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Phytochemistry, antibacterial and antioxidant properties from *Adinandra hongiaoensis* and *Polyspora huongiana* (Theaceae)

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Abstract

Adinandra hongiaoensis and *Polyspora huongiana* are the rare and native species found in Vietnam. Firstly, the present studies demonstrated the chemical components, antioxidant and antibacterial effects of the acetone extracts from these plants. Here, a total of 54 constituents are identified in the leaf extracts of 2 studied species using the gas chromatography/mass spectrometry (GC/MS) method. The *P. huongiana* extract displayed activity against 3 *Staphylococcus* strains, including *S. saprophyticus, S. aureus* ATCC 29213 and *S. aureus* ATCC 25923, while the *A. hongiaoensis* extract was not active against any studied bacteria. In addition, *A. hongiaoensis* and *P. huongiana* extracts was found effective against the DPPH radical scavenger with IC_{50} values of 213.31 and 47.60 µg/mL, respectively. The results of this report are expected to provide a foundation for further research aimed at applying these species in the development of pharmaceutical products in the future.

Keywords

Adinandra hongiaoensis; Polyspora huongiana; GC/MS; antibacterial; antioxidant properties

Introduction

Theaceae includes about 480 species and 9 genera mainly found in tropical and subtropical regions of East and Southeast Asia (1-5). In Vietnam, about 80 species and 11 genera have been recorded (6-9). *Adinandra* and *Polyspora* are the 2 genera belonging to the Theaceae family. The first genus comprises over 100 species widely distributed across China, India, Bangladesh, Japan, tropical Africa and Southeast Asian countries and about 12 species in Vietnam (7, 10, 11). Whereas, the later one includes about 40 species mainly found in East and Southeast Asia (5), of which 13 species recorded in Vietnam (5-7, 9, 12-20). The members of *Adinandra* genus have been reported to possess so many bioactive compounds as well as the biological activities, including analgesic, antitumor, anti-toxicity and antibacterial. In addition, the extracts from leaves of some *Adinandra* plants have been consumed as beverage for hundreds of years (21-26). Meanwhile, because of their beautiful tree shape, especially, blooming in winter, the *Polyspora* plants have been reported to have the ornamental value and they, thus, are usually used as street or shade

trees in gardens (27-29). Moreover, studies have also demonstrated the medicinal value of Polyspora plants, highlighting their natural antioxidant effects (30, 31) and cytotoxic properties (32-34).

Adinandra hongiaoensis Son & L.V.Dung and *Polyspora huongiana* Orel, Curry & Luu are 2 new species belonging to Theaceae family which have been recently described from Vietnam (11, 16). The type specimens of the first species were collected from Bidoup Nui Ba National Park, Lam Dong province, Vietnam. The morphological features of this species were characterized by having: trees up to 10 m tall, cordate leaf blade base, short pedicel length and pubescent petal pubescence (11). Similarly, the later plant was also found in Bidoup Nui Ba National Park. It is distinguished from other *Polyspora* species by its narrowly elliptic mature leaves, stamens arranged in three concentric circles, filaments basally joined to the inner petal whorl, and a densely tomentose ovary (16). So far, both species have been known as the rare species and only found in the type locations (11, 16). The present study, thus, demonstrated the chemical components, antioxidant and antibacterial activities of the acetone extracts from *A. hongiaoensis* and *P. huongiana* for the first time.

Materials and Methods

Bacterial strains

Eight bacterial strains were used to identify the antibacterial activity of the studied species, including 4 Gram-negative strains (*Salmonella typhimurium* ATCC 13311, *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 9199, *Enterobacter hormaechei* ATCC 700323) and 4 Gram-positive strains (*Staphylococcus saprophyticus* BAA750, *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 13883, *S. aureus* ATCC 25923).

Plant materials

The leaves of *Adinandra hongiaoensis* and *Polyspora huongiana* were collected from Bidoup Nui Ba National Park, Lam Dong province, Vietnam, at the coordinates of 12°10'59"N and 111°27'49"E; 12°11'00"N and 111°27'47"E, respectively. The vouchered specimens of *A. hongiaoensis* (BDNP24012021) and *P. huongiana* (BDNP25012021) were deposited in the Herbarium of Faculty of Natural Resources and Environment, Vietnam National University of Forestry at Dongnai.

