

**INTERNATIONAL JOURNAL OF ADVANCES IN  
PHARMACY, BIOLOGY AND CHEMISTRY****Research Article****Antimicrobial Activity of Volatile oil of  
*Eucalyptus globulus Labill*****Arti Dixit\*<sup>1</sup>, Ankur Rohilla<sup>1</sup>, Jyoti Dixit<sup>2</sup>, Vijender Singh<sup>2</sup>.**<sup>1</sup>Department of Pharmaceutical Sciences, Shri Gopi Chand Group of Institutions,  
Baghpat, UP, India–250609.<sup>2</sup>BBS Institute of Pharmaceutical and Allied Sciences,  
Knowledge Park-III, Greater Noida, UP, India**Abstract**

The present study was designed in order to conduct the antimicrobial activity of *Eucalyptus globulus Labill*. The potent antimicrobial activity of the essential oil extracted from the leaves of *Eucalyptus globulus Labill*. was evaluated against 6 antimicrobial strains, including 4 bacterial and 2 fungal strains; namely *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*. The results of the antimicrobial activity tests revealed that the essential oil of *E. globulus* has rather a strong antimicrobial activity, especially against *Bacillus subtilis*, *Staphylococcus aureus*, and *Aspergillus niger*. The maximum inhibitory activity was found with 100% concentration of volatile oil against bacteria *Bacillus subtilis* and fungus *Aspergillus niger*.

**Keywords:** *Eucalyptus globulus Labill*, Essential oil, Antimicrobial activity**INTRODUCTION**

Essential oils from herbal plants have gained a force to act as functional ingredients in foods, drinks, and cosmetics worldwide 1. The essential oils have been known to possess various pleiotropic effects due to the presence of bioactive components in them, and thus, the use of essential oils in pharmaceutical industries requires a systematic examination of the plant extracts. The *Eucalyptus* genus comprises over 500 species of aromatic trees and shrubs around the world 2. *Eucalyptus globulus Labill.*, a plant from family myrtaceae, commonly known as blue gum, grows well in Nilgiris, Annamalai, Palni and Simla hills 2-3. *Eucalyptus globulus* is a rich source of phytochemical constituents which contain flavonoids, alkaloids, tannins and propanoids, which are present in the leaf, stem and root of the plant. Numerous phytochemicals have been found to be associated with the plant which include 1,8-cineole,  $\alpha$ -gurjunene, globulol,  $\beta$ -pinene, pipertone, borneol, bornylacetate, camphene, caproic acid, citral,

eudesmol, fenchone, p-menthane, myrecene, myrtenol,  $\alpha$ -terpineol, verbinone, asparagine, cysteine, glycine, and ornithine 4-7. The *Eucalyptus* species extracts have a known history of herbal use for the treatment of cold, chest pain, or cough. Also, the *Eucalyptus* leaf extracts have been reported to treat influenza, chest problems, and skin rashes alongwith their vapours inhaled to brawl inflammation. *Eucalyptus globulus Labill.* contain large amounts of essential oils, that have an important role in medicine, aromatherapy, and perfumes. In addition, when the essential oils have been used at concentrations below their minimum inhibitory concentrations (MIC), they exert rubefacient, local anaesthetic, spasmolytic, antiphlogistic, and secretolytic effects, contributing to their therapeutic potential 8-10. Thus, the present study has been designed to determine the antimicrobial activity against the bacterial and fungal strains by the cylinder plate method.

## MATERIALS AND METHODS

### Collection of Plant material

Fresh leaves of *Eucalyptus globulus* commonly known as Blue gum were collected from Greater Noida and authenticated at Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is preserved in the herbarium section of Phytochemistry laboratory, Faculty of Pharmacy, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, Uttar Pradesh.

### Isolation of Volatile oil

The fresh leaves (2 kg) were hydrodistilled for three hours according to the method recommended in the British Pharmacopoeia 2003. The light yellowish green coloured oil (6 ml) was obtained. The collected volatile oil was dried over an anhydrous sodium sulphate and stored at 40°C in the dark.

### Microbial Strains

The strains were obtained from the microbiology laboratory, Department of Pharmacy, and the identification of each culture was done by conventional methods. Pure Gentamycin (50 mg/ml) and Nystatin (50 IU) were used as standards for comparison of antibacterial and antifungal activity, respectively. The selected test organisms are *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Candida albicans*.

### Preparation of Media

#### (a) Preparation of Media (for bacteria)

Media was prepared in distilled deionized (DI) water to produce 1000 ml by dissolving agar (15 g), peptone (6 g), beef extract (1.5 g), dextrose (1 g), yeast extract (3 g), casin enzymic hydrolysate (4 g). Then sufficient quantity of 1M NaOH or 1 M HCl was added. The pH was adjusted to  $8.3 \pm 0.05$  and then sterilized for 15 minutes at 15 lb pressure in an autoclave.

#### (b) Preparation of Media (for fungi)

Media was prepared in distilled deionized (DI) water to produce 1000 ml by dissolving Mixture of Peptic digest of animal tissue and Pancreatic digest of casein in 1:1 ratio (6 g), dextrose (1 g), and agar (15 g). Then sufficient quantity of 1M NaOH or 1 M HCl was added. The pH was adjusted to  $5.6 \pm 0.2$  and then sterilized for 15 minutes at 15 lb pressure in an autoclave.

