Ethnobotanical Studies of Women Specific Diseases among the Local Communities of District Swat

BY

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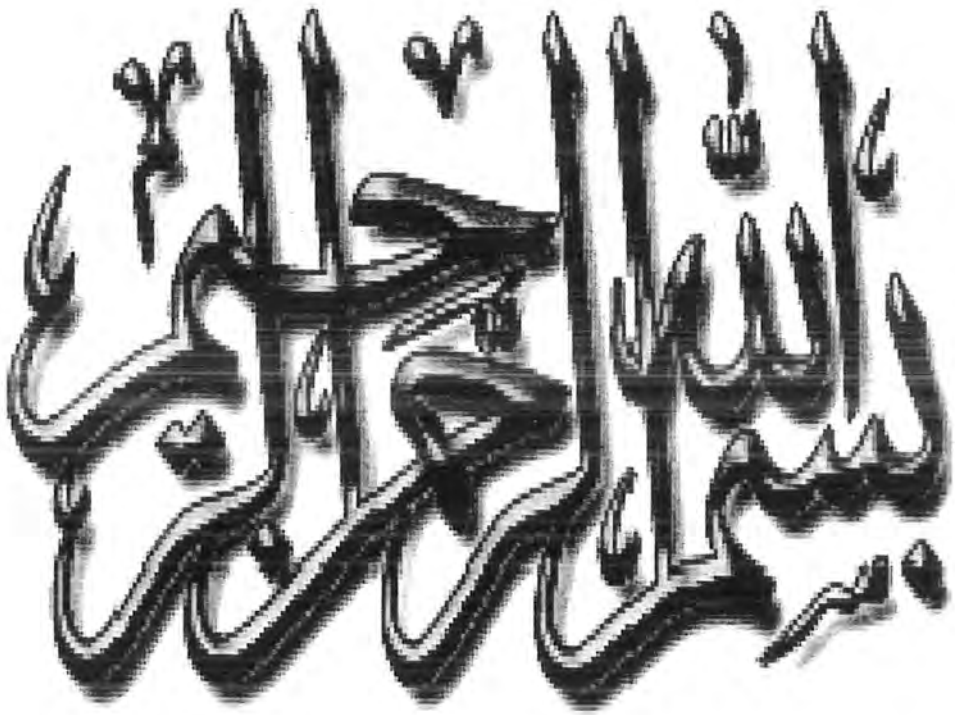
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APPROVAL CERTIFICATE

This thesis submitted by **Miss Sarwat is** accepted in its present form by the Department of **Plant Sciences**, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in **Molecular Systematics and Applied Ethnobotany.**

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The whole of the experimental work including lab and field described in this thesis was carried out by me in the, Department of Plant Sciences, Quaid-i- Azam University Islamabad, except where acknowledged. The findings and conclusion are of my own investigation with discussion of my supervisor Dr. Zabta Khan Shinwari, No part of this work has been presented previously for any other degree.

SARWAT

DEDICATION

"Dedicated to my beloved **PARENTS** because whatever I am today could never be possible without their continuous guidance, effort, sincerity and prayers. I am heartily thankful to them…."

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List of Abbreviations

%	Percentage
>	Greater than
°C	Degree Celsius
AMP	Ampicillin
C. albicans	*Candida albicans*
CFU/ml	Colony-forming units per milliliter
Co	Cobalt
Cr	Chromium
Cu	Copper
DMSO	Dimethyl Sulfoxide
E. caryophyllata	*Eugenia caryophyllata*
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
F. tenacissima	*Forsskalea tenacissima*
Fe	Iron
FSH	Follicle-stimulating hormone
Gm	Gram
GNRH	Gonadotropin releasing hormone
K	Potassium
K. pneumonia	*Klebsiella pneumoniae*
LH	Luteinizing hormone
MBC	Minimum Bactericidal Concentration
mg/l	Miligram per liter
MHA	Muller Hinton Ager
MIC	Minimum Inhibitory Concentration
Ml	milli liter
Mn	Manganese

NCCLS	National Committee for Clinical Laboratory Standards
Ni	Nickel
O.limbata	*Otostegia limbata*
P. aeruginosa	*Pseudomonas aeruginosa*
Pb	Lead
PIMS	Pakistan Institute of Medical Sciences
PPM	Parts per million
Q.aucher	*Quercus aucher*
r/h	Refluxes per hour
RTIs	Reproductive tract infections
S. aureus	*Staphylococcus aureus*
S. emarginatus	*Sapindus emarginatus*
S. typhi	*Salmonella Typhi*
Spp	Species
STIs	Sexually transmitted infections
W/V	Weight per volume
WHO	World Health Organization
Zn	Zinc
Ml	Micro liter

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Abstract

Medicinal plants are usually rich in various metabolites, having antimicrobial activities. Ethnobotany provides scientific rationale in revealing medicinally important plant species especially for finding new drugs that play vital role in the treatment of different diseases. This ethnobotanical survey of Swat, Khyber Pakhtunkhwa (KPK) was carried out to identify different medicinally important plant species. The aims of present study were to conduct ethnobotanical survey of Swat District to identify plants having medicinal properties which were used traditionally to treat Gynecological disorder and infectious disease and to study their antimicrobial potential against pathogens that cause infections in females. The Antimicrobial activities were investigated by using well diffusion method against four different bacterial strains (*Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa*) and one fungal strain (*Candida albicans*). Our results revealed that out of 12 plants that were studied, 7 plants exhibited inhibitory effect against selected pathogenic strains. *Woodfordia fruticosa, Quercus dilatata, Erythrina varigata, Ficus religiosa, Berberis lycium* showed high Antifungal activity against *C. albicans* with MIC values of 5, 2.5, 1.25, 0.625, 0.3125 mg/ml and MBC values of 5, 2.5, 1.25, 2.5, 0.625 mg/ml respectively. Both *Woodfordia fruticosa* and *Quercus dilatata* showed significant Antimicrobial activities against *E. coli* and *K. pneumoniae* with MIC values of 2.5 mg/ml and MBC values of 5 mg/ml. Plants exhibiting inhibitory potential against *S. aureus* were *Woodfordia fruticosa, Quercus dilatata, Azadirachta indica* and *Curcuma longa.* These plant extracts possessed the similar MIC values of 5 mg/ml and MBC values of 2.5 mg/ml respectively. None of the plants showed Antimicrobial activity against *Pseudomonas aeruginosa.* Proximate analysis revealed that in comparative assessment of the various species, *Zanthoxylum alatum* was found to have high concentration of fat and energy values compared to other species. In conclusion, the results of antifungal activity were quite impressive than Antibacterial activity. The result studied confirmed that *Woodfordia fruticosa* can be used as therapeutic potency in the treatment of infectious diseases. The results of selected medicinal plants analyzed for proximate analysis indicated that *Zanthoxylum alatum* most significant species have higher concentration of fat and energy value as compared to other species. *Zanthoxylum alatum* have the potential to provide essential nutrients to the human beings.

Chapter No. 1

Introduction

Introduction

Ethnobotany is the relationship that exists between people and particular environment. Ethnobotanical uses are not only limited to food, shelter, medicine, clothing, hunting and religious ceremonies but mainly used for health care (Aumeeruddy, 1996). Ethnobotany allows interaction between researchers with the local people that have the knowledge about use of plants. These people handle and preserve amounts of biological resources useful for industry and world community (Hussain and Sher, 2005; Ozcan, 2005). Mehmood *et al.*, (2011); Shinwari *et al.*, (2011); documented the traditional knowledge as well as conservation and sustainable use of medicinal plants of Pakistan.

Pakistan is fairly large country gifted with a variety of climates, ecological zones and topographical regions. The northern parts of mountainous regions of Pakistan are rich in biodiversity as they are situated at the junction of three mountain ranges (Shinwari *et al.*, 2000; Shinwari *et al* 2011). Pakistan has an altitude ranging from 0 to 8611 m, therefore, has a variety of climatic zones and distinctive biodiversity. The flora of Pakistan is varied, diverse and highly captivating. Pakistan host about 6000 species of flowering plants, out of which about 2000 species have medicinal, aromatic and economic values (Karki and Walliams, 1999).

Most of the medicinally important plant species in Pakistan are chiefly used by Tibbi Dawakhanas (medical centers of indigenous physicians known as Hakims) but unfortunately, very little concentration and interest has been paid to the local traditional uses of plants as hakims are only source which are concerned with the floral and vegetative parts of medicinal plant species without any regard to their botanical characteristics, or distribution in the various ecological zones of Pakistan (Hamayun *et al.*, 2005). More than 50,000 traditional herb practitioners prescribed traditional medicines which are being used by about 75% of the population in Pakistan (Gill, 2003). The indigenous knowledge of medicinal plant species is passing down from generation to generation of herb practitioners and local inhabitants (Ahmad, 2004).

In prehistoric times, people had knowledge of medicinal plants. There were about several hundred species which were used by the people for the treatment of different diseases in indigenous system as herbal products of medicines that used the whole plant or

plant extraction. Medicinal plants were collected by local people and practitioners because they knew the indigenous traditional knowledge and most were not engaged with the trade of these medicinal plants (Shinwari and Khan, 1999).

The discovery of different medicinal plants and their practice is as old as the history of plants for food (Ibrar, 2002). In the development of drug ethnobotany provides useful information thus saving time and money (Sher *et al.*, 2010; Joshi, 1982). Through ethnobotanical survey from local people and practitioners, indigenous knowledge was collected in order to obtain drugs against infectious diseases. Medicinal properties of plants and their potential as source for new drugs are determined by indigenous knowledge (Parekh and Chanda, 2007a).

According to World Health Organization about 80% of the population used medicinal plants as herbal medicine for primary healthcare (Farnsworth *et al.*, 1985). The properties of medicinal plant species in traditional herbal therapies have made an outstanding position in its origin and evolution. The indigenous traditional knowledge about the folklore systems have started vanishing with the passage of time due to lack of written documents, preservation and relatively due to low earnings in these traditions (Chandra *et al.*, 2006). Therefore, ethnobotanists play key roles in rescuing and documenting this disappearing knowledge to local communities (Rao and Henry, 1997).

The different systems of traditional medicines which include Ayurvedic, Unani and homeopathy are completely based on indigenous knowledge of medicinal plants. In Pakistan, China, India, Japan, Sri Lanka and Thailand the practice of traditional herbal medicine is extensively used (Hasan *et al.*, 2007). Today herbal products and extracts have become popular so these are widely used for the treatment of various human pathogenic infections (Srinivasan *et al.*, 2006). The demand of medicinal plant extracts and other alternative forms of medical treatments are increasing day by day (Cowan, 1999).

Medicinal Plants are used by local communities since centuries (Shinwari, 2010). Pakistan has more than 6,000 species of higher plants, out of which 12% are used medicinally (Shinwari, 2011). Different parts of plants are used in order to treat various forms of diseases and infections. Traditional medicine is one of the oldest systems of curing

diseases and infections. A variety of plants have been used in different parts of the world to treat human diseases and infections (Nweze *et al.,* 2004; Vineela and Elizabeth, 2005; Ekpo and Etim, 2009).

According to WHO about 350 million cases of feminine diseases occur each year (WHO, 2007). Reproductive cycle of women is very clinical and complex. The medicinal properties of plant species are used as a chief source for drug development to treat various health disorders including gynecological disorders. Many native plants are used against gynecological diseases like *Menorrhea, White discharge, Irregular periods, Dysmenorrh*eal and other infectious diseases including *gonorrhea, reproductive tract infection* (RTI) (Mohapatra and Sahoo, 2008).

Women of tribal areas hesitate to discuss their feminine problems to doctors due to psychological, social and religious barriers. Therefore, they have been trying to improve their fertility and regulate their reproductive cycles throughout the history by practicing ethnomedicinal remedies. Gynecological disorders and other infectious diseases greatly effects women health therefore the first priority of local women in Himalayan region is medicinal plants because of cheap therapies and quality products as compared to western pharmaceuticals which are costly, unaffordable and in most cases unavailable (Jan *et al.,* 2009). In 1959 WHO estimated that more than 340 million cases of curable sexually transmitted infections (STIs) like *Treponema pallidum, Chlamydia trachomatis Neisseria gonorrhoeae* and *Trichomonas vaginalis* predictably occurred worldwide (WHO, 2007).

In developing countries reproductive tract infections (RTIs) are documented as a public health problem and rank second after maternal morbidity and mortality as the cause of healthy life loss among women of reproductive age (Jindal *et al.,* 2009). Reproductive tract infections which comprise endogenous infections, iatrogenic infections and sexually transmitted infections (STIs) greatly effects women health (Muula and Geubbels, 2006).

Vaginitis which is one of the most general infections of the vagina caused by *Candida albicans, Neisseria gonorrhoeae, Chlamydia trachomatis* affects about 90% of patients (Friedrich, 1985). If reproductive tract infections (RTIs) are not treated carefully it can cause the severe consequences such as infertility, ectopic pregnancy, cervical cancer,

menstrual disorders and low birth weight babies. The existence of RTIs (especially ulcer-causing sexually transmitted infections) can endorse the attainment and transmission of the human immunodeficiency virus (HIV) (Rabiu *et al.*, 2010).

In addition to infectious problems which greatly affect women, hormonal disorders account for a number of female complications. Hormones which are involved in menstrual cycle has different role in the body and due to imbalance of these hormones many complications are raised like estrogen dominance. Hormone imbalance is related with many different diseases including uterine fibroids; endometriosis, adenomyosis and migraine are associated with the menstrual cycle (Weiderpass, *et al.*, 1999).

This menstrual process, requires clear communication between the participating glands which is regulated in the body parts by complex changes in the concentrations of five hormones i.e. gonadotropin releasing hormone (GNRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone (Berkow, 1987; Smith and Schiff, 1989; Vander *et al.*, 1990). All these hormones related to control the cyclic process are released in short bursts or pulses at intervals of 1 to 3 hours, so that constant levels are not observed in the circulation. (Berkow, 1987; Snow, 1996; Vander *et al.*, 1990). Hormonal imbalance causes many medical conditions like obesity, lactation, autoantibody production and tumors.

Antibiotics make available the main basis for the therapy of microbial (bacterial and fungal) infections. Although antibiotics take key role in the alleviation of different diseases but it also have side effects like appearance of multidrug resistant pathogens and the spread of the new infections (Abdalla, 2011). The use of plant extracts for medical treatments is enjoying great fame since 1990s when people realized that the effectual antibiotic life span is limited and over recommendation and mistreatment of traditional antibiotics are causing microbial resistance (Alam *et al.*, 2009). One of the major threats encountered currently is antibiotic resistance. The frequency of antibiotics resistance is increasing to most antimicrobial agents and make their use and control of infectious diseases complicated. There is no antimicrobial agent which is fully effectual against dormant facultative bacteria (Jouenne *et al.*, 1998).

Medicinal plants must be tested for microbiological contamination and foreign materials to assure quality (Kruti *et al.*, 2011). Recently, microbiologists have paid more attention towards some higher plant products to investigate some phytochemicals for their exploitation as antimicrobials. Such plant products would be biodegradable and safe to human health (Kumar *et al.*, 2008; Sugar *et al.*, 2008; Krishnamurthy *et al.*, 2008; Wang *et al.*, 2010).

Currently most of the drugs used to treat bacterial and other infections were isolated from natural resources including ethnomedicinal plants. New resources of therapeutic agents are provided by plants against multidrug resistant bacterial infections. A large variety of secondary metabolites are produced by medicinal plants which are either used as a precursors or lead compounds in the pharmaceutical industry and it is expected that extracts of medicinal plants showing target sites other than those antibiotics used will be active against drug resistant microbial pathogens (Shokeen *et al.*, 2009).

In traditional medication many plants been claimed for their effective or superior properties over synthetic drugs, like medicinal plant species such as *Bixa orellana* species, and *Bidens* species, have been claimed more competent to cure infectious diseases than synthetic antibiotics by traditional healers (Rojas, 2006). So it has become necessary to assess the properties and scientific base for the potential use of folk medicine for the curable treatment of infectious diseases produced by common pathogens. For the treatment of infectious diseases in non-severe cases medicinal plants might represent an alternative source (Shah, 2005).

The antimicrobial screening of higher plants extracts and products has shown that these plants have potential for new anti-infective agents (De Smet, 1997; Cowan, 1999; Kelmanson *et al.*, 2000; Srinivasan *et al.*, 2001). Medicinal plants extracts are of great significance due to their high potential as antimicrobial compounds, especially for curing of various infectious diseases caused by pathogens (Nasir and Chanda, 2006).

