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Chemical Composition, Antioxidant and Antibacterial Activities of *Balanophora latisepala* (V. Tiegh.) Lecomte in An Giang, Vietnam

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ABSTRACT

Balanophora latisepala is a species of angiosperm, which does not contain chlorophyll. This species has compulsory parasitic life on host trees and has been found in Bay Nui area, An Giang, Vietnam. This study was aimed to examine the phytochemical constituents, antioxidant and antibacterial activities of the *Balanophora latisepala* whole stem. Phytochemical screening was done using previously described method, the antioxidant activity was determined using DPPH free radical scavenging method while the antibacterial activity was evaluated by the agar well diffusion method. Preliminary phytochemical screening identified the following constituents; alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, amino acids and proteins, saponins, sterols, tannins, terpenoids, quinones, phytosterols, gum and resin, xanthoproteic, coumarins and essential oils. With respect to the antioxidant activity, the aqueous extract of *Balanophora latisepala* was comparable to the methanol extract in neutralizing DPPH free radical, with half-maximal effective concentration (EC_{50}) of 107.65 ± 4.90 µg/mL and 101.50 ± 2.37 µg/mL, respectively. These two extracts were able to neutralize DPPH radical more significantly than the ethanol extract (EC_{50} = 806.30 ± 23.52 µg/mL) ($p < 0.05$). For the antibacterial activity, at a concentration of 400 mg/mL, the aqueous extract of *B. latisepala* was most active against *Salmonella sp*. compared to the other bacterial strains including *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The methanol extract at 400 mg/mL was the least active against *P. aeruginosa* and *Salmonella* sp. These preliminary results showed that *B. latisepala* is a rare species that has low antioxidant and low antibacterial activities.

Keywords: Antibacterial, Antioxidant, *Balanophora latisepala*, Phytochemical composition.

Introduction

The family Balanophoraceae Rich. contains multiple species that have been used as supplements in traditional medicine for general well-being, joint inflammation relief, sexual performance, digestive support, and blood glucose balance.[1] In Vietnam, there are six plant species recorded in the genus *Balanophora*. These species have been exploited and used by local people as medicinal herbs in folklore.[2] Several biological activities of Balanophoraceae species have been recognized such as the antioxidant capacity of *B. laxiflora*, the ability to inhibit the HIV activity of *B. japonica* and *B. laxiflora*,[3] the hypoglycemic effect of *B. polyandra*, the analgesic and anti-inflammatory effects of *B. involucrata*.[3] Nevertheless, the medicinal properties of these species have not been reported the Vietnamese pharmacopoeia.[4] The *B. latisepala* is endemic to Vietnam, which was recorded in the Bay Nui Mountains areas - An Giang province and named *Moc Ba Hue* (in Vietnamese).[1] This obligatory parasitic plant has been recognized by indigenous people as having medicinal properties like other species of the same genus, leading to overexploitation of this species. Therefore, this study was conducted to determine the chemical composition, antibacterial and antioxidant capacity of *B. latisepala*, thereby providing **scientific evidence for the proper exploitation and conservation of this plant.**

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Materials and Methods

Experimental materials

B. latisepala whole stem was collected at 10°29'08.4"N, 104°58'59.8"E with an altitude of about 200 - 450 m in Cam Mountain - An Giang, Vietnam from June to September, 2020 (Figure 1). The sample was identified by Dr. Luu Hong Truong (Southern Institute of Ecology - Vietnam Academy of Science and Technology). The voucher specimens (Blatis082020-AGVN) was kept at Plant Lab, Biology Department, School of Education, Can Tho University.

