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Antimicrobial activity of *Hygrophila auriculata* (Schumach.) Heine and *Pergularia daemia* Linn.

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The antimicrobial efficiency and minimum inhibitory concentration of the extracts of *Hygrophila auriculata* (Schumach.) Heine (Acanthaceae) and *Pergularia daemia* Linn. (Apocyanaceae) were evaluated against nine bacterial species like (*Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhi*, *Proteus vulgaris* and *Shigella flexneri*) and two fungal species (*Aspergillus niger* and *Candida albicans*). The susceptibility of the microorganism to the extracts of these plants were compared with each other and with selected antibiotics. All these plants were effective against three or more of the pathogenic microorganisms. This *in vitro* study corroborated the antimicrobial activity of the selected plants used in folklore medicine.

Key words: Activity index (AI), total activity (TA), disc diffusion methods, microbial pathogens, folklore medicine.

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day (Yadav and Khan, 2012). Several synthetic antibiotics and drugs are employed in the treatment of the microbial infections and communicable diseases; but, the microbial pathogens develop resistance to the synthetic antibiotics. The increasing incidence of resistance to antibiotics and their side effects on the functioning of different parts of the body organ systems necessitate to finding out substitutes for the antibiotics (Sasikumar et al., 2007). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are often found with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs (Babu and Subhasree, 2009).

Natural products are important sources for biologically active drugs. There has been an increasing interest in the study of medicinal plants as natural products in different parts of the world. Medical plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases (Panchavarnakili et al., 2012). Many medicines like strychnine, aspirin, vincristine and taxol are of plant origin. According to World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. In developing countries, people of low income group such as farmers, inhabitants of hamlets and native communities use folk medicine for the treatment of common infectious diseases (Ratha et al., 2012). Among the estimated 2,50,000 to 5,00,000 plant species, only a small percentage has been investigated phytochemically

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and the fraction submitted to biological or pharmacological screening is even smaller (Mahesh and Satish, 2008). Rural communities in particular tribes of Trichy District, Tamilnadu, depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade.

Hygrophila auriculata, a perennial *angiosperm* of Acanthaceae, widely distributed semi-aquatic herb in India, is being used as vegetable in some states like Odisha, Chhattisgarh and West Bengal. The pre-flowering or flowering succulent aerial parts are boiled and consumed by the rural people of these states to increase the haemoglobin level. This herbal remedy does not have any side effects with proven effectiveness. This plant contains various groups of phyto-constituents viz. phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, glycosides, etc. and is useful in the treatment of anasaraca, diseases of urinogenital tract, dropsy of chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhoea, gonorrhea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagea, etc. (Rastogi and Mehrotra 1993; Annonymous, 2002; Sharma et al., 2002; Asolkar et al., 2005; Nadkarni, 2007).

Pergularia daemia (Forsk.) Chiov (Apocyanaceae), commonly known as utaran (Hindi), Dustapuchettu (Telugu), Uttamarani (Sanskrit) is a slender, hispid, fetid smelling laticiferous twiner found in the plains throughout the hot parts of India. *P. daemia* is said to have more magical application than medical application as it posses diverse healing potential for a wide range of illnesses. Some of the Folklore people use this plant to treat jaundice, as laxative, anti-pyretic, expectorants and also in infantile diarrhea. The leaf latex is locally used as pain killer killer and for relief from toothache (Hebbar et al., 2010), the sap expressed from the leaves are held to cure sore eyes in Ghana. The plant reduces the incidence of convulsion and asthma. It is used to regulate the menstrual cycle and intestinal functions. The root is useful in treating leprosy, mental disorders, anemia and piles (Omale et al., 2011). We report here the results of the antimicrobial properties of extracts from the leaves of *H. auriculata A.longifolia* and *P. daemia*.

