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COMPARATIVE STUDY ON ANTIMICROBIAL ACTIVITIES OF MANGROVES GROWING IN POLLUTED AND NON-POLLUTED SITES OF NORTHERN ARABIAN SEA

MALIK WAJID HUSSAIN CHAN[1], JAWAID AKRAM[2], SALMAN GULZAR[2], UROOJ ZAFAR[3], SADAR ASLAM[1], PIRZADA JAMAL AHMED SIDDIQUI[1], SHAKEEL AHMED KHAN[4] AND MUNAWWER RASHEED[1,5*]

[1]Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan
[2]Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi-75270, Pakistan
[3]Department of Microbiology, Faculty of Science, University of Karachi, Karachi-75270, Pakistan
[4]Barrett Hodgson University, NC-24, Deh Dih Korangi Creek, Karachi-74900, Pakistan
[5]Department of Chemistry, Faculty of Science, University of Karachi, Karachi-75270, Pakistan
[*]Corresponding author's email: rasheed.munawwer@uok.edu.pk

Abstract

The aims of this study were to evaluate and compare the antibacterial and antifungal activities of botanicals extracted using 70% methanol from leaf, bark and pneumatophore/root of four different species of mangroves growing in different polluted and non-polluted sites along the Northern coast of Arabian sea. Plants produce bioactive compounds under stress, therefore in-vitro activities of extracts of mangroves growing in polluted (*Avicennia marina*) and non-polluted (*A. marina*, *Aegiceras corniculatum*, *Rhizophora mucronata* and *Ceriops tagal*) sites were assessed. Activities were performed against five Gram-positive and seven Gram-negative bacteria and twelve fungi including ten molds (eight saprophytes and two dermatophytes) and two yeasts. Well diffusion and disc diffusion methods were used in case of bacterial strains and former was found more efficient, therefore, it was used for analyzing fungal strains. Dilution method was used for the determination of the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of extracts. MIC values were observed from 0.3125 to 40 mg/mL. MIC value of 0.3125 mg/mL was observed from bark of *A. corniculatum* (NBCA) against *Pseudomonas aeruginosa*. Various botanicals showed MIC value of 1.25 mg/mL against different species of bacteria. In general leaf extracts were comparatively more active and antibacterial spectrum of extracts from *R. mucronata* was broader. In case of antifungal activity, MIC values were ranging from 5 to 20 mg/mL. MIC value of 5 mg/mL was observed in various tests, including extracts obtained from non-polluted bark of *A. marina* (NBMA, against *Aspergillusniger* and *Fusariumoxysporum*); NBCA, against *A. niger* and *Candida albicans*; roots of *A. corniculatum* (NRCA, against *A. niger* and *Chrysosporium*sp); polluted pneumatophores of *A. marina* (PPMA against *Paecilomycesvariotii* and *Aspergillusterreus*); polluted bark of *A. marina* (PBMA against *Penicillium*sp). Polluted leaf (PLMA) and non-polluted pneumatophores (NPMA) of *A. marina*, and roots of *C. tagal* (NRTC) also showed MIC, 5 mg/mL against *C. albicans*. In general root extracts were more active and *Aegicerascorniculatum*showed broader spectrum. *A. marina* was the only mangrove found growing both in polluted and non-polluted habitat. Polluted stands of *A. marina* showed broader and higher antibacterial and antifungal activity indicating that the pollution stress has stimulated the physicochemical changes to produce botanicals responsible for antimicrobial activity.

Key words: *Caesalpinia crista*, Seed coat, Natural polyphenols, Antioxidant, Antibacterial, Solid phase extraction, HPLC.

