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Bio-Activity Investigations of Extracts of Different Parts of *Lumnitzera littorea* Voigt

Luksamee Vittaya[1]*, Sutkanung Na Ranong[1], Uton Charoendat[1], Sittichoke Janyong[1], Nararak Leesakul[2]

[1]*Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Sikao, Trang, Thailand*
[2]*Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand*

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ABSTRACT

The identification of active natural compounds from plants for use as therapeutic agents for aquatic diseases could reduce the use of environmentally harmful chemicals. *Lumnitzera littorea* is a plant in the Combretaceae family that thrives among mangroves. Although well known in traditional medicine, there are only a few reports of the activity of various parts of this plant against aquatic pathogenic bacteria. Therefore, this work studied the phytochemical composition of the stem-bark, leaf, twig, and flower of *L. littorea* extracts and further investigated the antioxidant and antibacterial activities. The qualitative and quantitative analyses of phenolic and flavonoid contents were carried using the Folin-Ciocaltue and the colorimetric aluminium chloride methods, respectively. Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts (ABTS) methods. The extracts were screened for antibacterial activity against different pathogens (*Streptococcus agalactiae*, *Aeromonas hydrophila*, *Vibrio harveyi* and *V. parahemolyticus*) using the hole-plate diffusion method. The results showed the presence of phenolic and flavonoid components in all extracts. Bark and twig extracts had greater total phenolic and flavonoid contents than those from flower and leaf extracts. Significant antioxidant activity was expressed by the bark and twig extracts. All extracts showed a wide range of antibacterial activity against the tested organisms. Bioactive substances present in *L. littorea* extracts exhibited antioxidant and antibacterial activities. *L. littorea* could be a potential source of natural bioactive compounds that could be used to develop safe and environmentally friendly pharmaceuticals against bacterial pathogens in aquatic animals.

Keywords: *Lumnitzera littotea*, Phenolic, Flavonoid, Antioxidant, Antibacterial.

Introduction

Plants are a great source of potentially beneficial chemical compounds. They produce a wide variety of substances that are used for biological functions and for preventing diseases caused by pathogens, fungi and other sources. Traditionally, it has been a common practice to treat microbial infections such as whooping cough, or skin ailments, and inflammations with crude extracts or decoctions derived from plants. However, there has been information of phenolic or flavonoid acting as bioactive substance but none of an obvious experimental result to determine the type of bioactive substance helping to treat the symptoms. Several previous works reported that different parts of plants including bark, leaves, fruits, flowers, and seeds have phytochemical constituents with a variety of pharmacological properties.[1-4] These phytochemicals contain naturally bioactive compounds that are formed during a plant's normal metabolic processes and which are often referred to as secondary metabolites. These metabolites commonly include alkaloids, terpenoids, tannins, saponins, phenolics and flavonoids. These are widely used as lipase inhibitors, antioxidants and antimicrobial agents.[1-6] To identify bioactive compounds, phytochemical screening is the first and important step in isolating the type of secondary metabolites that may be responsible for biological activity.[9-11] *Lumnitzera littorea* Voigt (Figure 1) is a mangrove tree belonging to the family Combretaceae. It is a medium to tall tree, growing from 10 to 30 meters high with rough, grey to brown bark and red flowers. Previous reports indicated the use of *L. Littorea* leaf extract against human pathogenic bacteria[11] and also confirmed cytotoxic activity.[12] In addition, extracts of *L. littorea* fruit and flower were a good source of natural antioxidants that acts as free radicals scavengers. This activity was supported by the existence of phytochemicals like phenolic and flavonoid components.[13] During our search for antibacterial activity among mangrove plants used in traditional Thai medicine; we found that a crude methanol extract from the bark, leaves, twig and flower of *L. littorea* exhibited moderate antibacterial activity against aquatic pathogenic bacteria.