MATERIALS AND METHODS

Plant materials

Fresh plant leaves were collected randomly from the gardens and villages of Trichy district, Tamilnadu from the natural stands. The botanical identity of these plants was confirmed by Dr.V.Sampath Kumar, Scientist – C, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu. The voucher specimens are deposited at the Department of Botany, National College (Autonomous), Tiruchirapalli-620 001, Tamilnadu, India.

Preparation of extracts

Aqueous extraction

Hundred grams of dried powder were extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through eight layers of muslin cloth and centrifuged at 5000 rpm 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to one-fifth of the original volume.

Solvent extraction

Hundred grams of dried plant powdered samples were extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4°C in airtight bottles for further studies.

Antimicrobial activity

Microorganisms

Microorganisms were obtained from the Microbial Type Culture Collection centre (MTCC), Chandigarh, India. Amongst eleven microorganisms investigated, nine were bacterial strains viz.,*Staphylococcus aureus* MTCC 3160, *Bacillus cereus* MTCC 442, *Streptococcus pneumonia* MTCC 655, *Escherichia coli* MTCC 598, *Pseudomonas aeruginosa* MTCC 42642 *Klebsiella pneumoniae* MTCC 7407, *Salmonella typhi* MTCC 3917, *Proteus vulgaris* MTCC 742 and *Shigella flexneri* MTCC 1457, while the other two were fungal strains viz. *Aspergillus nige*r MTCC 2546, *Candida albicans* MTCC 183. All the microorganisms were maintained at 4°C on nutrient and potato dextrose agar slants.

Disc diffusion method

Antimicrobial activity was carried out by the disc diffusion method. The antimicrobial assays of aqueous and methanolic extracts were performed by Bauer et al. (1966). Each plant extract was tested at two different concentrations (100 and 200 µg/ml) to see their inhibitory effects against microbial pathogens. Sterile paper discs (6 mm in diameter) prepared from Whatman No. 1 filter paper was impregnated with drug, containing solution placed on the inoculated agar. The inoculated plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone for the test microorganisms.

The potato dextrose agar plates were inoculated each with fungal culture by point (10 days old cultures) inoculation. The filter paper discs loaded with 100 and 200 µg/ml concentrations of the extracts were placed on test organism- seeded plates. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm (Taylor et al., 1995). Chloramphenicol and Fluconazole are used as standard antibiotics.

Minimum inhibitory concentration (MIC)

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes for each bacterium. Different concentrations of plant extracts ranging from 0.125 to 8 $\mu g/ml^{-1}$ concentration were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculums of respective bacteria (10^5 CFU ml^{-1}) and kept at 37°C for 24 h. The test tube containing the lowest concentration

Table 1. Effect of methanol and aqueous extracts of *H. auriculata.*

S/N	Name of the strain	Zone of Inhibition (mm)				
		Methanol (µg/ml)		Aqueous (µg/ml)		Synthetic drug (Chloramphenicol)
		100	200	100	200	
1	*Staphylococcus aureus*	11	18	8	10	22
2	*Streptococcus pneumoniae*	10	12	-	-	20
3	*Bacillus cereus*	9	11	-	9	17
4	*Escherichia coli*	10	12	-	8	21
5	*Pseudomonas aeruginosa*	8	12	-	8	18
6	*Klbseillae pneumoniea*	-	10	-	-	17
7	*Salmonella typhi*	-	9	-	-	16
8	*Proteus vulgaris*	8	10	-	-	20
9	*Shigella flexneri*	-	10	-	9	16
Antifungal activity						**Synthetic drug (Fluconazole)**
10	*Candida albicans*	-	9	-	-	15
11	*Aspergillus niger*	-	9	-	-	17

