Antimicrobial and Antioxidant Activity of Five Medicinal Plants Against Different Microbes

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The present study aims to assess the antimicrobial and antioxidant activity of selected medicinal plants (Achyranthes bidentata, Linum usitatissimum, Pedalium murex, Sphaeranthus indicus and, Terminalia bellirica) extracts against seven different microorganisms Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, and Streptococcus mutants. Leaf, root, and flower extracts of plants were prepared in different solvents like methanol, distilled water, dichloromethane, ethanol, ethyl acetate, chloroform, petroleum ether, propanol, benzene, and hexane. All the prepared extracts showed very good antimicrobial activity except distilled water extract. Most of the extracts were found to have antimicrobial potential against pathogens but Linum usitatissimum and T. bellirica leaf and seed extract prepared in methanol and chloroform solvents show a higher zone of inhibition against E. coli. Only Linum usitatissimum shows activity against Candida albicans. Minimum Inhibitory Concentration for Achyranthes bidentata extracts varied from 150µl/ml to 200µl/ml in different solvents. Antioxidant studies were carried out in methanolic extracts of all the plants. The maximum scavenging activity of methanolic leaf extracts was observed between 80 - 100 µg/ml concentrations.

Keywords: Antimicrobial Activity; Antioxidant Activity; Leaf extracts; Medicinal plants; Solvents.

The Medicinal plants are paving their way into the field of pharmaceuticals, nutraceuticals along cosmetics. Plants used in medicine have a huge range of constituents. These plants have been in use to treat a wide range of diseases for generations now. Their application is not just limited to curing common infectious illness but also play a significant part in healing some chronic diseases as well. Human-beings have been using plants for treating common bacterial and fungus infections1. These plants act as a rich source of micro-organic agents2. Due to the presence of antimicrobial molecules in abundance, a wide range of herbal plant extracts are used to treat infections worldwide. In Ayurveda, some of these bioactive compounds are used as raw materials after in vitro and in vivo screening3. Herbs have an upper-hand over synthetic drugs and also have very little or almost no side effects. Some of these benefits have grabbed the attention of experts and has turned it towards phytomedicines4. However multi-drug resistance property of bacteria

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and pathogens have diverted researchers and pharmacists into thinking towards the practical application of these restorative plants due to their antimicrobial potential. Furthermore, these herbs have been a wellspring of bioactive mixtures of pharmacological significance for individuals. Therefore, scientific testing is important for determining the adequacy of plants and traditional medicines. The biological assays (antimicrobial, antioxidant, and anti-inflammatory activities) related to skin and other diseases and their safety assessment have been affirmed. Numerous reports recorded on the natural exercises, phytochemistry, and security of numerous therapeutic plants being utilized in South Africa ordinary medication against skin conditions have not been used to its full extent. Recently, many researches have been done on numerous botanicals used by Batswana conventional experts for treating skin-related infections in the Ngaka Modiri Molema District Municipality, North West Province, South Africa.

MATERIALS AND METHODS

Collection of plant material
Five disease-free randomly selected medicinal plants were collected from the Tau Devi Lal Herbal Park near Khizrabad highway at Churpur in district Yamunanagar, Haryana, India. These plants were *Achyranthes bidentata*, *Linum usitatissimum*, *Pedalium murex*, *Sphaeranthus indicus*, and *Terminalia billerica*. Leaves and flowers of selected plants were shade dried and powdered in a mixer grinder.

Preparation of the plant extracts
The powder was submerged in various organic solvents such as methanol, chloroform, dichloromethane, distilled water, benzene, n-hexane, petroleum ether, and ethanol in the ratio of 1:10 (20gm in 200ml solvent) for 72 hours at room temperature. The extracts were filtered using Whatman filter paper no.1 after the incubation period of 72 hours and then total evaporation of the solvent was observed in the water bath at the boiling temperature of the respective solvent.

