Full Length Research Paper

Antimicrobial activity of crude methanolic extract of *Periploca aphylla*

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*Periploca aphylla* is used in the treatment of many diseases ethno pharmacologically in Pakistan. In the present manuscript, we demonstrate the antimicrobial effects of *P. aphylla*. Six different bacteria (*Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) are used in the screening of extract through agar well diffusion technique as well as four different strains of fungi (*Fusarium solani*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*) are characterized. Methanolic fraction of *P. aphylla* showed significant inhibition of bacterial and fungal growth. The potency of these activities is due to the presence of bioactive flavonoids, saponins and phenolic compounds.

**Key words:** *Periploca aphylla*, antimicrobial activity, *Escherichia coli*.

INTRODUCTION

According to World Health Organization, to get the potent herbal drugs, medicinal plants of different species are the best source throughout the world. It is noted that about 80% of individuals use traditional medicine from developed countries of the world, having potent compounds basically derived from medicinal plants. Therefore, such plants should be screened to obtain good safe antimicrobial agents for future use against the multidrug resistant strain of microbes. It is reported that with known antimicrobial properties, the plant extracts and phytochemical are processed for the prevention of chemical cardiotoxicity and different chronic diseases (Khan et al., 2011). It is on the record that medicinal plants provide 30% of worldwide drugs in the form of natural products isolated from them (Khan et al., 2009). Due to the antimicrobial properties, plants have been used for the treatment of different diseases, the compounds of which are synthesized during secondary metabolism of the plant. Also, due to the traditional uses and for the development of new anti-infectious agents, medicinal plants are important and rich source for them. A number of bioactive molecules are produced by the medicinal plants and thus they are the good rich source of different types of medicine. From very long time, the higher plants which are the good sources of medicinal compounds may play a very positive role in the maintenance of human health. Plants are basic source of knowledge of modern medicine. Due to the indiscriminate use of commercial antibiotics against infectious diseases, it is investigated that microorganism have developed multiple restively against the used antibiotics. It is also reported clinically that some antibiotics have negative effects on the host, such as hypersensitivity, immune-suppression and allergic reactions. Therefore, for the treatment of infectious diseases, alternative antimicrobial drug development is needed.

Natural potent antimicrobial agents can be isolated from barks, stems, leaves, flowers and fruits of plants. Those substances which inhibit different pathogens and have little toxicity to the host cells are considered to be candidates for developing new potent antimicrobial drugs. The alternative antimicrobial agents of different plant sources showed a positive result against the multidrug resistant strain of different microorganisms. Due to the good pharmacological results, medicinal plants have
become the target of intense study in the traditional system of the world. It is noted clinically, that the synthetic drugs are very costly and not suitable for the treatment of various diseases and they showed severe side effects and with serious adulterations. Therefore, to control the severe microbial infections, new infection fighting agents are required to search out/ explore (Khan et al., 2009, 2010a, b and c, 2011a, b).

Biochemical analysis of plant

Due to the presence of bioactive compounds, medicinal plants have special properties of treatment of various chronic diseases. Periploca aphylla is used medicinally in the treatment of different diseases as “alternative medicine”. Due the ethno pharmacological uses of the plant, it is of very much important to explore the biochemical composition of the plant.

Plant collection

Plant of *P. aphylla* was collected from the main Township campus of University Of Science and Technology Bannu Khyber Pakhtunkhwa Pakistan in the month of July, 2010. The plant was identified by Taxonomist Prof: Abdur–Rehman, Chairman Department of Botany Govt: Post Graduate College Bannu. The plant materials were washed by the de-ionized water and were shade dried at room temperature for tow weeks, chopped and grinded mechanically of mesh size 1 mm.

Preparation of plant extract

2 kg powder of *P. aphylla* was extracted in 3 liter of 70% methanol by random shaking. After a week, the extract was filtered by using Whitman filter paper No. 1. After filtration, the filtrate was further concentrated by using rotary vacuum evaporator at 38°C in order to get the methanolic crude extract of the plant. The methanolic crude extract was stored at 4°C in the refrigerator for further phytochemical studies in vitro investigation.

