Pak. J. Bot., 44(3): 1103-1109, 2012.

SELECTED MEDICINAL PLANTS USED IN HERBAL INDUSTRIES; THEIR TOXICITY AGAINST PATHOGENIC MICROORAGANISMS

HINA FAZAL[1,3*], NISAR AHMAD[2], BILAL HAIDER ABBASI[2] AND NAAZ ABBASS[4]

[1]*Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320 Pakistan*
[2]*Department of Biotechnology, Quaid-i-Azam University, Islamabad 45320 Pakistan*
[3]*Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar 25100, Pakistan*
[4]*Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Lahore, Pakistan*
**Corresponding author E-mail: hina_fazalso@yahoo.com*

Abstract

Multi-drug resistant strains of fungi and bacteria are imposing the need for new drugs. Reliable natural sources with minor side effects are needed to control anti-human pathogenic invaders specially bacteria. Given the demands for natural products that are inherently safe and environmentally compatible, the advancement in antimicrobial potential has provided a better alternative to synthetic resistance antibiotics. In the present investigation such types of medicinal plants were selected for analyses that are used by local herbal practioners for multiple diseases. Thirty three extracts of *Achillea millefolium, Acorus calamus, Arnebia nobilis, Fumaria indica, Gymnema sylvestre, Origanum vulgare, Paeonia emodi, Peganum harmala, Psoralea corylifolia, Rauwolfia serpentina* and *Vetiveria zizanioides* in chloroform, ethanol and hexane were investigated for their antimicrobial potential. These extracts were tested against eight microorganisms including four gram negative bacterial strains viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi*, three gram positive bacterial strains *Staphylococcus aureus, Bacillus subtilis and Bacillus cereus* and a fungal strain viz., *Candida albicans.* Majority of the extracts showed marked antimicrobial potential against the tested microorganisms.

Introduction

The incidence of severe infections in humans caused by pathogenic microorganisms has increased globally and is a key cause of morbidity and mortality in developing countries (Al-Bari *et al*., 2006). In recent years, resistance of human pathogenic microorganisms to drug has been frequently and extensively reported (Mulligen *et al*., 1993, Davis, 1994, Robin *et al*., 1998; Tumah, 2005). These resistant species of bacteria and fungi unceasingly appearing, that is commanding the need for a long-lasting search and production of modified new drugs (Silver, 1993). The exercise of complementary and alternative medicine is upraised in most of the developing countries in response to World Health Organization directives, culminating in several pre-clinical and clinical studies. These have provided the scientific basis for the efficacy of many plants or plant tissues used in folk medicine to treat infections (Vijaya & Ananthan, 1997; Dilhuydy, 2003). Similarly maximum of crude drugs are obtained from natural sources (Plants) or semi synthetic derivatives of natural products and is commonly used in traditional systems of medicine (Sukanya *et al.,* 2009). Medicinal plants extracts and active phytochemical contents with known antimicrobial efficiency can be of pronounced implication in therapeutic cures. Plant-based antimicrobial agents may be found in plant leaves, flowers, bark, stems, roots or fruits (Ahmad *et al*., 2012; Beuchat *et al.,* 1994; Anon, 1998). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2003). Therefore, plant scientists are progressively turning their devotion to folk medicine, looking for efficient drugs against microbial infections (Benkeblia, 2004). Medicinal plants are the inexpensive and harmless alternative homes of antimicrobials (Pretorius & Watt, 2001; Sharif & Banik, 2006; Doughari *et al*., 2007). According to Mitscher *et al*., (1987), plants have shown to be a potential source for the new antimicrobial agents. Active compounds of interest present in the medicinal plants have continuously been of great concern to the scientists (Ahmad *et al*., 2010; 2011a; 2011b; 2011c; 2012). In recent years this interest to explore plants having antibacterial efficiencies for various diseases is growing (Clark & Hafford, 1993). Today, people all over the world are trying to keep away from chronic stress, pollution and synthetic drugs (Perumalsamy *et al*., 1998). Keeping man-made drugs away from the human body and nature is really difficult, their supply is hard and expensive, microorganisms that showed resistant to these synthetic agents arise day by day and an increase in their number is detected. All these negativities have brought natural agents to the front and have brought alternative and complementary medicine up to date (Dulger *et al*., 1999, Rawat & Uniyal, 2003). Medicinal plants can be used against *E. coli* which causes diarrhea due to production of eneterotoxins, *S. typhi* is the cause of gastroenteritis, enteric fever, typhoid and septicemia (Fazal *et al*., 2011a). Similarly, many cases of diarrhea caused by *P. aeruginosa* are reported by Fazal *et al*., (2011b). *K. pneumoniae* causes emphysematous prostatic abscess (Fazal *et al*., 2011a) *S. aureus, B. subtilis, B. cereus,* and *C. albicans* are causing various diseases of gastro-intestinal tract. Clinical microbiologists, biotechnologist, botanist and biochemist are interested in the field of antimicrobial plant agents; the reason is that plants phytochemical will find their way into the production of new antimicrobial drugs prescribed by physicians as some of these are already being tested in humans. Furthermore the indigenous based knowledge of these selected medicinal plants is valued for applications as antimicrobial agents and for other diseases due to its phytochemical contents (Gilani *et al*., 2007; Hussain *et al*., 2009; Gilani *et al*., 2010; Walter *et al*., 2011; Shinwari *et al*., 2009; Shinwari, 2010).