Introduction

There is a worldwide interest in identifying botanicals as natural antimicrobial agents due to the spread of multidrug resistant (MDR) strains and undesirable side effects of pharmaceutical drugs (Bartfay *et al*., 2012; Qasim *et al*., 2019). Plants growing under stress may have higher activities due to the synthesis of secondary metabolites (Basile *et al*., 2010; Nadir *et al*., 2013; Schippmann *et al*., 2002) such as phenols and flavonoids, which play key roles in plant-environment interactions (Muhammad *et al*., 2015; Qasim *et al*., 2017; Rhodes, 1994). Studies proved that such compounds appear to be correlated with high bioactivities in plant extracts in response to pollution stress (Basile *et al*., 2010; Rezanejad, 2009).

Malir and Lyari rivers deposit high levels of contamination to the coastal estuary system (Nergis *et al*., 2012; Chan *et al*., 2019; Siddique *et al*., 2009). Approximately 30% of this waste is generated from municipal sources while 70% is generated by industries (Saleem and Kazi, 1998). Mangrove habitats have become dumping grounds for domestic sewage and industrial effluents receiving more than 500 MGDs from various sources with toxic chemicals including solvents, paints, dyes and heavy metals (M.F.F. Pakistan, 2016). Environmental stress could elicit biochemical changes in plants resulting in production of bioactive secondary compounds (Basile *et al*., 2010; Rezanejad, 2009) as a response to the physiochemical adaptations to survive under stress (Navami & Jaya, 2013). *A. marina* has shown considerable resilience to environmental stresses, acting as a huge sink for these contaminants. However, these are at high risk of being wiped out due to the increasing levels of pollutants. Typically, microbial community of tropical mangrove forests is represented by 91% bacteria and fungi (Xu, 2015; Bibi *et al*., 2019).

The coastline of Pakistan (~1050 km) is bestowed with numerous natural resources including mangrove forests from Karachi to the Indus Delta, in eastern coast line and in discrete patches in west towards Iran. Mono-specific stands of *A. marina* occur in Sandspit while *A. marina, Aegeceras corniculatum* and *Rhizophora mucronata* are habitant of the Indus Delta. The

mangroves in Sonmiani Bay, Balochistan (covering 31 km²) nominated as a Ramsar site (Beg *et al*., 1984; Khan *et al*., 1999) harbors *A. marina*, *Ceriopstagal* and *Rhizophora mucronata*. Sonmiani Bay is a small subtropical lagoon (363 km²) with a mangrove cover of 31 km² located along the North Arabian Sea coast of Pakistan, about 100 km west of Karachi (Saifullah and Rasool, 2002; Spalding *et al*., 1997; Manilal *et al*., 2016) studied the antimicrobial effects of whole plant *A. marina* extracts. However, little information is available on the antibacterial and antifungal activities from extracts of individual plant parts from mangrove populations along Pakistan coast. Estuaries along the Sindh Delta reportedly once harbored eight species of mangroves. Now *A. marina* is dominating among the four existing species and it is the only survivor particularly in highly polluted sites (Khan & Aziz, 2001).

The present study deals with comparison of antimicrobial activity of mangroves' botanical extracted from their different parts, growing in polluted and non-polluted sites. The three mangroves' stands selected for study included Sandspit (a polluted site), Indus Delta near Keti Bandar and Sonmiani Bay (both relatively non-polluted sites). In Pakistan, mangroves occupy about 0.26 million ha of deltaic region mostly (98%) comprised of *A. marina* (Qureshi, 1993). The aims of the present study were to assay and compare the antimicrobial activity of different botanicals using agar well-diffusion and disc-diffusion methods and to find out whether the mangroves growing in stress polluted sites show any difference in activity compared with those growing in non-polluted site. The hypothesis that plant extracts growing in polluted site will show higher bioactivity against microbes compared to those from non-polluted site and that aerial parts (particularly leaves) will have lower activities than submersible or submerged parts (pneumatophores/ roots).