Antifungal assay

To check the antifungal activity of methanolic crude extract of *P. aphylla*, Protocol of Khan et al. (2010b) was used with some modification.

Requirements

Methanolic crude extract of *P. aphylla*, DMSO, terbinafine, dis: water, micropipette, tips, flask, beakers, ruler, test tubes, incubator, autoclaver, SDA, electronic digital balance, luminar flow, and microorganisms used were; *Fusarium solani* (0300), *Aspergillus niger* (0198), *Aspergillus flavus* (0064) and *Aspergillus fumigatus* (66).

Preparation of samples

The stock solution of 1 mg/ml of methanolic crude extract of *P. aphylla* was prepared in DMSO. From this stock solution, further 1 ml solution of µ 200 µg/ml was prepared with DMSO as a diluting solvent. Similarly, the stock solution of 1 mg/ml of terbinafine (positive control/ antifungal agent) was prepared in the DMSO. From this stock solution, further 1 ml solution of required concentration, 200 µg/ml was prepared for dilution. Similarly, 1 ml DMSO was taken from the bottle used as a negative control.

Media for antifungal assay

Sabouraud dextrose agar (MERCK) was used to grow fungus for inoculums preparation. It was composed of peptone complex 10 g/L, glucose 40 g/L and agar 15 g/L.

Assay procedure

To prepare media for fungus growth, 6.5 g of SDA was dissolved in 100 ml distilled/ autoclave water in flask and autoclaved at 121°C for 20 min. 4 ml of this media solution was poured in all the autoclaved test tubes marked up to 10 cm in the laminar flow for four fungal strains. Then 67 µl of extract solution from the required concentration (200 µl) was put in all the 8 test tubes specified in duplicate for four fungal strains by micropipette. Similarly, 67 µl of terbinafine solution (positive control) of the required concentration (200 µl) was put in all the four test tubes. Similarly, 67 µl of DMSO was put in another set of four test tubes (one for each) of four fungal strains. After this, all the test tubes were placed in the slanting position in the Lumina flow in order to solidify the media in the test tubes at room temperature. After solidification, 10 spores from 7 day old culture of each fungal strain were placed in all the test tubes, packed air tightly and placed in the incubator at 28°C for 7 days. After 7 days, measured growth and calculated “Percentage inhibition” with the reference to negative control by using the formula:

\[
\text{Percentage inhibition growth} = \left( \frac{\text{dc} - \text{dt}}{\text{dc}} \right) \times 100
\]

where ‘c’ is used for negative control growth and ‘t’ is used for test tube/sample growth.

Antibacterial assay

The antibacterial activity of methanolic crude extract of
\textit{P. aphylla} was carried out through the modified protocol of (Khan et al., 2010b).

\textbf{Requirements}

Methanolic crude extract of \textit{P. aphylla}, DMSO, Cefixime-USP(Cefix), Roxithromycine (Rox), nutrient agar medium, nutrient broth medium, NaCl, dis:water, Petri plates, cork bore, incubator, autoclave, laminar flow, flask, beakers, micropipette, tips, microorganisms used were, \textit{Micrococcus luteus} (ATCC10240), \textit{Staphylococcus aureus} (ATCC10240), \textit{Escherichia coli} (ATCC15224), and \textit{Pseudomonas aeruginosa}.

\textbf{Media for bacteria}

Two types of media were used in the antibacterial assay, the nutrient broth medium and nutrient agar medium. The nutrient broth medium was composed of peptone (5 g/L) and meat extract (3 g/L) was used in bacterial strain inoculums preparation. The nutrient agar medium (MERCK) was composed of peptone (5 g/L), meat extract (3 g/L) and agar-agar (12 g/L) was used for bacterial growth in the Petri plates. Nutrient broth medium was prepared by dissolving 0.8 g/100 ml of distilled water while nutrient agar medium was prepared by dissolving 2 g/100 ml of distilled water of pH 7.0 and was autoclaved.

