

## ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF THE LEAF EXTRACTS OF *EUCALYPTUS GLOBULUS*

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### ABSTRACT

**Objective:** To investigate the antimicrobial activity and phytochemical analysis of *Eucalyptus globulus* leaves.

**Methods:** The antimicrobial activity of the leaves of *E. globulus* extracted with acetone, ethanol and petroleum ether was studied using well diffusion method against the selected bacterial and fungal isolates. The antimicrobial susceptibility of the bacteria and fungi to various concentrations of the extract was evaluated by the micro broth dilution method. The preliminary phytochemical screening was performed in the acetone extract of *E. globulus*. The presence of functional groups in the acetone extract of the leaves of *E. globulus* was determined by Fourier transform infrared (FT-IR) spectroscopy.

**Results:** Results suggested that the acetone extract exhibited maximum antimicrobial activity against the tested bacteria and fungi when compared with ciprofloxacin and fluconazole respectively (standard antibiotics). Minimum inhibitory concentration (MIC) showed that the wells containing a concentration of 12.5 mg/ml and 6.25 mg/ml of acetone extract inhibited the bacteria and fungi effectively. Phytochemical screening of acetone extract of *E. globulus* showed the presence of alkaloids, flavonoids, phenols, tannins, quinones, glycosides, steroids, terpenoids and leucoanthocyanides. FT-IR spectral analysis of the leaf extract of *E. globulus* showed the presence of functional groups such as -OH, -NH, C=O and -COOH.

**Conclusion:** Thus to conclude, different plants and their formulations have been used in ayurveda to cure diseases for several decades. The findings of the study indicate the potential to exploit the leaves of *Eucalyptus globulus* in the treatment of microbial diseases.

**Keywords:** *Eucalyptus globulus*, Phytochemical analysis, Antimicrobial activity, Microbroth dilution method, FT-IR spectroscopy

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### INTRODUCTION

Medicinal plants a boon to human beings contain a wide range of bioactive compounds or phytochemicals which are used to treat various ailments [1-3]. They are used worldwide in folk medicine and screening of such plants may result in the discovery of novel effective compounds against pathogenic microbes. Now-a-days miscellaneous usage of synthetic drug leads to countless side effects and this converges an idea to use medicinal plants as an alternative [4]. The plant based technique of medicine being natural does not cause any serious side effects or problems [5]. Almost all parts of plants are used to treat various diseases which are known to have diverse medicinal properties [6].

During last three decades, numerous antibiotics have been produced, but the clinical efficiency of these existing antibiotics is being threatened by the emergence of multidrug resistant pathogens [7]. According to World Health Organization (WHO), it was reported that medicinal plants would be the best source to obtain a variety of drugs [8]. Antibiotic resistance has become a global concern in recent decades and there is a growing concern regarding the search for new antimicrobial substances from different sources of medicinal plants [9]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [10].

*Eucalyptus globulus*, an important medicinal plant of Myrtaceae are used in many parts of the world for the treatment of a wide variety of diseases. It acts as an antiseptic agent, and the leaf extracts are used to treat influenza, skin rashes, chest problems and their vapour is inhaled to fight inflammation. It stimulates the release of saliva and helps loosen a cough and used to treat lung infections and gastro intestinal ulcers. *E. globulus* oil has useful antipyretic properties to reduce fever and decreases blood sugar level. *E. globulus*-also contains many aromatic principles which find out its

existence in the composition of many products such as shampoos, toothpaste, soaps, ointments, air fresheners etc. [11].

Thus the present study is sought to evaluate the *in-vitro* antimicrobial activity and phytochemical analysis of *Eucalyptus globulus*.

### MATERIALS AND METHODS

#### Plant material

The leaves of *E. globulus* Labill were collected during June 2016 from Kariyamandu, Ooty, Nilgiris district with the help of tribal people dominating that area. The leaves were air-dried in shade at ambient temperature and stored in double layer paper bags at room temperature, powdered and protected from the direct light, until further analysis. The plant specimen was identified at the Department of Botany, Avinashilingam University for Women, Coimbatore, Tamilnadu, India.

#### Extract preparation

The leaf extracts were prepared using the solvents namely acetone, petroleum ether and ethanol. The dry leaf sample (10g) was weighed and homogenized with 100 ml of the respective solvents separately. The crude preparation was left over night in the shaker at room temperature. The leaf extract was then transferred to a pre weighed beaker and the extract was concentrated by evaporating the solvent at 60 °C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide. The extraction yield was expressed as

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the dry extract (g)}}{\text{Weight of the sample used for the extraction (g)}} \times 100$$

#### Antimicrobial screening

In order to evaluate the antimicrobial activity of *E. globulus*, the bacterial strains used for the present study are *Escherichia coli*,

*Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Vibrio cholera*, *Streptococcus epidermis*, *Bacillus cereus*, *Staphylococcus aureus* and *Proteus vulgaris*. The fungal strains used were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium* species, *Trichoderma* species and *Rhizopus* species.