Extracts from the twigs were purified and a new compound 12-hydroxycorniculatolide A and other known compounds were obtained and all extracts showed antibacterial activity against 11 bacterial strains.[14] Literature search has shown that the activity of different parts of *L. littorea* against aquatic pathogenic bacteria is sparsely reported. Therefore, the bark, leaf, twig and flower extracts of *L. Littorea* were subjected to qualitative phytochemical screening and quantitative determination of major components like phenolics and flavonoids. The plant's antioxidant and antibacterial activity against four pathogenic aquatic bacteria were also studied. Our aim was to identify any correlation between biological activities of *L. littorea* and the phenolic and flavonoid constituents of the plant.

*Corresponding author. E mail: nokluksamee@hotmail.com
Tel: +66 896539180

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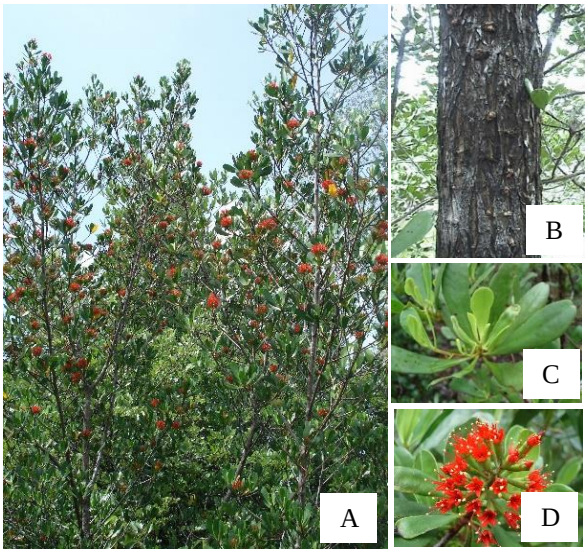

Figure 1: Twig (A), bark (B), leaf (C) and flower (D) of *L. littorea*

Materials and Methods

Standards and reagents

Gallic acid, rutin, 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid diammonium salts) (ABTS), butylated hydroxy toluene (BHT), butylated hydroxyanisole (BHA) and ascorbic acid (Vit.C) were purchased from Sigma-Aldrich. Folin-Ciocalteu reagent and potassium persulfate were purchased from LobaChemie. All chemicals and reagents used in the study were of analytical grade.

Plant material and extraction

Fresh bark, leaf, twig and flower from *L. littorea* were collected from Trang Province in Thailand (October 2015) and identified by Assistant Professor Sittichoke Junyoung. The specimen voucher BKF no. 194809 was deposited in the Forest Herbarium, Thailand. All parts were washed under tap water, cut into small pieces and dried for a week. About 1 kg each of the bark, leaf, twig and flower of *L. littorea* were macerated in 5 L of methanol for a week. The resulting extracts were filtered with Whatman No. 1 filter paper and the filtrates concentrated by rotary evaporator in a vacuum at 45^{o}C to give dry residues which were kept in a refrigerator until use. The percentage yields and color of bark, flower, leaf, and twig crude extracts are presented in Table 1. The extracts were then analyzed to determine the phenolic and flavonoid contents as well as antioxidant and antibacterial activities.

Phytochemical screening

Phytochemical screening was carried out on the extracts to test for the presence of anthraquinones, terpenoids, flavonoids, saponins, phenolic compounds and alkaloids using standard methods.[15]

Total phenolic contents (TPC)

The total phenolic content (TPC) in all extracts of *L. Littorea* were determined by Folin-Ciocalteu method with modification.[16] The concentration of all extracts was fixed at 1 mg/mL and 0.2 mL aliquots of samples in 2.5 mL of distilled water were mixed with 0.2 mL of Folin-Ciocalteu reagent. After 1 min, 2.0 mL of 7% sodium carbonate solution was added to the reaction mixture. It was then kept away from light for 60 min. The absorbance of all samples was measured at 765 nm against the blank. TPC was expressed as micrograms of gallic acid equivalents (GAE) per gram of dried crude extract (mg GAE/g crude extract) through the calibration curve of gallic acid ($y = 0.003x + 0.015$) with its linearity ($R^2 = 0.998$).