Figure 1: *Balanophora latisepala*

Preparation of plant materials

The collected *B. latisepala* was washed and dried at 60°C to a constant weight. It was then ground into powder. The powdered sample was extracted by decoction (10% w/v, powder: water) for 1 hour. The extract was filtered and evaporated using a rotary evaporator (RV 10 digital V-C, IKA, Germany).[5] The aqueous extract obtained after vacuum filtration was freeze-dried and dissolved in water at adequate concentrations for biological assays as described by Félix-Silva *et al*.[6] Similarly, the powdered plant sample was macerated in methanol (10% w/v, powder: methanol) and ethanol (10% w/v, powder: ethanol) for 3 hours and then filtered. After filtration, the marc was re-extracted following the same procedure (this was done three times). The extract was pooled together and evaporated using a rotary evaporator (RV 10 digital V-C, IKA, Germany) at 50°C. The concentrated extract was stored at 4°C.

Determination of chemical composition

The powdered plant sample (10 g) was placed in a flask and mixed with 100 mL each of the following solvents; water, methanol, ethanol, diethyl ether, and chloroform. The mixed solutions were left for 24 h on a shaker at room temperature. After filtration, the solvent in the solution was evaporated using a rotary evaporator until 50 mL remained. The mixture was stored in the refrigerator (4°C) until analysis. The chemical composition of the plant was determined as described by Abegunde (2015)[7] and Narasinga (2012).[8]

Antioxidant assay

The ability of the extract to neutralize DPPH radical was examined using the improved method of Shekhar and Anju (2014).[9] Extract samples were dissolved in methanol at different concentrations. To the extract solution (950 µL) was added 50 µL of DPPH solution (500 µg/mL). The mixtures were shaken vigorously and incubated in the dark for 30 minutes at room temperature. The absorbance of the mixture was measured at a wavelength of 517 nm with three replications. Ascorbic acid (mixed in methanol) was used as the standard. The free radical scavenging activity was calculated using the formular below.

$$\text{Percentage free radical scavenging activity} = \frac{Abs_{control} - Abs_{extract}}{Abs_{control}} \times 100$$

Antibacterial activity

The antibacterial activity of the extract samples was determined using the agar well diffusion technique as described by Jaradat *et al*.[10] Six bacterial strains were used for the study. These include *Bacillus subtilis* (ATCC23857™), *Bacillus cereus* (ATCC14579™), *Escherichia coli* (ATCC 25922™), *Salmonella* sp., *Staphylococcus aureus* (ATCC 25923™), and *Pseudomonas aeruginosa*.

Each plate of agar medium was spread with 50 µL of bacterial solution (density, 10^6 CFU/mL), drain and punched with 7 holes to form 7 wells. Twenty microliters (20 µL) of serial dilutions (50, 100, 200, 300, and 400 mg/mL) of the extract in DMSO (10%) was added to each well. The same volume of DMSO (10%) and Amoxicillin (1 µg/mL) were used as negative and positive controls, respectively. Each test was carried out in triplicate and the experiment was repeated twice. After 24 h, the sterile zone diameter was measured.

Statistical analysis

All data were analyzed using ANOVA with the aid of IBM SPSS 22 for Windows. Data obtained were displayed as mean ± SD. Multiple comparisons of the means were done using the Duncan Multiple Range Test at 5% probability level. P values < 0.05 were regarded as significant.

Results and Discussion

Chemical composition of B. latisepala

Plant chemicals are secondary metabolites produced from plant parts that can be used to treat diseases.[11] Analysis and identification of plant chemicals are important to determine their medicinal value.[12] In this study, the chemical constituents of *B. latisepala* were identified in five different solvent extracts, including polar solvent (water, methanol, ethanol) extracts and non-polar solvent (chloroform and diethyl ether) extracts. From the analysis, 16 chemical compounds were identified in *B. latisepala* including alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, amino acids and proteins, saponins, sterols, tannins, terpenoids, quinones, phytosterols, gum, xanthoproteic acid, coumarin, and essential oils (Table 1). Oxalates, phlobatannins, glycosides, anthocyanins, leucoanthocyanins, cholesterol, and emodins were not detected when extracted with the five solvents. Ethanol showed an ability to extract the most compounds (11 compounds), followed by methanol (10 compounds), and diethyl ether (9 compounds). Water and chloroform extracted the fewest compounds (8 compounds). In the polar solvent extract were identified 14 compounds, while in the non-polar solvent extract were identified only 2 compounds, phenol and amino acid/protein.

