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Exploring Phenolic Compounds and Alpha-Glucosidase Inhibition Potential of *HEDERA NEPALENSIS* in the High Mountains of Northern Vietnam

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Abstract: ***In the high mountain regions of Vietnam, Hedera nepalensis is extensively dispersed and has been shown to have a variety of phytochemicals. This study's major goals were to identify phenolic acids and flavonoidscompounds, andevaluate α-glucosidase inhibitory activity of H. nepalensis in the mountainous regions of four northern Vietnamese provinces, including Ha Giang, Lao Cai, Lai Chau, and Lang Son. All H. nepalensiscrude extracts were subjected to the UHPLC-Q-TOF-MS/MS technique, which was used in both the negative and positive electrospray ionization modes to identify 5 flavonoids and 6 phenolic acids. The moderate α-glucosidase inhibitory activity of these samples was also discovered at the tested concentration.***

Keywords: *Hedera nepalensis*, α-glucosidase, UHPLC-Q-TOF-MS/MS, flavonoids, phenolic acids

1. Introduction

Hedera nepalensis is a member of the Araliaceae family's *Hedera* genus, which is recognized to have therapeutic potential in some cases[1]. *H. nepalensis* has been found in several European nations, the Himalayas, China, India, and parts of Vietnam's high mountains[2,3]. According to recent findings from research on medicinal plants in Vietnam's northern highland regions, *H. nepalensis* is relatively widely dispersed in the provinces of Ha Giang, Lao Cai, and Lai Chau. *H. nepalensis* is still the subject of relatively little investigation, nevertheless[3].

The important phytochemicals identified in the crude methanol extract of *H. nepalensis* belong to the classes of flavonoids, phenolic acids, cardiac glycosides, steroids, alkaloids, and saponins[1,4-7]. UHPLC-QTOF-MS/MS has become an important and more efficient method for the determination of chemical structures[8].This technique has been widely used to analyse chemical constituents and metabolites of medicinal herbs, and the findings are remarkable[9-13]. Our research group has published 4 new triterpene saponin structures from *H. helix*[14] and 8 new structures from *H. nepalensis* applying dereplication[15].

Besides, many researchers have paid attention to the bioactivities of *Hedera nepalensis*. In 2014, according to Saleem et al., triterpenoid lupeol in *H.nepalensis* extracts had significant dipeptidyl peptidase-4 (DPP-4) inhibitory action[5]. In 2015, from the 95% ethanol extract of *H.nepalensis*, Li et al. extracted and identified two anticancer chemicals, which were pulsatilla saponin A and hederagenin 3-O-L-arabinopyranoside[16].In 2016, Jafri et al. suggested that extract and fractions of *H. nepalensis* and its isolated compound lupeol showed potent cancer chemo-preventive and cytotoxic potential[17]. In 2017, according to Jafri et al., *H. nepalensis* had a significant amount of phenol chemical components, which have significant antioxidant potential[6]. In 2022, Duong et al. reported that saponin extracts of *H.nepalensis* leaves exhibited a potential antimicrobial activity[18].

This study aims to explore flavonoids and phenolic acids composition of *Hederanepalensis* using UHPLC-Q-TOF-MS/MS and evaluate α-glucosidase inhibition activity of this medicinal plant collected in four northern provinces of Vietnam including Ha Giang, Lao Cai, Lai Chau, and Lang Son.

2. Materials and Methods

2.1. Chemicals and reagents

α-glucosidase (Lot 0000209485), p-nitrophenyl-α-D-glucopyranoside (PNPG), acarbose,dimethyl sulfoxide (DMSO), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) werepurchased from Sigma-Aldrich Chemical Co. (Singapore). Deionized water for HPLC; HPLC grade acetonitrile, methanol; analytical grade formic acid (≥98%) were obtained from Scharlau (Barcelona, Spain);

2.2. Sample preparation

Hedera nepalensis was collected from northern mountainous areas in Ha Giang, Lao Cai, Lai Chau, and Lang Son provinces, Vietnam. The plants were identified by botanist Tran Huu Dang MSc, Southern Institute of Ecology (VAST). Voucher specimens (Code: NaPro0621-24) were deposited in the Center for Research and Technology Transfer (VAST).

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After being gently washed, the leaves were cut into small pieces and left to naturally dry before being used for further experiments.For each *H. nepalensis* sample, about 10.0 (g) of leaves was filled with 25.0 (mL) of methanol–water (1:1, v/v). After being ultrasonicated for ten minutes at 50°C, the extracted solution was evaporated to dryness using a rotary evaporator system (BÜCHI R-300, Switzerland) following centrifugation. The extraction step continued with the residue. After five time of extraction, the crude extracts were collected. For UHPLC-Q-TOF-MS/MS analyses, precisely weighed 5.0 (mg) of each crude extract was completely dissolved with 10.0 (mL) of methanol–water (1:1, v/v) solvent. The samples were filtered through 0.45 (µm) filter membranes before being injected for analyses. For testing α-glucosidase inhibition activity, the crude extracts were dissolved in DMSO 10% at a concentration of 1000 (ppm).

