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SCREENING OF POTENTIAL MEDICINAL PLANTS FROM DISTRICT SAWAT SPECIFIC FOR CONTROLLING WOMEN DISEASES

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Abstract

Ethnobotany provides a scientific rationale to identify 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 (KP) was carried out to identify medicinally important plant species that are traditionally used to treat gynecological disorders and infectious diseases, and to study their antimicrobial potential against pathogens that cause infections in females. The antimicrobial activities were investigated using the well diffusion method against four different bacterial strains and one fungal strain. Results showed that out of 12 plants studied, seven plants exhibited inhibitory effects against *Candida albicans, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli. Woodfordia fruticosa, Quercus dilatata, Erythrina variegata, Ficus religiosa* and *Berberis lycium* showed high antifungal activity against *C. albicans* with minimum inhibitory concentration (MIC) values of 2.5, 1.25, 0.625, 1.25, 0.3125 mg/ml and minimum bactericidal concentration (MBC) values of 5, 2.5, 1.25, 2.5, 0.625 mg/ml, respectively. Both *Woodfordia fruticosa* and *Quercus dilatata* showed antimicrobial potential against *E. coli* and *K. pneumoniae* with similar 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* and all of them possessed 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 showed that in comparative assessment of the various species, *Zanthoxylum alatum* had the highest fat and energy values.

Introduction

Plants are used ethnobotanically for food, shelter, medicine, clothing, hunting and religious ceremonies, but their primary use is for health care (Aumeeruddy, 1996). Ethnobotanical studies document local knowledge about the uses of plants. Local people have a tremendous amount of knowledge about using, harvesting, and sustaining medicinal plants, and this knowledge is useful for industry and the world community (Hussain & Sher, 2005; Ozcan, 2005). In Pakistan, China, India, Japan, Sri Lanka and Thailand the practice of traditional medicine is widespread (Hasan *et al*., 2007).

In the field of drug development, ethnobotany also provides useful information, thus saving time and money (Sher *et al.*, 2010; Joshi, 1982). Through ethnobotanical surveys, indigenous knowledge from local people and practitioners has been documented in order to identify plants that may provide drugs against infectious diseases. Medicinal plants have been used by local communities for centuries (Shinwari, 2010). Pakistan has more than 6,000 species of higher plants, out of which 12% are used medicinally (Shinwari & Qaiser, 2011). Different plant parts are used for the treatment of various forms of diseases and infections. Traditional medicine is one of the oldest systems of curing diseases and infections and a variety of plants have been used in different parts of the world to treat human diseases and infections (Nweze *et al.,* 2004; Vineela & Elizabeth, 2005; Ekpo & Etim, 2009). According to a World Health Organization report, about 350 million cases of feminine diseases occur each year (Anon., 2007). Gynecological disorders and other infectious diseases greatly affect women's health, so the first priority of local women in the Himalayan region is medicinal plants because of immediate and cheap therapies as compared to other synthetic drugs, which are costly and in most cases unavailable (Jan *et al*., 2009).

An ethnobotanical survey and literature review of medicinal plants used in the treatment of gynecological disorders were carried out among the rural people in Swat district. Swat is located in Khyber Pakhtunkhwa province of Pakistan. The Swat valley lies between 34°40' and 35° North latitude and 72° to 74°6' East longitude at an altitude of 2000 m above sea level and is enclosed by the sky-high mountains. Swat was selected as the study area. As women of tribal areas hesitate to expose their feminine problems to doctors due to psychological, social and religious barriers, they have been trying to improve their fertility and regulate their reproductive cycles by practicing ethnomedicinal remedies. So far, 350 species have been reported from Swat District (Shinwari *et al*., 2003). Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases (Shah, 2005). Antibiotics are the main therapy for microbial (bacterial and fungal) infections. Although antibiotics play a vital role in the treatment of different diseases, they also have side effects. Antibiotic failure is due to the appearance of multidrug resistant pathogens and the spread of the new infections (Abdalla, 2011).

