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EVALUATION OF ANTIMICROBIAL ACTIVITY OF TWO MEDICINAL PLANTS- EUCALYPTUS (*EUCALYPTUS GLOBULUS*) AND NEEM (*AZADIRACHTA INDICA)*

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ABSTRACT

Medicinal plants, since times immemorial, have been used virtually in all cultures as a source of medicine. Medicinal plants play a key role in world health care systems. Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. In our study we have selected two medicinal plants which are naturally highly available and are quite common: Eucalyptus (*Eucalyptus globulus*) and Neem (*Azadirachta indica*). In the present investigation, the methanolic bark and leaf extracts of Eucalyptus and Neem were evaluated for antimicrobial activity against common human pathogens.

KEYWORDS: Medicinal plants, antimicrobial activity, Eucalyptus and Neem etc.

INTRODUCTION

Among the nearly 15,000 flowering plants documented, many of them are used as sources of medicine. In the developing nations, almost 80% people depend on these plants for medicine because of their easy availability and low cost of treatment. The modern allopathic system of medicine is known to produce serious side effects and resistance against antibiotics which make these drugs non-potent.[1] The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Screening of medicinal plants for antimicrobial activities and photochemical is important for finding potential new compounds for therapeutic use.[2, 3]

Plants are one of the most important sources of medicine. Today the large number of drugs in use is derived from plants, like morphine from *Papaver somniferum*, Aswagandha from *Withania somnifera*, Ephedrine from *Ephedra vulgaris*, Atropine from *Atropa belladonna*, Reserpine from *Roulphia serpentina* etc. The medicinal plants are rich in secondary metabolites (which are potential source of drugs) and essential for therapeutic importance.[4, 5]

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the Synthesis of useful drugs. Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in drug development in the pharmaceutical industry.[6]

Neem (*Azadirachta indica*) is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to India, Myanmar, Bangladesh, Sri Lanka and Pakistan growing in tropical and semi-tropical regions. Neem is a fast-growing tree that can reach a height of 15-20 m (about 50-65 feet), rarely to 35-40 m (115-131 feet). It is evergreen but in severe drought it may shed most or nearly all of its leaves. The branches are wide spread. The fairly dense crown is roundish or oval and may reach the diameter of 15-20 m in old, free-standing specimens.[7,8]

Azadirachta Indica belongs to the family Meliaceae, commonly known as neem. It is used in traditional medicine as a source of many therapeutic agents. *Azadirachta Indica* (leaf, bark and seed) are known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B viruses. Different parts of neem (leaf, bark and seed oil) have been shown to exhibit wide pharmacological activities including; antioxidant, antimalarial, antimutagenic, anticarcinogenic, anti-inflammatory, antihyperglycaemic, antiulcer and anti-diabetic properties. The biological activities are attributed to the presence of many bioactive compounds in different parts.[9,10]

Neem leaf extract has been prescribed for oral use for the treatment of malaria by Indian ayurvedic practitioners from time immemorial. Recently a clinical trial has been carried out to see the efficacy of neem extract to control hyperlipidemia in a group of malarial patients severely infected with *P. falciparum*, the lipid level, especially cholesterol was found to be lower during therapy when compared to non-malaria patients.[11]

Eucalyptus is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in many other countries including India. 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. Eucalyptus camaldulensis is an important ethno medicinal plant belonging to the family, Myrtaceae. It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Essential oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorants. Topical ointments containing eucalyptus oil have also been used in traditional Aboriginal medicines to heal wounds and fungal infections. Eucalyptus oil obtained by steam distillation and rectification of the fresh leaves has Eucalyptol (1, 8-cineole) as its active ingredient and this is responsible for its various pharmacological actions.[12,13]

The antimicrobial activities of the methanolic extracts of E. camaldulensis have also been reported. The emergence of bacterial resistance to the currently available antimicrobial drugs necessitates further research in the discovery of new safe and effective antibacterial agents. The investigation of certain indigenous plants for their antimicrobial activity is therefore of utmost importance. This study is aimed at investigating the antimicrobial activity of Eucalyptus camaldulensis against Gram-positive and Gram-negative bacteria thereby establishing it as a potential antimicrobial agent.[14,15]

MATERIALS AND METHODS

To perform the study of antimicrobial activities of sample medicinal plants i.e. Eucalyptus (*Euacalyptus globulus*) and Neem (*Azadirachta Indica)* the plant samples were collected from Jayanti Kunj, Rewa M. P. and grown in Nursery of Department of Botany, Govt. Model Science College, Rewa, M. P. Bacterial strains *Pseudomonas aeruginosa*, *Bacillus amiloliquifaciens, Staphyllococcus aureus and Eschirichia coli* were taken from Clongen Biotechnology, Pvt. Ltd., Noida, and U.P.

