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Bioactive potential of essential oil extracted from the leaves of *Eucalyptus globulus* (Myrtaceae)

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Abstract
Eucalyptus oil is the important source of medicine which is traditionally used in the treatment of various diseases and in healthcare. The present investigation was carried out to study the bioactive potential of an essential oil extracted from the leaves of *E. globulus* in terms of antibacterial activity against *S. aureus* and *P. aeruginosa*, antioxidant potential against DPPH radicals and anticancer activity against human lung cancer cells (A549). The pharmacological evaluation of extracted essential oil, revealed the ethnomedicinal value of eucalyptus oil for antibacterial and anticancer activities.

Keywords: *Eucalyptus globulus, Essential oil,* anticancer activity, bioactive potential

Introduction
Plants have been used as a source of medicines since ancient times. At present, almost 50% of medicines derived from plants or their synthetic derivatives which continuously creating interest in the plant world (Tuorkey, 2015) [1]. Eucalyptus oil is ethanomedicinally important and widely used in medicine as antiseptic, insect repellant, sedative yet stimulant, encephalitis, enteritis, erysipelas, fever, flu, inflammation, anesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, arthritis, asthma, boils, bronchitis, burns, cancer, diabetes, diarrhea, laryngitis, leprosy, malaria, mastitis, miasma, pharygnitis, phthisis, febrifuge, fumigant, hemostat, inhalant, preventitive, rubefacient, vermifuge, for a folk remedy for abscess, diphtheria, dysentery laryngalgia, rhinitis, sores, sore throat, spasms, trachalgia, worms, and wounds (Bachir & Benali, 2012) [2] Moreover, it is highly used in the soap and cosmetic industries (Patil & NItave. 2014) [3]. However, its antioxidant effect and anticancer effect on human lung cancer cells have not very well reported before.
In present study, we investigated the potential antibacterial, antioxidant and anticancer effect of eucalyptus oil extracted from the leaves of *Eucalyptus globulus (E. glubulus)*. Antibacterial potential of extracted oil was evaluated against pathogenic bacteria *Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa (P. aeruginosa)*. Antioxidant potential was evaluated against DPPH free radicals. Anticancer potential was evaluated against human lung cancer cell (A549) by MTT assay.

Materials and Methods
Plant material and essential oil extraction
Fresh plant leaves of *E. globulus* were collected during the flowering stage was subjected to steam distillation using a Clevenger-type apparatus. Leaves were immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapour and finally collected after decantation. The distillate was isolated and dried in a Rota-vapor to giving greenish-yellow oil. The collected oil was stored at 4 °C for antimicrobial and antioxidant assay (Ait-Ouazzou *et al*., 2011; Song *et al*., 2009) [4, 5]. The extracted essential oil was diluted with DMSO at the concentrations of 20, 40, 60, 80, 100% (v/v).

Antibacterial assays
Bacterial strains
Antibacterial activities of extracted eucalyptus oil from *E. globulus* were carried out against Gram positive: *S. aureus* and Gram negative: *P. aeruginosa*. Both bacterial strains were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained on nutrient agar. Bacterial cultures were prepared by transferring a single colony into a fresh medium and grown overnight at 37 °C. Turbidity of the culture was adjusted with sterile saline solution to match 0.5 Mc Farland standard 10^8 colony forming units/ml (CFU/ml) (Adnan *et al*., 2017) [6, 10].

Agar cup/well diffusion method

Antibacterial activity of eucalyptus oil were analyzed by agar cup/well diffusion method against *S. aureus* and *P. aeruginosa* on Muller Hinton Agar (MHA) (Hi-Media, India). A well was made on the plate with the help of gel puncture and 50 µl of eucalyptus oil (20%, 40%, 60%, 80%, 100%) were inoculated into the well and plates were incubated at 37 ˚C for 24 hours. On the next day, zone of inhibitions were discussed. Gentamycin standard antibiotic was used as the positive control. The diameters of the inhibition zones were measured in millimeters (Adnan *et al*., 2018a) [7].

