Screening of selected medicinal plants from Swabi for antimicrobial activities

ABSTRACT

The present study was conducted to test and compare the antibacterial activity of thirty extracts prepared from five medicinal plants namely Eryngium caeruleum (arial parts), Malvestrum tricuspedatum (arial parts), Tulipa stellata (arial parts), Ranunculus muricatus (arial parts) and Conyza bonariensis (arial parts). The activity of these plant extracts were tested against three Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis and Bacillus atropeus) and four Gram-Negative bacteria (Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi) using disc diffusion method. The efficacy of extracts was compared to the commercially prepared antibiotic discs. Majority of the extracts showed the highest antimicrobial potential against microorganisms. The most active plant among the selected medicinal plants was T. stellata (TS), at the concentration of 2 mg/12 µl, its crude extract was more active against E. coli and showed maximum activity (28.3 mm). M. tricuspedatum (MT) showed significant zone of inhibition in ethyl acetate extract against E. coli. The hexane extract of R. muricatus (RM) at the concentration of 2 mg/12 µl showed highest activity (22.5 mm) against B. subtilis.

Key words: Antimicrobial activity, agar disc diffusion method, Swabi medicinal plants.

INTRODUCTION

Nature has been a valuable source of medicine and has helped human in the maintenance of his health since time immemorial. The world has rich wealth of medicinal plants. Without the plant kingdom, humans cannot survive on this earth because the plant products and their active constituents play an important role in their survival (Balunas and Kinghorn, 2005).

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The green medicines are healthier and safer than synthetic ones. A number of herbal medicines are used for the management of various diseases. They have minimal toxicity, are cost effective, pharmacologically active and provide an easy remedy for many human ailments as compared with the synthetic drugs which are a subject of adulteration and side effects (Wang et al., 2007). Medicinal plants are considered as an important source of new chemical substances with potential therapeutic effects and that can be used to treat chronic and infectious diseases. Natural products from plants can be templates for new drug development and have many interesting biological activities such as antidiabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic, gastro protective effects etc. All parts of plant can be used as herbal medicine such as leaf, stem, flower, bark, fruit, Peel, rhizome, essential oil, latex, bud, etc. The medicinal values of plants lie in phytochemicals present in them that produce a definite physiological action on the human body (Woodford and Livermore, 2009). The development and spread of resistance to currently available antibiotics is a global concern. The crucial fact about the treatment of bacterial infections is the ability of bacteria to develop resistance to antimicrobial agents (Sengupta and Chattopadhyay, 2012).

Indiscriminate use of antibiotics appears to promote
development of pathogens showing resistance to multiple drugs. The multiple drug resistance not only increases morbidity and mortality but also increase expenditure on patient management and implementation of infection control measures (Woodford and Livermore, 2009). There is continuous and urgent need to discover new antimicrobial compound with diverse chemical structures and novel mechanism of action. Therefore, action must be taken to control the use of antibiotics, to better understand the genetic mechanism of resistance and to continue studies of developing new drugs. At present, clinically important microorganisms are characterized not only by single drug resistance, but also by multiple drug resistance. It is now common practice to use a combination of two or more antibiotics with different mode of action in an effort to prevent the expansion of antibiotic resistance and improve the outcome of therapy. Synergistic effects of antimicrobial agents may be due to certain complex formations that become more effective than individual in the inhibition of microorganisms (Mahboubi and Bidgoli, 2010).

Medicinal plants play a vital role in the healthcare provision of numerous countries and they have been used as a drug treatment of major human diseases (Latheef et al., 2008). These plants have been classified by World Health Organization (WHO) as the best source of drugs (Wolabi et al., 2007). In fact, important drugs, such as morphine, codeine, digitoxin, and quinine, are all of plant origin (Osman et al., 2013).

**MATERIALS AND METHODS**

**Plant materials**

The aerial parts of *Conyza bonariensis*, *Tulipa stellata*, *Ranunculus maricatus*, *Eryngium caeruleum* and *Malvestrum tricaspidatum* were collected during the flowering season from District Swabi Pakistan. The specimens were deposited in the herbarium of PCSIR (Pakistan Council of Scientific and Industrial Research) laboratories, Peshawar Khyber Pakhatoonkhwa with voucher specimen numbers.