The overall objective of the current study was to evaluate the antimicrobial potential of these important medicinal plants traded frequently in the local market.

Materials and Methods

Plant materials: Various parts of eleven medicinal plants belonging to different families of angiosperms i.e., *Achillea millifolium, Acorus calamus, Arnebia nobilis, Fumaria indica, Gymnema sylvestre, Origanum vulgare, Paeonia emodi, Peganum harmala, Psoralea corylifolia, Rauwolfia serpentine* and *Vetiveria zizanioides* were procured from local market of Peshawar. These plants were authenticated by the experts of Medicinal Botanical Centre, PCSIR laboratories Complex, Peshawar Pakistan by consulting different pharmacopoeias, WHO monographs and other available literature.

Preparation of solvent extractions: For antimicrobial activity, active parts of the plants were powdered; 25 g of each powdered materials were extracted with hexane; ethanol and chloroform. Briefly, 150 ml of each solvent were separately added to thimble containing powdered plant. For best extraction a Soxhlet extractor was used for 48 h. After Soxhlet extraction, each extract was concentrated through rotary evaporator. After thorough evaporation of solvent, plant extracts were stored at 4^{o}C in airtight small bottles after accurate weighed. For stock solution preparation, 100mg of extract was dissolved in DMSO (0.5 ml) as a solvent and were used as the test extracts for antimicrobial assay.

Test microorganisms: Pathogenic strains of bacteria viz., *Staphylococcus aureus (*ATCC # 6538), *Pseudomonas aeruginosa* (ATCC # 9721), *and Escherichia coli* (ATCC # 25922) were obtained from PCSIR Laboratories, Lahore. Clinical isolate of *Klebsiella pneumoniae, Salmonella typhi, Bacillus subtilis*, *Bacillus cereus* from Microbiology laboratory, Quaid-i-Azam, Islamabad and a fungal strain *Candida albicans* was obtained from Hayatabad Medical Complex, Peshawar . These microorganisms were maintained on nutrient agar medium at 4^{o}C.

Determination of antibacterial activity: Antibacterial activity of solvent extracts; hexane, ethanol and chloroform were determined by Disc-diffusion method on nutrient agar medium in terms of diameters of inhibition zone (Bakht *et al.,* 2011) against four gram negative, three gram positive and one fungus pathogens. Nutrient agar media (2.8 g 100 ml^{-1}) and nutrient broth (1.3 g 100ml^{-1}) were prepared by dissolving in distilled water. The nutrient broth was dispensed in test tubes (7-8 ml) and flasks (20-25ml). All of the media and apparatus were sterilized at 121^{o}C and 15 psi pressure for 20minutes. Agar media was poured into Petri plates and incubated overnight at 37ºC to check any contamination. The stock cultures were freshened by streaking on fresh agar plates and incubated overnight. Next day these cultures were inoculated into flasks containing broth media. They were then kept in the shaking water bath (Model; GLSC-SBR-04-28) for 16 hrs at 200 rpm at 37ºC.

On the following day microbial cultures were standardized in test tubes by comparing with 0.5 McFarland (turbidity) Standard. 100 µl of standardized microbial cultures were spread on each nutrient agar plate. These impregnated plates were then kept for absorption (15 minutes) in a refrigerator. Whattman filter paper-I discs were placed on these agar media plates.

The stock solutions of all the extracts in different concentrations of 1, 2 and 3 mg disc^{-1} in 6, 12 and 18 µl volume were applied on these discs in triplicates. Antibiotics including Azithromycin, Ciprofloxacin, and Clotrimazole were applied as positive control on separate plates against gram positive bacteria, gram negative bacteria and *Candida albicans* respectively, while DMSO used for making stock solution was applied as negative controls. These plates were then incubated at 37ºC overnight. Antimicrobial potential was recorded for each extract in terms of mm of zones of inhibition around each disc.

Results

Many microorganisms, which cause damage to human health, exhibit drug resistance due to inadequate use of antibiotics. Thus, there is a need for the discovery of new antimicrobial agents from natural sources, including plants. To search for traditionally used medicinal plants with potent antimicrobial properties against gram negative and gram positive bacteria, eleven medicinal plants were screened in different solvents which were very effective against some bacteria and showed the highest activity. The curative properties of medicinal plants are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins and sterols. The extracts of root, stem, bark and seeds of different plants have revealed the presence of these metabolites. Thus the preliminarily screening tests are useful in the detection of the bioactive principles and may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds (Mallikharjuna *et al.,* 2007).

Majority of the plant extracts were effective against most of the bacterial and fungal species and a few showed the highest activity.

Hexane extracts of aerial parts of *Achillea millifolium* showed best activity against *K. pneumoniae* (17 mm), *S. aureus* (16.5 mm) and *B. subtilis*, (14.8 mm), while ethanol extracts showed best activity against *E. coli* (19 mm), followed by *P. aeruginosa* (18 mm), *S. typhi* (16 mm) and *S. aureus* (15 mm). Chloroform extract of *Achillea millifolium* showed (13.5 mm) activity against *B. subtilis* while anticandidal activity has zero results. Whereas hexane and ethanol extracts had shown (10.5 mm) and (12.6 mm) activity against *C. albicans* (Fig. 1).