Determination of DPPH free radical scavenging activity

Antioxidant activity of eucalyptus oil was measured against DPPH free radicals in terms of radical scavenging ability (Brand *et al*., 1995). Different concentrations of extracted eucalyptus oil diluted in phosphate buffer (pH 7.4) (20%, 40%, 60%, 80%, 100%) were added in a tube containing 2 ml of 6×10^{-5} M of DPPH solution in DMSO. All the tubes were incubated up to 1 hour in dark. At the end of incubation, decrease in absorbance was measured at 517 nm. Phosphate buffer was used as blank. DPPH solution without extracted eucalyptus oil was used as a control. Ascorbic acid was used as a standard. All determinations were carried out in triplicate. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging activity (%) = (A0 – A1) /A0 ×100

Where, A0 = absorbance of the control

A1 = absorbance of the sample

Cytotoxic assay (MTT assay)

A549 cell line (lung cancer cell line) was seeded in 96-well plates and incubated in humidifier atmosphere containing 5% CO_2 at 37 °C up to adherence. Cells were then treated with different concentration of extracted eucalyptus oil (20%, 40%, 60%, 80%, 100%). Cells were washed with PBS solution and subjected with 100 µl of MTT solution (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (5mg/ml) and incubated for 4 hours. Finally, the medium was removed and 100 µl of DMSO was added to solubilize the formazan crystals. Amount of formazan crystal was determined by measuring the absorbance at 570 nm using ELISA reader. 5 – Flourouracil was used as a positive control. All assays were done in triplicate (Adnan *et al*., 2018a) [7].

Results

Antibacterial potential of extracted eucalyptus oil

Extracted eucalyptus oil was studied for its antagonistic potential against pathogenic bacteria like *S. aureus* and *P. aeruginosa*. Results of antibacterial activity are represented in the form of zone of inhibition (Figure 1 & 2). Results revealed that, the extracted eucalyptus oil showed antibacterial activity with varying magnitudes, depending on the size of inoculums and on the concentration. Extracted eucalyptus oil has higher antagonistic activity against *S. aureus* compare to *P. aeruginosa*. Largest zone of inhibition was obtained for *S. aureus* with 100% concentration.

