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Antioxidant and antimicrobial activity of a mangrove plant Avicennia marina (Forsk.)

Pooja Moteriya, Ashish Dalsaniya, Sumitra Chanda*

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot 360005, Gujarat, India

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ABSTRACT

Objective: To evaluate the antioxidant and antimicrobial potential of different parts (leaf, stem and pneumatophore) of a mangrove plant *Avicennia marina* (Forsk.) Vierh (Avicenniaceae). **Methods:** The extraction was done by cold percolation method using solvents of hexane, ethyl acetate, acetone, methanol and water. Total phenol and flavonoid content were measured by Folin-Ciocalteu's reagent and aluminium chloride colorimetric method respectively. The antioxidant activity was evaluated using antioxidant assays of 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity, superoxide radical scavenging assay, 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid, ferric reducing antioxidant power and reducing capacity assessment. The antibacterial activity was done by agar well diffusion method against four Gram-positive, four Gram-negative bacteria and four fungi.

Results: Different antioxidant assays showed different levels of activity in different parts and different solvent extracts. Overall, acetone extract of stem showed the best antioxidant activity. The Gram-positive bacteria were more susceptible than Gram-negative bacteria and fungi. **Conclusions:** The results indicated extract of Avicennia maring can be used as a promising

Conclusions: The results indicated extract of *Avicennia marina* can be used as a promising source of natural antioxidant.

1. Introduction

Free radicals are produced continually in various metabolic processes and exist in biological systems. They are important for maintaining normal physiological functions. In fact, the human body is constantly exposed to free radicals such as hydroxyl radical, superoxide radical, peroxyl radical, alkoxyl radical, nitric oxide, hydrogen peroxide, hypochlorous acid, singlet oxygen, ozone, peroxynitrite, *etc*.

An antioxidant is a substance that can inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and tert-butylhydroquinone are in use but their usage is getting restricted because of their possible toxic properties for human health and environment[1,2]. Hence, the development of alternative antioxidants from natural origin is the need of the hour. Therefore, it is imperative to evaluate antioxidant activity of the plants used in herbal medicine in order to elucidate the mechanism of their pharmacological action and provide information on their antioxidant activity.

The rise of antibiotic resistant microorganisms is one of the severe problems in health care systems of the world. In addition to this problem, antibiotics are sometimes associated with adverse

E-mail: svchanda@gmail.com

effects on the host including hypersensitivity, immune suppression and allergic reactions. Therefore, drugs with novel and new antimicrobial properties have to be found in order to combat such diseases. The medicinal plants are endowed with many secondary metabolites such as alkaloids, flavonoids, lignins, phenols, sterols, saponins, tannins and terpenes, and they are well known as a major source of antibacterial, antioxidant and anticancer agents[3,4].

Mangroves are the unique plant communities that inhabiting the estuarine and intertidal regions of both tropical and subtropical coasts and largely confined to the region between 30° north and south of the equator. Mangrove plants are salt-tolerant (up to 500 mmol/L NaCl) plants and they produce novel metabolites unique to the environment with various important economic and environmental functions[5]. Mangroves usually grow in estuarine swamps which have unique adaptations to combat environmental stress conditions like high salinity, high temperature, low nutrient and excessive radiation. They are well adapted to these ecological hostile conditions by alterations in their physiological processes which result in the synthesis of novel chemical compounds that offer protection to these plants against various biotic and abiotic stresses mentioned above[6]. Mangroves and mangrove associates contain biologically active antiviral, antibacterial and antifungal, antiplasmodial and hepatoprotective activities[7,8].

Avicennia marina (Forsk.) Vierh. (A. marina) is commonly known as the grey or white mangrove plant resident in the tropical and subtropical regions. It belongs to the family Avicenniaceae. Considering the above, in the present work, an attempt was made to

^{*}Corresponding author: Sumitra Chanda, Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot 360005, Gujarat, India.

evaluate the antioxidant and antibacterial potential of different parts of *A. marina*.