Ethanolic extracts of rhizome of *Acorus calamus* had shown best inhibition against *P. aeruginosa* (20 mm), followed by *Salmonella typhi* (18.5 mm) and *C. albicans* (17.7). Hexane and ethanolic extracts of *Acorus calamus* were equally active against *B. subtilis* and (15.5 mm) zone of inhibition was recorded. Whereas, hexane and chloroform extracts of *Acorus calamus* have shown no activity against *C. albicans* and *K. pneumoniae* in the present study. Others results of antimicrobial activities of *Acorus calamus* are given in (Fig. 2).

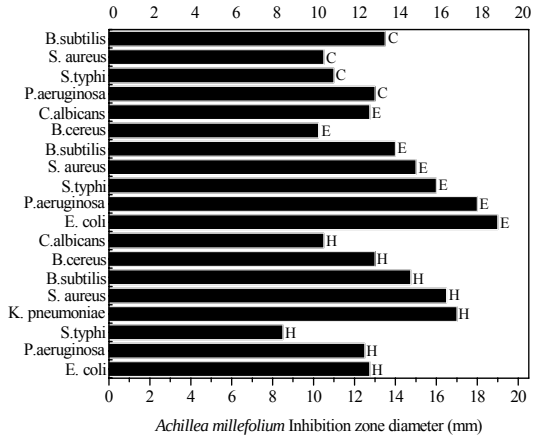

Fig. 1. Antimicrobial potential of hexane (H), ethanol (E) and chloroform (C) extracts of *Achillea millefolium* against different bacteria and fungus. Values are the means of three replicates.

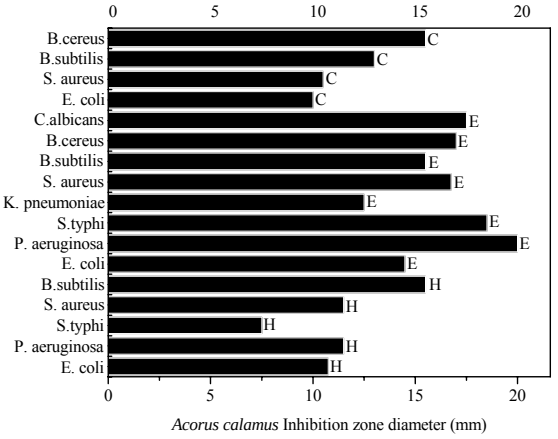

Fig. 2. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Acorus calamus* against different bacteria and fungus. Values are the means of three replicates.

In case of *Arnebia nobilis* root and root bark, the ethanolic extract was the most effective anticandidal extract, showing (17.8 mm) inhibitory zone, while the other two extracts have similar effects viz., (14 mm) in each case. While hexane, chloroform and ethanol extracts have shown best activities against *B. subtilis* (16.8, 17.5 and 14.5 mm). Chloroform extracts against *P. aeruginosa* have shown (16 mm) and against *B. cereus* (15.3 mm) zones (Fig. 3).

Ethanol extract of aerial parts of *Fumaria indica* have inhibitory activities against *S. aureus* (17.5 mm) *S. typhi* (16 mm) and *K. pneumoniae* (15 mm), While hexane and chloroform extracts have 15.8 mm and 16 mm activity against *B. subtilis* and *P. aeruginosa* respectively. Whereas chloroform and ethanol extracts have 15.8 mm and 11.5 mm inhibitory activity against *C. albicans* respectively (Fig. 4). Previously, Parekh & Chanda (2007) determined the antimicrobial activities of aqueous and ethanolic extracts of seeds of *Fumaria indica* against *Klebsiella pneumonia* and *Proteus mirabilis* and given positive results.

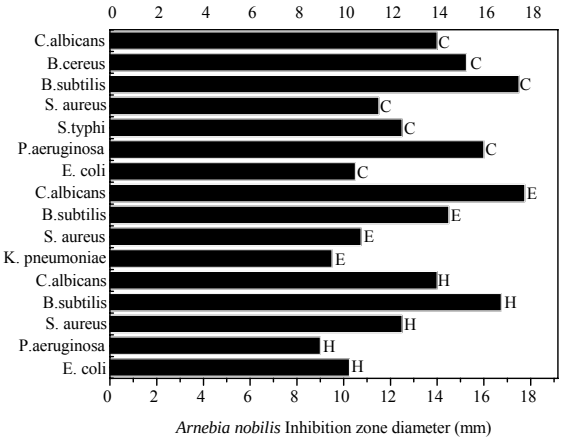

Fig. 3. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Arnebia nobilis* against different bacteria and fungus. Values are the means of three replicates.

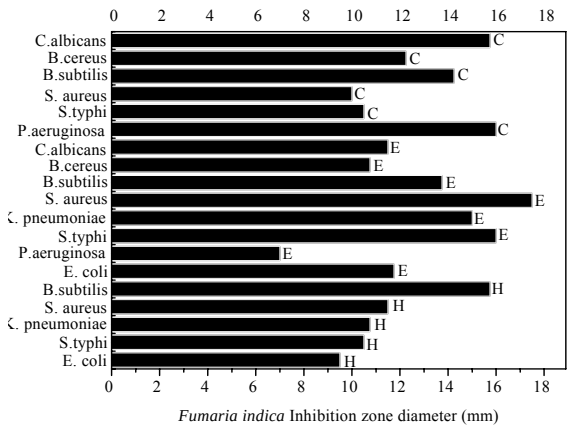

Fig. 4. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Fumaria indica* against different bacteria and fungus. Values are the means of three replicates.

