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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF CRUDE HERBAL EXTRACTS AGAINST DIFFERENT MICROORGANISMS

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ABSTRACT
Antimicrobial activity of extracts of four plants viz., *Ocimum tenuiflorum, Emblica officinalis, Coriandrum sativum and Azadirachta indica* were evaluated against four microorganisms viz., *Escherichia coli, B. amyloliquifaciens, Staphylococcus aureus and Pseudomonas aeruginosa.* The extract of *Ocimum tenuiflorum* was found to be active in all three concentrations (25, 50 and 75 $\mu g/\mu L$) against *E. coli,* two concentrations (50 and 75 $\mu g/\mu L$) against *P. aeruginosa* and *S. aurius. Emblica officinalis* was effective against almost all three concentrations of all microorganisms except 25 $\mu g/\mu L$ of *S. aurius. Coriandrum sativum* showed the maximum zone of inhibition against *S. aurius* (26 mm) and. *P. aeruginosa* (16 mm). *Azadirachta indica* showed best effect against *P. aeruginosa* (MZI-16mm), and to some extent against *E. coli* and *S. aurius*.

INTRODUCTION

Medicinal plants have a great economic importance throughout the world. They are the major source of novel drugs with antimicrobial activity. Plants based traditional medicines have made considerable contributions to human health and plants provide a natural blueprint for the development of new drugs (Cragg *et al*., 1997; Iwu *et al.,* 1999). They are effective in the affordable treatment of infectious diseases and mitigate many of the side effects associated with synthetic antimicrobials (Murray, 1995; Iwu *et al.,* 1999).

The *Emblica officinalis* is highly valued in traditional Indian medicine (Scartezzini *et al*., 2006). Earlier studies have demonstrated the potent antimicrobial properties of *E. officinalis* (Ahmed *et al*., 1998) and it is used as antiviral for cold and flu. In the respiratory infections, it has an antibiotic activity against a wide range of bacteria, used traditionally in the treatment of lungs (Chopra and Simon, 2000). It also has shown antifungal activity *in vitro* (Dutta *et al*., 1998).

Coriander has traditionally been referred to as antidiabetic (Gray and Flatt, 1999), anti-inflammatory and cholesterol lowering (Chithra and Leelamma, 1997). Flavonoides of coriander include quercitin, kaempferol, rhamnetin and epigenin and it also contains active phenolic acid compounds including caffeic and chlorogenic acid. It has been reported that the volatile oils found in the leaves of *C. sativum* plant may have antimicrobial properties against food borne pathogens such as *Salmonella* species (Isao *et al.,* 2004).

Tulsi leaves also possess anti-fungal and anti-viral activity (Rajeshwari, 1992). Oil extracted from leaves of this plant was found to have significant insecticidal properties (Nanasombat and Lohasupthawee, 2005). The extensive studies have been made for therapeutic potentials in various areas like immuno-stimulation, anticancer antioxidant, as adjuvant to radiotherapy, antiulcer, analgesic and antidiabetic (Hammer *et al*., 1999).

Azadirachta indica has wide range of medicinal properties. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties.

The use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times in India (Bhattacharjee, 1998). Natural products are known to play an important role in both drug discovery and chemical biology. From the above facts, it is obvious that the search for new antimicrobials is the need of the hour.

The present investigation was carried out to evaluate the antimicrobial activity of crude extracts prepared from the leaves of selected medicinal plants against selected microorganisms.

MATERIALS AND METHODS

Collection of Medicinal plant samples

Fresh plant or plant parts were collected randomly from the region of Nursery. Fresh plant materials were washed under running tap water, air dried and then homogenized with ethanol to fine paste. Then, filtered with Whatman filter paper

and allowed to dry in hot air oven.

Preparation of cultures

A100μL of each *Pseudomonas auroginosa, Bacillus amyloliquifaciens, Staphylococcus aureus* and *Escherichia coli* were inoculated in the four flasks having 30 mL each Nutrient broth and kept into shaker overnight for growth.

Plant extract preparation

All the sample 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 4.00g and then crushed in 70% ethanol in the ratio of 1:8 in the mortar pestle and grinded properly. Crushed samples were filtered through Whatman filter paper in a flask/ beaker. Filtrates were placed in a hot air oven at 40ºC in a flask/beaker till it completely dried for 2-4 days.Dried filtrate was dissolved in 5ml of 1X Tris saline buffer and stored in refrigerator.

Preparation of agar plates

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

In order to check the antimicrobial activity against selected microbes (*Bacillus amyloliquifaciens, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*), three wells were formed in each of the culture plates by 1000 mL tip and were filled with 25, 50, and 75μL of each plant extract.