2. Material and methods

2.1. Plant collection

A. marina (Forsk.) plant parts leaf, stem and pneumatophore were collected in the month of August, 2014 from Jodiya, Jamnagar, Gujarat, India. They were thoroughly washed, and shade dried. The dried plant parts (leaf, stem and pneumatophore) were crushed to fine powder and stored in air tight bottles which were later used for solvent extraction.

2.2. Individual cold percolation method

The dry powder of leaf, stem, and pneumatophore of *A. marina* was extracted individually by cold percolation method[9] using five different solvents (hexane, ethyl acetate, acetone, methanol and water) with different polarity.

2.3. Determination of total phenol content (TPC) and total flavonoid content (TFC)

The amount of total phenol and flavonoid content was determined by Folin-Ciocalteu's reagent method[10] and aluminium chloride colorimetric method[11] respectively. The procedure followed is as described earlier[12].

2.4. Antioxidant assays

The antioxidant activity of the different solvent extracts was evaluated by four different *in vitro* antioxidant assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, superoxide anion radical scavenging activity, 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) cation free radical scavenging activity and ferric reducing antioxidant power (FRAP). The procedure followed is as described earlier[12].

2.5. Antimicrobial activity

Antimicrobial activity was done by agar well diffusion method[9,13] against Gram-positive bacteria, Gram-negative bacteria and fungal strains. The procedure followed is as described earlier[14].

2.6. Microorganisms tested

The microorganisms were obtained from National Chemical Laboratory, Pune, India. The Gram-positive bacteria studied were Bacillus cereus ATCC 11778 (B. cereus), Bacillus subtilis ATCC 6633 (B. subtilis), Staphylococcus aureus ATCC 29737 (S. aureus) and Corynebacterium rubrum ATCC 14898 (C. rubrum). The Gram-negative bacteria were Escherichia coli NCIM 2931 (E. coli), Pseudomonas aeruginosa ATCC 27853 (P. aeruginosa), Salmonella typhimurium ATCC 23564 (S. typhimurium) and Klebsiella pneumonia NCIM 2719 (K. pneumonia). The fungal strains were Candida albicans ATCC 10231 (C. albicans), Cryptococcus neoformans NCIM 3542 (C. neoformans), Candida glabrata NCIM 3448 (C. glabrata) and Candida apicola NCIM 367 (C. apicola).

2.7. Statistical analysis

Each sample was analyzed individually in triplicate and the results

were expressed as the mean value $(n = 3) \pm SEM$.

3. Results

3.1. Extraction yield

The extractive yield of different solvent extracts was given in Figure 1. The extractive yield was different in different solvents and parts. Amongst the three parts, maximum extractive yield was in leaf (Figure 1A). Irrespective of the part, methanol and water extracts had maximum yield. The non-polar and semi-polar solvent extracts (hexane and ethyl acetate) had minimum and very less extractive yield (Figure 1). Further, acetone though a polar solvent like methanol, had very less extractive yield as compared to methanol.

3.2. TPC and TFC

In all the three parts, irrespective of the solvents, TPC was more than TFC except in ethyl acetate extract of leaf (Figure 2). In leaf, TPC was maximum in water extract followed by methanol and acetone extract respectively (Figure 2A). In stem, on the other hand, maximum TPC was in acetone extract followed by methanol and water extracts (Figure 2B). The TPC was almost same in methanol and water extracts. In stem also like leaf, ethyl acetate extract had minimum TPC. An entirely different trend was found in pneumatophore. Maximum TPC was in methanol extract followed by ethyl acetate extract and acetone extract respectively (Figure 2C). Minimum TPC was in water extract. Among the three parts, maximum TPC was in stem. Different parts showed different levels of TPC in different solvent extracts.

In all the three parts, TFC was maximum in ethyl acetate extract, maximum being in leaf (Figure 2A). In leaf, TFC was maximum in ethyl acetate extract followed by both polar solvent extracts (methanol and acetone). Minimum TFC was in water extract. In stem also, maximum TFC was is ethyl acetate extract followed by acetone extract (Figure 2B). Methanol extract had comparatively less TFC than acetone extract and it was almost negligible in water extract. In pneumatophore, the TFC content in all the solvent extracts was similar to that of stem (Figure 2C).