The hexane extract of *Gymnema sylvestre* leaves was most effective against *B. cereus* and formed (17.5 mm) zone of inhibition, while (16.5 mm) in the case against *B. subtilis.* Ethanol extract had activities against *S. aureus* (15.5 mm) and chloroform against *B. subtilis* (16.5 mm). However chloroform extract of *Gymnema sylvestre* was most effective against fungus and formed 12.3 mm zone (Fig. 5).

All the three extracts of aerial parts of *Origanum vulgare* have shown anticandidal activity (chloroform 13.5 mm, hexane 12.5 and ethanol 9.5 mm). The most effective was the hexane extract which formed 19.5 mm zone of inhibition against *P. aeruginosa*. The ethanolic extract formed (19 mm), chloroform, (16.8 mm) and hexane (16 mm) zones against *B. subtilis.* However the ethanol extract was also effective against *S. typhi* (15.5 mm) (Fig. 6).

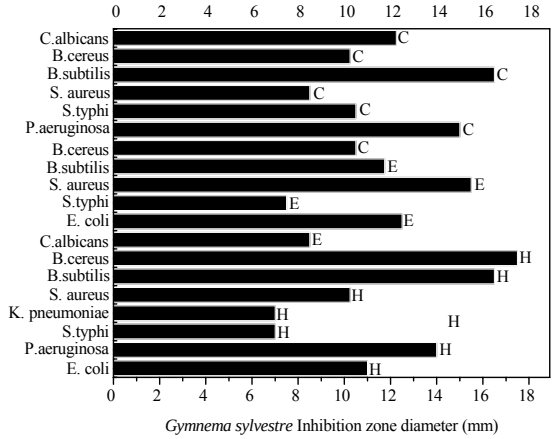

Fig. 5. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Gymnema sylvestre* against different bacteria and fungus. Values are the means of three replicates.

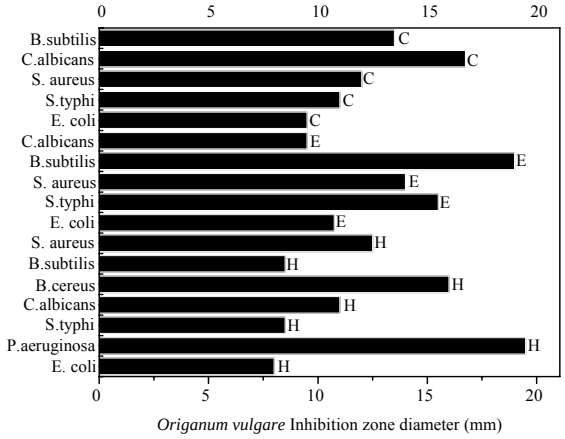

Fig. 6. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Origanum vulgare* against different bacteria and fungus. Values are the means of three replicates.

Paeonia emodi chloroform extract showed best activity against *B. cereus* (17.8 mm) and 16 mm activity against *P. aeruginosa,* whereas the ethanol and hexane extracts had best inhibitory activities against *S. typhi* (17 mm) and *B. subtilis* (15 mm) respectively. The best anticandidal extract of *Paeonia emodi* was that of ethanol forming 17 mm zone followed by chloroform (11 mm) while hexane extract had no activity against fungus (Fig. 7).

According to Arshad *et al*., (2008), *Peganum harmala* can minimize *E. coli* infection in poultry. In present studies the ethanolic and hexane extracts were equally effective against *E. coli*, forming 10.8 mm inhibitory zones. *P. harmala* extracts in ethanol and hexane exhibited best activity against *K. pneumonia* (25.8 mm and 22.5 mm) respectively. The chloroform extract was best effective against *S. aureus* (24.5 mm). However (18.3 mm) zone of inhibition was observed against fungus by ethanolic extract of *P. harmala*, whereas (10.8 mm) and (9 mm) anticandidal zones were formed by chloroform and hexane extracts respectively (Fig. 8).

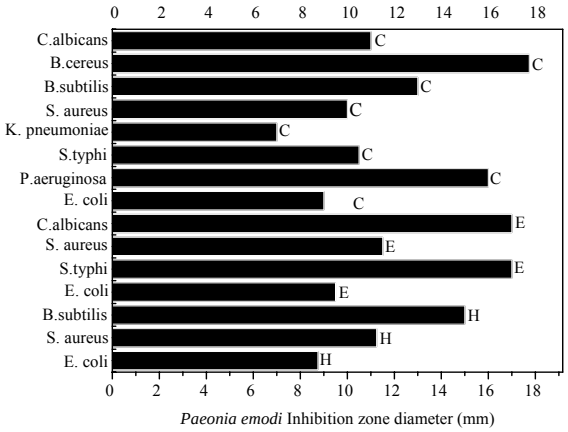

Fig. 7. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Paeonia emodi* against different bacteria and fungus. Values are the means of three replicates.