Total flavonoid content (TFC)

The total flavonoid content (TFC) of bark, flower, leaf, and twig crude extracts were determined using the aluminum chloride method as previously reported[16] with modification using rutin as the standard. 200 µL of a 1 mg/mL concentration of each sample were placed in a centrifuge tube and 0.5 mL of 5% $NaNO_2$ was added. The reaction mixture was left to stand for 6 min at room temperature before 0.2 mL of $AlCl_3$ was added. After 5 min, 0.5 mL of 1 M NaOH was added and the total volume was made up to 1.5 mL with distilled water. The solution was again mixed well and the absorbance was measured against a blank at 510 nm with a UV-Visible spectrophotometer. The total flavonoid content of each sample was calibrated alongside the standard curve of rutin at concentrations of 50-600 mg/mL and expressed in terms of rutin equivalents (RU) per gram of dried crude extract (mg RU/g crude extract) through the calibration curve of rutin ($y = 0.001x – 0.016$) with its linearity ($R^2 = 0.998$).

Antioxidant activity

DPPH Free Radical Scavenging Assay

The free radical scavenging capacity of extracts was determined by DPPH assay following a previous method with slight modifications.[17] The stock solution of 0.15 mM DPPH was prepared in methanol. Butylated hydroxyl toluene (BHT) and ascorbic acid (Vit C) were used as standards and a series of standards and samples (bark, leaf, twig and flower extracts) were prepared in the concentration range of 4 – 50 mg/mL. Briefly, 2 mL of each extract and standard were placed in different test tubes. For each tube, 2 mL of 0.15 mM DPPH solution was added. The tubes were allowed to stand at room temperature away from light for 30 min and their absorbances were measured at 517 nm. The control was prepared as above without extract. The absorbance of the solutions was measured at 517 nm against the blank. BHT and ascorbic acid were used as positive controls. The free radical scavenging activity of both standard and samples was calculated for each concentration using the formula:

$$(1– (A_{sample}–A_{sample\ blank})/A_{control}))*100$$

Where A_{sample} is the absorbance of the test sample with DPPH solution,

$A_{sample\ blank}$ is the absorbance of the test sample only, and

$A_{control}$ is the absorbance of DPPH solution. The graphs were plotted. The antioxidant activity of the sample was expressed as the effective concentration (mg/mL) of the extract required to scavenge 50% of free radicals. All measurements were performed in triplicates and expressed as average values.

ABTS Free radical scavenging assay

In this study, radical scavenging capacity was measured using the $ABTS^+$ solution (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonate) radical cation. The assay was performed as described in a previous report.[18] The stock solution of ABTS radical cation ($ABTS^+$) was prepared by mixing 50 mL of 7 mM ABTS with 880 mL of 140 mM $K_2S_2O_8$ (potassium persulfate). The mixture was allowed to react for 16 hours at room temperature in darkness. The $ABTS^+$working solution was prepared by diluting the stock solution with methanol to get an absorbance of 0.700 ± 0.025 units at 734 nm using a spectrophotometer. A series of standards and extracts was prepared in the range of 1–30 mg/mL. Aliquots of 0.1 mL of sample extracts and standards were placed in different test tubes then 0.9 mL of diluted $ABTS^+$ working solution was added to each test tube. The reaction mixture was shaken and left to incubate for 6 min at room temperature. After incubation, the absorbance was measured at 734 nm. The free radical scavenging assay was carried out in triplicates. ABTS radical scavenging capacity was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_{control}–A_{sample})/A_{control}] * 100,$$

Where $A_{control}$ is the absorbance of the extract without $ABTS^+$ working solution and A_{sample} is the absorbance of the extract with $ABTS^+$ working solution. The lowest concentration at which the extract scavenged 50% of free radicals was interpreted as IC_{50}.

