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Screening of Antimicrobial Activities of Traditional Indian Medicinal Plant Eucalyptus Globules

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Abstract: The essential oil of Eucalyptus globules is endowed with antimicrobial properties established in vitro and unequivocal. In present study antimicrobial activity of methanolic extracts of Eucalyptus globules were investigated by disc diffusion assay against a panel of bacteria and fungi. Methanolic extract of the plant showed potent anti-microbial activity against B. cereus with MIC 1.56 µg/ml and showed potent antifungal activities against A. flavus with MIC 1.56 µg/ml.
Key Words- Antimicrobial Activities, Antifungal Activities, Eucalyptus globules.

I. INTRODUCTION

Eucalyptus is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in many other countries including India.[1-4] The genus name Eucalyptus comes from the Greek word eucalyptos, meaning "well-covered," and refers to its flowers that, in bud, are covered with a cup-like membrane.[2] Though native to Australia, its therapeutic uses have been introduced and integrated into traditional medicine systems, including Chinese, Indian Ayurvedic, and Greco-European. Its volatile oil is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branches.[5] Eucalyptol (1, 8-cineole) is the active ingredient of the eucalyptus oil, responsible for its various pharmacological actions. Pharma copoeial grade dried eucalyptus leaf must contain at least 2.0% (v/m) volatile oil, composed mainly of 1, 8-cineole.[3] The Indian Pharmacopoeia requires not less than 60% w/w of cineole.[5]

The essential oil of *eucalyptus* is commonly used in traditional medicine because of its expectorant and balsamic activity. The leaves could also be a promising source of phenolic compounds, which can be used for possible applications in food, pharmaceutical and cosmetic industries[6].

Because of their antioxidant activity leaf extracts of E. globules have been proposed as food additives. Therefore, this species might be a good candidate for further development as a nutra ceutical. However, detailed information has not been published about the phenolic composition of leaves of this species. In other works[7], reported the characterization of 55 phenolic constituents in fruits of E. globules, including gallic acid, hydrolysable tannins and flavonols using extracts with acetone/water (70:30 v/v) and 0.5% acetic acid. The study on the phenolic content of the leaves of E. globules were performed and reported the existence of 39 phenolic compounds, including 26 compounds that have not previously been detected in leaves of E. globulus, extracted with 70% acetone containing 0.5% acetic acid. The following phenolic constituents were detected gallic, ellagic and methylellagic acids, eucaglobulin, quercetin derivatives and others[7-9].

In this study, the antimicrobial activities of Eucalyptus globulus leaf extracts were investigated.

II. MATERIALS AND METHODS

E. globulusleaves were collected since March a June of 2015 around Desh Bhagat University, Mandi Gobindgarh (Punjab), India. The plant material was air dried for 10 days and stored at ambient temperature (25±1ºC) without exposure to direct sunlight.

A. Extraction method

The collected plant material was shade dried at room temperature. And after proper dryness of plant material it converted into powder. Prepared powdered plant material (2g, 4g and 6 g respectively) was transferred to dark-coloured flasks and mixed with 40 ml of solvents with different polarities (water, methanol, acetone) respectively and stored at room temperature. After 24, infusions were filtered through What man No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 ^{0}C using Rotatory evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 ^{0}C.

B. Antimicrobial Screening

The anti-bacterial activities of extract were tested against strains isolated from animal byproducts. The strains include three Gram-positive bacteria (S. aureus, L. monocytogenes and B. cereus) and three Gram-negative bacteria (E. coli, P. aeruginosa and S. typhimurium) using Muller Hinton agar medium (Oxoid). The anti-fungal activities of the compounds were tested against two fungi (C. albicans and A. flavus) using Sabouraud dextrose agar medium (Oxoid).

C. Paper Disc Diffusion Technique

The sterilized medium [10] (autoclaved at 120 °C for 30 min) (40–50 °C) was inoculated (1 ml/100 ml of medium) with the suspension (105 cfu/ml) of the micro-organism (matched to 0.9 McFarland barium sulphate standard) and poured into a Petri dish to give a depth of 3–4 mm. The paper impregnated with the test extract was placed on the solidified medium. The plates were pre-incubated for 2 h at room temperature (24˚C) and incubated at 37–28 °C for 24 h for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin and Fluconazole were used as standard for anti-bacterial and anti-fungal activity, respectively.

D. Inhibitory Concentration (MIC)

MIC [26] of the extract was determined by agar streak dilution method. A stock solution of the extract in DMSO was prepared and graded quantities of the test compounds incorporated in specified quantity of Muller Hinton agar for anti-bacterial activity and Sabouraud dextrose agar medium for anti-fungal activity. A specified quantity of the medium (40°C) containing the compound was poured into a Petri dish to give a depth of 3 mm and allowed to solidify.