Materials and Methods

Sample collection: Fresh plant parts (leaves, bark, roots/pneumatophores) were collected from three different mangroves enriched sites located at Sandspit (24° 49' 49" N; 66° 55' 41" E), Indus Delta (24° 93' 33" N; 67° 27' 43.54" E) and Sonmiani Bay (25° 30' 55.76" N'; 66° 32' 29.41" E) during monsoon seasonof 2016. Mangroves were identified by Dr. Muneeba Khan (Taxonomist, Herbarium, Karachi University Herbarium, Center for Plant Conservation, University of Karachi. Herbarium numbers, mentioned in parenthesis, were *A. marina* (91869), *A. corniculatum* (93323), *C. tagal* (91867), *R. mucronata* (91868). Plant materials were thoroughly washed with tap water to remove any adhering soil and debris, followed by brief rinsing with distilled water, air dried at room temperature in shade for about 3 to 5 weeks. Cut and chopped into small pieces manually and soaked in 70% methanol in glass jars for 15 days (thrice). The extracts were combined, filtered and was evaporated under reduced pressure using a rotary vacuum evaporator to yield thick hard flakes with cracks. All crude extracts were stored at 4°C in sealed jars.

Antimicrobial assay: A number of methods have been used to evaluate antimicrobial activity, however, disc diffusion and well diffusion are used most commonly, due to their utility as rapid, simple and cost effective methods (Balouiri *et al*., 2016). The results obtained by agar well diffusion were more sensitive than disc diffusion method due to larger growth, and greater zone of inhibition (Valgas *et al*., 2007.).

Disc diffusion and well diffusion methods used, were as described by Bauer *et al*., 1966; Magaldi *et al*., 2004) and Valgas *et al*., 2007). The antimicrobial activities of extracts were tested against five Gram-positive and seven Gram-negative bacteria (Table 1). Well diffusion method showed higher activities against bacterial strains and was used against twelve fungal species including ten molds (eight saprophytes and two dermatophytes) and two yeast species (Table 2).

Pure bacterial and fungal cultures maintained in the Department of Microbiology, University of Karachi, were revived for the study. Stock solution (20 mg/mL) of each plant extracts was prepared in sterilized dimethyl sulfoxide (DMSO). Sterile DMSO was used as a negative control, Ciprofloxacin (CIP) as a positive control for bacteria, while Polymyxin was used as a positive control for fungi. Cell suspension was prepared by inoculating 24 h old bacterial and yeast culture in 5 mL saline solution. Turbidity of bacterial suspension was matched with 0.5 McFarland standard, which is equivalent to 1.5×10^8 CFU/mL cellular load. Confluent lawn of each bacterial culture was made on Mueller-Hinton Agar (MHA, Oxoid UK) plate. Similarly, fungal spore suspension (5×10^5 spores/mL) was prepared from 5 d old molds. Fungal lawn of each fungal culture was made on Sabouraud's Dextrose Agar (SDA) plate. Lawns were allowed to diffuse for 5-10 min. 6 mm wells were dug and 20 µL of stock solution of extracts was introduced into the wells and allowed to diffuse into media for 15-20 min. Plates were incubated at 37°C for 24 h for bacteria and at ambient temperature (25°C) for 5 d for fungi (Cauwelier *et al*., 2004; Magaldi *et al*., 2004; Valgas *et al*., 2007).

For disc-diffusion assay, sterile cotton swab was immersed in culture suspension and pressed against wall of test tube to get rid of excess culture suspension. Lawn was made by streaking the swabs evenly on the MHA. Plates were allowed to stand for 5 min. Filter paper discs (6 mm dia.) were impregnated with 10 µL of plant extract stock solution at room temperature (25°C) for 15 to 20 min (Selvamohan *et al*., 2012). Discs autoclaved for 15 min at 121°C and 15 psi, were placed and pressed slightly on agar surface and plates were incubated at 37°C for 24 h (Fiebelkorn *et al*., 2003). All experiments were repeated thrice or more to obtain statistical consistent results.