Extraction procedures

The fresh samples were dried at 50 °C and ground into fine powder. 100 g of powder was soaked in 500 mL of 99% acetone (Thermo Fisher Scientific, USA) for 72 h and then the extract was filtered using Whatman paper. The residue material was subjected to 2 additional acetone extractions and the final product was obtained by pooling all the filtrates. Solvent removal was performed at 45 °C using a vacuum evaporator, resulting in the brown extracts (26).

Gas chromatography/mass spectrometry (GC/MS)

Chemical components in different extracts were first analyzed using Thermo Scientific™ ISQ™ 7000 GC-MS (Thermo Fisher Scientific, USA) system with the stationary phase Agilent DB-5MS column. Running parameters were set as previously described (25). The chemical compositions were determined based on the NIST 2017 library.

Antibacterial activity

Agar disk diffusion method was used to determine the extract's antibacterial effect as recommended by CLSI (35). Bacterial cultures of 0.5 McFarland standard equivalent were spread on sterile MHA plates and different acetone extracts diluted with DMSO 15 % into concentrations of 100, 150 and 200 mg/mL were subsequently added to the standard sterile disc. The plates were incubated at 35 ± 2 ° C for about 18 h for measurement of the zone of inhibition. Gentamicin disc (10 µg, Nam Khoa BioTek, Vietnam) was used as positive control, whereas DMSO 15 % was used as negative control. The zone of inhibition of studied sample was calculated by the formula

$$A = D-d \qquad \text{(Eqn. 1)}$$

Where, D is a zone of inhibition; d is the diameter of disc (6 mm). The experiment was done in triplicate and data were presented as mean ± standard deviation (SD). Fisher's least significant difference (LSD) and one-way analysis of variance (ANOVA) were employed for statistical analysis.

Antioxidant activity

Antioxidant effect was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay followed with minor modifications (25). The extract was completely dissolved in 99.8% methanol (Thermo Fisher Scientific, USA) to obtain different concentrations. Then, 0.3 mL was mixed with 3.7 mL of 0.1 mM DPPH. The mixture was incubated for 30 min in the dark at room temperature. The absorbance was subsequently measured at 517 nm using a UV-Vis spectrophotometer (Genesys 20, USA). The antioxidant effect of the extract was determined by IC_{50} value in comparison with ascorbic acid, which was used as a control. Antioxidant activity was calculated as:

$$\% \text{ DPPH} = \frac{A_o - A_i}{A_o} \times 100 \qquad \text{(Eqn. 2)}$$

Where, Ao and Ai are the absorbance of the blank (DPPH solution) and the sample (DPPH solution and extract sample) respectively.

Statistical analysis

The experiments were carried out 3 times and the results were reported as the mean ± standard deviation (SD). The data were analyzed using a one-way analysis of variance (ANOVA) to compare different groups, with Fisher's least significant difference (LSD) procedure ($p<0.05$) used for multiple comparisons. Statistical analysis was done with Statgraphics Centurion XV (version 15.1.02, Statgraphics Technologies, Inc., USA).

Results

Chemical compositions from two studied extracts

The chemical compositions of the acetone extracts from the leaf of *A. hongiaoensis* and *P. huongiana* were shown in Table 1 and Fig. 1. Accordingly, the *A. hongiaoensis* extract was found to be rich in linolenic acid (16.40 %), neophytadiene (9.76 %), γ-sitosterol (9.73 %), phytol (9.51 %), 2-pentanone, 4-hydroxy-4-methyl- (7.47 %), l-(+)-ascorbic acid 2,6-dihexadecanoate (7.24 %), tetracosan-10-yl acetate (5.31 %), labda-8(20)-13-diene-15,19-dioic acid, (E)- (4.89 %). The *P. huongiana* extract was characterized by the predominance of 1,2,3-benzenetriol (18.02 %), neophytadiene (13.40 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (12.94 %), vitamin E (8.81 %), squalene (5.72 %), benzoic acid, 4-hydroxy- (4.94 %).

Antibacterial properties of acetone extracts from two Theaceae species

The antibacterial effect of the acetone extracts from 2 studied plants were conducted using agar disc diffusion method. Overall, the *P. huongiana* extract was found to be effective against 3 out of 8 bacterial strains, including *B. cereus, S. aureus* ATCC 29213 and *S. aureus* ATCC 25923, while no active agent was found in the *A. hongiaoensis* extract (Table 2). At dose of 100 mg/mL, the *P. huongiana* extract showed a weaker effect than those at dose of 150 and 200 mg/mL, whereas there was no significant difference in the zone of inhibition between the concentrations of 150 and 200 mg/mL (Table 2).