From different parts of the world antimicrobial properties of medicinal plants are being increasingly reported (Saxena, 1997; Nimri *et al.*, 1999; Saxena and Sherma, 1999). Many researchers have examined the importance of medicinal plants, but only a few studies

have lead to these ethnobotanical findings with laboratory job to confirm the real antimicrobial property of these plants (Bhattarai *et al.*, 2008; Shakya *et al.*, 2008).

The present research work is focused on determining the antimicrobial activities of some selected traditional medicinal plants which are affective against female infectious diseases. The antimicrobial activities of the selected plants were checked against different fungal and bacterial strains. The proximate analyses (moisture, proteins, ash, fiber, crude fats and carbohydrates contents) were also checked.

Ethnobotanical survey of medicinally important plants against feminine diseases was conducted in Swat Khyber Pakhtunkhwa situated 160 kilometers (99 miles) from Islamabad. The Swat valley lies in the north between 34° 40 to 35° 55° North latitude and 72° to 74° 6 East longitudes at an altitude of 2000 meter above sea level and is enclosed by the sky-high mountains. The region is humid having mild summer with average annual rainfall exceeding 1000 mm and mean annual temperature of about 18 °C (Adnan *et al.*, 2006).

The total area of Swat is 5,337 km^2 (2,060.6 sq miles). The Swat District contains about 1550 taxa of flowering plants and 55 Pteridophytes. There are 7 types of forests from tropical dry deciduous to alpine. There are various reports about ethnobotanically important medicinal plants; the number varies from 55 to 345 species in Swat (Ahmad and Sirajuddin, 1996). The flora of swat is very diverse and unique as the area is a nexus of three big mountain ranges namely Karakorum, Hindukush and Himalayas (Hamayun *et al* 2006). So far, 350 species have been reported from Swat District (Shinwari *et al.*, 2004).

Through ethnobotanical survey and literature review 12 different medicinal plants species were collected from different areas of Swat which were used in folklore medicine to analyze their antimicrobial activities against few common gynecological diseases. To check the antimicrobial activities of plants extracts, well diffusion method was used. In menstrual cycle hormones are involved therefore proximate analysis and nutrient composition of medicinal plants were performed to check the effectiveness of selected medicinal plants against menstrual disorders. In addition to pharmacologically important, each medicinal plant species has its own nutrient composition. These nutrients are important for the physiological functions of human body. Such nutrients and biochemicals such as carbohydrates, fats and proteins play an essential role in satisfying human needs for energy

and life processes (Hoffman *et al.*, 1998; Mathews *et al.*, 1999; Dingman, 2002). Some of the medicinal plant species are being used alone but most of them are being used in combination with other plants.

Aims and Objectives

1. To conduct the Ethnobotanical survey and enlist the plants which are medicinally important in female specific diseases of Swat District and understand the indigenous people dependence on natural remedies.

2. To investigate the antimicrobial activities of the medicinal plant extracts against different bacteria and fungi causing female specific infections

3. To determine the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of plant extracts that proves to be prospective in initial screening.

4. To analyze the proximate composition of selected medicinal species.

Chapter No. 2

Literature Review

Literature Review

2.1: Literature Review on Ethnobotany

In Pakistan a lot of studies on ethnobotany have been made by (Hayat *et al.*, 2008; Jan *et al.*, 2008; Shah & Hussain; 2008; Wazir *et al.*, 2007; Ahmad *et al.*, 2003, 2006; Durrani *et al.*, 2006; Qureshi *et al.*, 2006; Zabihullah *et al.*, 2006; Sultana *et al.*, 2006; Badshah *et al.*, 2006; Hussain *et al.*, 2006; Arshad *et al.*, 2004; Saeed *et al.*, 2004). On some parts of Swat ethnobotany has also been reported (Hussain *et al.*, 1995; Hussain & Sher.1998; Sher *et al.*, 2003, 2004; Hussain *et al.*, 2004, 2005). Shinwari *et al.*, (2003) reported the useful medicinal and other useful plants of District Swat Pakistan.

Hazrat *et al.*, (2011) investigated the ethnobotanical importance of wild plants of Dir Kohistan Valley, Khyber Pakhtunkhwa, Pakistan. The main purpose of the study was to explore the wealth of ethnobotanicaly important medicinal plants. A total of 40 species, belonging to 25 families of wild herbs, shrubs and trees were found to be used by the local people in the valley to the treat various diseases.

Ali *et al.*, (2011) investigated economically important plants in various parts of Malam Jabba Swat. The main objective was to prepare an enthnobotanical inventory of plant resources in the area, as well as the conservation status of important medicinal and aromatic plants. Study on 90 species of ethnobotanically important have been documented, out of which 71 species were used as medicinal plants, 20 species as fodder plant, 10 species as vegetables, 14 species as wild fruit, 18 species as fuel wood, 9 species as furniture and agricultural tools, 9 species as thatching, fencing and hedges, 4 species as honey bee, 2 species as evil eyes, 2 species as religious and 3 species as poison. From this study improvement in the sound effects of resources misuse especially of medicinal and aromatic plants was suggested. Therefore such type of survey may helpful to understand local forest resources and the potential of medicinal and aromatic plants.

Sher *et al.*, (2010) studied the ethnopharmaceutically important medicinal plants of Shawar valley, Swat District. The investigation revealed that 87 plants species belonging to 58 families have ethnomedicinal importance. These include 50 dicotyledonous families, 3 monocotyledons families (Aliaceae, Iridacea and Poaceae), 2 gymnosperms families

(Pinaceae and Taxaceae) 2 Pteridophytes families (Polypodiaceae and Pteridaceae) and family Halvelaceae of fungi. These species were used for the treatment of various human pathogenic ailments in indigenous system of medicines. These species have been used for the curing of gastro-intestinal problems and antispasmodic.

Khan *et al.*, (2009) conducted an ethnobotanical survey in Bannu during 2007-2008 which indicated that 50 plant species are being used by local inhabitants for medicinal and other purposes. Poaceae and Moraceae are the largest families each with 5 species. The only fungus used as food was *Agaricus campestris*.

Khan *et al.*, (2007) also have reported some traditional and ethnobotanically important medicinal and aromatic plants of Buner, Swat and Chitral District. They investigated the plants for further intense use by scientist, pharmacologist and chemist. The results revealed 15 indigenous species which are of much importance and valuable mostly used by local inhabitants for diseases like muscular pain, cough, fever rheumatism and diabetes.

Ibrar *et al.*, (2007) collected ethobotanical information on 97 plant species which were classified for their therapeutic, traditional medicinal and economic uses from Ranyal Hills Shangla, District Pakistan. Many of these plants are being used to cure one or more human ailments. The results revealed that plants have medicinal uses as well as many other uses including 37 fuel species, 37 forage/fodder species, 31 medicinal species, 18 edible species, 12 species used for making shelter, 10 vegetables species, 9 poisonous species, 7 ornamental species, 6 timber wood species, 4 furniture wood species, 4 species used for fencing, 4 honey bee plants, 3 species for agricultural tools, 2 species used as flavoring agents, 2 species for making mats and baskets, 2 species used with religious belief, 2 species for cleaning teeth, 1 species as tea substitute, 1 fiber yielding species, 1 species as adhesive, 1 irritant species and 1 species for making pens.

A survey was conducted by Zabihullah *et al.*, (2006) of Kot Manzaray Baba Valley which revealed that local inhabitants use 82 plant species for different purposes which includes 52 medicinal species (65%), 16 fuel species (20%), 11 fodder species (12%), 5 honeybee species (6%), 7 fruit species (8%), 8 timber species (9.80%), 6 potherb species

(7%). For snake bite *Arisaema jacqumontii* is traditionally used as antidote. Other uses of plants include making of agricultural tools, furniture wood, hedges, game tools, fencing and thatching. Due to deforestation and overgrazing vegetation of the area is under severe biotic pressure.

2.2: Literature Review on Antimicrobial Activities

Shandesh and Dinesh (2011) reported the antimicrobial activities of the six extracts (two each of methanol, chloroform and hexane) of leaves and flowers of *Woodfordia fruticosa* against 14 microorganisms by disk diffusion method. Among six extracts examined, 66% extracts exhibited antimicrobial properties against *Bacillus subtilis*, 50% extracts against *Staphylococcus aureus, Salmonella Typhi, Salmonella paratyphi, Citrobacter frendii* 33% extracts against pathogenic strains including *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Shigella dysenteriae* and 16% extracts against *Enterobacter* species. *Acenitobacter* species. Two human pathogenic fungi, *Candida albicans* and *Aspergillus* species. showed inactivity against the extracts tested.

Joshi *et al.*, (2010) assayed *in vitro* antibacterial against used four plants employing cup diffusion method. All the plants were ineffective against *E. coli* and *K. pneumonia*. *Achyranthes bidentata* was found to be ineffective against all the tested organisms. *E. caryophyllata* produced largest zone of inhibition (22 mm) against *S. typhi* and minimum bactericidal concentration (MBC) value of 5 mg/l was obtained with *Azadirachta indica* against *S. typhi*. *K. pneumoniae* and *E. coli* were found to be resistant with all the plant extracts.

Keskin *et al.*, (2010) investigated the antimicrobial activities in 10 Turkish medicinal plants against different pathogenical strains by using well diffusion method. The extracts of *Alchemilla officinalis, Hibiscus* species, *Melissa officinalis, Silybum marianum, Camellia sinenses, Rosmarinus officinalis* and *Foeniculum vulgare* showed broad spectrum of antibacterial activities by forming zones of inhibition ranging from 4-32 mm except *Erica vulgare*. The most resistant microorganism was *E. coli*.

Aqueous, methanol and chloroform extracts from the leaves of *Ficus religiosa, Thespesia populnea* and *Hibiscus tiliaceus* for antibacterial and antifungal activities was

screened by Hemaiswarya *et al.,* (2009). Zone of inhibition of 10 to 21 mm was produced by the chloroform extract of *F. religiosa* at very low MIC values. Against bacterial strains the moderate antibacterial activitie was possessed by methanolic extracts. Using of aqueous extracts showed less antibacterial activity or inactivity. Against *Aspergillus niger* and *Penicillium notatum* the extracts of *F. religiosa* were found to be sensitive. The extracts of the leaves of selected plants exhibited significant and variable inhibitory effects against the pathogenic strains tested.

Parekh and Chanda (2007b) have screened 34 medicinal plants, belonging to 28 different families, for potential of antibacterial activities against six bacterial pathogenic strains. The methods which have been used in this study were agar disc diffusion and agar well diffusion. The most active extracts have been ethanol/methanol than aqueous extracts for all the plants studied. *K. pneumoniae* was found the most susceptible bacterium, while *S. typhimurium* and *E. coli* were the most resistant bacteria. *Woodfordia fruticosa* Kurz, showed encouraging results of antibacterial activities.

Ten medicinal plant species were screened for antibacterial activity against pathogenic bacterial strains by Nair and Chanda (2007). Aqueous and ethanol extracts were screened for antibacterial activity by using agar disc diffusion and agar well diffusion methods. The most resistant strains were *P. aeruginosa* and *S. typhimurium* while the most susceptible bacterial strains were *B. cereus*, *P. mirabilis* and *E. officinalis* showed strong inhibiting activity against all the microorganisms tested.

Çıkrıkçı *et al.,* (2008) worked on turmeric and curcumin isolation and biological assessment against standard bacterial and mycobacterial strains such as *E. coli, S. aureus, E. feacalis, P. aeruginosa, M. smegmatis, M. simiae, M. kansasii, M. terrae, M. szulgai* and the fungi *C. albicans*. For the turmeric extracts and pure curcumin antibacterial and antifungal activities have been determined. Inactivity against the gram-negative bacteria *E. coli* and *P. aeruginosa* was showed by the extracts and curcumin. Little activity was showed by the isolated turmeric extracts and pure curcumin against the studied mycobacteria.

Panthi and Chaudhary (2006) studied 18 plant species used in folklore medicine in west Nepal for their antibacterial activities by the disk diffusion method. The tested bacteria

used include *S. aureus*, *E. coli*, *P. aeruginosa* and *S. boydii*. Extracts of 8 plants showed encouraging result against three bacterial strains while other showed activities against 1 or 2 strains. Present findings support the traditional knowledge of local users.

Nair *et al.*, (2005) screened 9 plant species i.e. *Sapindus emarginatus, Hibiscus rosa-sinensis, Mirabilis jalapa, Rheo discolor, Nyctanthes arbortristis, Colocasia esculenta, Gracilaria corticata, Dictyota* spps. and *Pulicaria wightiana* for antibacterial activities against selected 6 bacterial strains, *P. testosteroni, S. epidermidis, K. pneumoniae, B. subtilis, P. morganii,* and *M. flavus* by using agar disk diffusion and Agar ditch diffusion method. *P. testosteroni* and *K. pneumoniae* were found the most resistant bacterial strains. *S. emarginatus* showed broad degree of antibacterial activities against the bacterial strains tested.

Sakar *et al.*, (2005) found ethyl acetate extract of *Q. aucheri, Jaub* and Spach was found effective against *Candida* species. Voravuthikunchai *et al.*, (2004) found ethanolic extract of *Q. infectoria* having very good activities against enterohaemorrhagic *E. coli* strains.

2.3: Literature Review on Proximate Analysis

Hussain *et al.*, (2011) conducted a study of 4 medicinal plants namely *Aerva javanica, Calotropis procera, Datura alba,* and *Nepeta suavis* traditionally used as medicine by local people in the northwest Pakistan. They investigate the nutritional value and mineral contents of the selected plant species. The results conducted from proximate analysis of plant sample determines that protein and ash contents were highest in *Datura alba* i.e. (21.353%) and (18.803%), carbohydrate content (70.123%) in *Aerva javanica.* The energy (398.496 K cal/100 g), fats (12.595%) and fibre (40.150%) were reported highest in *Nepeta suavis,* while in *Calotropis procera* moisture conten (11.255%) was highest. The overall results revealed that *Nepeta suavis* is the most important species having higher concentrations of fat, fiber and energy values compared to the other species. Atomic Absorption Spectrometric method have been used for analyzing essential elements such as Fe, Cd, Cu, Mn, Pb, Cr, Mg and Na from the medicinal plants in variable range.

Ogbe *et al.*, (2011) conducted study on the leaves of *Moringa oleifera* for proximate and mineral analysis. The results of proximate analysis revealed the presence of high crude

protein (17.01% ± 0.1) and carbohydrate (63.11% ± 0.09). The leaves also contained appreciable amounts of crude fiber (7.09% ± 0.11), ash (7.93% ± 0.12), crude fat (2.11% ± 0.11) and fatty acid (1.69% ± 0.09). The total minerals showed were Ca (1.91% ± 0.08), K (0.97% ± 0.01), Na (192.95 ± 4.4), Fe (107.48 ± 8.2), Mn (81.65 ± 2.31), Zn (60.06 ± 0.3) and P (30.15 ± 0.5) parts per million (ppm). The magnesium (0.38% ± 0.01) and copper (6.10% ± 0.19) contents were the least. The presence of these essential nutrients and minerals implies *Moringa oleifera* leaves from Lafia, Nasarawa State could be utilized as a source of feed supplement to improve growth performance and health status of poultry. The benefits of essential nutrients and minerals in maintaining good health were also highlighted in this study.

Adnan *et al.*, (2010) studied proximate composition and nutrient contents of five medicinal plant species collected from northwest Pakistan. The plants *Bupleurum falcatum* and *Valeriana officinalis* belongs to humid regions, while *Forsskalea tenacissima, Lavandula angustifolia* and *Otostegia limbata* belongs to sub-humid regions. By following methods of Association of Official Analytical Chemist proximate analysis (total protein, fats, carbohydrate, ash, and moisture contents) were carried out. Macronutrients (Ca, Mg, Na, K) and micronutrients (Fe, Cu, Pb, Zn, Ni, Cr, Co, Mn) were also analyzed by using Atomic Absorption Spectrometric method. Results showed higher concentration of macronutrients in *F. tenacissima* and micronutrients in *O. limbata*. From the results it was concluded that sub-humid region's species are having higher nutritional value than humid region species.

Hussain *et al.* (2009) studied the proximate parameters and micronutrients composition of fourteen medicinal plant species of different areas of Pakistan and compared these plants with medicinal plants used in Mussafeen and Itreeful ustokhudus which are the two herbal products made by Qarshi Industry Pvt Ltd. The results showed that proximate composition of each medicinal plant used in herbal products were different with the exception of *Fumaria offcinalis* which have higher to moderate values of carbohydrates, ash, fat and protein contents as compared to other plant species used in the herbal formulations. The results obtained from proximate analysis of herbal products revealed that Mussafeen yield high value of fats, ash, proteins, and fibers as compared to Itreeful ustokhudus. The elemental analysis showed that Co, Zn, Fe, Cd, Ni and Pb percentage was higher in *Sphaeranthus hiritus, Fumaria offcinalis* and *Cuscuta reflexa* as compared to other materials

in the herbal products. The herbal product Itreeful ustokhudus has highest composition of almost all nutrients namely Cu, Zn and Cd, while Fe, Pb and Ni showed highest concentration in herbal product Mussafeen. The result of present study showed that the concentrations which were found are however below the standards mentioned by the WHO.