Study of the antimicrobial activity

Culture collection
Microbial cultures of pathogens were obtained from the Institute of Microbiology and Technology (IMTECH) Chandigarh. *Candida albicans* (MTCC NO. 3017), *Escherichia coli* (43), *Pseudomonas aeruginosa* (2295), *Staphylococcus aureus* (3160), *Staphylococcus epidermidis* (9041), *Staphylococcus hominis* (4435), and *Streptococcus mutans* (1943) were used in this study. The highly pure cultures of bacteria were transferred and maintained on nutrient agar plates for better vegetative growth.

Preparation of extract dilutions
A small quantity (5 g of leaf/flower/root) of powder was weighed out. It was soaked in 20 ml of solvents for 3 days, filtered in the new flask and the residue was discarded. The filtrate was then put into a water bath at 45-50ºC, after which all the solvent was evaporated from the filtrate. Then solvent got extracted, and DMSO was used to dissolve residual powder and left it in the refrigerator at 4°C for further analysis. Agar well diffusion method was used for the evaluation of antimicrobial potential on Mueller Hinton Agar medium. The antimicrobial potency of the extract was assessed in the form of the diameter of inhibition zones (mm). The mean value of inhibition zones of the triplicates was taken as the final result.

The Minimum inhibitory concentration of plants crude extract
The Minimum inhibitory concentration (MIC) of plant crude extracts in various solvents was carried out using the standard MIC analysis method.

Antioxidant activity of selected plants extracts

DPPH radical scavenging activity
The IC50 value was determined. DPPH assays done in all the plant samples in methanolic extracts and ascorbic acid were taken and used as standard. The antioxidant activity of the extracts was measured. A similar amount of DPPH and methanol was used as control and methanol was taken as blank. Different concentrations of the ascorbic acid standard (0.1 ml) (10 to 100ug/ml) were prepared and 3ml of DPPH was added in whole concentration. The test solution was also prepared in different concentrations (10 to 100ug/ml). Both standard and samples were incubated in dark conditions for half-hour. Absorbance was recorded at 517nm using a spectrophotometer. From this absorbance, the percentage of inhibition activity was calculated. The IC50 value of extract was expressed as the free radical scavenging
activity. This IC50 defines the inhibition of DPPH radical by 50% through different concentrations of the plant extract.

\[ \text{% Scavenging activity} = \left(\frac{(A0-A1)}{A0}\right) \times 100 \]

A0 = Absorbance of control
A1 = Absorbance in the presence of the extract

RESULTS AND DISCUSSION

The antimicrobial activity of plant extracts against microbes

*A.bidentata* root extract showed the maximum antimicrobial activity within dichloromethane and chloroform extracts. The chloroform extract of roots showed maximum inhibition zone diameters (22mm) against *S. mutans* while there was no activity against the *S. hominis* and *C. albicans* [Table 1]. The maximum zone of inhibition of *Achyranthes bidentata* methanol extract was 21.2mm, when tested against *S. aureus*, observed in some other researches\(^\text{14}\).

*L. usitatissimum* flower extracts contain antimicrobial activity in all the solvents except distilled water. The activity was better in chloroform extract of leaves against strains *S. hominis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and no activity was seen against *S. mutans* and *C. albicans*. Maximum inhibition zone diameters of 26 mm against *S. hominis* were recorded while no activity was observed against *S. mutans* and *C. albicans* [Table 2]. Different zone of inhibition was observed in same plants in different studies which revealed that *L. usitatissimum* plant shows significant activity against *S. aureus* and least

### Table 1. Zone of inhibition diameter (in mm) of *A. bidentata* root extract in different solvent extracts against various pathogenic strains

| Solvents          | E.coli | P.aeruginosa | S.mutans | S.aureus | S.epidermidis | S.hominis | C.albicans |
|-------------------|--------|--------------|----------|----------|---------------|-----------|------------|
| Methanol          | 13     | 15           | 22       | 14       | 15            | -         | -          |
| Dichloromethane   | 12     | 16           | 21       | 13       | 16            | 17        | -          |
| Distilled water   | -      | -            | -        | -        | -             | -         | -          |
| Ethanol           | 13     | 16           | 21       | 12       | 14            | 16        | -          |
| Ethyl acetate     | 13     | 15           | -        | 14       | 14            | 12        | -          |
| Petroleum ether   | 11     | -            | 20       | 14       | 15            | -         | -          |
| Propanol          | 14     | 12           | 22       | 18       | 16            | -         | -          |
| Chloroform        | 14     | 13           | 25       | 21       | 16            | -         | -          |
| Benzene           | 11     | 19           | 19       | 19       | 14            | -         | -          |
| Hexane            | 13     | 11           | 22       | 16       | 15            | -         | -          |

