4. Conclusion

In summary, five compounds were isolated from the ethyl acetate fraction of the whole plant of *P. lanceolata* (L.) Farw. Their structures were identified as vitexin (**1**), naringenin 7-*O*-(2-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside (**2**), naringin (**3**), neoeriocitrin (**4**), and hypolaetin-7-*O*-*β*-D-glucopyranoside (**5**). Compounds **1-5** are firstly reported from *P. lanceolata* (L.) Farw.

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PHYTOCHEMICAL ANALYSIS AND BIOACTIVITY EVALUATION OF *BOEICA POROSA* C.B. CLARKE FROM LAM BINH MOUNTAINOUS REGION, TUYEN QUANG PROVINCE

Pham Thi Thu Trang[1], Nguyen Trung Thanh[1], Nguyen Thu Uyen[1], Nguyen Thi Thu Minh[1], Nguyen Thi Hai[2], Hua Van Luong[3], Chu Quynh Mai[4], Doan Thi Phuong Ly[4], Nguyen Thi Minh Hue[4], Tran Thi Thanh Van[4], Ngo Thi Thuy Ngan[5], Nguyen Thi Thu Thuy[6], Nguyen Tien Dat[1,*], Do Hoang Giang[1,*]

[1]Center for Research and Technology Transfer, Vietnam Academy of Science and Technology;

[2]Ministry of Education and Training, Vietnam;

[3]Lam Binh Forest Protection Office, Tuyen Quang Province, Vietnam;

[4]Tan Trao University, Vietnam;

[5]Department of Fundamental Science, Thai Nguyen University of Medicine and Pharmacy, Vietnam;

[6]Joint Vietnam-Russia Tropical Science and Technology Research Center, Vietnam

*Corresponding author: ntdat@ctctt.vast.vn or dhgiang@ctctt.vast.vn

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Summary

Phytochemical Analysis and Bioactivity Evaluation of *Boeica porosa* C.B. Clarke from Lam Binh Mountainous Region, Tuyen Quang Province

For the first time, a phytochemical and bioactivity analysis of *Boeica porosa* was reported. The total contents of phenolic, flavonoid, saponin, and alkaloid compositions were analysed and the antioxidant, anti-inflammatory effects, α-amylase inhibition and cytotoxicities of extracts and subfractions from *B. porosa* in Lam Binh mountainous area, Tuyen Quang province, were evaluated. Total extracts and subfractions from the aerial part of the plant had higher chemical amounts and exhibited stronger bioactivities than those of the underground part. Ethyl acetate subfraction of the aerial part had

considerable total phenolic and flavonoid contents at 440.5 mg GAE/g and 286.6 mg QE/g, respectively, and exhibited significantly the antioxidant effect and α-amylase inhibition. All of the samples demonstrated weak NO production inhibition in LPS-stimulated RAW264.7 macrophage cells and cytotoxic activity against HepG2 and A549 cancer cells. These findings provided a promising direction for further isolation of bioactive compounds from *B. porosa.*

Keywords: *Boeica porosa, α-Amylase, Antioxidant, Anti-inflammation, Cytotoxic.*

1. Introduction

Boeica porosa C.B. Clarke is a species belonging to the Gesneriaceae family. The plant is a small subshrub and grows on rocky slopes in subtropical mountain forests in northern Vietnam, Southern China, Northern India, and Myanmar [1],[2]. Till now, no study on phytochemicals and bioactivities of *Boeica porosa* C.B. Clarke has been conducted. A previous study detected several classes of compounds, such as phenolics, flavonoids, tannins, quinones, alkaloids, and steroids from the species *Boeica filiformis*, however, very little attention to the chemical constituents and bioactive effects of *Boeica* species has been paid [3]. This study presented for the first time the evaluation of chemical compositions, antioxidant, anti-inflammation, and cytotoxicity of extracts from *B. porosa* collected in the Lam Binh mountainous region of Tuyen Quang province. The plant could be found widely in the mountainous forests with an elevation of 300-500 meters in Tuyen Quang province, thus, this could be a valuable resource for the investigation of bioactive products.