Multiple Drug Resistance

Culture preparation

120 ml of nutrient broth was prepared and poured in each conical flask. The broth was then autoclaved and after autoclaving they were left to cool at room temperature in laminar air flow chamber. 100µl each of *Pseudomonas aeruginosa, Bacillus amyloliquifaciens,*

Staphylococcus aureus and *Escherichia coli* were inoculated into the four flasks. The inoculated culture was then kept in shaker overnight for growth.

Plant Extract preparation

Washing and drying of all the sample plants leaves were washed with distilled water and dried in hot air oven for 1-2 days to reduce the moisture content. Each of dried plant samples were weighed 4.00gm, and then crushed in 70% ethanol in the ratio of 1:8 in the mortar pestle and grinded properly then crushed samples were filtered through Whatmann filter paper 1 in a flask/beaker. Filtrates were placed in hot air oven at 40˚C in a flask/beaker till it completely dry for 2-4 days. Dried filtrate was dissolved in 5ml 0f 1X tris saline buffer and stored in refrigerator.

Preparation of agar plates

Nutrient Agar media was prepared and autoclaved then it was poured in autoclaved petriplates, then it was left for 15-20 minutes to solidify.50 µlitre of culture (*Pseudomonas aeruginosa*, *Bacillus amiloliquifaciens, Staphylococcus aureus* and *Escherichia coli*) were spread it into nutrient agar plates respectively.

MDR with standard drugs

Here, to get the standard reference values, the tetracycline, chloramphenicol drugs were taken. Different concentration (25, 50 and 75 µg) of these drug's are poured into the wells of *Pseudomonas aeruginosa*, *Bacillus amiloliquifaciens, Staphylococcus aureus* and *Escherichia coli* plates respectively.

Testing with plant sample

In order to check the antimicrobial activity against selected microbes (*Pseudomonas aeruginosa*, *Bacillus amiloliquifaciens, Staphylococcus aureus* and *Escherichia coli*), three wells were made in each of the culture plates by 1000ml tip of micropipette and were filled with 25, 50, 75µl of each plant extract. All the petriplate were kept in an incubator at 37˚C for 24 hrs (not in an inverted position). After proper time of incubation growth of microbes was checked in all the Petri plates. After incubation for 24 hrs the plates were observed for zone of inhibition, the zone of inhibition was measured with scale and the observation was recorded on table.

Minimum Inhibitory Concentration (MIC)

To perform the MIC experiment we took six test tubes, washed and dried them and poured 3 ml nutrient broth to each test tube and autoclaved them. 1ml plant extract was added to the first test tube, mixed it properly then 1ml mixture of this tube was added to the next (second) test tube. Likewise taken 1ml from second test tube and added it to the third test tube, then repeated the procedure till the sixth test tube. Discarded 1ml from the last test tube then 40 µl bacterial cultures were added to each test tube and incubated for overnight in shaker, then after incubation taken optical density in spectrophotometer at 595nm.

RESULTS AND DISCUSSIONS

Multiple Drug Resistance

Different chemical compounds present in the plant extract are mainly responsible for the antimicrobial activity. These compounds are diffused through the agar medium and depending on their concentration form the zone of inhibition (inhibition ring) and inhibit the growth of microorganism. Zone of inhibition can be known by measuring the diameter of inhibition ring in mm.

