



## Original Research Article

**Evaluation of Antimicrobial Potential of *Eucalyptus camaldulensis* L.****Bhawana Pandey\*, Sheetal Singh**

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**ABSTRACT**

The *in vitro* antimicrobial activity of acetone, methanol and water extracts of leaf, stem and bark *Eucalyptus camaldulensis* L. (*Myrtaceae*.) It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Antimicrobial was examined against six bacterial species *Bacillus megaterium*, *B.subtilis*, *Staphylococcus*, *S. aureus*, *Micrococcus luteus* and *E.coli* using the agar well diffusion method. Phytochemical screening was carried out for phenols, flavonoids and tannins. Results showed that the plant extracts exhibited a dose-dependent inhibition of microorganisms. The acetone and methanol extracts of leaf and stem bark of *E. camaldulensis* displayed maximum antibacterial activity against all the bacterial species studied. *E. camaldulensis* extracts contained phenols, flavonoids and tannins at varying levels. The ability of the crude extracts of the test plant to inhibit the growth of bacteria is an indication of its broad spectrum. The antibacterial activity of the leaf extracts of *E. camaldulensis* can be attributed to the action of the phytochemical compounds it contains. There was no significant difference in the antimicrobial activity of the extracts on Gram-negative and Gram positive bacteria despite the differences in their cell wall components. The crude and pure methanol extract of *E. camaldulensis* has been found to be effective against *Staphylococcus aureus* (0.9cm) and *Bacillus megaterium* (3.1.cm) diameter zone of inhibition.

**Keywords:** *Eucalyptus camaldulensis*; Phytochemicals; Antimicrobial activity; Agar well diffusion method; Zone of inhibition

**INTRODUCTION**

In recent years multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious

diseases, uncommon infections are a serious medical problem [1]. Therefore the scientists of the 21st century are generally reviving our traditional knowledge [2] and are screening various parts of plants scientifically used in the folklore medicine in

search of newer lead compounds having antimicrobial efficacy.

Eucalyptus oil obtained by steam distillation and rectification of the fresh leaves has as its active ingredient and this is responsible for its various pharmacological actions [3]. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial [4], antifungal and antiviral agents with significant activity against infective microorganisms [5].

Antibacterial properties of Indian medicinal-plants against *Micrococcus luteus* was studied by scientists [6]. Medicinal plants are known to produce bioactive molecules which react with organisms inhibiting bacterial or fungal growth and protect the human body against pathogens [7,8].

*Eucalyptus camaldulensis* is a genus of tropical and subtropical evergreen trees of family *Myrtaceae*, contain chemical constituent such as saponins, tannins and glycosides [9].

Various species of *E. camaldulensis* have been known to possess antimicrobial [10], analgesic [11] and antihypertensive [12] activities. However, there is insufficient information regarding the antimicrobial activity of *E. camaldulensis* L. In this paper, the antimicrobial property of crude and pure extract of the leaf, stem and bark of *E. camaldulensis* L. has been studied.

## MATERIALS AND METHODS

### Plant materials and preparation of extract

The leaves stem and bark of and *Eucalyptus camaldulensis* L. were collected from Amarkantak Hills (M.P.), India. The plant materials were dried under shade and crushed in a grinder and stored for further use.

The air dried, powdered plant materials were extracted in the Soxhlet apparatus successively with different solvents in the increasing order of polarity like Acetone, Methanol and Water. Each time before extracting with the next solvent, the

powdered materials were dried in a hot air oven at 40°C. Finally, the materials were macerated using hot water with stirring for 10 hours and the water extracts were filtered.

The different solvent extracts were concentrated, vacuum dried and weighed. The percentage yield was expressed in terms of air dried sample. The extracts were dried over anhydrous sodium sulphate, stored in sealed vials in refrigerator (5-8°C) until analysis.

### Test microorganisms

The bacteria used in this study *Bacillus megaterium*, *B.subtilis*, *Staphylococcus*, *S. aureus*, *Micrococcus luteus* and *E.coli* were obtained from the culture collections of Nitza Pvt. Ltd., Hyderabad, India. Bacteria were cultured in Mueller Hinton Broth (MHB) for 37°C.

### Antimicrobial bioassay

The antimicrobial activity of the crude extracts was determined in accordance with the agar well diffusion method [13]. Bacteria were cultured overnight at 37°C in Mueller Hinton Broth (MHB). Subsequently, using a sterile borer, well of 9 mm diameter was made in the inoculated media. Negative control was prepared using the same solvent employed to dissolve the extracts. Gentamycin (50 µg/ml) and Ampicilin (100 units/disc) were used as positive control. The test plates were incubated at 37°C for 24 hours depending on the incubation time required for a visible growth [11]. The diameter of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured.

### Preliminary phytochemical analysis

The extracts of the plant were screened for phenols, flavonoids and tannins using the following procedure:

### Test for Saponins

Foam test- Samples were dissolved in distill water and shaken vigorously. A layer of foam on top layer was formed which is stable, indicates the presence of saponins in the sample.