**Extraction and fractionation**

The plants materials after collection shade dried for 18 days (Philip et al., 2009). The dried plants materials were then powdered in grinder machine. The powdered material was soaked in methanol for 24 h with shaking. Methanol was filtered using vacuum rotary evaporator (Philip et al., 2009). The process was repeated thrice. As a result, a crude Methanolic extract was obtained. A solution of the Methanolic extract was prepared in distilled water with vigorous shaking. Then its liquid - liquid extraction was done using hexane, chloroform, ethyl acetate and butanol respectively. Dry extract was dissolved in distill water (200 ml) and was transferred to separating funnel. Hexane (150 ml) was added to the separating funnel and shaken well. The hexane soluble substances were dissolved in it. After 10-15 min, aqueous portion and hexane portion were separated from each other, because water and hexane are not mixable with each other. Due to high density aqueous portion was beneath the hexane. This was then washed with hexane three times. All the hexane fractions were combined, concentrated and completely dried on vacuum rotary evaporator under reduced pressure at a temperature ranging from 40-50°C. The same procedure was repeated for Isobutanol, chloroform and ethyl acetate solvents included in the study. All these fractions of the selected plants along with the crude extracts were subjected to biological activity using various bacteria and fungi (Benhammamou et al., 2008).

**Determination of antimicrobial activities**

Nutrient agar media were used for the culturing and growth of all microorganisms used in the present study. They were used for inoculation, shaking incubation and standardization of these microorganisms. Chloroform, hexane, ethyl acetate, aqueous, Butanol and Methanolic crude extracts of these medicinal plants were evaluated for antimicrobial potential. The dried crude extracts of all the plants were diluted and adjusted to 1 mg 6µl⁻¹ in dimethylsulfoxide (DMSO), chloroform, hexane, ethyl acetate, aqueous, butanol and methanolic crude extracts depending on their solubility. The stock solution was prepared and each 6 µl of the solution contained 1 mg of the plant extract (Fazal et al., 2011). The antimicrobial activity of selected medicinal plants extracts was evaluated using disc diffusion method. For the determination of antimicrobial activities, the culture of bacteria were justified to 0.5 Mc farland standards and then inoculated to nutrient agar (Aida et al., 2001). Sterile filter paper discs impregnated with plant extracts of 6 and 12 µl in volume were applied on plate's discs. The required amounts of nutrient agar media (2.8 g 100 ml⁻¹) and nutrient broth (1.3 g 100 ml⁻¹) were dissolved in distilled water in flasks. The nutrient broth media approximately 20 ml per test tube were taken. All the apparatus and media, viz., Petri plates, blue tips, yellow tips, and Whitman filter paper discs used in the activity were sterilized at 1.5 pounds pressure and 121°C for 1 h. After sterilization, agar media were poured into the Petri-plates in a laminar flow cabinet, allowed to solidify and placed in an incubator at 37°C to avoid any contamination during test application. The microbial stock cultures were freshened by streaking with sterile inoculation loop on the nutrient agar plates in a laminar flow hood. These cultures were incubated at 37°C for 24 h (Oboh and Obasuyi, 2007). The first streaked cultures reinoculated on fresh agar media plates and again
incubated at 37°C for 24 h. The second streaked cultures were inoculated into the sterilized nutrient broth in flasks containing approx. 20-25 ml broth media which were then incubated in the shaking water bath (Model; GLASC-SBR-04-28) for 18 h at 200 rpm at 37°C. The microbial cultures from flasks were diluted in test tubes containing sterilized nutrient broth for standardization by comparing with 0.5 McFarland (turbidity) Standard (Fazal et al, 2011). 50 µl of standardized microbial cultures were spread on each nutrient agar plate with the help of a glass spreader. These impregnated plates were then placed in a refrigerator for absorption for about 15 min. The impregnated Petri-plates containing standardized microbial inoculums were again brought to laminar flow after 15 min absorption. Whitman filter paper discs (6 mm in diameter) were placed on agar media with the help of sterilized forcep (Bakht et al, 2011). Then the chloroform, hexane, ethyl acetate, aqueous, butanol and methanolic extract in different concentrations of 1, and 2 mg disc⁻¹ in 6 and 12 µl volume were applied on the discs. Antibiotic (Azithromycin, Ciprofloxacin, Clotrimazole) were applied (6 µl disc) on separate plates as positive control for gram positive bacteria, gram negative bacteria and C. albicans, respectively. All the solvents used for making stock solution were also applied (6 µl disc⁻¹) on the discs as negative controls. These plates were then incubated at 37°C.