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Antibacterial properties
Inoculum preparation
The antibacterial activity of the *L. littorea* extracts was tested against four bacterial strains produced from tested inoculums of *Streptococcus agalactiae* SAAQ001, *Aeromonas hydrophila* AHAQ001, *Vibrio harveyi* VHAQ001 derived from Kasetsart University, and *Vibrio parahemolyticus* derived from Songkhla Aquatic Animal Health Center, Thailand. All tested inoculums were produced through the modification of a previous method.[19] The determination of antibacterial activity and minimum inhibitory and bactericidal concentrations (MIC/MBC) used adjusted concentrations at $1x10^8$ CFU mL^{-1} in NaCl solution and $1x10^6$ CFU mL^{-1} in Mueller Hinton broth (MHB). In addition, the culture medium for the *Vibrio* spp. subculture was enhanced with 1.5% NaCl.

Screening of antibacterial activity
The bark, leaf, twigs and flower extracts of *L. littorea* were screened in quadruplicate for their antibacterial properties. A modified hole-plate diffusion method was used following a reported procedure.[20] All petri dishes containing Mueller Hinton agar (MHA) were swabbed with a cotton swab soaked with $1x10^8$ CFU mL^{-1} of each tested inoculum. After drying the swabbed agar for 5 min, holes (6 mm in diameter) were made in the agar with a sterilized tube and samples of extracts at a concentration of 100 $mg.mL^{-1}$in DMSO were introduced aseptically into the holes. Oxolinic acid and DMSO were introduced into other holes as positive and negative controls, respectively. The agar plates were incubated at 35^{o}C for 18-24 h and the antibacterial activity of each extract sample was evaluated by measuring the zone of inhibition surrounding the hole.

Determination of MIC and MBC
The preparation of four pathogenic bacteria, *S. agalactiae* SAAQ001, *A. hydrophila* AHAQ001, *V. harveyi* VHAQ001, and *V. parahemolyticus* as stock solutions was carried out for the tested inoculums at $1x10^6$ CFU mL^{-1}.[19] The stock solutions of each extract and control were prepared with an initial concentration of 100 $mg.mL^{-1}$, and serially diluted two-fold with MHB to obtain a concentration range from 0.01 to 50000 $mg.mL^{-1}$. Fifty µL of each concentration were added in 96-well plates containing 50 µL of MHB. The inoculums, standardized at $1x10^6$ CFU/mL, were poured into each well by multichannel auto-pipette and gently mixed in order to produce a final concentration of 5 x 10^5 $CFU.mL^{-1}$ in each well. The plates were covered with a sterile plate sealer and incubated at 35^{o}C for 24 h. The turbidity of the solutions was then checked, and *p*-Iodonitrotetrazolium chloride (INT) was added to each well to confirm bacterial growth by discoloration of the mixture. The presence of microorganisms reduced the yellow dye to a pink color. The MIC was taken as the lowest concentration of the extract that prevented color change in the well: thus showing complete inhibition of bacterial growth. A negative growth control of DMSO was also included in every test in quadruplicate following the previously mentioned method and four wells of inoculums were set up as a negative control.[21] In addition, MBC was determined by streak plate technique performed after a broth microdilution test. Briefly, one loopful of the clear solution presenting in the broth micro-dilution test was streaked onto agar plates of MHB. The agar plates were incubated at 35^{o}C for 24 h. The MBC value was estimated from the appearance of bacterial colonies on the agar plates, on which antibacterial agent concentrations were specified. The lowest concentration that presented no bacterial colony on the plate was the MBC.[21,22]

Statistical analysis
The data were presented as means ± SD for triplicate and quadruplicate determinations of total TPC and TFC and antibacterial activity. Statistical analysis was carried out using ANOVA. The correlation between phenolic and flavonoid contents and antioxidant activity (DPPH and ABTS) was determined using one way ANOVA and the Pearson correlation coefficient (r) at the significant levels of 0.01 and 0.05.

