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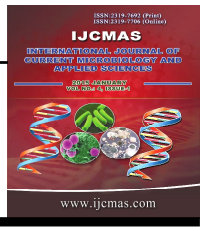

Original Research Article

A Comparative Study of Effect of Essential Oil and Alcoholic Extract of Eucalyptus Leaves on Medically Important Bacterial and Fungal Isolates

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Keywords

Antimicrobial activity, Hydro distillation, GC/MS, Essential oil extract, Zone of Inhibition

A B S T R A C T

Eucalyptus is a common widespread dominant tree found ecologically and economically under wide range of climatic condition. The study is focused to evaluate the medicinal property of the plant and its product which was traditionally used by the people of Asian countries like Nepal and India. The essential oil was obtained through hydro-distillation using Clevenger apparatus for 5-6 hrs and the oil percentage was found to be 2%. The alcoholic and aqueous extracts of leaves were prepared in different concentration. The antimicrobial test was studied against fungal and bacterial pathogens of plant as well as animal through agar well diffusion. The results were found to be positive on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes, Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Alternaria alternata, Penicillium chrysogenum* and *Malassezia furfur*. The essential oil shows higher inhibition levels as compared to aqueous and alcoholic extract of root and leaves.

Introduction

Eucalyptus species grow in a wide range of climatic and edaphic condition in their natural habitats. They consist of high potential of allochemical and also essential oil. The oil consists of 16 components out of which five compounds α-pinene, δ -3carene, β-phellandrene, 1–8 cineole and p-cymene (Iqbal *et al.*, 2005). The plant extract of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory traits (Satish *et al.*, 1999; Shariff *et al.*, 2006; Mohana and Raveesha *et al.*, 2006). Plant metabolite and plant based medicine appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumer in contrast to the synthetic pesticides (Verma and Dubey *et al.*, 1999). Due to the development of resistance against pathogen, there is an urgent need to screen a large no of plants for antifungal and antibacterial activity against important seed borne *Aspergillus sps*, plant based disease organism and human pathogen causing skin and hair infection, with the ultimate aim of developing plant based medicine, extract, compound formulation for plant disease management, human skin disease management and safe storage of grains (Satish *et al.*, 1999). Discovery of new compound is required to combat fungal

infection (Ravindra *et al.*, 2008; Prakash and Ragavan, 2008; Etienne *et al.*, 2008).

A previous study report stated that *Eucalyptus sps – E. camaldulensis* leaf essential oil contained bioactive compound that displayed antibacterial, analgesic and anti-inflammatory effect, anti-termitic activity, antioxidative and antiradcal activities, larvicidal and mosquito repellent activities (Pornpun Siramon *et al.,* 2013). Another study supports that the antimicrobial activity of the essential oil of *Eucalyptus sps* shows positive effect on *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Penicillium citrinum* (Pornpon *et al.*, 2012; Bachir Raho Ghalem and Benali Mohammed, 2008).

In this study, medicinal value of Eucalyptus plant has been screened. The test microbial cultures were of pathogenic bacteria and fungi origin. The importance of the work can be supported by the fact that natural product and medicinal agents derived from plants play vital role in health care system of 20% of the population in developed countries and more than 50% of all drugs in clinical use and have a natural product origin (Harami *et al.,* 2006).

Materials and Method

Collection of plant material and isolation of essential oil and preparation of extracts-

For isolation of Oil, Leaves sample were collected from Dhanusha, the oil was isolated by following procedure- 100gm of leaves sample were added to Clevenger apparatus under hydro distillation for 5 to 6 hr, 2ml of essential oil was obtained.

For the preparation of extract of the plant leaves & roots were collected from Chanakyapuri garden, Delhi and root from nursery, Greator Noida. Leaves were dried and methanolic and aqueous extract of different concentration were prepared

Test Microorganism

The test organisms were obtained from Codon Biotech Pvt. Ltd., Noida. Laboratory like *E. coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Paenibacillus polymyxa* and *Clostridium sp.* and some fungal samples were isolated from plant, some from human and some from hospital like *Aspergillus niger*, *A. flavus*, *Aspergillus oryzae*, *Alternaria alternata*, *Penicillium citrinum* and *Malassezia furfur* (Dandruff) and yeast sample *Saccharomyces cerevisiae* from curd and *Geotrichum candidum*

Antibacterial activity: The antimicrobial effectiveness was screened by Agar well diffusion method. The different pure bacterial strains such as *E. coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Paenibacillus polymyxa* and *Clostridium sp.* were taken and swabbed with sterile cotton over the entire surface of the pre-set solidified NA petri plate. The two wells were already made before bacterial swabbing. One well was filled with 50µl/80µl of extract or essential oil and second was filled with sterile distilled water and the plates were incubated for 24 hrs at 37°C and zone of inhibition was observed.