2.3. UHPLC-Q-TOF-MS/MS analyses

The ExionLC™ UHPLC system coupled to an $X500_R$ QTOF mass spectrometer (AB SCIEX, USA) via an electrospray ionization (ESI) interface enabled the acquisition of high-resolution MS and MS/MS spectra in both negative and positive ion modes. Chromatographic separation process and MS parameters were set up as mentioned in our previous study[13]. Briefly, chromatographic separation was carried out on a Hypersil GOLD C18 column (150 x 2.1 mm, 3µm) (Thermo Fisher Scientific, USA) at room temperature. Water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) were used as mobile phase with a linear gradient elution (0–4 min, 2-20% B; 4-30 min, 20-68% B; 30-32 min, 68-98% B; 32-40min, 98% B). The flow rate was set at 0.4 (mL/min). The sample inject volume was 5.0 (µL).The QTOF operating parameters were optimized as follows: the ion source temperature, 500°C; curtain gas, 30 psi; nebulizer gas (GS 1), 45 psi; heater gas (GS 2), 45 psi. For the negative mode, ion spray voltage was set at −4.5 kV, the declustering potential (DP) was −70 V, the collision energy (CE) was performed at −20 eV and the collision energy spread (CES) was 10 eV. For the positive mode, ion spray voltage was set at 5.5 kV, the DP was 80 V, the CE was 20 eV and the CES was 10 eV. The mass range for TOF MS scan was set at m/z 70–2000. For TOF MS/MS scan, the mass range was set at m/z 50–1500.

Raw data were recorded and processed with SCIEX OS software version 1.2.0.4122 (AB SCIEX, USA).

2.4. α-Glucosidase inhibition activity

α-Glucosidase inhibition activity of *H. nepalensis* samples was performed as described by our previous study[19]. In short, the reaction buffer was sodium phosphate buffer at 100.0(mM) and pH of 6.8. In eachwell, a mixture of 20 (µL) of test sample (or DMSO 10% for the negative controls), 20 (µL) of α-glucosidase at 0.33 (U/mL) in water, and 140 (µL) of buffer were incubated at 37°C for 20 min. Then, 20 (µL) of PNPG substrate at 2.5 (mM) in water (or water for the blanks) was added to each well. Acarbose was used as positive control. After 30 min of incubating at 37°C, the reaction products were measured at 405 (nm) using a thermostatically controlled PowerWave HT microplate spectrophotometer (BioTek, USA).

The percentage of α-glucosidase inhibition was calculated as follows:

$$\text{Inhibitory activity (\%)} = (A_0\text{-}A_1)/A_0 \text{x } 100\%$$

Where A_0 is the absorbance of the negative control; A_1 is the absorbance of the sample.

2.5. Data analysis

Bioactivity data were processed using Rstudio (version 1.4.1717) software. The obtained data were compared by one-way ANOVA and Tukey's HSD post hoc test. If $p \leq 0.05$, adifference was considered statistically significant.

3. Results and Discussion

3.1. UHPLC-Q-TOF-MS/MS analyses

After processing the mass spectrometry data**(Table 1)**, five flavonoids and six phenolic acids were identified. Surprisingly, all these phytochemicals were found in all four *H. nepalensis* samples in Ha Giang, Lao Cai, Lai Chau, and Lang Son areas.

3.1.1. Flavonoids

There were many scientific studies reported the present of flavonoids, a diverse group of polyphenolic compounds that possess a wide range of biological activities, in *Hedera* species[6, 20-22]. The fragmentation pattern of flavonoids in the negative ion mode was more difficult to interpret than in the positive ion mode[23]. In this study, five flavonoids **(Figure 1)** were identified in *H. nepalensis* samples based on the MS/MS and comparison with literature data[23-25].

