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Antimicrobial Activity of five medicinal plants of Bangladesh

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The antimicrobial activity of five medicinal plants was evaluated by disc diffusion method. Among those, some fraction of plant extracts (400 µg /disc) exhibited potent antimicrobial activity against five gram positive and seven gram negative bacteria and three fungi. Among the test samples of *O. mungos,* the carbon tetrachloride soluble fraction exhibited 12.0 mm and 13.0 mm zone of inhibition against *B. megaterium* and *Aspergillus niger* respectively. The crude methanol extract of *S. nodiflora* exhibited 14.0 mm zone of inhibition against *Bacillus cereus* whereas the carbon tetrachloride soluble fraction revealed 16.0 mm against *Shigella boydii.* The chloroform soluble fraction of *P. sagitatta* exhibited 16.0 mm zone of inhibition against *Staphylococcus aureus* and *Shigella boydii.* Among the test samples of *M. macrophylla*, the carbon tetrachloride soluble fraction showed 15.0 mm zone of inhibition against *Salmonella paratyphi* and 13.0 mm zone of inhibition against *Aspergillus niger.* The crude methanol extract of *G. philippensis* exhibited 18.0 mm zone of inhibition against *Bacillus cereus*.

Keyword: *Ophiorrhiza mungos* L, *Synedrella nodiflora* L, *Parabaena sagittata* Miers, *Mussaenda macrophylla* Wall, *Gmelina philippensis* Cham., disc diffusion method.

1. Introduction

In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of modern antibiotics[1-7]. Therefore, the demand for new and effective antimicrobial agents with broad-spectrum activities from natural sources is increasing day by day. In order to identify plant species having potential antimicrobial principles, the pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions *of Ophiorrhiza mungos* L., *Synedrella nodiflora* L., *Parabaena sagittata* Miers., *Mussaenda macrophylla* Wall. and *Gmelina philippensis* Cham. were screened by disc diffusion method[8].

Ophiorrhiza mungos L. (Bengali name: Ronjonkali) belonging to the family Rubiaceae is a flowering plant, adapted to many environments. *O. mungos* is an annual herb attaining a height of 30 cm and is distributed all over Bangladesh. Traditionally, this plant is used in wound healing[9] and snake bites[10].

Synedrella nodiflora L. (Synonym: *Verbesina nodiflora*, Local name: Sinderella weed, Pig grass, Node weed) is a flowering herb which belongs to the family Asteraceae. *S. nodiflora* grows well in different environments and mainly found in Bangladesh, India, Japan, Span, China and England[11]. The whole plant is diuretic and laxative[12]. The anti-inflammatory[13],

insecticidal[14] and analgesic[15] activities of the plant have also been reported.

Parabaena sagittata Miers. Belonging to the family Menispermaceae is a lofty climber, indigenous to Chittagong, Bangladesh[16]. It is also found in northern part of Thailand and used by the hill tribes of this region for medical purposes. A decoction of stems and leaves affords a treatment for jaundice, indigestion and painful intestinal disturbances. All parts of the plant may be used as febrifuge and tonic[17]. The leaf paste of P.sagptatta is boiled in coconut oil and is aplied on incision[18].

Mussaenda macrophylla Wall. (Local name: Magballi, Dhobi tree, Family: Rubiaceae) is a flowering shrub which is distributed in central and eastern Nepal to about 1800 m in moist places in association with herbs and other shrubs. It is also found in northern India, southern China and Myanmar.[19] Traditionally the bark of this plant is used in snake bite[20]. Previous studies with *M. macrophylla* revealed antibacterial, anticoagulant, anti-inflammatory and hepatoprotective activities[21]. The plant is also active against oral pathogens[22].

Gmelina philippensis Cham. (Synonym**:** *Gmelina hystrix*, Bengali name: Badhara, Korobi, Family: Verbenaceae) is a small tree with pendant branches. It is native to Philippine islands, India and south-east Asia and also distributed in United States, Australia, Vietnam, Thailand, Malaysia, Indonesia, Myanmar and Bangladesh[23]. In the Philippines, the fruit-juice is applied to eczema of the feet while root-juice is used as a purgative and in treating fatigue in Indo-China. In Peninsular Malaysia, the fruit pounded with lime is applied as a poultice to the throat as a remedy for coughs. The extract of the roots is used internally as a stimulant, resolvent and in treating diseases of the joints and nerves. Likewise, an extract of the leaves is employed externally[24].