Antifungal activity: The fungal strains were isolated from soil, infected leaf of tomato and hair dandruff and were inoculated in PDA media. Antifungal activity was also done by above mentioned agar well diffusion method. The different fungal strains such as *Aspergillus niger*, *A. flavus*, *Aspergillus oryzae*, *Alternaria*

alternata, Penicillium citrinum and Malassezia furfur and in yeast sample *Saccharomyces cerevisiae* from curd and *Geotrichum candidum* were taken for testing of antifungal activity. The PDA media were poured on petri plate. Two to three wells were made in which one well was filled with control and other two filled with sample extract or oil.

Result and Discussion

Among 15 samples taken, 6 fungal samples, 2 yeast samples and 7 bacterial samples were found to be positively inhibited by the essential oil extracted. The result of in vitro antimicrobial test shown by the extract and essential of *Eucalyptus sps* with reference to control is shown in table.

The results clearly indicate that the antimicrobial agent in Eucalyptus tree is extracted in non- polar solvents. Thus, the inhibitory effect of oil as compared to alcoholic extract was far different and greater than alcoholic extract.

The result shows that the wide variation in the antimicrobial properties of plant essential oil with respect to type of plant extract. The oil shows its antibacterial and antifungal activity against all the microbes i.e., bacteria or fungal pathogens. The efficiency of essential oil of *Eucalyptus sps*. was found to be potent against all the sample including fungi, bacteria and yeast. It was also found effective on anaerobic bacteria like *Clostridium acetobutylicum*. The amount of extracted oil in agar well diffusion depends on the sample as in bacterial sample 10-20µl is taken where as in fungus 50-80µl is required to determine the effectiveness. In fungal sample, the oil shows highest inhibition on Aspergillus niger i.e. 2.3cm. The present study therefore compared the inhibitory effect of *E. sps* essential oil and was an attempt to established the basis for the use of oil in the treatment of Aspergillosis, candidiasis, Urinary tract infection and wound infection.

Table.1 Result of in vitro susceptibility test of bacterial isolate against essential oil and extract

Name of isolate	**Zone of inhibition shown in cm**		
	Essential oil	**Aquous extract**	**Alcoholic extract**
Staphylococcus aureus	1.4	0	0.6
E. coli	2.1	0	1.1
Bacillus subtilis	1.4	0.8	1
Enterobacter aerogenes	1.6	0.7	1.2
Paenibacillus polymyxa	1.3	0.8	0.6
Pseudomonas. aeruginosa	1.2	0	0.4
Clostridium acetobutylicum	1.4	0.9	1.1

Table.2 Result of in- vitro susceptibility test of fungal isolate against essential oil and extracts

Fungal Strain	Zone of inhibition shown in cm		
	Essential oil	Aqueous Extract	Alcoholic Extract
Aspergillus niger	2.3cm	1	1.5
Aspergillus flavus	1.2	0	1.0
Aspergillus oryzae	1.5	0.4	1.0
Alternaria alternata	2	0.7	1.3
Penicillium chrysogenum	1.7	0.5	0.9
Malassezia furfur	1.6	0	0.9
S. cerevisiae	1.8	0	0.6
G. candidum	1.5	0	0.4

Figure.1 Bar diagram showing antibacterial activity of oil and extracts against bacterial cultures