Chapter No. 3

Materials and Methods

Materials and Methods

The research work was carried out in the Laboratory of Molecular Systematics and Applied Ethnobotany, Quaid-i-Azam University, Islamabad. *In vitro* antimicrobial activities were investigated using some selected medicinal plant species. Proximate composition (moisture, ash, fiber, crude fats, proteins and carbohydrates) were also carried out.

3.1: Ethnobotanical survey

An ethnobotanical survey and literature review of medicinal plants used in the treatment of gynecological disorders was c arried out among the rural people in Swat District. A large variety of plants which were used as ethnomedicinally against feminine disorders have been reported but uses of folk medicines in large number all over the world have also remained endemic to certain tribal areas.

3.1.2: Plants collection

Twelve different plant species i.e. *Artemisia vulgaris, Azadirach indica, Berberis lycium, Curcuma longa, Erythrina variegate, Ficus religiosa, Hibiscus rosa-sinensis, Quercus dilatata, Trachyspermum ammi, Valeriana jatamansii, Woordfordia fruticosa and Zanthoxylum alatum* were collected from different areas of Swat. These plant species were identified by plant taxonomist. Under running tap water fresh plant materials (whole or parts) were washed, air dried and then ground into fine powder with electric blender (Geepas, Model no GCG 289). The powder of each plant was stored in airtight bottles until required for further analysis. The powdered samples were also processed for proximate analysis Table **3.1**.

Table 3.1. *Plants with their correct nomenclature were arranged alphabetically by botanical name following vernacular name, parts used and Ethnomedicinal uses.*

Sr.no	Botanical name	Local name	Parts used	Family	Ethnomedicinal uses
1	*Artemisia vulgaris*	Chaagu	Leaves	Asteraceae	Leaves are helpful in suppressed menses. Young women take syrup before and after the full moon by just starting menses. It is also used for insomnia and nervousness kills parasitic worms internally.
2	*Azadirach indica*	Neem	Leaf and seeds	Meliaceae	A plant is used to control irregular periods and as a contraceptive. The extracts of leaves are used to stop excessive menstrual bleeding. Seed oil is helpful if applied externally in uterus as a contraceptive before copulation.
3	*Berberis lycium*	Kwaray, kashmal	Root	Berberidaceae	The dried roots bark in powder form is used as tonic in rephorlogical complaints. It is used as astringent in gynecological disorders and also used in jaundice.
4	*Curcuma longa*	Tumeric or curcumin	Rhizome	Zingiberaceae	It is used in the powdered form and mixed with milk for the relief of menstrual pain
5	*Erythrina variegate*	Flame trees	Leaves	Fabaceae	To cure irregular periods red flower of the plant (four or five no.) fried with desi ghee should be taken in the morning daily.
6	*Ficus religiosa*	Peepal	Wood, bark and fruit	Moraceae	The paste of the bark (10 g) taken with water (one glass) twice daily for one month is helpful to cure white discharge.
7	*Hibiscus rosa-sinensis*	Shoe flower	Flower	Malvaceae	For about two-three months white flower of the plant (five no.) taken in the morning in empty stomach to cure white discharge. Red flower of the plant (four-five no.) fried with desi-ghee taken in the morning daily to treat irregular periods.
8	*Quercus dilatata*	Toor banj	Fruit	Fagaceae	The fruits in the powdered form are used to treat gonorrhea and urinary tract infections.
9	*Trachyspermum ammi*	Ajwain	Seeds	Apiaceae	Herb is used to control menstrual discharge. Seeds in powder form mixed with sugar and butter are taken orally for 3 days once daily to normalize menstrual discharges. To clear uterus and regulate menstrual cycle after birth this recipe is also used.
10	*Valeriana jatamansii*	Murma	Stem and roots	Papilionaceae	Root juice of *Valeriana jatamansii* is applied internally for the treatment of painful menstruation, hypertension, cramps and irritable bowel syndrome.
11	*Woordfordia fruticosa*	Dhawai	Flower	Lythraceae	Flowers of *Woodfordia fruticosa* in the powder form (half spoon) are mixed with honey and taken daily during menstrual period.
12	*Zanthoxylum alatum*	Laighunay timber	Shoots and roots	Rutaceae	The powder fruit of *Zanthoxylum alatum* is used to treat gonorrhea and urinary tract infections.

3.1.3: Sample preparation

In solvent extraction the shade dried powder (5 g) was mixed with 500 ml of methanol in a conical flask and kept it for 24 h. After 24 h, extracts were filtered through Whatman no 1 filter paper and centrifuged at 5000 g for 10 min. The filtrates were dried until a constant dry weight of each extract was obtained. The supernatant was collected and the solvent was evaporated through rotary evaporator.

3.1.4: Microorganisms Tested

A total of 5 microbial cultures were investigated during this study. Three gram-negative bacterial strains *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* along with one gram- positive bacterial strain *Staphylococcus aureus*, were used to check the antibacterial potential of the plant extracts. One fungal strain *Candida albicans* was also used to assess antifungal properties. The identified microorganisms were obtained from Pakistan Institute of Medical Sciences (PIMS). On nutrient agar slope the organisms were maintained at 4 °C and sub-cultured before use.

3.1.5: Requirements

Test samples, Muller Hinton agar, nutrient broth, McFarland Barium Sulphate turbidity standard, cultures of microbial (bacterial and fungal) strains, sterile normal saline solution (0.9 % NaCl w/v), organic solvent DMSO, standard antibiotic disks Amphicillin, spirit lamp, laminar flow hood, autoclave, incubator (37 °C), test tubes, falcon tubes, sterile cork borer, micropipettes, Petri plates (small and large).

3.1.6: *In vitro* Antimicrobial assay

The antimicrobial activities was determined by agar well diffusion method (Irobi *et al.*, 1994; Shinwari *et al.*, 2009).

3.1.7: Preparation of sample dilution

The extract (5 mg) was dissolved in 1 ml of dimethyl sulfoxide (DMSO) and was allowed until completely dissolved. The stock solution was again diluted and 5 mg/ml

concentration of extract was prepared. Antibiotics discs were used as positive controls and DMSO as a negative control.

3.1.8: Preparation of media

Muller Hinton agar (MHA) and nutrient agar was used for *in vitro* antimicrobial activity. The Muller Hinton agar medium was prepared by dissolving 3.8g agar in 100 ml of distilled water. Nutrient agar was prepared by dissolving 2.0 g in 100 ml of distilled water.

3.1.9: Preparation of McFarland turbidity standard

By adding specific volumes of 1% sulfuric acid and 1.175% barium chloride McFarland was prepared. In this study McFarland 0.5 standards were used which contained 99.5 ml of sulphuric acid (1%) and 0.5 ml of barium chloride (1.175%). Solution was dispensed into tubes comparable to those used for inoculums preparation. The McFarland 0.5 standard provides turbidity comparable to a bacterial suspension containing 1.5×10^8 cfu/ml (NCCLS, 2002).

3.1.10: Preparation of saline

Sodium chloride (9g) was dissolved in 100 ml of distilled water for the preparation of solution.

3.1.11: Preparation of inoculums

Using sterilized wire loop, touch 3-5 well isolated colonies (twenty-four hours old cultures) of test organisms and emulsified in 3-4 ml of physiological normal sterile saline solution until by adjusting it to turbidity; equivalent of 0.5 McFarland standards at which the number of cells is assumed to be 1.5×10^8 cfu/ml.

3.1.12: Preparation of seeded agar plates

In petri plate (20 x 90 mm) Muller Hinton agar medium was poured and allowed to solidify for 5 min and were incubated at 37 °C for 24hrs in incubator to check contamination. Using a sterile cotton swab Muller Hinton agar plate was inoculated. Cotton swab was dipped in the prepared inocula and excess fluid was removed by pressing the swab against the side of

the tube above the level of suspension. In three dimensions the cotton swab was streaked evenly over the surface of the medium rotating the plate to ensure even distribution.

3.1.13: Pouring of test solutions; incubation and measurement of zone of inhibition

Sterile cork borer was used for making the wells of about 8 mm in diameter in the agar plates. Plant extract of 50 µl was loaded in each well with the help of micropipette. With the help of sterilized needle antibiotics disc ampicillin (AMP) were placed on the inoculated plate about15 mm from the edge of the plate. To ensure its contact with the agar the disc was pressed lightly down. The disc was not moved once place and was incubated at 37 °C for 24 hrs. Using a ruler on the underside of plate the diameter of each zone of inhibition in mm was measured and the results were recorded.

3.1.14: Determination of the minimum inhibitory concentration (MIC)

The MIC of the crude extracts was determined by using the method prescribed by Akinpelu and Kolawale (2004). Extracts of the plants (5 mg/ml) were poured into nutrient broth in test tubes and this concentration of 5 mg/ml was taken as the initial. Four tubes of 2 ml nutrient broth were set up and 2 ml of 5 mg/ml of the extract was taken. Two-fold dilution was used for the four tubes of nutrient broth forming concentrations of 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml and 0.3125 mg/ml. Normal saline was used for the preparation of suspensions of the pathogenic strains. If the bacterial suspension is not of the same density as the McFarland 0.5, the turbidity can be adjusted by adding sterile saline or by adding more bacterial colonies. The turbidity was then compared with 0.5 McFarland's standard by visual comparison. At that point the cells were assumed to be 1.5×10^8 cfu/ml. The cell suspension of about 0.1 ml was then inoculated into each tube and the tubes were incubated at 37°C for 24 hrs in incubator. The lowest concentration of the extract at which no growth of the microbes was observed was taken as the minimum inhibitory concentration (MIC).

3.1.15: Determination of the minimum bactericidal concentration (MBC)

Following the Spencer and Spencer (2004) method the minimum bactericidal concentration (MBC) of the plant extract against the pathogenic microbes was determined. The tubes which have low MIC value were sub-cultured on fresh nutrient agar medium. The

plates were then incubated at 37 °C for 24 hrs. The MBC was taken as the lowest concentration of the extract that showed no growth on the nutrient agar medium.

3.2: Proximate analysis

The total protein, fat, carbohydrate, moisture and ash of the plant sample were determined by proximate analysis and were reported as the percentage of the product. By using mortar and pestle all the samples were crushed into powdered form and these samples were used for proximate analysis. Following Association of Official Agricultural Chemists (AOAC) proximate analysis of the samples for moisture, ash, crude fibers, crude fats, proteins and carbohydrates were carried out. Micro Kjeldahl method described by Pearson, (1976) nitrogen was determined. The nitrogen content was converted to protein by multiplying by a factor of 6.25. The total Carbohydrate content was determined by difference method and all the proximate values were determined in percentage (AOAC, 2000; AOAC, 2003).

3.2.1: Determination of Moisture

In a petri dish (3g) of the selected plant material was taken and placed in the oven at 105 °C for 12 hrs (Fig. 1). It was then removed, cooled in a desicator and weighed. The sample in the oven was heated again for another two hours and the process was repeated, untill a constant weight was achieved. By using the following formula the moisture content was calculated. The process was repeated two times more for getting a more precise data. The apparatus used in the moisture determination were oven, desiccators and analytical balance (AOAC, 2000, AOAC, 2003).

Calculation

$$\text{Moisture Content (\%)} = \frac{(B-A) - (C-A)}{(B-A)} 100$$

Where

$W_1 = A$ = weight of clean, dry petri dish (g)

$w_2 = B$ = weight of petri dish + wet sample (g)

$w_3 = C$ = weight of petri dish + dry sample (g)

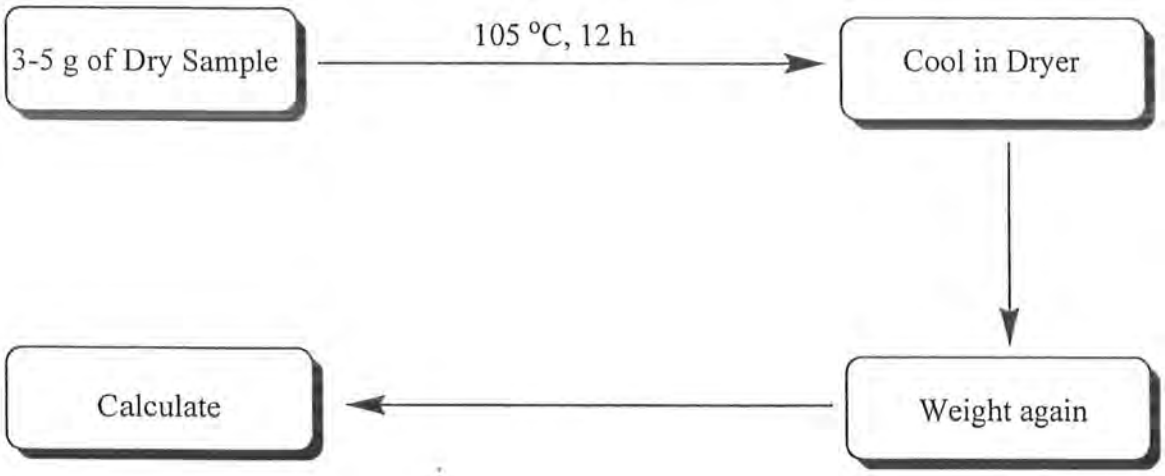

Fig 1: Diagrammatical presentation of humidity content in feed ingredients

3.2.2: Determination of Ash

In a crucible dried sample (1 g) was taken and was charred over a low flame. The crucible was then kept in a muffle furnace set at 550 °C and left until white ash was obtained. The ash obtained was moistened with water, dried on steam and then on hot plate. The crucible was again placed in the muffle furnace at 550 °C,untill a constant weight was obtained. The resultant ash was calculated by the formula. The apparatus used in the ash determination were muffle furnace, hot plate, and desiccators (Awan and Salim, 1997; AOAC, 2000; AOAC, 2003; Okwu and Morah, 2004).

Calculations:

$$\text{Ash (\%)} = \frac{W_1 \times 100}{W_2}$$

W_1 = Weight of sample after ashing

W_2 = Weight of sample

3.2.3 Crude Fat

In this method, using petroleum ether, fats were extracted from the sample and evaluated as a percentage of the weight before the solvent was evaporated.

Method

First of all extraction flasks from the kiln were taken without touching them with the fingers then cool in a dryer and weighed (mg). After weighing 3 to 5 g of dry sample (mg) in an extraction thimble, handling it with tongs and placed in the extraction unit. Connected the flask containing petroleum ether at 2/3 of total volume to the extractor. Than boiled and adjust heat to obtain about 10 refluxes per hour (r/h) (Fig. **2**). When finished, the petroleum ether was evaporated by distillation or in a rotavapor. The flasks were cooled in a dryer and weighed again. The extraction length will depend on the quantity of lipids in the sample. Very fatty materials will take 6 hours and the defatted sample can be used in determining crude fibers. The apparatus used in the determination of fat were heating mantle, soxhlet extraction, desiccators and oven (Awan and Salim, 1997; AOAC, 2000; AOAC, 2003; Okwu and Morah, 2004).

