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Original Article

# STUDIES ON *IN VITRO* EVALUATION OF ANTIBACTERIAL ACTIVITIES OF *EUCALYPTUS GLOBULUS* LABILL LEAF

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**ABSTRACT**

**Objective:** The objective of the present investigation was to evaluate the antibacterial potential of n-hexane and methanolic leaf extracts of *Eucalyptus globulus* Labill.

**Methods:** The antibacterial potential of the leaf extracts were tested against some human pathogenic bacteria causing several diseases such as *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, *Bacillus subtilis*, *Micrococcus luteus*, *Salmonella typhimurium*, *Escherichia coli*, *Bacillus circulans*, *Streptococcus mitis*, *Enterococcus faecalis* by using agar well diffusion method. The concentration of test plant extracts was 20 mg/ml. The inhibitory activity of the leaf extracts was compared with streptomycin as reference antibiotic (RA). The concentration of (RA) was 0.5 mg/ml.

**Results:** The result of the study revealed that n-hexane extract of *Eucalyptus globulus* Labill. Leaf was highly effective against *Micrococcus luteus* (19.66±0.94 mm) and least effective against *Shigella flexneri* (12.33±2.05 mm) whereas the methanolic extract had high inhibition effect against *Vibrio cholerae* (17.66±1.24 mm) and least against *Pseudomonas aeruginosa* (10 mm). The zone of inhibition of reference antibiotics ranged between 28.33±0.94 mm (*Streptococcus mitis*) to 21.66±3.09 mm (*Escherichia coli*).

**Conclusion:** The leaf extract of *E. globulus* may be useful as an alternative antimicrobial agent in natural medicine for the treatment of numerous infectious diseases.

**Keywords**: Agar well diffusion, Antibacterial activity, *Eucalyptus globulus* leaf, n-Hexane, Methanol, Streptomycin


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## INTRODUCTION

Phytomedicines that derived from plants have shown great promise in the treatment of infectious diseases including viral infections [1]. Single and polyherbal preparations have been used throughout history for the treatment of various diseases. Many studies have been carried out to extract various natural products for screening antimicrobial activity but attention has not been focused intensively on studying the combinations of these products for their antimicrobial activity [2-5].

*Eucalyptus globulus* Labill (Family Myrtaceae) is an aromatic tree. The previous work on over 500 species of *Eucalyptus* genus suggests that some species counteract influenza viruses; others are antimalarial or highly active against bacteria [6]. The extracts of some species of *Eucalyptus* are now entering into common herbal use for the treatment of cold, chest pain, or a cough. Its leaf extracts have been used to treat influenza, skin rashes and chest problems, while their vapor is inhaled to fight inflammation [7]. Due to the bioactive components of the plant, their essential oil is indeed promising in view of their use as an effective antibacterial, antifungal, and antioxidant agents. With the growing interest for the use of essential oils in both food and pharmaceutical industries, a systematic examination of the plant extracts has become increasingly important [8]. Leaf extracts of *Eucalyptus* have been approved as food additives and cosmetic formulations. Research data has demonstrated that the extracts exhibited various biological effects, such as antibacterial, ant hyperglycemia [9] and antioxidant [10] activities. Mota at al., (2015) evaluated the *in vitro* antimicrobial activity of the *Eucalyptus globulus* essential oil, xylitol and papain substances against some micro-organisms and concluded that its oil has antimicrobial activity and can appear to be a viable alternative as germicidal agent [11].

In the present investigation, n-hexane and methanolic extracts of *Eucalyptus globulus* Labill leaf have been evaluated for their antimicrobial activities.

## MATERIALS AND METHODS

### Collection and identification of plant material

The plant *Eucalyptus globulus* Labill was collected from the "Chandaka reserve forest" area near Bhubaneswar, Odisha in the month of March, 2014. Identification of the voucher specimen was done by available literature [12]. The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India. The leaves were collected in bulk amount, washed in running tap water, dried under shade and made to coarse powdered form.

