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Optimization of antimicrobial activity of medicinal plants (*Coriandrum sativum*, *Ocimum tenuiflorum* and *Phyllanthus emblica*) against MDR pathogens

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Abstract

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa's or viruses etc. main classes of antimicrobials are antibiotics, antiviral and antifungal. In this study the ethanolic and aqueous extract of three medicinal plants i.e. *Coriandrum sativum*, *Ocimum tenuiflorum* and *Phyllanthus emblica* were investigated for their antimicrobial activities against four bacterial strains i.e. *Pseudomonas aeruginosa*, *Bacillus amiloliquifaciens*, *Staphyllococcus aureus* and *Escherichia coli*. Multiple drug resistance and minimum inhibitory concentration were analyzed of these medicinal plants for above pathogens.

Key-Words: MDR, MIC, Antimicrobial activity, Pathogen

Introduction

Medicinal plants are indeed the most important source of life saving drugs for the majority of the world's population. For this study it is important to select suitable biotechnological tools that would be helpful to multiply and conserve the critical genotypes of medicinal plants. 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 (Bajaj and Williams, 1995). 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 (Ved *et al*., 1998). A large number of secondary metabolites such as tannins, alkaloids, phenolics and terpenes responsible for the valuable pharmacokinetic properties of medicinal plants and nucleic acids (Tanaka, 1988).

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The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections (Nagendra *et al*., 2006).

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 (Kritikar and Basu, 2000). 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 (Dutta *et al*., 1998).

Coriander is a valuable herb in treating digestive disorders. One or two teaspoons of coriander juice, added to fresh buttermilk, is highly beneficial in treating indigestion, nausea, dysentery, hepatitis and ulcerative colitis. It is also helpful in typhoid fever (Ebo *et al*., 2006; and Chitra, 1997). Tulsi has got diverse healing properties. It has also been proved to be effective in reducing cholesterol levels. Having anti

bacterial and anti parasitic properties makes it suitable for combating infectious diseases of various types. Recent findings have indicated that the Tulsi may well provide protection from radiation poisoning. It has also been indicated that Tulsi possesses anti cancerous properties. There has come up a belief that a Tulsi leaf swallowed daily will ensure protection from cancer (Sandhu and Heinrich, 2005; Archana, 2009; Hammer, 1999). Amla is one of the richest natural sources of vitamin C, its fresh juice containing nearly twenty times as much vitamin C as orange juice. Clinical tests on patients suffering from pulmonary tuberculosis have shown that this high concentrate is more quickly assimilated then the synthetic vitamin. It is an ingredient of many Ayurvedic medicines and tonics, as it removes excessive salivation, nausea, vomiting, giddiness, spermatorrhoea, internal body heat and menstrual disorders (Jose, 2001; Antoni *et al*., 2006, Hanafy and Hatem, 1991).

Material and Methods

To perform the study of antimicrobial activities of sample medicinal plants i.e. *Coriandrum sativum*, *Ocimum tenuiflorum* and *Phyllanthus emblica*, the plant samples were collected from Neha Nursery, Noida, U.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 mortor pestle and grinded properly then crushed samples were filtered through whattman 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 petriplates 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 Concenteration (MIC)

To perform the MIC experiment we took six test tubes, washed and dried them. 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. 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 Discussion

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 (*Coriandrum sativum*, *Ocimum tenuiflorum* and *Phyllanthus emblica*) 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*

Zone of inhibition of ethanolic plant extract *Coriandrum sativum* (Table-2)

Coriandrum sativum showed very low resistance towards the bacterial strains, but gave good result against *pseudomonas aeruginosa*.

Zone of inhibition of ethanolic plant extract *Ocimum tenuiflorum* (Table-3)

Ocimum tenuiflorum gave good results for *Escherichia coli* and *Staphylococcus aureus.*

Zone of inhibition of ethanolic plant extract *Phyllanthus emblica* (Table-4)

Ethanolic extract of *Phyllanthus emblica* indicated maximum resistance for *Pseudomonas aeruginosa* and *Escherichia coli.*

Minimum Inhibitory Concenteration (MIC)

It is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Agar diffusion techniques are used widely to assay plant extracts for antimicrobial activity. Factors affecting MIC are variation in incubation time, Variation in temperature, Variation in pH of broth etc.

Conclusion

From above study of three medicinal plants i.e. *Coriandrum sativum, Ocimum tenuiflorum and Phyllanthus emblica*, which are used in traditional medicine we got that they are active against bacterial strains but there were great variation in their antimicrobial activities. The results showed that *Ocimum tenuiflorum* and *Phyllanthus emblica* indicated maximum activity against *P. aeruginosa* with 20±0.32 and 25±0.25 mm zone of inhibition respectively and also exhibited potent antimicrobial action against all bacterial isolates tested.