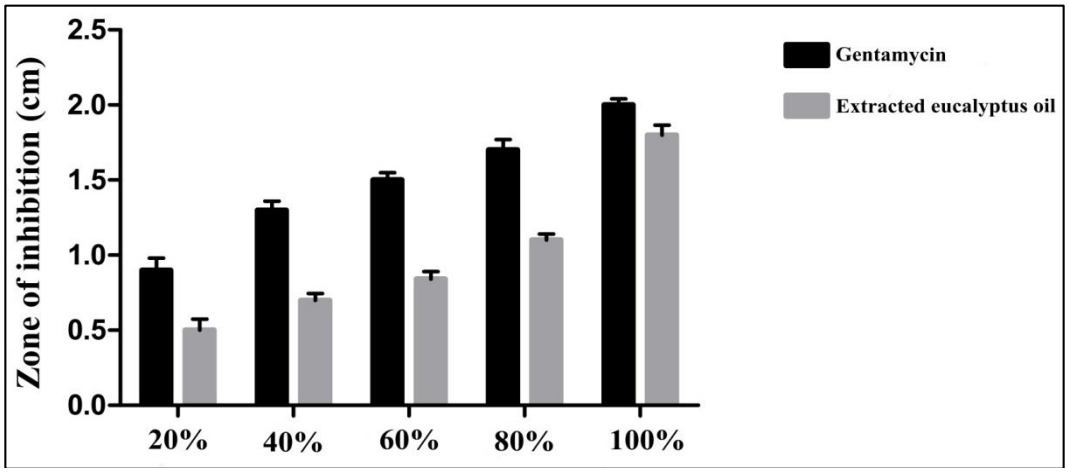

Fig 1: Antibacterial assay of extracted eucalyptus oil against *S. aureus.*

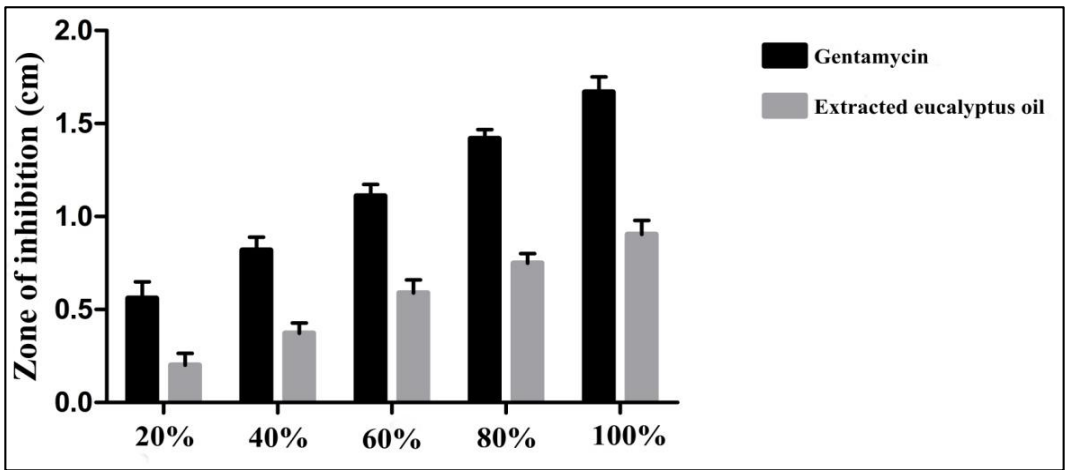

Fig 2: Antibacterial assay of extracted eucalyptus oil against *P. aeruginosa.*

Antioxidant potential of extracted eucalyptus oil

Antioxidant potential was studied against DPPH molecules in comparisons to ascorbic acid. Extracted eucalyptus oil exhibited good radical scavenging capacity against DPPH molecules. Extracted eucalyptus oil reflected dose dependence of the antioxidant potentials as there was increase in their concentration (20%, 40%, 60%, 80%, 100%), antioxidant potential was also increased (Figure 3). The scavenging effects of all concentration of extracted eucalyptus oil were found to be relatively less than ascorbic acid.

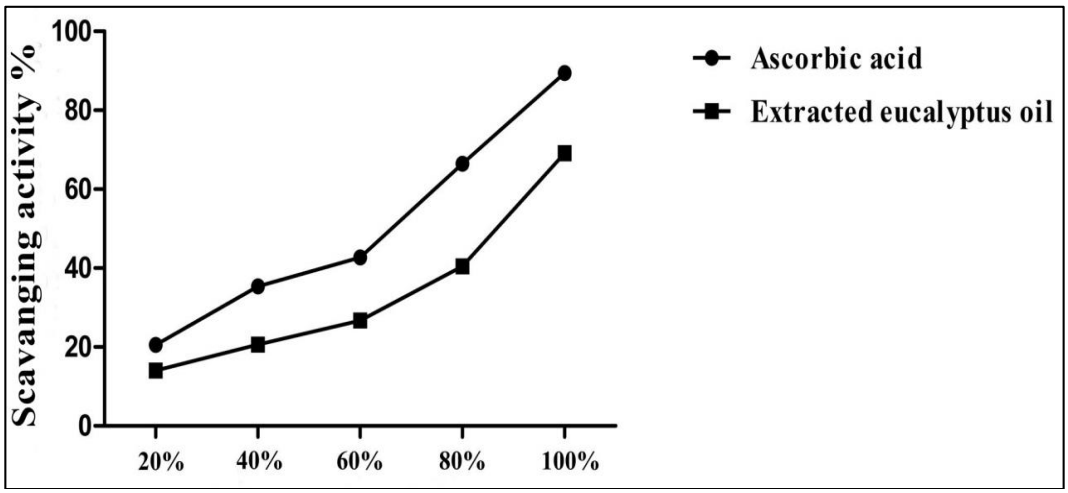

Fig 3: Antioxidant potential of extracted eucalyptus oil against DPPH free radical.

Cytotoxic potential of extracted eucalyptus oil

A different concentration of extracted eucalyptus oil was tested for its cytotoxic effect against A549 cells. Extracted eucalyptus oil showed dose-dependent cytotoxic activity on A549 cell lines. Cytotoxic activity of extracted eucalyptus oil was lower than the standard chemotherapy drug Fluorouracil (5FU) (Figure 4).

