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Lupeol, oleanic acid & steroids from *sonneratia alba* j.e. Sm (sonneratiaceae) and antioxidant, antibacterial & cytotoxic activities of its extracts

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ABSTRACT

Secondary metabolites obtained from medicinally important plants are priceless natural resources for new drug development. Focused on traditional medicinal uses of *Sonneratia alba* J.E, present study was to isolate, purify secondary metabolites and scientifically evaluate the biological activities of its methanolic extract and different fractions. After collection of the plant sample, it was washed, sundried and extracted with methanol. From the crude methanolic extract different chemical compounds were isolated from partitionates by adopting Vacuum Liquid Chromatography, Thin Layer Chromatography and other standard separating process. In this consequence five compounds *i.e.* Lupeol (**1**), Oleanic acid (**2**), β-Sitosterol (**3**), β- stigmasterol (**4**) and Sitost-4-en-3-one (**5**) were isolated and characterized by studying ^{1}H NMR spectroscopic data and comparing with authentic reported data. The crude extract of *S. alba* was partitioned by following modified Kupkan method to evaluate biological activities. Among all the fractions, chloroform and aqueous soluble fractions showed the significant free radical scavenging activity with IC_{50} value (15.58 ± 0.55) µg/ml and (15.06 ± 0.35) µg/ml respectively in comparing with Butyl hydroxyl toluene (BHT) which revealed the IC_{50} value 34.25 ± 2.36µg/ml. In case of antimicrobial test, carbon tetrachloride, chloroform and aqueous soluble fractions inhibited the bacterial growth having the zone of inhibition ranging from 7-9 mm, 7-10 mm and 7 mm, respectively at a concentration of 400µg/disc. On the other hand, carbon tetra chloride soluble fraction showed good cytotoxic activity with LC_{50} value 7.94 ± 0.450 µg/ml. So it is required further investigate to isolate important secondary metabolites which could give us a new voyage for ailment of human with harmless natural resources

KEY WORDS: *Soneneratia alba* J.E. Sm., Lup-20(29)-en-3-ol, Oleanic acid, β-Sitosterol, β- stigmasterol, Sitost-4-en-3-one, antioxidant activity, antimicrobial activity and cytotoxic activity.

INTRODUCTION

Folk medicinal uses of different medicinal plants are very common in Bangladesh. The tribal people practice traditional treatment system for their ailment. There are lots of medicinally important mangrove plants are available in sundorbon area in Bangladesh. One of the important mangrove plants is *Sonneratia alba* J.E which is tree to 20 m broadly spreading evergreen plant belongs to the family

Sonneratiaceae and genus *Sonneratia* [Little S A. et al., 2004]. Previous biological investigation shows that it has antidiabetic property [Nancy J. Morada et al., 2011], cytotoxic and anmicrobial activity [Milon et al., 2012] and nutitional value [Priya D. Patil, 2012]. Generally, phytochemistry deals with the plants secondary metabolites [Harborne, 1998]. Bioactive natural products are mainly secondary metabolites which are used by the host as defensive and protective mechanism against their enemies and predators. Focused on bioactivity of this plant it was undertaken to isolate, purify important secondary metabolites and screening their biological and pharmacological activity in laboratory.

MATERIALS AND METHODS

PHYTOCHEMICAL ANALYSIS

General Experimental Procedure

^{1}H NMR spectra were recorded using a Bruker AMX 500 (500 MHz) instrument and the spectra were referenced to the residual deuterated solvent ($CDCl_3$) signal. Gel permeation chromatography was conducted over Sephadex (LH-20). Preparative TLC was carried out by using normal phase Si-gel 60 F254 (Merck) on glass plates (20×20 cm) of 0.5-mm thickness. Spots on TLC and preparative TLC plates were visualized after spraying the developed plates with 1% vanillin in sulfuric acid, followed by heating at 110 °C for few minutes.

Collection of Plant Material

Plant samples of *S. alba* J. E. Sm. were collected from Sunderban area in September 2010 from Bangladesh. This plant was identified by the taxonomist of the National Herbarium, Dhaka and a sample specimen has been deposited (Accession Number 31303).

