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ORIGINAL ARTICLE

Phytochemical Profiling and Bioactivity of A Mangrove Plant, *Sonneratia apetala*, from Odisha Coast of India

Jayanta Kumar Patra[1,2], Swagat Kumar Das[1], and Hrudayanath Thatoi[1]

ABSTRACT **Objective:** To test the antioxidant, antidiabetic, anticancer and antibacterial activities along with phytochemicals of *Sonneratia apetala* Buch.-Ham. **Methods:** The antibacterial activity was determined by agar well diffusion method. The antioxidant activity was determined by standard assay. The antidiabetic activity was evaluated by α-glucosidase inhibition assay and *in vivo* anticancer property was determined against Ehrlich ascites carcinoma (EAC) cells in Swiss Albino mice. Further partial characterization of the methanol extracts was carried out by thin layer chromatography, high performance liquid chromatography, ^{1}H nuclear magnetic resonance spectroscopy and Fourier transform-infra red spectrum spectral analysis. **Results:** Four solvent extracts (acetone, ethanol, methanol and aqueous) of leaf and bark possess strong antioxidant properties. *In vivo* anticancer activity of methanol extract leaf indicated positive activity showing 34% inhibition against EAC cells in Swiss Albino mice. All extracts exhibited α-glucosidase inhibitory activity in a dose-dependent manner indicating presence of promising antidiabetic properties. The extracts possess strong antibacterial activity against the selected pathogenic bacteria (minimal inhibitory concentration ranging from 1.25–5.00 mg/mL). The partial characterization of the methanol extracts of leaf and bark revealed the presence of phenolics as the lead compound responsible for studied bioactivities of the plant extracts. **Conclusion:** *Sonneratia apetala* extracts have potent antibacterial, antioxidant, antidiabetic and anticancer properties which can be further exploited for its pharmaceutical applications.

KEYWORDS bioactive compounds, chromatography, Ehrlich ascites carcinoma, mangrove, *Sonneratia apetala*

Plant form a valuable source of medicines and despite remarkable progresses in synthetic medicinal chemistry, over 25% of the prescribed drugs are derived directly or indirectly from plants.[1] The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (photochemical). Systematic endogenous knowledge gathering on the utilization of known plants among communities may result in the discovery of novel and effective compounds. In India, indigenous herbal remedies such as Ayurveda and other Indian traditional medicine have since ancient times used plants for treatment of various diseases including cancer and diabetes. Even though, these traditional practices are empirical in nature, over 200 million people in India with limited access to primary healthcare centers, depend on traditional needs.[2] Therefore, current researches have been devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them.

Recently much attention has been focused on reactive oxygen species and free radicals which lead to oxidative stress.[3,4] Oxidative stress is an important contributor to a variety of pathological conditions including cardiovascular dysfunction, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury, cancer, diabetes and neurodegenerative diseases.[5] Almost all organisms are well protected against free radical damage by antioxidative enzyme system such as superoxide dismutase and catalase or non enzymatic antioxidant system such as α-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione.[6] However, these systems are frequently insufficient to totally prevent the damage, especially under the conditions of severe oxidative stress, resulting in progression of diseases.[7] This has prompted investigations in the use of natural antioxidants as a complementary therapeutic approach. The antioxidant properties

1. Department of Biotechnology, College of Engineering and Technology, Biju Patnaik University of Technology, Bhubaneswar (751003), India; 2. School of Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk (712749), Republic of Korea
Correspondence to: Dr. Hrudayanath Thatoi, Tel: 91-9437306696, E-mail: hnthatoi@gmail.com

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of plant extracts have been attributed to their polyphenolic content which has gained considerable importance due to their potential as antioxidative, antidiabetic, anticarcinogenic, antibacterial, antiallergic, antimutagenic and anti-inflammatory activities.[8,9] The implication of free radicals in different steps of carcinogenesis is well documented.[10] Phytochemicals containing antioxidant properties have shown capacity to inhibit carcinogenesis.[11] The antioxidants have been reported to reduce the risk of diabetes onset, improve glucose disposal and improve diabetic associated complications. Therefore, medicinal plants with high antioxidant potential could be used effectively to manage diabetes and its associated complications.[12]

Sonneratia apetala Buch.-Ham. is a mangrove tree belonging to the family Lythraceae, abundantly grown in the coastal areas in India, Bangladesh, Malaysia, Australia, etc. It is a fast growing mangrove used in the reforestation of salinity affected areas. According to Bandaranayake,[13] fruits and barks of the plants belonging to genus Sonneratia have remedial activity against asthma, febrifuge, ulcers, swellings, sprains, bleeding, and hemorrhages. There are few reports on the antibacterial and antioxidant activities of fruit of *S. apetala*[14] and other Sonneratia species.[15] However, detailed study on the biological evaluation of the leaf and bark of this plant has not been done so far. Keeping the above factors in consideration, the present study was aimed to evaluate the antioxidant, cytotoxic, antidiabetic and antibacterial activities of various extracts of both leaf and bark of *S. apetala* with a view to characterize the potential bioactive compound present in this plant.

METHODS

Collection and Processing of Plant Material

Fresh, young and tender leaves and bark of *Sonneratia apetala* Buch.-Ham. (Lythraceae) were collected from the mangrove growing areas outside Bhitarkanika sanctuary along Odisha Coast in between 2010–2012. The specimen was identified at Department of Natural Products, Institute of Minerals and Materials Technology, Bhubaneswar (RRL-B), Odisha, India and voucher specimen (VS No. RRL-B-12563) was deposited. The leaves and bark were dried for 15 days and then pulverized into fine powder using mechanical grinder. Fine powder (25 g) of each plant was added to a conical flask along with 100 mL of different polar and non-polar solvents (acetone, ethanol, methanol and water) and was kept in shaker incubator for 48 h. The liquid extracts were evaporated to dryness by vacuum distillation and stored at 4 ℃ for further analysis. Percentage yield was calculated from the dry powdered plant materials.

Bioactivity Evaluation

Screening of Antibacterial Activity

In vitro antibacterial activity of leaf and bark extracts of *S. apetala* was carried out against nine human pathogenic bacteria obtained from Institute of Microbial Technology, Chandigarh and others were lab isolates [*Staphylococcus aureus* (MTCC 1144), *Shigella flexneri* (Lab isolate), *Bacillus licheniformis* (MTCC 7425), *Bacillus brevis* (MTCC 7404), *Vibrio cholerae* (MTCC 3904), *Pseudomonas aeruginosa* (MTCC 1034), *Staphylococcus epidermidis* (MTCC 3615), *Bacillus subtilis* (MTCC 7164) and *E.coli* (MTCC 1089)]. Antibacterial evaluation of the solvent extracts was undertaken by agar cup plate method of Khalid, et al[16] and minimal inhibitory concentration (MIC) was determined by 2-fold microdilution method.[17] Streptomycin (35 μg/disc) was used as the standard antibiotics. The results were expressed and analyzed on the basis of MIC values as described by Kuete and Efferth.[18] The results of antibacterial activity of all the solvent extracts are categorized into three types: significant (MIC <2 mg/mL), moderate (MIC: 2–5 mg/mL) and weak (MIC >5 mg/mL).