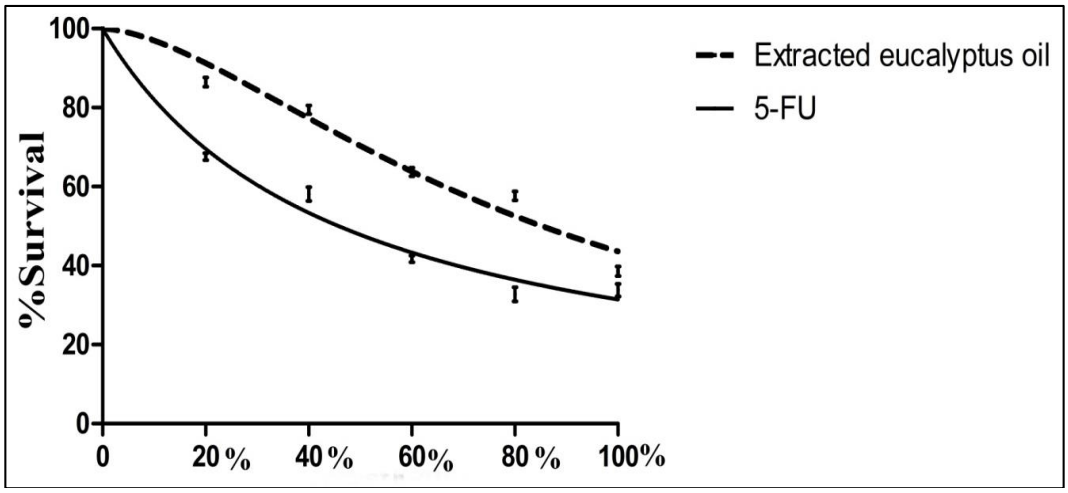

Fig 4: Cytotoxic activity of extracted eucalyptus oil against A549 cell line.

Discussion

From the ancient times, natural products has been proven as a promising source for the discovery of bioactive compounds, important for the development of new pharmaceuticals for fighting against infection, inflammation, cancer and various other diseases (Adnan *et al*., 2017a,b; 2018a,b) [6, 7]. Therefore, traditional medicines and natural products have been the most successful source of potential drug leads.

Previous literature revealed that eucalyptus oil is an important source of many pharmacologically and medicinally important compounds especially of terpenoids which have been use in aromatherapy. Moreover, different pharmacological activities like analgesic, antifungal, anti-inflammatory, antibacterial, antidiabetic, antioxidative, antiviral, antitumor, antihistaminic, anticancer cytochrome p450 inhibitor and hepatoprotective properties of eucalyptus oil has been reported due to the presence of components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, β- pinene, γ-terpinene, α-terpinol, alloocimene and aromadendrene (Nezhad *et al*., 2009) [11].

Present study also revealing the medicinal importance of extracted eucalyptus oil in terms of antibacterial, antioxidant and anticancer activity. From the above experiment it can be inferred that extracted eucalyptus oil suggests significant growth inhibiting effects on *S. aureus* and *P. aeruginosa*. The efficacy of extracted eucalyptus oil against these microorganisms may provide a scientific ground for the application of it in prevention and treatment of bacterial infections caused by various pathogenic bacteria which have developed resistance to antibiotics. High antioxidant potential of extracted eucalyptus oil against DPPH molecules indicated its use in to the treatment of various diseases like atherosclerosis, diabetes and inflammatory joint disease which are caused by free radicals within the body. Cytotoxic

effect of extracted eucalyptus oil against A549 cells indicated its possible application in the treatment of lung cancer.

In conclusion, it was observed that extracted eucalyptus oil contains a wide variety of secondary metabolites that hold good antibacterial, antioxidant and anticancer capacity based on the experiments performed which add scientific evidence to conduct further studies, investigate the lead compounds present in the plant, evaluate its bioactive potential on *in vivo* animal models and put forward an attempt to carry out trails on human beings.

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