Multiple drug resistance with standard drugs

For standard reference values, the tetracycline and chloramphenicol drugs were taken. Different concentrations (25, 50 and 75μg) of these drugs were poured into the wells of *Bacillus, Staphylococcus, E. coli and Pseudomonas* plates respectively. All the Petri plates were kept in an incubator at 37ºC for 24h. After proper time of incubation, growth of microbes was checked in all the Petri plates.

Minimum inhibitory concentration

All the plant extracts were subjected to individual microbiological tests to ascertain their antimicrobial activity against four species of microorganisms: *Escherichia coli, B. amyloliquifaciens, Staphylococcus aureus and Pseudomonas aeruginosa*. The antimicrobial activity of the extracts was determined by measuring the diameter of zone of inhibition (ZI) exhibited by the extracts.

RESULTS AND DISCUSSION

Multiple drug resistance

The extracts from the four different plant species were investigated. Zone of Inhibition was observed in plant extracts against microbes. However, standard antibiotics Tetracycline and Chloramphenicol were also used to compare their activity against microbes with that of plants extract activity. The plant extracts and standard antibiotics showed different antimicrobial activity against the all four microorganisms (Table 1).

The extract of *Ocimum tenuiflorum* was found to be active in all three concentrations (25, 50 and 75 μg/μL) against *E. coli,* two concentrations (50 and 75 μg/μL) against *P. aeruginosa* and *S. aurius* however it was not found effective against *B. amyloliquifaciens* in any concentration. The similar results were also obtained where essential oils extracted from the leaves of *Ocimum sanctum L.* found to inhibit *in-vitro* growth of *E. coli,* and *P. aeruginosa* showing its antibacterial activity (Rajeshwari, 1992).

Emblica officinalis was active against almost all three concentrations of all microorganisms except 25 μg/μL of *S. aurius*. The result of the present study supports the report of Khanna and Nag (1973) that constituents of *E. officinalis* have been found to be active against a range of bacteria including *Staphylococcus aureus* and *Escherichia coli*. Saeed and Tariq (2007) found that aqueous infusion and decoction of *Emblica officinalis* exhibited potent antimicrobial activity against *Staphylococcus aureus* however the aqueous infusion and decoction of coriander did not show any antimicrobial activity against G -ve urinary pathogens as well as against *Candida albicans*.

Coriandrum sativum found to be effective against *P. aeruginosa* and *S. aureus but not against E. coli and B. amyloliquifaciens*. Chaudhary and Tariq (2006) found that the aqueous decoction of coriander did not possess any antibacterial potential. In contrary, some workers have found that coriander has strong antibacterial activity against *S. aureus* and *E. coli* (Al-Jedah *et al*., 2000).

Azadirachta indica showed best effect against *P. aeruginosa,* to some extent against *E. coli* and *S. aurius* but no effect against *B. amyloliquifaciens*. All the microorganisms were susceptible

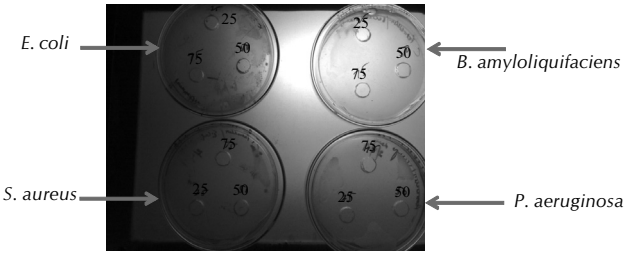

Figure 1: Multiple drug resistance of Tetracycline against *E. coli, B. amyloliquifaciens, S. aureus* and *P. aeruginosa*

Table 1: Antimicrobial activity of plants extract and comparison with standard antibiotics

Microorganisms	Minimum inhibition zone (mm)																	
	Ocimum tenuiflorum			*Emblica officinalis*			*Coriandrum sativum*			*Azadirachta indica*			Standard antibiotics Tetracycline			Chloramphenicol		
Microbe conc.(μg/μl)	25	50	75	25	50	75	25	50	75	25	50	75	25	50	75	25	50	75
E. coli	12	14	18	12	16	19	-	-	-	14	-	16	18	24	23	32	36	38
P. aeruginosa	-	18	20	12	15	17	12	14	16	16	16	18	14	20	23	-	15	25
S. aurius	-	12	16	-	13	12	26	20	16	-	12	14	21	26	30	32	34	36
B. amyloliquifaciens	-	-	-	13	16	20	-	-	-	-	-	-	11	16	23	-	-	-