Results and Discussion

Yield of extracts and phytochemical screening
Yields of *L. littorea* bark, leaf, twig, and flower extracts ranged from 19% to 28% (Table 1). The highest yield was produced from methanol flower extract and the lowest from leaf extract. Preliminary phytochemical analysis revealed the presence of phenolic, flavonoids and saponins in all the extracts, alkaloids were limited to the bark and twigs extracts, while terpenoids were found only in the twigs extract (Table 1). No anthraquinone was detected in any of the extracts. The bark, leaf, twigs and flower extracts contained phenolic and flavonoid metabolites which are known to have pharmacological potential in medicine.[23] These findings provided evidence to support the investigation of these extracts as potential pharmaceuticals. Present in all parts of *L. Littorea*, saponins exhibited antimicrobial activity characteristic of mangrove plants and are extremely noxious to cold-blooded animals.[24] They have also been found to exert antibacterial activity against a wide range of microorganisms *in vitro*.[11] Alkaloids have previously been reported to inhibit microbial growth[25] and possess anti-inflammatory properties.[26]Although terpenoid secondary products were observed only in the twigs extract, terpenoids have been reported to exert activity against cancer and bacteria.[27-29]

Total phenolic and flavonoid contents
The TPC of the extracts of *L. Littorea* was determined by the Folin-Ciocalteu method and values were between 567.22 to 2361.11 mg GAE/g crude extract. The extracts of bark and twig contained greater amounts of phenolics (2361.11 and 2160.56 mg GAE/g crude extract) compared to the flower and leaf extracts (1320.56 and 567.22 mg GAE/g crude extract) (Table 2). It has been hypothesized that the phenolics present in bark, flower, leaf, and twig extracts of *L. littorea* may play an important role in the plant's biological activity.[30,31] The TFC of all extracts was investigated by colorimetric aluminum chloride assay. The TFC showed values between 793.33 to 3949.00 mg RU/g in the various crude extracts. The bark and twigs extracts contained higher contents of flavonoids (3949.00 and 3171.67 mg RU/g crude extract) than the leaf and flower extracts (793.33 and 1071.67 mg RU/g crude extract). This consistent variation could result from differences in the polyphenolic and flavonoid contents of the different parts of this plant (Table 2) which showed as statistically significant ($P < 0.05$). In addition, the higher TPC and TFC of *L. littorea* may play an important role in the antioxidant activity.[32] There was a statistically significant correlation between the total phenolic and flavonoid contents in the *L. littorea* fractions at significant levels of 0.01 (Table 3).

Antioxidant activity
The antioxidant capacities of the extracts were studied with 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid diammonium salts (ABTS) assays. Generally, the DPPH method is used to measure free radical scavenging activity by testing the oxidation reaction of crude extracts when reacted with DPPH, which is a stable purple free radical at room temperature. When DPPH accepts electrons or hydrogen free radicals, the color changes from purple to the yellow color of DPPH:H. The ability of the DPPH free radical to undertake reduction by an antioxidant is measured by the decrease in its absorbance at 517 nm. In our work, the free radical scavenging effects of bark, leaf, twig and flower extracts increased from 10% to 93% with increase in the concentration of the extracts (Figure 2). The scavenging activity of DPPH ranged from 94% to 97% across all the extracts. The methanol extracts of the bark and twigs showed the highest antioxidant activity with IC_{50} values of 6.49 ± 0.53 and 7.38 ± 0.04 mg/mL, respectively. The $ABTS^+$ assay is a good tool to determine antioxidant activity. The decolourization of the $ABTS^+$ radical indicates the capacity of an antioxidant species to give electrons or hydrogen atoms to inactivate radical species. In this work, the samples exhibited reduced absorbance with moderate scavenging activity, ranging from 2% to 71% when the concentration of the extract was increased (Figure 2). There was good agreement between the results of DPPH and ABTS assays in our study. The methanol extracts of bark (3.32 ± 0.06