2. Experimental

2.1. Samples and extraction

Boeica porosa mature plants were collected in Lam Binh mountainous area, Tuyen Quang province, Vietnam. The aerial part of the plant included mature and immature leaves, as well as flowers. The samples were identified by Dr Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. The plants were cleaned under water taps, separated the aerial and underground parts, dried at 50-55°C, and then powdered. Next, 50 g of each sample was extracted in triplicate with 1 L of methanol in a sonication bath at 60°C. The solution was filtered and evaporated to yield the total extract of the aerial part (BPA_Total, 6.15 g) and the underground part (BPU_total, 4.23 g). 3 grams of each total extract was suspended in water and successively partitioned in n-hexane, followed by EA to yield the hexane and EA solutions. Next, the water layers were separately adsorbed on the Diaion HP-20 columns which were washed with distilled water and then eluted with methanol. All of the organic layers were concentrated to give the hexane (H), ethyl acetate (EA), and water (W) subfractions, respectively, of the aerial (BPA) and underground (BPU) parts.

2.2. Instruments and chemicals

UV-Vis absorbance was measured on a BioTek Synergy HTX multimode reader, Agilent, US. The incubator and the sonication bath were supplied by Daihan Scientific, Korea. Micropipette and pipette tips were supplied by Isolab, Germany. The solvents: methanol (MeOH), dichloromethane (DCM), ethyl acetate (EA), n-hexane, DMSO, water, together chemicals, such as $AlCl_3$, $NaNO_2$, Na_2CO_3, K_3PO_4, K_2HPO_4, KH_2PO_4, NaCl, NaOH, and HCl (36%) were purchased from Daihan Scientific, Korea. Folin-Ciocalteu reagent, gallic acid, lipopolysaccharides from *Escherichia coli* O26:B6 (LPS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-amylase, 3,5-dinitro salicylic acid (DNSA), sodium potassium tartrate tetrahydrate, reduced nicotinamide adenine dinucleotide (NADH), trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), N^G-Methyl-L-arginine acetate salt (L-NMMA), Griess reagent, catechin, and quercetin were supplied by Merck, Germany.

2.3. Total phenolic assay

The total phenolic contents (TPC) of the samples were evaluated by the Folin - Ciocalteu method [4]. The standard solutions were prepared with several concentrations of gallic acid (10-400 μg/mL). The analytes were prepared by dissolving the extracts or subfractions in methanol at certain concentrations. Each 100 μL of the sample or standard solution was mixed with 900 μL of Folin - Ciocalteu reagent 10% and 1000 μL of Na_2CO_3 6% and incubated for 15 min at 40 °C. Its absorbance was measured with a UV-Vis. spectrophotometer at 750 nm. The total phenolic content was calculated as mg gallic acid equivalent (mg GAE/g) by using the gallic acid calibration curve.

2.4. Total flavonoid assay

The total flavonoid contents (TFC) of the

samples were examined using a previous method [4]. The calibration standards were prepared with different concentrations of quercetin (10-200 μg/mL). The analytes were prepared by the same process as the total phenolic assay. Mix 100 μL of $NaNO_2$ 5% to 500 μL sample or standard solution at room temperature for 5 min, then add 100 μL of $AlCl_3$ 10% and 500 μL NaOH 1M to the mixture. Keep the mixture at room temperature for 15 min. Its absorbance was measured with a UV-Vis spectrophotometer at 510 nm. The total flavonoid content was calculated as mg quercetin equivalent (mg QE/g) by using the quercetin calibration curve.

2.5. Total alkaloid assay

The total alkaloid contents (TAC) of the samples were quantified by a previous method [5]. 5 grams of each sample was suspended in 100 mL of water, acidified to pH 2 by HCl 1N and partitioned with EA (100 mL × 3 times). The organic phase was removed and the water phase was basified by 1N NaOH to pH 11 and then extracted with CH_2Cl_2 (3 times × 200 mL). The combined CH_2Cl_2 extracts were evaporated under reduced pressure to give total alkaloid mixtures. Total alkaloid content was measured by the weight percentage of the alkaloids in the sample.

2.6. Total saponin assay

The total saponin contents (TSC) of the samples were determined by vanillin-sulphuric acid assay [6]. 50 μL of each extract or subfraction solution was incubated with 50 μL of 8% (w/v) vanillin in ethanol and 1500 μL of sulphuric acid 72% (v/v) for 15 min at 70 °C, the standards and the reagent blank made up with various concentrations of aescin and the solvent, respectively. Next, the samples were cooled at ambient temperature for 5 minutes. The absorbance of the standards and samples was measured against the blank at 560 nm.

2.7. Antioxidant assay

The antioxidant activities of extracts and subfractions were evaluated by DPPH, superoxide, and hydroxyl radicals scavenging assays.