### Test for Glycosides

Hansch Test- In aqueous extract conc.  $H_2SO_4$  was added from the side walls and formation of a brown ring suggested the presence of carbohydrates.

### Test for Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

## RESULTS AND DISCUSSION

The percentage yield of extracts and the phytochemical constituents of the plant are shown in and Table 1, respectively. The maximum per cent yield was registered in the methanol extract of stem bark of *E. camaldulensis*. Methanol and water were more efficient to extract antioxidant compounds from *Phellinus baumii* [14]. Similarly, it was showed that methanol extract of stem bark of *E. camaldulensis* had a maximum extractable value [15], so for antimicrobial test methanol extract of stem bark of *E. camaldulensis* were used in crude and pure form.

**Table 1: Phytochemical Screening of Different Solvent Extracts of *E. camaldulensis* L**

Sample	Extraction medium	Saponins	Glycosides	Tannins
Leaf	Acetone	++	+++	+++
	Methanol	+++	++	+++
	Aqueous	++	+	++
Stem Bark	Acetone	+++	++	+++
	Methanol	+++	+++	+++
	Aqueous	+	+	++
Fruit	Acetone	++	++	++
	Methanol	++	+++	+++
	Aqueous	+	+	+

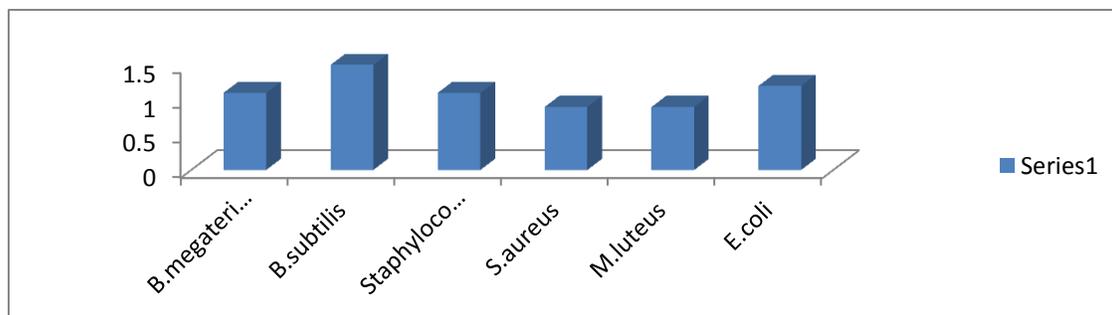
The antimicrobial activity of crude and pure extracts of *E. camaldulensis* shown in Table 2 and 3. The plant extracts showed a dose-dependent inhibition of micro-organisms. Among the extraction medium, methanol extracts of stem bark of *E. camaldulensis* displayed maximum antibacterial activity against *B.subtilis* bacterial species.

### Eucalyptus(Crude extract)

Methanol is used for the soxhlet extraction of *E. camaldulensis*. The in vitro antibacterial activity of crude extracts on Gram-negative and Gram positive organisms were tested and tabulated as in Table 2.

**Table 2: Antibacterial activity of crude extracts of *E. camaldulensis* L (in cm)**

Organisms	<i>B.megaterium</i>	<i>B.subtilis</i>	<i>Staphylococcus</i>	<i>S.aureus</i>	<i>M.luteus</i>	<i>E.coli</i>
Plant	1.1	1.5	1.1	0.9	0.8	1.2
<i>Eucalyptus camaldulensis</i>						



**Fig 1: Antibacterial activity of crude extracts of *E. camaldulensis* L**  
**X-axis= Organisms ,Y-axis =Readings**

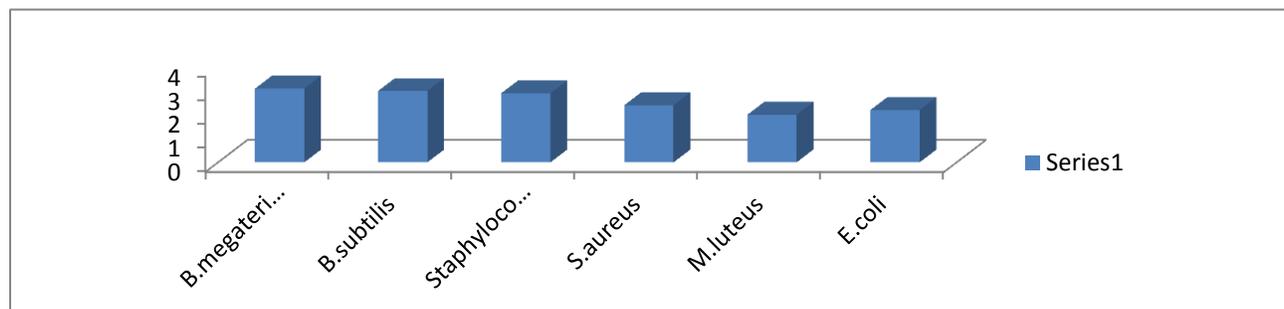
The maximum zone of inhibition of crude extract of *E. camaldulensis* was shown by *B.subtilis* i.e. 1.5cm and minimum zone of inhibition was shown by *M.luteus* i.e. 0.8 cm.