Suspension of the micro-organism was prepared to contain approximately 105 cfu/ml and applied to plates with serially diluted compounds in DMSO to be tested and incubated at 30°C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

E. Antimicrobial activity

Methanolic Extract was screened for anti-microbial activities by paper disc diffusion technique[10]. The anti-bacterial activity of the compound was tested against strains isolated from animal byproducts and were accused of being a direct cause of food intoxication in human[25]. The strains include three Gram-positive bacteria (S. aureus, Legionella monocytogenes and Bacillus cereus) and three Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa and Salmonella typhimurium) using Muller Hinton agar medium (Oxoid)[11]. The anti-fungal activities of the compounds were tested against two fungi (Candida albicans and Aspergillusflavus) using Sabouraud dextrose agar medium (Oxoid)[11]. The observed data on the anti-microbial activity of the compound and standard drugs are given in table.

TABLE

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT

Tested Extracts	In vitro Activity-zone of Inhibition in mm (MIC in µg/ml)							
	Tested Strains							
	E. coli O157 (F)	S.Ttyphimurium (3)	L.Monocytogenes (A)	S. Aureus	P.Aaeruginosa (C)	B.Ccereus (E)	C. Albicans	A. Flavus
WE-1g/20ml	10 (45)	15 (3.215)	14 (30)	16 (1.56)	12 (45)	9 (45)	−ve	12 (25)
WE-1g/10ml	14 (25)	12(45)	13 (30)	13 (25)	15 (25)	16 (1.56)	10 (35)	12 (15)
WE-1g/6.7ml	14 (20)	13 (45)	16 (3.215)	15 (25)	13 (30)	12 (45)	12 (20)	26 (0.38)
ME-1g/20ml	10 50)	11 (35)	11 (>45)	12 (45)	11(45)	18 (1.7)	10 (30)	21 (1.56)
ME-	11 (>45)	10 (>50)	13 (30)	15 (25)	13 (20)	12 (35)	15 (12)	17 (1.56)

1g/10ml								
ME-1g/6.7ml	12 (30)	15 (12)	12 (45)	11 (50)	11(20)	16 (1.56)	14 (12)	15 (12.5)
AE-1g/20ml	11 (45)	18 (0.82)	14 (13)	11 (25)	12 (45)	13 (30)	12 (25)	15 (18)
AE-1g/10ml	15 (35)	12 (45)	14(12.5)	16 (12.5)	12 (45)	16 (1.56)	10 (40)	17 (9.5)
AE-1g/6.7ml	11(45)	11(>45)	9 (>45)	10 (>45)	11(>45)	15 (10)	10 (45)	30 (0.39)
Ciprofloxacin (100 µg/ml)	19 (1.56)	20 (0.38)	17 (1.56)	17 (1.56)	19 (0.88)	20 (0.37)	–	–
Fluconazole (100 µg/ml)	–	–	–	–	–	–	18 (1.56)	21 (0.78)
DMSO	−ve	−ve	−ve	−ve	−ve	−ve	−ve	−ve

WE- Water Extract; ME- Methanolic Extract ; AE- Acetone Extract

III. RESULTS AND DISCUSSION

All the prepared plant extract were screened for their anti-bacterial activity against S. aureus, L. monocytogenes, B. cereus, E. coli, P. aeruginosa and S. typhimurium and anti-fungal activity against C. albicans and A. flavus[11]. The minimum inhibitory concentrations (MIC) of all compounds were also determined. The anti-bacterial Data revealed that all tested plant extract of this investigation are moderate to good in activity against all the tested pathogenic bacteria. As compared to the standard drug Cipro floxacin with MICs 1.56, 0.39, 1.56, 1.56, 0.78 and 0.38 µg/ml against E. coli, S. typhimurium, L. monocytogenes, S. aureus, P. aeruginosa, and B. cereus respectively, different extracts showed very promising activity. WE- 1g/20ml showed significant anti-microbial activity against S. aureus, S. typhimurium with MICs 1.56 µg/ml and 3.215 µg/ml respectively. Sample AE- 1g/20ml showed potent anti-microbial activity against S. typhimurium with MIC 0.88 µg/ml. Also compounds AE- 1g/10ml, WE- 1g/10ml and ME- 1g/6.7ml showed potent anti-microbial activity against B. cereus with MIC 1.56 µg/ml.

The screening data of anti-fungal activity of this extract shows wide range of anti-fungal activity. It is of interest that sample ME-1g/10ml was found to exhibit the most potent in vitro anti-fungal activity with MICs of 12 and 1.56 µg/ml against C. alb cans and A. flavus. Also compounds WE- 1g/6.7ml and AE- 1g/6.7ml showed pronounced anti-fungal activity against A. flavus with MIC 0.39 µg/ml. Extract ME- 1g/20ml showed potent anti-fungal activity against A. flavus with MIC 1.56 µg/ml.