### Preparation of Standard Solutions

For the preparation of standard solution, pure Gentamycin and Nystatin were dissolved in DMSO.

### Preparation of Test Solutions

The volatile oil of leaves of *Eucalyptus globulus* was dissolved in dimethyl formamide (DMSO). The three different concentrations (10% v/v, 50% v/v and 100% v/v) have been used for antimicrobial activity.

### Preparation of Inoculum

Fresh suspension of test organism were prepared from freshly grown agar slant, by transferring the test organism aseptically in sterilized nutrient agar media contained in test tube. The test tubes were then incubated at  $37 \pm 2^{\circ}\text{C}$  for bacteria 24 hours &  $25 \pm 2^{\circ}\text{C}$  for fungus for 24 hours. The suspensions were used within 24 hours of their preparation.

### Antimicrobial Screening

The antimicrobial screening was performed by using Cylinder-Plate method, commonly known as Cup Plate Method. All the apparatus used for the experiment were thoroughly cleaned, dried and wrapped suitably before sterilization and were sterilized by heating in a hot air oven at  $150^{\circ}\text{C}$  for 1 hour.

#### (A) Preparation of Agar Plates

The test tubes containing nutrient agar media were heated to melt the agar media. Each test tube was inoculated with 0.2 ml of the suspension of test organism and was rotated in between the palms to distribute the test organism uniformly in the melted agar media. The inoculated nutrient agar media was then transferred immediately to the sterilized petri dish, using the aseptic techniques.

#### (B) Assay Method

The cup plate method was used for evaluating antimicrobial activity. The antimicrobial activity of the volatile oil was studied against four bacterial and two fungal strains. The activity of volatile oil was tested against at various concentrations in dimethyl formamide against all the micro-organism. Gentamycin (50 mg/ml) and Nystatin (50 IU) were used as standards for comparison of antibacterial and antifungal activity, respectively. The antimicrobial activity of volatile oil was screened by the agar well diffusion method. Nutrient agar plates were swabbed with the respective broth culture of the respective broth culture of the organism and kept for 15 minutes in laminar chamber for absorption to take place. Wells were made in agar plates using a sterile cork borer and 10  $\mu\text{l}$  of different concentrations of volatile oil were added to different wells. The plates were incubated at  $37 \pm 2^{\circ}\text{C}$  for bacteria for 24 hours and 25

$\pm 2^{\circ}\text{C}$  for fungus for 24 hours. The diameter of the zone of inhibition was measured in millimeter.

### RESULT AND DISCUSSION

The hydrodistillation of *Eucalyptus globulus* for three hours according to the method recommended in the British Pharmacopoeia 2003 yielded light yellowish green coloured volatile oil. The volatile oil of leaves of *Eucalyptus globulus* collected from Neha nursery, Greater Noida region, displayed significant inhibitory activity against gram positive *Bacillus subtilis*, *Staphylococcus aureus* and gram negative bacteria *Pseudomonas aeruginosa*, and *Escherichia coli*. In addition, they were found to be active against the fungi, *Candida albicans* and *Aspergillus niger*. All the three dilutions showed significant antimicrobial activity in comparison to their respective standards used. The maximum inhibitory activity was found with 100% concentration of volatile oil against

bacteria *Bacillus subtilis* (15 mm) and fungus *Aspergillus niger* (20mm) (Table 1).

### CONCLUSION

The essential oil from the fresh leaves of *Eucalyptus globulus* Labill showed significant inhibitory activity against gram positive *Bacillus subtilis*, *Staphylococcus aureus* and gram negative bacteria *Pseudomonas aeruginosa*, and *Escherichia coli*. Also, the essential oil was found to be active against the fungi, *Candida albicans* and *Aspergillus niger*. Every single dilution showed significant antimicrobial activity in comparison to the respective standard solutions. Moreover, the maximum inhibitory activity was found with 100% concentration of volatile oil against bacteria *Bacillus subtilis* (15mm) and fungus *Aspergillus niger* (20mm). Further studies are needed in this area in order to investigate the antimicrobial activity of essential oil present in the different parts of *Eucalyptus globulus* Labill.

**Table 1. Antimicrobial activity of Volatile oil of *Eucalyptus globulus* Labill leaves.**

S. No.	Test Organism	Zone of Inhibition (mm) <sup>a</sup>				
		Dilution of volatile oil in Acetone		DMSO (v/v)	Standards	
		10%	50 %		100 %	Gentamycin 50 mg/ml
1	<i>Bacillus subtilis</i>	10	12	15	45	–
2	<i>Escherichia coli</i>	11	11.5	13	32	–
3	<i>Staphylococcus aureus</i>	9	12.2	14	33	–
4	<i>Pseudomonas aeruginosa</i>	9.2	11.1	14.4	42	–
5	<i>Aspergillus niger</i>	17	19	20	–	42
6	<i>Candida albicans</i>	10	12	13	–	32

Gentamycin - Against bacterial strains only  
Nystatin - Against fungal strains only

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