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mg/mL) and twig (3.61 ± 0.04 mg/mL) presented better antioxidant activity than flower (5.62 ± 0.05 mg/mL) and leaf (16.42 ± 0.11 mg/mL) extracts (Figure 3). The lower the IC_{50} values, the better the antioxidant activity. From the above values, it shows that the plant extracts show better antioxidant activity compared to BHT (IC_{50} = 10.39 ± 0.00 and 10.73 ± 0.12 mg/mL for DPPH and ABTS assays, respectively). In summary, the methanol extracts of the bark and twig were always the more active extracts, which may well be related to their phenolic and flavonoid components (Table 2). In addition, the pure compounds of macrocyclic lactone and coumarin derivatives from the twig of *L. littorea* could scavenge free radicals because their structures contained many conjugated double bonds and oxygen as a substituent group, resulting in highly efficient free radical capture with lower IC_{50} values.[14] To increase the understanding of the antioxidant effect of methanol extracts of *L.littorea* bark, flower, leaf and twig, their phenolic and flavonoid compounds should be isolated and purified in future studies.

Correlation between antioxidant activity, total phenolic and total flavonoid contents

Correlation analysis was used to explore the relationships between TPC and TFC in *L. littorea* and antioxidant activity measured by DPPH and ABTS assays (Table 3). In this work, a significant linear correlation was found between the free radical scavenging activity determined by DPPH assay and total phenolic at significant levels of 0.05. However, the correlative value found between ABTS and total polyphenolic compounds of *L. littorea* was significant at 0.05 for phenolic and 0.01 for flavonoid. The phytochemical study showed that the methanol extracts of *L. littorea* contained many bioactive compounds. This result justifies the use of this plant in traditional medicines. Its bioactive compound can scavenge free radicals implicated in several disease conditions.[7,33]

Antibacterial screening

Phenolic and flavonoid phytochemical compounds have been previously reported for their antibacterial activity.[34] The bark, leaf, twig and flower extracts from *L. Littorea* were screened for antibacterial activity (Table 4) and compared with the effect of the antibiotic agent oxolinic acid (oxo). The effective growth inhibition of the bioactive component present in these extracts showed as clear areas in a hole-plate test. The diameter of the inhibition zone was slightly different among extracts. They were in a range from 11 to 22 mm against four pathogenic bacterial strains: the Gram positive *S. agalactiae* and the Gram negative *V. harveyi*, *A. hydrophila* and *V. parahaemolyticus*. Generally, a methanol solvent extracts more phenolic and polyphenolic compounds than other solvents, obtaining high quantities of bioactive components[13,15] that give rise to clear antibacterial properties.[35] Moreover, antibacterial activity was slightly different when incubation time was increased to 48 h. The MIC of the studied *L. littorea* extracts against the four organisms ranged between 390.6 and 6250 $mg.mL^{-1}$ and the MBC of the extracts against the four organisms ranged between 781.2 and 12500 $mg.mL^{-1}$ (Table 5). All extracts exhibited stronger antibacterial activity against the Gram negative strains especially the bark and twig extracts which were more active against *V. parahaemolyticus*, with low MIC values of 0.3 $mg.mL^{-1}$ for both extracts. The higher concentrations of phenolic and flavonoid compounds in the twig and bark extracts could also inhibit the cell protein synthesis of bacteria.[34,36] Flavonoids contains one carbonyl group in its molecule which exhibits antibacterial activity by permeating cell walls and soluble proteins.[34] The antibacterial property of the twig extract is consistent with the strong free radical scavenging activity of macrocyclic lactone and coumarin derivatives isolated from the twig of *L. littorea*.[14] Interestingly, the flower extract of *L. littorea* showed good inhibitory effect against both Gram positive and Gram negative bacterial strains with lower MIC and MBC values. Generally, Gram positive bacteria are believed to be weaker having only an outer peptidoglycan layer, which is not an effective impermeable barrier. Gram negative bacteria, however, have an outer phospholipid membrane carrying the structural lipopolysaccharide compound and the presence of both a peptidoglycan and a phospholipid layer makes the cell membranes of these bacteria impermeable to pharmaceutical constituents. Even in the face of this barrier, phytochemical constituents effectively inhibited the growth of these pathogenic strains.[34] Therefore, in the search for new compounds, the isolation and purification of the flower extract of *L. littorea* is an attractive prospect to our group, which may further explain this activity. The results of this study indicated that the extracts of the bark and twig of *L. littorea* are potential sources of antibacterial agents against pathogenic aquatic bacteria.