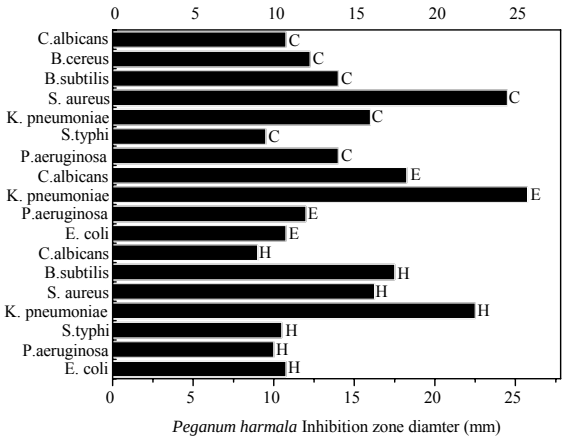

Fig. 8. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Peganum harmala* against different bacteria and fungus. Values are the means of three replicates.

Hexane and ethanol extracts of seeds of *Psoralea corylifolia* formed best inhibitory zones against *B. subtilis* (16 mm and 17 mm) respectively, 15 mm and 14.3 mm zones of inhibition were observed against *K. pneumonia* and *S. aureus.* Chloroform extracts have shown no anticandidal activity; however ethanol and hexane extracts were effective against *C. albicans* (7.5 mm and 17 mm) (Fig. 9). According to Anwar (2006) no significant activity was recorded in *E. coli* and *K. pneumonia* and showed some activity against *S. aureus.* The extracts also have positive antifungal activity against *C. albicans.*

Ethanolic extract of roots of *Rauwolfia serpentina* exhibited the best anticandidal activity 22.5 mm, while hexane, ethanol and chloroform have also shown good inhibitory effects against *B. subtilis* (17 mm, 16.5 mm and 16.3 mm) respectively (Fig. 10). In contrast Jigna *et al.,* (2005) performed the antibacterial activity of ethanolic extracts of leaves of *R. serpentina.* According to his observations 12 mm, 10 mm, 15 mm, 10 mm zones of inhibition were formed against *B. cereus, B. subtilis, K. pneumonia and P. aeruginosa* respectively, while no zone was formed against *S. typhi* and *S. aureus.* It also showed anticandidal activity.

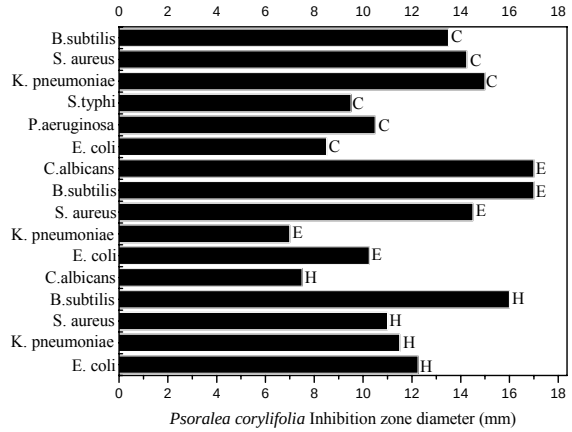

Fig. 9. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Psoralea corylifolia* against different bacteria and fungus. Values are the means of three replicates.

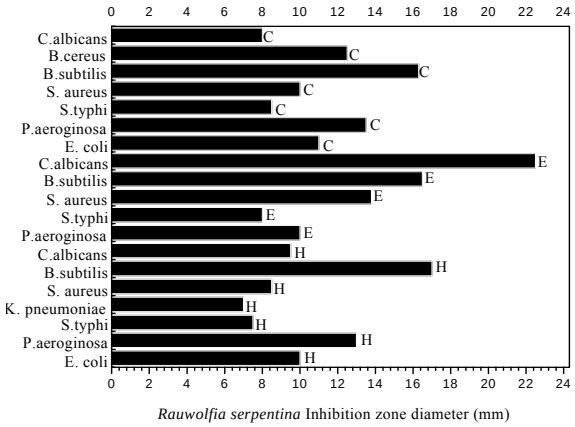

Fig. 10. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Rauwolfia serpentina* against different bacteria and fungus. Values are the means of three replicates.

In case of *Vetiveria zizanioides,* the aerial parts were extracted with different solvents and checked for antimicrobial potential. The best antibacterial extracts were ethanolic and chloroform extracts exhibiting (20 mm) and (19.5 mm) zones of inhibition against *P. aeruginosa,* seconded by chloroform and ethanolic extracts (18 mm) and (17 mm) respectively against *B. subtilis.* However the ethanol and chloroform extracts were also effective against *S. aureus* and *B. cereus* (16.5 mm and 15 mm) respectively, but the hexane extracts have no effect against fungus, whereas chloroform and ethanol extracts formed (12.5 mm) and (11.5 mm) zones against *C. albicans* (Fig. 11).

Discussion

As modern medicines are quite expensive in the developing countries, thus investigation on antimicrobial activities of ethno medicinal plants is the call of the day. These phytochemicals will find their way in the arsenal of antimicrobial drugs prescribed by physicians.