The effectual lifespan of an antibiotic is limited and over-recommendation and mistreatment with traditional antibiotics have caused microbial resistance to develop (Alam *et al.,* 2009). The increasing frequency of resistance to most antimicrobial agents complicates their use and the control of infectious diseases. There is no antimicrobial agent which is fully effective against dormant facultative bacteria (Jouene *et al.,* 1998). Before the development of Western medicine, traditional medicinal plants were used as remedies for the treatment of various diseases. Currently, most of the drugs used to treat bacterial and other infections were isolated from natural resources, including medicinal plants. New resources of therapeutic agents are provided by plants against multidrug resistant bacterial infections. A large variety of secondary metabolites is produced by medicinal plants; these are either used as precursors or lead compounds in the pharmaceutical industry and it is expected that medicinal plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. (Shokeen *et al.*, 2009).

The main purpose of the current study was to assess the inhibitory activities of selected medicinal plants against some human pathogenic microbes that cause infectious diseases, such as vaginitis caused by *Candida albicans*. Through an ethnobotanical survey and literature review, twelve different medicinal plants were selected that were effective against gynecological disorders and infectious diseases. Plants exhibiting antimicrobial potential against the microbial pathogens were further analyzed to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Since many plants are a good source of edible fats, proteins, and carbohydrates and thus have nutritive and calorific values, proximate analysis was also performed for the selected plants.

Materials and Methods

Plant collection: Twelve different plant species were collected from different areas of Swat (Table 1). These plant species were identified by a plant taxonomist. Fresh plant materials (whole or parts) were washed under running tap water, air dried and then ground into fine powder with an electric blender. The powder was stored in airtight bottles until required for further analysis. The powdered samples were also processed for proximate analysis.

Table 1. List of 12 medicinal plants used for female specific diseases, including scientific names, local names, parts used, family and uses.

S. No.	Scientific name	Local name	Parts used	Family	Ethnomedicinal uses
1.	*Artemisia vulgaris*	Chaagu	Leaves	Asteraceae	Leaves are helpful to suppress menses. Young women take syrup before and after the full moon when just starting menses. It is also used for insomnia and nervousness and kills parasitic worms internally
2.	*Azadirachta indica*	Neem	Leaf and seeds	Meliaceae	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 to uterus as a contraceptive before copulation
3.	*Berberis lycium*	Kwaray, kashmal	Root	Berberidaceae	The dried root bark in powder form is used as a tonic in nephrological complaints. It is used as an 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 variegata*	Flame trees	Leaves	Fabaceae	To cure irregular periods, red flowers of the plant (four or five) 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 flowers of the plant (five) taken in the morning on empty stomach to cure white discharge. Red flowers of the plant (four-five) 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. This recipe is also used to clear uterus and regulate menstrual cycle after birth
10.	*Valeriana jatamansii*	Murma	Stem and roots	Valerianaceae	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 powdered fruit of *Zanthoxylum alatum* is used to treat gonorrhea and urinary tract infections

Sample preparation: The plant materials were dried under shade and ground into fine powder using an electric blender. For solvent extraction, 5g of air dried powder was mixed with 500ml of organic solvent (methanol) in a conical flask, plugged with cotton and then kept for 24h. After 24h, it was 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 a rotary evaporator.

Microorganisms tested: Five different microbial cultures were used in this study. Three Gram-negative bacterial strains, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*, along with one Gram-positive bacterium, *Staphylococcus aureus*, were used to check the antibacterial potential of the plant extracts. One fungal strain, *Candida albicans*, was used to assess antifungal properties. The identified microorganisms were obtained from the Pakistan Institute of Medical Sciences (PIMS) Islamabad.

Antibacterial activity: The antibacterial activity was assessed by the agar well diffusion method. Muller Hinton agar medium was prepared by using 10g/l agar dissolved in distilled water. Muller Hinton agar medium was poured into each petri plate of 20 x 90mm and allowed to cool to 45°C to solidify. Culture suspension of each bacterial strain was prepared in normal saline by adjusting turbidity to an equivalent of 0.5 McFarland standard, at which the number of cells was assumed to be 1.5×10^8 cfu/ml. Wells of 8 mm diameter were made in the agar with a sterile cork borer. Approximately 50µl of plant extract was loaded in each well. The plates were incubated at 37°C for 24h. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded.

