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# The Antimicrobial Activities of Methanolic Extracts of *Eucalyptus camaldulensis* Against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*

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**ABSTRACT**
Methanolic extract of leaves of *Eucalyptus camaldulensis* was studied for in vitro microbial activities by agar dilution method. The phytochemical analysis of the crude extract of the medicinal plant revealed the presence of saponin, saponin glycosides, steroid, cardiac glycoside, tannins, volatile oils, phenols and balsam (gum). The methanolic extract of the plant inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* (ATCC103207 and Clinical strain respectively) but had no inhibitory effects on and *Escherichia coli*. The minimum inhibitory concentration (MIC) of the extract ranged from 1.25g/ml to 5g/ml. The results obtained suggest that *Eucalyptus camaldulensis* can be used in treating diseases caused by the test organisms.

***Keywords:*** Antimicrobial, *Eucalyptus camaldulensis*, Pathogenic microorganisms, Inhibitory activity

## 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 (Ebana *et al.*, 1991; Williams, 1996; Pamplona–Roger, 1999; Manna and Abalaka, 2000). Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive.

However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001). 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 (Shariff, 2001). There is therefore the need to search for plants of medicinal value. The plant used in the present study is *Eucalyptus camaldulensis* (Myrtaceae), which are 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 *E. camaldulensis*.

The antimicrobial activity of the active fractions of the extract was also determined.

## MATERIALS AND METHODS

*Collection of plant materials*

*Eucalyptus camaldulensis* was collected at the garden of University of Tehran, Iran in the month of September, 2008. The plant was identified by of Faculty of Biology, Payame Noor University Iran. Source of microorganisms The organisms used were *Bacillus subtilis*, *Escherichia coli* (ATCC 10412), *Staphylococcus aureus* (ATCC 103207), The organisms were obtained from the Pharmaceutical Microbiology Unit of Islamic Azad university.

Standardization of microorganisms Exactly 0.2 ml of overnight cultures of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 3 – 5h to standardize the culture to 106 cfu/ml.

A lapful of the standard cultures was used for the antimicrobial assay (Collins *et al.*, 1995).

*Preparation of plant materials and extract*

The old leaves were picked from stem 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 aluminum 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 plants were screened for using the methods of Harbone (1984) and Trease and Evans (1989).

The components analysed for are saponins, saponin glycosides, steroid, glycosides, anthraquinones, tannins, flavonoids alkaloid, volatile oils, phenols and balsam (gum).

*Screening for antibacterial 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 vortexed 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 37oC. 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.

*Separation of extracts of E. camaldulensis into fractions using accelerated gradient chromatography (AGC)*

Three grams (3.0g) of leaf extract of the plant was dissolved in small quantity of distilled methanol. Five g of silica gel was added to the mixture and stirred thoroughly after which it was transferred to a mortar and mixed thoroughly with pestle. The components of the mixture were then separated using AGC machine (FMI LAB Pump Model QD. ALITEA. AB. Stockholm, Sweden). The fractions were collected in sterile test tubes for further analysis.

*Separation of components of fractions using thin layer chromatography (TLC)*

TLC plate was prepared and spotted with fractions of the medicinal plants separated by the AGC. The spotted plate was then placed in TLC tank containing the solvent system: Butanol: Acetic acid: water in the ratio 60: 15: 25 respectively for *E. camaldulensis* fractions. The separated components on the TLC plate was viewed using ultraviolet light (Eagle Scientific Ltd., Great Britain) at 365 nm wavelength. Fractions from the AGC with similar components on TLC were combined and used for antimicrobial assay.

*Antimicrobial activity of combined fractions of E. camaldulensis on pathogenic microorganisms*

The combined fractions of *E. camaldulensis* were tested for activity on the test organisms using the method of Collins *et al.* (1995) as described in screening for antibacterial.

## RESULTS

Phytochemical screening of crude extracts of *E. camaldulensis* indicated that plant had saponins and tannins. However *E. camaldulensis* had volatile oils. The components, anthraquinones, hydrolysable tannin, flavonoid, alkaloid and glycosides were not detected in the crude extract of the plant tested (Table 1).

Table1. Phytochemical components of the *Eucalyptus camaldulensis*

| *Phytochemical components* | |
|---|---|
| Saponin glycosides | - |
| Saponins | + |
| Steroid | - |
| Glycosides | - |
| Digitalis glycosides (cardiac) | - |
| Anthraquinones | - |
| Tannins | + |
| Hydrolysable tannins | - |
| Flavonoids | - |
| Alkaloid | - |
| Volatile oils | + |
| Phenols | - |
| Balsam (gum) | + |

+: Present; -: Absent

*Antimicrobial activity of the crude extract*

The results (Table 2) revealed that the crude extract of the plant exhibited antimicrobial effects on some test organisms. plant extract inhibited the growth of *B. subtilis*, *S. aureus* (ATCC 103207 and clinical) but were unable to inhibit the growth of *Escherichia coli* (Table 2).

Table 2. Antimicrobial activities of the crude extract on pathogeni microorganisms at 2000 g/ml concentration

| Organisms | |
|---|---|
| *Bacillus subtilis* | + |
| *Staphylococcus aureus* (ATCC103207) | + |
| *Escherichia coli*(ATCC 10418) | - |

*The minimum inhibitory concentration (MIC) of the crude extracts*

The MIC of *E. camaldulensis* extract for *S. aureus* (clinical and typed) and *B. subtilis* was 5g/ml.The MIC was 1.25 g/ml for S. aureus (clinical and typed) and 5 g/ml for *B. subtilis* when the extract of, *E.*

*coli* exhibited resistance to all the concentrations of plant extract used in this study.

*The antimicrobial activity of combined fractions*

Result shows the results of the antimicrobial activity of combined fractions of *E. camaldulensis*. Combination of the fractions was based on similarity in Rf values. The results revealed that fractions 1 – 4 and 5 – 7 were unable to inhibit the growth of any of the test organisms. Fractions 8, 11 – 14 and 15 inhibited the growth of *Staphylococcus* strains and B. subtilis.. None of these fraction combinations inhibited the growth of *E. coli*. The rest of the fractions exhibited no antimicrobial activity on the test organisms.

*Phytochemical screening of active fractions of the extract*

The results of the phytochemical screening of active fractions in extract of *E. camaldulensis* revealed the presence of alkaloids, volatile oils and balsam (gum). Saponins, flavonoids, steroids were not detected in the active fractions of plant tested.

## DISCUSSION

The crude extract of medicinal plant studied was found to contain one or more of the following phytochemical compounds saponins, saponin glycosides, steroid, cardiac 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, Combretaceae and Myrtaceae, to which the plant used in the present study belong. 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. camaldulensis* 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 and glycosides in fractions of *E. camaldulensis*. 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. camaldulensis 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 (Nguji, 1988; Dalziel, 1995). The lemon scented volatile oil in *E. camaldulensis* may be incorporated in pharmaceuticals e.g. *Eucalyptus* syrup, anticough solutions and suppositories for its strong antibacterial action. It could also find much use as expectorants and decongestants.

## CONCLUSION

*E. camaldulensis* 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* and *Bacillus subtilis* at low concentration. They are however not effective against, *Escherichia coli.*

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