Calculations

$$\text{Crude lipid content (\%)} = \frac{(B-A)}{C} 100$$

$A = W_1$ = weight of clean dry flask (g)

$B = W_2$ = weight of flask with fat (g)

$C = W_3$ = weight of sample (g)

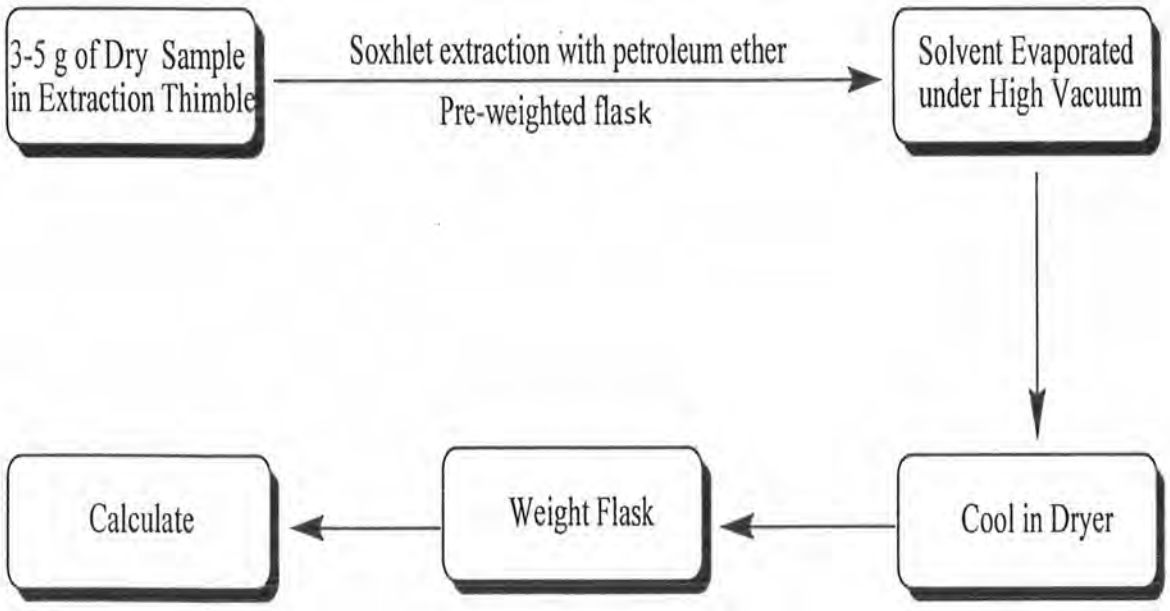

Fig 2: Diagrammatical presentation of lipids by soxhlet's method

3.2.4: Determination of Crude Fiber

The defatted plant material (1 gm) was taken. In a beaker and the sample was then placed and boiled in 200 ml of sulphuric acid (1.25%) for half an hour. The contents were filtered through linen cloth in the fluted funnel and washed with distilled water to neutralize the contents. After filtration the contents were transferred to the beaker and boiled in 200 ml of sodium hydroxide (1.25%) for half an hour. After that, contents were again filtered, washed with distilled water for neutralization. A gooch crucible was prepared with an asbestos mat and contents of beaker were placed on the mat and washed with of ethyl alcohol (15 ml).

The crucible was dried in an oven at 110 °C to a constant weight. The crucible having crude fiber was cooled and weighed (W_1). The apparatus used in the determination of fiber were muffle furnace, oven and suction pump. The contents of the crucible were ignited over a low flame until charred and then kept in a muffle furnace at 550 °C and weighed (W_2) (Awan and Salim, 1997; AOAC, 2000; AOAC, 2003; Okwu and Morah, 2004).

Formula

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2 \times 100}{W_3}$$

Where

W_1 = Weight of sample before ignition

W_2 = Weight of sample after ignition

W_3 = Weight of sample

3.2.5: Determination of Protein by Micro Kjeldahl Method

The protein determination is divided into three steps:

1. Digestion
2. Distillation
3. Titration

3.2.1. Digestion

In a digestion flask 0.5 g of dried plant material was taken. About 1 g of the digestion mixture (copper sulphate, potassium sulphate and ferrous sulphate, 1:18:0.2) and about 20 ml of concentrated sulphuric acid was added. The solution was heated until the solution became clear and frothing ceased. Solution was further boiled for another 2 hrs.cooled and about 50 ml water was added in 5 ml portion with mixing to the digest. The digest was transferred to a 100 ml volumetric flask and made the volume up to the mark (Awan and Salim, 1997; AOAC, 2000; AOAC, 2003; Okwu and Morah, 2004).

3.2.2. Distillation

For distillation Parnas Wagner distillation assembly was arranged. About 100 ml of 4% boric acid was used and 1 drop of methyl red indicator was added, and pink color appeared. Five milliliter of the digest was transferred to the distillation assembly and 10 ml of 50% sodium hydroxide solution was added to the digest in the assembly. The distillation was completed in 6 min, and the color of boric acid change to yellow due to the formation of ammonium borate.

3.2.3. Titration

The boric acid was titrated with 0.1 N hydrochloric acid. The boric acid having ammonia changed again to pink from yellow. The percent protein was calculated by the formula. The instrument used in the determination of protein was micro Kjeldahl, burette, hot plate and digestion.

Calculations

$$\% \text{ protein} = \frac{[(\text{volume used for sample} - \text{volume used for blank}) \times 1.4007 \times \text{E.N})]}{\text{Weight of sample taken}} \times 6.25$$

Where:

1.4007 = Weight of nitrogen expressed in gram in the formula

6.25 = Protein factor

E.N = Exact Normality

3.2.6: Determination of Carbohydrate

By using the following formula Carbohydrate was determined by difference method.

Formula

Carbohydrate (in grams) = 100 - (% Moisture + % crude fat + % Ash + % protein)

3.2.7: Determination of Energy Values

Energy values were determined by means of the following formula,

Formula:

K calories / 100 gm: [(9 x % crude fats) + (4 × % carbohydrates) + (4 × % proteins)].

Chapter No. 4

Results

Results

Literature review and ethnobotanical survey of twelve plant species namely *Artemisia vulgaris, Azadirach indica, Berberis lycium, Curcuma longa, Erythrina variegate, Ficus religiosa, Hibiscus rosa-sinensis, Quercus dilatata, Trachyspermum ammi, Valeriana jatamansii, Woordfordia fruticosa and Zanthoxylum alatum*) were collected and screened against five pathogenic strains (3 gram-negative bacterial strains *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* and 1 gram-positive bacterial strain *Staphylococcus aureus* along with 1 fungal strain *Candida albicans*). The selected medicinal plants were also analyzed for proximate parameters (i.e. fat, carbohydrate, protein, ash, moisture, energy and fiber) by using Association of Official Agricultural Chemists method (AOAC, 2000, AOAC, 2003).

4.1: Antimicrobial Activity

4.1.1: Antifungal Activity against *Candida albicans*

The results revealed that out of twelve plants, seven plants exhibited antimicrobial activities against one or more of the selected strains using concentration of 5 mg/ml. The highest antifungal activity was observed for methanol extract of *Woodfordia fruticosa* (27.086 ± 2.970) followed by *Berberis lyceum* (22.36 ± 1.018), *Quercus dilatata* (21.97 ± 2.632), *Azadirach indica* (14.46 ± 0.546), *Ficus religiosa* (12.88 ± 0.265). The lowest zone of inhibition at 5 mg/ml was observed for *Erythrina variegate* (11.66 ± 0.299) against *Candida albicans*. The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) value ranged from 5-0.3125 mg/ml respectively. *Berberis lyceum* was found to have low MIC and MBC value of 0.625 and 0.3125 mg/ml respectively. (Fig **4.1**).

4.1.2: Antibacterial Activity against *Escherichia. coli*

The methanolic extracts of *Woodfordia fruticosa* and *Quercus dilatata* at a concentration of 5 mg/ml showed significant inhibition against *E. coli* where the highest zone of inhibition was noted (13.55 ± 1.130) and (11.22 ± 1.045).respectively. The MIC and MBC value of *Woodfordia fruticosa* were 5 and 2.5 mg/ml respectively. The MIC and MBC value of *Quercus dilatata* were 2.5 and 1.25 mg/ml respectively. (Fig **4.2**).

4.1.3: Antibacterial Activity against *Staphylococcus aureus*

Only four plant extracts showed promising results against *S. aureus*. The methanolic extract of *Woodfordia fruticosa* showed (13.29 ± 0.550) highest zone of inhibition while *Curcuma longa* (12.7 ± 1.031), *Quercus dilatata* (8.67 ± 0.529) and *Azadirach indica* showed (7.96 ± 0.314) zone of inhibition at a 5 mg/ml concentration. *Woodfordia fruticosa, Curcuma longa, Quercus dilatata* and *Azadirach indica* showed an analogous MIC and MBC values of 5 and 2.5 mg/ml respectively. (Fig **4.3**).

4.1.4: Antibacterial Activity against *Klebsiella pneumoniae*

Only two plants showed high degree of inhibition against *K. pneumoniae*. The methanolic extracts of *Woodfordia fruticosa* showed (10.48 ± 0.660) and *Quercus dilatata* showed (8.846 ± 0.853) zone of inhibition at 5 mg/ml concentration against *K. pneumoniae. Woodfordia fruticosa* and *Quercus dilatata* extracts showed comparable MIC and MBC values of 5 and 2.5 mg/ml respectively. (Fig **4.4**).

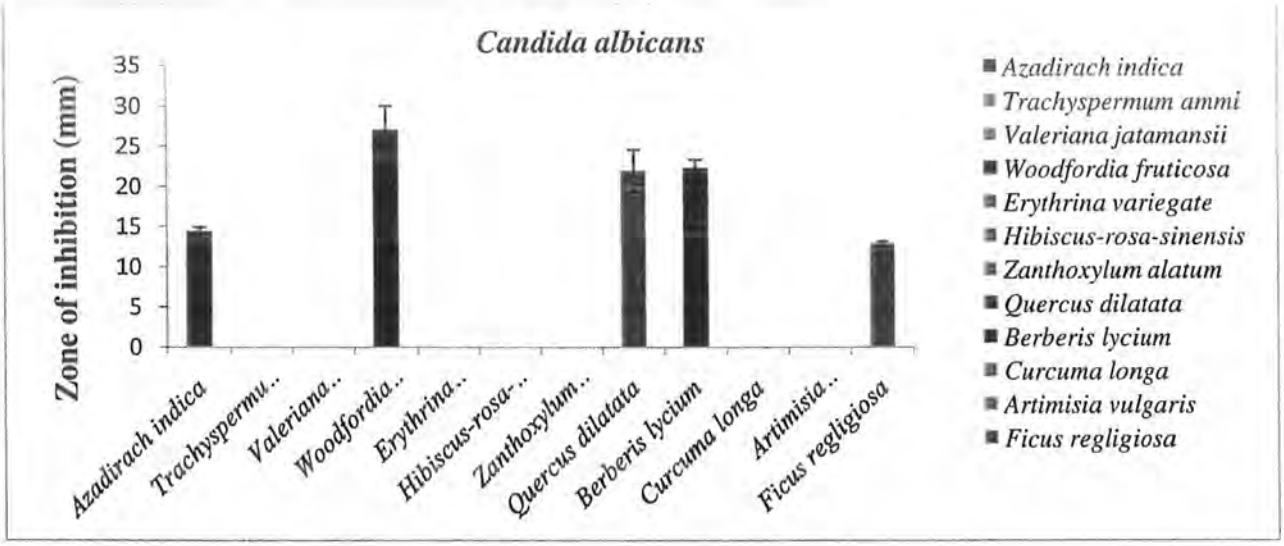

Fig: 4.1. Antifungal Activity of various medicinal plants against *C. albicans* at 5 mg/ml concentration.

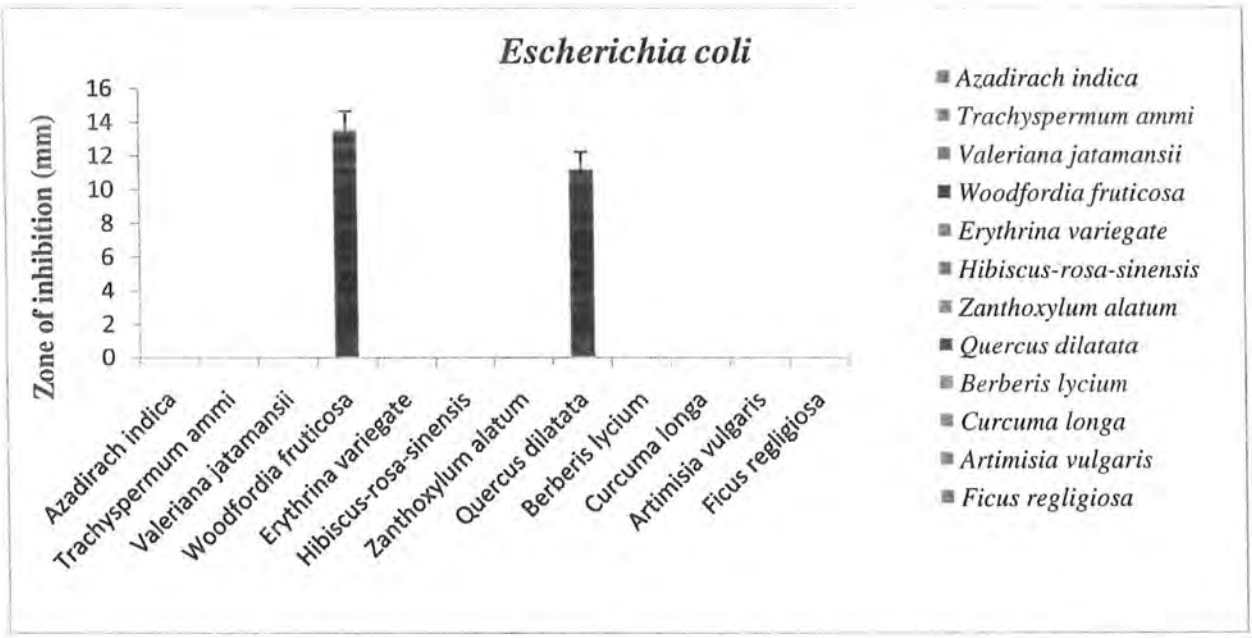

Fig: 4.2. Antibacterial Activity of various medicinal plants against *E. coli* at 5 mg/ml concentration.

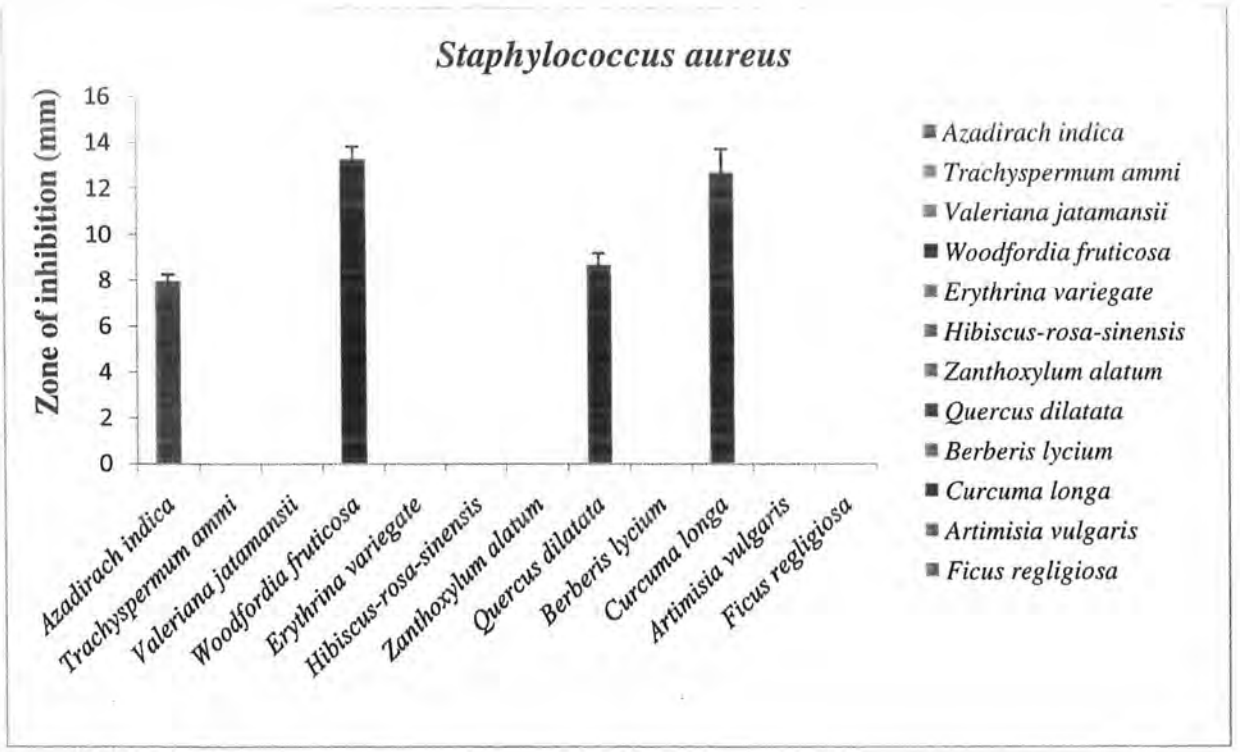

Fig: 4.3 Antibacterial Activity of various medicinal plants against *S. aureus* at 5 mg/ml concentration.