DPPH radical-scavenging activity was conducted by modifying a previous method [7]. Briefly, each sample (20 μL) was mixed with 380 μL of DPPH in methanol and then dark incubated at 37°C for 20 minutes. The absorbance was measured at 517 nm. Ascorbic acid was used as a positive control.

Meanwhile, the superoxide radical scavenging activity was measured by the reduction of nitro blue tetrazolium (NBT) [8]. Briefly, various concentrations of each sample were mixed with phosphate buffer (20 mM, pH 7.4), NADH (70 μM), NBT (50 μM), and PMS (15 μM), then, incubated for 5 min at the ambient temperature. The quantity of formazan generated was determined by measuring the absorbance at 562 nm against an appropriate blank. Catechin was used as a positive control.

The hydroxyl radical scavenging assay was measured based on quantification of the degradation product of 2-deoxyribose by condensation with thiobarbituric acid [9]. 50 μL of the test sample was incubated with 100 μL of the phosphate buffer 50 mM pH 7.8, 100 μL of deoxyribose 2.8 mM, and 100 μL of $Fe(NH_4)_2(SO_4)_2$ 500 μM for 1 h at 37 °C. Next, 250 μL of trichloroacetic acid (10%, w/v) and 250 μL of thiobarbituric acid (1%, w/v) were added, and the reaction mixture was boiled for 15 min in a water bath. The colour development was measured at 532 nm. Catechin was used as a positive control.

The IC_{50} value of each extract or subfraction was measured based on experiments in several concentrations. The statistically significant difference in IC_{50} values was evaluated by one-way ANOVA and Tukey's HSD post hoc analysis.

2.8. α-amylase inhibition assay

The α-amylase enzyme inhibitory activity was evaluated by the DNSA method [10] with some modifications. The substrate was prepared by boiling 1 g of starch in 100 mL of phosphate buffer (pH 7.0) for 10 minutes, then it was left to cool down at room temperature. DNSA reagent was prepared by mixing 12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitro salicylic acid solution. The extracts and subfractions were dissolved in DMSO 10% was further dissolved in buffer ($(Na_2HPO_4/NaH_2PO_4$ (0.02 M), NaCl (0.006 M) at pH 6.9) to give various concentrations. Next, 200 μL of each sample solution was mixed with 200 mL of α-amylase solution (2 units/mL) and incubated for 3 minutes at 37°C. Thereafter, 200 μL of the substrate was added, and the solution was incubated at 37°C for 15 minutes. The reaction was terminated by the addition of 200 μL of the DNSA reagent and was boiled for 10 min in a water bath at 85 - 90°C. The mixture was cooled to ambient temperature and measured the absorbance at 650 nm by using the microplate reader. Acarbose was used as a positive control.

2.9. NO production inhibition assay

The effects of extracts on the NO production in LPS-stimulated RAW264.7 macrophage cells were examined as a reported method [11]. The cells (ATCC, MD, US) were cultivated in 96-well plates at 2 × 105 cells/well and incubated for 18 h. The plates were treated with the extracts (from 0,1 µg/mL to 100 µg/mL) for 30 min and then incubated for another 24 h with or without 1 µg/mL LPS. 100 µL of the culture supernatant was transferred to another 96-well plate, and 100 µl of Griess reagent was added. The absorbance of the reaction solution was read at the wavelength 570 nm. The remaining cell solutions in a cultured 96-well plate were used to evaluate cell viability by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as below [12]. L-NMMA, a well-known NO production inhibitor [13], was used as a positive control.

2.10. Cytotoxicity assay

The viability of cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method [12]. A549 and HepG2 cancer cells (ATCC, MD, US) were separately cultivated in 96-well plates at a concentration of 1×10^5 cells/well and treated with various concentrations of the samples. The plates were incubated under a humidified 5% CO_2 atmosphere at 37 °C. After 48h, a solution of MTT 500 µg/mL was added to each well and the plates were incubated for another 4h. After removing the supernatant, formazan crystals were dissolved in isopropanol and the OD values were measured at 570 nm using a microplate reader. Ellipticine was used as a positive control for the evaluation of cytotoxicities of the extracts and subfractions against the cancer cells.