MDR with standard drugs

The results of zone of inhibition of sample ethanolic plant extracts Eucalyptus (*Euacalyptus globulus*) and Neem (*Azadirachta Indica)* for four bacterial species *Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphylococcus aureus* and *Escherichia coli* through standard antibiotics (tetracycline and chloramphenicol).(Table-1)

Where S = *Staphylococcus aureus,* B= *Bacillus Amyloliquifaciens,*

P= *Pseudomonas aeruginosa and* E= *Escherichia Coli*

Table-1 Multiple Drug Resistance with standard drugs

Antibiotic Conc(µg)	**25**				**50**				**75**			
	Diameter of Zone of Inhibition in mm											
Antibiotic/Microorganism	S	B	P	E	S	B	P	E	S	B	P	E
Tetracycline	21	11	14	18	26	16	2	24	3	23	22	23
Chloramphenicol	32	-	-	32	34	-	15	36	36	-	25	38

Zone of inhibition

Total two extracts from two different plant species were investigated. Zone of Inhibition was observed in some plant extracts against microbes were tested**.** However; standard antibiotics Tetracycline, ampiciline and chloramphenicol were also used to compare their activity

against microbes with that of plants extract activity. The results of zone of inhibition are shown in table:

Zone of inhibition of ethanolic plant extract *Eucalyptus globulus*

Eucalyptus globulus showed very low resistance towards the bacterial strain *Staphylococcus aureus*, but gave good result against *pseudomonas aeruginosa*, *Bacillus Amyloliquifaciens* (table2,3,4 and 5).

Table 2: Zone of inhibition of ethanolic plant extract of Eucalyptus against *Staphylococcus aureus*

S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Eucalyptus globulus*	25	1.5	*Staphylococcus aureus*
2	*Eucalyptus globules*	50	1.9	*Staphylococcus aureus*
3	*Eucalyptus globulus*	75	1.78	*Staphylococcus aureus*

Table 3: Zone of inhibition of ethanolic plant extract of Eucalyptus against *Bacillus amyloliquifaciens*

S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Eucalyptus globulus*	25	1.6	*Bacillus amyloliquifaciens*
2	*Eucalyptus globules*	50	1.8	*Bacillus amyloliquifaciens*
3	*Eucalyptus globulus*	75	2.0	*Bacillus amyloliquifaciens*

Table 4: Zone of inhibition of ethanolic plant extract of Eucalyptus against *Pseudomonas aeruginosa*

S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Eucalyptus globulus*	25	1.5	*Pseudomonas aeruginosa*
2	*Eucalyptus globules*	50	1.6	*Pseudomonas aeruginosa*
3	*Eucalyptus globulus*	75	2.0	*Pseudomonas aeruginosa*

Table 5: Zone of inhibition of ethanolic plant extract of Eucalyptus against *Escherichia Coli*

S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Eucalyptus globulus*	25	1.5	*Escherichia Coli*
2	*Eucalyptus globules*	50	1.6	*Escherichia Coli*
3	*Eucalyptus globulus*	75	2.0	*Escherichia Coli*

Zone of inhibition of ethanolic plant extract *Azadirachta indica*

Azadirachta indica showed very good results towards the bacterial strain *Staphylococcus aureus and Bacillus amyloliquifaciens* but gave low resistance against *pseudomonas aeruginosa* and *Escherichia Coli* (table 6,7,8and 9).

Table 6: Zone of inhibition of ethanolic plant extract of *Azadirachta indica* against *Staphylococcus aureus*

S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Azadirachta indica*	25	1.4	*Staphylococcus aureus*
2	*Azadirachta indica*	50	1.5	*Staphylococcus aureus*
3	*Azadirachta indica*	75	1.6	*Staphylococcus aureus*

Table 7: Zone of inhibition of ethanolic plant extract of *Azadirachta indica* against *Bacillus Amyloliquifaciens*