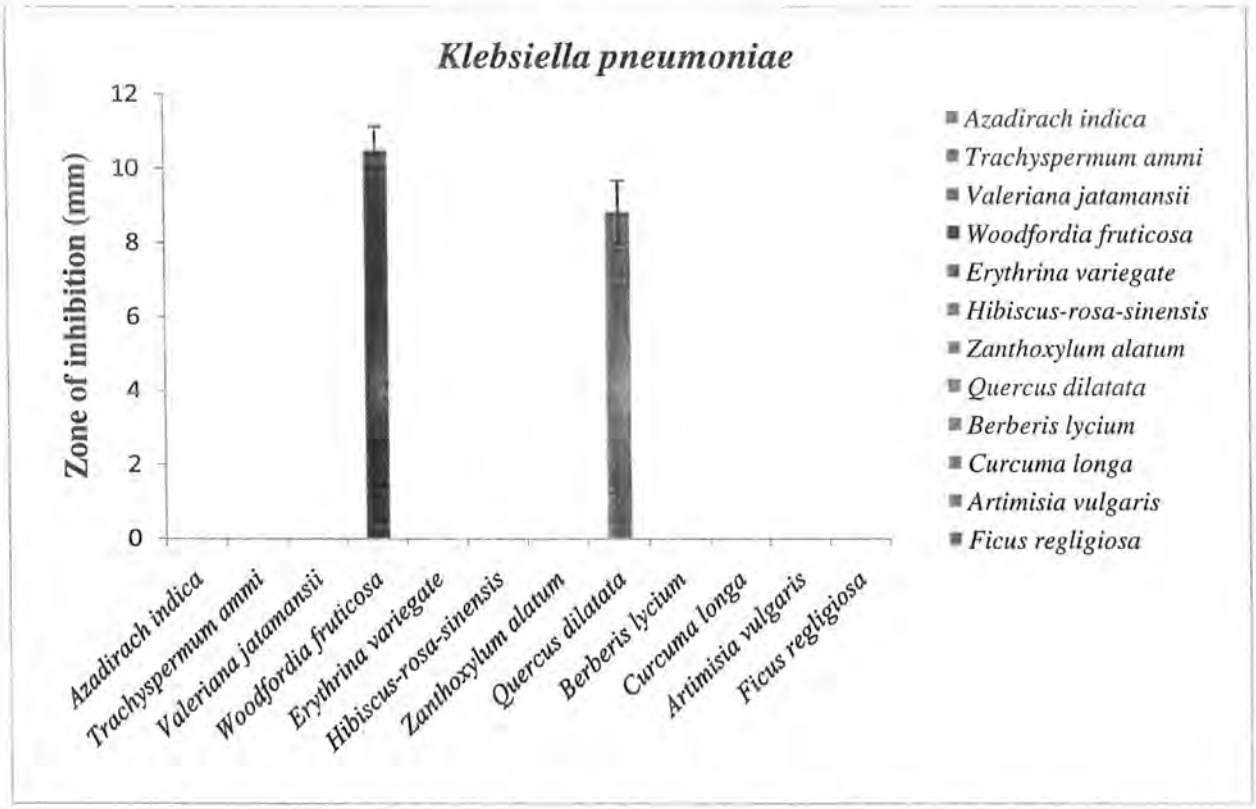

Fig: 4.4 Antibacterial Activity of various medicinal plants against *K. pneumoniae* at 5 mg/ml concentration.

4.2: Proximate Analysis

4.2.1. Moisture Contents

The highest moisture contents was observed in *Hibiscus rosa-sinensis* (11.424 ± 0.6900) followed by *Zanthoxylum alatum* (8.577 ± 0.7684), *Erythrina variegate* (6.813 ± 0.6060), *Curcuma longa* (6.541 ± 0.7541), *Quercus dilatata* (6.298 ± 0.3021), *Artemisia vulgaris* (6.76 ± 0.5770), *Azadirach indica* (4.724 ± 0.0375), *Ficus religiosa* (4.700 ± 0.5174), *Berberis lycium* (4.233 ± 0.1527), *Trachyspermum ammi* (3.390 ± 0.3699) and *Woordfordia fruticosa* (3.146 ± 0.I404). The lowest moisture contents were observed in *Valeriana jatamansii* (2.483 ± 0.0429) (Fig **4.5**).

4.2.2. Ash Contents

The highest percentage of Ash contents was present in *Valeriana jatamansii* (37.29 ± 0.2607) followed by *Woordfordia fruticosa* (28.441 ± 0.1860), *Azadirach indica* (17.432 ± 0.1905), *Trachyspermum ammi* (17.432 ± 0.1905), *Quercus dilatata* (7.629 ± 0.1279), *Ficus*

religiosa (6.805 ± 0.6164), *Artemisia vulgaris* (5.8 ± 0.1516), *Curcuma longa* (4.078 ± 0.2407), *Zanthoxylum alatum* (3.979 ± 0.2371), *Hibiscus rosa-sinensis (2.881 ± 0.1579), Berberis lycium* (2.8511 ± 0.5751). *Erythrina variegate* (2.484 ± 0.2889) showed the lowest Ash contents (Fig **4.6**).

4.2.3 Fat Contents

The ranking of fat contents in all the selected medicinal plants were *Zanthoxylum alatum* (19.799 ± 0.1741), *Quercus dilatata* (7.796 ± 0.025), *Azadirach indica* (7.732 ± 0.0668), *Woordfordia fruticosa* (7.632 ± 0.03), *Erythrina variegate* (6.0288 ± 0.01247), *Valeriana jatamansii* (5.409 ± 0.0484), *Hibiscus rosa-sinensis* (4.788±0.1504), *Trachyspermum ammi* (4.642 ± 0.010), *Berberis lycium* (3.818± 0.06133), *Artemisia vulgaris* (2.62 ± 0.0596), *Curcuma longa* (2.605 ± 0.0559) and *Ficus religiosa* (2.5± 0.0769) (Fig **4.7**).

4.2.4 Fiber Contents

The order of percentage composition of fiber contents was observed to be *Trachyspermum ammi* (33.892 ± 0.0251), *Hibiscus-rosa-sinensis* (32.62 ± 1.5291), *Artemisia vulgaris* (32.486 ± 0.109), *Erythrina variegate* (18.76 ± 0.924), *Curcuma longa* (12.673 ± 0.1713), *Valeriana jatamansii* (9.879 ± 0.1075), *Quercus dilatata* (7.465 ± 0.7203), *Azadirach indica* (4.451 ± 0.0996), *Ficus religiosa* (2.084 ± 0.001), *Zanthoxylum alatum* (1.752± 0.0776), *Berberis lycium* (1.44± 0.508) and *Woodfordia fruticosa* (0.786 ± 0.0095) (Fig **4.8**).

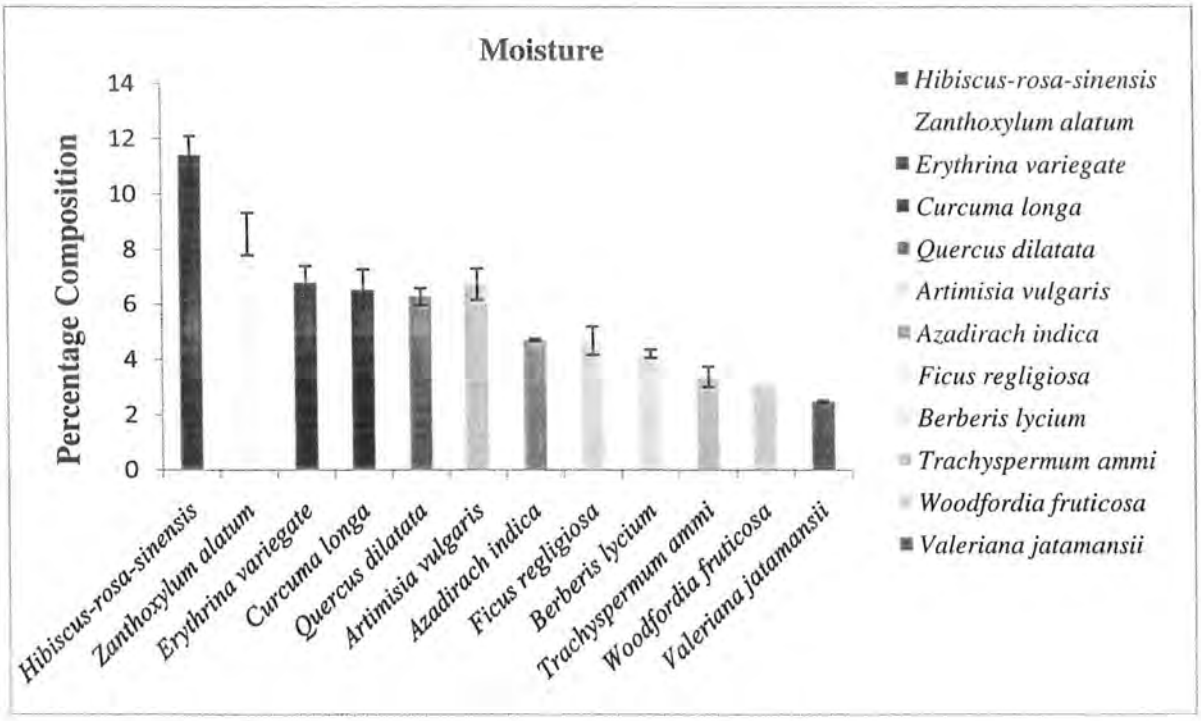

Fig 4.5. Percentage composition of moisture contents.

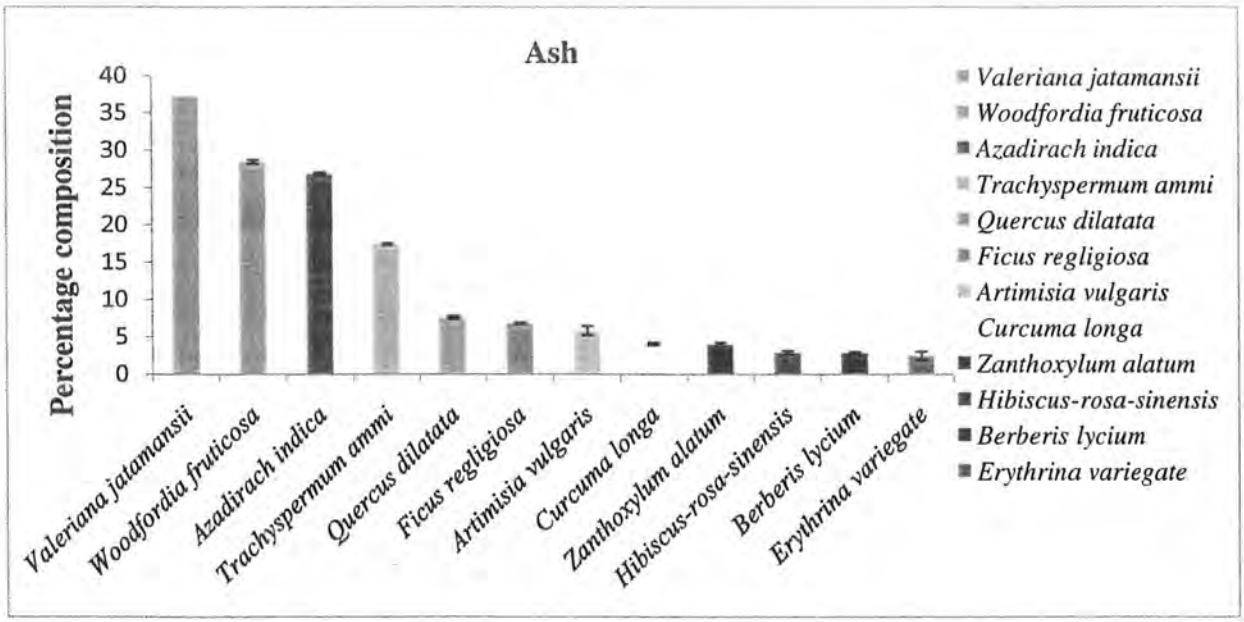

Fig 4.6. Percentage composition of Ash contents.

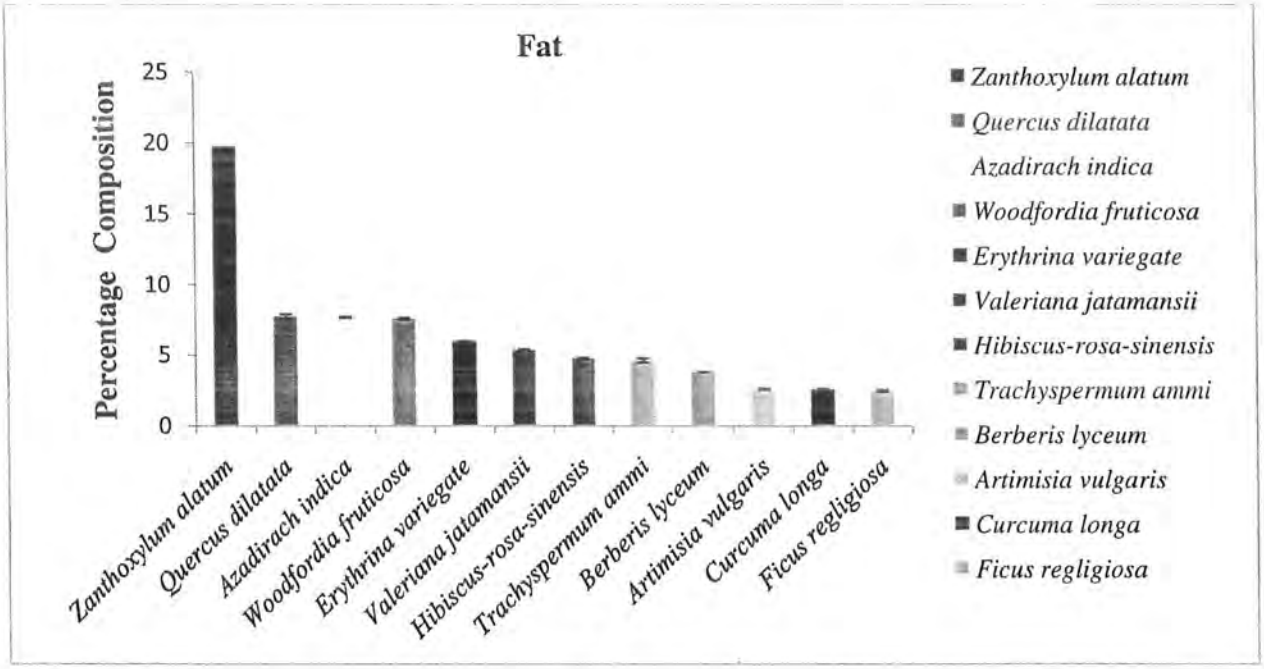

Fig 4.7. Percentage composition of fat contents.

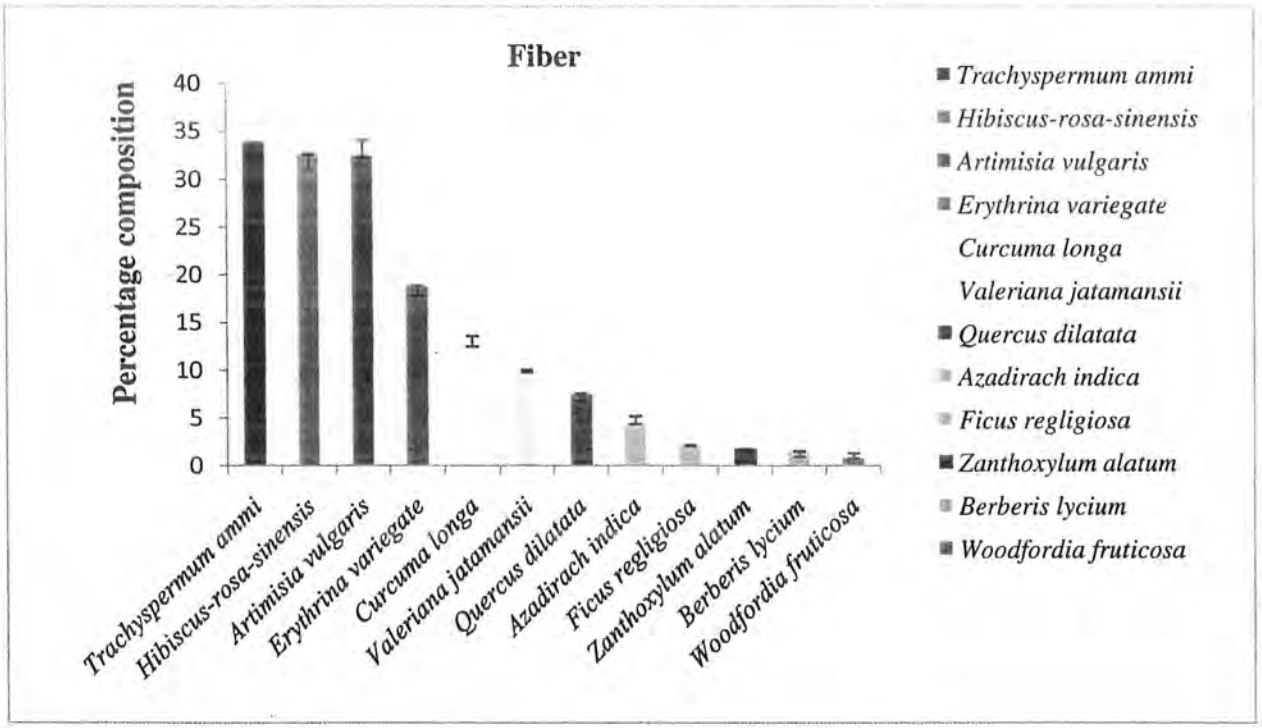

Fig 4.8. Percentage composition of Fiber contents.