Determination of the minimum inhibitory concentration (MIC): The MIC of the crude extracts was determined using the method described by Akinpelu & Kolawale, (2004). Five mg/ml of each of the extracts were reconstituted into nutrient broth in test tubes and the 5mg/ml was taken as the initial concentration. Four more tubes of 2ml nutrient broth were set up and 2ml of 5mg/ml of the extract were taken and used for two-fold dilution of the four tubes of nutrient broth, forming concentrations of 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml and 0.3125mg/ml. Normal saline was used to prepare turbid suspensions of the microbes. If the bacterial suspension was not of the same density as the McFarland 0.5 standard, the turbidity was decreased by adding sterile saline or increased by adding more bacterial culture. The turbidity was matched with 0.5 McFarland standard by visual comparison. At that point, the cells were assumed to be 1.5×10^8 cfu/ml. The cell suspension (0.1 ml) was inoculated into each of the tubes. All the tubes were incubated at 37°C for 24 h. The lowest concentration of the extract that showed no growth of the microbes was taken as the minimum inhibitory concentration (MIC) (Table 2).

Table 2. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of plant extracts against pathogenic bacteria and fungus.

Plants	Organisms tested at 5 different concentration mg/ml.																			
	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	+	++															

W.F = *Woodfordia fruticosa*, Q.D = *Quercus dilatata*, E.V = *Erythrina variegata*, B.L = *Berberis lycium*, A.I = *Azadirachta indica*, C.L, *Curcuma longa*, F.R = *Ficus religiosa*.
Where (**-**) = growth, (+) = light growth, (++) = moderate growth, (+++) = high growth, (y) = MIC and (x) = MBC

Determination of the minimum bactericidal concentration (MBC): The minimum bactericidal concentration (MBC) of the plant extract against the microbes was determined using the method of Spencer and Spencer, (2004). The tubes of the MIC that showed no growth of the microbes were sub-cultured on fresh nutrient agar medium. The plates were incubated at 37°C for 24h. The MBC was taken as the lowest concentration of the extract that produced no growth on the medium (Table 2).

Proximate analysis: Following the Association of Official Agricultural Chemists (Anon., 2000, 2003) methods, proximate analysis of the samples for moisture, ash, crude fibers, crude fats, proteins and carbohydrates was carried out. By using mortar and pestle, all the samples were crushed into powdered form and were used for proximate analysis. The moisture and ash were determined using a weight difference method. Fiber content was estimated from the loss in weight of the crucible and its content on ignition. The crude fat content of the samples was determined by the Soxhlet extraction method. Carbohydrate was determined by difference of the sum of the all percent compositions of moisture, ash, fat and protein from 100. The nitrogen value, which is the precursor for proteins of a substance, was determined by the micro Kjeldahl method (Pearson, 1976), involving digestions, distillation and finally titration of the sample. The nitrogen content was converted to protein by multiplying by a factor of 6.25. All the proximate values were reported in percentages.

Results

The ethnobotanical survey of medicinal plants traditionally used to treat female specific diseases conducted in Swat district collected useful information. During the present study, ethnomedicinal data on 12 plant species (perennials, biennials and annuals) were collected. Information regarding their botanical name, vernacular name, family, part used and their ethnomedicinal uses are listed in Table 1.

Each medicinal plant showed a different degree of inhibition against different microorganisms. Among the 5 pathogenic microbes used in the present study; the most susceptible microbe was *Candida albicans.* Antimicrobial screening of individual plants against fungus (*C. albicans*) showed that the microbe was sensitive to only 6 plants extracts. It was most sensitive to *Woodfordia fruticosa* (27.08 ± 2.97 SD) followed by *Berberis lycium* (22.36 ± 1.01 SD), *Quercus dilatata* (21.97 ± 2.63 SD), *Azadirachta indica* (14.46 ± 0.54), *Ficus religiosa* (12.88 ± 0.26 SD) and *Erythrina variegata* (12.57 ± 1.48 SD). The organism was found to be resistant to the rest of the plant extracts.

Plant extracts exhibiting antimicrobial potential against *C. albicans* were analyzed to determine their MIC and MBC. *Quercus dilatata* and *Ficus religiosa* showed similar MIC and MBC values of 1.25 and 2.5mg/ml, respectively. MIC of *Woodfordia fruticosa* was 2.5 and MBC was 5mg/ml. MIC and MBC of *Erythrina variegata* were 0.625 and 1.25 mg/ml. The lowest MIC (0.3125mg/ml) was shown by *Berberis lycium* (MBC 0.625mg/ml).