As a part of our continuing investigation of medicinal plants of Bangladesh[25,26], the crude methanol extracts of *O. mungos, S. nodifloara*, *P.* sagittata, *M. macrophylla* and *G. philippensis* and their aqueous and organic soluble fractions were screened for antimicrobial activity for the first time in Bangladesh.

2. Materials and Methods

2.1 Plant Materials

The whole plant of *O. mungos* and *S. nodiflora,* leaves of *P. sagittata, M. macrophylla* and *G. philippensis* were collected from Dhaka and voucher specimen (DACB 35632, 35644,35546, 35633 and 35547 respectively) for each of the plant sample has been deposited in Bangladesh National Herbarium for future reference.

2.2 Extraction

The collected pant parts were cleaned, sun dried for several days and then oven dried for 24 hours at 40°C to facilitate grinding. The powdered materials (500 gm each) were separately macerated in 2.5 liters of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extracts were concentrated with a rotary evaporator at low temperature (40-45°C) and reduced pressure. The concentrated methanol extracts were partitioned by the modified Kupchan partition protocol[27] and the resultant partitionates were evaporated with rotary evaporator to yield pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions (Table 1). The residues were then stored in a refrigerator until further use.

Table 1: Kupchan partitionates of *O. mungos, S. nodiflora.M. macrophylla, G. philippensis* and *P. sagittata* obtained from 5 gm of crude extract.

Crude/ extract/ Fraction	*O. mungos* (gm)	*S. nodiflora* (gm)	*P. sagittata* (gm)	*M. macrophylla* (gm)	*G. phillippensis* (gm)
Me	5.0	5.0	5.0	5.0	5.0
PESF	2.0	1.0	1.75	1.75	1.5
CTCSF	1.0	1.0	1.2	1.2	1.0
CSF	0.5	0.5	1.0	1.0	0.5
AQSF	0.3	0.5	0.25	0.25	0.5

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF= Aqueous soluble fraction

2.3 Antimicrobial screening

Antimicrobial activity of the crude extract, its fractions and the compounds were determined against five gram positive bacteria (Table 2) and seven gram negative bacteria (Table 3) and three fungi (Table: 4) by the disc diffusion method.[8]

Measured amount of the test samples were dissolved in definite volume of solvent (chloroform or methanol) and applied to sterile discs and carefully dried to evaporate the residual solvent. The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. In this investigation, ciprofloxacin (30 µg/disc) disc was used as the reference.

2.4 Statistical analysis:

Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

3. Results and Discussion

The crude methanol extracts of *O. mungos, S. nodiflora P. sagitatta, M. macrophylla,* and *G. philippensis* and their pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble extractives were screened for antimicrobial activity. The results of the antimicrobial screening are presented in Table 2, 3 and 4.

The test samples of *O. mungos* exhibited zone of inhibition ranging from 5.0 to 13.0 mm against the test organisms. The highest 12.0 mm zone of inhibition was exhibited against *B. megaterium* by carbon tetrachloride soluble fraction. This fraction also showed 13.0 mm zone of inhibition against *Aspergillus niger.*

Among the test samples of *S. nodiflora,* the crude methanol extract exhibited 14.0 mm zone of inhibition against *Bacillus cereus*. The *S. nodiflora* extractives exhibited zone of inhibition ranging from 6.0 to 16.0 mm against gram negative bacteria. The carbon tetrachloride soluble fraction revealed 16.0 mm against *Shigella boydii*. This fraction also showed 13.0 mm zone of inhibition against *Candida albicans.*

The test samples of *P. sagitatta* exhibited zone of inhibition ranging from 6.0 to 16.0 mm against gram positive bacteria. The highest (16.0 mm) zone of inhibition was demonstrated by the chloroform soluble fraction against *Staphylococcus aureus.* This fraction also showed 16.0 mm zone of inhibition against gram negative bacteria *Shigella boydii.* The carbon tetrachloride soluble extractives of *P. sagitatta* revealed 13.0 mm zone of inhibition against *Candida albicans.*