Antioxidant properties of acetone extracts from two Theaceae species

The antioxidant effect of acetone extract from *A. hongiaoensis* and *P. huongiana* were investigated using the DPPH radical scavenging test with IC_{50} value of 213.31 and 47.60 µg/mL, respectively (Table 3).

Discussion

The chemical components and biological properties of the different solvent extracts isolated from other Adinandra species from Vietnam have been reported by prior reports (22-26). For instance, the methanol extract of Adinandra poilanei stem showed 5 compounds, including tyrosol; scopoletin; lupeol; 2,6-dimethoxy-1,4-benzoquinone and 2β-hydroxypomolic acid (26). The chemical compositions and biological properties of the ethanol extract and its fractions, ethyl acetate and dichloromethane, isolated from Adinandra glischroloma stem were also investigated. Accordingly, the ethanol extract contained some chemical groups such as flavonoids, polyphenolic and coumarins (22). Moreover, the dichloromethane fraction from this species was found to be effective against B. subtilis and L. plantarum. The antioxidant activities of the dichloromethane, ethyl acetate fractions and ethanol extract were quantified with EC50 values of 86.7, 88.5 and 111.2 µg/mL, respectively. The ethanol extract also possessed effect against gastric, lung and breast cancer cell lines with IC50 values of 59.1, 63.23 and 67.48 µg/mL, respectively (22). Similarly, the ethanol extract and its

Table 1. The chemical components of acetone extracts from *A. hongiaoensis* (AHO) and *P. huongiana* (PHU).

RT	Compounds	AHO (%)	PHU (%)
2.45	2-Pentanone, 4-hydroxy-4-methyl-	7.47	4.00
4.62	3-Hexenoic acid, (E)-	0.21	-
4.67	cis-1,2-Dihydrocatechol	-	0.27
5.84	Cyclohexene, 4-ethenyl-1,4-dimethyl -	-	0.09
5.92	Eucalyptol	-	0.19
7.22	Alpha-l-rhamnopyranose	-	0.70
7.24	Phenethylamine, 3-benzyloxy-2-fluoro-á-hydroxy	0.09	-
7.44	Paromomycin	0.09	-
8.22	12,15-Octadecadiynoic acid, methyl ester	0.04	-
8.56	5-Hydroxymethylfurfural	-	2.77
9.50	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	0.09	-
9.87	Pyrogallol	-	18.02
10.02	Melezitose	0.32	2.27
10.16	Vanillin lactoside	0.05	-
10.20	Octahydrochromen-2-one	0.11	-
10.85	Benzoic acid, 4-hydroxy-	-	4.49
11.19	cis-Vaccenic acid	0.12	-
11.51	1,3-Benzenediol, 4-propyl-	0.34	-
11.57	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	0.25	-
11.82	Benzenepropanol, 4-hydroxy-3-methoxy-	-	0.24
12.00	7-Oxo-2-oxa-7-thiatricyclo[4.4.0.0 (3,8)]decan-4-ol	-	0.73
12.14	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl) phenol	0.31	-
12.37	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	0.73	-
12.42	Tetradecanoic acid	0.30	-
12.88	Neophytadiene	9.76	13.40
12.90	1-Hexadecanol, 2-methyl-	0.28	-
13.11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3.61	12.94
13.49	n-Hexadecanoic acid	-	2.29
13.51	l-(+)-Ascorbic acid 2,6-dihexadecanoate	7.24	-
13.70	trans-Sinapyl alcohol	0.61	-
14.27	Phytol	9.51	3.45
14.40	Linolenic acid	16.40	2.12
14.48	Octadecanoic acid	0.97	-
14.88	9-Hexadecenoic acid	0.32	-
15.26	Ethyl iso-allocholate	0.23	-
15.80	Kauren-19-oic acid	1.10	-
16.07	17-Pentatriacontene	3.70	-
16.19	Palmitin, 2-mono-	1.15	0.46
16.30	1-Heptatriacotanol	0.32	-
16.67	17-Pentatriacontene	0.96	-
17.37	Tetracosan-10-yl acetate	5.31	-
17.41	Linolenin, 1-mono-	-	0.31
18.05	4,6-Androstadien-3β-ol-17-one, acetate	0.98	-
18.38	Squalene	2.84	5.72
19.23	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	-	0.67
19.28	Labda-8(20)-13-diene-15,19-dioic acid, (E)-	4.89	-
21.12	Ethyl iso-allocholate	-	0.36
22.15	17-Pentatriacontene	0.93	-
22.52	Vitamin E	-	8.81
25.03	Stigmasterol	2.06	5.56
26.09	γ-Sitosterol	9.73	-
26.91	Stigmast-7-en-3-ol, (3β,5α)-	-	2.52
27.37	α-Amyrin	-	5.47
28.07	dl-α-Tocopherol	3.14	-
	Total	**96.56**	**97.85**

Table 2. The antibacterial activity of acetone extracts from *A. hongiaoensis* and *P. huongiana*.