Screening of Antioxidant Activity

The *in vitro* antioxidant activity of the solvent extracts was evaluated by the following experiments. Total phenolic content was estimated according to the method of Slinkard and Singleton[19] using catechol as standard phenolic compound. The ascorbic acid content was estimated following the methods of Barros, et al[20] with slight modifications. Total antioxidant capacity of plant extracts was determined by Prieto, et al.[21] The reducing power of solvent extracts was determined by the method of Oyaizu.[22] The 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging effect was determined by following modified method of Hatano, et al.[23] The ferrous ion chelating activity was assessed as described by Zhao, et al.[24] Nitric oxide scavenging activity was assessed by Griess reaction method.[25] The ABTS free radical scavenging activity of the mushroom extracts were determined by the following method of Thaipong, et al.[26] Butylated hydroxyl toluene (BHT) was used as

reference standard. The result for DPPH, ABTS, Ion chelating and reducing power assays were expressed as effective concentration showing 50% scavenging potential (EC_{50}). In case of the reducing power, the extracts effective concentration (EC), providing 0.5 absorbance (EC_{50}) was calculated from the graph of absorbance registered at 700 nm against the corresponding extract concentration.[27]

Screening of Antidiabetic Potential

The α-glucosidase inhibition assay was performed according to the method of Apostolidis, et al.[28] α-Glucosidase (1 U/mL) was premixed with 0.1, 0.5 and 1.0 mg of extract and made up to 500 μL with 50 mmol/L phosphate buffer solution (PBS) at pH 6.8, while in positive controls acarbose replaced the plant extracts. Then it was incubated for 5 min at 37 ℃; 1 mmol/L of paranitrophenyl alpha D glucopyranoside (pNPG, 200 μL) in 50 mmol/L of phosphate buffer was added to initiate the reaction and the mixture was terminated by the addition of 500 μL of 1 mol/L sodium carbonate and the final volume was made up to 1.5 mL with water. α-Glucosidase activity of the mixture was determined by measuring the quantity of nitrophenol released from pNPG. The absorbance of the mixture at 405 nm was measured using a multimode plate reader (Biorad). The results were expressed in concentration of extract required to inhibit 50% of α-glucosidase (IC_{50}). The percent inhibitory activities of α-glucosidase was calculated using the following formula: Inhibition (%) = $(Abs_{control}–Abs_{sample})/Abs_{control} \times 100$. Where, $Abs_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample) and Abs_{sample} is the absorbance of the test sample. All the experiments were carried out in triplicates.

Screening of Anticancer Activity

The evaluation of anticancer activity of methanol extract of leaf of *S. apetala* was determined *in vivo* as described by Debnath, et al[29] against Ehrlich Ascites carcinoma (EAC) cells in Swiss Albino mice (10 weeks old; 18–20 g). The extracts were administered intra-peritoneally at a dose of 0.2 mmol/kg body weight. EAC cells were maintained *in vivo* in Swiss Albino mice (10 mice for each set of experiment), by passaging every 10 days. EAC cells of 9 day old were used for the screening. Plant extract (25 μg/mL) was suspended in PBS (pH 7.2) using 2% Tween 80. On day 1, 100 μL of EAC cells (10×10^6 cells/mL of isotonic saline) per 10 g body weight of the animals was injected intra-peritoneally. Seven doses of the plant extracts (0.2 mmol/kg body weight, 100 μL/10 g body weight) were injected intra-peritoneally from day 2 to day 8 in the test group. Control animals received only vehicle. After a 6 h fasting condition, on day 9, all animals were sacrificed. The fluid in the peritoneal cavity were collected and re-diluted 10 times and the cell viability was counted in homocytometer. Mitomycin C at a dose of 1 mg and 10 mg/kg body weight respectively were used as standard.

Chemical Characterization

Phytochemical Analysis

A qualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, cardiac glycosides, sterols, anthroquinone glycosides, carbohydrates and protein was carried out using standard procedures[30] and quantitative phytochemical tests for determination of proteins, carbohydrates, flavonoid, alkaloids, riboflavin, thiamine, tannins and free amino acid[30,31] and nitrogen, potassium, phosphorous, potassium pentoxide, potassium dioxide[32] were carried out using standard procedures.

Chromatographic Characterization

For Thin layer chromatography profile of the methanol extract of leaf of *S. apetala* was undertaken by standard procedure as described by Kumar, et al[33] and Sadasivam and Manickum.[31] The procedure was followed on various solvent-solvent ratios (n-butanol: acetic acid: water in 12:3:5, 12:1:1, 10:1:1, and 4:1:1, respectively) until good resolution was noticed. Catechol was used as reference standard and the retention factor (Rf) values of all the spots were determined. For column chromatography, the extract was adsorbed onto silica gel by triturating in a mortar and left for about 10 h to dry. The column (20 cm × 25 cm) was packed with a solution of silica gel with n-butanol using the wet slurry method. About 20 fractions are eluted and collected in dry glass bottles.[34]

Bioautography Screening

Antibacterial screening of the separated column fractions were carried out by agar well diffusion method against standard test microorganisms (*B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. flexneri*) as described by Patra, et al.[35] For TLC plate bioautography, the chromatogram developed by thin layer chromatography (TLC) was placed in a

sterile bioassay petri dish and an inoculum of different bacterial strains containing CFU/mL in molten nutrient agar was distributed over the plates and incubated for 24 h at 37 ℃. Clear zones around the TLC plates confirmed the antibacterial property of the fraction.[36] Separated compounds on TLC plates were sprayed with 1,1-diphenyl 2-picrylhydrazl (methanolic DPPH) to detect the presence of antioxidant active compound. It gives an orange spot or a deep yellow colored spot on a purple background.[37]

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) analysis of methanol extracts of both leaf and bark of *S. apetala* was performed by HPLC analyser (Shimadzu Corporation, Japan). The column used was Phenomenex Luna 5 μ C18 (2) 100 A (250.0 mm × 4.6 mm) at ambient temperature. The solvent system optimized for the analysis was methanol: acetonitrile: water in the ratio of 25:35:40. The flow rate was 1 mL/min and detection wave length was set at 232 nm.