\textbf{Preparation of samples}

Methanolic crude extract of 1 mg/ml, 3 mg/ml and 5 mg/ml solution was prepared in the dimethyl sulfoxide (DMSO). Similarly 1 mg/ml solutions of cefixime-USP and roxithromycin, used as positive control, were also prepared in the DMSO. DMSO was also used as a negative control.

\textbf{Microorganisms/Preparation of inoculums}

Four strains of bacteria, \textit{S. aureus} (ATCC6538), \textit{M. luteus} (ATCC10240), \textit{E. coli} (ATCC15224), and \textit{P. aeruginosa} were used in this assay. 24 h old culture in nutrient broth (MERCK) of selected bacterial strain was mixed with physiological saline (0.9% NaCl w/v,) and turbidity was corrected by adding sterile physiological saline until a McFarland 0.5 BaSO$_4$ turbidity was corrected by adding sterile physiological saline (0.9% NaCl w/v.) and nutrient broth (MERCK) of selected bacterial strain was used.

\textbf{Assay procedure (agar diffusion method)}

2 g of nutrient agar was dissolved in 100 ml of distilled water (pH 7.0) in flask and autoclaved at 121°C for 20 min. After this, it was allowed to cool up to 45°C. Then suitable amount of this agar medium was put in to the autoclaved Petri plates of 10 cm and placed in laminar flow to solidify. Then a loop of bacterium streaked on the surface of agar media of Petri plates very carefully. After this, six wells were dug in the agar media of Petri plates at equal distance by the help of sterile cork bore (8 mm). Then 100 µl from 1, 3 and 5 mg/ml of extract solution was put in the respective wells by micropipette. Similarly 100 µl of cefixime-USP and roxithromycin were put in their respective wells. Then 100 µl of DMSO was also put in a well. Finally, all the Petri plates were packed tightly in the laminar flow and incubated at 37°C for 24 h. After 24 h of incubation, the zone of inhibition of all the extracts and controls were measured in mm and compared with the control.

\section*{RESULTS}

\textbf{Antibacterial activity of \textit{P. aphylla} methanolic extracts (PAME)}

Different concentrations (1 to 5 mg/ml) of \textit{P. aphylla} methanolic extract (PAME), cefixime-USP and roxithromycin (1 mg/ml) positive control, were used for screening of antibacterial activity. \textit{P. aphylla} methanolic extracts (PAME) exposed low activity (15.5 mm) against \textit{S. aureus}, followed by \textit{P. aeruginosa} (17 mm) while highest activity of the four tested strains was shown against \textit{E. coli} (22 mm), however comparatively high activity (21 mm) was shown against \textit{M. luteus} as in Table 1. Similarly, the cefixime-USP, positive control indicated low activity (18 mm) against \textit{M. luteus} followed by \textit{P. aeruginosa} (20 mm) while highest activity of this positive control was shown against \textit{E. coli} (29 mm), however comparatively high activity was shown against \textit{S. aureus} (27 mm). Similarly, Roxithromycin, positive control indicated low activity (19 mm) against \textit{E. coli} followed by \textit{P. aeruginosa} (21 mm) while highest activity for this control was shown against \textit{M. luteus} (26 mm), however comparatively high activity was shown against \textit{staphylococcus aureus} (23 mm).

\textbf{Antifungal activity of \textit{Periploca aphylla} methanolic extracts (PAME)}

67 µl (200 µg/ml) of \textit{P. aphylla} methanolic extracts (PAME), 67 µl DMSO (99.9%) and terbinafine 67 µl (200 µg/ml) were used for screening of antibacterial activity. The \textit{Periploca aphylla} methanolic extracts (PAME) indicated low activity (11.11%) against \textit{A. niger} followed by \textit{A. fumigatus} (28.57%) while highest activity was shown against \textit{A. flavus} (89.58%), however comparatively high activity was shown against \textit{F. solani} (29 mm).
Table 1. Antibacterial activity of *Periploca aphylla*.