Table 2. The MIC index of methanol and aqueous extracts of *H. auriculata.*

S/N	Name of the strain	Methanol			Aqueous		
		MIC (µg/ml)	MBC (µg/ml)	MIC_{index}	MIC (µg/ml)	MBC (µg/ml)	MIC_{index}
1	*S. aureus*	0.125	0.250	2	4	4	1
2	*S. pneumoniae*	0.250	0.500	2	-	-	-
3	*B. cereus*	0.500	0.500	1	4	4	1
4	*E. coli*	0.500	0.500	1	-	-	-
5	*P. aeruginosa*	2	2	1	-	-	-
6	*K. pneumoniae*	4	4	1	-	-	-
7	*S. typhi*	2	2	1	-	-	-
8	*P. vulgaris*	0.500	1	2	4	4	1
9	*S. flexneri*	0.500	0.500	1	-	-	-
10	*C. albicans*	2	2	1	-	-	-
11	*A. niger*	2	2	1	2	2	1

of extract which showed reduction in turbidity when compared with control was regarded as MIC of that extract (Muhamed et al., 2011).

Total activity (TA) determination

Total activity is the volume at which test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g (Sharma and Kumar, 2009).

AI = Activity Index (IZ developed by extract/IZ developed by standard).

RESULTS AND DISCUSSION

The results reveal variability in inhibitory nature of each extract against specific bacteria. The inhibition of bacterial growth was dose dependent since the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains as evidenced by the higher zone of inhibitions at higher concentrations of each extract. Antimicrobial activity (assessed in terms of inhibition zone, total activity and activity index) of the crude extracts, tested against selected microorganisms are recorded.

Both crude methanol and aqueous extracts of *A. longifolia* exhibited varying degrees of antimicrobial activities against the test organisms. The 200 µg/ml crude methanol extract showed higher inhibition zone than crude aqueous extract against *S. aureus*, *S. pneumoniae*, *E. coli* and *P. aeruginosa*, respectively (Tables 1 and 3). Similarly 200 µg/ml methanol extract of *P. daemia* exhibited inhibition zone of 15 mm (AI = 0.818) for

Table 3. Antimicrobial activity index of crude extracts of *H. auriculata.*

S/N	Name of the strain	Methanol			Aqueous		
		Activity index		Total activity (ml/g)	Activity index		Total activity (ml/g)
		100	200		100	200	
1	*S. aureus*	0.5	0.818	4	0.363	0.454	0.175
2	*S. pneumoniae*	0.5	0.6	2	-	-	-
3	*B. cereus*	0.529	0.647	1	-	0.529	0.175
4	*E. coli*	0.476	0.571	1	-	0.380	-
5	*P. aeruginosa*	0.444	0.666	0.25	-	0.444	-
6	*K. pneumoniae*	-	0.588	0.125	-	-	-
7	*S. typhi*	-	0.562	0.25	-	-	-
8	*P. vulgaris*	0.4	0.5	1	-	-	-
9	*S. flexneri*	-	0.625	1	-	0.562	0.175
10	*C. albicans*	-	0.6	0.25	-	-	-
11	*A. niger*	-	0.529	0.25	-	-	-

Table 4. Effect of methanol and aqueous extracts of *P. daemia* on microbes.

S/N	Name of the Strains	Zone of Inhibition (mm)				
		Methanol (µg/ml)		Aqueous (µg/ml)		Synthetic drug (Chloramphenicol)
		100	200	100	200	
1	*Staphylococcus aureus*	10	15	10	12	22
2	*Streptococcus pneumoniae*	10	11	-	9	20
3	*Bacillus cereus*	9	10	-	10	17
4	*Escherichia coli*	10	12	-	-	21
5	*Pseudomonas aeruginosa*	8	10	-	8	18
6	*Klbseillae pneumoniae*	-	8	-	-	17
7	*Salmonella typhi*	-	10	-	8	16
8	*Proteus vulgaris*	-	9	-	-	20
9	*Sheigella flexneri*	8	10	-	-	16
Antifungal activity						**Synthetic drug (Fluconazole)**
10	*Candida albicans*	-	-	-	-	15
11	*Aspergillus niger*	-	-	-	-	17

S. aureus and 12 mm (AI = 0.571) for *E. coli* respectively. The aqueous extract showed highest inhibition zone of 12 mm in (AI = 0.454) for *S. aureus* and 10 mm (AI = 0.529) for *B. cereus* (Tables 4 and 6).