Among bacterial pathogens, the most sensitive was *Staphylococcus aureus*, which was inhibited by the plant extracts. The most effective plant extract that demonstrated significant activity was *Woodfordia fruticosa* (13.29 ± 0.55 SD), while *Quercus dilatata* (8.67 ± 0.52 SD), *Azadirachta indica* (7.96 ± 0.35 SD) and *Curcuma longa* (12.7 ± 1.15 SD) showed activity by producing zones of inhibition. Plant extracts exhibiting inhibitory potential against *S. aureus* possessed similar MIC values of 5mg/ml and MBC values of 2.5mg/ml.

Two plants extracts, *Woodfordia fruticosa* (13.55 ± 1.13 SD) and *Quercus dilatata* (11.22 ± 1.04 SD), produced zones of inhibition against *E. coli*. *Woodfordia fruticosa* (10.48 ± 0.66 SD) and *Quercus dilatata* (8.84 ± 0.85 SD) also showed promising activity against *Klebsiella pneumoniae.* MIC and MBC against *E. coli* and *K. pneumoniae* were similar and ranged from 5-2.5mg/ml. None of the 12 plants showed activity against *Pseudomonas aeruginosa.* The medicinal plants *Zanthoxylum alatum, Trachyspermum ammi, Artemisia vulgaris, Valeriana jatamansii* and *Hibiscus rosa–sinensis* showed no activity against the selected pathogens (Fig. 1).

In comparative assessments of the proximate analysis of various species, the results showed that *Zanthoxylum alatum* was the most significant, with a high concentration of fat and energy values compared to other species. The second highest fat and energy values were determined in *Artemisia vulgaris*, followed by *Trachyspermum ammi*, while other species like *Ficus religiosa* had a minor proportion of carbohydrates (Fig. 2). Out of 12 plant extracts, only 7 were found to be effective against *C. albicans, S. aureus, E. coli* and *K. pneumoniae. P.aeruginosa* showed no sensitivity to any of the 12 plant extracts.