In the present studies 33 different extracts viz., (Chloroform, Ethanol and Hexane) of 11 medicinal plants were assessed for their antimicrobial potentials. Extracts were more effective against bacteria than fungi. All of the plant extracts tested for antibacterial potential showed varying degree of antibacterial activities against the Gram-positive, Gram-negative bacterial species and fungus (Figs. 1-11). The best activity was recorded for the ethanolic extract of seeds of *Peganum harmala* which formed 25.8 mm zone of inhibition against K. *pneumonia,* followed by the chloroform extract of the same plant which was effective against *S. aureus* and formed 24.5 mm zone. The hexane extract of *P. harmala* was best effective against *K. pneumonia* and ethanolic extract of root of *R. serpentina* against *C. albicans,* each forming 22.5 mm zone as shown in Fig. 10. This could be attributed to the concentration of the active substance causing the inhibitory effect which could have been higher in these parts of plants. In present study ethanolic extract of *Achillea millifolium* showed the best activity of 19 mm against *E. coli* and 15 mm against *S. aureus* as shown in Fig. 1. Previously Souza *et al.,* (2006) evaluated the antibacterial activity of ethanolic extract of *A. millefolium* leaves against the same bacteria but exhibited negative results. Similar antimicrobial activity was also reported by Fazal *et al*., (2011a; 2011b).The antimicrobial activities of *Acorus calamus* are evaluated by different workers, Kar & Jain (1971) reported that the essential oil of *A. calamus* has inhibitory effect against *B. subtilis* and *S. aureus.* According to the study of Phongpaichit *et al.,* (2005), crude methanolic extract of *A. calamus* showed 9.2 mm and 6.8 mm inhibition zone against *S. aureus* and *E. coli* respectively, while no zone was formed against *P. aeruginosa* and *C. albicans* showed 9.3 mm inhibitory zone. While the present results showed that ethanolic extracts of *A. calamus* had shown inhibitory activities against all the tested organisms viz., *E. coli* (14.5 mm), *P. aeruginosa* (20 mm), *S. typhi* (18.5 mm) and *S. aureus* (16.8 mm) as shown in Fig. 2. Ethanolic extract of aerial parts of *Fumaria indica* exhibited inhibitory activities against *S. aureus* (17.5 mm) *S. typhi* (16 mm) *and K. pneumoniae* (15 mm) as shown in Fig. 4. Parekh & Chanda (2007) evaluated the antimicrobial activities of aqueous and ethanolic extracts of seeds of *Fumaria indica* against *K. pneumonia* and *Proteus mirabilis*. Sheng *et al.,* (2004) isolated three prenyiflavonoids, namely corylifols A-C (1-3) from the seed of *P. corylifolia* that showed antibacterial activity against *S. aureus* and *S. epidermid*is. The present study also reflects that all the three extracts of *P. corylifolia* were effective against *S. aureus* while all of the three extract have zero activity against *B. cereus. Rauwolfia serpentina* have maximum inhibitory activity of 20 mm in ethanol extract against *P. aeruginosa.* All the three extracts of *Origanum vulgare* have no activity against *K. pneumonia* while 19 mm zone of inhibition was observed when ethanolic extract of *Origanum vulgare* was used against *B. subtilis* as shown in Fig. 6. *Vetiveria zizanioides* chloroform extract exhibited 18 mm against *B. subtilis. Gymnema sylvestre* hexane extract and *Paeonia emodi* chloroform extract had maximum inhibitory activity of 17.5 mm and 17.8 mm against *B. cereus* as shown in Fig. 5 and 7 respectively.

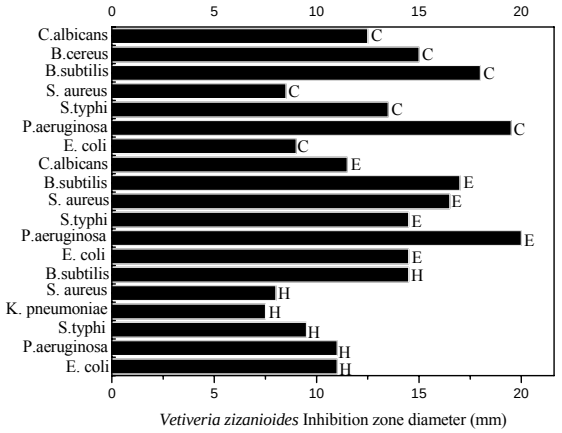

Fig. 11. Antimicrobial potential of hexane, ethanol and chloroform extracts of *Vetiveria zizanioides* against different bacteria and fungus. Values are the means of three replicates.

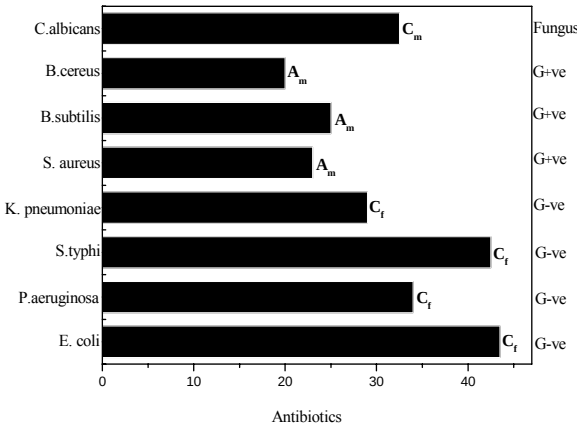

Fig. 12. Antimicrobial potential of standard antibiotics against different bacteria and fungus. Values are the means of three replicates.

The use of hexane, ethanol and chloroform as extracting solvents proved to be more efficient in extracting the active compounds .This could be ascribed to the alcoholic aqueous environment which promotes easy extraction as reported by Nostro *et al.,* (2000). The antibacterial activities of the hexane, ethanol and chloroform extracts compared favorably with that of two standard antibiotics (Ciprofloxacin and Azithromycin) and have appeared to be broad spectrum as its activities were independent of gram reaction, whereas the three extracts were also compared with standard antibiotics Clotrimazole against fungus *C. albicans* (Fig. 12).