3.3. Antioxidant activity

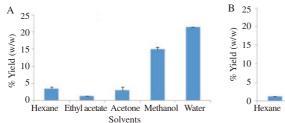
In the present study 4 different antioxidant capacity assays with different mechanism of action were done to evaluate the antioxidant capacity of different solvent extract of different parts of *A. marina*.

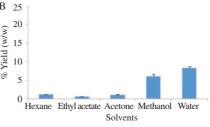
3.4. DPPH radical cation scavenging activity

DPPH radical cation scavenging activity of leaf, stem and pneumatophore of A. marina was given in Table 1. Amongst different parts and different solvent extracts of A. marina, the acetone extract of stem (110 μ g/mL) and pneumatophore (108 μ g/mL) had the lowest IC₅₀ value, followed by methanol extract of leaf (127 μ g/mL). In stem, maximum TPC was in acetone extract and correspondingly acetone extract showed the lowest IC₅₀ value i.e. it had a direct correlation. This was not evident in pneumatophore and leaf

3.5. Superoxide anion radical scavenging assay

The IC_{50} values of superoxide anion radical scavenging activity was given in Table 1. Amongst different parts and different solvent extracts of *A. marina*, the water extract of leaf had the lowest IC_{50}





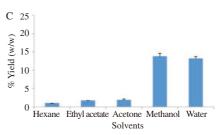
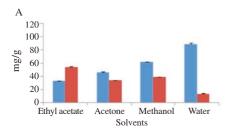
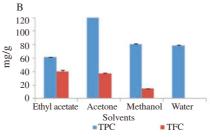


Figure 1. The extractive yield of different solvents extracts of *A. marina*. A: Leaf; B: Stem; C: Pneumetophore.





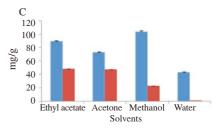


Figure 2. TPC and TFC of different solvent extracts of *A. marina*. A: Leaf; B: Stem; C: Pneumetophore.

value (230 µg/mL) followed by water extract of stem (352 µg/mL). The other solvent extracts of different part showed varied level of superoxide activity. The IC₅₀ values ranged from 376–560 µg/mL. The ethyl acetate extracts of all the three parts showed poor activity, and the IC₅₀ value was more than 1000 µg/mL. The best superoxide activity was shown by water extract of leaf and the activity was comparable with that of standard gallic acid (IC₅₀ value = 185 µg/mL).

3.6. ABTS radical cation scavenging activity

Amongst different parts and different solvent extracts of *A. marina*, the acetone extract of stem and water extract of pneumatophore had the lowest IC₅₀ value (40 μ g/mL) *i.e.* it showed the best ABTS activity followed by methanol extract of leaf (52 μ g/mL) (Table 1). The other solvent extracts showed poor activity.

3.7. FRAP

The FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue colored complex of ferrous ion (Fe²⁺)

and tripyridyl-triazine. Prior to this, a colorless ferric ion (Fe³⁺) gets oxidized to ferrous ion (Fe²⁺) by the action of electron donating antioxidants. The FRAP activity of leaf, stem and pneumatophore of *A. marina* was given in Table 1. Among the three parts, maximum FRAP activity was in stem. There was a direct correlation between TPC and FRAP activity in all the solvent extracts in all the three parts. In leaf, maximum TPC was in water extract and maximum FRAP activity also in water extract. All the other solvent extracts showed a direct correlation between TPC and FRAP activity. In stem, unlike maximum TPC was in acetone extract and maximum FRAP activity. In stem also, other solvent extracts showed a direct correlation with TPC. In pneumatophore, maximum TPC and FRAP activity was in methanol extract and other solvent extract followed a similar trend. It can be concluded that there is a direct correlation between TPC and FRAP activity.

3.8. Antimicrobial activity

Five solvent extracts of *A. marina* of leaf, stem and pneumatophore showed little antimicrobial activity against Gram-negative bacteria and fungi. The Gram-negative *S. typhimurium* and *E. coli* and the

 Table 1

 Antioxidant activity of different parts of A. marina.

