Phytochemical analysis and antimicrobial activity of methanolic extract of *Eucalyptus globules*


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ABSTRACT

Methanolic extract of leaves of *Eucalyptus globulus* was studied for in vitro microbial medicinal plant revealed the presence of saponin, saponin glycosides, steroid, tannins, volatile oils, phenols and balsam (gum). The methanolic extract of the plant inhibited the growth of Staphylococcus aureus, Candida albicans but had no inhibitory effects on Escherichia coli. The minimum inhibitory concentration (MIC) of the extract ranged from 1.25g/ml to 5g/ml. The results obtained with the help of HPLC analysis it is concluded that the concentration of *E.globulus* extract inhibited the growth of bacterial strains, blank solution which do not contain bacteria. HPLC analysis reveals that *E.globulus* extract is more active due to the presence of the above phytochemical components present in the solution and shows variable peaks in graphs it suggest that Eucalyptus can be used in treating diseases caused by the test organisms. The main objective of this study is to examine the antimicrobial activities of the methanolic extracts of *Eucalyptus globulus*. The antimicrobial activity of the active fractions of the extract was also determined.

Keywords: Steroids, Saponins, Candida albicans, Pseudomonas aeruginosa, Anti-inflammatory

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and since the beginning of man. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity. Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. There is therefore the need to search for plants of medicinal value. The plant used in the present study is *Eucalyptus globulus*, which is used traditionally for the treatment of wounds, boils and other ailments with which the test organisms are routinely associated. The main objective of this study is to examine the antimicrobial activities of the methanolic extracts of *Eucalyptus globulus*. The antimicrobial activity of the active fractions of the extract was also determined.

Action of the leaves of *Eucalyptus globulus*:

- Anti-bacterial [an agent that destroys bacteria; bactericide]
- Antioxidant [contributing to the oxidation of free radicals which are believed to contribute to premature aging and dementia]
- Antiseptic [an agent for inhibiting the growth of microorganism on living tissue or destroying pathogenic bacteria]
- Anti-inflammatory [an agent to ease inflammation].
MATERIALS AND METHODS

Collection of plant materials:
The fresh leaves of *Eucalyptus globulus* were collected from the Sheikh Forest, Hoshiarpur, Punjab, India

Isolation of micro-organisms:
1) Collection of samples
   (a) Waste water was collected from the Leather Industry, Maqsudan, Jalandhar, Punjab
   (b) Soil sample was collected from the garden area of CT Institute.
   (c) Another sample of water was collected from the Pond water.

2) Further nutrient agar plates prepared using pour plating method were used for spreading of the dilutions. Colonies were isolated and streaked for bacteria and yeast for isolation of pure culture using their respective growth media.

The micro-organisms used were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Preparation of plant materials and extract:
The fresh leaves of *E.globulus* were picked and air dried over a period of two weeks. 50.0g each of the dried leaves were used for the extraction.

Extract preparation:
The method of Okogun (2000) was used to obtain the plant extract. Fifty grams (50g) of dried plant material were extracted with 200ml of solvent (in the ratio of 9:1ml distilled methanol: water respectively). The leaves were completely submerged and then covered with aluminium foil. Extraction was allowed to proceed for 48h. The extract was decanted and the solvent removed by evaporation at room temperature (28±2°C) to obtain the extract. The air dried extract was stored for 48h in sterile universal bottles at room temperature. The sterility of the extract was tested before use.

Phytochemical screening of crude extracts:
The phytochemical components of the medicinal plant were screened for using the methods of Harbone (1984) and Trease and Evans (1989). The components analysed were saponins, saponin glycosides, steroid, glycosides, tannins, flavonoids, alkaloids, volatile oils, phenols.

Screening for microbial activity:
The method of Collins *et al.* (1995) was used to test for antimicrobial activity of the plant extracts. 0.2g of the extract was reconstituted in5ml sterile distilled water and vortexes for homogeneity. 1ml of the reconstituted extract was added to Petri dishes having sterile molten nutrient agar (Oxoid) to make a final concentration of 2000 g/ml. The plates were prepared in duplicates and allowed to set at room temperature (28±2°C). A loopful each of the standardized culture of test organisms was streaked on the solidified medium and incubated for 24h at 37°C. Control plates comprising extract without inoculum and inoculum with extract were made in parallel.

Determination of minimum inhibitory concentration (MIC) of extracts:
The MIC of the plant extract was determined on solid medium (Nutrient agar) using the method of Collins *et al.* (1995). The range of concentration used was 0.0625 – 5.0mg/ml.

RESULTS

Phytochemical screening of crude extracts of *E. globulus* indicated that plant had alkaloids, flavonoids, phenolics and tannins. However, *E.globulus* had volatile oils. The components steroids, glycosides and saponins were not detected in the crude extract of the plant tested.