Fourier Transform-Infra Red Spectrum Analysis

The methanol extract of leaf and bark of the plant (10 mg) was grinded and dispersed with 90 mg of micronized dried IR grade potassium bromide (KBr). The disc was then scanned over a wave number range of 4000 to 400 cm^{-1} using IR Affinity-1 Fourier transform-infra red (FT-IR) spectrometer (Shimadzu, Japan) and the spectra were recorded in the transmittance mode from 4000 to 400 cm^{-1} using IR solution software. The wave numbers of different components present in active sample were analyzed and the possible stretches of functional groups were identified.

Nuclear Magnetic Resonance Spectroscopy Analysis

^{1}H nuclear magnetic resonance spectroscopy (NMR) spectra of methanol extract of leaf of *S. apetala* was recorded on a NMR-400 MHz (with multi-nuclei analysis from ^{1}H, ^{19}F to ^{15}N) and chemical shifts were recorded as δ values. The result graph was compared with the reference chart and possible functional group present in the plant was determined.[38]

Statistical Analysis

Experiments were carried out in triplicates and the data was expressed as mean ± standard deviation. Where applicable, the means of all the parameters were examined for significance by two way analysis of variance (ANOVA) and the differences between samples were determined by Duncan's Multiple Range test using GenStat discovery (edition 3) statistical software package. Differences were considered significant at a probability level of $P<0.05$.

RESULTS

Bioactivity Evaluation

Screening of Antibacterial Properties

The leaf and bark extracts of *S. apetala* showed antibacterial activity against most of the tested pathogenic bacteria (Table 1). Among all the four solvent extracts, the acetone extract in both leaf and bark samples showed significant antibacterial activity with the zone of inhibitions ranging from 12.0 ± 0.5 to 22.0 ± 1.4 mm (data not shown) whereas all other solvent extracts (ethanol, methanol and aqueous) showed antibacterial activity against a few microorganisms such as *B. licheniformis*, *B. brevis* and *V. cholerae*, but no activity against the bacterial strains such as *S. flexneri*, *S. epidermidis* and *E. coli*. The results were statically analyzed and the least significant difference (LSD) values were calculated for leaf sample (LSD: 4.305) and bark sample (LSD: 4.313) separately at $P<0.05$. The MIC values in all the solvent extracts of both leaf and bark of *S. apetala* ranged from 1.25 to 5.0 mg/mL (Table 1) similarly the minimum bactericidal concentration (MBC) values varied from 2.5 mg/mL up to values greater than 5.0 mg/mL (Table 1). It was found that, the acetone leaf extract showed significant antibacterial activity against *S. aureus* and *V. cholera* with MIC values of 1.25 mg/mL whereas all other solvent extracts of both leaf and bark sample showed moderate or weak antibacterial activity (Table 1). The antibiotic, streptomycin (35 μg/disc) showed antibacterial activity against all the text pathogen (22.0 ± 0.7 to 32.0 ± 0.7 mm). In case of the standard antibiotics, the MIC <10 μg/mL is significant, moderate antibacterial values are with MIC of 10–100 μg/mL. On these criteria, the standard antibiotics showed significant antibacterial activity with MIC less than 10 μg/mL against almost all the tested bacteria (Table 1).

Screening of Antioxidant Activity

About seven antioxidant assays were performed on different solvent extracts of *S. apetala* to study the antioxidant potential of the plant parts (Tables 2 and 3). The ethanol extract of the bark showed maximum phenol and ascorbic acid content (8.63% ± 0.02% and 14.4% ± 0.14% dry weight, respectively) as compared to all other three extracts (Table 2). The total antioxidant

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Table 1. MIC and MBC of Solvent Extracts of *S. apetala* against Nine Bacterial Pathogens (mg/mL)

Strains	Extract	Acetone		Ethanol		Methanol		Aqueous		Streptomycin (μg/mL)	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	Leaf	1.25	2.5	–	–	2.5	5	2.5	5	17.50	35.00
	Bark	2.5	5	2.5	5	5	>5	–	–		
S. flexneri	Leaf	2.5	5	–	–	2.5	5	2.5	5	4.32	8.75
	Bark	2.5	5	2.5	5	5	>5	–	–		
B. licheniformis	Leaf	2.5	5	5	>5	2.5	5	5	>5	8.75	17.50
	Bark	2.5	5	5	>5	5	>5	5	>5		
B. brevis	Leaf	2.5	5	5	>5	5	>5	5	>5	1.08	2.16
	Bark	5	>5	5	>5	5	>5	5	>5		
V. cholerae	Leaf	1.25	2.5	5	>5	5	>5	–	–	1.08	2.16
	Bark	5	>5	–	–	2.5	5	5	>5		
P. aeruginosa	Leaf	2.5	5	–	–	2.5	5	5	>5	8.75	17.50
	Bark	2.5	5	5	>5	>5	>5	5	>5		
S. epidermidis	Leaf	2.5	5	–	–	2.5	5	–	–	8.75	17.50
	Bark	2.5	5	–	–	–	–	–	–		
B. subtilis	Leaf	2.5	5	–	–	2.5	5	>5	>5	4.32	8.75
	Bark	2.5	5	>5	>5	5	>5	–	–		
E. coli	Leaf	2.5	5	–	–	2.5	5	–	–	1.08	2.16
	Bark	2.5	5	–	–	–	–	–	–		

Notes: – Not detected

Table 2. Antioxidant Potential of Solvent Extracts in Leaf and Bark of *S. apetala*

Plant species	Extract	Acetone	Ethanol	Methanol	Aqueous
Total phenol content	Leaf	5.23 ± 0.18	5.75 ± 0.04	5.08 ± 0.06	3.18 ± 0.01
(in % dry weight)	Bark	6.17 ± 0.0	8.63 ± 0.02	7.35 ± 0.05	4.40 ± 0.04
Ascorbic acid content	Leaf	8.50 ± 0.14	9.40 ± 0.14	10.65 ± 0.07	8.25 ± 0.21
(in % dry weight)	Bark	11.54 ± 0.05	14.40 ± 0.14	13.15 ± 0.07	4.10 ± 0.28
Total antioxidant capacity	Leaf	133.41 ± 1.85	271.52 ± 3.85	222.59 ± 7.27	128.63 ± 4.06
(in mg/gm dry weight)	Bark	138.2 ± 2.68	52.30 ± 6.48	70.39 ± 1.20	151.68 ± 3.43

capacity was found out to be more in the ethanol leaf extract (271.52 ± 3.85 mg/g dry weight) as compared to other extracts and bark sample (Table 2).