Solvents	DPPH IC ₅₀ (µg/mL)			Sup	eroxide I	C ₅₀ (µg/mL)	Α	BTS IC	₅₀ (μg/mL)	F	ity (mol/g)		
	Leaf	Stem	Pneumatophore	Leaf Stem		Pneumatophore	Leaf	Stem	Pneumatophore	Leaf	Stem	Pneumatophore	
Ethyl acetate	460	200	150	> 1 000	> 1000	940	247	67	72	2.41	5.06	8.91	
Acetone	450	110	108	> 1000	376	584	73	40	> 1 000	2.80	12.16	6.76	
Methanol	248	168	127	480	400	480	52	57	61	4.36	7.04	11.57	
Water	580	840	> 1000	230	352	560	180	75	40	5.60	6.28	2.18	

 Table 2

 Antimicrobial activity of A marina leaf mm.

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Solvents	(Gram-posit	ive bacteri	a		Gram-ı	negative bacteri	a	Fungi			
	B. cereus	$B.\ subtilis$	C. rubrum	S. aureus	E. coli	P. aeruginosa	K. pneumonia	S. typhimurium	C. albicans	C. glabrata	C. neoformans	C. apicola
Hexane	8.5	-	9.5	-	9.0	-	-	13	-	-	-	-
Ethyl acetate	9.0	8.5	9.5	11.0	10.5	-	-	12	10	-	-	-
Acetone	10.5	8.5	9.5	8.5	-	-	-	12	11	-	-	-
Methanol	9.0	8.5	10.5	9.5	-	-	-	-	10	-	-	-
Water	-	8.5	11.5	9.5	-	-	-	-	-	-	-	-

Table 3 Antimicrobial activity of *A. marina* stem. mm.

Solvents	Gram-positive bacteria					Gram-n	egative bacter	ia	Fungi			
	B. cereus	B. subtilis	C. rubrum	S. aureus	E. coli	P. aeruginosa	K. pneumonia	S. typhimurium	C. albicans	C. glabrata	C. neoformans	C. apicola
Hexane	8.0	-	9.0	9.0	11.5	-	-	-	-	-	10	-
Ethyl acetate	10.0	8.5	10.5	10.5	10.0	-	-	-	10	-	10	-
Acetone	10.0	9.0	11.5	11.0	10.5	-	-	-	11	-	-	-
Methanol	9.0	-	10.0	10.0	9.0	-	-	-	10	-	10	-
Water	8.5	-	9.0	-	-	_	-	-	-	_	-	-

 Table 4

 Antimicrobial activity of A. marina pneumatophore. mm.

Solvents	(Gram-posit	ive bacteri	ia		Gram-r	negative bacteri	a	Fungi			
	B. cereus	B. subtilis	C. rubrum	S. aureus	E. coli	P. aeruginosa	K. pneumonia	S. typhimurium	C. albicans	C. glabrata	C. neoformans	C. apicola
Hexane	9.0	8.5	8.5	-	8.5	-	-	-	-	-	-	-
Ethyl acetate	9.0	8.5	9.5	9.5	8.5	-	-	-	-	-	-	-
Acetone	8.5	8.5	8.5	10.0	-	-	-	-	-	-	-	-
Methanol	-	8.5	9.5	-	-	-	-	-	-	-	11	-
Water	-	8.5	9.0	9.0	-	-	-	-	-	-	-	-

fungi *C. albicans* and *C. neoformans* were slightly inhibited. In Gram-positive bacteria, *C. rubrum* was most susceptible bacteria, it was inhibited by all the five solvent extracts of all three parts. Ethyl acetate, acetone and methanol extracts showed antimicrobial activity against all the four Gram-positive bacterial strains.

4. Discussion

Extraction method and extraction solvents greatly influence the extractive yield of medicinal plants. Each plant material and solvent system behave differently because each plant or plant parts possess different phytochemicals in different concentrations. However, it is true that higher yield do not indicate higher biological activity. In the present study maximum extractive yield was in leaf. The difference in the yield of various solvent extracts can be attributed to the polarity of different compounds in different organs or different plants. Difference in yield in different solvent extracts of different organs of same plants was also reported by Khlifi *et al.*[15] and Moteriya *et al.*[16].

