**Alcoholic and Aqueous Extract of *Eucalyptus globulus* Posses Antimicrobial Activity and Antifungal Property Confined only to Alcoholic Extract**

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***Abstract***

*Alcoholic and aqueous extract of leaves of Eucalyptus globulus was studied for in vitro for its antimicrobial and antifungal activity. The study was done against gram-positive bacteria (Staphylococcus aureus), gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and fungus (Candida albicans). The evaluation was done by determining its inhibition zone. Result demonstrated that alcoholic extract has broad spectrum antibacterial and antifungal activity on gram-positive bacteria, gram-negative bacteria and fungus, whereas the aqueous extract has only broad spectrum antibacterial activity. Our study confirms the antibacterial and antifungal property of E. globules.*

***Keywords:*** *Eucalyptus globules, anti-bacterial, amoxicillin, ciprofloxacin*

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**INTRODUCTION**

Resistances of microorganism towards conventional antimicrobial agents are a serious problem worldwide. This necessitates the search of a novel chemical agent which can substitute the conventional antimicrobial agents.

*Eucalyptus globules* (Family: Myrtaceae) is a tall and evergreen tree native to Australia and Tasmania but successfully introduced worldwide including India [1–4]. It is one such plant which symbolizes all that is wondrous in nature because, the whole plant has been used as traditional medicine for household remedy against various human ailments from antiquity. Although, it is native to Australia, its therapeutic effect have been introduced and integrated into traditional system of medicine of India, China and Greco-Europe. In India, it is described in Ayurveda for more than 1250 preparations containing eucalyptus oil [5]. Many amongst these preparations have been successfully used in various infectious conditions. Eucalyptus oils have a history of wide application including pharmaceutical use. Moreover, report suggests that it has antibacterial effect but proper scientific evidence is missing. Hence, the aim of our study was to investigate the antimicrobial activity of *Eucalyptus globules* extracts on some microbial stains.

**MATERIALS AND METHODS**

**Plant Materials**

The fresh leaves of *Eucalyptus globulus* were collected on October 2014 from the local areas of Bangalore, India. The sample was authenticated in the research and development laboratory of Natural Remedies Pvt. Ltd., Bangalore of Karnataka State, India, by comparing the sample with authentic sample. A voucher specimen has been preserved at the laboratory for further reference. The collected fresh leaves of *E. globulus* were air dried over a period of two weeks and the air-dried leaves were grinded into powder.

**Extract Preparation**

***Alcoholic Extract***

About 50 g of dried plant material were extracted with 200 ml of solvent (in the ratio of 9:1 ml distilled methanol: water respectively). The leaves were completely submerged and then covered with aluminum foil. Extraction was allowed to proceed for 48 h. 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 48 h in sterile universal bottle at room temperature. The sterility of the extract was tested before use.

***Aqueous Extract***

Aqueous extract were prepare by maceration in distilled water for 4 days at 4°C with intermediate shaking then the extracts were filtered through cotton then kept at 4°C till use.

**Preliminary Phytochemical Analysis**

The preliminary phytochemical screening revealed the presence of steroids, alkaloids, saponins, terpenes, tannins, phenolic substances, carbohydrates, volatile oil and mucilage [6, 7]. Quantitative estimations revealed the presence of 3% of volatile oil in fresh leaves and 16.52% of total mucilage content in the dried leaf powder.

**Micro Organisms**

Standard microbial strains *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida albicans* were obtained from the microbiology department, T John College of Pharmacy. Bacterial strains were cultured on nutrient agar medium at 37°C for 2 days. While yeast and moulds strain were cultivated and tested on Sabouraud’s dextrose agar medium at 25°C for 2 days.

The organisms were identified by cellular, cultural and biochemical characteristics. All species of *Staphylococcus* are gram positive cocci. On nutrient agar, they tend to be white, circular, entire, convex colonies. On blood agar, *S. aureus* showed hemolysis of the agar in the area around the colony.

***IN VITRO* SCREENING FOR ANTIBACTERIAL ACTIVITIES**

**Antibacterial Activity [8–12]**

Antibacterial activity of the synthesized compounds was determined, using a slightly modified cup plate method.

Muller Hinton agar was used for the growth of bacterial strains such as gram-positive (*S. aureus*) and gram-negative (*E. coli* and *Pseudomonas aeruginosa*) and fungal stain (*Candida albicans)*. Each organism was suspended in normal saline solution and transmittance (T) of 75 to 77% at 530 nm was made, which is equal to 106 CFU/ml. All the test compounds were dissolved in DMSO at a concentration of 1 mg/ml. Each was inoculated with 20 µl of microbial suspension. 100 µl of the test compounds was added to each cup. The plates containing bacteria were incubated at 37°C for 24 h, the positive antimicrobial activity were read, based on the growth inhibition zone and compared with amoxicillin and ciprofloxacin drugs.

**RESULTS AND DISCUSSION**

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 used. Other investigator has reported the presence of these components in members of the families, Combretaceae and Myrtaceae, to which the plant used in the present study (Table 1).