Bacterial strains	Concentration (mg/mL)			Gentamicine	Negative control
	100	150	200		
E. coli	-	-	-	13.5 ± 0.87	-
S. typhimurium	-	-	-	17.67 ± 0.58	-
S. flexneri	-	-	-	14.67 ± 1.15	-
E. hormachei	-	-	-	15.33 ± 1.53	-
B. cereus	-	-	-	13.17 ± 0.76	-
S. saprophyticus	-	6.33 ± 1.53[a]	5.67 ± 0.76[a]	20.67 ± 0.58[b]	-
S. aureus ATCC 29213	3.25 ± 0.25[a]	6.17 ± 1.26[b]	5.17 ± 0.29[b]	18.33 ± 1.15[c]	-
S. aureus ATCC 25923	3.75 ± 0.43[a]	7.40 ± 1.15[b]	6.83 ± 0.76[b]	18.50 ± 0.87[c]	-

Different superscript lower-case letters in the same row denote significant differences ($p<0.05$). - not active.

Table 3. The antioxidant activity of acetone extracts from *A. hongiaoensis* and *P. huongiana*.

	A. hongiaoensis	*P. huongiana*	Ascorbic Acid
IC_{50} (µg/mL)	213.31	47.60	2.41

fractions, dichloromethane and ethyl acetate fractions, from the Adinandra megaphylla stem were also reported to consist of some bioactive compounds and biological activity. As a result, the ethanol extract possessed polyphenolic and coumarins compounds (24).The dichloromethane and ethyl acetate fractions of this species had an inhibitory effect on B. subtilis, L. plantarum and E. coli. The ethyl acetate fraction and ethanol extract were found effective against the DPPH radical scavenger with EC50 values of 32.0 and 28.9 µg/mL, respectively. The ethanol extract also showed cytotoxic effects against several cell lines, including lung, gastric, and breast, with IC50 values of 43.52, 56.54, and 58.24 µg/mL, respectively (24).

A study showed a new triterpene compound, lupan-3β,20-dihydroxy-28-carbaldehyde and 11 known compounds from the methanol extracts of the stem obtained from *Adinandra hainanensis* grown in Vietnam (23). Furthermore, 10 out of 12 compounds, including a new compound, isolated from this species were also found to possessed the cytotoxicity (KB, Hep-G2, LU, MCF-7 cell lines) and α-glucosidase inhibitory effects (23). The ethanol extract from the leaf of *Adinandra bockiana* collected from Vietnam also contained some chemical groups like tannin, polyphenol and coumarin (21). Also, ethanol extract and its fractions, dichloromethane and ethyl acetate, isolated from *A. bockiana* displayed activity against *B. subtilis*, *L. plantarum* and *S. macescens* as well as effective against DPPH radical scavenger with EC_{50} values of 0.2, 0.47 and 5.7 µg/mL, respectively. The ethanol extract of this plant also showed cytotoxic activities against breast, lung, gastric cell lines with IC_{50} values of