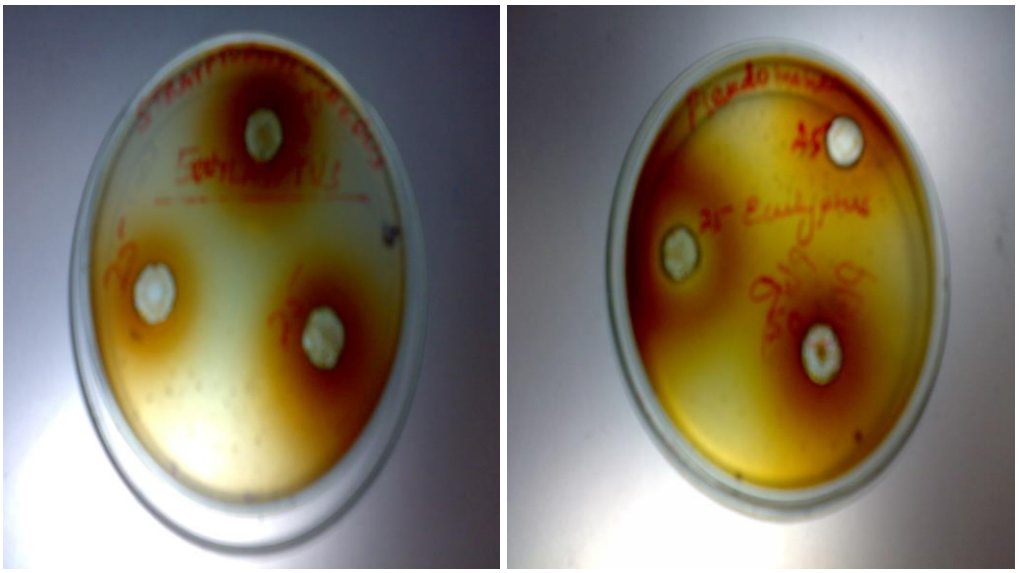
S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Azadirachta indica*	25	1.2	*Bacillus amyloliquifaciens*
2	*Azadirachta indica*	50	1.5	*Bacillus amyloliquifaciens*
3	*Azadirachta indica*	75	1.8	*Bacillus amyloliquifaciens*

Table 8: Zone of inhibition of ethanolic plant extract of *Azadirachta indica* against *Pseudomonas aeruginosa*

S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Azadirachta indica*	25	-	*Pseudomonas aeruginosa*
2	*Azadirachta indica*	50	1.0	*Pseudomonas aeruginosa*
3	*Azadirachta indica*	75	1.3	*Pseudomonas aeruginosa*

Table 9: Zone of inhibition of ethanolic plant extract of *Azadirachta indica* against *Escherichia Coli*

S. NO.	Sample	Concentration	Zone of inhibition	Microorganism
1	*Azadirachta indica*	25	1.0	*Escherichia Coli*
2	*Azadirachta indica*	50	1.4	*Escherichia Coli*
3	*Azadirachta indica*	75	1.5	*Escherichia Coli*



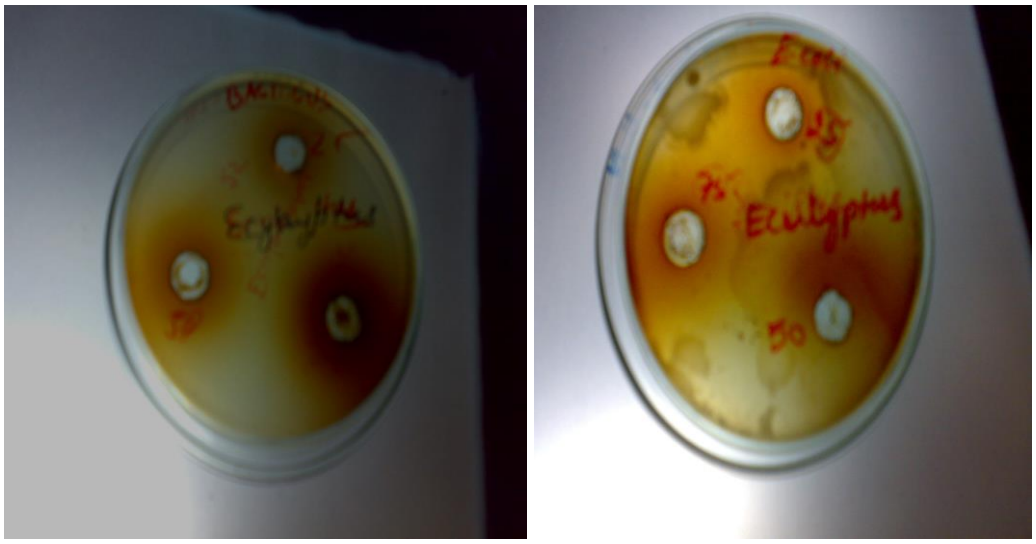


Fig 1: MDR of Eucalyptus against *S. aureus, B. amyloliquifaciens, P. aeruginosa and E. coli,*

