reported by Wu *et al.*, (2007 that flavonoids at a concentration range of 1–100 µg/mL improved cell viability and inhibited cellular leakage of hepatocyte aspartate aminotransferase (AST) and alanine aminotransferase (ALT) caused by CCl_4. Similarly in the *in vivo* experiment various doses of flavonoids has significantly reduced the levels of AST, ALT, total protein, and albumin in serum as well as hydroxyproline and sialic acid levels in liver. Histopathological examinations also revealed the improvement in damaged liver tissues with the treatment of extracts having flavonoid (Hollman *et al.*, 1997; Wu *et al.*, 2007).

These plants extracts have protective effects on hematology parameters as it is prove by significant activities against hemolysis obtained in study. Results indicates that after administration of methanolic and chloroform leaves, fruit and flower extracts as well as standard drug (Silymarine), levels of these parameter was increased and became closed to normal values . However, improvement in level of RBC, WBC and platelets count was dose depended manner. It was reported by Adedapo *et al.*, (2009) and Osuigwe *et al.*, (2007) that rapid increases or decreases in hematological parameters causes weakness in immune systems of animals and prolong untreated condition may results serious consequence (Okonkwo *et al.*, 2004). CCL_4 reduces the count in blood analysis (RBC, WBC and Platelets) while good results with protective effects were showed by these plants extracts by decreasing levels of enzymes and raised level of RBC, WBC and Platelets, which were similar to the results of normal and olive oil control group. Above results proved that the phytochemicals present in DSM and IHM detoxify ROS and particularly flavonoids which were proven to have protective effects (Liu, *et al.*, 2011).

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood of animals. It can also be used to explain blood relating functions of chemical compound/plant extracts (Murray *et al.*, 2000; Okonkwo *et al.*, 2004; Yakubu *et al.*, 2007). The liver may be considered as most important organ as it is major site of metabolism and elimination of foreign substances. The oxidative stress that is due to free radical or reactive oxygen species causes liver injury and its treatment is important and challenging task for health care professions (Jiwon, 2005). The growing body evidence indicates that oxidative stress plays an important role in pathogenesis of many clinical conditions (Sasaki and John, 2017). Antioxidants both enzymatic and non enzymatic prevents free radical induced tissue damage by preventing the formation of radicals scavenging them or by promoting their decomposition (Young and Woodside, 2001).

The antioxidant system of mammalian cells mainly possessed three enzymes like GPx (Glutathione peroxidase), CAT (Catalase) and SOD (Superoxide dismutase) and these three enzymes collectively prevent reactive oxygen species in the cells during aerobic metabolism (Jia *et al.*, 2011). Carbon tetrachloride has been used to induce toxicants in animal model for evaluating organs injuries as it breakdowns by cytochrome into trichloromethyl radical and proxy trichloromethyl radical leads to start lipid peroxidation and processed to an accumulation of lipid oxidation products finally caused the injury. Increased level of reactive oxygen species damages the cells and plant extracts which possessed antioxidant properties prevents various diseases derived from ROS. Unsaturated fatty acids (oleic acid) and phenolic acids present in plants extracts were reported to possess antioxidant activity and these properties found to prevent oxidative damage to liver (Dong *et al.*, 2011: Palipoch *et al.*, 2014).

Carbon tetra chloride (CCl_4) is a contaminant that damages the organs. Toxicity was created by CCl_4 in liver and was focused on the assessment of protective effects of plants extracts. With increasing concentration of plant extracts protective effect were increased. Damaged cellular organization, hepatocyte pycnosis, inflammation along with extreme degeneration of liver tissues were observed in CCL_4 group. Microscopic examination revealed that CCl_4 group highly damaged was in liver, kidney and spleen of animals. Liver alteration such as cirrhosis which leads to necrosis, fibrosis and nodules and enlarged spleen was observed in cirrhotic mice in CCl_4 group as which was also reported in previous studies (Das *et al.*, 2014).

Changes in cellular organization of above three organs were notably reduced by administration of plant extracts and found no fibrosis in *Debregeasia salicifolia* methanol, *Myrsine africana* leaves methanol, *Debregeasia salicifolia* chloroform, *Myrsine africana* leaves chloroform, and *Indigofera heterantha* methanol group which was also proven by some earlier studies (Simeonova *et al.*, 2014: Chan *et al.*, 2016).Standard drug silymarine recovered the cellular organization when it was damaged by toxicants. These results demonstrate significant relationship between serum enzymes, biochemical parameters and hepatic enzymes. The study revealed that an increased in ALT, AST, ALP, antioxidants enzymes, blood cells, total proteins and direct bilirubin in the blood of experimental animals those received CCl4 alone indicates considerable cellular lesion which was further confirmed by histopathology.