Among the four scavenging experiments, both the leaf and bark extracts showed significant dose dependent DPPH free radical activity with the EC_{50} values ranging from 39.9 to 54.48 μg/mL (Table 3). Out of the four solvent extracts, the methanol extracts in both leaf and bark sample showed higher activity (EC_{50} 39.9 ± 0.47 μg/mL). In case of ABTS free radical scavenging assay, all the four solvent extracts were comparatively active with EC_{50} values ranging from 39.30 to 56.57 μg/mL. In case of the nitric acid scavenging assay (NOX), the EC_{50} values ranged between 58.84 to 103.29 μg/mL. All the four extract showed less metal chelating (MC) activity with EC_{50} values ranging from 384.07 μg/mL to 3436.42 μg/mL. It was found that the methanol extracts of both leaf and bark sample showed comparatively higher reducing potential than other solvent extracts with EC_{50} values 585.35 and 491.39 μg/mL respectively (Table 3). The LSD values of individual experiments for leaf and bark samples were determined statistically as 1.475 and 2.634 for DPPH assay, 1.231 and 2.112 for ABTS assay, 0.2599 and 0.2527 for NOX, 0.2068 and 0.3113 for MC assay and 0.00299 and 0.00287 for reducing assay, respectively. The results of all the four solvent extracts of both leaf and bark samples of the plant concluded that the methanol fraction possesses comparatively better antioxidant potential. The result depicted that the solvent extracts were able to scavenge the free radicals effectively at a lower concentration (EC_{50} 39.9 μg/mL), however they were not so much active

Table 3. EC_{50} Values of All Antioxidant Assays by *S. apetala* Extracts and Standard BHT (μg/mL)

Antioxidant assay	Extract	Acetone	Ethanol	Methanol	Aqueous	BHT
DPPH free radical scavenging assay	Leaf	41.32 ± 4.29	41.12 ± 0.59	39.90 ± 0.47	41.92 ± 0.48	151.67 ± 0.20
	Bark	40.78 ± 0.86	40.81 ± 0.47	40.64 ± 0.23	54.48 ± 1.38	
Nitric oxide scavenging assay	Leaf	63.54 ± 0.14	103.29 ± 0.22	66.95 ± 0.36	58.84 ± 0.29	84.64 ± 1.06
	Bark	63.83 ± 0.29	79.81 ± 0.51	60.01 ± 0.14	62.72 ± 0.22	
ABTS scavenging assay	Leaf	41.12 ± 0.56	39.30 ± 0.85	41.80 ± 0.61	44.75 ± 0.95	51.37 ± 0.49
	Bark	40.43 ± 1.30	39.04 ± 0.67	49.59 ± 1.34	56.57 ± 1.99	
Metal chelating assay	Leaf	1501.5 ± 3.33	3436.42 ± 1.45	0 ± 0	384.07 ± 0.98	519.83 ± 0.53
	Bark	780.24 ± 2.98	3012.04 ± 1.66	857.63 ± 0.39	0 ± 0	
Reducing power	Leaf	681.62 ± 2.46	572.04 ± 1.74	585.35 ± 0.69	1910.03 ± 0.43	159.10 ± 0.42
	Bark	884.39 ± 0.51	1573.58 ± 2.54	491.39 ± 1.69	870.24 ± 0.28	

Table 4. IC_{50} Values for Yeast α-Glucosidase Inhibition by *S. apetala* Extracts and Standard Acarbose ($\bar{x} \pm s$, mg/mL)

Extract	Acetone	Ethanol	Methanol	Aqueous	Acarbose
Leaf	0.608 ± 0.007	0.359 ± 0.006	0.286 ± 0.022	0.493 ± 0.020	0.017 ± 0.003
Bark	0.637 ± 0.008	0.439 ± 0.005	0.432 ± 0.010	0.435 ± 0.017	

for chelating metal ions as well as with less reducing power.

Screening of Antidiabetic Potential

As shown in Table 4, It was found that the methanol extract of both the leaf and bark sample showed comparatively better antidiabetic potential with IC_{50} values of 0.286 ± 0.022 and 0.432 ± 0.01 mg/mL, respectively (Table 4). Under the experimental conditions, the standard glucosidase inhibiting drug, acarbose inhibited α-glucosidase enzyme with IC_{50} value 0.017 ± 0.003 mg/mL. The methanol extracts of leaf had the highest α-glucosidase inhibitory potential among the extracts.

In vivo Screening of Anticancer Potential

The *in vivo* anticancer property of the methanol extract of leaf of *S. apetala* showed 34.0% inhibition against EAC. Mitomycin C at a dose of 1 mg and 10 mg/kg body weight respectively was used as standard, which showed 100% inhibition at all times at all cases.

Chemical Characterization

Phytochemical Analysis

All the four solvent extracts of *S. apetala* showed the presence of almost all the phytochemicals except cardiac glycosides which were only present in acetone and ethanol extracts of both the plant samples and saponins, which are only present in ethanol and aqueous extracts of the bark (Table 5). The quantitative phytochemical analysis of the dry plant powder of *S. apetala* also showed the presence of primary and secondary metabolites as well as vitamins and minerals in moderate quantity (Table 6). The result showed that both the leaf and bark of the plant are rich in carbohydrate and protein as well as all the secondary metabolites studied. However, they showed comparatively less thiamine and riboflavin content along with the minerals.

Chromatograpic Profiling

In order to obtain some information on the active components and functional groups present in crude extract of *S. apetala*, different types of chromatographic techniques viz. paper chromatography, TLC, column chromatography along with HPLC, ^{1}H NMR and FTIR studies were undertaken. Prior to the chromatographic separation, the solvent system n-butanol: acetic acid: water in the ratio 4:1:1 was selected as the appropriate solvent system for chromatography. The TLC chromatogram showed two clear and distinct spots/bands with the selected solvent system at ratio 4:1:1 with Rf 0.178 and 0.296 as against other three ratios of the solvent system which gave unclear spots with varying Rf values (Table 7). The presence of phenolic compounds in the extract in the TLC plate was conformed when compared with the reference standard (catechol). In bioautography study, only the upper spot in the TLC chromatogram showed selective antibacterial activity (Table 8). Likewise the column fractions also showed

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Table 5. Screening of Phytochemicals of Leaf and Bark of Different Solvent Extracts of *S. apetala*