Cell suspension (0.5 McFarland Index standard) was used to make Confluent lawn MHA plates and allowed to stand for diffusion for 5-10 minutes. Wells (6 mm) were dug and two fold serially diluted extracts were introduced into the wells. Plates were incubated as detailed above. MBC was determined by streaking the inoculums taken from the ZOI experiments, having the lowest

concentration of plant extracts. Plates were incubated for 24 to 48 h at 37°C. The absence of growth from the highest dilution was then considered as MBC. MBCs or MFCs were determined by sub-culturing the test dilution (showing no visible turbidity) on to freshly prepared nutrient agar media plates. After the incubation period, the lowest concentrations, which did not show any visible growth of bacteria or fungi were taken as MBCs or MFCs (Valgas *et al*., 2007, Anon., 1998).

Results for anti-bacterial based on the zone of inhibition (ZOI in mm) were interpreted as follows: For well-diffusion method; 09-12 mm = low activity; 13-15 mm = moderate activity and >15 mm = significant activity, whereas, for disc-diffusion method; 07-10 mm = low activity; 11-13 mm = moderate activity; and >13 mm = significant activity. Extracts showing ZOI ≥ 15 mm or above diameter in preliminary screening were proceeded for determination of MICs, MBCs, and MFCs (Hudzicki, 2009).

Table 1. Preliminary screening of antibacterial activity of botanicals obtained from plants growing in polluted (P) and non-polluted (N) sites, from leaf (L), root (R), pneumatophore (P) and bark (B); of *A. marina* (MA), *A. corniculatum* (CA), *C. tagal* (TC) or *R. mucronata* (MR), by well-diffusion / disc-diffusion method using 10 µl of 40 mg/mL extract per well/disc.

	Zone of Inhibition (mm)														
Codes	**NLMR**	**NBMR**	**NRMR**	**NLCA**	**NBCA**	**NRCA**	**NLTC**	**NBTC**	**NRTC**	**NLMA**	**NBMA**	**NPMA**	**PLMA**	**PBMA**	**PPMA**
Gram-positive bacteria															
Micrococcus luteus	12/-	12/-	12/-	-	-	-	13/-	-	-	-	-	-	16/28	-	8/-
Bacillus cereus	-	-	-	12/-	14/-	-	-	-	-	12/-	11/-	12/-	11/-	-	-
Staphylococcus aureus	12/-	12/-	12/-	16/11	14/-	15/12	11/-	19/16	15/12	-	8/-	8/-	12/-	12/-	18/13
Methilcillin-resistant Staphylococcus aureus (MRSA)	-	-	-	-	15/-	12/-	11/-	-	-	14/-	-	-	11/-	-	-
Staphylococcus saprophyticus	21/32	15/36	15/30	32/30	17/30	-	13/-	28/28	38/32	13/-	13/-	12/-	-	-	18/28
Gram-negative bacteria															
Escherichia coli	15/10	18/20	16/10	-	-	-	18/10	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	20/41	-	-	-	21/14	24/22	12/-	-	-	11/-	13/-	11/-
Shigella sp.	14/-	15/-	-	-	-	-	-	24/-	24/-	-	-	-	-	15/34	18/30
Salmonella typhi	14/-	15/10	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	16/26	17/26	16/30	-	14/17	18/26	18/25	19/28	19/31				-	-	-
Citrobacter sp.	13/-	13/-	12/-	-	-	-	12/-	-	-	-	-	-	-	-	-
Proteus mirabilis	17/18	18/23	13/12	13/10	14/11	14/9	13/10	9/9	10/9	18/14	18/21	16/12	15/20	14/11	16/20

(-) represents ZOI <8 mm

Table 2. Preliminary screening of antifungal activity of botanicals obtained from plants growing in polluted (P) and non-polluted (N) sites, from leaf (L), root (R), pneumatophore (P) or bark (B); of *A. marina* (MA), *A. corniculatum* (CA), *C. tagal*(TC) or *R. mucronata* (MR), by well-diffusion method using 10 µL of 40 mg/mL extract per well.