Extraction and Isolation

Powdered plant materials (leaves) having a weight of 700 gm were taken in a reagent container (5 L) and soaked in 3 L of methanol. The container with its contents was sealed by cotton plug and aluminum foil and kept for a period of 2 weeks with occasional shaking and stirring. The whole mixtures were then filtered through cotton and finally with a Whatman No. 1 filter paper. The volume of the filtrate was then reduced using a Buchii Rotavapour at low temperature and pressure. The Weight of the dried crude extract was 37.8 gm. The crude extract (37 gm) was fractionated with the help of Vacuum Liquid Chromatography (VLC) method by using the mixture of pet. Ether; ethyl acetate and finally methanol; ethyl acetate and got a number of fractions. Depending on the TLC pattern of the entire VLC fractions, VLC fractions obtained by eluting 50% ethyl acetate and pet ether solvent system, 100% ethyl acetate and 5% methanol in ethyl acetate were done Gel Permeation Chromatography for fractionation using Sephadex (LH-20). A number of column fractions were obtained by using pet ether and chloroform solvent system. Preparative thin layer chromatography of the column fractions yielded five compounds. The Rf value of compound 1 as Lup-20(29)-en-3-ol (Lupeol) was 0.458 in 100% toluene system, compound 2 as Oleanic acid was 0.26 in 100% toluene system, compound 3 as β-Sitosterol was 0.44 in 95 % toluene- ethyl acetate system, compound 4 as β-stigmasterol was 0.33 in 95 % toluene-ethyl acetate and compound 5 as Sitost-4-en-3-one was 0.5 in 50% toluene- n-hexane system respectively.

EVALUATION OF BIOLOGICAL ACTIVITY

Preparation of Plant Samples

An aliquot (5.0 g) of the concentrated aqueous methanol extract was fractionated by the modified Kupchan partitioning protocol into pet ether, carbon tetrachloride, chloroform and aqueous soluble fractions respectively. [Van Wagenen *et al*., 1993] Subsequent evaporation of solvents afforded pet ether (1.5 g), carbon tetrachloride (1.15 g), chloroform (0.5 g) and aqueous soluble (0.65 g) materials. Partitionates obtained by this way were subjected to different biological assays.

Evaluation of Antioxidant Activity

Brand-Williams [Brand-Williams *et al.* 1995] method was used to estimate free radical scavenging

activities of the methanolic extracts of leaves of *S. alba* J.E. on stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Crude methanolic extract and different fractions (2 mg of each) were dissolved in methanol to prepare 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.8125 µg/ml, 3.91 µg/ml, 1.95 µg/ml and 0.98 µg/ml concentrated test sample by serial dilution technique. Each test sample (50 µl) was mixed with 5 ml of a DPPH-methanol solution at a concentration 40µg/ml. The reaction mixture was vortexed thoroughly and left in the dark place at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm and from these values the corresponding percentage of inhibitions were calculated by using the following equation:

Percent of inhibition = $[1- (Abs_{sample} / Abs_{control})] \times 100$ %

Where Abs_{sample} is the absorbance of the sample material and $Abs_{control}$ is the absorbance of the control reaction (containing all reagents except the test material). Then percent inhibitions were plotted against respective concentrations. IC_{50} values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph. Tert-butyl-1-hydroxytoluene (BHT) was used as positive control. The experiment was performed thrice and the result was expressed as mean ± Standard Error of Mean (SEM) in every case.

Antimicrobial Screening

Antimicrobial screening was performed using disc-diffusion method [Rizvi *et al,* 2011]. Crude methanolic extract and different fractions (8 mg of each) were dissolved in methanol to obtain desired test samples at different concentration in aseptic condition. Sterilized filter paper discs were taken in a blank Petridis under laminar hood. Then discs were soaked with solutions of test samples and dried. Standard Ciprofloxacin (30 µg/disc) discs were used as positive control and blank discs were used as negative controls. The sample discs, standard antibiotic discs and control discs were placed gently on marked zones in the agar plate's pre-inoculated with test bacteria, protozoa and fungi. The plates were then kept in a refrigerator at 4^0C for about 24 hours to allow sufficient diffusion of materials from discs to surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours. The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka.

Evaluation of Cytotoxic Activity

The evaluation of cytotoxic activity was done by the Brine shrimp lethality bio-assay [Meyer *et al,* 1982]. In this experiment simulated sea water is prepared by dissolving 38gm of sea salt in 1L of distilled water. Brine shrimp eggs were collected and hatched in a tank containing sea water. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. With the help of a Pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of seawater.