From the results of the qualitative phytochemical screening, it is evident that *B. latisepala* is capable of synthesizing many secondary metabolites that are beneficial to humans. The results of this study were similar to those of Wang *et al*. and Nguyen *et al.* where flavonoids, tannins, coumarin and sterols were detected in *B. latisepala* plant.[3,13] Whereas the other eleven compounds synthesized in *B. latisepala* plant including alkaloids, carbohydrates, cardiac glycosides, phenols, amino acids and proteins, saponins, quinones, phytosterols, gum, xanthoproteic acid and essential oils were not recorded in the above mentioned previous studies. In this study, the presence of phenol in the plant samples was different from the findings of Nguyen *et al.*[13] This may be attributed to environmental factors as the differences in environmental conditions and host differences have been shown to affect the presence and content of compounds in plants.[14] The presence of many biologically active secondary metabolites shows potential for the use of *B. latisepala* as a medicinal herb.[15] It is possible to use alkaloids in cancer treatment because of its cytotoxic and analgesic properties.[16] Cardiac glycosides actively support functions of the heart, help to prevent cardiac arrhythmias.[17] Flavonoids inhibit tissue damage, induce vasodilation, antimicrobial and increase the effectiveness of the immune system. It is considered an antibiotic, analgesic, anti-allergic, antiviral, anti-cancer, and anti-diarrhea drug.[18] Courmarin has potential for use as an antimicrobial (antifungal, and antiviral),[19] anticancer, antihypertensive, anti-aging, anticonvulsant, antiallergic, hypoglycemic, analgesic, and antipyretic agent.[20] Terpenoid possesses many biological activities including antibacterial, anti-parasitic, antiviral, antifungal, antispasmodic, hypoglycemic, anti-inflammatory, immunomodulatory, anti-cancer, and anti-tuberculosis activities.[21] Saponin has many biological activities, in particular, this compound is considered to be effective in preventing diabetes.[11]

The analysis of the chemical composition of *B. latisepala* showed that the ethnomedicinal use of plants of the genus *Balanophora* and *B. latisepala* in particular as tonics, treatment of intestinal diseases, and in the relief of pain is justifiable. Furthermore, the chemical composition identified in *B. latisepala* showed the potential of application of this species in the prevention or treatment of many diseases such as diabetes or cancer.

Antioxidant activity

The DPPH free radical scavenging activity of the plant extracts was evaluated spectrophotometrically by measurement of decrease in the absorbance of the DPPH solution.[9] DPPH free radical neutralizing effect of *B. latisepala* whole stem was investigated in three solvents (water, methanol, and ethanol) extracts. From the DPPH free radical scavenging ability of the extract, the EC_{50} value (µg/mL) was determined using the regression equation (Table 2), of which, the higher the EC_{50} value, the lower the antioxidant activity, and vice versa. As shown in table 3, the antioxidant activity of the water, methanol, and ethanol extracts of *B. latisepala* was lower than that of ascorbic acid ($P < 0.05$). Among the three extracts, water extract (EC_{50} = 107.65 ± 4.90 µg/mL) and methanol extract (EC_{50} = 101.50 ± 2.37 µg/mL) exhibited higher antioxidant activity while ethanol extract showed less antioxidant activity than the other extracts with EC_{50} = 806.30 ± 23.52 µg/mL ($P < 0.05$).