	Zone of inhibition (mm)														
Code	**NLMR**	**NBMR**	**NRMR**	**NLCA**	**NBCA**	**NRCA**	**NLTC**	**NBTC**	**NRTC**	**NLMA**	**NBMA**	**NPMA**	**PLMA**	**PBMA**	**PPMA**
Dermatophytes															
Trichophyton mentagrophytes	-	-	8	-	-	15	-	-	11	-	17	-	-	-	-
Microsporum gypseum	-	-	-	-	-	23	-	-	-	-	13	-	-	-	-
Saprophytes															
Aspergillus flavus	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-
Aspergillus niger	-	17	-	13	21	19	6	-	-	-	22	-	-	17	-
Aspergillus terreus	-	-	-	28	-	-	-	13	-	-	-	14	13	-	19
Aspergillus terricola	-	-	-	26	18	14	20	-	-	-	10	-	-	-	-
Chrysosporium spp.	-	-	-	-	-	21	-	-	-	-	-	20	12	-	15
Fusarium oxysporum	-	10	-	-	-	-	-	-	-	-	22	-	-	-	11
Paecilomyces variotii	12	-	-	16	-	-	-	-	23	-	-	7	-	-	21
Penicillium sp.	13	-	-	-	-	-	-	15	-	17	11	-	-	18	-
Yeasts															
Candida albicans	15	-	17	21	16	-	23	11	16	-	18	20	34	23	14
Saccharomyces cerevisiae	12	14	18	20	13	11	-	15	10	16	10	-	10	25	21

(-) represents ZOI <8 mm

Table 3. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of mangrove extracts, which showed ZOI ≥ 15 mm.

	Extracts	MIC (mg/ml)	MBC (mg/ml)
Gram-positive bacteria			
M. luteus	PLMA	1.25	1.25
S. aureus	PPMA	I.25	I.25
	NLCA	1.25	1.25
	NRCA	10	10
	NBTC	2.5	2.5
	NRTC	5	5
MRSA	NBCA	40	40
S. saprophyticus	PPMA	40	40
	NLCA	1.25	1.25
	NBCA	2.5	2.5
	NBTC	1.25	1.25
	NRTC	1.25	1.25
	NLMR	1.25	1.25
	NBMR	1.25	1.25
	NRMR	1.25	1.25
Gram-negative bacteria			
E. coli	NLTC	5	5
	NLMR	1.25	1.25
	NBMR	1.25	1.25
	NRMR	1.25	1.25
K. pneumoniae	NLCA	1.25	1.25
	NBTC	5	5
	NRTC	1.25	1.25
Shigella sp.	PBMA	1.25	1.25
	PPMA	1.25	1.25
	NBTC	1.25	1.25
	NRTC	1.25	1.25
	NBMR	20	20
S. typhi	NBMR	20	20
P. aeruginosa	NBCA	0.313	0.313
	NRCA	5	5
	NLTC	2.5	2.5
	NBTC	2.5	2.5
	NRTC	2.5	2.5
	NLMR	1.25	1.25
	NBMR	5	5
	NRMR	2.5	2.5
P. mirabilis	PLMA	40	40
	PPMA	20	20
	NLTC	10	10
	NBTC	20	20
	NRTC	10	10
	NLMR	1.25	1.25
	NBMR	20	20

Results

The data for the antibacterial effects of 70% methanolic extracts of botanicals extracted from various parts of each mangrove species is presented in Tables 1 to 4. The botanicals showing maximum antibacterial activity (Tables 1 and 3) against Gram positive species (*Staphylococcus saprophyticus*) was obtained from the roots of *C. tagal* (NRTC) with ZOI, 38 mm and MIC, 1.25 mg/mL. NRTC also showed maximum activity in the case of Gram negative species, *Klebsiella pneumoniae* (ZOI, 24 mm and MIC, 1.25 mg/mL). Activity of similar magnitude was also observed with barks and roots of *C. tagal* against *Shigella sp*. All the extracts except one from non-polluted leaves of *A. marina* were found active against *Staphylococcus aureus*.