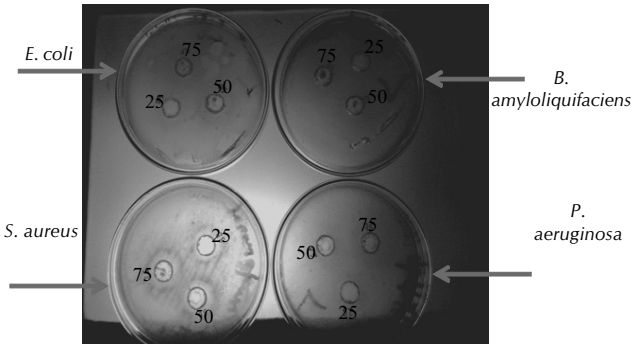

Figure 2: Multiple drug resistance of *Ocimum tenuiflorum* against *E. coli*, *B. amyloliquifaciens*, *S. aureus* and *P. aeruginosa*

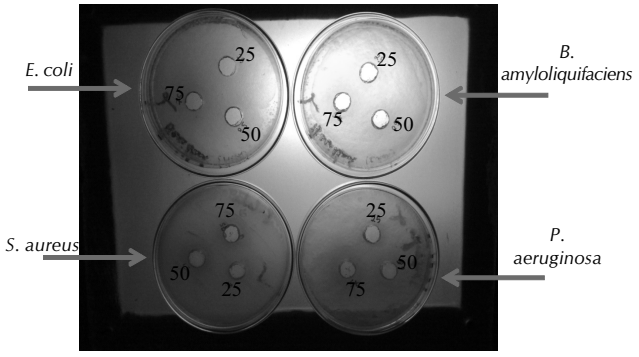

Figure 3: Multiple drug resistance of *E. officinalis* against *E. coli*, *B. amyloliquifaciens*, *S. aureus* and *P. aeruginosa*

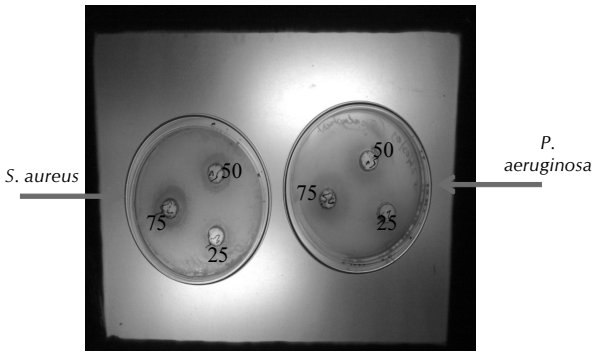

Figure 4: Multiple drug resistance of *Coriandrum sativum* against *P. aeruginosa, S. aureus*

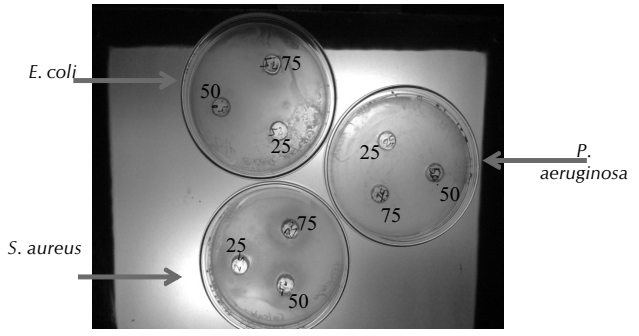

Figure 5: Multiple drug resistance of *Azadirachta indica* against *P. aeruginosa, S. aureus* and *E. coli*

to *Tetracycline*. *B. amyloliquifaciens* found to be resistant to Chloramphenicol however *E. coli* and *S. aurius* were very susceptible *and P. aeruginosa* was least susceptible. According to Rastogi and Mehrotra (2002), the plant extracts and their components show hydrophobicity, which help the lipids of the bacterial cell membrane and mitochondria to get partitioned, making them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death. This may be the possible reason why plant extracts in the present work have shown antimicrobial activities. Bhuiyan *et al*. (1997) reported that the aqueous extract (5%w/v) showed no detectable antimicrobial activities on agar, the acetonic axtract (5%w/v) at the same concentration, however, produced appreciable antimicrobial effects. The acetonic extract from the bark of Neem was bactericidal at concentrations ≤ 1% (w/v). The oils of *Azadirachta indica,* exhibited moderate activity and that of *Ocimum sanctum,* demonstrated comparatively low activity against *A. niger* and *A. fumigatus* as compared to control.. These results support the plant oils can be used to cure mycotic infections and plant oils may have role as pharmaceutical and preservatives (Bansod and Rai, 2008).

Based on the results, it is concluded that plant extracts have great potential as antimicrobial compound against microorganisms and they can be used in treatment of infectious diseases caused by resistant microorganisms. *E. officinalis* showed stronger activity than the other plants against all the tested bacterial strains. Therefore, *E. officinalis* can be selected for further analysis. It can be used to discover bioactive natural products that may serve as lead in the development of new pharmaceuticals that address unmet therapeutic needs. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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