The agar well diffusion method was employed to determine the antimicrobial activity of the leaf extracts of *E. globulus*. Briefly, the above-mentioned bacteria were cultured on the nutrient broth (Himedia, Mumbai, India) at 37±0.1 °C while the fungi were grown on Sabouroud dextrose broth (Himedia, Mumbai, India) at 28±0.1°C. The well grown bacterial and fungal cultures (100 µl) were spread plated/swabbed on sterile Muller Hinton and Sabouroud dextrose agar media separately. Five wells were made on each plate and to each well 20 µl of the leaf extracts, ciproflaxin (bacteria) and fluconazole (fungi) which served as positive control/reference antibiotics and DMSO (negative control) were added and incubated at 37 °C for 24 h (bacteria) and at room temperature for 5 d (fungi) respectively. After the incubation period, the diameter of the inhibition zones was measured.

#### Microbroth dilution assay

The antimicrobial susceptibility of the bacteria and fungi to various concentrations of the extract was evaluated by the micro broth dilution method [12]. The bacterial and fungal cultures were grown in sterile nutrient broth and Sabouraud dextrose broth separately. A volume of 99 µl of the medium with respective microbes was added to the wells of microtitre plates separately. The concentration of the plant extract was 100 mg/ml. The different concentrations of the extract were prepared and then the wells were inoculated with 1 µl of the selected extracts separately. The plates were incubated at 37°C for 24 h (bacteria) and at room temperature for 5 d (fungi) respectively. The activity was observed as negative if growth was there and positive if the medium appeared clear without any growth of the microbes and the minimum inhibitory concentration was noted.

#### Phytochemical analysis

The acetone extract of *E. globules* leaf was subjected to preliminary phytochemical tests namely carbohydrates, amino acids, phenols, steroids, glycosides, quinones, alkaloids, tannins, anthocyanin, volatile oils, terpenoids, coumarins and leucoanthocyanides as per the standard methods [13].

#### FT-IR analysis

The powdered acetone extract of *E. globules* (1µg) leaves were remixed with 2.5 mg of dry potassium bromide and grounded well using mortar and pestle. The powder was filled in a 2 mm internal diameter micro-cup and loaded onto FT-IR spectrophotometer at 26°C±1°C. The samples were scanned using infrared rays in the range of 4000-750 cm<sup>-1</sup> by Fourier Transform Infrared Spectrophotometer. The spectral data were compared with the reference chart to identify the functional groups responsible for biological activities in the sample [14].

#### RESULTS

It was observed that acetone extraction produced the maximum yield of 28.1%, whereas in ethanol extraction the yield was 17.5% and petroleum ether extraction yielded only 10.2%. The results of the antimicrobial activity were summarized in table 1. *E. globulus* leaf extracts showed potent antimicrobial activity in acetone extract when compared with ethanol and petroleum ether extract. The acetone extract of *E. globules* exhibited a maximum zone of inhibition against *Streptococcus epidermis* (27 mm) followed by *E. coli* (26 mm) and minimum zone of inhibition was noticed in *Shigella flexneri* (17 mm). Similarly, the Gram positive bacteria *Bacillus cereus* and *Staphylococcus aureus* exhibited of the zone of inhibition of 24 mm against acetone extract. Followed by acetone extract, ethanol extract exhibited moderate activity and petroleum ether extract of *E. globulus* revealed minimum activity (table 1) when compared with the standard antibiotic, ciprofloxacin (19-28 mm) which served as positive control.

Table 1: Antibacterial activity of the leaf extracts of *E. globulus*

Bacterial isolates	Zone of inhibition in diameter (mm)				
	ACE	ETH	PE	CPF	DMSO
<i>Streptococcus epidermis</i>	27	25	21	28	-
<i>Vibrio cholera</i>	23	15	12	24	-
<i>Pseudomonas aeruginosa</i>	25	17	15	27	-
<i>Shigella flexneri</i>	17	14	9	19	-
<i>Escherichia coli</i>	26	23	20	27	-
<i>Proteus vulgaris</i>	19	15	10	20	-
<i>Klebsiella pneumoniae</i>	22	18	13	25	-
<i>Bacillus cereus</i>	24	20	14	26	-
<i>Staphylococcus aureus</i>	24	20	13	26	-

ACE-Acetone Extract ETH-Ethanol Extract PE-Petroleum Ether Extract, CPF -Ciproflaxin (Positive control) DMSO-Dimethyl Sulphoxide (Negative control)

The inhibitory activity of *E. globulus* leaf extract against the tested fungal isolates was depicted in table 2. Acetone extract of *E. globulus* leaf exhibited a maximum zone of inhibition against *Rhizopus stolonifer* (23 mm) followed by *Aspergillus niger* (22 mm) and minimum zone of inhibition was noticed in *Aspergillus flavus* (10 mm). Petroleum ether extract of *E. globulus* possessed

minimum activity and moderate zone of inhibition was observed in ethanol extract against the tested fungal isolates when compared with fluconazole (standard antibiotic/positive control) which produced the inhibition zone of 13-25 mm. The negative control (DMSO) showed no zone of inhibition against the tested bacterial and fungal isolates.