2.11. Statistics

One-way ANOVA, Tukey's HSD posthoc analysis, and correlation analysis were performed in R Package. Values of $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Phytochemical characterization of extracts and subfractions of Boeica porosa

The contents of phenolics, flavonoids, saponins, and alkaloids of the total extracts and subfractions of aerial and underground parts of *Boeica porosa* were evaluated. The data were shown in 0. In general, *B. porosa* aerial part had higher phytochemical contents than the underground one. In both two parts, the total phenolic contents of EA and W subfractions were remarkably higher than those of the total extracts, whereas the hexane residues had the lowest TPC values. For the *B. porosa* underground part (BPU), the subfraction EA had the highest content of phenolics at 191.5 mg GAE/g, followed by the subfraction W at 167.4 mg GAE/g, while TPC values of the total extract and subfraction H were at 134.8 and 41.3 mg GAE/g, respectively. For the aerial part, the TPC of the subfraction EA reached the highest value at 440.5 mg GAE/g. The data for the water residue was 251.4 mg GAE/g, which was slightly higher than the total extract (245.3 mg GAE/g), meanwhile, subfraction H had the lowest phenolic content at 35.1 mg GAE/g. Besides, the TFC values showed a similar trend to the TPC which the EA subfractions had the highest total flavonoid contents, followed by water residues, total extracts, and hexane subfractions. These revealed that phenolic and flavonoid compositions were enriched from the total extracts to the subfractions EA and W.

On the other hand, total saponin and alkaloid contents showed a different trend to the TPC and TFC. In both cases of the aerial and underground parts, the water residues contained the highest amounts of saponins and alkaloids, followed by the ethyl acetate subfractions. These were higher than those of the total extracts. Saponins and alkaloids could not be detected in the hexane subfractions. Meanwhile, the TSC of other fractions of the aerial part varied from 32.7 mgAes/g to 72.3 mgAes/g, which were gently higher than those of the underground part. Similarly, the alkaloid contents of the extracts and subfractions from the underground part were slightly lower than the total extract and corresponding fractions of the aerial part.

Table 1. Total phenolic (TPC), flavonoid (TFC), saponin (TSC) and alkaloid (TAC) contents of the extracts and subfractions of the aerial part (BPA) and the underground part (BPU) of B. porosa. "total", "H", "EA", and "W" represented the total extract, hexane, ethyl acetate, and water subfractions, respectively, of each part.

Sample	TPC(mg GAE/g)	TFC(mg QE/g)	TSC (mgAes/g)	TAC (%)
BPA_total	245.3±20.4	128.9±10.4	32.7±5.1	1.8%±0.1%
BPA_H	35.1±6.5	11.3±1.5	N.D.	N.D.
BPA_EA	440.5 ± 26.9	286.6 ± 15.0	58.9±4.2	2.7%±0.3%

Sample	TPC(mg GAE/g)	TFC(mg QE/g)	TSC (mgAes/g)	TAC (%)
BPA_W	251.4±23.1	182.8±10.2	72.3±8.0	3.1%±0.6%
BPU_total	134.8±12.6	98.3±7.7	24.9±2.2	1.1%±0.2%
BPU_H	41.3±5.7	14.6±2.1	N.D.	N.D.
BPU_EA	191.5 ± 15.4	163.5 ± 9.9	27.6±3.1	1.6%±0.3%
BPU_W	167.4±13.8	115.7±9.8	58.2±4.5	2.0%±0.2%

N.D.: Not detected at the limit of the method.

3.2. Antioxidant activities of the extracts and subfractions of B. porosa

Antioxidant activity of *B. porosa* total extracts and subfractions were evaluated by DPPH, hydroxyl and superoxide radicals scavenging effects. Data were shown in 0. For each part of the plant, the ethyl acetate fraction was the most effective DPPH radical scavenger. The ethyl acetate fraction of the aerial part (BPA_EA) demonstrated the strongest DPPH scavenging effect with IC_{50} at 18.8 µg/mL, which was lower than the value of ascorbic acid, the positive control (IC_{50} 29.8 µg/mL). The total extract (BPA_total) and water fraction (BPA_W) of the aerial part and the ethyl acetate residue of the underground part (BPU_EA) also exhibited considerable DPPH scavenging activity with IC_{50} at 35.4, 30.3, and 37.2 µg/mL, respectively. Meanwhile, only total extracts, ethyl acetate and water subfractions of *B. porosa* aerial part demonstrated weak scavenging effects of hydroxyl and superoxide free radicals. The ethyl acetate residue showed remarkable activities in which the IC_{50} values were gently higher than those of catechin, the positive control while the rest of the fractions exhibited weak inhibition of hydroxyl and superoxide free radicals.