Conclusion

The present results (Figs. 1-11) clearly demonstrated that most of the plants had great potential for antibacterial activities against seven bacterial strains and one fungal strain tested. The antibacterial activities of three extracts of 11 species against pathogenic microorganisms were examined in the present study and their potency was compared with each other and antibiotics by the presence or absence of inhibition zones and zone diameter. The tested plant showed the highest activity against certain bacteria, indicating that these plants are good source of antibiotics for the treatment of certain bacterial diseases. However, further experimental and research efforts on these plants and their extracts are needed to specify their pharmacological implication. Other details needed will include tests using other solvents, infrared spectrometry, MS and NMR of the constituents of the extracts.

References

Ahmad, N., H. Fazal, B.H. Abbasi and M. Iqbal. 2011a. *In vitro* larvicidal potential against *Anopheles stephensi* and antioxidative enzyme activities of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous*. *Asian Pacific Journal of Tropical Medicine,* 4: 169-175.

Ahmad, N., H. Fazal, B.H. Abbasi, I.U. Rahman, S. Anwar, M.A. Khan, A. Basir, H. Inayat, R. Zameer, R.S.A. Khalil and K.Y. Khan. 2011b. DPPH-scavenging antioxidant potential in regenerated tissues of *Stevia rebaudiana*, *Citrus sinensis* and *Saccharum officinarum*. *Journal of Medicinal Plants Research,* 5: 3293-3297.

Ahmad, N., H. Fazal, B.H. Abbasi, M. Rashid, T. Mahmood and N. Fatima. 2010. Efficient regeneration and antioxidant potential in regenerated tissues of *Piper nigrum* L. *Plant Cell, Tissue and Organ Culture,* 102: 129-134.

Ahmad, N., H. Fazal, B.H. Abbasi, S. Anwar and A. Basir. 2012. DPPH free radical scavenging activity and phenotypic difference in hepatoprotective plant (*Silybum marianum* L.). *Toxicology and Industrial Health doi:* 10.1177/0748233712436637.

Ahmad, N., H. Fazal, M. Ayaz, I. Mohammad and L. Fazal. 2011c. Dengue fever treatment with *Carica papaya* leaves extracts. *Asian Pacific Journal of Tropical Biomedicine,* 330-333.

Al-Bari, M.A., M.A. Sayeed, M.S. Rahman and M.A. Mossadik. 2006. Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis* a novel species collected in Bangladesh. *Res. J. Med. Sci*., 1: 77-81.

Anonymous. 1998. Naturally occurring antimicrobials in food. *Task Force Report.,* nr 132. Ames, Iowa: CAST.

Anwar, M. 2006. The pharmacognostic and pharmacological studies on medicinal valued herbal drugs, *Erythrina variegata* var *orientalis, Matricaria chamomilla, Psoralea corylifolia and Chenopodium album.* Ph.D thesis, Department of Pharmacognosy, University of Karachi, Pakistan.

Arshad, N., C. Neubauer, S. Hasnain and M. Hess. 2008. Efficacy of *Peganum harmala* Extract. Immunology, Health and Disease, *Poultry Science*, 87: 240-249 doi:10.3382/ps. 2007-00341.

Bakht, J., H. Ali, M.A. Khan, A. Khan, M. Saeed, M. Shafi, A. Islam and M. Tayyab. 2011. Antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* by disc diffusion method. *African Journal of Biotechnology,* 10(85): 19825-19835.

Benkeblia, N. 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and Garlic (*Allium sativum*). *Lebensmwiss u-Technol.,* 37: 263-268.

Beuchat, L.R., R.W. Brackett and M.P. Doyle. 1994. Lethality of carrot juice to *L. monocytogenes* as affected by pH, sodium chloride, and temperature. *J. Food Prot.,* 57: 470-4.

Clark, A.M. and C.D. Hafford. 1993. Discco and development of novel prototype antibiotics for opportunistic infection related to acquired immunodeficiency syndrome. In; human medical agents from plants. American Chemical Society (ACS Symposium series), Washington D.C: 228-241.

Davis, J. 1994. Inactivation of antibiotic and the dissemination of resistance genes. *Science,* 264: 375-382.

Dilhuydy, J.M. 2003. Patients attraction to complementary and alternative medicine (CAM): a reality which physicians can neither ignore nor deny. *Bull. Cancer,* 90: 623-628.

Doughari, J.H., A.M.E. Mahmood and S. Manzara. 2007. Studies on the antibacterial activity of root extracts of *Carica papaya* L. *Afri. J. Microbiol. Res.,* 037-041.

Dulger, B., M. Ceyhan, M. Alitsaous and E. Ugurlu. 1999. *Artemisia absinthium* L. (Pelin)'un antimikrobiyal aktivitesi. *Turk. J. Biol*., 23: 377-384.

Fazal, H., N. Ahmad and M.A. Khan. 2011b. Physico-chemical, phytochemical evaluation and DPPH-scavenging antioxidant potential in medicinal plants used for herbal formulation in Pakistan. *Pak. J. Bot.,* 43: 63-67.

Fazal, H., N. Ahmad, I. Ullah, H. Inayat, L. Khan and B.H. Abbasi. 2011a. Antibacterial Potential in *Parthenium hysterophorus, Stevia Rebaudiana* and *Ginkgo Biloba*. *Pak. J. Bot.,* 43: 1307-1313.