Antibiotics chloramphenicol and fluconazole have shown moderate inhibition zone diameter than that of plant extracts. It had the inhibition zone in the range of 15 to 22 mm. The zones of inhibition produced by the tested extracts against *Aspergillus niger* and *Candida albicans* ranged between 8 to 9 mm. The highest zone of inhibition was produced by methanol extracts of *H. auriculata* while that of *P. daemia* did not inhibit the growth (Tables 1 and 4).

Methanol extract of *P. daemia* showed least MIC value that is, 0.500 µg/ml (MBC = 0.250 µg/ml) against *S. aureus* while aqueous extract had moderate activity at 0.500 µg/ml (MBC = 1.0 µg/ml) concentration (Table 5). Similarly the *H. auriculata* methanol extract was found to be highly effective as it has shown very low MIC value (0.125 µg/ml) against *S. aureus* (Table 2). The total activity was highest for methanol extracts of both plants (4.0 and 1.1 ml/g) against *S. aureus* (Tables 3 and 6). Our results support this view as methanol extracts had comparatively more inhibition action than aqueous extracts (Hugo et al., 2005).

Several reports have shown the antimicrobial properties of plant extracts under laboratory conditions (Doss et al., 2009a; Doss et al., 2009b; Venkataswamy et al., 2010; Anand et al., 2001). Normally Gram-positive bacterial strains are found to be more susceptible to the extracts than Gram negative bacteria. This is attributed to the fact that these two groups differ by their cell wall

Table 5. The MIC index of methanol and aqueous extracts of *P. daemia.*

S/N	Name of the strain	Methanol			Aqueous		
		MIC (µg/ml)	MBC (µg/ml)	MIC_{index}	MIC (µg/ml)	MBC (µg/ml)	MIC_{index}
1	*S. aureus*	0.500	0.250	0.500	0.500	1.0	2
2	*St. pneumoniae*	1.0	2.0	1	2.0	2.0	1
3	*B. cereus*	0.500	1.0	1	1.0	1.0	1
4	*E. coli*	2.0	2.0	1	-	-	-
5	*P. aeruginosa*	-	-	-	-	-	-
6	*K. pneumoniae*	-	-	-	-	-	-
7	*S .typhi*	-	-	-	-	-	-
8	*P. vulgaris*	-	-	-	-	-	-
9	*S. flexneri*	4.0	2.0	0.5	-	-	-
10	*C. albicans*	-	-	-	-	-	-
11	*A. niger*	-	-	-	-	-	-

Table 6. Antimicrobial activity index of crude extracts of *P. daemia.*

S/N	Name of the Strains	Methanol			Aqueous		
		Activity index		Total activity (ml/g)	Activity index		Total activity (ml/g)
		100	200		100	200	
1	*S. aureus*	0.454	0.681	1.1	0.454	0.545	1
2	*St. pneumoniae*	0.5	0.55	0.55	-	0.45	0.25
3	*B. cereus*	0.529	0.588	1.1	-	0.588	0.5
4	*E. coli*	0.476	0.571	0.275	-	-	-
5	*P. aeruginosa*	0.444	0.555	-	-	0.444	-
6	*K. pneumoniae*	-	0.470	-	-	-	-
7	*S. typhi*	-	0.625	-	-	0.5	-
8	*P. vulgaris*	-	0.45	-	-	-	-
9	*S. flexneri*	0.5	0.625	0.137	-	-	-
10	*C. albicans*	-	-	-	-	-	-
11	*A. niger*	-	-	-	-	-	

components and their thickness (Doss et al., 2009a). In conclusion, the methanol extracts of both plants possess broad spectrum of antibacterial activity against the test bacteria species. The results obtained from this work gives high hope for the development of new antibacterial agents.

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