Conclusion

Drugs discovery from medicinal plants still remains an important area of human health. The possible benefits of plant based medicines have led to unscientific exploitation of the natural resources, a trend that is being observed globally. Therefore drug discovery from natural source involves a complicated approach combining botanical, phytochemical, biological and molecular techniques. Current study provides details of ethnobotanical based biological activities of seven medicinally important plants from Murree hills areas located in North East of Pakistan. The study was carried out to collect and document information regarding traditionally utilization of these medicinal plants as those were not reported earlier. According to results various plant extracts comprised of valuable phenolic and flavonoids (quercetin, rutin and *p*-cumeric acid) as well as saturated and unsaturated fatty acids and contained antioxidants / electron scavenging activities, antimicrobial, anti tuberculosis activities, anticancer and hepatoprotective activities. The findings revealed that these plants possessed biologically active secondary metabolites can be further isolated in future pharmacological studies. These plants need to examine more to obtain new compounds, might be useful for preparation of new drugs required to control various diseases of human and animals.

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The *In Vitro* and *In Vivo* Biological Activities of the Leaf of Cape Myrtle, *Myrsine africana* L.

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The cape myrtle, *Myrsine africana* L., is a widely used medicinal plant, which has not been well investigated. We assessed the *in vivo* hepatoprotective and *in vitro* antiproliferative and antioxidant effects of leaf extracts of *M. africana* chemically profiled using high-performance liquid chromatography. Three flavonoids were quantified, and gas chromatography–mass spectrometry analysis revealed the presence of common fatty acids. The animal study was conducted on mice treated with CCl4, using three doses each of the methanol and chloroform extract (100, 200 and 300 mg/kg b.w.),with silymarin as a positive control. Hepatoprotective effects were determined by analyzing blood for liver marker and antioxidant enzymes, direct bilirubins and total proteins. The methanol extract (300 mg/kg b.w.) showed the strongest hepatoprotective effects against abnormalities produced by CCl4. The *in vivo* hepatoprotective effects correlated well with the *in vitro* antioxidant and antiproliferative activities and with high levels of flavonoids in the extracts. Finally, molecular docking studies of the constituent quercetin were undertaken *in silico* and several sites of binding to human estrogen receptor (ER) protein, linked with alkaline phosphatase, identified. Copyright © 2017 John Wiley & Sons, Ltd.

Keywords: Myrsine africana; hepatoprotective; antioxidant; antiproliferative; quercetin; molecular docking.

INTRODUCTION

Myrsine africana L. (Myrsinaceae), the Cape myrtle or Babrang, is also known as khokhal. The fruits are edible and are traditionally used as a carminative, appetizer, flavoring agent or fragrance, and as an anthelmintic. The leaf is used as a 'blood purifier', and in treatment of diarrhea, rheumatism, toothache, bronchitis, cough and pulmonary tuberculosis, and as an antiviral and fungicidal agent. Antibacterial effects have been described for the leaf extract of *M. africana* (Ahmad *et al.*, 2016), and the methanol extract and the isolated constituent myrsigenin have been shown to be antispasmodic (Azam *et al.*, 2011). The current work was conducted to analyze the leaf extract using high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS), to explore its proliferative, hepatoprotective and antioxidant activities, and investigate the molecular docking of the constituent quercetin on liver enzymes.

MATERIALS AND METHODS

Chemicals and reagents. Enzyme and protein lysis kits were obtained from AMS (S.p.A. Italy). Quercetin, 1,1 diphenyl 1–2-picryl-hydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) were obtained from Sigma-Aldrich Chemical Co. (USA). High-performance liquid chromatography solvents were of analytical grade.

Collection and preparation of leaf samples. Leaves of *M. africana* were collected from KotliSattian (Rawalpindi). A specimen (voucher no.137) has been deposited at the Herbarium of Plant Sciences Quaid-i-Azam University, Islamabad. The samples were shade and sun dried followed by oven drying overnight at 60° C, ground to a powder, sieve (80mesh). One hundred grams of dried leaf was extracted with distilled water, methanol, ethanol, chloroform and n-hexane using soxhlet apparatus and a rotary evaporator.

Determination of phytochemicals. Total flavonoids, phenols and tannins were estimated using a modified Folin–Ciocalteu colorimetric procedure. Alkaloids and saponins were determined as described by Abbasi *et al.* (2015).

High-performance liquid chromatography analysis. High-performance liquid chromatography analysis was performed using a Shimadzu HPLC system (Tokyo, Japan), C18 column (25 mm × 4.5 mm, 5 µm) and UV/visible detector. The compounds were eluted using a gradient of acetonitrile and 0.1% phosphoric acid (36:64). The injection volume for all samples was

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