Table 1: Yield and qualitative phytochemical analysis of *L. littorea* extracts

Used part	Yield (%)	Constituents					
		Anthraquinone	Terpenoid	Alkaloid	Flavonoid	Phenolic	Saponin
Bark	25.10	-	-	+	+	+	+
Leaf	19.50	-	-	-	+	+	+
Twig	21.03	-	+	+	+	+	+
Flower	28.13	-	-	-	+	+	+

Note: "+" means presence and "-" means absence

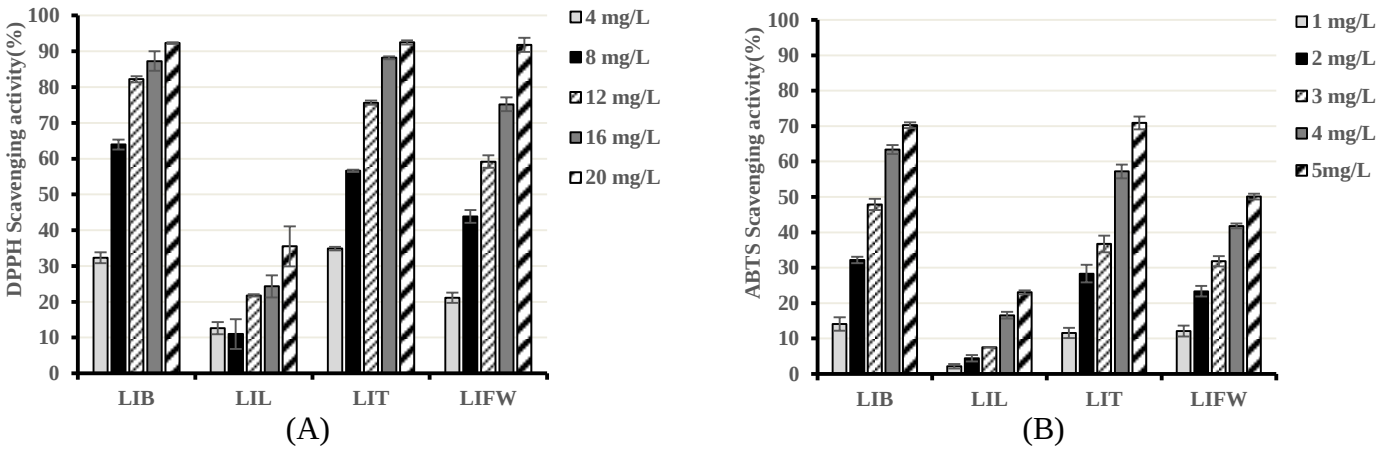

Figure 2: The percentage of free radical scavenging activity with vary concentration of crude extracts by (**A**) DPPH and (**B**) ABTS assays. LIB, LIL, LIT and LIFW are bark, leaf, twig and flower of *L. littorea*, respectively. Data are expressed as mean ± SD, n = 3.

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Table 2: Total phenolic, total flavonoid and antioxidant activity of bark, leaf, twig and flower extracts of *L. littorea*

Plant	Part Used	Total phenolic content (mg GAE)/g CE)	Total flavonoid content (mg RU)/g CE	IC_{50} (µg/mL)	
				DPPH	ABTS
L. littorea	Bark	2361.11 ± 98.63[d]	3949.00 ± 63.84[d]	6.49 ± 0.53[b]	3.32 ± 0.06[b]
	Leaf	567.22 ± 31.90[a]	793.33 ± 5.77[a]	28.17 ± 0.18[d]	16.42 ± 0.11[e]
	Twig	2160.56 ± 109.36[c]	3171.67 ± 52.99[c]	7.38 ± 0.04[b]	3.61 ± 0.04[b]
	Flower	1320.56 ± 49.90[b]	1071.67 ± 42.53[b]	10.33 ± 0.17[c]	5.62 ± 0.05[c]
BHT		-	-	10.39 ± 0.00[c]	10.73 ± 0.12[d]
Ascorbic acid		-	-	1.41 ±0.00[a]	2.66 ± 0.10[a]

Data are presented as mean ± SD from analysis of three samples, in triplicate; superscripts a – e indicate the difference compared with the others (in the same column) is significant at 0.05 level TPC and TFC from higher to lowest values.