### Processing of plant material and preparation of extract

The collected leaves were shade dried and ground to form coarse powder and had been successively extracted with the solvent n-hexane and methanol by Soxhlet apparatus [13] and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

### Evaluation of the extracts for antibacterial activity

The *in vitro* antibacterial screening was carried out against selected four gram-positive, eight gram-negative and two gram-variable bacterial pathogens causing various diseases in human. The bacterial pathogens were *Staphylococcus aureus* (MTCC-1430), *Vibrio cholerae* (MTCC-3906), *Pseudomonas aeruginosa* (MTCC-1035), *Shigella flexneri* (MTCC-95430), *Salmonella typhi* (MTCC-733), *Klebsiella pneumoniae* (MTCC-109), *Salmonella paratyphi* (MTCC-3220), *Bacillus subtilis* (MTCC-1305), *Micrococcus luteus* (MTCC-1809), *Salmonella typhimurium* (MTCC-98), *Escherichia coli*

(MTCC-614), *Bacillus circulans* (MTCC-490), *Streptococcus mitis* (MTCC-2897), *Enterococcus faecalis* (MTCC-459). These species were procured from Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India. The remaining bacterial species were procured from Post Graduate Department of Microbiology, OUAT, Bhubaneswar, Odisha. These organisms were identified by standard microbial methods [14]. The antibacterial screening of the extracts was carried out by determining the zone of inhibition using agar well diffusion method [13] for bacteria.

**Standard drugs used and preparation of doses for antibacterial assay**

Streptomycin was used as Reference Antibiotics (RA). The stock solution of RA was prepared in sterile distilled water to give a concentration of 0.5 mg/ml.

**Agar well diffusion assay**

Agar well diffusion method [12] was followed to determine the zone of inhibition of microbes in Nutrient Agar (NA, HiMedia Laboratories Ltd., Mumbai). Plates were swabbed (sterile cotton swabs) with 8 hr old broth culture of bacteria. Wells (8 mm diameter) were made in each of these plates using sterile cork borer. A stock solution of plant extracts was prepared at a concentration of 20 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 h. Control treatments comprising inoculums without plant extract were set up. The plates were incubated at 37 °C for 24 h for bacterial pathogens. Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and statistical analysis was carried out.

## RESULTS AND DISCUSSION

**Antibacterial screening**

The result of antibacterial screening of the leaf extracts against the test organism revealed that the n-hexane extract was more effective to inhibit the growth of test organisms than methanolic extract. In the present investigation n-hexane leaf extract of *E. globulus*, a potential medicinal plant showed maximum inviting activity against *Micrococcus luteus* (19.66±0.94 mm) followed by *Salmonella typhimurium* (19.33±2.05 mm), *Salmonella typhi* (18.66±1.69 mm), *Klebsiella pneumoniae* (18.66±1.69 mm), *Vibrio cholerae* (18.33±1.24 mm), *Salmonella paratyphi* (16.66±1.69 mm), *Streptococcus mitis* (16.33±1.24 mm), *Pseudomonas aeruginosa* (15.66±0.47 mm), *Escherichia coli* (15.33±1.24 mm), *Bacillus circulans* (15±2.16 mm), *Bacillus subtilis* (15±1.63 mm), *Enterococcus faecalis* (14.66±1.24 mm), *Staphylococcus aureus* (14±2.16 mm) and *Shigella flexneri* (12.33±2.05 mm). The methanolic extract exhibited highest zone of inhibition against *Vibrio cholerae* (17.66±1.24 mm) followed by *Micrococcus luteus* (17±0.81 mm), *Escherichia coli* (15.66±1.69 mm), *Salmonella typhimurium* (15.33±1.24 mm), *Staphylococcus aureus* (15±0.81 mm), *Streptococcus mitis* (13.66±0.47 mm), *Shigella flexneri* (12.66±0.94 mm), *Klebsiella pneumoniae* (12.33±1.69 mm), *Salmonella paratyphi* (12.33±1.24 mm), *Bacillus circulans* (11.66±1.24), *Salmonella typhi* (11.33±0.47 mm), *Enterococcus faecalis* (10.33±1.24 mm), *Bacillus subtilis* (10.33±0.47) and least against *Pseudomonas aeruginosa* (10 mm). The result of these two (n-hexane and methanolic) extracts were compared with reference antibiotic streptomycin and the zone of inhibition of streptomycin was found to range from 28.33±0.94 mm (*Streptococcus mitis*) to 21.66±3.09 mm (*Escherichia coli*) (table 1 and fig. 1).