Clean test tubes were taken. These test tubes were used for preparing ten different concentrations (one test tube for each concentration) of test samples. Again ten test tubes were taken for ten concentrations of standard drug Vincristine and another one test tubes for negative control test. Four mg of all the test samples (Pet ether soluble fraction, carbon tetrachloride soluble fraction, DCM and the ethyl acetate soluble fraction) were taken and dissolved in 200µl of pure di methyl sulfoxide (DMSO) in vials to get stock solutions. Then 100µl of Solution was taken in test tube each containing 5ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 100µl sample was added to test tube and fresh 100µl DMSO was added to vial. Thus ten

different test tubes had different concentrations of test samples. The concentrations in ten different test tubes were 400µg/ml, 200µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml and 0.78125 µg/ml respectively. In the present study Vincristin sulphate is used as the positive control. Measured amount of the Vincristin sulphate is dissolved in DMSO to get an initial concentration of 40µg/ ml from which serial dilutions are made using DMSO to get 20µg/ml, 10µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml and 0.078125µg/ml. Then the positive control solutions are added to the pre marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups. For the preparation of negative control, 100 µl of DMSO was added to each of three pre marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii was added to each vial. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

After 24 hours, the vials were inspected using a magnifying glass and the number of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration- mortality data were analyzed statistically by using Microsoft Excel program. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

STATISTICAL ANALYSIS

Values for analgesic activity were expressed as "mean increase in latency after drug administration ±SEM" in terms of seconds. The significance of difference between means was determined by t-test values of $p<0.05$ were considered significant.

RESULTS

Repeated chromatographic separation and purification of the methanolic crude extract of the powdered leaf of the plant *S. alba* J.E. of yielded five compounds and the structures of those were solved by extensive analyses of ^{1}H NMR data as well as by comparing with published data and co-TLC with authentic samples.

Characterization of Compound 1as Lupeol

It was white amorphous solid appeared as a yellow spot on TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110^0 C for 5-10 minutes. The ^{1}H NMR (500 MHz, CDCl3) spectrum of compound 1 showed a double doublet (*J* = 11.0, 5.0 Hz) at δ 3.18 which could be assigned for H-3 in the triterpene nucleus. The presence of six tertiary methyl groups at δ 0.74, 0.81, 0.92, 0.95, 0.96 and 1.03, assignable to methyl protons at H-24, H-28, H-25, H-27, H-23 and H-26 respectively. The multiplet of one proton intensity at δ 2.33 was assigned to H-19. An isopropenyl side chain by signals at δ 1.68 (3H, s, H-30) and two broad signals at δ 4.56 and 4.72 (each 1H, s, H-29a and H-29b) manifesting its relation with lupane-type of triterpenoids. The above spectral features were similar to the ones reported for lupeol [Chaiyadej et al, 2004]. On this basis compound 1 was characterized as Lup-20(29)-en-3-ol. The identity of compound 1 was further confirmed by comparing its spectral data with previously reported values as well as Co-TLC with an authentic sample of lupeol (Figure 1).

Characterization of Compound 2 as Oleanic Acid

It was white amorphous solid appeared as a pinkish purple color band on TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110^0 C for 5-10 minutes. The ^{1}H NMR (500 MHz, CDCl3) spectrum of compound 2 revealed an olefinic proton at δ of 5.26 (1H, b s, *J*= 3.5 Hz), a carbinolic proton at δ of 3.21 (1H, dd, *J*=4.5 Hz and 11.5 Hz) suggesting it's axial and α orientation and δ of 2.80 (1H, bd, *J*= 11.0 Hz) along with seven tertiary methyl groups at δ 0.73,

0.75, 0.89, 0.89, 0.91, 0.97 and 1.11 (each 3H, s). The singlet signals for seven methyl groups in the ^{1}H NMR spectrum indicated the presence of 12-oleanane skeleton in the compound and it belongs to an oleanane-type triterpene having a carboxylic function. The compound was identified unambiguously as oleanolic acid by comparison with an authentic sample and the reported oleanolic acid (Figure 2).

Characterization of Compound 3 as β-Sitosterol

It was colorless crystal appeared as a blue color band on TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110^0 C for 5-10 minutes.