4.2.5. Protein Contents

The ranking of protein contents was observed to be *Azadirach indica* (55 ± 1), *Valeriana jatamansii* (43.71 ± 0.073), *Ficus religiosa* (35 ± 1), *Curcuma longa* (29.74 ± 0.0105), *Quercus dilatata* (24.4 ± 0.1), *Zanthoxylum alatum* (19.233 ± 0.0152), *Woodfordia fruticosa* (13.5± 1.112), *Erythrina variegate* (7.034 ± 0.1143), *Trachyspermum ammi* (5.1606 ± 0.4605), *Hibiscus-rosa-sinensis* (3.853 ± 0.0906), *Berberis lycium* (3.72 ± 0.0636) and *Artemisia vulgaris* (2.945 ± 0.0542) (Fig **4.9**).

4.2.6. Carbohydrate Contents

The ranking of carbohydrate contents was noted to be *Berberis lycium* (81.09 ± 1.0198), *Valeriana jatamansii* (70.679 ± 0.4685), *Hibiscus-rosa-sinensis* (67.548 ± 0.8634), *Quercus dilatata* (54.912 ± 0.3241), *Trachyspermum ammi* (54.05 ± 1.2886), *Curcuma longa* (53.746 ± 0.2266), *Ficus religiosa* (51.871 ± 0.2304), *Woodfordia fruticosa* (43.243 ± 0.6608), *Azadirach indica* (40.533 ± 2.6855), *Zanthoxylum alatum* (37.195 ± 0.7848), *Erythrina variegate* (23.276 ± 0.8899) and *Artemisia vulgaris* (19.2 ± 0.0057) (Fig **4.10**).

4.2.7. Energy value (K cal/100 g) Contents

The ranking of energy value (K cal/100 g) contents was noted to be *Woodfordia fruticosa* (448.771 ± 4.526), *Quercus dilatata* (392.951 ± 1.228), *Trachyspermum ammi* (390.567 ± 1.928), *Erythrina variegate* (383.479 ± 0.9463), *Hibiscus-rosa-sinensis* (370.577 ± 2.929), *Zanthoxylum alatum* (366.72 ± 1.906), *Berberis lycium* (362.88 ± 1.484), *Valeriana jatamansii* (339.911 ± 2.17), *Azadirach indica* (318.992 ± 12.026), *Curcuma longa* (311.813 ± 0.592), *Ficus religiosa* (267.956 ± 1.208) and *Artemisia vulgaris* (55.6 ± 29.04) (Fig **4.11**).

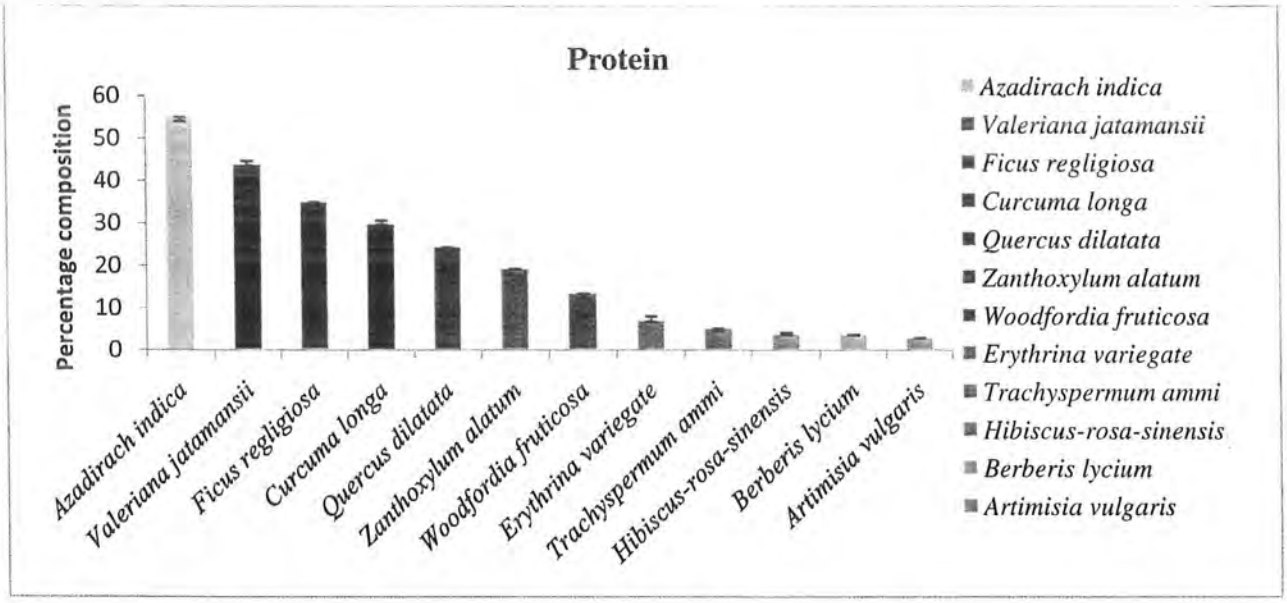

Fig 4.9. Percentage composition of Protein contents.

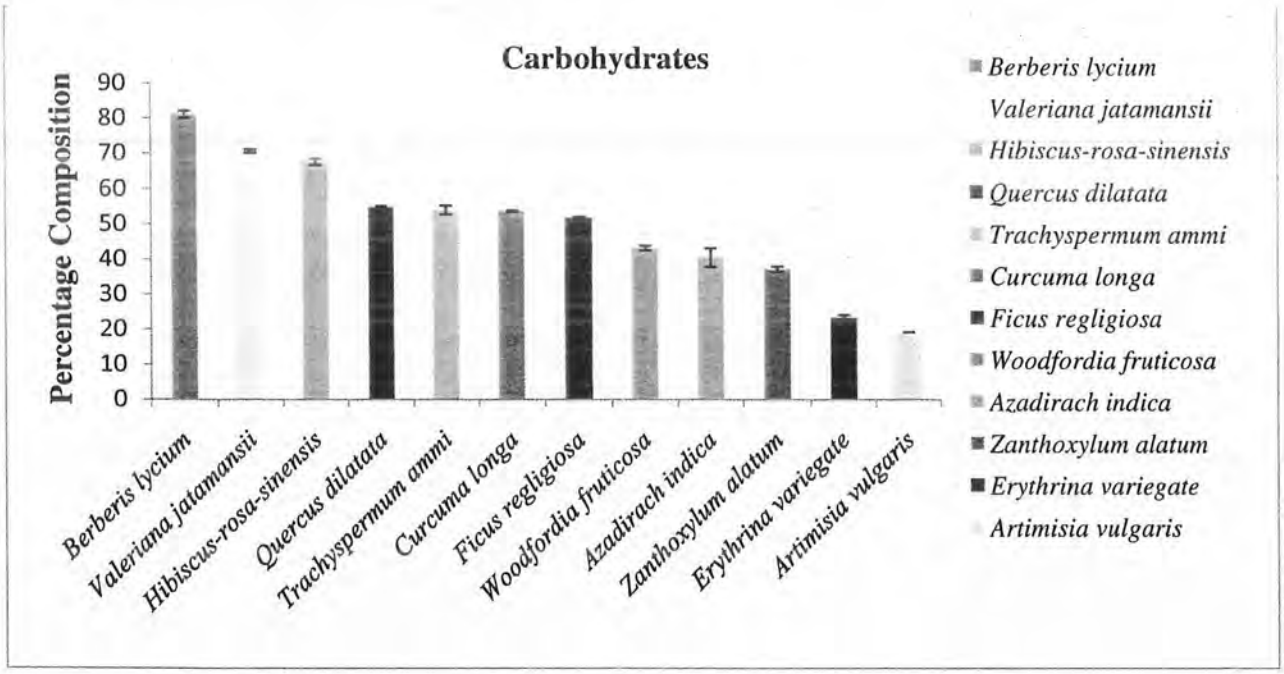

Fig 4.10. Percentage composition of Carbohydrate contents.

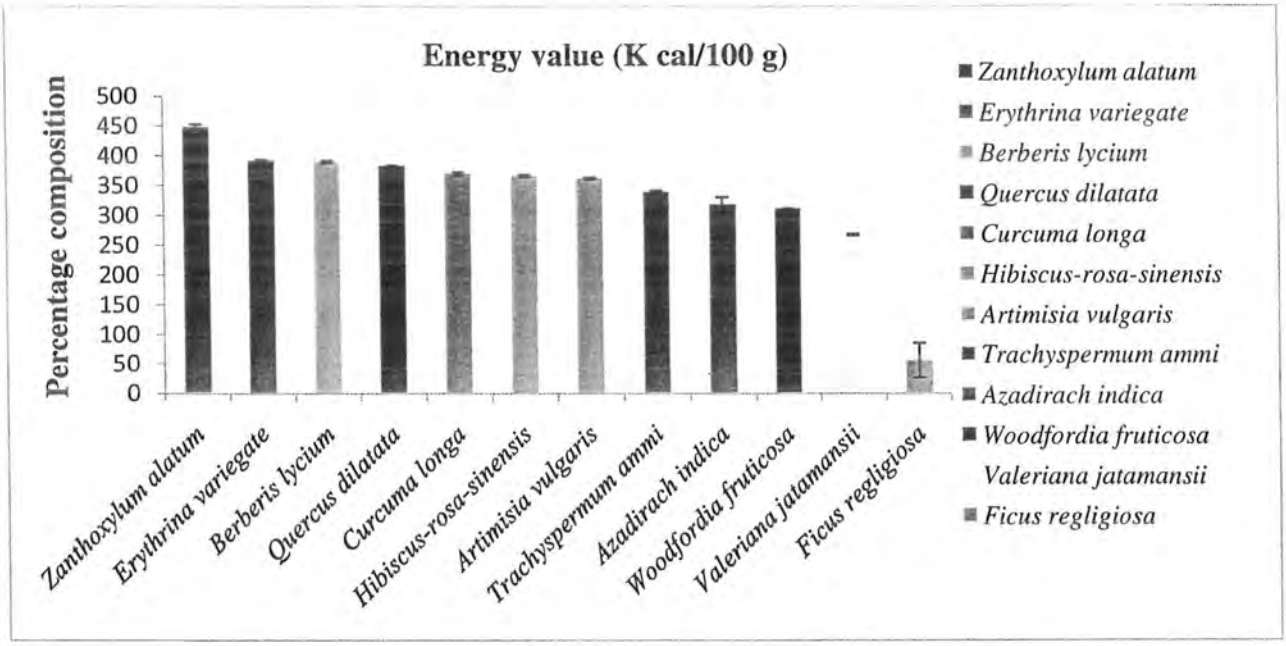

Fig 4.11. Percentage composition of Energy value (K cal/100 g) contents.

Table 4.1. *Some promising plants having antimicrobial activity against multidrug resistant strains.*

Species name	Microorganism			
	C. albicans	*S. aureus*	*E. coli*	*K. pneumoniae*
Woodfordia fruticosa	27.086 ± 2.970	13.29 ± 0.550	13.55 ± 1.130	10.48 ± 0.660
Berberis lycium	22.36 ± 1.018	0 ± 0	0 ± 0	0 ± 0
Quercus dilatata	21.97 ± 2.632	8.67 ± 0.529	11.22 ± 1.045	8.846 ± 0.853
Azadirach indica	14.46 ± 0.546	7.96 ± 0.314	0 ± 0	0 ± 0
Ficus religiosa	12.88 ± 0.265	0 ± 0	0 ± 0	0 ± 0
Erythrina variegate	11.66 ± 0.299	0 ± 0	0 ± 0	0 ± 0
Trachyspermum ammi	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Valeriana jatamansii	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Hibiscus-rosa-sinensis	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Zanthoxylum alatum	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Curcuma longa	0 ± 0	12.7 ± 1.031	0 ± 0	0 ± 0
Artemisia vulgaris	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ampicillin	26 ± 0.1	33 ± 1.4	48 ± 0.1	21 ± 0.31
DMSO	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 4.2. *Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of plants extracts against pathogenic bacteria.*

Plants	Organisms tested at 5 mg/ml concentration																			
	Candida albicans					*Staphylococcus aureus*					*Escherichia coli*					*Klebsiella pneumoniae*				
	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125	5	2.5	1.25	0.625	0.3125
W.F	y	x	+	++	+++	y	x	+	++	+++	y	x	+	++	+++	y	x	+	++	+++
Q.D	-	y	x	+	++	y	x	+	++	+++	y	x	+	++	+++	y	x	+	++	+++
E.V	-	-	y	x	+															
B.L	-	-	-	y	x															
A.I						y	x	+	++	+++										
C.L						y	x	+	++	+++										
F.R	-	y	x	+	++															

Woodfordia fruticosa (W.F), Quercus dilatata (Q.D), Erythrina variegate (E.V), Berberis lycium (B.L), Azadirach indica (A.I), Curcuma longa (C.L), Ficus religiosa (F.R).

Where (-) = growth, (+) = light growth, (++) = moderate growth, (+++) = high growth, (y) = MIC and (x) = MBC

The minimum inhibitory concentration (MIC) of the extracts against pathogenic strains ranging from 2.5-0.3125 mg/ml and with a minimum bacterial concentration (MBC) ranging from 5-0.625 mg/ml.

Table 4.3. *Proximate analytical data of the selected medicinal plant species in percentage (%).*

Species Name	Moisture	Ash	Fat	Fiber	Protein	Carbohydrate	Energy value (K cal/10 g)
Azadirach indica	4.72 ± 0.05	26.86 ± 0.10	7.73 ± 0.06	32.48 ± 0.10	5.1 ± 0.46	40.5 ± 2.68	318.9 ± 12.02
Trachyspermum ammi	3.39 ± 0.36	17.43 ± 0.19	4.64 ± 0.01	12.67 ± 0.17	3.8 ± 0.09	70.6 ± 0.46	339.9 ± 2.17
Valeriana jatamansii	2.48 ± 0.04	37.29 ± 0.26	5.40 ± 0.04	9.87 ± 0.10	2.9 ± 0.05	51.8 ± 0.23	267.9 ± 1.208
Woodfordia fruticosa	3.146 ± 0.140	8.44 ± 0.18	7.63 ± 0.03	33.8 ± 0.02	7.03 ± 0.1	53.7 ± 0.22	311.8 ± 0.59
Erythrina variegate	6.813 ± 0.606	2.48 ± 0.28	6.02 ± 0.01	7.4 ± 0.72	29.7± 0.1	54.9 ± 0.32	392.9 ± 1.228
Hibiscus-rosa-sinensis	11.42 ± 0.69	2.88 ± 0.15	4.78 ± 0.15	4.45 ± 0.09	43.7± 0.7	37.1 ± 0.78	366.7 ± 1.906
Zanthoxylum alatum	8.57 ± 0.76	3.97 ± 0.23	19.79 ± 0.17	2.08 ± 0.00	24.4± 0.1	43.2 ± 0.66	448.7 ± 4.526
Quercus dilatata	6.29 ± 0.30	7.62 ± 0.12	7.79 ± 0.025	0.78 ± 0.09	55 ± 1	23.2 ± 0.88	383.4 ± 0.9463
Berberis lyceum	4.23 ± 0.15	2.85 ± 0.57	3.81 ± 0.061	1.752 ± 0.07	35 ± 1	54.0 ± 1.28	390.56 ± 1.92
Curcuma longa	6.54 ± 0.75	4.07 ± 0.24	2.60 ± 0.05	1.44 ± 0.50	19.23 ± 01	67.54 ± 0.8	370.57 ± 2.929
Artemisia vulgaris	6.76 ± 0.57	5.78 ± 0.151	2.62 ± 0.059	32.62 ± 1.52	3.72 ± 0.6	81.0 ± 1.01	362.88 ± 1.48
Ficus religiosa	4.70 ± 0.51	578 ± 0.616	2.5 ± 0.076	18.76 ± 0.92	13.5 ± 1.1	19.20 ± 0.0	55.60 ± 29.04

Where ± SD is standard deviation

Table 4.4. *Descriptive Statistics of selective species.*

Plant	Sum	LO 95 % CI	Mean	UP 95 % CI	SD	Variance	SE Mean	CV %
Azadirach indica	956.98	289.12	318.99	348.87	12.026	144.63	6.9433	3.7701 %
Trachyspermum ammi	1019.7	334.52	339.91	345.30	2.1701	4.7093	1.2529	0.6384 %
Valeriana jatamansii	803.87	264.95	267.96	270.96	1.2086	1.4607	0.6978	0.4510 %
Woodfordia fruticosa	935.44	310.34	311.81	313.28	0.5921	0.3506	0.3418	0.1899 %
Erythrina variegate	1178.9	389.90	392.95	396.00	1.2282	1.5085	0.7091	0.3126 %
Hibiscus-rosa-sinensis	1100.2	361.99	366.72	371.46	1.9060	3.6329	1.1004	0.5197 %
Zanthoxylum alatum	1346.3	437.53	448.77	460.02	4.5269	20.493	2.6136	1.0087 %
Quercus dilatata	1150.4	381.13	383.48	385.83	0.9463	0.8955	0.5463	0.2468 %
Berberis lycium	1171.7	385.78	390.57	395.36	1.9282	3.7179	1.1132	0.4937 %
Curcuma longa	1111.7	363.30.	370.85	377.85	2.9291	8.5794	1.6911	0.7904%
Artemisia vulgaris	1088.7	360.03	362.89	365.74	1.1485	1.3190	0.6631	0.3165 %
Ficus religiosa	166.80	16.540	55.600	127.74	29.040	843.33	16.766	52.230 %

Where SD is standard deviation, SE = standard error, CV = cumulative variance

Chapter No. 5

Discussion

Discussion

Due to limited modern health services in the developing countries like Pakistan most of the people are dependent on plant based medicines. From the most primitive times the mankind had been fascinated by medicinal plants in an attempt to treat diseases and alleviate physical suffering. According to World Health Organization survey 65% patients in Srilanka, 60% in Indonesia, 75% in Nepal, 85% in Myanmar, 80% in India and 90% in Bangladesh are treated by traditional healers. In Pakistan about 60% populations of villages are getting primary health care by traditional practitioners (Hakeems), who recommend herbal preparations (Haq, 1983).