The ZOI in case of antifungal activity of extracts are listed in Table 2 while MIC and MFC values are given in Table 4. Almost all the extracts, except those obtained from non-polluted leaves of *A. marina* and roots of *A. corniculatum* gave significant ZOI against *Candida albicans*. Polluted leaves of *A. marina* showed maximum ZOI, 34 mm with MIC value 5 mg/mL. In the same way, except from non-polluted pneumatophores of *A. marina* and leaves of *C. tagal* (NLTC), remaining extracts showed significant zones of inhibition (<20 to >15 mm) against *Saccharomyces cerevisiae*. Being non-pathogenic, the MICs and MFCs of *S. cerevisiae* were used for comparison only. In case of antifungal activity, the most active extracts were obtained from roots of *A. corniculatum* (ZOI, 23 mm and MIC 10 mg/mL against *Microsporum gypseum*) and leaves of *A. corniculatum* NLCA (ZOI, 28 mm and MIC 10 mg/mL against *Aspergillus terreus* and *Aspergillus terricola*, respectively).

Discussion

Unfavorable conditions can induce secondary metabolite production in plants to higher levels than in plants growing in pristine or less polluted habitats (Qasim *et al*., 2019). Strong antimicrobial activities were observed from the *A. marina* growing in polluted site compared to non-polluted site indicated by Zoufan *et al*., (2017). The species selected for antimicrobial activity in this study could help in indicating some physiological patterns of activities from a variety of microbial taxa (Booyens & Thantsha, 2014).

Fifteen extracts of four mangrove species collected from polluted and non-polluted sites were evaluated for the antibacterial and antifungal activities (Tables 1-4). The antimicrobial activities of the botanicals extracted from leaves, roots (or pneumetaphores), and bark of these.

Species were compared. Extracts obtained from *A. marina*, growing in non-polluted environments were also studied.

Extracts of *R. mucronata* (leaves, bark and roots) from non-polluted sites were relatively more potent against bacterial strains (Tables 1 and 2), which are in accordance with the earlier studies on methanol extracts of bark and leaf of *R. mucronata* with strong activity against bacterial strains and it may be due to the presence of various secondary metabolites (Fennell *et al*., 2004; Padmakumar, 1988; Vlachos *et al*., 1996). These results were almost in agreement with Joel and Bhimba (2010) and Kumar *et al*., (2009), who used leaf and bark extract on various microbes using well and disc diffusion methods, respectively. Among the four mangroves in this study, antibacterial spectrum was found broader in contrast to the antifungal spectrum of the botanicals from *R. mucronata* (Tables 3 and 4).

Table 4. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of mangrove extracts, which showed ZOI ≥ 15 mm.

Microganism	Code	MIC (mg/mL)	MFC (mg/mL)
Dermatophytes			
T. mentagrophytes	NBMA	10	10
	NRCA	20	20
M. gypseum	NRCA	10	10
Saprophytes			
A. niger	PBMA	10	10
	NBMA	5	5
	NBCA	5	5
	NRCA	5	5
Penicillium sp.	PBMA	5	5
	NLMA	20	20
	NBTC	20	20
P. variotii	PPMA	5	5
	NLCA	20	20
	NRTC	20	20
Chrysosporium sp.	PPMA	10	10
	NPMA	10	10
	NRCA	5	5
F. oxysporum	NBMA	5	5
A. terreus	PPMA	5	5
	NLCA	10	10
A. terricola	NLCA	20	20
	NBCA	10	10
	NLTC	10	10
Yeast			
C. albicans	PLMA	5	5
	PBMA	20	20
	NBMA	10	10
	NPMA	5	5
	NLCA	10	10
	NBCA	5	5
	NLTC	20	20
	NRTC	5	5
	NLMR	10	10
	NRMR	20	20

Bark extracts obtained from *A. corniculatum* showed the highest antibacterial activity with MIC, 0.3125 mg/mL against *P. aeruginosa*. Leaf extracts of *A. corniculatum* showed activity against *S. aureus* comparable to those reported earlier (Bakhshi and Chaudhri, 2014). Bark and leaf extracts of *A. corniculatum* also showed maximum ZOI (30 mm) both against *S. saprophyticus*. In case of antifungal activity, MIC value of 5 mg/mL was observed for botanicals obtained from bark of *A. corniculatum* against *A. niger* and *C. albicans* and root of *A. corniculatum* against *A. niger* and *Chrysosporium* sp. *A. corniculatum* showed broader spectrum against fungi as compared to *R. mucronata*., *C. tagal*, and *A. marina*.