Table 2: Antifungal activity of the leaf extracts of *E. globulus*

Fungal isolates	Zone of inhibition in diameter (mm)				
	ACE	ETH	PE	FLU	DMSO
<i>Aspergillus niger</i>	22	18	16	23	-
<i>Penicillium species</i>	17	13	11	18	-
<i>Aspergillus fumigatus</i>	16	14	13	17	-
<i>Rhizopus stolonifer</i>	23	20	17	25	-
<i>Aspergillus flavus</i>	10	9	8	13	-
<i>Trichoderma species</i>	20	15	10	21	-

ACE-Acetone Extract ETH-Ethanol Extract PE-Petroleum Ether Extract FLU-Fluconazole (Positive control) DMSO-Dimethyl Sulphoxide (Negative control)

The screening for minimum inhibitory concentration (MIC) showed that the wells containing a concentration of 12.5 mg/ml and 6.25 mg/ml of acetone extract inhibited the bacteria and fungi respectively, followed by ethanolic extract (25 mg/ml) and petroleum ether extract (50 mg/ml).

The phytochemical analysis in the acetone extract of *E. globulus* leaves showed the presence of phytoconstituents such as alkaloids, flavonoids, phenols, tannins, quinones, glycosides, steroids, terpenoids, volatile acids and leucoanthocyanides (table 3).

Table 3: Phytochemical analysis of *E. globulus*

S. No.	Phytochemicals	Acetone extract
1	Alkaloids	-
	Dragendroff's reagent	-
	Wagner's reagent	+
2	Flavonoids	+
3	Phenols	-
	Ferric chloride test	+
	Lead acetate test	-
4	Amino Acids	-
	Ninhydrin test	-
	Biuret test	-
5	Tannins	-
	Lead acetate test	+
6	Quinones	-
	Sodium hydroxide test	+
7	Carbohydrates	-
	Fehling's test	-
8	Glycosides	-
	Legal's test	+
9	Steroids	-
	Salkowski test	+
10	Starch	-
	Iodine test	-
11	Antocyanin	-
12	Volatile Oils	+
13	Terpenoids	+
14	Coumarins	-
15	Leucoanthocynidines	+

FT-IR spectral analysis of acetone extract of *E. globulus* leaf revealed the presence of different functional groups (fig. 1). Spectral data of the extract confirmed the presence of functional groups such as -OH, NH, C=O and -COOH. The peak at 3441.01 cm<sup>-1</sup> was assigned to the O-H stretching vibration, the peak appeared in the range of 1165-879.54 cm<sup>-1</sup> is mainly attributed to the stretching vibration of C-O. The characters of peak intensities and position at 3332.99 and

3248.13 cm<sup>-1</sup> in the IR spectrum display the characteristic absorption of H bonded OH stretching. The hydroxyl peak at 3379.29 cm<sup>-1</sup> and the peak situated at 1705.07 cm<sup>-1</sup> was assigned to the absorption of carbonyl (-C=O stretching). Peaks in the range between 3650-3300 cm<sup>-1</sup> correspond to stretching vibrations of OH groups as well as from amides. The peak at 2916.37, 2854.65, 2800.64 and 2754.35 cm<sup>-1</sup> belongs to the C-H stretching vibrations.