Throughout the world for thousands of years the most important source of providing primary health care are medicinal plants. However, in the middle of twenth century, researchers have privileged the use of synthetic drugs for alleviation of different diseases therefore the contribution of medicinal plants was decreased by approximately one fourth. Scientists are moving back to traditional remedies due to important active compounds present in medicinal plants that are chemically balanced, valuable, effective and least injurious with none or much reduced side effects as compared to synthetic chemicals. Herbal medicines in the Western society are getting fame along with other important therapies namely homeopathy, traditional Chinese medicine and osteopathy. Indigenous remedies which are more effective, harmless and economical were in common use among both rural and urban areas. In general, people are becoming more alert of the detrimental side effects of synthetic drugs and are realizing the benefits of a more natural way of life.

The elected pathogenic strains used for the study were three gram-negative namely *Escherichia coli, Klebsiella pneumonia* and *pseudomonas aeruginosa* and one gram-positive bacteria *Staphylococcus aureus* along with one fungal strain *Candida albicans.* The pathogens *Candida, Cryptococcus* and *Aspergillus* are the main causing agents of fungal infections (Richardson, 2005). *C. albicans* the major fungal pathogen affecting human's health has been a serious problem, especially in those individuals whose immune system have been weakened (Odds *et al.,* 2006). *C. albicans* possess the capability to colonize skin and mucosal surfaces of healthy people and these pathogens are inhibited in the gastrointestinal tract, oral cavity and vagina, often causing external infections (Mavor *et al.,* 2005).

Majority of infections are caused by *E. coli* such as gastroenteritis, meningitis, urinary tract infections, pneumonia, infected bones, joints, skin and soft tissue infections (Todar, 2007). *K. Pneumoniae* causes lethal infections like pneumonia and urinary tract infections (UTI). For maximum extraction of plants methanol was used in present studies as it is considered best solvent (Srivastava *et al.*, 2001). Nutrient agar was used extensively for culturing of microorganisms. The antimicrobial activity of selected medicinal plants was determined by well diffusion method.

Since long *Woodfordia fruticosa* has been thought as an important medicinal plant locally it is known as Dhawai. Commonly its leaves and flowers were preferred in traditional remedies in south East Asian countries. The bark and twigs have also been reported for other uses also. The potential of *Woodfordia. Fruticosa* has been reported by Parekh and Chanda (2007); Das *et al.*, (2007). *Woodfordia fruticosa* showed maximum activity against *C.albicans* which was the most susceptible fungus followed by bacteria *S. aureus*, *E. coli* and *K. pneumoniae* while the most resistant bacteria were *P. aeruginosa.*

Curcuma longa (curcumin), member of the *Zingiberaceae* family, is a perennial herb and has many traditional uses in the Chinese and Ayurvedic systems of medicine. The rhizome of *Curcuma longa* contains natural medicinal properties, including antibacterial, anti-inflammatory, antineoplastic, and analgesic due to the presence of moniterpenoids, sesquiterpenoids, and Curcuminoids (Tang and Eisenbrand, 1992; Fang *et al.*, 2003). Insecticidal activities have also been reported by Chander *et al.*, (1991). In addition, *Curcuma longa* also have wound healing and detoxifying properties (Joe *et al.*, 2004). During our studies the methanol extract of *curcuma longa* showed inhibitory activity against *S. aureus* by producing zone of inhibition (12.7 mm)

Quercus dilatata also known as Toor banj by the local people. Its fruits and leaves are used for ethnomedicinal purposes. *Quercus* spps. having antimicrobial potential has been used in many problems like skin, wounds, and gastrointestinal ailments (Viegi *et al.* 2003), along with mild antiseptic, astringent, small cuts (Leporatti and Ivancheva, 2003), and mouth gargles (Aburjai *et al.*, 2007). Our studies results showed that *Quercus dilatata* exhibited maximum antifungal activity against *C. albicans* (21.97 mm) at 5 mg/ml. The ranking of

antibacterial activities of *Quercus dilatata* against three pathogenic strains was *E. coli* > *K. pneumoniae* followed by *S. aureus.*

Berberis lycium locally known as Kwaray, kashmal has a number of traditional uses. An alkaloid berbamine isolated from *B. lycium* has hypotensive effect (Khan *et al.*, 1969). *B. lycium* is medicinal plant traditionally used for prevention of various ailments including diabetes mellitus, particularly by the local inhabitants of Hamaliya region, (Muhammad *et al.*, 2006). For reducing serum cholesterol in broilers *B. lycium* had been used (Chand *et al.*, 2007), *B. lycium* has been used to cure hepatitis, stomach ache, a cooling agent and wound healing (Hassan *et al.*, 2010). *B. lycium* showed antifungal activity against *C. albicans* only by producing zone of inhibition (22.36 mm) at a concentration of 5 mg/ml.

Azadirachta indica is traditionally used medicinal plant belongs to family Meliaceae and found in southern Asia (Akula *et al.*, 2003). *Azadirachta indica* is used in treatment of skin diseases, leprosy, stomach ulcers, rheumatism, respiratory tract infections, sore gums and throat, eye and ear infections, and diabetes (Isman *et al.*, 1990; Kaura *et al.*, 1998; Akula *et al.*, 2003). A variety of herbal products were used by the local communities to cure different infectious diseases (Mann *et al.*, 2008). The results conducted from our research work revealed that *Azadirachta indica* exhibited maximum inhibitory activities against *S. aureus* and *E. coli* by producing zone of inhibition (14.46 mm) and (7.96 mm) at 5 mg/ml.

Ficus religiosa of family Moraceae is used for the treatment of diseases. Some of the *Ficus* species. have been used as a therapy for diabetes, visceral obstructive disorders, leprosy, respiratory disorders and certain skin diseases (Chopra *et al.* 1950), and also as an absorbent for inflammatory swellings and burns (Bhattacharjee, 1998). Bark of different *Ficus* species. (*F. religiosa, F. bengalensis, F. glomerata, F. infectora* and *Abizzia lebbeck*) have been used to cure female genital tract infections (Palep *et al.* 2003). The results revealed that *Ficus religiosa* inhibited *C. albicans* by producing zone of inhibition (12.88 mm) at 5 mg/ml.

Erythrina variegate of Fabaceae family is used as antiseptic as well as anthelmintic in the treatment of joint pain and inflammation (Patil, 2003). In India, China, and Southeast Asia, The bark and leaves of *Erythrina variegate* are used to cure wind-damp obstruction

syndrome manifested as rheumatic joint pain, and to stimulate lactation and menstruation for women (Whistler and Craig, 2004). *Erythrina variegate* showed significant activity against *C. albicans* with zone of inhibition (11.66 mm) at a concentration of 5 mg/ml.

None of the twelve plants showed sensitivity against *Pseudomonas aeruginosa*. The medicinal plants *Zanthoxylum alatum, Trachyspermum ammi, Artemisia vulgaris, Valeriana jatamansii and Hibiscus rosa–sinenses* which was ethnobotanically explored was observed to be inactive against the selected pathogenic strains.

The MIC of the plants extracts of *Woodfordia fruticosa* and *Quercus dilatata, Erythrina varigata,* and *Berberis lycium* have wide effect against *C. albicans* with concentration of 2.5, 1.25, 0.625 and 0.3125 mg/ml respectively. MBC of these plants ranged from 5, 2.5, 1.25 and 0.625 mg/ml respectively. *Woodfordia fruticosa, Quercus dilatata, Azadirachta indica* and *Curcuma longa* showed MIC and MBC against *S. aureus* with concentration ranged from 5-2.5 mg/ml. MIC and MBC against *E. coli* ranged from 5-2.5 mg/ml. Against *K. pneumoniae* only *Woodfordia fruticosa* and Quercus *dilatata* showed efficacy ranged from 5-2.5 mg/ml.

Against *C. albicans* some of the selected medicinal plants showed maximum inhibitory activities. *Woodfordia fruticosa* and *Quercus dilatata* exhibited maximum antimicrobial activities against the four pathogenic strains except *P. aeruginosa* which was resistant against these plants.

In the developing countries including Pakistan where contagious diseases are common, therefore it is needed to investigate and explore plant base medicines. Mostly people use synthetic drugs to treat different ailments but now they are becoming aware of the potency and side effects of synthetic drugs therefore people are diverting more towards traditional medicines. Due to toxicity and side effects of allopathic medicines herbal medicines are becoming more popular and has lead increase in the number of herbal drug manufacturer (Agarwal, 2005).

Most of herbal products are used orally, so, the proximate and nutrient analysis of the herbal preparations and raw material play vital role in determining the nutritional importance

and health effects (Kochhar, 2006; Pandey, 2006; Taiga, 2008). According to WHO proximate and micronutrients analysis is important to standardize herbal drugs. So, the herbal formulations have to pass through standardization processes (Niranjan and Kanaki, 2008; Ojokoh, 2008).

Carbohydrates, fats and protein are the necessary nutrients of life. The proteins quality and quantity in the seeds are essential factors and important for the choice of plants for nutritive value, systematic classification and plant improvement programs (Nisar *et al.*, 2009). Proximate and nutrient analysis of edible fruits and vegetables plays a key role in assessing their nutritional importance. Along with their medicinal benefits, a variety of medicinal plant species are also used as food. Evaluating their nutritional significance can help to understand the importance and worth of these plants species (Pandey *et al.*, 2006). Therefore, twelve medicinal plants species were selected to analyze their nutritional values during our studies.

Zanthoxylum alatum of family Rutaceae, which is locally known as Laighunay or timber. The powdered seeds of Zanthoxylum *alatum* are being used as an aromatic tonic, stomachic and for fever, dyspepsia and cholera. Our results showed that *Zanthoxylum alatum* was the most significant species having high concentration of fat and energy values compared to other species. The ranking of proximate analysis in *Zanthoxylum alatum* is energy > carbohydrate > protein > fat > moisture >ash > fiber.

Erythrina has been used in traditional medicine to treat insomnia, malarial fever, asthma, veneral disease, tooth-ache and antihelminthic. The alkaloid erythroidine isolated from *Erythrina variegate* has been used as a muscle relaxant. The alkaloid haemoerythrina was investigated for anti-cancer activity (Payne, 1991). *Erythrina variegate* was observed to have high energy and carbohydrate content. The ranking of proximate analysis in *Erythrina variegate* is energy > carbohydrate > protein > fiber > moisture > fat > ash.

Woodfordia fruticosa another species found in South East and Far East Asia (Malaysia, Indonesia, Sri Lanka, China, Japan and Pakistan) as well as tropical Africa (Kirtikar, 1935). Treatment of female specific disorders such as leucorrhea and dysmenorrhea with flower based preparations is well known among the tribes. Herbal composition

containing *Woodfordia fruticosa* has been used to treat gynecological disorders. It is believed to treat anemia due to excessive bleeding associated with menstrual disorders (Katiyar *et al.*, (2002). Result of proximate analysis of *Woodfordia fruticosa* showed high content of fiber, ash and less quantity of protein.

Artemisia vulgaris (Asteraceae) a valuable medicinal plant commonly used by local inhabitants in folk medicines. Some *Artemisia* species namely *A. absinthium A. annua* or *A. vulgaris* have been integrated into the pharmacopoeias of several European and Asian countries (Proksch, 1992). *Artemisia* species also have pharmacological actions by protecting liver, eliminating fever, lowering the blood pressure, sedation and anti-inflammation, antibacteria, antipathogenic microbes and antitumor activity (Yao, 2007). Proximate analysis of *Artemisia vulgaris* showed high percentage of carbohydrate (81.09 ± 1.0198) and energy (362.88 ± 1.484) content.

Ficus another species have been uses in traditional folk medicines as astringents, carminatives, stomachics, vermicides, hypotensives, anthelmintics and anti-dysentery drugs (Trivedi *et al.* 1969). *Ficus religiosa* had minor proportion of carbohydrates (19.20 ± 0.0057) and energy content (55.60 ± 29.040).

Curcuma longa (Zingiberaceae) is being used to treat bronchitis, dropsy, burns, vertigo, skin diseases, boils, hysteric effects, fevers, swellings, chronic gonorrhea, bruises, small pox, chicken pox, liver infections, scorpion snake and leech bites, congestions, scabies, dyspepsia, ring worm, etc. Curcuminoids have been isolated from the roots of *Curcuma longa* which is a group of phenolic compounds is known to have beneficial and valuable effects on health and the ability to cure certain diseases (Joe *et al.*, 2004). Due to its usefulness as pesticide, fungicide and bactericide importance of turmeric has significantly increased (Velayudhan *et al*, 1994). The ranking of proximate analysis in *curcuma longa* is energy > carbohydrate > protein > moisture > ash > fat > fiber.

In Pakistan *Berberis lycium* is found in northern areas including Gilgit, Swat, Baltistan and Kashmir. The ranking of proximate analysis is energy > carbohydrate > protein > moisture > fat >ash > fiber.

Azadirach indica is also well known medicinal plant. In India, most of the people rely on medicinal plants and *Azadirach indica* is popularly known as the village dispensary (Akula *et al.*, 2003). The ranking of proximate analysis is energy > carbohydrate > fiber > ash > fat > protein > moisture.

Ajwain local name of *Trachyspermum ammi* is generally used for medicinal purposes as a digestive stimulant or to treat liver disorders. The major phenolic compound thymol present in Ajwain has been reported to have a germicide, antispasmodic, and antifungal agent (Nagalakshmi *et al.*, 2000). Results of *Trachyspermum ammi* showed high proportion of energy > carbohydrates > ash > fiber > fat > moisture > protein content.

Valeriana jatamansii, locally known as Murma belongs to family Papilionaceae. Present results showed highest percentage of ash contents as compared to other species. The order of proximate analysis in *Valeriana jatamansii* is energy > carbohydrate > ash > fiber >fat > protein > moisture content.

Hibiscus-rosa-sinensis also known as (Shoe flower) belongs to family Malvaceae. Mostly flowers are traditionally preferred. The ranking of proximate analysis is energy > carbohydrate > protein > moisture > fat > fiber > ash. *Hibiscus-rosa-sinensis* was found to have lower ash content.

Quercus dilatata belongs to Fagaceae family and used in the treatment of urinary tract infections. The fruits of *Quercus* species after buried for some period of time are then eaten with intent to cure diabetes and as anti hyperlipidemic agent; to lower plasma cholesterol and triglycerides levels (Pieroni *et al.*, 2005). The proximate analysis ranking of *Quercus dilatata* showed energy > protein > carbohydrate > fat > ash > moisture > fiber. The results showed that *Quercus dilatata* do not have any fiber contents.