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Table 1: Phytochemical constituents of *B. latisepala* whole stem

No.	Compounds	Extract				
		Water	Methanol	Ethanol	Diethyl ether	Chloroform
1	Alkaloid	+	+	+	+	+
2	Carbohydrates	+	+	+	+	-
3	Cardiac glycosides	+	+	+	-	+
4	Flavonoids	+	-	+	+	+
5	Phenol	+	+	+	-	-
6	Amino acids and proteins	+	-	-	-	-
7	Saponin	+	-	-	-	-
8	Sterol	-	+	-	-	+
9	Tannin	+	+	+	-	-
10	Terpenoid	-	+	+	+	+
11	Quinones	-	-	+	+	-
12	Oxalate	-	-	-	-	-
13	Phlobatannin	-	-	-	-	-
14	Phytosterol	-	+	+	+	-
15	Gum	-	+	+	+	+
16	Fat	-	-	-	-	-
17	Glycoside	-	-	-	-	-
18	Xanthoproteic	-	-	-	-	+
19	Anthocyanin	-	-	-	-	-
20	Coumarin	-	+	+	+	+
21	Leucoanthocyanin	-	-	-	-	-
22	Cholesterol	-	-	-	-	-
23	Emodins	-	-	-	-	-
24	Oil	X	X	X	+	X
25	Carotenoid	X	X	X	-	X
26	Polyuronids	-	-	-	X	X

Note: "+": detected; "-": undetected; "X": not tested.

Table 2: Regression equation and EC_{50} value (µg/mL) for DPPH free radical scavenging activity of *B. latisepala* whole stem extracts

Treatments	Regression equation	R^2	EC_{50} (µg/mL)
Aqueous extract	y = 0.0045x + 0.0195	0.9871	107.65 ± 4.90[b]
Methanol extract	y = 0.0042x + 0.0682	0.971	101.50 ± 2.37[b]
Ethanol extract	y = 0.0006x - 0.0026	0.9854	806.30 ± 23.52[c]
Control: ascorbic acid	y = 0.1526x - 0.0584	0.9518	3.65 ± 0.26[a]

EC_{50} values, shown in mean ± SE, in a column that have the same letter are not significantly different (Duncan, P > 0.05).

When compared with *B. laxiflora*, a species of the same genus, the aqueous extract of *B. latisepala* had significantly higher antioxidant activity (EC_{50} = 107.65 ± 4.90 µg/mL) as against EC_{50} of 2037.4 ± 238.18 µg/mL for *B. laxiflora*.[22]

Antibacterial activity

The antibacterial activity of the whole plant extract of *B. latisepala* was investigated on 6 bacterial strains including *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella* sp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results showed that the whole plant extract of *B. latisepala* was inactive against strains of bacteria including Gram-negative bacteria (*B. cereus*, *B. subtilis*, *E. coli*, *Salmonella* sp.) and Gram-positive bacteria (*S. aureus*, *P. aeruginosa*). It was observed that the higher the concentration of the extracts, the higher the antibacterial activity. However, the ethanol extract did not show good antibacterial activity against all the tested organisms. It has been found that *E. coli* often causes chronic and acute diarrhea, urinary tract infection, sepsis, and meningitis.[23] *P. aeruginosa* has been associated with increased mortality in bloodstream infection compared to infections such as urinary tract infection, hospital-acquired pneumonia which are caused by other gram-positive organisms.[24] *Salmonella* infection causes gastroenteritis and severe septicaemia. *S. aureus* is a common cause of a wide range of acute and chronic skin superficial infections, pneumonia, osteomyelitis, and mastitis.[25]

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Table 3: Antibacterial activity of *B. latisepala* whole stem extracts