Test	Acetone		Ethanol		Methanol		Aqueous	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Alkaloid	+	+	+	+	+	+	+	+
Cardiac glycoside	+	+	+	+	–	–	–	–
Anthraquinone glycoside	+	+	+	+	+	+	–	–
Tannin	+	+	+	–	+	–	+	+
Steroids	+	+	+	+	+	+	+	–
Saponins	–	–	–	+	–	–	–	+
Flavonoids	+	+	+	+	+	+	+	+
Gums and mucilages	+	+	+	+	+	+	–	+
Carbohydrates	+	+	+	+	+	+	+	–
Proteins and amino acids	+	+	+	+	+	+	+	+
Terpinoid	+	+	+	+	+	–	+	+

Notes: "+" means present; "–" means absent

Table 6. Quantitative Phytochemical, Vitamins and Minerals Content of Crude Plant Powders of *S. apetala* ($\bar{x} \pm s$, % of dry weight)

Content	Leaf	Bark
Primary metabolites		
Carbohydrate content	9.85 ± 0.04	9.68 ± 0.25
Protein content	10.20 ± 0.07	10.12 ± 0.01
Total free amino acid	0.01 ± 0.01	0.01 ± 0.00
Secondary metabolites		
Phenol	1.74 ± 0.04	1.84 ± 0.06
Flavonoid	1.80 ± 0.04	1.38 ± 0.10
Tannin	2.15 ± 0.04	2.11 ± 0.14
Alkaloid	1.58 ± 0.06	1.25 ± 0.03
Saponin	13.80 ± 0.03	7.20 ± 0.07
Vitamins		
Thiamine	0.19 ± 0.00	0.198 ± 0.00
Riboflavin	0.02 ± 0.00	0.019 ± 0.01
Minerals		
Nitrogen content	1.64 ± 0.20	1.62 ± 0.19
Phosphorous content	0.18 ± 0.01	0.16 ± 0.02
Potassium content	1.02 ± 0.03	1.03 ± 0.01
P_2O_5 content	0.41 ± 0.03	1.22 ± 0.24
K_2O content	0.39 ± 0.01	1.23 ± 0.03

Table 7. TLC of Methanol Extract of Leaf of *S. apetala* Using Different Solvent System

Solvent System	No. of bands	RF value
N:A:W=12:3:5	1	A=0.416
N:A:W=10:1:1	–	–
N:A:W=12:1:1	1	A=0.324
N:A:W=4:1:1	2	A=0.178, B=0.296

Notes: "N:A:W" means n-butanol : acetic acid : water

Table 8. Bioautography of Methanol Extracts of Leaf of *S. apetala* against the Pathogenic Strains

Strains	Activity
S. aureus (MTCC 1141)	No inhibition
S. flexneri (lab isolated)	No inhibition
P. aeruginosa (MTCC 1034)	Only upper portion
B. subtilis (MTCC 7461)	Only lower portion
E. coli (MTCC 1089)	No inhibition

potent antibacterial activity (Table 9).

The HPLC analysis of leaf of *S. apetala* gave 4 peaks with the maximum height at 2.567 min of retention time whereas in the bark extract only 2 peaks were observed with maximum height at 2.480 min retention time (Figure 1). The HPLC chromatogram of the plant extract was compared with the HPLC chromatogram of standard catechol which also showed characteristics peak at about 2.5 min retention. ^{1}H NMR analysis of methanol extract of leaf of *S. apetala* showed presence of β-substituted aliphatic and α-mono substituted aliphatic group of compounds (data not shown). FT-IR spectra of methanol extract of leaf and bark of *S. apetala* exhibited similar characteristic absorption bands as compared to standard catechol at 3412, 1741, 1263, 1112 and 826 cm^{-1} (Figure 2). The leaf extract showed 9 peaks stretching from 3412.22 to 826.53 cm^{-1} (Figure 2A). Likewise the bark extract showed 7 peaks ranging from 3814.76 to 1073.43 cm^{-1} (Figure 2B).

DISCUSSION

The present investigation demonstrates that

Table 9. Antibacterial Screening of Column Fractions of Methanol Extract of Leaf of *S. apetala*

Strains	Column fractions									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
S. aureus (MTTC-1141)	+	–	–	–	–	–	+	–	–	–
S. flexneri (lab isolated)	–	+	–	–	–	+	+	–	–	–
P. aeruginosa (MTCC-1034)	–	+	–	–	–	+	+	+	–	–
B. subtilis (MTCC-7461)	+	+	–	–	+	+	+	+	–	–
E. coli (MTCC-1089)	–	–	–	–	–	+	–	+	–	–

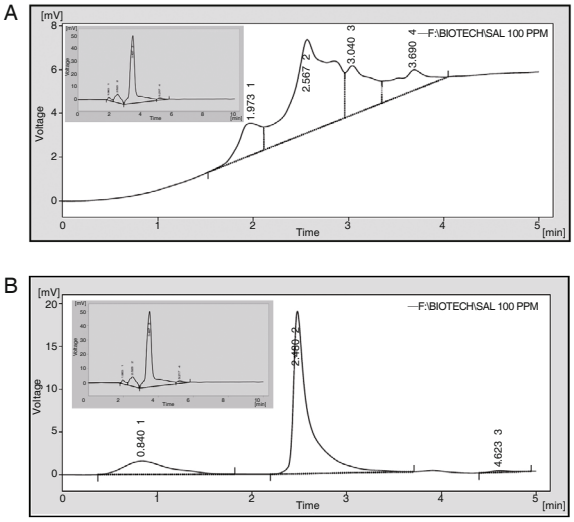

Figure 1. HPLC Analysis of Methanol Extract of Leaf and Bark of *S. apetala*

Notes: A: leaf; B: bark

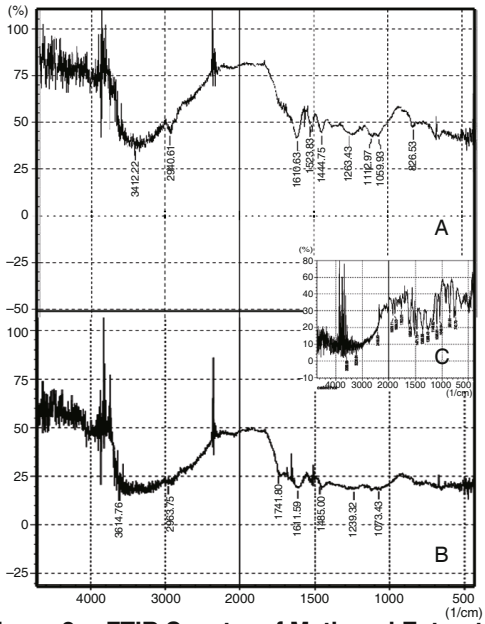

Figure 2. FTIR Spectra of Methanol Extract of Leaf and Bark of *S. apetala*

Notes: A: leaf; B: bark; C: standard catechol

acetone, ethanol, methanol and aqueous extracts derived from *S. apetala* (both barks and leaves) possess antibacterial, antioxidant, antidiabetic and anticancer properties. Although some reports exist on antibacterial and antioxidant study of *S. apetala*, the present study is different with respect to extracts used, strains employed for the antibacterial assay and antioxidant assays conducted. The present study along with earlier reports validates the ethnomedicinal uses of the *S. apetala* for cure of different diseases.[39]