### Table 2. Inhibitory zone diameters (in mm) of *L. usitatissimum* of flower extract in different solvents against various microbes

| Solvents          | E.coli | P.aeruginosa | S.mutans | S.aureus | S.epidermidis | S.hominis | C.albicans |
|-------------------|--------|--------------|----------|----------|---------------|-----------|------------|
| Methanol          | 26     | 23           | 24       | 20       | -             | 21        | 20         |
| Dichloromethane   | 18     | 19           | 20       | 16       | 20            | 12        | 18         |
| Distilled water   | -      | -            | -        | -        | -             | -         | -          |
| Ethanol           | 24     | 23           | 16       | 18       | 19            | 14        | 12         |
| Ethyl acetate     | 19     | 18           | 17       | 16       | 15            | 11        | 14         |
| Petroleum ether   | 23     | 18           | 13       | 20       | 21            | 20        | 13         |
| Propanol          | 20     | 19           | 11       | 21       | 16            | 18        | 14         |
| Chloroform        | 28     | 26           | 24       | 23       | 18            | 16        | 12         |
| Benzene           | 10     | 12           | 15       | 14       | 11            | 14        | 11         |
| Hexane            | 19     | 14           | 13       | 13       | 12            | 12        | 12         |
Table 3. Inhibition zone diameter (in mm) of *P. murex* leaf crude extract in different solvents against various pathogenic strains

| Solvents      | *E. coli* | *P. aeruginosa* | *S. mutans* | *S. aureus* | *S. epidermidis* | *S. hominis* | *C. albicans* |
|---------------|-----------|-----------------|-------------|-------------|-----------------|-------------|--------------|
| Methanol      | 16        | 16              | -           | 14          | 16              | -           | -            |
| Dichloromethane | 12      | 13              | -           | 11          | 11              | -           | -            |
| Distilled water | -        | -               | -           | -           | 12              | -           | -            |
| Ethanol       | 15        | 12              | -           | 12          | 12              | -           | -            |
| Ethyl acetate | 12        | -               | -           | 12          | 12              | -           | -            |
| Petroleum ether | 13      | -               | -           | 13          | -               | -           | -            |
| Propanol      | -         | 13              | -           | 13          | 13              | -           | 14           |
| Chloroform    | 15        | 12              | -           | 15          | 16              | -           | -            |
| Benzene       | 12        | 12              | -           | 12          | 14              | -           | -            |
| Hexane        | 13        | -               | -           | 13          | 12              | -           | -            |

Table 4. Inhibition zone diameters (in mm) of *S. indicus* leaf crude extract in different solvents against various pathogenic strains

| Solvents      | *E. coli* | *P. aeruginosa* | *S. mutans* | *S. aureus* | *S. epidermidis* | *S. hominis* | *C. albicans* |
|---------------|-----------|-----------------|-------------|-------------|-----------------|-------------|--------------|
| Methanol      | 19        | 21              | 20          | 24          | 26              | 24          | -            |
| Dichloromethane | 14      | 13              | -           | 11          | 23              | -           | -            |
| Distilled water | -        | -               | -           | -           | -               | -           | -            |
| Ethanol       | 24        | 23              | -           | 23          | 20              | 23          | -            |
| Ethyl acetate | 18        | 16              | -           | 19          | 18              | -           | -            |
| Petroleum ether | 16      | 14              | -           | 18          | 26              | -           | -            |
| Propanol      | 15        | 13              | -           | 16          | 21              | -           | -            |
| Chloroform    | 20        | 23              | -           | 23          | 24              | -           | -            |
| Benzene       | 14        | 16              | -           | 16          | -               | 14          | -            |
| Hexane        | 12        | 14              | -           | 19          | -               | -           | -            |