Conclusion

People from all over the world prefer herbal products as source of drugs for the prevention and treatment of infectious diseases because they have fewer side effects, cheaper and easily available as compared to synthetic medicines. People used herbal drugs as therapy from ancient times. This is because plants contain a number of active compounds which are responsible for having potentially useful therapeutic values. Emergence of multiple drug resistantence has become far more serious problem therefore it is necessary to search for complementary and alternative medicines. Medicinal plants used in the present study were selected on the basis of traditional and ethnomedicinal uses. The selected plants were used for the treatment of Gynecological disorders like menorrhea, gonorrhea, irregular periods etc, but in the present research work same plants were used to check their antimicrobial activity for the treatment of infectious diseases like vaginitis caused by *Candida albicans*. The results of antifungal activity were quite impressive than antibacterial activity. The results of antifungal activity have indicated that methanolic extract of *Woodfordia fruticosa* greatly inhibit the growth of *Candida albicans*. Thus the results obtained confirm that *Woodfordia fruticosa* can be used as therapeutic potency in the treatment of infectious diseases. *Woodfordia fruticosa* also showed activity against *E.* coli, *Klebsiella pneumonia* and *pseudomonas aeruginosa.* The plant species are also used as food supplements along with its oral decoction. The results of selected medicinal plants analyzed for proximate analysis indicated that *Zanthoxylum alatum* most significant species have higher concentration of fat and energy value as compared to other species. *Zanthoxylum alatum* have the potential to provide essential nutrients to the human beings.

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Annexure

Fig: methanolic extract of samples

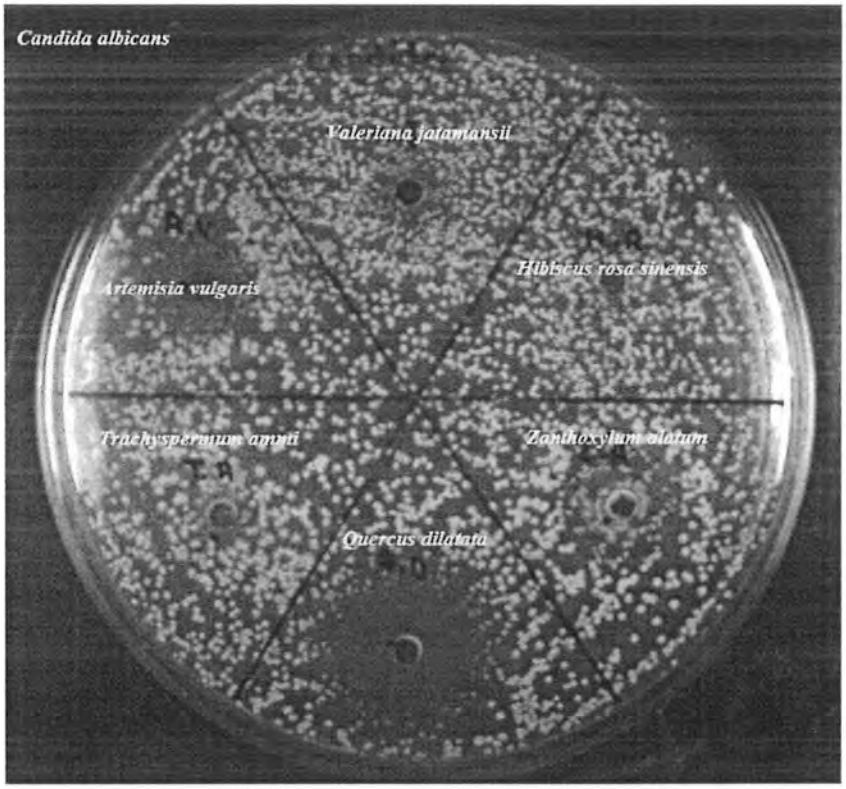


Fig: Plates Showing Antibacterial Activity of *Quercus dilatata* against *Candida albicans*

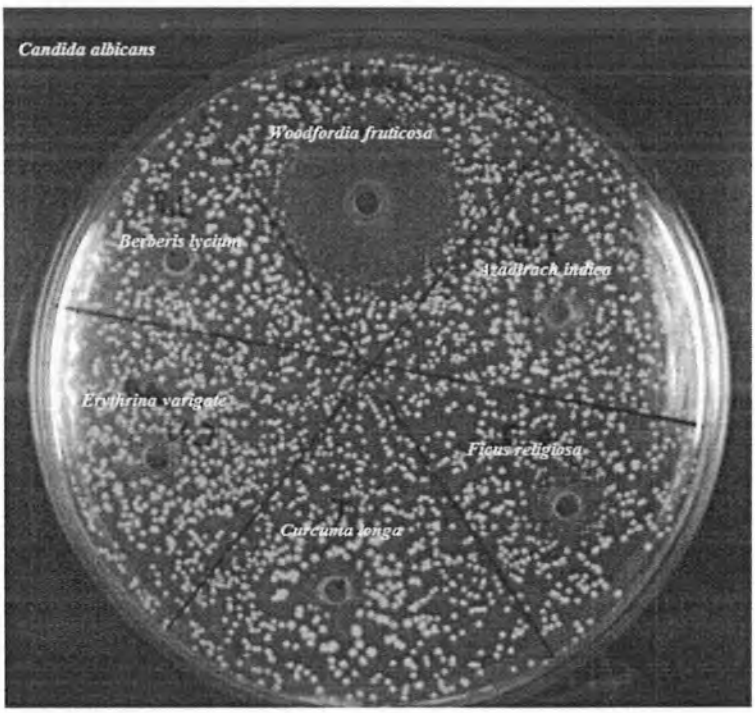
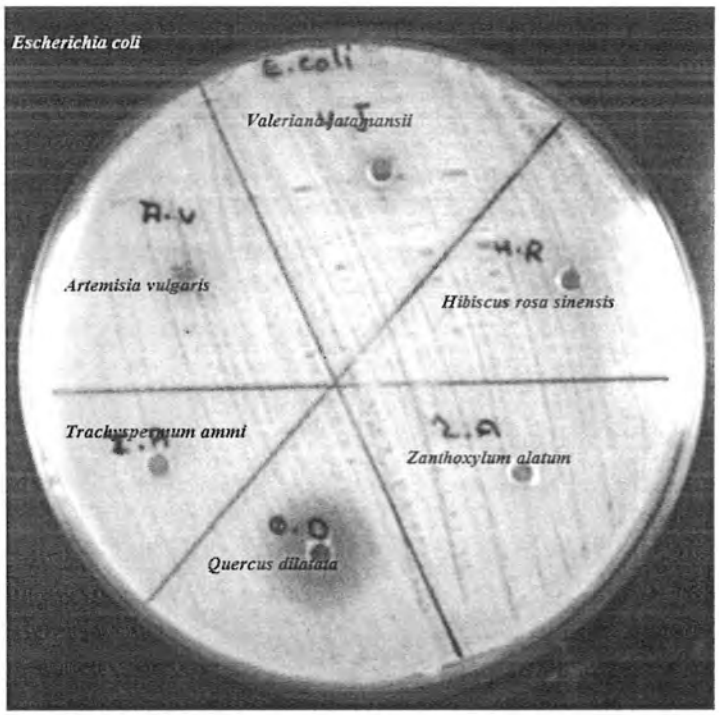


Fig: Plates Showing Antibacterial Activity of *Woodfordia fruticosa* against *Candida albicans*



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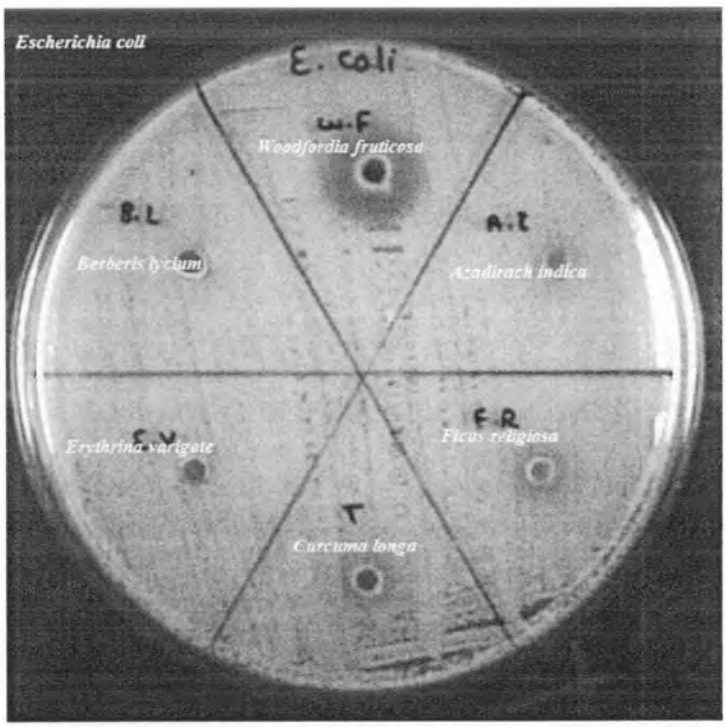

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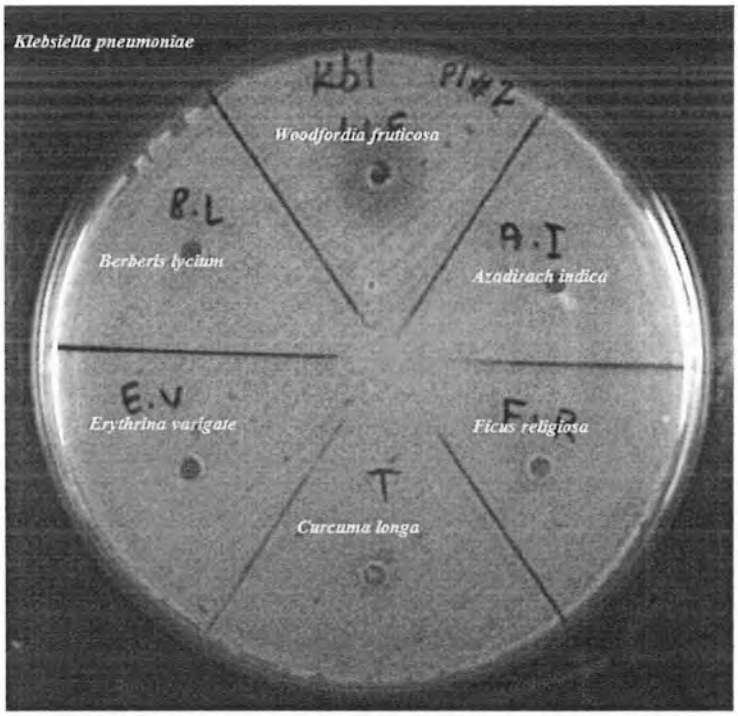
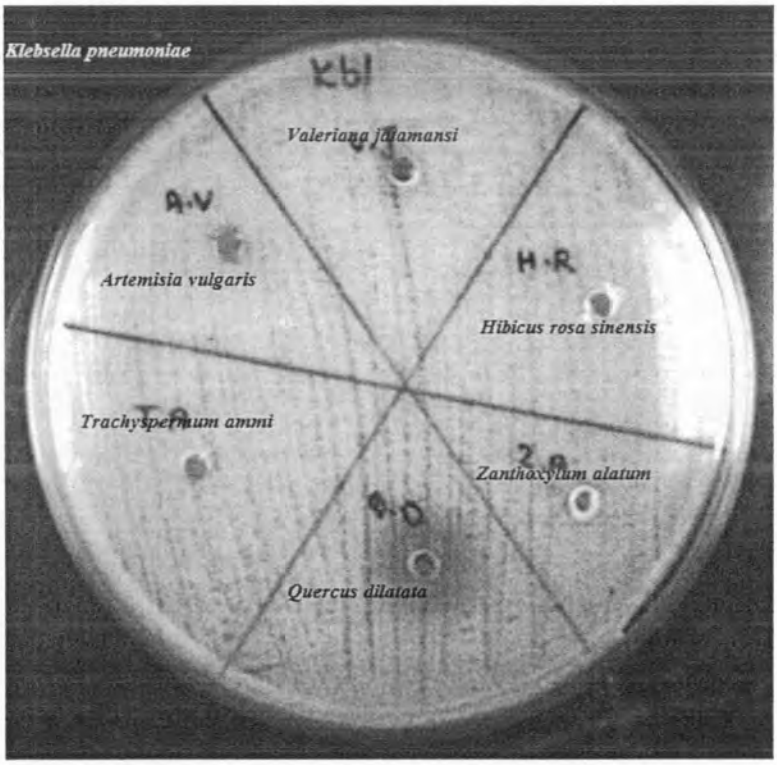


Fig: Plates Showing Antibacterial Activity of *Woodfordia fruticosa* against *Klebsiella pneumoniae.*



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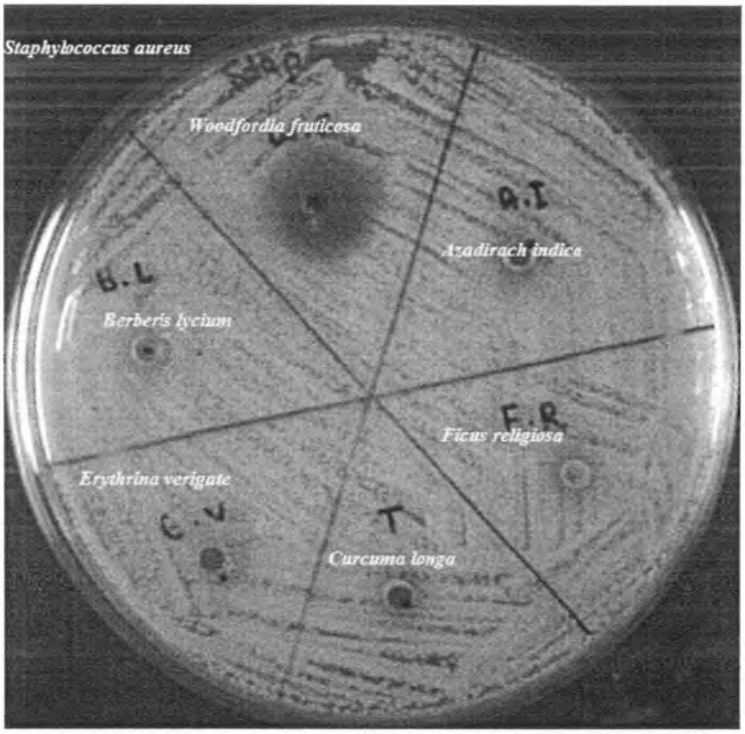

Fig: Plates Showing Antibacterial Activity of *Woodfordia fruticosa* against *Staphylococcus aureus*

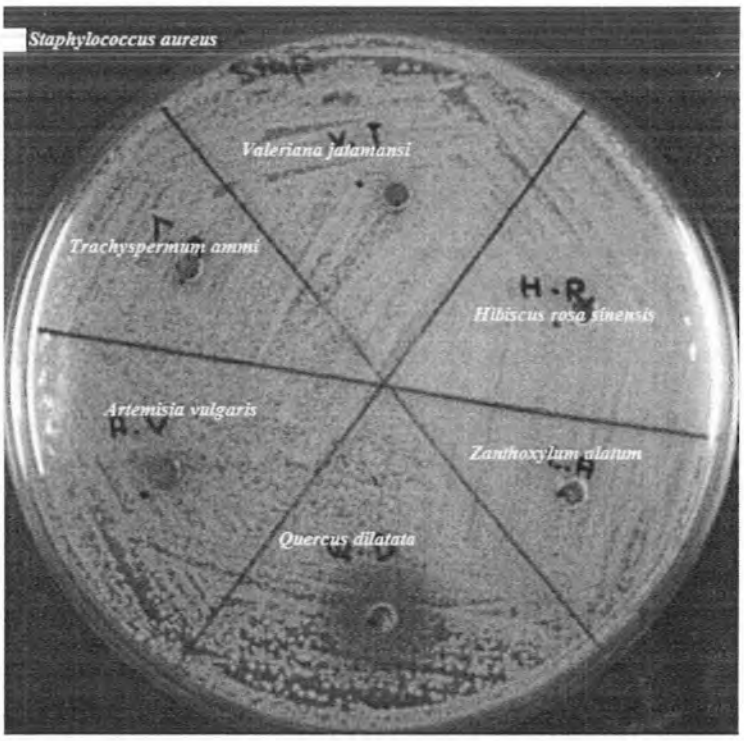

Fig: Plates Showing Antibacterial Activity of *Quercus dilatata* against *Staphylococcus aureus*