### Positive controls

**Against Gram positive bacteria:** Azithromycin 50 µg 6 µ⁻¹

**Against Gram negative bacteria:** Ciprofloxacin 30 µg 6 µ⁻¹

**Against C. albicans:** Clotrimazole 50 µg 6 µ⁻¹

### RESULTS

Results obtained in the present study showed that the tested five medicinal plants extracts possess potential antibacterial activity against *B. subtilis*, *E. coli* *K. aeruginosa*, *K. pneumonia* *S. aureus* *P. aeruginosa* *E. coli* *S. aureus* *K. pneumonia* *P. aeruginosa* *E. coli*. When tested using disc diffusion method, the crude extract of *T. stellata* showed significant activity against *B. subtilis* and *E. coli* of about 28 mm. The highest antibacterial activity of 20 mm in *B. subtilis* and least activity 14 mm in *E. coli* were recorded. Aerial parts of *T. stella* showed highest activity in hexane extract against *C. Albicans* 22 mm, while lowest activity was observed against *S. typhi*. Isobutanol extract of *T. stellata* showed maximum activity against *S. aureus* 23.4 mm, followed by *E. coli* 18.2 mm, *P. aeruginosa* 13.4 mm, *S. typhi*, 17.4 mm, and *K. pneumonia* 16.4 mm.

*Conyza bonariensis* showed almost similar zone of inhibition against all the tested bacteria except *S. typhi*, *B. subtilis* and *B. atropoeus*. The highest activity was recorded in hexane extract against *E. coli* 22 mm (*Table 2*), followed by *S. aureus* in chloroform extract. *E. caeruleum* showed no activity against *S. typhi* in most of the plant extracts. The highest activity was recorded against *E. coli* 25 mm, followed by *C. albicans* 16 mm in ethyl acetate. *P. aeruginosa* and *K. pneumonia* exhibited moderate activity against tested bacteria and fungi. At both the concentrations, 1 mg/6 µl and 2 mg/12 µl *E. coli* and *S. typhi* recorded no activity in chloroform.

### Table 1: Antimicrobial activity of *T. stellata* against Gram positive bacteria, Gram negative bacteria and fungi.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Crude (1mg/6µl)</th>
<th>Hexane (2mg/12µl)</th>
<th>Chloroform (2mg/12µl)</th>
<th>Ethyl acetate (1mg/6µl)</th>
<th>Isobutanol (1mg/6µl)</th>
<th>Aqueous (2mg/12µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>22±0.7</td>
<td>11.2±0.4</td>
<td>14.5±0.3</td>
<td>12.3±0.2</td>
<td>20.6±0.4</td>
<td>24.3±0.3</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>17.1±0.3</td>
<td>8.1±0.2</td>
<td>10.4±0.2</td>
<td>15±0.4</td>
<td>20.2±0.3</td>
<td>22.1±0.3</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>23±0.2</td>
<td>12±0.3</td>
<td>14.5±0.3</td>
<td>18±0.5</td>
<td>16.2±0.3</td>
<td>20.1±0.4</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>10.1±0.4</td>
<td>13±0.2</td>
<td>14.2±0.2</td>
<td>17±0.3</td>
<td>13.3±0.3</td>
<td>15.7±0.2</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>25.1±0.5</td>
<td>15.2±0.2</td>
<td>18.3±0.2</td>
<td>10±0.3</td>
<td>23±0.1</td>
<td>26.5</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>22.1±0.2</td>
<td>10±0.3</td>
<td>14.2±0.5</td>
<td>8±0.2</td>
<td>20±0.3</td>
<td>25.3±0.5</td>
</tr>
<tr>
<td><em>B. atropoeus</em></td>
<td>17.1±0.2</td>
<td>1±0.3</td>
<td>0.0±0.0</td>
<td>10±0.5</td>
<td>8.3±0.7</td>
<td>12.5±0.4</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>16.2±0.2</td>
<td>22.4±0.2</td>
<td>17.2±0.4</td>
<td>13.4±0.3</td>
<td>10±0.2</td>
<td>14.3±0.2</td>
</tr>
</tbody>
</table>
The lowest zone of inhibition was observed against B. Atrophus 10 mm at both the concentrations. Ethyl acetate, crude and aqueous extracts were also less active against C. albicans. P. aeruginosa and K. pneumonia showed no activity at both the concentrations. R. muricatus showed significant activity against all the tested bacterial strains except B. subtilis. The most significant zone of inhibition was shown by E. coli 23 mm, followed by S. typhi 19 mm and C. albicans 15 mm, respectively. The lowest activity was observed against K. pneumonia 10 mm (Figures 3 and 4).