***Table 1:*** *Phytochemical Screening of Eucalyptus globules.*

|  |  |
| --- | --- |
| Alkaloids | + |
| Tannins | + |
| Glycosides | - |
| Saponins | - |
| Steroids | - |
| Flavonoids | + |
| Phenolics | + |

***+,*** *Positive;* ***-,*** *Negative.*

The result of antibiogram (Table 2) suggest that *S. aureus* was highly sensitive to chloramphenicol, nitroxoline and pristinamycin; intermediate resistant towards amoxicillin, erythromycin whereas resistant towards ampicillin and griseofulvin. *E. coli* was highly sensitive to amoxicillin, erythromycin; intermediate resistant towards chloramphenicol, nitoxoline, pristinamycin and ampicillin, whereas resistant towards griseofulvin. *P. aeruginosa* was highly sensitive to amoxicillin, erythromycin; intermediate resistant towards chloramphenicol, nitoxoline, pristinamycin; whereas resistant towards ampicillin and griseofulvin. *C. albicans* was sensitive only for griseofulvin. Table 3 shows the result obtained for the antimicrobial effect of *E. globules*. The observation suggests that all the bacterial stains of *S. aureus, E. coli and P. aeruginosa* show no effect on lower concentration up to 2 μl of both aqueous and alcoholic extract of *E. globules*. All bacterial stains responded to the extracts at 5 μl dose, suggesting that the aqueous and the alcoholic extract is sensitive to both gram positive and gram negative bacteria. On the other hand, alcoholic extract but not the aqueous extract inhibits *C. albicans* growth suggesting that the active principle responsible for antifungal activity is present in the alcoholic extract. All the bacterial and fungal stain shows dose dependent bacterial inhibition in response to the aqueous and alcoholic extract of *E. globules*. It is observed that both aqueous and alcoholic extracts consist of the active principles responsible for antimicrobial effect. It is also been observed that the alcoholic extract possess more inhibition zone than the aqueous extract suggesting that the component responsible for antimicrobial property are present more in the alcoholic extract.

The result of the study suggests that both, aqueous and alcoholic extracts used in the present study have antibacterial effects which are effective against both gram positive and gram negative bacteria. It also suggests that the antifungal principles of *E. globules* are confined only to the alcoholic extracts.

***Table 2:*** *Antibiogram Results of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotics** | **Zone of Inhibition (cm)** | | | |
| ***S. aureus*** | ***E. coli*** | ***P. aeruginosa*** | ***C. albicans*** |
| Ampicillin | 0 | 1.2 | 0 | 0 |
| Amoxicillin | 1.5 | 2.8 | 2.7 | 0.2 |
| Chloramphenicol | 2.7 | 1.7 | 1.2 | 0.4 |
| Erythromycin | 1.3 | 2.3 | 2.2 | 0.4 |
| Nitroxoline | 2.1 | 1.3 | 1.2 | 0.5 |
| Pristinamycin | 2.6 | 1.2 | 1.2 | 0 |
| Griseofulvin | 0.2 | 0.2 | 0.3 | 2.7 |

***Table 3:*** *Antimicrobial Activity of Crude Extract of Eucalyptus globules.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Microbial Species** | ***S. aureus*** | | | | | | |
| The Dose (μl) | | 1 | 2 | 5 | 7.5 | 10 | 20 |
| Inhibition zone diameter (cm) | Aqueous Extract | 0 | 0 | 1.3 | 1.8 | 2.1 | 2.9 |
| Alcoholic Extract | 0 | 0 | 1.6 | 2.5 | 2.9 | 4 |
| Microbial Species | ***E. Coli*** | | | | | | |
| The Dose (μl) | | 1 | 2 | 5 | 7.5 | 10 | 20 |
| Inhibition zone diameter (cm) | Aqueous Extract | 0 | 0 | 0.9 | 1.1 | 1.4 | 1.7 |
| Alcoholic Extract | 0 | 0 | 1.2 | 1.5 | 2 | 2.4 |
| Microbial Species | ***P. aeruginosa*** | | | | | | |
| The Dose (μl) | | 1 | 2 | 5 | 7.5 | 10 | 20 |
| Inhibition zone diameter (cm) | Aqueous Extract | 0 | 0 | 0.8 | 1 | 1.2 | 1.6 |
| Alcoholic Extract | 0 | 0 | 1.1 | 1.3 | 1.9 | 1.3 |
| Microbial Species | ***C. albicans*** | | | | | | |
| The Dose (μl) | | 1 | 2 | 5 | 7.5 | 10 | 20 |
| Inhibition zone diameter (cm) | Aqueous Extract | 0 | 0 | 0 | 0.3 | 0.9 | 1.0 |
| Alcoholic Extract | 0 | 0 | 0.3 | 1.2 | 1.6 | 2.1 |

**CONCLUSION**

The present study confirms the antimicrobial and antifungal property of *E. globules* that showed significant inhibition in the growth of bacterial stain *S. aureus, E. coli and P. aeruginosa* and fungal stain *C. albicans.* The encouraging results suggest that *E. globules* might be exploited as natural antibiotics for the treatment of several infectious diseases.

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