The ^{1}H NMR (500 MHz, CDCl3) displayed a proton multiplet at δ 3.50, the position and multiplicity of which was indicative to H-3 of a steroid nucleus. The typical olefinic H-6 of the steroidal skeleton was evident as a doublet (*J*=5.2 Hz) at δ 5.30 that integrated for one proton. The spectrum also revealed signals at δ 0.68 and δ 1.01 (3H each) assignable to two tertiary methyl groups at Me-13 (H3-18) and Me-10 (H3-19), respectively. The ^{1}H NMR spectrum also showed two doublets (*J*=7.0 Hz) centered at δ 0.83 and δ 0.85 which could be attributed to the methyl groups at Me-25. The doublets (*J*=6.4 Hz) at δ 0.92 was assignable to the methyl group at Me-20. On the other hand, the triplet (*J*=6.5 Hz) of three proton intensity at δ 0.81 could be ascribed to the primary methyl group attached to Me-28.The above spectral features were in close agreement to those observed for β-sitosterol [Morales et al., 2003]. (Figure 3)

Characterization of Compound 4 as β-stigmasterol

It was needle shaped off white crystals appeared as a purple spot on TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110^0 C for 5-10 minutes. The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) revealed a one proton multiplet at δ 3.51, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. The typical signal for the olefinic H–6 of the steroidal skeleton was evident from a multiplet at δ 5.34 integrating one proton. The olefinic protons (H–22 and H–23) appeared as characteristic downfield signals at δ 5.16 and δ 5.03 respectively in the ^{1}H NMR spectrum. Each of the signal was observed as double of doublets (*J* = 15.0 Hz, 6.5 Hz) which indicated couplings with the neighboring olefinic and methane protons. The spectrum further revealed signals at δ 0.67 and δ 1.00 (3H each) assignable to two tertiary methyl groups at C–13 and C–10, respectively. The ^{1}H NMR spectrum showed two doublets centered at δ 0.83 (*J* = 6.0 Hz) and 0.85 (*J* = 6.0 Hz) which could be attributed to the methyl groups at C–25. The doublet at δ 0.91 (*J* = 6.4 Hz) was demonstrative of a methyl group at C–20. On the other hand, the triplet (*J* = 6.5 Hz) of three-proton intensity at δ 0.83 could be assigned to the primary methyl group attached to C–28. The above spectral features are in close agreement to those observed for β-stigmasterol [Ikan, 1991]. On this basis compound 4 was characterized as β- stigmasterol. The identity of compound 4 was further confirmed by comparing its spectral data with previously reported values as well as Co-TLC with an authentic sample of β-stigmasterol. (Figure 4).

Characterization of Compound 5 as Sitost-4-en-3-one

It was colorless crystal appeared as a pale blue color band on TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110^0 C for 5-10 minutes. The ^{1}H NMR (500 MHz, $CDCl_3$) spectrum revealed a proton singlet at δ 5.72, the position and multiplicity of which was indicative of H-4 of the steroidal nucleus. The spectrum also revealed signals at δ 0.72 and δ 1.16 (3H each) assignable to two tertiary methyl groups at Me-13 (H-18) and Me-10 (H-19), respectively. The ^{1}H NMR spectrum also showed two doublets (*J*=6.6 Hz) centered at δ 0.82 and δ 0.80 which could be attributed to the methyl groups at Me-25. The doublets (*J*=6.4 Hz) at δ 0.92 was assignable to the methyl group at Me-20. On the

other hand, the triplet (*J*=6.6 Hz) of three proton intensity at δ 0.85 could be ascribed to the primary methyl group attached to Me-28. The spectral features are in close agreement to those observed for Sitost-4-en-3-one [Morales et al., 2003] (Figure 5).