IV. CONCLUSION

Studies confirm and extend the previously reported antimicrobial activities of Plant methanolic extracts[12]. Most previous studies of Eucalyptus globules have reported on the antimicrobial activity of oils[13-15] with variable results. This study uses methanolic extracts to overcome the problems associated with the insolubility of oil components in agar gels. The broad range of microbial susceptibilities indicates the potential of these extracts as a surface disinfectant as well as for medicinal purposes and possibly as food additives to inhibit spoilage. However, further studies are needed before these extracts can be applied to these purposes. In particular, toxicity studies are needed to determine the suitability of these extracts for the use as antiseptic agents[15].

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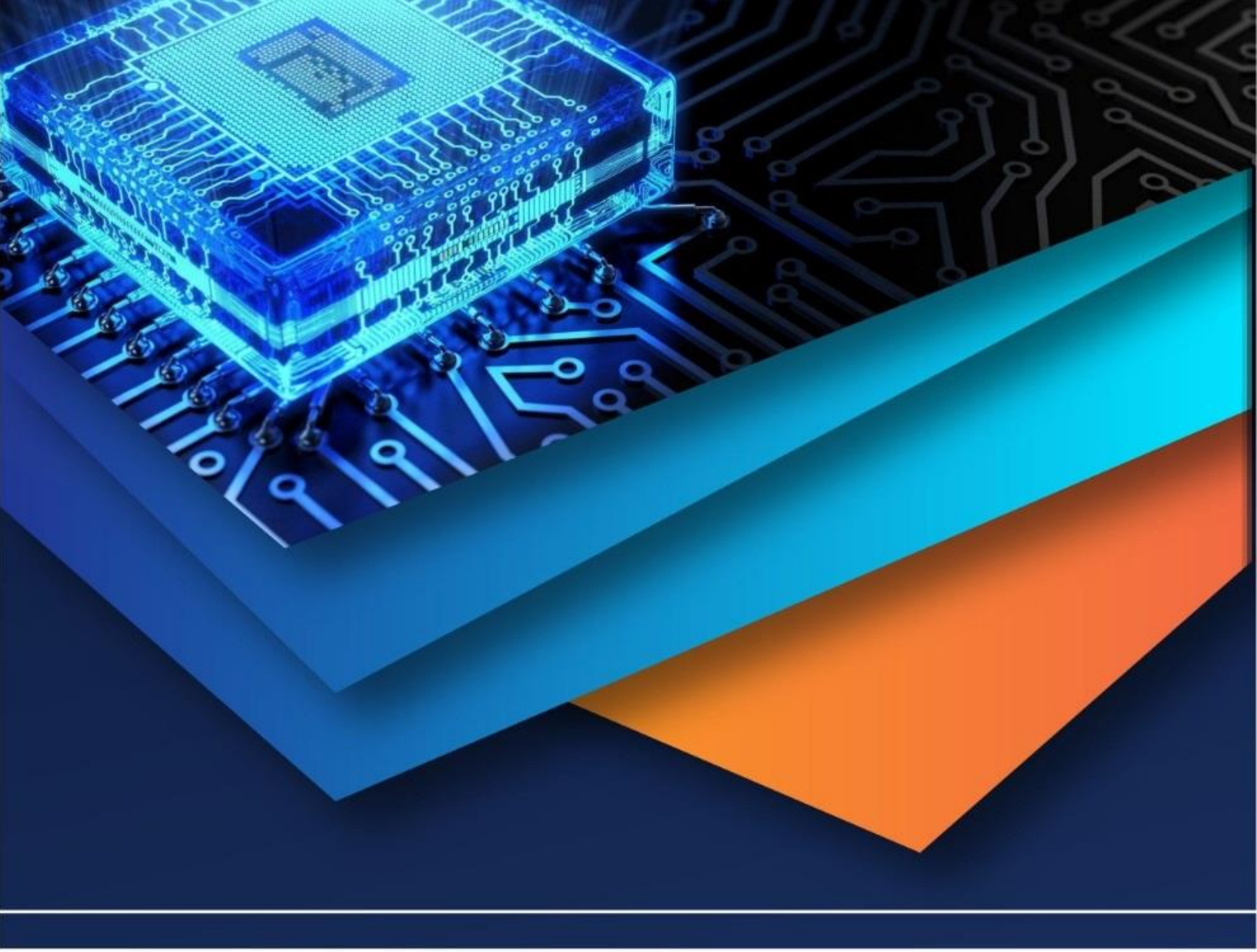

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