Table 2. Antioxidant activities of *B. porosa* extracts and subfractions

Sample	DPPH (IC_{50}, µg/mL)	Hydroxyl (IC_{50}, µg/mL)	Superoxide (IC_{50}, µg/mL)
BPA_total	35.4±2.8	87.0±6.2	89.6±9.2
BPA_H	>100	>100	>100
BPA_EA	18.8±2.7	38.7±3.4	36.2±2.8
BPA_W	30.3±4.1	45.0±3.2	56.7±6.0
BPU_total	56.4±6.3	>100	>100
BPU_H	>100	>100	>100
BPU_EA	37.2±2.7	>100	>100
BPU_W	46.1±5.32	>100	>100
Ascorbic acid *	29.8±1.6	-	-
Catechin **	-	24.5±1.6	30.2±2.8

*, ** Positive controls.

Antioxidant activities of the samples were strongly correlated with the levels of phenolics and flavonoids. These compounds were reducing agents which could easily react to active free radicals. In this study, the subfractions BPA_EA, BPA_W and the extract BPA_total exhibited considerable DPPH, hydroxyl and superoxide free radicals scavenging activities that could relate strictly to their high phenolic and flavonoid contents.

3.3. α-amylase, NO-production inhibitory, and cytotoxic effects of B. porosa extracts and subfractions

α-amylase is the enzyme that catalyses the hydrolysis of starch to smaller chains. Activations of the enzyme may cause an increase in postprandial blood glucose which could be harmful to diabetic patients. α-amylase inhibitors could reduce the activation of the enzyme which could delay the increase of blood glucose levels. α-amylase inhibitory activities of *B. porosa* extracts and subfractions were evaluated (0). As shown, the ethyl acetate subfraction from the aerial part (BPA-EA) exhibited considerable inhibition of the enzyme α-amylase with IC_{50} at 106.6 µg/mL which was lower than acarbose, a common oral antidiabetic drug (IC_{50} at 108.3 µg/mL). Meanwhile, the other extracts and fractions demonstrated weak inhibitions of the enzyme. Since the fraction BPA_EA had the highest contents of phenolics and flavonoids, while its total saponin and alkaloid contents were the second highest among the analytes, α-amylase inhibitory effects could be affected simultaneously by these four phytochemical constituents. Both α-amylase and α-glucosidase digest the carbohydrates that increase the

postprandial glucose level in diabetic patients. Inhibiting the activity of these two enzymes can control postprandial hyperglycemia, and reduce the risk of developing diabetes [14]. Previously, inhibitions of *B. porosa* to these enzymes have not been investigated. In this study, the EA subfraction of *B. porosa* exhibited significant inhibition of α-amylase. Thus, α-glucosidase inhibition of this subfraction and the others should be further evaluated.

Table 3. α-amylase, NO production inhibitions and cytotoxicity against A549 and HepG2 cells of B. porosa extracts and subfractions

Sample	a-amylase inhibition (IC_{50}, µg/mL)	NO inhibition (IC_{50}, µg/mL)	A549 (IC_{50}, µg/mL)	HepG2 (IC_{50}, µg/mL)
BPA_total	157.1 ± 20.2	44.2 ± 1.4	> 100	> 100
BPA_H	>200	>100	>100	>100
BPA_EA	106.6 ± 9.3	26.7 ± 2.4	72.4 ± 6.8	88.1 ± 7.9
BPA_W	140.4 ± 12.9	40.9 ± 4.1	94.4 ± 8.5	>100
BPU_total	>200	58.6 ± 5.2	> 100	> 100
BPU_H	>200	>100	>100	>100
BPU_EA	167.8 ± 16.0	49.5 ± 4.9	>100	>100
BPU_W	171.2 ± 14.4	43.7 ± 5.8	>100	>100
Acarbose	108.3 ± 9.53	-	-	-
L-NMMA	-	8.48 ± 0.78	-	-
Ellipticine			0.43 ± 0.03	0.47 ± 0.02