Gilani, S.A., A. Kikuchi, Z.K. Shinwari, Z.I. Khattak and W.N. Watanabe. 2007. Phytochemical, pharmacological, and ethnobotanical studies of *Rhazya stricta* Decne. *Phytother. Res.,* 21, 301-307.

Gillani, S.A., Y. Fujii, Z.K. Shinwari, M. Adnan, A. Kikuchi and K.N. Watanabe. 2010. Phytotoxic studies of medicinal plant species of Pakistan. *Pak. J. Bot.,* 42(2): 987-996.

Hussain, J., H. Hussain, Z.K. Shinwari, I. Ahmad, S.T. Hussain and V. Ahmad. 2009. Antibacterial activity of the chemical constituents from *Ranunculus laetus. Chemistry of Natural Compounds,* 45(5): 720-721.

Jigna, P., N. Rathish and C. Sumitra. 2005. Preliminary screening of some folklore medicinal plants from Western India for potential antimicrobial activity. *Indian J. Pharmacol*., 37(6): 408-409.

Kar, A. and S.R. Jain. 1971. Antibacterial evaluation of some indigenous medicinal volatile oils. *Qual., Plant.Mater. Veg*., 10(3): 231-237.

Mallikharjuna, P.B., L.N. Rajjana, Y.N. Seetharam and G.K. Sharanasappa. 2007. Phytochemical studies of *Strychnos potatorum* L.f.- A Medicinal Plant. *E-Journal of Chemistry*, 4(4): 510-518.

Mitscher, L.A., S. Drake, S.R. Golloapudi and S.K. Okwute. 1987. A modern look at folkloric use of antiintective agents. *Journal of Natural Products*, 50: 1025-1040.

Mulligen, M.E., K.H. Murry-Leisure, B.S. Ribner, H.C. Standiford, J.F. John, J.A. Karvick, C.A. Kauffman and V.L. Yu. 1993. Methicillin resistant *Staphylococcus aureus*. *The American Journal of Medicine,* 94: 313-328.

Nostro, A., M.P. Germano, V.D. Angelo, A. Marino and M.A. Cannatelli. 2000. Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity. *Lett. Appl. Microbiol.,* 30: 379-384.

Parekh, J. and S. Chanda. 2007. *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. *African Journal of Microbiology Research*, 1(6): 092-099.

Perumalsamy, R., S. Ignacimuthu and A. Sem. 1998. Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharm*., 62: 173-182.

Phongpaichit, S., N. Pujenjob, V. Rukachaisirikul and M. Ongsakul. 2005. Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn. *Songklanakarin J. Sci. Technol.*, 27(Suppl. 2): Thai Herbs.

Pretorius, C.J. and E. Watt. 2001. Purification and identification of active components of *Carpobrotus edulis* L. *J. Ethnopharmarcol*. 76: 87-91.

Rawat, R.B.S. and R.C. Uniyal. 2003. National medicinal plants board committed for overall development of the sector. *Agro. Bios. Med. Plants,* 1: 12-16.

Robin, E.H., W. Anril, M. Alexander, M. Loeto and K. Keith. 1998. Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* Type b in children under 5 years of age in Botswana. *International Journal of Infectious Diseases,* 3(1): 18-25.

Rojas, R., B. Bustamante and J. Bauer. 2003. Antimicrobial activity of selected Peruvian medicinal plants. *J. Ethanopharm.,* 88: 199-204.

Sharif, M.D.M. and G.R. Banik. 2006. Status and utilization of medicinal plants in Rangamati of Bangladesh. *Res. J. Agric. Biol. Sci.,* 2(6): 268-273.

Sheng, Y., C.Q. Fan, Y. Wang, L. Dong and J.M. Yue. 2004. Antivacterial prenylflavone derivatives from *Psoralea corylifolia* and their structure. Activity relationship study. Biooranii is medicinal chemistry, 12: 4387-4392.

Shinwari, Z. K., I. Khan, S. Naz and A. Hussain. 2009. Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases. *African Journal of Biotechnology*, 8(24): 7082-7086.

Shinwari, Z.K. 2010. Medicinal plants research in Pakistan. *J. Med. Pl. Res*., 4(3): 161-176.

Silver, L.L. 1993. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrob. Agents Chemother.,* 37: 377-383.

Souza, T.M., V.L.B. Rangel, R.C.L. Rr. Pietro, L.E. Santos and R.R.D. Moreira. 2006. Phytochemical screening of *Achillea millefolium* harvested at Araraquara-SP. Rev. *Bras. Pl. Med., Botucatu,* 8, n.esp: 151-154.

Sukanya, S.L., J. Sudisha, P. Hariprasad, S.R. Niranjana, H.S. Prakash and S.K. Fathima. 2009. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology,* 8(23): 6677-6682.

Tumah, H. 2005. Fourth-generation cephalosporins: *In vitro* activity against nosocomial gram-negative bacilli compared with β-lactam antibiotics and ciprofloxacin. *Chemotherapy,* 51(2-3): 80-85.

Vijaya, K. and S. Ananthan. 1997. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. *J. Altern. Complement Med.*, 3: 13-20.

Walter, C., Z. K. Shinwari, I. Afzal and R.N. Malik. 2011. Antibacterial activity in herbal products used in Pakistan. *Pak. J. Bot.*, 43(Special Issue): 155-162.

(Received for publication 17 March 2011)