Antioxidant Activity

The antioxidant activity of the different fractions of *S.alba* was measured on the basis of its DPPH scavenging activity. In this investigation, chloroform soluble fraction and aqueous soluble fraction showed the significant free radical scavenging activity with IC_{50} value (15.58 ± 0.55) µg/ml and (15.06 ± 0.35) µg/ml respectively. The positive control used was-Butyl hydroxyl toluene (BHT) and for which the IC_{50} values were found to be 34.25±2.36µg/ml. (Table 1)

Antimicrobial Activities

Crude methanolic extract and different fractions were subjected for in vitro screening of antimicrobial activity in comparing with standard Ciprofloxacin. The zone of inhibition produced by the carbon tetrachloride, chloroform and aqueous soluble fraction of the maethanolic extract of *S. alba* were ranged from 7-9 mm, 7-10mm and 7 mm, respectively at a concentration of 400 µg/disc. In case of, carbon tetrachloride soluble fractions the growth of *Shigella dysenteriae (*gram negative bacteria) and *Bacillus subtilis, Bacillus megaterium, Bacillus cereus* (gram positive bacteria) were inhibited moderately. On the other hand chloroform soluble fractions the growth of *Bacillus megaterium and Bacillus cereus* (gram positive bacteria) were inhibited moderately. In case of, aqueous soluble fractions the growth of *Bacillus megaterium and Staphylococcus aureus* (gram positive bacteria) were inhibited moderately. From the obtained data it is seen that *Sonneratia alba* J. E contains some important secondary metabolites which are active against gram negative bacteria. (Table 2)

Cytotoxic Activity

The lethal concentration LC_{50} of the test samples after 24 hour was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. Vincristine sulfate (VS) was used as positive control and the LC_{50} was found 0.451 µg/ml for VS. Compared with the negative control VS (positive control) gave significant mortality and the LC_{50} values of the different extractives were compared to this positive control. Among all the fractions carbon tetrachloride soluble fraction showed significant lethal concentration 7.94 ± 0.450 µg/ml. (Table 3)

DISCUSSION

Methanolic extract and different fractions of leaves of *Sonneratia alba* J.E. Sm were subjected to phytochemical screening and evaluation of different biological activities in the laboratory. By following different isolation and purification techniques, five secondary metabolites were isolated and characterized which are commonly found in plants. It is well established that these types of secondary metabolites were isolated previously from the plants of Sonneratiaceae. Bioactivities, bioactive compounds and chemical constituents of mangrove plants [W.M. Bandaranayake, 2002], Phytochemical Investigation& Antimicrobial Activity of Methanolic Extract of *Sonneratia apetala* Buch-Ham. Areal parts [Sangram Keshari Panda, 2012], Oleanolic acid - an a-Glucosidase inhibitory and antihyperglycemic active compound from the fruits of *Sonneratia caseolaris* [Ashok Kumar Tiwari et al, 2010].

Furthermore crude methanolic extract and different fractions showed significant antioxidant activity due to the presence of phenolic types of compounds. It also revealed significant cytotoxic activity. Different types of biological investigation showed that plants of this family have important biological activities. An Evaluation of Antihyperglycemic and Antinociceptive Effects of Methanol Extract

of *Cassia Fistula* L. (Fabaceae) Leaves in Swiss Albino Mice [Zahidul Islam Khan et al., 2010]. So this study evident that *Sonneratia alba* J.E. Sm is medicinally important plant from which important secondary metabolites can be isolated which could be used as medicinal entity.

CONCLUSION

From the investigated plant, five secondary metabolites has been isolated and characterized. These types of secondary metabolites are very essential and common to plants. The isolated compounds were characterized by extensive study of ^{1}H NMR spectroscopic data and comparing with authentic published data and doing co-TLC with reliable samples. Characterization was concisely done because we know that ^{1}H NMR is the best way to elucidate structure of different compounds. So the isolated compounds are authentic because it is well known that 1HNMR is the best way to elucidate chemical structure. On the other hand it has significant biological property due to the presence of medicinally important secondary metabolites. So further study concerning this plant, there might be new pathway for mitigation of human sufferings.

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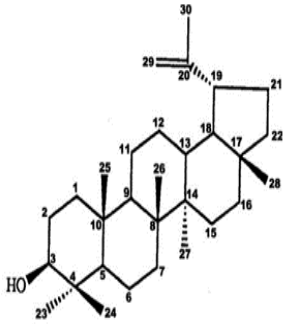