Bacteria	Solvents	Antibacterial ring diameter (mm)						
		50 mg/mL	100 mg/mL	200 mg/mL	300 mg/mL	400 mg/mL	Amoxicillin	DMSO 10%
B. cereus	Aqueous	1.00 ± 1.00^{bc1}	2.00 ± 1.00^{b1}	3.67 ± 0.57^{a1}	3.67 ± 0.57^{a1}	3.67 ± 0.57^{a1}	-	-
	Methanol	-	1.00 ± 1.00^{b12}	2.67 ± 2.08^{ab1}	3.67 ± 2.08^{a1}	4.33 ± 2.08^{a1}	-	-
	Ethanol	-	-	-	1.00 ± 1.00^{b1}	2.67 ± 1.53^{a1}	-	-
B. subtilis	Aqueous	1.00 ± 1.00^{bc1}	2.00 ± 1.00^{b1}	3.67 ± 0.58^{a1}	3.67 ± 0.58^{a1}	3.67 ± 0.58^{a1}	-	-
	Methanol	-	0.68 ± 0.58^{b2}	1.67 ± 0.58^{b2}	4.33 ± 2.08^{a1}	5.67 ± 2.52^{a1}	-	-
	Ethanol	-	-	-	-	-	-	-
E. coli	Aqueous	3.33 ± 1.16^{d1}	5.00 ± 1.00^{c1}	8.33 ± 0.58^{b1}	9.33 ± 0.58^{ab1}	10.00 ± 1.00^{a1}	9.67 ± 1.16^{ab}	-
	Methanol	-	-	1.00 ± 1.00^{c2}	1.33 ± 1.16^{c2}	5.0 ± 1.73^{b2}	9.00 ± 1.00^{a}	-
	Ethanol	-	-	-	-	-	10.33 ± 1.16^{a}	-
Salmonella sp.	Aqueous	7.00 ± 1.73^{d1}	11.33 ± 0.58^{c1}	14.67 ± 1.16^{ab1}	16.33 ± 1.16^{a1}	15.00 ± 1.00^{ab1}	13.67 ± 2.08^{b}	-
	Methanol	-	-	1.00 ± 1.00^{cd2}	5.33 ± 1.16^{b2}	8.67 ± 0.58^{a2}	15.00 ± 1.00^{a}	-
	Ethanol	-	-	-	-	-	13.67 ± 2.08^{a}	-
P. aeruginosa	Aqueous	2.33 ± 0.58^{e1}	4.33 ± 0.58^{d1}	7.33 ± 0.58^{c1}	8.00 ± 0.00^{b1}	9.00 ± 0.00^{a1}	-	-
	Methanol	1.00 ± 1.00^{d2}	3.00 ± 0.00^{c2}	4.33 ± 0.58^{b2}	7.00 ± 1.00^{a1}	7.67 ± 0.58^{a2}	-	-
	Ethanol	-	-	0.67 ± 0.58^{b3}	2.33 ± 0.58^{a2}	2.00 ± 0.00^{a3}	-	-
S. aureus	Aqueous	2.67 ± 2.31^{cd1}	5.33 ± 1.16^{bc1}	6.67 ± 1.16^{bc2}	9.00 ± 1.00^{ab1}	9.00 ± 0.00^{ab2}	13.0 ± 5.20^{a}	-
	Methanol	2.00 ± 2.00^{de1}	3.33 ± 3.06^{d1}	8.00 ± 0.00^{c1}	9.67 ± 0.58^{bc1}	11.00 ± 1.00^{b1}	17.33 ± 0.58^{a}	-
	Ethanol	-	-	-	-	1.67 ± 1.16^{b3}	16.00 ± 1.73^{a}	-

Note: "-" undetected
The means ± standard deviation with the same letter in the same row (corresponding to each *concentration*) and same number in the same column (corresponding to each *solvent*) are not significantly different (Duncan, $p > 0.05$).

The aqueous and methanol extracts of *B. latisepala* were highly active against these bacteria strains. However, the ethanol extract of *B. latisepala* showed low antibacterial and antioxidant activities. This may be attributed to the presence of gum in the sample which may have affected the extraction of other substances contained in the plant extract due to the insoluble nature of the gum in ethanol.

Conclusion

The phytochemical analysis of *B. latisepala* whole stem extracts reveal the presence of sixteen (16) chemical compounds, including alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, amino acids and proteins, saponins, sterols, tannins, terpenoids, quinones, phytosterols, gum, xanthoproteic, coumarin, and essential oils. *B. latisepala* whole stem water and methanol extracts had better free radical scavenging activity than the ethanol extract. Aqueous and methanol extracts of *B. latisepala* whole stem also showed antibacterial activity.

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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