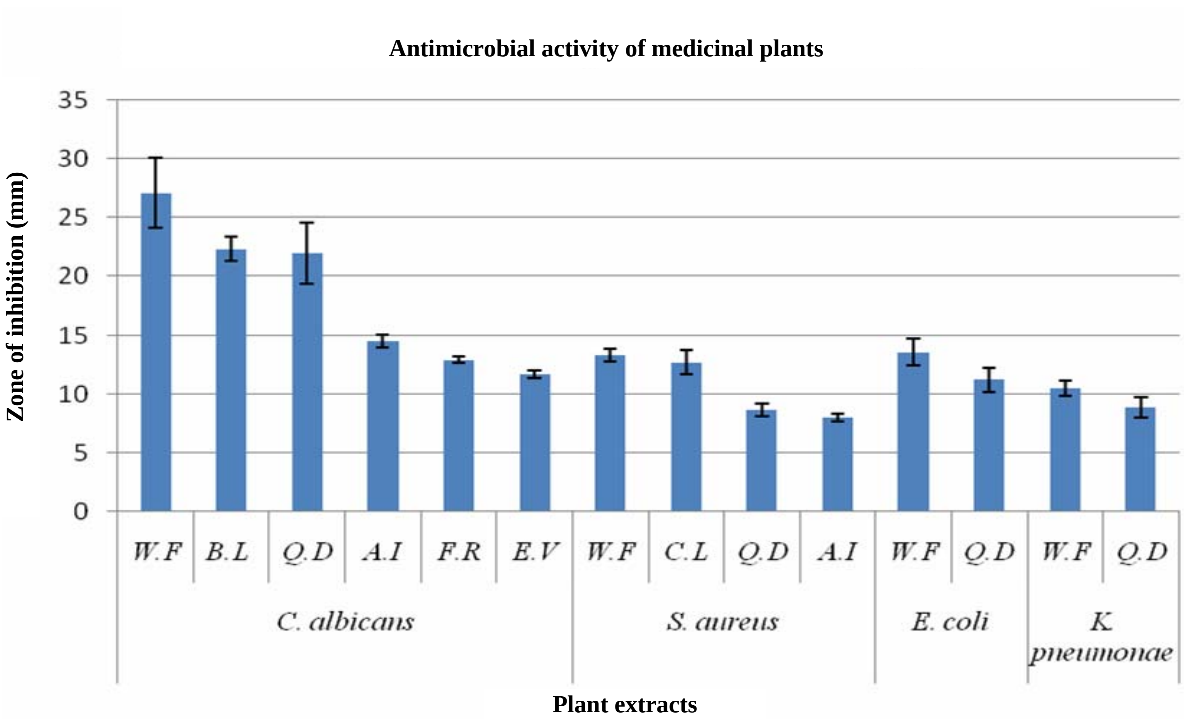

Fig. 1. Antimicrobial activity of ethnobotanically selected medicinal plants against five microorganisms.
W.F = Woodfordia fruticosa, A.I = Azadirachta indica, Q.D = Quercus dilatata, B.L = Berberis lycium, F.R = Ficus religiosa, E.V = Erythrina variegata, C.L = Curcuma longa.

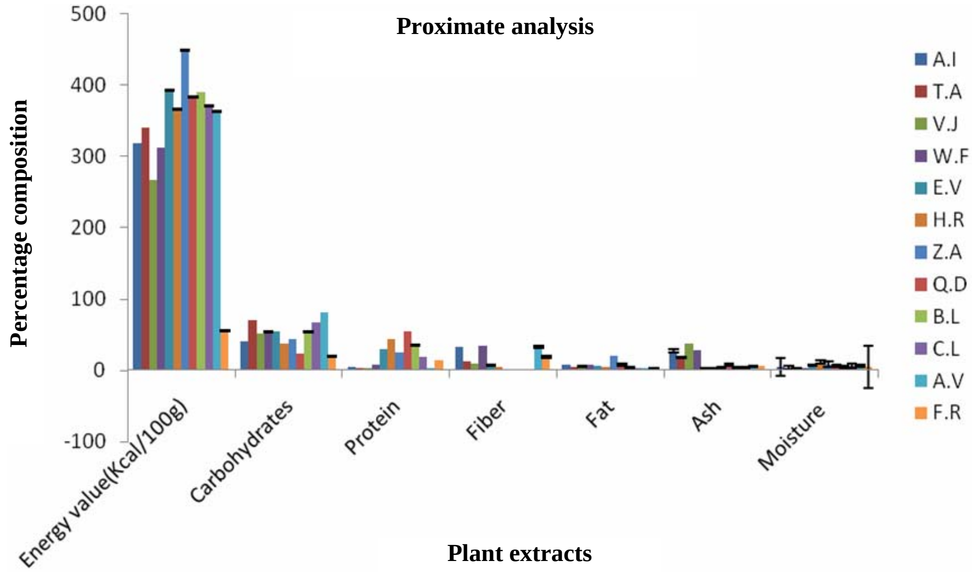

Fig. 2. A comparison of nutritional contents in the selected medicinal plant species.
W.F = Woodfordia fruticosa, A.I = Azadirachta indica, Q.D = Quercus dilatata, B.L = Berberis lycium, F.R = Ficus religiosa, E.V = Erythrina variegata, C.L = Curcuma longa, A.V = Artemisia vulgaris, Z.A = Zanthoxylum alatum, H.R = Hibiscus rosa sinenses, V.J. = Valeriana jatamansii, T.A = Trachyspermum ammi.