**DISCUSSION**

In the present study, 6 different extracts including chloroform, hexane, butanol, ethyl acetate, aqueous and crude extracts of the aerial parts of five selected medicinal plants *E. caeruleum*, *M. tricuspedatum*, *T. campestre*, *R. muricatus* and *C. bonariensis* were assessed for their antimicrobial potential against three Gram positive bacterial species, that is, *B. subtilis*, *S. aureus* and *B. atropoeus*, four Gram negative bacterial species, that is, *E. coli*, *P. aeruginosa*, *S. typhi* and *K. pneumonia* and fungi *C. albicans* using disc diffusion method. *E. caeruleum* aerial parts were used against all tested microorganisms. *E. caeruleum* showed best zone of inhibition in ethyl acetate extract against all tested microorganisms (Table 3). Maximum zone of inhibition was observed against *E. coli*. Chloroform, hexane and crude extract of *E. caeruleum* were not effective against bacteria but were most effective against *C. albicans*. Thiem et al. (2010) reported the antimicrobial activity of ethanolic extracts of leaves and roots of *Erangium planum, Erangium campestre and Erangium maritimum* against two species of bacteria (*S. aureus* and *B. subtilis*) and five species of fungi (*C. albicans, Candida glabrata, Cryptococcus neoformans, Aspergillus niger and Trichophyton mentagrophytes*). The results indicated that ethanolic extract of leaves and roots of *E. planum, E. campestre* and *E. maritimum* exhibited high antifungal activity, while the antibacterial activity was of moderate order. *C. bonariensis* showed best zone of inhibition in isobutanol extract against all tested microorganisms, highest activity was recorded against *P. aeruginosa*, followed by *S. aureus* and lowest activity was observed against *B. subtilis*. Crude extract was also active against all tested microorganisms except *B. subtilis*, and the highest activity was recorded against *K. pneumonia*. Aqueous, chloroform and hexane extracts were not effective against bacterial species but were quite effective against *C. albicans*. Jack and Okorosaye (2008) reported the phytochemical analysis and antimicrobial activity of the leaf extracts of *C. sumatrensis* and showed the presence of some substances such as tannins, flavonoids, steroids and glycosides. It was shown that the antimicrobial tests of *C. sumatrensis* leaf extract were not effective against the bacterial species *P. aureginosa, S. aureus, Bacillus* sp. and *E. coli*, but inhibited the growth of the fungus *A. niger*.

*M. tricuspedatum* aerial parts were tested against Gram positive bacteria, Gram negative bacteria and fungal species. The highest zone of inhibition was recorded against *E. coli* in ethyl acetate extract. Islam et al. (2007) studied the antimicrobial and
Figure 1: Antimicrobial activity of *M. tricuspidatum* at 1mg/6µl concentration of plant extract.

Figure 2: Antimicrobial activity of *M. tricuspidatum* at 2mg/12µl concentration of plant extract.
Figure 3: Antimicrobial activity of *R. muricatus* at 1 mg/6 µl concentration of plant extract.