The test samples of *M. macrophylla* exhibited zone of inhibition ranging from 6.0 to 11.0 mm against gram positive bacteria. The carbon tetrachloride soluble fraction showed 11.0 mm zone of inhibition against *Staphylococcus aureus*. This fraction also revealed 15.0 mm zone of inhibition against *Salmonella paratyphi* and 13.0 mm zone of inhibition against *Aspergillus niger.*

Among the test samples of *G. philippensis,* the crude methanol extract exhibited 18.0 mm zone of inhibition against *Bacillus cereus*. The *G. philippensis* extractives exhibited zone of inhibition ranging from 6.0 to 11.0 mm against gram negative bacteria. The carbon tetrachloride soluble fraction revealed 11.0 mm against *Escherichia coli*. This fraction also showed 13.0 mm zone of inhibition against *Aspergillus niger.*

Table 2: Antimicrobial Activity of *O. mungos, S. nodiflora, P. sagitatta, M. macrophylla and G. philippensis a*gainst gram Positive Bacteria

Diameter of zone of inhibition (mm)					
Test Sample	***Bacillus cereus***	***B. megaterium***	***B. subtilis***	***Staphylococcus aureus***	***Sarcina lutea***
O. mungos					
ME	8.0±0.32	5.0±0.82	8.0±0.80	8.0±0.70	9.0±0.88

PESF	-	-	8.0±0.33	-	8.0±0.95
CTCSF	10.0±0.53	12.0±0.32	10.0±0.72	11.0±0.32	8.0±0.76
CSF	9.0±0.78	7.0±0.33	9.0±0.62	8.0±0.32	7.0±0.53
AQSF	8.0±0.05	8.0±0.58	-	-	-
S. nodiflora					
ME	14.0±0.82	11.0±0.32	8.0±0.57	9.0±1.03	8.0±0.30
PESF	-	-	-	-	-
CTCSF	10.0±0.82	8.0±0.55	9.0±0.67	5.0±1.02	8.0±0.25
CSF	4.0±0.12	10.0±0.92	8.0±0.27	-	7.0±0.25
AQSF	8.0±0.52	7.0±0.72	-	-	5.0±0.30
P. sagitatta					
ME	6.0±0.22	8.0±0.12	9.0±0.85	12.0±0.02	8.0±0.92
PESF	-	-	-	10.0±0.44	-
CTCSF	11.0±0.92	10.0±0.88	9.0±0.65	14.0±0.22	8.0±1.12
CSF	10.0±1.4	13.0±0.92	10.0±0.85	16.0±0.39	11.0±0.86
AQSF	-	-	-	9.0±0.05	-
M. macrophylla					
ME	9.0±0.41	5.0±0.87	8.0±0.80	8.0±0.72	8.0±0.08
PESF	-	-	8.0±0.30	-	7.0±0.95
CTCSF	8.0±0.51	10.0±0.86	7.0±0.70	11.0±0.12	8.0±0.78
CSF	6.0±0.71	-	9.0±0.68	8.0±0.40	7.0±0.56
AQSF	7.0±0.55	-	-	-	-
G. philippensis					
ME	18.0±0.82	12.0±0.41	8.0±0.57	9.0±1.44	6.0±0.78
PESF	-	-	-	-	-
CTCSF	10.0±0.86	7.0±0.50	5.0±0.11	8.0±1.33	8.0±0.25
CSF	8.0±0.33	6.0±0.22	8.0±0.35	-	9.0±0.65
AQSF	8.0±0.58	6.0±0.74	-	-	3.0±0.40
Ciprofloxacin (30 µg / disc)	45.0±2.01	42.0±1.17	42.0±0.73	42.0±0.56	42.0±0.13

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Table 3: Antimicrobial activity of *O. mungos, S. nodiflora, P. sagitatta, M. macrophylla and G. philippensis* against gram Negative Bacteria

Diameter of zone of inhibition (mm)							
Test Sample	***Escherichia coli***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***S. paratyphi***	***Shigella boydii***	***S. dysenteriae***	***Vibrio parahemolyticus***
O. mungos							
ME	7.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32
PESF	6.0±0.32	8.0±0.32	-	-	-	-	-
CTCSF	7.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32
CSF	-	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	-