The root and bark extracts of *C. tagal* were the most active, both showing activity against *K. pneumoniae* with ZOI 21 and 24 mm, respectively. Bark and leaf extracts of *C. tagal* also showed ZOI of 24 mm against *Shigella*sp. In case of fungi, root and bark extracts of *C. tagal* showed maximum activity against *S. saprophyticus* with ZOI, 38 and 28 mm respectively. NRTC also showed MIC, 5 mg/mL against *C. albicans*. A little work has been reported in literature related to the antimicrobial activity of *C. tagal*.

The antibacterial spectrum of *A. marina* was comparatively narrow. However, the ZOI studies showed that the botanicals obtained from different parts of plant growing in polluted areas are more effective than that of botanicals from plants growing in non-polluted area. Similarly, pneumatophores showed more potential followed by leaf or bark. Leaf extracts of *A. marina* showed activity against *E. coli* and *S. aureus* comparable to those reported by Bakhshi and Chaudhri (2014). Considering antifungal potentials, bark extracts of *A. marina* from non-polluted plants showed strong activity against eight different fungal species. MIC value of 5 mg/mL was observed from the extract of bark of *A. marina* grown in non-polluted site against *A. niger* and *F. oxysporum*; pneumatophores of polluted site against *P. variotii* and *A. terreus*; bark of polluted site against *Penicillium* sp. leaf of polluted and pneumatophores of non-polluted sites against *C. albicans*. Mahasneh (2002) has also reported that leaf extracts of *A. marina* were active against *C. albicans* and *A. flavus*. *A. marina* was the only mangrove found growing in polluted as well as non-polluted habitat. It has fluorish in the Sandspit backwater channels that receive untreated sewage and industrial waste from the Lyari River. Polluted stands of *A. marina* showed broader and higher antibacterial and antifungal activity indicating that the pollution stress has stimulated the physicochemical changes to produce botanicals responsible for antimicrobial activity.

These fifteen extracts (Table 1) have also been used in another separate study against *Mycobacterium bovis*, *Acholeplasmalaidlawii* and *Mycoplasma capri*, causative agents of Mycoplasmosis in buffaloes. Statistically, the mean values of polluted bark of *A. marina*, non-polluted leaf of *A. marina* and leaf of *A. corniculatum* showed significant differences in antimicrobial activities among *M. bovis*, *A. laidlawii* and *M. capri*, while polluted leaf of *A. marina*, polluted pneumatophores of *A. marina*, non-polluted bark of *A. marina*, non-polluted pneumatophores of *A. marina*, non-polluted bark of *A. corniculatum* and root of *A. corniculatum* showed non-significant relation (Fareed, 2018).

In general, methanol extracts of mangroves were more effective against Gram-positive bacteria than Gram-negative (Manilal *et al*., 2016). MIC values were observed from 0.3125 to 40 mg/mL. Various botanicals showed MIC value of 1.25 mg/mL against different species of bacteria. Leaf extracts of botanicals from *R. mucronata* were comparatively more active with a broader antibacterial spectrum than those of other mangrove species. In case of antifungal activity, MIC values were between 5 to 20 mg/mL. MIC value of 5 mg/mL was obtained in various tests in current study. Root extracts of mangroves were relatively more active than other plant parts. *Aegiceras corniculatum* showed broader spectrum of activities among the species tested. The results obtained from this antimicrobial study indicated that the mangroves of Pakistan coast possess antibacterial and anti-fungal activity suggesting the possibility of finding potent antimicrobial agents from mangrove.

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