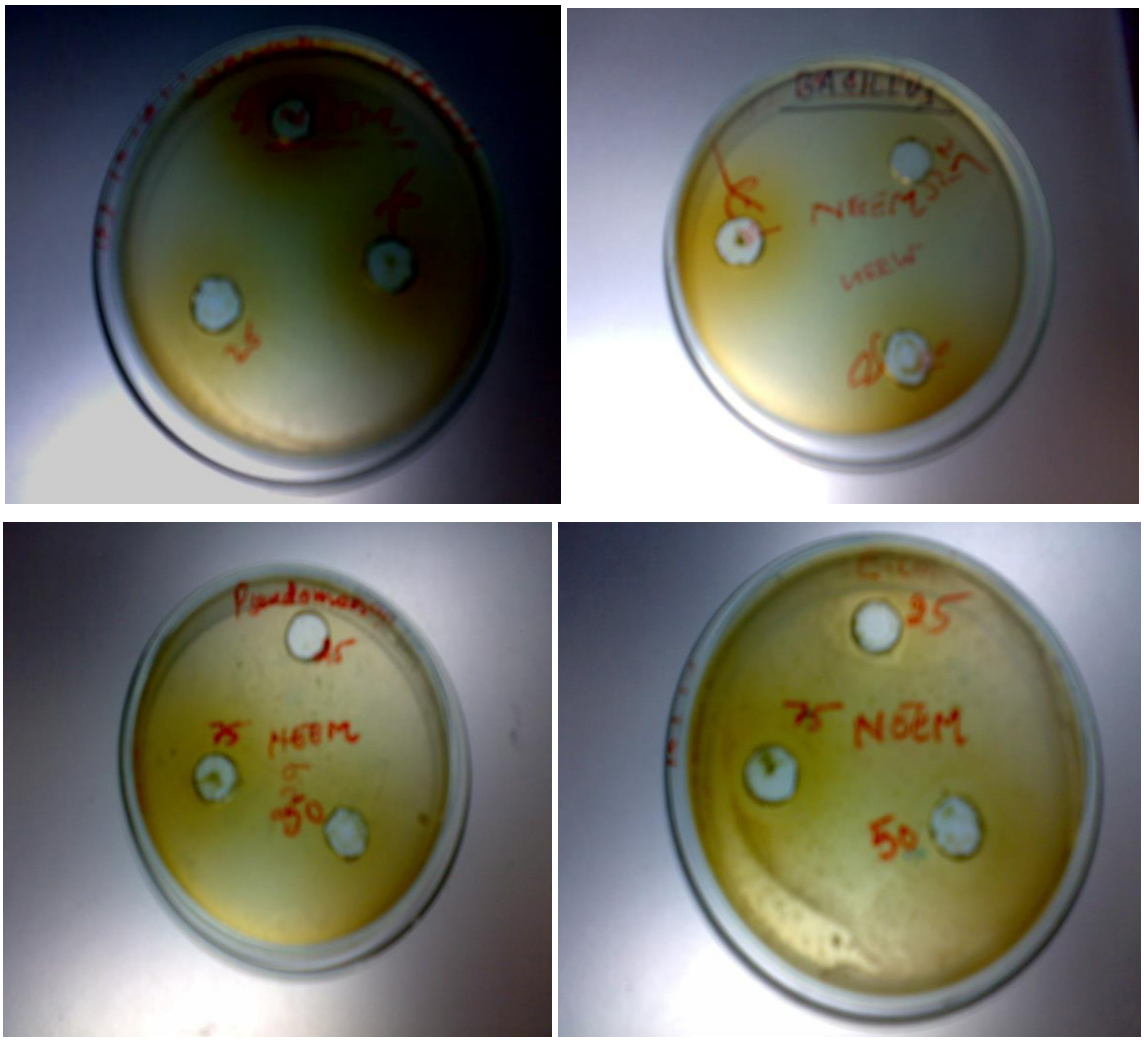


Fig 2: MDR of *Azadirachta Indica* against *S. aureus, B. amyloliquifaciens, P. aeruginosa and E. coli,*

CONCLUSION

This investigation was carried out to find out antibacterial activities from leaves (bark) of two *Eucalyptus globulus* and *Azadirachta indica* was determined against *Staphylococcus aureus* Gram (+) and *Escherichia coli* Gram (-) bacteria, *Pseudomonas aeruginosa and Bacillus Amyloliquifaciens*. From above study of two medicinal plants i.e. *Eucalyptus globulus* and *Azadirachta indica* which are used in traditional medicine we got that they are active against bacterial strains but there were great variation in their antimicrobial activities.

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