AQSF	-	-	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32	8.0±0.32
S. nodiflora							
ME	7.0±0.55	8.0±0.12	10.0±0.84	7.0±0.22	7.0±0.11	6.0±0.30	9.0±0.12
PESF	-	-	7.0±0.32		-	-	6.0±0.12
CTCSF	10.0±0.44	14.0±0.12	-		16.0±0.33	8.0±0.33	6.0±0.55
CSF	8.0±0.32	-	7.0±0.38		10.0±0.58	12.0±0.05	10.0±0.67
AQSF	-	7.0±0.32	7.0±0.08		-	-	7.0±0.52
P. sagitatta							
ME	8.0±0.57	10.0±0.61	11.0±0.57	9.0±0.94	13.0±0.36	-	9.0±0.21
PESF	-	8.0±0.72	-	-	9.0±0.56	-	-
CTCSF	14.0±0.95	10.0±0.84	-	15.0±1.15	11.0±0.54	13.0±0.36	-
CSF	11.0±0.95	-	-	12.0±0195	16.0±0.55	10.0±0.95	-
AQSF	-	-	7.0±0.15	-	6.0±0.90	11.0±0.74	-
M. macrophylla							
ME	-	-	-	9.0±0.94	-	10.0±0.95	-
PESF	-	10.0±0.72	13.0±0.36	-	6.0±0.56	-	-
CTCSF	8.0±0.95	9.0±0.84	11.0±0.57	15.0±1.15	12.0±0.54	-	8.0±0.36
CSF	12.0±0.95	-	7.0±0.15	12.0±0195	10.0±0.55	-	7.0±0.21
AQSF	-	-		-	-	6.0±0.74	-
G. philippensis							
ME	7.0±0.51	7.0±0.61	10.0±0.94	6.0±0.57	7.0±0.36	8.0±0.31	8.0±0.21
PESF	-	-	-	-	7.0±0.16	-	-
CTCSF	11.0±0.95	-	8.0±1.00	7.0±1.10	-	-	7.0±0.16
CSF	7.0±0.55	6.0±0.15	-	-	-	-	-
AQSF	-	-	-	-	-	-	-
Ciprofloxacin (30 µg / disc)	42.0±0.43	42.0±1.11	45.0±0.73	47.0±2.33	34.0±0.58	42.0±0.22	35.0±0.44

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Table 4: Antimicrobial activity of *O. mungos, S. nodiflora, P. sagitatta, M. macrophylla and G. philippensis* against Fungi.

Diameter of zone of inhibition (mm)			
Test Sample	***Candida albicans***	***Aspergillus niger***	***Sacharomyces cerevacae***
O. mungos			
ME	11.0±1.32	10.0±0.30	7.0±0.22
PESF	-	-	6.0±0.36
CTCSF	11.0±0.65	13.0±0.85	7.0±0.88
CSF	7.0±0.22	8.0±0.52	-
AQSF	-	-	-
S. nodiflora			
ME	8.0±1.30	8.0±0.31	10.0±0.12
PESF	-	-	6.0±0.36
CTCSF	13.0±0.65	12.0±0.15	10.0±0.18

CSF	8.0±0.22	-	9.0±0.18
AQSF	9.0±0.52	7.0±0.72	-
P. sagitatta			
ME	11.0±1.32	-	-
PESF	-	-	-
CTCSF	13.0±0.65	13.0±0.85	7.0±0.88
CSF	10.0±0.22	8.0±0.52	11.0±0.22
AQSF	-	-	11.0±0.92
M. macrophylla			
ME	12.0±0.32	7.0±0.32	11.0±0.32
PESF	-	-	6.0±0.36
CTCSF	11.0±0.60	13.0±0.85	7.0±0.88
CSF	13.0±0.22	8.0±0.51	-
AQSF	-	-	-
G. philippensis			
ME	11.0±1.32	8.0±0.30	9.0±0.22
PESF	-	-	6.0±0.36
CTCSF	10.0±0.60	13.0±0.85	7.0±0.88
CSF	6.0±0.22	7.0±0.50	-
AQSF	-	-	-
Ciprofloxacin (30 µg / disc)	38.0±0.49	37.0±0.64	38.0±0.30

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF= Aqueous soluble fraction

4. Conclusion

The extractives of *O. mungos*, *P. sagitatta, S. nodifloara, P. sagitatta, M. macrophylla* and *G. philippensis* demonstrated varying degrees of antimicrobial activity against five gram positive and seven gram negative bacteria and three fungi. Further work especially bioassay-guided fractionation is warranted in order to isolate and characterize the active constituents responsible for the antimicrobial property.

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