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

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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 drugs 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, is that 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 number 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 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 enterotoxins, 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; Shiwari et al., 2009; Shiwari, 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 zizanoides 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°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°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°C and 15 psi pressure for 20minutes. Agar media was poured into Petri plates and incubated overnight at 37°C 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 37°C.

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. Whatman 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 37°C 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 extracts of Achillea millifolium showed (13.5 mm) activity against B. subtilis while antacidial 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).
In case of *Arnebia nobilis* root and root bark, the ethanolic extract was the most effective antifungal 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.

The hexane extract of *Gymnema sylvestre* leaves was most effective against *B. cereus* (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 antifungal 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).
against *S. aureus*. 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 antifungal activity 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) antifungal zones were formed by chloroform and hexane extracts respectively (Fig. 8).

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 antifungal 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*.
have no effect against fungus, whereas chloroform and ethanol extracts formed (12.5 mm) and (11.5 mm) zones of inhibition against K. pneumonia and S. aureus. These phytochemicals will find their way in the arsenal of antimicrobial drugs prescribed by physicians. 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 millefolium 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 prenylfлавonoids, namely corylifols A-C (1-3) from the seed of P. corylifolia that showed antibacterial activity against S. aureus and S. epidermidis. 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 ethanolic 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. 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.
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**


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.


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