Polyphenol is a broad term used to define substances that possess a benzene ring bearing one or more hydroxyl groups, including functional derivative[17]. Phenols and flavonoids biosynthesized in different parts are known for their antioxidant properties[18,19]. Polyphenolics and flavonoid are natural antioxidants based on their abilities to scavenge free radicals and reactive oxygen species[20]. The solvents of different parts showed different levels of TPC. A definite influence of solvents was envisaged. Ethyl acetate extract of pneumatophore had maximum TPC while acetone extract of stem had maximum TPC. On the other hand, leaf solvent extracts had almost same amount of TPC. Hsouna et al.[21] reported the maximum TPC was in ethyl acetate extract of Ceratonia siliqua while Sasikala et al.[22] reported the maximum TPC was in acetone extract of Passiflora leaves, fruit and root. In the present study, the aqueous extract of pneumatophore had the minimum amount of TPC while the aqueous extract of other two parts had higher amounts of TPC. Polarity of extracting solvents greatly influences the extraction of phenols from plant material[23].

Studies of different researchers revealed that there was a direct correlation between TPC and antioxidant activity[24] or TFC and antioxidant activity of plant extracts[25]. In the present study, the maximum TPC was in polar solvent extracts as also reported by Ammar *et al.*[26] and Chanda *et al.*[27] and the maximum TFC was in ethyl acetate extracts. Padalia *et al.*[28] also reported the maximum

flavonoid content in ethyl acetate extracts of different flowers. The acetone extract of stem had the maximum TPC, hence it can be considered as a good source of antioxidants.

The antioxidant activities of plant extracts vary with assay methods because of the complex nature of phytochemicals present in them, the solvent used for extraction, *etc*.[29]. Hence, any single method cannot correctly evaluate the antioxidant efficacy of natural plant extracts since the assays differ in their mechanism of action and in the way the end products are measured[30].

The DPPH antioxidant activity showed a direct correlation between TPC and acetone extract of stem. This was not evident in pneumatophore and leaf. It can be concluded that in the same plant, different parts show different levels of activity and different correlation indication that phenolics are not the only components in the extracts that could possess antioxidant activity. Similar results were reported by Padalia et al.[28] and Pellegrini et al.[31]. There was a direct correlation between TPC and superoxide activity in leaf while in stem and pneumatophore there was no such correlation. Xiao et al.[32] reported a positive correlation between TPC and superoxide activity. In this study, ABTS antioxidant activity showed a correlation with TPC similar to that of DPPH antioxidant activity with TPC. Floegel et al.[33] also reported a strong correlation between DPPH and ABTS antioxidant activities and with TPC. The results suggested that acetone extract of stem and water extract of pneumatophore are more effective in the termination of free radical reactions and this is related to its phenolic content as also reported by Marazza et al.[34]. In the present study, TPC and FRAP activity had direct correlation. Similar results were reported by Katalinic et al.[35] and Banerjee et al.[36].

Different solvent extracts of the three parts of *A. marina* could inhibit Gram-positive bacteria to some extent but did not inhibit Gram-negative bacteria or fungi. The reason may be the crude nature of the solvent extracts and the phytoconstituents needed to inhibit the microorganism may not be fully extracted by cold percolation method since the biological activity of any plant extract depends on the nature of the solvent and extraction method.

In the present study, different solvent extracts of different parts were capable of scavenging a wide range of free radicals, though the best antioxidant activity was shown by stem. The mangrove plant *A. marina* showed considerable antioxidant activity in spite of the crude nature of the extracts evaluated. However, they could inhibit only Gram-positive bacteria to a limited extent. However, they can be further exploited to elucidate their exact mechanism of action and

can be considered as a good source of antioxidant compounds. The antioxidant nature can be exploited as biopreservatives in foods to enhance the shelf life of perishable.

Conflict of interest statement

We declare that we have no conflict of interest.

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