Discussion

Two aspects of ethnobotanically explored medicinal plants were investigated in this study. The first aspect was antimicrobial activity of the plants against selected pathogenic microbes and the second aspect was proximate analysis. The antimicrobial results demonstrated that extracts of *Quercus dilatata, Woodfordia fruticosa, Erythrina variegata, Berberis lycium, Azadirachta indica, Ficus religiosa* and *Curcuma longa* were effective against the selected pathogens. The methanolic extract of *Woodfordia fruticosa* showed activity against all four pathogenic strains investigated except *P. aeruginosa*. Maximum activity was seen against the fungus *C. albicans*, followed by bacteria *S. aureus*, *E. coli* and *K. pneumoniae*, while the most resistant bacterium was *P. aeruginosa* .Shandesh and Dinesh, (2011) reported the antimicrobial activity of the methanolic extracts of flowers of *Woodfordia fruticosa* against *E. coli*, *K. pneumoniae*, *S .aureus* and *P. aeruginosa* at a concentration of 50mg/ml. Against *C. albicans*, *Woodfordia fruticosa* did not show any activity (Shandesh & Dinesh, 2011). However, in the present study, the growth of *C. albicans* was remarkably inhibited by methanolic extracts of flowers of *Woodfordia fruticosa* at the concentration of 5mg/ml. This suggests that *Woodfordia fruticosa* is effective at a concentration of 5mg/ml rather than 50mg/ml. The antimicrobial potential of *Woodfordia fruticosa* has already been reported (Parekh & Chanda, 2007; Das *et al*., 2007). Grover *et al.,* (2011) reported that the methanolic extracts of *Azadirachta indica* exhibited both antibacterial and antifungal properties. *Quercus dilatata, Berberis lycium* and *Erythrina variegata* also showed significant inhibitory activity against *C. albicans* at concentration of 5 mg/ml. *Quercus dilatata* showed efficacy against *E. coli*, *K. pneumoniae* and *S. aureus* by producing inhibitory activity while against *P. aeruginosa*, this plant did not show any activity. Panthi and Chaudhary, (2006) reported that *Zanthoxylum alatum* showed inhibition against *S. aureus* and *P. aeruginosa*, and *Valeriana jatamansii* showed inhibition against *S. aureus* and *E. coli*. In the present study, *Valeriana jatamansii* and *Zanthoxylum alatum* did not show any inhibitory activity against selected pathogenic microbes at a concentration of 5mg/ml. *Azadirachta indica* and *Curcuma longa* also showed activity against *S. aureus*. None of the twelve plants produced a zone of inhibition against *P. aeruginosa.* The medicinal plants *Trachyspermum ammi*, *Artemisia vulgaris*, and *Hibiscus rosa-sinensis* did not show any inhibitory activity against the selected pathogens. However, negative results do not mean that bioactive constituents are absent or that these plants are not effective against the microorganisms. The minimum inhibitory concentration (MIC) of the extracts against pathogenic strains ranged from 2.5-0.3125 mg/ml and minimum bacterial concentration (MBC) from 5-0.625 mg/ml. In the treatment of infectious diseases, the use of plants is common in traditional medicine. The study showed that the methanolic extracts had wide ranges of activity on the selected pathogenic strains. The results indicate that the methanolic extracts of all selected medicinal plants may contain active components that produced zones of inhibition against the selected pathogenic strains. The medicinal plants' value lies in chemical substances that produce a definite physiological action on the pathogens. The biologically active compounds present in plants have the potential for development as medicinal agents. There is a need for the development of new antimicrobial drugs from medicinal plants to treat infectious diseases; these may be less toxic to humans and have novel mechanisms of action. The plants which showed the highest antibacterial activity can be further subjected to isolation of the therapeutic antimicrobial compounds and evaluated pharmacologically. The study confirmed that the plants that showed maximum inhibitory activity can be used for the treatment of infectious diseases.

Since most of the herbal products are used orally, knowing proximate and nutrient analysis of the herbal products and raw material used therein plays an essential role in assessing nutritional significance and health effects (Kochhar *et al.*, 2006; Pandey *et al.*, 2006; Taiga *et al.*, 2008). The results of the proximate analyses were different for each species. *Zanthoxylum alatum* had the highest concentration of fat and energy values compared to other species. It was second highest in *Artemisia vulgaris* (81%) followed by *Trachyspermum ammi* (70%), while other species like *Ficus religiosa* (19%) had a minor proportion of carbohydrates. The protein contents were highest in *Quercus dilatata* (55%), followed by *Berberis lycium* (33%). A lesser proportion was found in *Valeriana jatamansii* (2%). *Woodfordia fruticosa* (33%) had the highest percentage composition of fiber contents, followed by *Artemisia vulgaris* (32%). *Quercus dilatata* showed no fiber contents. *Hibiscus rosa-sinensis* (11%) and *Zanthoxylum alatum* (8%) had highest concentrations of moisture. Ash results demonstrated that *Valeriana jatamansii* (37%) had the highest percentage, followed by *Woodfordia fruticosa* (28%).The lowest proportion was found in *Hibiscus rosa-sinensis* (2%).

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