**Table 1: *In vitro* antibacterial activity (zone of inhibition in mm) of different plant extracts of *Eucalyptus globulus* Labill**

| Test organisms | Zone of inhibition in mm | | |
|---|---|---|---|
| **Bacteria** | **n-Hexane extract (20 mg/ml)** | **Methanol extract (20 mg/ml)** | **Reference antibiotic streptomycin (0.5 mg/ml)** |
| *Staphylococcus aureus* | 14±2.16 | 15±0.81 | 28.33±1.24 |
| *Vibrio cholerae* | 18.33±1.24 | 17.66±1.24 | 24.66±1.69 |
| *Pseudomonas aeruginosa* | 15.66±0.47 | 10 | 24±0.81 |
| *Shigella flexneri* | 12.33±2.05 | 12.66±0.94 | 24.66±2.49 |
| *Salmonella typhi* | 18.66±1.24 | 11.33±0.47 | 24.33±2.05 |
| *Klebsiella pneumoniae* | 18.66±1.69 | 12.33±1.69 | 24±2.44 |
| *Salmonella paratyphi* | 16.66±1.69 | 12.33±1.24 | 27.33±1.24 |
| *Bacillus subtilis* | 15±1.63 | 10.33±0.47 | 26.66±0.47 |
| *Micrococcus luteus* | 19.66±0.94 | 17±0.81 | 23±0.81 |
| *Salmonella typhimurium* | 19.33±2.05 | 15.33±1.24 | 22.66±1.88 |
| *Escherichia coli* | 15.33±1.24 | 15.66±1.69 | 21.66±3.09 |
| *Bacillus circulans* | 15±2.16 | 11.66±1.24 | 27±2.16 |
| *Streptococcus mitis* | 16.33±1.24 | 13.66±0.47 | 28.33±0.94 |
| *Enterococcus faecalis* | 14.66±1.24 | 10.33±1.24 | 22.66±1.69 |

Results expressed as mean±SD of three determinations

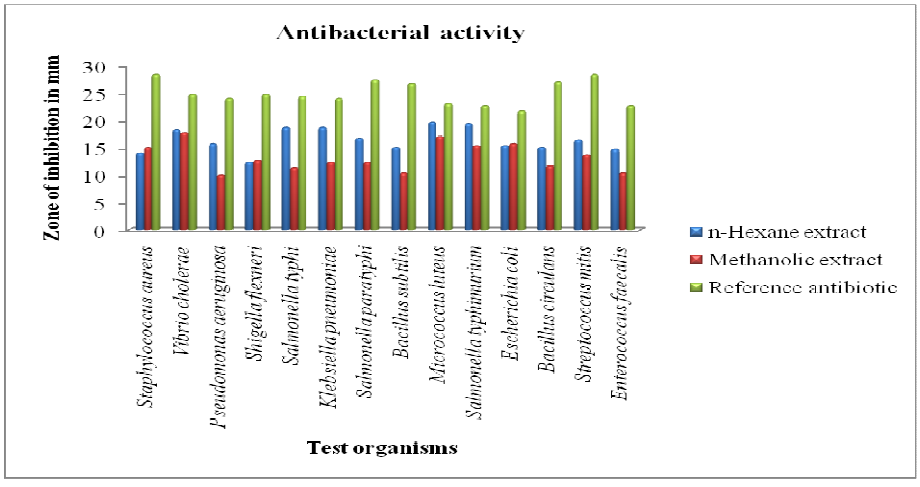


**Fig. 1: *In vitro* antibacterial activity (zone of inhibition in mm) of different plant extracts of *Eucalyptus globulus* Labill**

The standard drug streptomycin was found effective at a much lower concentration than the leaf extracts. However, this plant is commonly used by the local people traditionally against a large number of diseases. It is expected that this plant may have very less toxicity and one might conclude that the use of these plants would probably produce less effects of toxicity compared with a conventional chemotherapeutic agent.

According to a report of Damjanović-Vratnica *et al.*, (2011), the essential oil of *E. globulus* showed strong antimicrobial activity against *Streptococcus pyogenes*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumonia* [15]. The essential oils from leaves of *E. globulus* have been reported to have potential usefulness as a microbiostatic, antiseptic or as disinfectant agent [16]. The leaf extracts of *E. globulus* was found to be effective against micro-organisms that cause food poisoning, acne and athlete's foot [17]. A good antimicrobial activity of *E. globulus* crude extract against *Staphylococcus aureus, Streptococcus agalactiae, Listeria monocytogenes, Bacillus subtilis, Escherichia* coli, *Klebisiella pneumoniae* and *Salmonella typhi* was also reported by Enciso-Díaz *et al.* in 2012 [18].