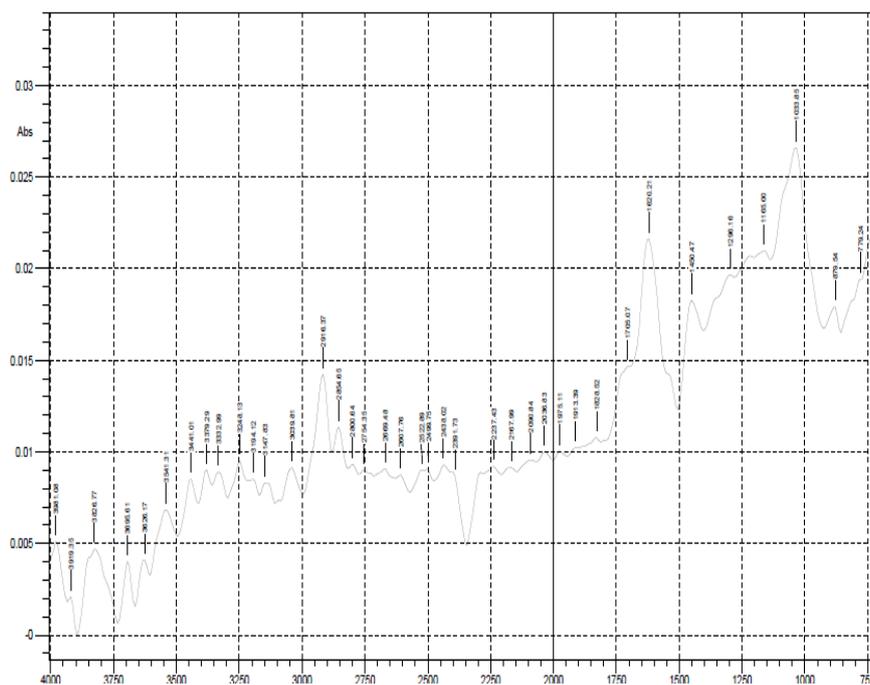


Fig. 1: FT-IR spectrum of the leaf extract of *E. globules*

## DISCUSSION

Medicinal plants serve to be a source of remedy for many diseases and place an important role in the health of an individual and society. *E. globulus* leaf extracts possessed a significant antimicrobial activity against the tested bacterial and fungal isolates. Among the tested extracts acetone proved to possess strong antimicrobial activity which suggests that it is a dipolar aprotic solvent of medicinal polarity and can dissolve a wide range of compounds [15].

Additionally, acetone possesses extra carbon than ethanol which aids in the extraction of other compounds and this may be the reason for better antimicrobial activity in acetone extract when compared with ethanol and petroleum ether extracts. *E. globulus* seems to be a potential remediant for the treatment of many infectious diseases and also be used as natural food preservative [16-18]. The inhibitory effect of the leaf extract of the candidate plant will be helpful to pharmacological industry to prepare antimicrobial drugs for treating various bacterial and fungal diseases. Hence *E. globulus* proves to be a promising agent to cure various infections/diseases and has a wide spread use in the pharmacological system.

Presence of phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, quinones, glycosides, steroids, terpenoids and leucoanthocyanidins served as a defence mechanism against microorganisms [19]. The presence of flavonoids, alkaloids, tannins, glycosides and terpenoids may disrupt the cell membrane of bacteria and forms a complex with extracellular soluble proteins and thereby inhibits its growth [20]. The flavonoids are more lipophilic and may also disrupt the microbial membranes. The presence of alkaloids may intercalate with the DNA of the microbes and fight against infectious diseases [21]. Similarly, the presence of steroids is known to have a cardio tonic effect and also possess antibacterial and insecticidal properties [22]. Tannins also precipitate the microbial proteins, thus making the nutritional proteins unavailable for microbial growth. In the same manner, the presence of polyphenols and secondary metabolites fight against the bacterial and fungal diseases [23]. Presence of cardiac glycosides may inhibit the Na<sup>+</sup>/K<sup>+</sup> pump and increases the availability of calcium ions for the contraction of heart muscles which reduces the congestive heart failure, cardiac arrhythmia and distension of heart [24, 25]. The present work reveals the presence of various phytochemical constituents in the plant extract which provides a scientific validation for its use in folk medicine in the selected area which is renowned for tribal community. Further advanced research activities on the isolation of the compounds in the plant extract can be explored.

In any bio-organic molecule, its functional groups influence its biological activity considerably as they contribute significantly to their inherent acid-base properties, solubility, partition coefficient, crystal structure and stereochemistry and so on. All these properties are supposed to influence the absorption, distribution, metabolic extraction and toxicity of bioactive molecules [26]. In the present work, FT-IR spectral analysis of the leaf extract of *E. globulus* illustrated the presence of phytochemicals carrying hydrogen bonded-OH, -NH, C=O and -COOH functional groups. The hydroxyl functional group seems to be an integral part of phenolic phytochemicals and also the presence of various biologically active functional groups, viz. alcoholic, amide, carbonyl and carboxylic groups confirms that the plant possesses bioactive phytochemicals. Thus to conclude the results of the above study clearly demonstrate that the acetone extract of *globules* exhibits moderate antibacterial and antifungal activities which might be helpful in preventing the progress of various infectious diseases and can be used in the alternative system of medicine. The effective biomolecules which act as antimicrobial substance have to be isolated and identified so that it could become the base for the development of medicine for the drug resistant microorganisms and also a natural blueprint for the development of new drugs.

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## AUTHORS CONTRIBUTION

Abirami and Nishanthini were involved in phytochemical and FT-IR spectral analyses of *Eucalyptusglobulus* leaf extract. Poonkothai contributed in antimicrobial activity of different extracts of *Eucalyptusglobulus*.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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