Table 5. Inhibitory zone diameter (in mm) of *T. bellirica* seed crude extract in different solvents against different pathogenic strains

| Solvents      | *E. coli* | *P. aeruginosa* | *S. mutans* | *S. aureus* | *S. epidermidis* | *S. hominis* | *C. albicans* |
|---------------|-----------|-----------------|-------------|-------------|-----------------|-------------|--------------|
| Methanol      | 25        | 28              | -           | 24          | 23              | 20          | -            |
| Dichloromethane | 20       | 23              | -           | 20          | 18              | 19          | -            |
| Distilled water | -        | -               | -           | -           | -               | -           | -            |
| Ethanol       | 23        | 24              | -           | 16          | 26              | 12          | -            |
| Ethyl acetate | 19        | 12              | -           | 11          | 12              | 16          | -            |
| Petroleum ether | 18       | 16              | -           | 18          | 12              | 14          | -            |
| Propanol      | 16        | 14              | -           | 17          | 14              | 13          | -            |
| Chloroform    | 24        | 23              | -           | 20          | 21              | 23          | -            |
| Benzene       | 12        | 12              | -           | 18          | 19              | -           | -            |
| Hexane        | 12        | 11              | -           | 16          | 15              | -           | -            |
Table 6. IC 50 Value of different plants against DPPH radicals

| Plant          | IC50 Value Mean±SD |
|---------------|-------------------|
| A.bidentata   | 57±2.5            |
| L. usitatissimum | 16.26±3.0        |
| P.murex       | 78.24±1.6         |
| S. indicus    | 75.67±2.3         |
| T. bellirica  | 94.67±0.6         |

Fig. 1. DPPH radical scavenging graph of plant extract

activity against K. pneumonia. It was observed that the plant extract showed antimicrobial activity against gram-positive bacteria and less activity against gram-negative bacteria\(^{15}\), whereas the present study result indicates the activity of ethanol and chloroform extract against both gram-positive and gram-negative bacteria\(^ {16-18}\).

*P. murex* leaf exhibited maximum antimicrobial activity in methanol and chloroform solvents. The extract showed antimicrobial activity against almost all pathogenic microbes while *P. murex* did not affect the growth of *S. hominis*, *S. mutans*, and *C. albicans* [Table 3]. According to previous research the *Pedalium murex*, ethyl acetate and petroleum ether extracts showed significant results against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*\(^ {19}\).

*S. indicus* leaf extract in methanol showed major inhibition zone against *P. aeruginosa* (26 mm). The dichloromethane and aqueous extracts were found less active against *S. hominis*, *S. mutans*, and *C. albicans* [Table 4]. Whereas some studies evaluated that the pathogenic bacterial growth was inhibited by *S. indicus* hexane extract of the stem. The extract prepared from methanol showed higher activity against *S. aureus* (25.67mm) but showed least activity against *P. aeruginosa* (9.83) which is opposite to our results.\(^ {20}\) Some past researches also reveals that the root extract inhibits a smaller number of bacterial isolates\(^ {21}\).

*T. bellirica* seed methanol extract contains maximum antimicrobial activity against all the pathogens except *S. mutans* and *C. albicans*. Their methanol extract showed a very high activity, observed against *P. aeruginosa* (28mm). These extracts prepared in various solvents showed a maximum inhibition zone against *P. aeruginosa E. coli*, *S. aureus*, and *S. epidermidis* as shown in [Table 5]. While work done by researchers revealed that the ethyl acetate extracts of this plant show significant activity towards microorganisms and aqueous extract of *Terminalia bellirica* maximum zone of inhibition of 6mm were observed which is very lowest as compared to our study\(^ {22}\).
The methanolic and chloroform extract of S. indicus leaves showed multiple resistance towards P. aeruginosa, S. aureus, and S. epidermidis while it exhibited no activity against C. albicans. The extract of L. usitatissimum showed the maximum antimicrobial activity against S. aureus, E. coli, S. hominis and, P. aeruginosa while no activity was observed against S. epidermidis, Streptococcus mutans, and C. albicans. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of plant extract is shown in [Fig:1]. The IC50 value of all plants A. bidentata, L. usitatissimum, P. murex, S. indicus and, T. billerica was found to be 57ug/ml, 16.26ug/ml, 78.24ug/ml, 75.67ug/ml, 94.67ug/ml respectively whereas IC50 value of ascorbic acid is 38.28ug/ml. All medicinal plant crude extracts give outstanding antioxidant activities over DPPH radical as shown in [Table 6].