## CONCLUSION

Although many works on antibacterial activities of the essential *Eucalyptus* oil and leaf extract has been extensively surveyed but its antimicrobial mechanisms have not been reported in great details. This study has shown that leaf extract of *Eucalyptus globulus* Labill. Possess rather a significant activity against different microorganisms, including human pathogens. These results confirm the potential use of *E. globulus* leaf extract in the food and pharmaceutical industries, which may be useful as an alternative antimicrobial agent in natural medicine for the treatment of numerous infectious diseases.

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## CONFLICT OF INTREST

All the authors have none to declare

## REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
2. Belaiche P. Traité de phytothérapie et d'aromathérapie. Tome I. Maloine SA. Editeur. Paris; 1979. p. 125-7.
3. Abu-Shanab B, Adwan G, Abu-Safiya D, Jarrar N, Adwan K. Antibacterial activities of some plant extracts utilized in popular medicine in palestine. Turk J Biol 2004;28:99-102.
4. Adwan G, Abu-Shanab B, Adwan K, Abu-Shanab F. Antibacterial effects of nutraceutical plants growing in palestine on pseudomonas aeruginosa. Turk J Biol 2006;30:239-42.
5. Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J Ethnopharmacol 2008;116:403-6.
6. Ody P. The Complete Medicinal Herbal. Dorling Kindersley. London; 1994. p. 61.
7. Musyimi DM, Ogur JA. Comparative assessment of the antifungal activity of extracts from *Eucalyptus globulus* and *Eucalyptus citriodora*. Res J Phytochem 2008;2:35–43.
8. Baratta MT, Dorman HJ, Deans SG, Figueiredo AC, Barroso JG, Ruperto G. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour Fragrance J 1998;13:235–44.
9. Gray AM, Flatt PR. Antihyperglycemic actions of *Eucalyptus globulus* (*Eucalyptus*) are associated with pancreatic and extrapancreatic effects in mice. J Nutr 1998;128:2319–23.
10. Lee KG, Shibamoto T. Inhibition of malonaldehyde formation from blood plasma oxidation by aroma extracts and aroma components isolated from clove and eucalyptus. Food Chem Toxicol 2001;39:1199–204.
11. Mota VDS, Turrini RNT, Poveda VDB. Antimicrobial activity of *Eucalyptus globulus* oil, xylitol and papain: a pilot study. Rev Esc Enferm USP 2015;49:215-9.
12. Saxena HO, Brahmam M. The Flora of Orissa. Vol. I-IV. Orissa Forest Development Corporation. Bhubaneswar; 1994-1996.
13. Khatoon A, Jethi S, Nayak SK, Sahoo S, Mohapatra A, Satapathy KB. Evaluation of *in vitro* antibacterial and antioxidant activities of *Melia azedarach* L. bark. IOSR. J Pharm Biol Sci 2014;9:14-7.
14. Sahoo S, Nayak D, Kumar GS, Jayakumari S. Antimicrobial investigation of *Ammania baccifra* Linn. against some urinary and gastro-intestinal tract infection-causing pathogens. Indian Drugs 2012;49:44-8.
15. Damjanović-Vratnica B, Đakov T, Šuković D, Damjanović J. Antimicrobial effect of essential oil isolated from *Eucalyptus globulus* Labill. from Montenegro. Czech J Food Sci 2011;29:277–84.
16. Ghalem BR, Mohamed B. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. Afr J Pharm Pharmacol 2008;2:211-5.
17. Takahashi T, Kokubo R, Sakaino M. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculate*. Lett Appl Microbiol 2004;39:60–4.
18. Enciso-Díaz OJ, Méndez-Gutiérrez A, De Jesús LH, Sharma A, Villarreal ML, Taketa AC. Antibacterial activity of *Bougainvillea glabra*, *Eucalyptus globulus*, *Gnaphalium attenuatum*, and propolis collected in mexico. Pharmacol Pharm 2012;3:433-8.

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