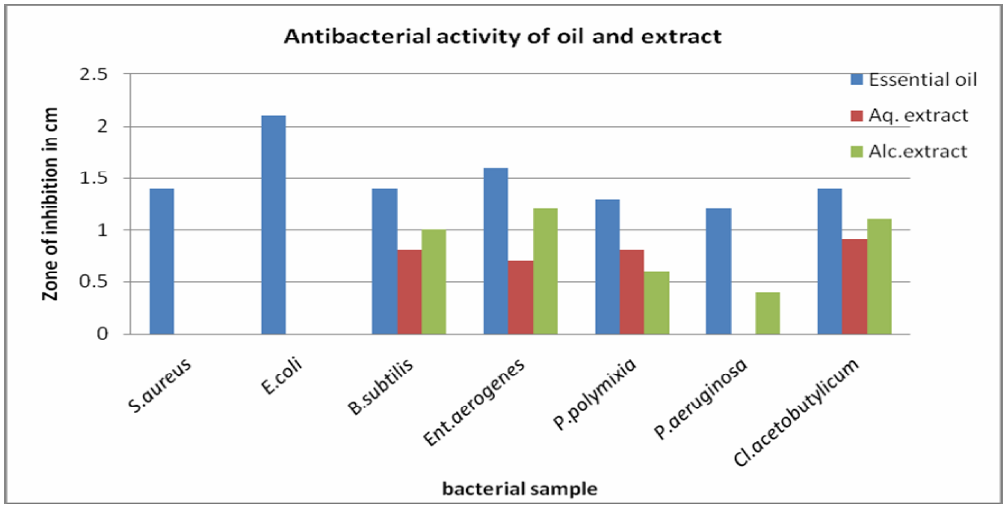
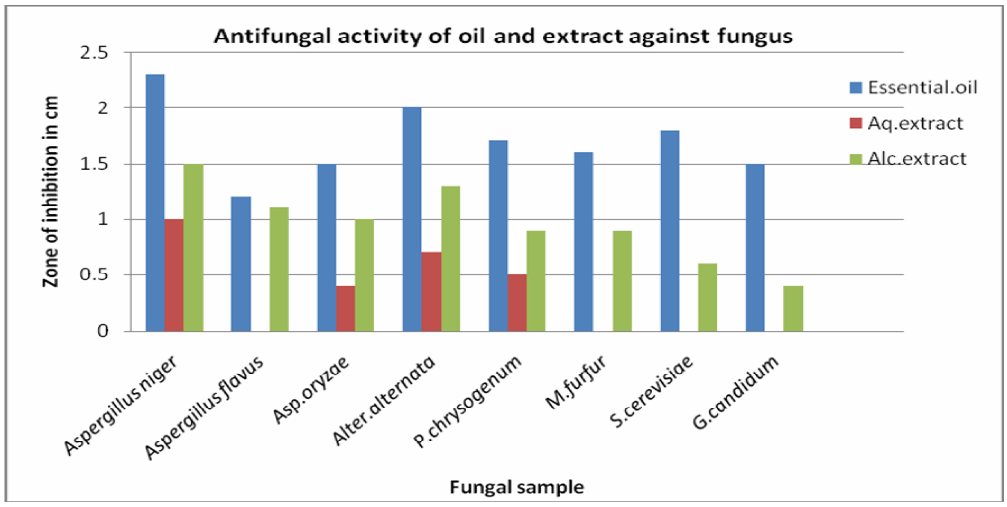


Figure.2 Bar diagram Graph showing antifungal activity of oil & extract



References

Etienne, A., Datry, A., Gaspar, N., *et al.* 2008. Successful treatment of disseminated *Geotrichumcapitatum* infection with a combination of caspofungin and voriconazole in an immune compromised patient. *Mycoses*, 51(3): 270.

Harami, M.A., Abayeh, O.J., NNEU, Ibok., Samual, Emgba Kafu. 2006. Antifungal activity of extracts of some Cassia, Detarium, and Zizipus species against dermatophytes. *Natural Product Radiance*, 5(5): 357–36.

Iqbal, S., Bhanger, M.I., Anwar, F. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chem.*, 93: 265–272.

Mohana, D.C., Raveesha, K.A. 2006. Anti-bacterial activity of *Caesalpinia coriaria* (Jacq.) Willd. against plant pathogenic *Xanthomonas pathovars*: an eco-friendly approach. *J. Agric. Technol.*, 2: 317–327.

Pornpun Siramon, Yoshito Ohtani, Hideaki Ichiura, 2013. Chemical composition and antifungal property of *Eucalyptus camaldulensis* leaf oils from Thailand. *Rec. Nat. Prod.*, 7(1): 49–53.

Prakash, N., Ragavan, B. 2008. Phytochemical screening and antifungal activity of *Cyperus esculentus L. Icfai Univ. Press*, 2(3): 32–37.

Bachir Raho Ghalem, Benali Mohammed, 2008. Antimicrobial activity of leaf essential oil of *Eucallylptus camaldulensis* and *Eucalyptus globlu. Afr. J. Pharm. Pharmacol.*, 2(10): 211–215.

Ravindra, Malabadi, Vijay Kumar, S. 2008. Evaluation of antifungal property of medicinal plants. *J. Phytol. Res.*, 21(1): 139–142.

Satish, S., Raveesha, K.A., Janardhana, G.R. 1999. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris pathovarst*. *Lett. Appl. Microbiol.*, 28: 145–147.

Shariff, N., Sudarshana, M.S., Umesha, S., Hariprasad, P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla and Physalis minima* leaf and callus extracts. *Afr. J. Biotechnol.*, 5: 946–950.

Verma, J., Dubey, N.K. 1999. Prospective of botanical and microbial products as pesticides of tomorrow. *Curr. Sci.*, 76: 172–179.