This outcome confirms that the crude extracts of the plants (Achyranthes bidentata, Linum usitatissimum, Pedalium murex, Sphaeranthus indicus, and Terminalia billerica) used in the study are the proficient and best solution for the hinder the development of dental caries microbes with its overflowing wellspring of auxiliary metabolites. This examination leads to the improvement of better treatment with natural herbs-based medicine to conquer the incidental effects brought about about anti-toxins/antibiotics. The outcome shows the strength of methanol and chloroform crude extracts of plants as antimicrobial agents. The current investigation gives a qualitative ratio of some potential plant species used for antimicrobial activity. For future preclinical and clinical investigations, the fundamental information on the harmfulness profile of ethanol and aqueous extract of this therapeutically significant plant was gathered from past examinations and results which is valuable in clinical work for treating microbial illness. Through this study, it was seen that in-vitro antimicrobial profiling of methanol extract of roots of Achyranthes bidentata was discovered to be very effective against the bacterial strains, Staphylococcus aureus, and Streptococcus mutans. A subjective phytochemical examination was done for the identification of carbohydrates, saponins, flavonoids, alkaloids, tannins, reducing sugars, steroids, gums. The photochemical analysis of alkaloids, flavonoids, tannins shows antimicrobial movement and saponin might be credited against tainting specialists where flavonoids showed antimicrobial action by complexing with the cell wall and also binds to adhesion. The current investigation was done to investigate the antibacterial adequacy of five therapeutic plants against a few bacterial strains which could be additionally utilized for characterization of the novel phytochemicals in the treatment of contagious diseases, particularly which works against drug-resistant microorganisms and lead to the development of more effective antimicrobial compounds. Therapeutic plants have been known for synthesis of active metabolites with established potential antimicrobial and antibacterial activities, which undoubtedly have framed the reason for their applications in drugs, alternative medicines, and normal treatments. Achyranthes bidentata showed a maximum zone of inhibition and for two pathogenic strains. Because of solvents’ polarity that confirms the type of reaction and solubility of compounds, the zonation differences are produced in each extract. Most all distillates have a better capacity which may be allocated to the ability to extract the natural antimicrobial chemicals such as flavonoids, alkaloids, terpenoids, and phenolic compounds. Thus, the examination guarantees the plants’ worth utilized in Ayurveda, which could be very significant for the improvement of new medications.

CONCLUSION

The result of the present study has addressed several new ways and different research problems in the present scenario. All the plant showed the specific antimicrobial activity against the pathogen used in the different solvents. Among the solvents used methanol and ethanol extracts showed the highest activity with regard to the inhibition of microbial growth while distilled water shows the minimum antimicrobial activity against different plant extracts. Very large inhibition zone is shown by A. bidentata, P. murex, Terminalia bellirica rest shows the satisfactory activity in various solvents against different pathogens. All plant exhibited antimicrobial activity but the highest activity is observed in the L. usitatissimum. The purified phytoneutrients fractions can be further used to isolate the lead compound from the fractions and then 3D examination studies of structure can
be done in the future. So that active ingredients can be further subjected to clinical research to provide a new drug. Some more studies and researches may be done on these plants to identify and isolate their bioactive compound causing antimicrobial and antioxidant activity. More studies need to be conducted using in vivo/in vitro models to find the precise molecular mechanisms and targets for cell growth inhibition which will allow the rational design for more effective chemicals for the eventual chemo preventive and/or therapeutic agent.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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