Antibacterial results in the present study showed that both leaves and barks of acetone, ethanol, methanol and aqueous extracts of *S. apetala* were able to inhibit the growth of gram-positive and gram-negative bacteria. However, there is no inhibition found in the aqueous extract of bark of *S. apetala* against *B. brevis*. It is a standard principle that crude solvent extracts of plants which shows a MIC values <8 mg/mL are considered potentially useful for therapeutics.[40] In the present study, all the extracts of both leaf and bark of *S. apetala* showed potent antibacterial activity against the test pathogens with the MIC values ranging 1.25 to 5 mg/mL, which are comparable with standard antibiotics streptomycin. Results of the present findings also corroborates with previous published reports.[41,42] The result further revealed that antibacterial potency of the bioactive compounds was not affected when extracted in hot conditions, indicating that the plant material contains thermo-stable bioactive compounds. The present findings are hence encouraging in recognizing a plant showing interesting antibacterial activity.

Mangrove plants and their parts are potent source of natural antioxidants as they survive in high environmental stress conditions. The antioxidant and free radical scavenging potential of acetone, ethanol, methanol and aqueous extracts of barks and leaves of *S. apetala* may be attributed to the nature of different chemical compounds present in them as confirmed by phytochemical tests. Hossain, et al[14] have reported that the antioxidant potential of fruit of *S. apetala* which

conforms from our findings that not only fruit but also leaf and bark of this plant are rich source of natural antioxidants (Tables 2 and 3). Many researchers have also reported positive correlation between free radical scavenging activity and total phenolic compound[43] which is also corroborated with the present finding. The free radical scavenging capacity of compounds has been attributed to various mechanisms such as prevention of chain reaction, chelating metals, reductive capacity and radical scavenging.[43] The distinct scavenging activities of different extracts can be due to the diverse chemical nature of various phytochemicals that react with different types of free radicals in unique way.[44] The extracts of both leaf and bark of *S. apetala* showed potent reducing power which increased with increase in the concentration of sample. The antioxidant activity of both the parts of the plant might be due to the inactivation of free radicals or complexion with metal ions or combination of the two.[44] Similarly the extract shows higher phenol content and polyphenolic compounds, which contribute directly to the antioxidative action of plant extracts because of their radical scavenging ability. The plant extracts possess ascorbic acid in considerable amount highlighting the potential antioxidant richness in the plant parts. The positive chelating and nitric oxide scavenging activity of extracts of both leaf and bark of *S. apetala* might be due to the presence of secondary metabolites like phenolics in the plant. The ethnomedicinal use of this plant by the local healers against diseases like febrifuge, ulcers, swellings, sprains, bleeding are well validated by its strong antioxidant properties as evidenced from the present results.

The antihyperglycaemic response was replicated in an *in vitro* model using α-glucosidase inhibitory activity. One therapeutic approach for treating diabetes is to decrease the postprandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes (α-glucosidase, α-amylase) in the digestive tract. Inhibitors of these enzymes delay and prolong carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise.[45] The present study revealed that all the four extracts (acetone, ethanol, methanol, aqueous) of leaf and bark inhibited α-glucosidase activity with as low as 0.286 mg/mL IC_{50} value in a dose dependent manner. Under the experimental conditions, the standard glucosidase inhibiting drug, acarbose inhibited α-glucosidase enzyme with IC_{50} value of 0.017 mg/mL. The present study revealed that the methanol extracts of leaf has comparatively higher α-glucosidase inhibitory potential among all the extracts. The inhibition of α-glucosidase enzyme by the plant extracts might be attributed to several possible factors such as fiber concentration, presence of inhibitors on fibers, encapsulation enzyme by the fibers present in the sample, etc. The results of *S. apetala* are in support of traditional uses of the species to reduce blood glucose level.[46] It is likely that the long term treatment may achieve the desired results with diabetes mellitus patients.

The present study revealed that only the methanol extract of leaves of *S. apetala* exhibited anticancer activity as compared to other extracts. Results represented in the current study showed that methanol extract of leaves of *S. apetala* inhibited the growth of EAC cells in mice in comparison with the standard drug mitomycin (Table 5). The anticancer activity of *S. apetala* may probably be due to presence of various secondary metabolites such as phenolics in the plant extract which are responsible for decrease in tumor volume and the ascite fluid content in experimental mouse. The anticancer potential of few mangrove plants has also been reported by various workers around the world.[47-49]

In order to obtain some information on the active components and functional groups present in crude extract of *S. apetala*, different types of chromatographic techniques viz. paper chromatography, TLC, column chromatography along with HPLC, ^{1}H NMR and FTIR studies were undertaken. These procedures enable recognition of known metabolites in extracts in the earliest stages of separation and thus economically very important. TLC chromatogram of the methanol extract of both leaf and bark of the plant showed the active compound as phenolic when compared with the standard catechol. The active column fractions showed antibacterial properties when tested against the pathogenic bacteria and antioxidant properties when tested against DPPH free radical, which might be due to the presence of phenolic compounds since phenols have been known to scavenge free radical with multiple biological activities.[50] Similarly the HPLC chromatogram of methanol extract of both the leaf and bark of *S. apetala* was compared with the HPLC chromatogram of standard catechol, which also showed a characteristics peak at 2.5 retention time which confirmed the presence of phenolic compounds in the plant extract.

The HPLC fingerprinting is the best way for chemical characterization of bioactive compounds from plants and their extracted fractions.[51,52] Due to the complexity of the chemical profile of the extracts, it was difficult to interpret the results and give a concrete conclusion, however, only qualitative and quantitative differences were noted. ^{1}H NMR analysis of the methanol extract of the leaf of *S. apetala* showed presence of aliphatic group of compounds (data not shown) as conformed by the standard chart of Silverstein, et al.[38] Partial identification of compounds of the plant parts were carried out by FTIR spectroscopy when run under IR region of 400–4000 cm^{-1}, showed variation in the peaks in both leaf and bark extracts of the plant.