On the other hand, the anti-inflammatory effects of the total extracts and subfractions were evaluated via NO production inhibition assay. As shown (0), all of the samples exhibited weaker inhibitions of NO production than L-NMMA, the positive control. BPA_EA demonstrated the strongest inhibition among the extracts and fractions with the IC_{50} at 26.7 µg/mL, which was higher than the IC_{50} of L-NMMA at 8.48 µg/mL. Hexane subfractions did not show considerable effects on the NO production, whereas, the rest of the samples exhibited gentle weak inhibitions. The NO production inhibitory effects showed a similar trend to the antioxidant effects of the samples, that the BPA_EA and BPA_W exhibited the strongest activities while the other fractions demonstrated weak effects. These could be affected by the high TPC and TFC values of these fractions. Previous studies also revealed that the antioxidant and anti-inflammatory effects had a strong correlation. In a body, when free radicals, the atom or molecules that carried one or many unpair electrons, were not balanced with the antioxidant agents, it might lead to various diseases, such as diabetes, inflammation, cancer, etc. [15]. Phenolics and flavonoids were well-natural antioxidant agents, thus, these contents might strictly affect the anti-inflammatory activity of the *B. porosa* EA and water subfractions. Moreover, cell viability assay results indicated that all of the samples had no cytotoxicity to the RAW264.7 cells (Data not shown).

Besides, cytotoxicities on the A549 (lung cancer) and HepG2 (liver cancer) cells of the samples were evaluated. Except for BPA_EA and BPA_W exhibited weak cytotoxicity (IC_{50} from 72.4 to 94.4 µg/mL), other samples had no significant cytotoxic against these cancer cell lines.

4. Conclusion

In this study, the chemical constituents and bioactivities of *B. porosa* were investigated for the first time. Total extracts of *B. porosa* had high contents of phenolics, flavonoids and certain amounts of saponins and alkaloids. The ethyl acetate subfraction of the aerial part of *B. porosa* not only had high contents of phytochemicals but exhibited considerable antioxidant, α-amylase, and NO production inhibitions. All of the samples demonstrated weak cytotoxicities on A549 and HepG2 cancer cells. These were remarkable results that further studies are needed to investigate the bioactive compositions of *B. porosa*.

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CHEMICAL CONSTITUENTS OF THE ENDOPHYTIC FUNGUS *PENICILLIUM SHEARII* ISOLATED FROM *PYRROSIA LANCEOLATA* (L.) FARW.

Ma Chi Thanh[1,*], Nguyen Thanh Dan[1], Ho Le Truc Linh[1], Ly Tu Loan[2]

[1]*Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam;*

[2]*Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam*

*Corresponding author: mcthanh@ump.edu.vn

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Summary

Chemical Constituents of the Endophytic Fungus *Penicillium shearii* Isolated from *Pyrrosia lanceolata* (L.) Farw.

Endophytic fungi are one of the typical endophytic microorganism groups that have been particularly interested in research recently because of their roles in many aspects; the most special thing is the ability to biosynthesize secondary substances with biological activity such as antioxidant, antibacterial, anti-tumor... The endophytic fungal strains were identified as *Penicillium shearii* CBS 290.48 by morphological microscopic and DNA sequencing based on the modified Sanger method. From the ethyl acetate fraction of the medium extract of *P. shearii*, four compounds were isolated and identified as ergosterol (**1**), dehydroxypaxilline (**2**), chrysophanol (**3**), and paspaline (**4**). Their structures were determined by means of spectroscopic methods (MS, 1D, and 2D NMR).

Keywords: *Penicillium shearii, Endophytic fungus, Pyrrosia lanceolata, DNA sequencing.*

1. Introduction

Endophytic fungi are one of the typical endophytic microorganisms, present in the majority of plant species and existing in many parts of plants such as roots, stems, leaves, flowers, fruits, and seeds [1]. The interaction relationship between endophytic fungi and host plants is very complex to ensure a sustainable symbiosis [2],[3]. Endogenous fungi could produce many secondary metabolites in different groups, such as alkaloids, flavonoids, glycosides, coumarins, lactones, chromones, quinones, steroids, terpenoids, xanthones, lignans, peptides, polyketides, organic acids, and phenolic compounds [4],[5],[6],[7],[8]. In addition, the compounds isolated from endophytic fungi exhibited remarkable biological activities as antioxidant [8],[9], antibacterial [10], and anti-tumor [11],[12], which is a potential source for new drug research. However, there are not many studies on endophytic fungi in Vietnam. Therefore, this study is conducted to expand research on potential substances from plant-endophytic fungi, and to contribute to the development of bioactive secondary metabolites for the treatment of several diseases. Our present study on phytochemical constituents from the ethyl acetate fraction of the endophytic fungus strains of *P. shearii* led to the isolation of four compounds. Their structures were identified as ergosterol (**1**), dehydroxypaxilline (**2**),