Table 1: Phytochemical components of the *Eucalyptus globulus*

<table>
<thead>
<tr>
<th>Component</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

+ : Positive, - : Negative.
Table 2: Antimicrobial activities of the crude extracts on pathogenic microorganisms at 2000 g/ml concentration:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Action of E.globulus Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
</tr>
</tbody>
</table>

**Antimicrobial activity of the crude extracts**

The table (2) revealed that the crude extracts of the plants exhibited antimicrobial effects on some test organisms. The plant extract inhibited the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* but had no effect on growth of *Escherichia coli*.
The minimum inhibitory concentration (MIC) of the crude extracts:
The MIC of E.globulus extract for *S. aureus*, *C. albicans*, *P. aeruginosa* was 5g/ml. The MIC was 1.25 g/ml for *S. aureus*, *P. aeruginosa* and 5 g/ml for *C. albicans* when *E. coli* exhibited resistance to all the concentrations of plant extract used in this study.

Phytochemical screening of active fractions of the extract:
The results of the phytochemical screening of active fractions in extracts of *E. globulus* revealed the presence of alkaloids, flavonoids, phenolics and tannins, saponins, glycosides, steroids were not detected in the active fractions of plant tested.

Growth assessment of bacterial in different concentrations of *E. globulus* The bacterial culture is checked for its growth in *E. globulus* extract containing media by spectrophotometer at 600 nm. Following results were obtained after taking absorbance.

<table>
<thead>
<tr>
<th>Concentration of E.globulus (ppm)</th>
<th>O.D. at 600 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ppm</td>
<td>0.1</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.2</td>
</tr>
<tr>
<td>150 ppm</td>
<td>0.3</td>
</tr>
<tr>
<td>200 ppm</td>
<td>0.4</td>
</tr>
<tr>
<td>250 ppm</td>
<td>0.5</td>
</tr>
<tr>
<td>300 ppm</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**HPLC Results:** HPLC of isolate 1 was performed. The following results were obtained which shows that *E. globulus* extract is capable of inhibiting bacterial colonies after 24hrs of incubation.

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Figure 1: The growth curve for bacterial strain in E.globulus containing media was obtained.
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Figure 2: Analysis of E.globulus (5 g/ml) with HPLC after six days incubation, as blank
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HPLC analysis it is concluded that the concentration of E.globulus extract inhibited the growth of bacterial strains, blank solution which do not contain bacteria. HPLC analysis reveals that E.globulus extract is more active due to the presence of the above phytochemical components present in the solution and shows variable peaks in graphs.

DISCUSSION

The crude extract of medicinal plant studied was found to contain one or more of the following phytochemical compounds saponin glycosides, steroid glycosides, tannins, volatile oils, phenols and balsam (gum). Other investigators (Ahmad et al., 1998; Pamplona – Roger, 1999; Shariff, 2001) have reported the presence of these components in members of the families, Combrtaceae and Myrtaceae, to which the plant used in the present study. The results of spectrophotometer at 600 nm and HPLC shows that E.globulus extract is effective on different bacterial strains and inhibit the growth of bacterial colonies. The inhibitory effects of this medicinal plant on the microorganisms may therefore, be due to the presence of the above phytochemical components. The results of the present study showed that the crude extract of E. globulus did not inhibit the growth of Escherichia coli. This means that the extract have no effect on these organisms. The results of the present study also showed the presence of alkaloids, tannins, phenolics in fractions of E. globulus. The loss of these phytochemical components may be due to fractionation. Harbone (1984) reported that the activity of plant extracts can sometimes change after fractionation and a pure crystalline compound may eventually be obtained which lacks the activity of the original extract. The occurrence of tannins in E. globulus shows that the plants may be useful in various industries. For example, tannin is useful in food, pharmaceutical and leather industries as well as in agriculture. The lemon scented volatile oil in E. globulus may be incorporated in pharmaceuticals e.g. Eucalyptus syrup, anticoagulants and suppositories for its strong antibacterial action. It could also find much use as expectorants and decongestants.

CONCLUSION

E. globulus have been found to be effective against some pathogenic microorganisms involved in wounds, burns and skin infections. Thus, the plant can be used in the treatment of these ailments. The extracts of the plants proved active against Staphylococcus aureus, Candida albicans, and Pseudomonas aeruginosa at low concentration. The lemon scented volatile oil in E. globulus may be incorporated in pharmaceuticals e.g. Eucalyptus syrup, anticoagulants and suppositories for its strong antibacterial action. It could also find much use as expectorants and decongestants. They are however not effective against Escherichia coli and the effect different concentrations of extract are still under process.

REFERENCES