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Table1: 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

Table 2: Zone of inhibition of ethanolic plant extract *Coriandrum sativum*

Microbes/conc.(µg/µl)	25	50	75
E. coli	14±0.01	-	16±0.23
P. aeruginosa	16±0.11	16±0.12	18±0.28
S. aureus	-	12±0.13	14±0.38
B. amyloliquifaciens	-	-	-

Table 3: Zone of inhibition of ethanolic plant extract *Ocimum tenuiflorum*

Microbes/conc(µg/µl)	25	50	75
E. coli	12±0.02	14±0.12	18±0.21
P. aeruginosa	-	18±0.24	20±0.32
S. aureus	14±0.05	12±0.25	16±0.25
B. amyloliquifaciens	-	-	10±0.21

Table 4: Zone of inhibition of ethanolic plant extract *Phyllanthus emblica*

Microbes/conc(µg/µl)	25	50	75
E. coli	15±0.05	16±0.12	19±0.32
P. aeruginosa	25±0.25	21±0.21	18±0.23
S. aureus	-	12±0.47	14±0.21
B. amyloliquifaciens	-	-	10±0.12

Table 4: Observations of minimum inhibitory concentrations for Coriander, Tulsi and Amla (at 595nm)

CORIANDER				
S.No.	***P. aeruginosa***	***S. aureus***	***E. coli***	***B.amyloliquifaciens***
1.	0.00	0.00	0.00	0.00
2.	2.500	2.549	1.639	1.910
3.	1.083	1.245	1.315	1.581
4.	1.205	0.534	1.195	1.824
5.	1.005	0.807	1.363	1.551
6.	0.678	0.731	1.049	1.724
TULSI				
1.	0.00	0.00	0.00	0.00
2.	1.210	1549	0.610	1.085
3.	0.978	0.907	0.423	0.960
4.	0.301	0.722	0.254	0.483
5.	0.432	0.638	0.413	0.784
6.	0.310	0.850	0.196	0.562
AMLA				
1.	0.00	0.00	0.00	0.00
2.	0.170	0.869	1.478	0.947
3.	0.267	0.614	1.414	0.747
4.	0.368	0.216	1.100	0.494
5.	0.012	0.451	1.621	0.664
6.	0.056	0.156	1.534	0.446

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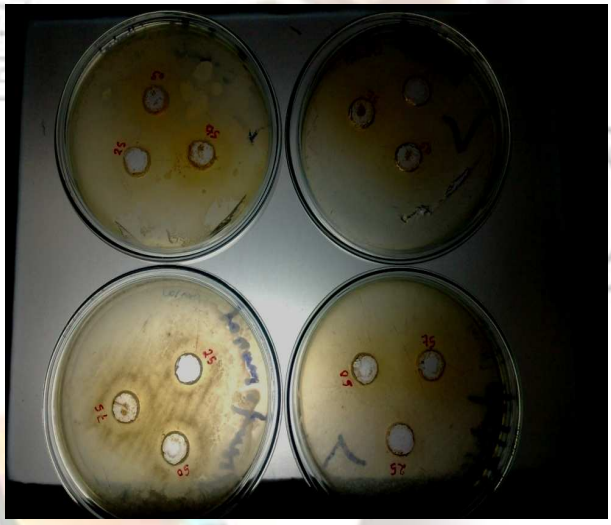

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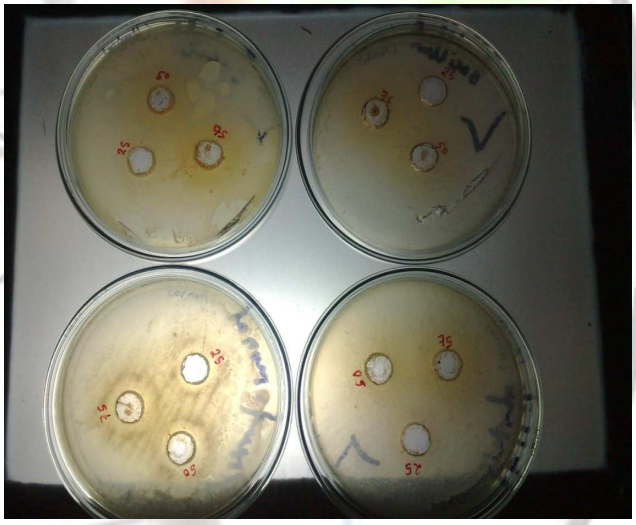

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Fig. 1: MDR of drug against *P. aeruginosa, E. coli, B. amyloliquifacien, S. aureus*
Fig. 2: MDR of amla against *E. coli, P. aeruginosa, S. aureus*
Fig. 3: MDR of coriander against *P. aeruginosa, E. coli, B. amyloliquifacien, S. aureus*
Fig. 4: MDR of tulsi against *E. coli, P. aeruginosa, S. aerues, B. amyloliquifaciens*