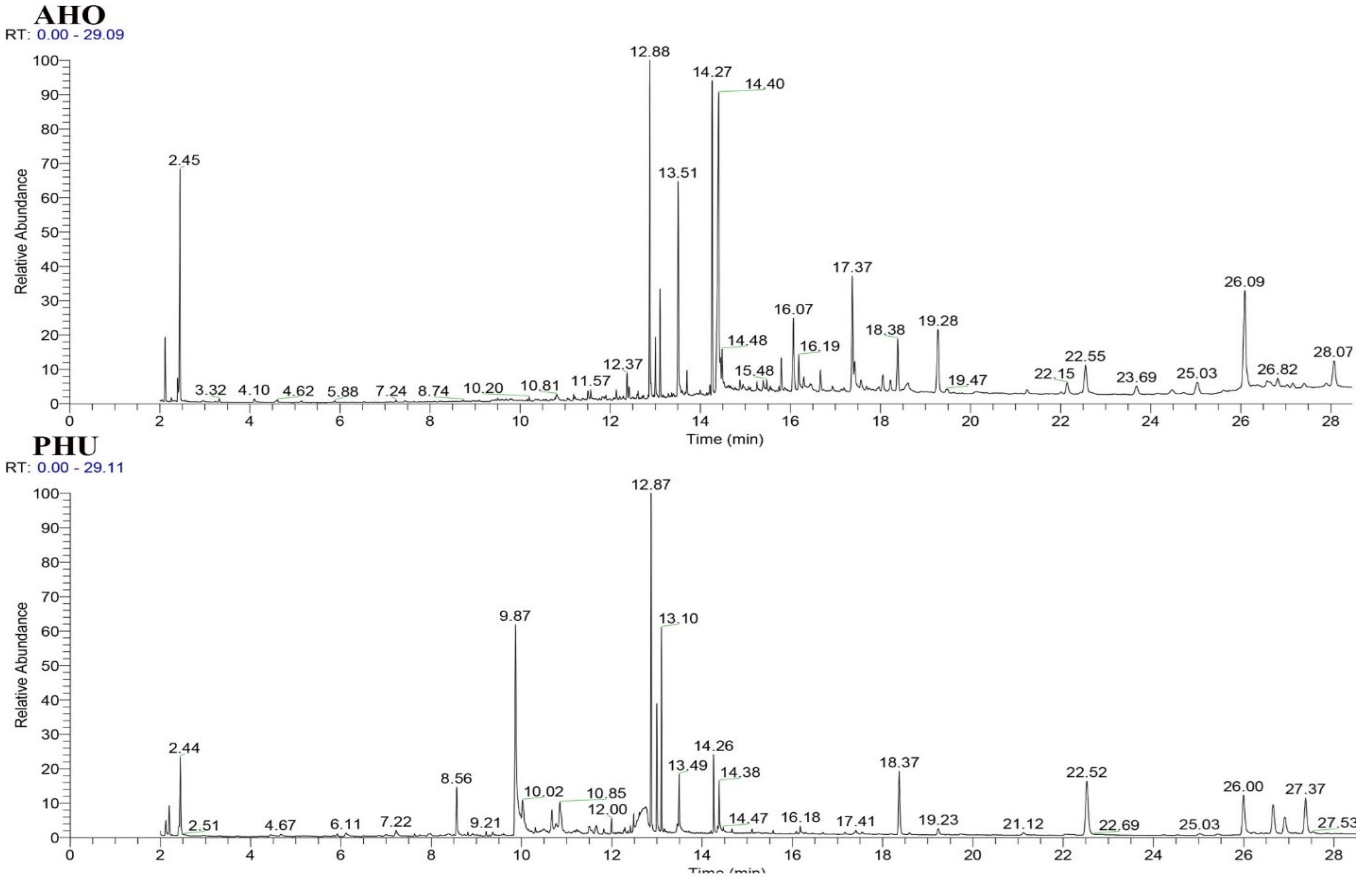

Fig. 1. The GC chromatogram of acetone extracts from *A. hongiaoensis* (AHO) and *P. huongiana* (PHU).

43.15, 48.73 and 58.62 µg/mL, respectively (21).

Previous studies have also provided insights into the chemical constituents of *Gordonia chrysandra*, a synonym for *Polyspora chrysandra*. For instance, 2 new flavanonol glycosides, chrysandrosides A and B, were obtained from *P. chrysandra* roots (36), whereas a new phenolic glycoside, chrysandroside A was also isolated from the stem of this species (37). A study demonstrated the polyphenolic compounds and antioxidant properties from the fruit extract of *Gordonia axillaris*, a synonym species of *Polyspora axillaris* (30). Accordingly, the fruit extract of this plant contained some polyphenolic constituents, including rutin, gallic acid, protocatechuic acid, epicatechin, 2-hydrocinnamic acid, p-coumaric acid, quercetin, epicatechin gallate and ferulic acid. The ABTS radical scavenging effect of the extract was 525.05 ± 14.34 µmol Trolox/g DW (30).

Conclusion

In this study, the phytochemical properties, antibacterial and antioxidant activities of the acetone extracts isolated from 2 Theaceae species, *Adinandra hongiaoensis* and *Polyspora huongiana* were investigated for the first time. Along with the applicability of many species of Theaceae family, hopefully, the result of this study may serve as a foundation for future research, exploring the use of these species in pharmaceutical and other fields.

Authors' contributions

TVP and HTV participated in the design of the study and performed the experiments and statistical analysis. TDN, TTTN, HTDN, NAN, QHN, TTL and VHN performed experiments and handled the research data. HTV and TVP drafted the manuscript and resolved all the queries of editors and reviewers. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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