Research Article

# Phytochemical and Antibacterial activity of *Eucalyptus*

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**ABSTRACT**

The methanol leaf extracts of *Eucalyptus* showed significant antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus pyrogens, Staphylococcus aureus.* The antibacterial potential of methanol extract of *Eucalyptus* was tested by using Agar well diffusion method. The (100mg/ml) leaf extract showed maximum inhibition against *Pseudomonas aeruginosa* (22mm). Phytochemical tests were performed and showed that the antibacterial activity of plant *Eucalyptus* leaves was due to the presence of phytochemical compounds like steroids, phenolic compounds, tannins, flavonoids, saponins.

**Keywords:** Methanol extract of *Eucalyptus*, Phytochemical Analysis, Antibacterial activity.

## INTRODUCTION

Infectious diseases are world's most important reason of untimely death, killing 50,000 people each day[1]. Resistance to antimicrobial agents is rising in a wide diversity of pathogens and numerous drug resistances are becoming common in diverse organisms[2]. The microbial fighting is mounting day by day and the viewpoint for the use of antimicrobial drugs in the prospect is still uncertain. Therefore, way to be taken to decrease this problem, for example, to control the use of antibiotic, build up research to enhance understand the genetic mechanisms of resistance, and to continue studies to develop new drugs either synthetic or natural. The final goal is to present suitable and well-organized antimicrobial drugs to the patient[3]. Infectious disease can become a threat to public health in this world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. Although ancient civilization recognized the antiseptic or antibacterial potential of many plant extracts, they failed to document the preservative and curative effects of plant extracts[4].

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases[5]. The plant extracts have been developed and proposed for use as antimicrobial substances[6]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine[7].

Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential.[8-10] The present study was aimed to evaluated the antibacterial potential of methanol extract of *Eucalyptus* against bacterial pathogens and phytochemical analysis was done.

## MATERIALS AND METHODS

### Collection and Drying of plant materials

Mature leaves of were collected *Eucalyptus* from Coimbatore in Tamil Nadu. The leaves were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

### Preparation of plant extract

10 g of powdered leaves were extracted successively with 100 ml of methanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use [11].

### Test microorganisms

Five pathogenic bacteria, *viz*., *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Klebsiella pneumonia*, *Vibrio cholera* and *Pseudomonas aeruginosa* were used during the present study and were obtained from MTCC, Chandigarh. The cultures were sub-cultured and maintained on nutrient agar slants and stored at 4°C.

### Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards.

### Determination of antibacterial activity (Agar well Diffusion)

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculums on the surface of the media. Wells (8 mm in diameter) were punched in the agar. Methanol extracts with same concentrations of 100 mg/ml were used. The plates were

incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

**Phytochemical analysis**

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

***Test for Alkaloids (Meyer's Test)***

The extract of*Eucalyptus* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent[12]. The samples were then observed for the presence of turbidity or yellow precipitation [13].

***Test for Glycoside***

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated Sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer [11].

***Test for Terpenoid and Steroid***

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids [11].

***Test for Flavonoid***

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavonoid [11].

***Test for Reducing sugars***

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

***Test for Triterpenes***

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

***Test for Phenolic Compounds (Ferric chloride test)***

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

***Test for Tannins***

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution wad added. Blue colour was observed for gallic tannins and green black for catecholic tannins [14].

***Test for Saponins***

2g of the powered sample was boiled in 20 ml of distilled water in a water bath. 10ml of the filterable was mixed with 5 ml of distilled water shaken vigorously for a stable persistent broth. The following was mixed with 3 drops of Olive oil and shaken vigorously and then observed for the formation of emulsion.

## RESULTS AND DISCUSSION

The present study aimed at testing the antibacterial activity of *Eucalyptus* leaves against five human pathogens and the findings were summarized. The leaves of *Eucalyptus* were collected from Coimbatore district. The collected leaves were dried and powdered. Powdered leaves were extracted successively using polar solvent viz., methanol. The extracts of *Eucalyptus* were tested against pathogenic bacteria like *klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyrogens* and *Staphylococcus aureus* by agar well diffusion method. The methanol extract of *Eucalyptus* (100mg/ml) showed maximum zone of inhibition (22mm) against *Pseudomonas aeurginosa. Streptococcus pyogens* showed (16mm) less zone of inhibition (Table 1). Antibacterial activity of *Eucalyptus* plant leaves is due to the presence of phytochemical compounds like phenolic compounds, tannins, steroids, flavonoids, saponins (Table 2).

**Table 1:** Antibacterial activity of *Eucalyptus*methanol extract against bacterial pathogens:

| Organism | Concentration of extract and zone of inhibition (mm) | | |
|---|---|---|---|
| | 50 µl | 75 µl | 100 µl |
| *Escherichia coli* | 17mm | 20mm | 21mm |
| *Pseudomonas aeruginosa* | 19mm | 20mm | 22mm |
| *Klebsiella pneumonia* | 16mm | 19mm | 20mm |
| *Streptococcuspyogens* | 16mm | 18mm | 18mm |
| *Staphylococcus* | 15mm | 17mm | 21mm |

**Table 2:** Phytochemical analysis of *Eucalyptus* extract

| Test | Result |
|---|---|
| Phenolic compounds | + |
| Tripenoid | - |
| Triterpenes | - |
| Tannins | + |
| Saponins | + |
| Steroids | + |
| Flavonoids | + |

## DISCUSSION

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organisation estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world population. There are about 45,000 plant species in India with capacity to produce a large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity[15,16]. The result of phytochemicals in the present investigation showed that the plant leaves contain components like tannins, saponins, steroids, phenolic compounds flavonoids. This study reports the presence of different phytochemicals with biological activity that can be valuable therapeutic index[17,18]. In the present study, we have found that the biologically active phytochemicals were present in the methanolic extracts of few medicinal plants. The antibacterial properties of these extracts may be due to the presence of above mentioned phytochemicals.

## CONCLUSION

Phytochemical and antibacterial activity of *Eucalyptus* extract showed that it is mainly due to the presence of phytochemical compounds such like tannins, saponins, glycosides, triterpenes and tripenoid. The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs.

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