FT-IR spectra of methanol extract of leaf and bark of *S. apetala* exhibited similar characteristic absorption bands as compared with standard catechol at 3412, 1741, 1263, 1112 and 826 cm^{-1}, respectively which are identified by following the references of Coates.[53] The leaf extract showed 9 peaks stretching from 3412.22 to 826.53 cm^{-1}. The band at 3412.22 cm^{-1} is ascribed to hydroxyl group, the band at 1610.63 cm^{-1} is ascribed to primary amine NH bend, the band at 1444.73 cm^{-1} corresponds to saturated alipathic group (methyl C-H bend), The signals at 1263.43–1059.93 cm^{-1} is due to the C-C stretch acetylenic compounds. Likewise the bark extract showed 7 peaks ranging from 3814.76 cm^{-1} to 1073.43 cm^{-1}. The band at 2863.75 cm^{-1} corresponds to methyl C-H asym whereas the band at 1611.50 cm^{-1} corresponds to organic nitrates. The band at 1073.43 cm^{-1} ascribed to cyclic ethers, C-O stretching. FTIR spectroscopy data represents the molecular adsorption and transmission, creating a molecular fingerprint of the compounds present in the sample. The curative properties of the plant extracts might be due to the presence of these aromatic and aliphatic groups of compounds which are mostly phenolics (phenols, flavonoids, tannins and saponins) and alkaloids, terpenes (aliphatic group).[54-58] The presence of such phytochemicals in the mangrove plant extracts may be correlated with the biological activities exhibited by the plants.

Based on the results it can be concluded that the acetone, ethanol, methanol and aqueous solvent extracts of leaves and barks of *S. apetala* possesses pronounced antibacterial, antioxidant, antidiabetic and anticancer potential which tend to support their use in the traditional medicines. The present study reveals that crude extracts have the potential as antibacterial compounds against microorganisms and may be utilized in the treatment of infectious diseases caused by resistant organisms. Further evaluation of the antibacterial properties of the plant extracts against a more extensive panel of microbial agents is reasonable. These extracts contain a plethora of chemicals that can scavenge free radicals, chelate metal ions, etc., which can be related to their enormous antioxidant potentials. Our results suggest that one of the targets for hypoglycaemic property of *S. apetala* is α-glucosidase enzyme inhibition. The anticancer study also revealed that they can also be a source for the development of novel anticancer drug leads. This investigation highlights the health benefits of *S. apetala* and lends some scientific support to their traditional use. These extracts contains series of chemicals mainly phenolics which showed anticancer and antidiabetic properties. Further bioassay-guided fractionation approaches will be required on these species to identify and/or purify the major active constituents. It should be noted that *in vivo* tests should also be carried out to investigate whether the *in vitro* results reported translate into activities that might support the traditional uses of this plant. This plant parts can be exploited in preparation of natural drugs for the treatment of various diseases with appropriate pharmaceutical approaches.

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Conflict of Interest

We declare that we do not have any conflict of interest.

Author Contributions

Patra JK has conceptualized and conducted the experiments and contributed immensely in the preparation of the manuscript. Das SK contributed in part of the bioactivity studies and the preparation of the manuscript. Thatoi HN has designed and edited the manuscript.

REFERENCES

1. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat Prod Rep 2000;17:175-285.
2. Edler C. Ayurveda for diabetes mellitus: a review of the biomedical literature. Altern Ther Health Med 2004;10:44-50.
3. Block G, Patterson B, Subar A. Fruits, vegetables, and cancer prevention: a review of the epidemiological evidence. J Nutri Cancer 1992;18:1-29.
4. Tsai TH, Tsai PJ, Ho SC. Antioxidant and anti-inflammatory activities of several commonly used spices. J Food Sci 2005;70:93-97.
5. Slater TF. Antioxidant, vitamins and carotene in disease prevention. Am J Clin Nutr 1991;53:189S-396S.
6. Betancur-Ancona D, Peraza-Mercado G, Moguel-Ordoñez Y. Fuertes-Blanco S. Physicochemical characterization of lima bean (*Phaseolus lunatus*) and Jack bean (*Canavalia ensiformis*) fibrous residues. J Food Chem 2004;84:287-229.
7. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993;90:7915-7922.
8. Altmann HJ, Orunow W, Mohr U, Richter-Reichhelm HB, Wester PW. Effects of BHA and related phenols on the fore stomach of rats. J Food Chem Toxicol 1986;24:1183-1188.
9. McCormick DL, May CM, Thomas CF, Detrisac CJ. Anticarcinogenic and hepatotoxic interactions between retinyl acetate and butylated hydroxytoluene in rats. Cancer Res 1986;46:5264-5269.
10. Halliwell B. Oxidative stress and cancer. Biochem J 2007;401:1-11.
11. Wang S, Meckling KA, Marcone MF, Kakuda Y, Tsao R. Can phytochemical antioxidant rich foods act as anti-cancer agents? Food Res Int 2011;44:2545-2554.
12. Hossain SJ, El-Sayed M, Aoshima H. Antioxidative and anti α-amylase activities of four wild plants consumed by pastoral nomads in Egypt. Orient Pharm Exp Med 2009;9:217-224.
13. Bandaranayake WM. Traditional and medicinal uses of mangroves. Mangroves Salt Marshes 1998;2:133-148.
14. Hossain SJ, Basar MH, Rokeya B, Arif KMT, Sultana MS, Rahman MH. Evaluation of antioxidant, antidiabetic and antibacterial activities of the fruit of *Sonneratia apetala* (Buch.-Ham.). Orient Pharm Exp Med 2013;13:95-102.
15. Saad S, Taher M, Susanti D, Qaralleh H, Awang AFIB. *In vitro* antimicrobial activity of mangrove plant *Sonneratia alba*. Asian Pac J Trop Biomed 2012;2:427-429.
16. Khalid F, Siddiqi R, Mojgani N. Detection and characterization of a heat stable bacteriocin (Lactocin LC-09) produced by a clinical isolate of Lactobacilli. Med J Islamic Acad Sci 1999;12:67-71.
17. Dewanjee S, Kundu M, Maiti A, Majumdar R, Majumdar A, Mandal SC. *In vitro* evaluation of antimicrobial activity of crude extract from plants *Diospyros peregrina*, *Coccinia grandis* and *Swietenia macrophylla*. Trop J Pharmacol Res 2007;6:773-778.
18. Kuete V, Efferth T. Cameroonian medicinal plants: pharmacology and derived natural products. Frontiers Pharmacol 2010;1:123.
19. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enology Viticult 1977;28:49-55.
20. Barros L, Ferreira MJ, Queiros B, Ferreira IC, Baptista P. Total phenol, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushroom and their antioxidant acitivities. Food Chem 2007;103:413-419.
21. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phodphomolybdenum complex: specific application to the determination of Vitamin E. Anal Biochem 1999;269:337-341.
22. Oyaizu M. Studied on product of browning reaction, prepared from glucose amine. Jpn J Nutr 1986;44:307-315.
23. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharma Bull 1988;36:2090-2097.
24. Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L, et al. Effect of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in Barely (*Hordeum vulgare* L.). J Agri Food Chem 2006;54:7277-7286.
25. Kiran G, Rajyalakshmi G, Baburao B, Venkateshwar Rao J, Sarangapani M. Free radical scavenging activity of some isatin-5-sulphonamide derivatives. Pharmacol Online 2009;1:540-545.
26. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal 2006;19:669-675.
27. Velez Z, Campinho MA, Guerra ÂR, García L, Ramos P, Guerreiro O, et al. Biological characterization of *Cynara cardunculus* L. methanolic extracts: antioxidant, anti-proliferative, anti-migratory and anti-angiogenic activities. Agriculture 2012;2:472-492.
28. Apostolidis E, Kwon YI, Shetty K. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov Food Sci Emerg Technol 2007;8:46-54.
29. Debnath B, Srikanth K, Banarjee S. 1,5-N,N'-disubstituted-2-(dubstituted benzenesulphonyl)-glutamamides as antitumor agents. Part 2. Synthesis, biological activity and

