

In vitro* Antibacterial Effect of certain Essential Oils against *Pseudomonas aeruginosa

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ABSTRACT

The antibacterial effect of various essential oils was investigated against *Pseudomonas aeruginosa*. The solvents used for extraction of essential oils were methanol, dichloromethane, hexane and chloroform. *In vitro* antibacterial activity was performed by disc diffusion method. The results obtained in the present study suggest that *Eucalyptus globulus* can be used in treating diseases caused by the test organism.

Key words: Essential oils, Antibacterial activity, Solvent, *Pseudomonas areuginosa*.

INTRODUCTION

Essential oils are found in various parts of plant body such as seeds, flowers, leaves fruits are concentrated in certain special cell and glands because of their properties. They are widely used in perfumes, flavorings and medicine. Essential oils are common group of natural product present in aromatic medicinal plants and are the most important source of life saving drugs for the majority of the world population. Medicinal plants represent a rich source of antimicrobial agents.

Plants are used medicinally in different countries are source of many potent and powerful drugs. The most common hospital infection is caused by opportunistic pathogen *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is a notorious nosocomial pathogen capable of surviving for extended periods in almost any liquid environment even in some disinfectant solution.

Pseudomonas aeruginosa infect people who are already ill or injured. It's not highly virulent but is a successful opportunistic pathogen because it has pilli for adherence and an extracellular slime layer that interferes with phagocytosis. Burned

patient are at particularly high risk because they have lost their skin production. *Pseudomonas aeruginosa* infection are extremely difficult to treat with antibiotics. The efficacy of various species of medicinal plants against a variety of pathogen has found out by a number of workers¹⁻¹⁰.

Chemotherapy is the main approach in the treatment of bacterial infection. A major problem with various antibiotics include toxicity at high cost and low efficacy. These problems therefore necessitate a constant search for antimicrobials. In the present study essential oils from seven medicinal plants which have been traditionally used as antimicrobial agent were examined for antimicrobial activities against *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Eucalyptus globulus*, *Mentha arvensis*, seeds of *Sapindus mukorossi*, *Myristica fragrans*, fruit of *Emblca officinalis*, fresh flowers of *Jasminum sambac*, *Rosa indica* were collected from the different location of Bhopal. Plants samples were washed, shade, dried at room temperature. The dried and ground plant parts were

extracted with different solvents (hexane, dichloromethane, methanol, chloroform) by steam distillation.

Extraction of essential oil

Raw plant material consists of leaves, flowers, fruits, seeds is put into distillation apparatus using soxhlet apparatus. Weight of plant material has been taken before loaded in the soxhlet apparatus and the water is heated so that the steam passes through the plant material vaporizing the volatile compounds. The vapour flow through a coil where they condense back to liquid which is then collected in the receiving vessel.

Maintenance of microbial culture

In the present study *Pseudomonas aeruginosa* is taken as the test microorganism. The cultures were maintained on nutrient agar slants and stored at 4°C. Stock culture were subculture at regular intervals.

Disc diffusion method

Antibacterial activity of essential oils of seven plant were tested against *Pseudomonas aeruginosa*. This bacteria grow in nutrient agar medium. Nutrient agar medium was poured into the plate to obtain uniform depth and allowed to solidify. The standard inoculums suspension

Table 1: Zone of inhibitory activity (in millimeter) of different plant extracts against *Pseudomonas aeruginosa*

Source	Extract	Zone of inhibition (mm)
<i>Emblica officinalis</i>	Methanol	10mm
	Dichloromethane	-
	Hexane	9mm
	Chloroform	8mm
<i>Eucalyptus globules</i>	Methanol	15 mm
	Dichloromethane	10 mm
	Hexane	4mm
	Chloroform	8mm
<i>Mentha arvensis</i>	Methanol	5 mm
	Dichloromethane	-
	Hexane	4mm
	Chloroform	-
<i>Jasminum sambac</i>	Methanol	1mm
	Dichloromethane	-
	Hexane	-
	Chloroform	-
<i>Sapindus mukorossi</i>	Methanol	6mm
	Dichloromethane	4mm
	Hexane	5mm
	Chloroform	-
<i>Myristica fragrans</i>	Methanol	9 mm
	Dichloromethane	7mm
	Hexane	7 mm
	Chloroform	-
<i>Rosa indica</i>	Methanol	3mm
	Dichloromethane	-
	Hexane	4mm
	Chloroform	-
Standarad	Penicillin	20mm

(10⁴CFU/MI) were streaked over the surface of the media. 4mm diameter discs were prepared with Whatman No.1 filter paper and used for the study 10µl of essential oil was diluted with volume of 5% ethanol and impregnated on the filter paper disc and placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the inoculated agar surface Penicillin (5µg /disc) was used as positive reference standard to determine the sensitivity of the tested strain. 5% Ethanol was used as negative control. Finally the inoculated plate were incubated at 37°C for 24 hrs and the inhibition zone was observed.(Table-1)

RESULTS AND DISCUSSION

The antibacterial activity of seven plant species extracts were assayed *in vitro* by agar disc diffusion method against *Pseudomonas aeruginosa*. Table-1 summarizes the *Pseudomonas* growth inhibition by different extracts of the screened plant species. A significant inhibition zone of *Pseudomonas aeruginosa* was found in all solvent extracts of *Eucalyptus globulus* showing 15,10,4,8mm inhibition. *Emblica officinalis* and *Myristica fragrans* presented moderate inhibition in methanol, dichloromethane, hexane, chloroform successfully inhibition against *Pseudomonas aeruginosa*.The highest antibacterial activity of methanol leave extract of *Eucalyptus globulus* was 15mm. and least antibacterial activity recorded in methanol flower extract of *Jasminum sambac* is 1mm.

The result of present investigation clearly indicate that *Eucalyptus globules* is potentially a good source of antimicrobial agent. It can be used as development of new therapeutic agent.

The present study was conducted to investigate the *in vitro* antimicrobial activity. However,

the plants differ significantly in their activity against nearly all the extracts of *Eucalyptus globulus* presented significant inhibition against *Pseudomonas aeruginosa*. The Gram negative strain *Pseudomonas aeruginosa* is a common bacterium that can cause diseases in animals and humans. Infection associated with *Pseudomonas aeruginosa* are pneumonia, septicemia, meningitis, brain abscesses, urinary tract infection. The most common hospital infection is caused by opportunistic pathogen of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* are extremely difficult to treat with antibiotics. In evidence from expt, plant such as *Eucalyptus globulus* showed high activity against *Pseudomonas aeruginosa*.

Many microorganisms which cause damage to human health exhibit drugs resistance due to inadequate use of antibiotics. Thus there is a need for the discovery of new substance from natural source including plants. Plants produce a diverse range of bioactive molecules making them rich source of different types of medicine. The present result show that the methanol leaf extract of *Eucalyptus globulus* showed more inhibitory effect than the other plant extracts.

CONCLUSION

In conclusion the present investigation comes out with the fact that methanol leaf extract of *Eucalyptus globulus* is potentially a good source of antimicrobial agent. Thus in the study a certain value of plants used in ayurveds which could be of considerable interest to the development of new drugs has been tested for their efficacy against pathogenicity. These promissory extract opens the possibility of finding new clinically effective antibacterial compounds.

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