Table 3: Antimicrobial activities of *E. caeruleum* against Gram positive bacteria, Gram negative bacteria and fungi.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Crude</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Isobutanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1mg/6µl</td>
<td>2mg/12µl</td>
<td>1mg/6µl</td>
<td>2mg/12µl</td>
<td>1mg/6µl</td>
<td>2mg/12µl</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>8.4±0.3</td>
<td>10.7±0.2</td>
<td>9.2±0.3</td>
<td>14.3±0.4</td>
<td>11±0.4</td>
<td>14.2±0.6</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>8.2±0.3</td>
<td>10±0.4</td>
<td>7±0.3</td>
<td>9.5±0.4</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>10±0.4</td>
<td>14±0.3</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>10±0.2</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>10.8±0.2</td>
<td>12.5±0.2</td>
<td>13.1±0.2</td>
<td>15.4±0.3</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>7±0.3</td>
<td>11±0.3</td>
<td>10.5±0.2</td>
<td>12.6±0.3</td>
<td>12±0.3</td>
<td>16.2±0.4</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>7±0.3</td>
<td>9.2±0.3</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>9±0.5</td>
<td>12.5±0.4</td>
</tr>
<tr>
<td><em>B. atropoeus</em></td>
<td>8.4±0.02</td>
<td>12.1±0.3</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>12±0.4</td>
<td>14.5±0.3</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>9.2±0.4</td>
<td>13</td>
<td>9±0.3</td>
<td>11±0.4</td>
<td>0.0±0.0</td>
<td>0±0.00</td>
</tr>
</tbody>
</table>
irritant activities of *Malva parviflora*, *Malvastrum coronandelianum* and *Amaranthus viridis*. Hexane, chloroform, and ethanol extracts of these plants were tested against bacterial and fungal species. The results showed that slight variation was found in the zone of inhibition between Gram positive bacteria and Gram negative bacteria. Chloroform extract showed prominent antibacterial activity as compared with that of other extracts. The antifungal activities of all the extracts were almost the same. While our result showed significant activity against Gram positive and Gram negative bacteria. *T. stillata* showed best zone of inhibition in crude extract against all microorganisms. Aqueous extract was active against Gram positive and Gram negative bacteria and *C. albicans*. The highest zone of inhibition was observed against *S. typhi* in aqueous extract. Banu et al. (2012) studied the antimicrobial potential of *T. sintenisii* (Baker) in ethanol, water, methanol and acetone. The extracts were checked on three bacteria using disc diffusion method. All extracts exhibited antimicrobial effect on *S. aureus, E. coli* and *Pseudomonas syringae*. The result showed that all extracts of *T. sintenisii* (Baker) showed the best antibacterial activity against microorganisms, while the results of the present study also showed best activity against the bacteria. *R. muricatus* arial parts in hexane extract showed highest activity against *B. subtilis*, followed by *S. aureus* and lowest activity was observed against *B. atropoeaeus*. Crude, chloroform and ethyl acetate extracts were also well significant against all microorganisms. *R. muricatus* in isobutanol extract showed best zone of inhibition against *P. aeruginosa, B. subtilis* and *S. aureus* but no zone of inhibition was observed against *B. atropoeaeus*. The aqueous extract showed significant zone of inhibition at all concentration except *B. subtilis* and *B. atropoeaeus*. Hussain et al. (2000) studied the phytochemical and antimicrobial bioassay of five medicinal plants, *Lepidium sativum, Nerium oleander, Ranunculus repens, Tecoma stans* and *Urtica dioca*, in chloroform and water on five bacterial species and two fungal species. The microorganisms were *B. subtilis, P. vulgaris, S. aureus, E. coli, P. aeruginosa, S. typhi, A. niger* and *C. albicans* using well diffusion method. The results indicated that all bacterial and fungal species showed no significant antimicrobial activities. While according to the findings of the present study, significant activities were recorded against both bacterial and fungal species.

**CONCLUSION**

The arial parts of selected medicinal plants extracts in organic solvents, such as hexane, chloroform, ethyl acetate, isobutanol and crude extracts, showed high biological activities against microorganisms. The selected medicinal plants contain active constituent which has a wide range of medicinal values and as such, these plants could be used as a potential source of natural antimicrobial drugs. This research helps to obtain active constituent from selected plants for the treatment of microbial infection. More ever additional study is needed against other pathogenic bacteria and fungi.

**RECOMMENDATION**

It is recommended that the above selected plants are important for their antimicrobial activity and therefore, more efforts are need to explore the uses of the medicinal plants. The extracts from arial parts of selected plants can be used as potential sources of chemotherapeutic agents. Enriched information about selected medicinal plants is documented which need to be explore further for human welfare.

**REFERENCES**