Table 3: The correlation coefficient (r) values of phytochemicals and antioxidant activity regression analysis

Assay	ABTS IC_{50} (µg/mL)	DPPH IC_{50} (µg/mL)	TFC (mg RU/g CE)	TPC (mg GAE/g CE)
TPC (mg GAE/g CE)				1.000
TFC (mg RU/g CE)			1.000	0.944**
DPPH IC_{50} (µg/mL)		1.000	-0.743	-0.910*
ABTS IC_{50} (µg/mL)	1.000	-0.744**	0.999**	-0.912*

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Table 4: Antibacterial activity of different parts of *L. littorea* against pathogens in aquatic animals

Samples	Zone of inhibition diameter (mm)							
	S. agalactiae		*A. hydrophila*		*V. harveyi*		*V.parahaemolyticus*	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Bark	21.38 ± 1.60	20.75 ± 1.19	17.81 ± 0.94	16.25 ± 2.25	19.75 ± 0.87	20.00 ± 0.54	17.12 ± 1.38	17.62 ± 0.63
Leaf	14.50 ± 3.67	14.88 ± 2.63	15.06 ± 2.31	13.38 ± 0.25	15.88 ± 1.83	15.63 ± 1.60	11.75 ± 3.40	12.00 ± 0.41
Twig	16.50 ± 1.17	15.88 ± 1.98	17.13 ± 1.89	16.25 ± 2.10	13.31 ± 0.69	13.31 ± 1.53	14.75 ± 1.94	14.38 ± 1.05
Flower	15.50 ± 1.22	15.56 ± 1.38	17.13 ± 2.50	16.25 ± 2.18	11.38 ± 1.38	11.63 ± 1.20	12.63 ± 2.06	13.81 ± 2.23
Oxo	11.63 ± 1.59	12.25 ± 2.35	29.50 ± 0.68	28.13 ± 0.78	11.63 ± 1.59	12.25 ± 2.35	11.62 ± 1.59	12.25 ± 2.35

Values are inhibition zone and expressed as mean (mm) ± S.D (n = 4) (Conc. 4 mg/hole).

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (µg.ml^{-1}) of the extracts from *L. littorea* against pathogens in aquatic animals

Samples	MIC and MBC (µg.mL^{-1})							
	S. agalactiae		*A. hydrophila*		*V. harveyi*		*V.parahaemolyticus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bark	1562.5	12500	1562.5	3125	1562.5	6250	390.6	6250
Leaf	3125	12500	3125	12500	1562.5	3125	1562.5	12500
Twig	6250	6250	781.2	6250	1562.5	6250	390.6	6250
Flower	1562.5	3125	781.2	781.25	1562.5	6250	781.2	12500
Oxo	195.31	3125	0.05	0.10	0.01	0.01	0.01	0.01

Values are minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) and expressed as mean (mm) ± S.D (n = 4); Oxo = oxolinic acid.

Conclusion

Biological activities of extracts of different parts of *Lumnitzera littorea* Voigt were studied *in vitro*. Antioxidant and antibacterial assays were performed to determine IC_{50} and minimum inhibitory and minimum bactericidal concentrations. Methanol extracts of various parts of *L. Littorea* were effective as antioxidant agents and against both Gram-negative and Gram-positive strains of pathogenic aquatic bacteria. These activities may be due to the presence of phenolic and flavonoid compounds which are well known for their antibacterial and other therapeutic properties. The methanol extracts of *L. littorea* could serve as repository for the discovery of potential candidates for the development of medicine for the treatment of diseases caused by aquatic pathogens.

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Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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