<table>
<thead>
<tr>
<th>Conc (mg/ml)</th>
<th><em>Micrococcus luteus</em> Zone (mm)</th>
<th><em>Escherichia coli</em> Zone (mm)</th>
<th><em>Pseudomonas aeruginosa</em> Zone (mm)</th>
<th><em>Staphylococcus aureus</em> Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/ml Cefix</td>
<td>18.3±0.34</td>
<td>29±1.6</td>
<td>20±0.97</td>
<td>27±2.21</td>
</tr>
<tr>
<td>1 mg/ml Rox</td>
<td>26.5±2.45</td>
<td>19.6±0.45</td>
<td>21±1.21</td>
<td>23±2.02</td>
</tr>
<tr>
<td>1 mg/ml PAME</td>
<td>17.1±2.12</td>
<td>13.5±0.74</td>
<td>13±2.01</td>
<td>11±1.09</td>
</tr>
<tr>
<td>3 mg/ml PAME</td>
<td>19.5±1.78</td>
<td>18.2±0.98</td>
<td>15±1.21</td>
<td>13±1.31</td>
</tr>
<tr>
<td>5 mg/ml PAME</td>
<td>21.7±1.32</td>
<td>22.5±1.34</td>
<td>17±1.11</td>
<td>15.5±1.01</td>
</tr>
</tbody>
</table>

Mean±SE (n=3).

Table 2. Antifungal activity of *Periploca aphylla*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Aspergillus fumigatus</em> Zone (mm)</th>
<th><em>Aspergillus flavus</em> Zone (mm)</th>
<th><em>Fusarium solani</em> Zone (mm)</th>
<th><em>Aspergillus niger</em> Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>00±0.00</td>
<td>00±0.00</td>
<td>00±0.00</td>
<td>00±0.00</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>88.57±2.7</td>
<td>87.75±4.1</td>
<td>96.66±2.6</td>
<td>95.55±3.3</td>
</tr>
<tr>
<td>Extract</td>
<td>28.57±2.4</td>
<td>89.58±2.9</td>
<td>86.66±3.1</td>
<td>11.11±1.9</td>
</tr>
</tbody>
</table>

Mean±SE (n=3).

(86.66%). The terbinafine, a positive control indicated low activity (87.75%) against *A. flavus* followed by *A. fumigatus* (88.57%) while highest activity was shown against *F. solani* (96.66%), however comparatively high activity was shown against *A. niger* (95.55%). Similarly, DMSO, a negative control indicated zero percent (0.00%) activity against all the four used fungal strain as shown in the Table 2.

DISCUSSION

Antimicrobial potency of *P. aphylla* extracts

Due to emergence of antibiotic resistant strains as well as side effects of synthetic drugs, investigation of potent antimicrobial drugs obtained from natural resources has been an objective of researchers and investigators. The findings of our results showed that *P. aphylla* methanolic extracts had significant antibacterial activity against all pathogenic strains. Our results agreed with results reported by Ndhlala et al. (2009) during antimicrobial characterization of the South African tree aloe (*Aloe barberae*) due the presence of bioactive polyphenolic constituents. Narod et al. (2004) studied the antimicrobial effects of *Toddalia asiatica* extracts against Gram-negative and Gram-positive bacteria which strongly justify our results. Fungi are significant destroyers of food material and grains; unfit them for human consumption by disturbing their nutritive value and often by producing mycotoxins (Janardhana et al., 1998). In our study replicate results of antifungal activity indicate that growth of various fungal strains had been markedly inhibited by *P. aphylla* methanolic extracts. Several studies attributed the inhibitory effect of plant extracts against microbes due to the presence of phenolic compounds (Sahreen et al., 2010, 2011) and might be the presence of saponins which have antifungal properties polyphenolic compound like catechin and tannins.

Conclusion

Methanolic extract of *P. aphylla* showed significant inhibition against bacterial and fungal strains. Therefore further research of isolation and purification of these bioactive antimicrobial constituents are in progress in our lab.

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