Figure 1: Lupeol

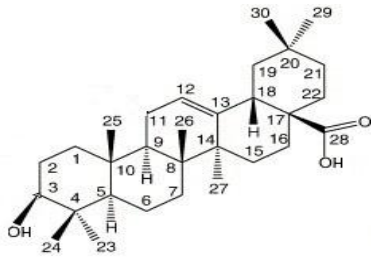

Figure 2: Oleanic acid

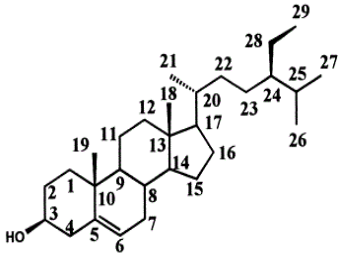

Figure 3: β sitosterol

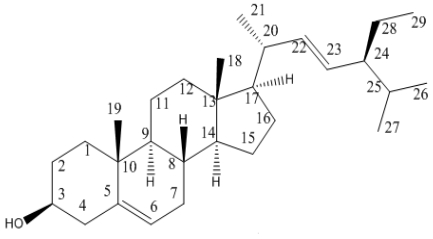

Figure 4: β- stigmasterol

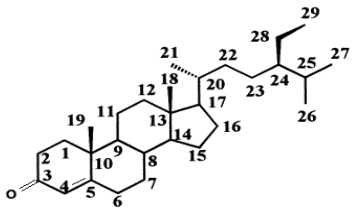

Figure 5: Sitost-4-en-3-one

Table 1: C_{50} values of the standard and partitionates of *Sonneratia alba*

Sample code	Test sample	IC_{50} value (µg/ml)
BHT	*tert*- butyl-1-hydroxytoluene	34.25±0.56
PE	Pet-ether soluble fraction of the methanolic extract of the leaves	82.93±1.05
CTC	Carbon tetrachloride soluble fraction of the methanolic extract of the leaves	154.17±0.256
CL	Chloroform soluble fraction of the methanolic extract of the leaves	15.58±0.49
AQ	Aqueous soluble fraction of the extract of the leaves	15.069±0.65

BHT = Butyl hydroxyl toluene, PE = Pet-ether Soluble Fraction, CTC = Crbon tetrachloride Soluble Fraction, CL = Chloroform Soluble Fraction and AQ = Aqueous Soluble Fraction

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): *P<0.05, All values are means of individual data obtained from five rats (*n* = 3)

Table 2: Antimicrobial activity of the test samples of *Sonneratia alba* J. E.

Test microorganisms	Diameter of zone of inhibition(mm)				
	PE	CTC	CL	AQ	Ciprofloxacin
Gram-negative bacteria					
Escherichia coli	---	---	---	---	46±0.34
Salmonella typhi	---	---	---	---	---
Pseudomonas aeruginosa	---	---	---	---	---
Klebsiella	---	---	---	---	---
Shigella dysenteriae	---	7	---	---	45±0.70
Shigella sonni	---	---	---	---	27±0.56
Shigella boydii	---	---	---	---	17±0.09
Shigella flexi	---	---	---	---	---
Gram positive bacteria					
Bacillus subtilis	---	9±0.45	---	---	45±0.56
Bacillus polymyxa	---	---	---	---	40±0.40
Bacillus megaterium	---	9±0.50	7	7	35±0.75
Sarcina lutea	---	---	---	---	20±0.25
Staphylococcus aureus	---	---	---	7	37±0.35
Bacillus cereus	---	7±0.65	10±0.305	---	40±0.05
Proteus spp.	---	---	---	---	40±0.65

PE= Pet-ether Soluble Fraction, CTC = Carbon tetrachloride Soluble Fraction, CL = Chloroform Soluble Fraction and AQ = Aqueous Soluble Fraction.

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): *P<0.05, All values are means of individual data obtained from five rats (*n* = 3)

Table 3: LC_{50} values of the test samples of *Sonneratia alba* J.E.

Test samples	Regression line	R^2	LC_{50} (μg/ml)
VS	Y = 30.79x+60.64	0.972	0.451±0.42
PE	Y = 33.62x+9.058	.0950	16.51±0.36
CTC	Y = 31.60x+21.57	0.946	7.94±0.66
CL	Y = 16.10x+9.908	0.969	309.16±0.15
AQ	Y = 19.12x+97.141	0.946	174.41±0.45

VS= Vincristine sulphate, PE= Pet ether Soluble Fraction, CTC = Carbon tetrachloride Soluble Fraction, CL= Chloroform Soluble Fraction and AQ = Aqueous Soluble Fraction

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): *P<0.05, All values are means of individual data obtained from five rats (*n* = 3)