QSAR study. Internet Elec J Mol Design 2002;1:488-502.
30. Edeoga HO, Okwa DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;4:685-688.
31. Sadasivam S, Manickam A, eds. Biochemical methods. New Age, International (P) Ltd. Publication: New Delhi; 2005.
32. Trivedy RK, Goel PK, Trisal CL, eds. Practical methods in ecology and environmental sciences. Karod: Enviromental Media Publication; 1988.
33. Kumar T, Ray S, Brahmacharya RL, Ghose M. Preliminary GC-MS analysis of compounds present in root exudates of three mangroves Sp. Acta Chromatograph 2009;1:117-125.
34. Tomer K, Singh V, Sethiya NK. Isolation and Characterization of new lanosteroid from ethanolic extracts of *Eclipta alba* Linn. J Pharm Res 2009;2:1635-1637.
35. Patra JK, Panigrahi TK, Rath SK, Dhal NK, Thatoi HN. Phytochemical screening and antimicrobial assessment of leaf extracts of *Excoecaria agallocha* L.: a mangal species of Bhitarkanika, Orissa, India. Adv Nat Appl Sci 2009;3:241-246.
36. Raouf NA, Ibraheem BM. Antibiotic activity of two *Anabaena* Sp. against fish pathogens. Afr J Biotechnol 2008;7:2244-2248.
37. Adeloye O, David AA, Obafemi AC. Studies on antimicrobial, antioxidant and phytochemical analysis of Urena lobata L. J Phy Nat Sci 2007;1:1-8.
38. Silverstein RM, Webster FX, Kiemle DJ, eds. Proton NMR spectrometry. Spectrometric identification of organic compounds. USA: John Wiley and Sons-Inc; 2005.
39. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol Manag 2002;10:421-452.
40. Fabry W, Okemo PO, Ansorg R. Antibacterial activity of East African medicinal plants. J Ethnopharmacol 1998;60:79-84.
41. Varaprasad B, Varahalarao V, Naidu KC. Mangrove plant *Sonneratia apetala* antimicrobial activity on selected pathogenic microorganisms. Ori J Chem 2009;25:445-447.
42. Jaimini D, Sarkar C, Shabnam AA, Jadhav BL. Evaluation of antibacterial properties of mangrove plant *Sonneratia apetala* Buch. Ham leaf. World Appl Sci J 2011;14:1683-1686.
43. Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, et al. Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. Food Chem Toxicol 2009;47:2083-2091.
44. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agri Food Chem 1995;43:1813-1815.
45. Ali H, Houghton PJ, Soumyanath A. Alpha amylase inhibitory activity of some Malaysian plants used to treat diabetes with particular reference to *Phyllanthus amarus*. J Ethnopharmacol 2006;107:449-455.
46. Sheela CG, Augusti KT. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J Exp Biol 1992;30:523-526.
47. Kathiresan K, Boopathy NS, Kavitha S. Coastal vegetation: An underexplored source of anticancer drugs. Nat Prod Rad 2006;5:114-119.
48. Khajure PV, Rathod JL. Potential anticancer activity of Acanthus ilicifolius extracted from the mangroves forest of Karwar, west coast of India. World J Sci Technol 2011;1:1-6.
49. Milon Md A, Muhit Md A, Goshwami D, Masud MM, Begum B. Antioxidant, cytotoxic and antimicrobial activity of *Sonneratia alba* bark. Int J Pharm Sci Res 2012;3:2233-2237.
50. Nagavani V, Ragahava RT. Evaluation of antioxidant potential and identification of polyphenols by RP-HPLC in *Michelia champacai* flowers. Adv Biol Res 2010;4:159-168.
51. Patra JK, Gouda S, Sahoo SK, Thatoi HN. Chromatography separation, ^{1}H NMR analysis and bioautography screening of methanol extract of *Excoecaria agallocha* L. from Bhitarkanika, Orissa, India. Asian Pac J Trop Biomed 2012;2:50-56.
52. Springfield EP, Eagles PKF, Scott G. Quality assessment of South African herbal medicines by means of HPLC fingerprinting. J Ethnopharmacol 2005;101:75-83.
53. Coates J. Interpretation of Infrared spectra, a practical approach. In: Meyers RA, ed. Encyclopedia of analytical chemistry. Chichester: John Wiley & Sons Ltd; 2000:10815-10830.
54. Bandaranayake WM. Survey of mangrove plants from Northern Australia for phytochemical constituents and UV absorbing compounds. Curr Top Phytochem 1995;14:69-78.
55. Polya G. Biochemical targets of plant bioactive compounds. London, New York, Washington DC: CRC Press, Boca Raton; 2003.
56. Chinou I, ed. Primary and secondary metabolites and their biological activity. In: Waksmundzka-Hajnos M, Sherma J, Kowalska T, eds. Thin layer chromatography in phytochemistry. Taylor and Francis Group, Boca Raton London New York: CRC Press;2008:59-76.
57. Waksmundzka-Hajnos M, Sherma J, Kowalska T, eds. Thin layer chromatography in phytochemistry. Boca Raton London New York: CRC Press, Taylor & Francis Group; 2008.
58. Patra JK, Dhal NK, Thatoi HN. *In vitro* bioactivity and phytochemical screening of *Suaeda maritima* (Dumort): a mangrove associate from Bhitarkanika, India. Asian Pac J Trop Med 2011;4:727-734.

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