**Eucalyptus (Purified extract)**

Hexane and ethanol (5ml:5ml) is used for the purification of *E. camaldulensis*. The in vitro antibacterial activity of purified extracts on Gram-negative and Gram positive organisms were tested and tabulated as in Table 3.

**Table 3: Antibacterial activity of pure extracts of *Eucalyptus camaldulensis* (in cm)**

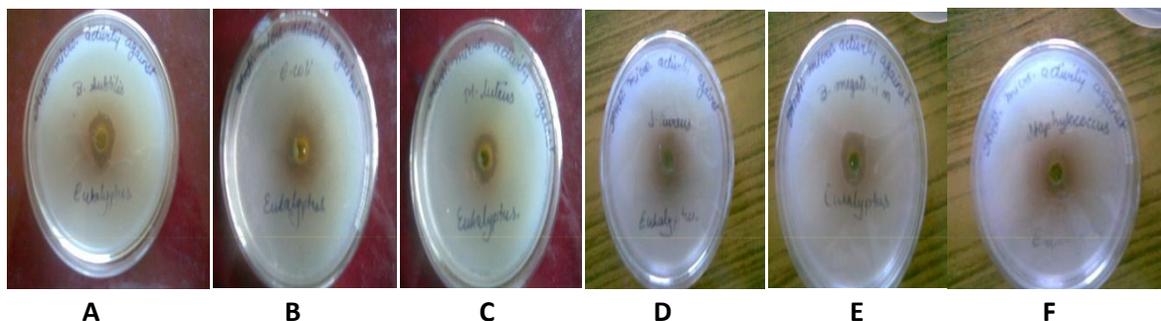
Organisms	<i>B.megaterium</i>	<i>B.subtilis</i>	<i>Staphylococcus</i>	<i>S.aureus</i>	<i>M.luteus</i>	<i>E.coli</i>
Plant <i>Eucalyptus camaldulensis</i>	3.1	3.0	2.9	2.4	2.0	2.2



**Fig. 2: Antibacterial activity of pure extracts of *E. camaldulensis* L**  
**X-axis= Organisms , Y-axis =Readings in cms**

The maximum zone of inhibition (Fig.3) of purified extract of *E. camaldulensis* was shown by *B. megaterium* i.e. 3.1 cm and minimum zone of inhibition was shown by *M. luteus* i.e. 2 cm. The antibacterial activity of the leaf extracts of *E. camaldulensis* L can be attributed to the action of

the phytochemical compounds it contains. There was no significant difference in the antimicrobial activity of the extracts on Gram-negative and Gram positive bacteria despite the differences in their cell wall components.



**Fig. 3: Antimicrobial activity shown by *Eucalyptus camaldulensis* L on (A) *B.subtilis* (B) *E.coli* (C) *M.luteus* (D) *S.aureus*. (E) *Staphylococcus* (F) *B.megaterium*.**

Saponins, glycosides and tannins present in the plants were known to be toxic to the microorganisms. Tannins from *Dichrostachys cinerea* root bark possessed antibacterial activities 24. In the present study, the phytochemical analysis of *E. camaldulensis* L solvent extracts revealed the presence of saponins, glycosides and tannins at varying intensity. The phytochemical characteristics possessed by *E. camaldulensis* L may be attributed to its antimicrobial properties.

The high inhibitory potential of acetone and methanol extracts of leaf and stem bark of *E. camaldulensis* L might be due to the high solubility of the phytoconstituents in the organic solvents. The phytoconstituents might be present in higher concentrations in the leaf and stem bark along with some new microbicidal agents reflecting its higher bactericidal and fungicidal potential. Presence of these phytoconstituents in the leaf and stem bark pointed towards the pharmacological activities of this plant and supported the claim of the traditional users.

In support of the present study, the results revealed that the methanol extracts were more potent than aqueous extracts of plants studied [16]. Similarly the methanol extracts of *E. camaldulensis* exhibited inhibitory activities that were found to be a little higher than aqueous extract on the bacterial species. Results of the present investigation agreed with the other reported results.

## CONCLUSION

The results of this study have shown that the leaf extracts of *E. camaldulensis* (*Eucalyptus*) have great potential as antimicrobial agents in the treatment of infectious organisms. Further detailed investigation of the active components of the plant for the exact mechanism of action will contribute greatly to the development new pharmaceuticals. The antibacterial activity of the leaf and bark extracts of *E. camaldulensis* can be attributed to the action of the phytochemical compounds it contains.

It is inferred from the current findings that the phytoconstituents might be present in high concentrations in the leaf of *E. camaldulensis* along with some new microbicidal agents reflecting its higher bactericidal potential. However, further studies are necessary to find out the active principles responsible for these activities which can be used as natural antimicrobial agents for human consumption and cure of infectious diseases.

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