At T_R = 7.97 min, there were two pseudomolecular ions $[M+H]^+$ of 465.1024 (**compound 3**) and 611.1598 (**compound 4**). **Compound 3** produced the MS/MS daughter ions at m/z 303 and 285, corresponding to the elimination of a glucose molecule followed by a water molecule. **Compound 4** was fragmented to ions at m/z 465 and 303, indicating the consecutive loss of one Rha and one Glc. The fragment ion at m/z 303 was determined to be quercetin. Therefore, **compounds 3** and **4** wereidentified asisoquercitrin (quercetin 3-*O*-glucoside) and rutin, respectively. In addition, at T_R = 8.73 min, there were two protonated ions $[M+H]^+$ of 449.1078 (**compound 5**) and 595.1652 (**compound 6**). These two compoundsshowed the same fragment at m/z 287, indicating the loss of a glucose (**compound 5**), a glucose and a rhamnose (**compound 6**) from kaempferol. As a result, **compounds 5** and **6** were tentativelycharacterized askaempferol-7-*O*-glucoside and kaempferol-7-*O*-neohesperidoside, respectively. Besides, **compound 7** at T_R=9.00 min exhibited a deprotonated ion $[M-H]^-$ at m/z 593.1520 and showed fragment ions at m/z 285 and 151. Hence,**compound 7** was determined as kaempferol-3-*O*-rutinoside.

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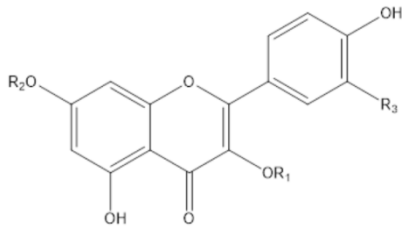

3. R_1=Glc, R_2=H, R_3=OH
4. R_1=Rha-Glc, R_2=H, R_3=OH
5. R_1=H, R_2=Glc, R_3=H
6. R_1=Rha-Glc, R_2=R_3=H
7. R_1=H, R_2= Rha-Glc, R_3=H

Figure 1: Chemical structures of identified flavonoids in *H. nepalensis*samples.

Table 1: Flavonoids and phenolic acids of *H. nepalensis* samples characterized by UHPLC-Q-TOF-MS/MS.

No.	RT	Precursor mass	Name	Formula	Exact mass	m/z	Ion	Error	MSMS
1	6.19	353.0875	Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	353.0873	M-H	0.68	191.0553 $[M-H-C_9H_6O_3]^-$; 173.0452 $[M-H-C_9H_8O_4]^-$; 161.0243 $[M-H-C_6H_8O_7]^-$; 135.0452 $[M-H-C_8H_{10}O_7]^-$; 93.0344 $[M-H-C_{10}H_{12}O_8]^-$
2	7.83	191.0563	Quinic acid	$C_7H_{12}O_6$	192.0634	191.0556	M-H	3.85	93.0427 $[M-H-CH_6O_5]^-$; 85.0344 $[M-H-C_3H_6O_4]^-$
3	7.97	465.1024	Isoquercitrin (quercetin 3-*O*-glucoside)	$C_{21}H_{20}O_{12}$	464.0955	465.1033	M+H	-1.95	303.0509 $[M+H-C_6H_{10}O_5]^+$; 285.0595 $[M+H-C_6H_{12}O_6]^+$
4	7.97	611.1598	Rutin	$C_{27}H_{30}O_{16}$	610.1534	611.1612	M+H	-2.32	465.1094 $[M+H-C_6H_{10}O_4]^+$; 303.0520 $[M+H-C_{12}H_{20}O_9]^+$; 147.0676 $[M+H-C_{21}H_{20}O_{12}]^+$
5	8.73	449.1078	Kaempferol-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	448.1006	449.1084	M+H	-1.31	287.0587 $[M+H-C_6H_{10}O_5]^+$
6	8.73	595.1652	Kaempferol-3-*O*-rutinoside	$C_{27}H_{30}O_{15}$	594.1585	595.1663	M+H	-1.85	287.0544 $[M+H-C_{12}H_{20}O_9]^+$; 147.0656 $[M+H-C_{21}H_{20}O_{11}]^+$
7	9.00	593.1520	Kaempferol-7-*O*-neohesperidoside	$C_{27}H_{30}O_{15}$	594.1585	593.1507	M-H	2.28	285.0622 $[M-H-C_{12}H_{20}O_9]^-$; 151.0151 $[M-H-C_{20}H_{26}O_{11}]^-$
8	9.31	515.1204	1,5-Dicaffeoylquinic acids	$C_{25}H_{24}O_{12}$	516.1268	515.1190	M-H	2.81	353.1150 $[M-H-C_9H_6O_3]^-$; 335.1071 $[M-H-C_9H_8O_4]^-$; 191.0705 $[M-H-C_{18}H_{12}O_6]^-$; 179.0490 $[M-H-C_{16}H_{16}O_8]^-$; 161.0367 $[M-H-C_{16}H_{18}O_9]^-$
9	9.32	353.0885	Neochlorogenic acid	$C_{16}H_{18}O_9$	354.0951	353.0873	M-H	3.51	191.0702 $[M-H-C_9H_6O_3]^-$; 179.0526 $[M-H-C_7H_{10}O_5]^-$; 161.0333 $[M-H-C_6H_8O_7]^-$
10	10.03	353.0885	4-*O*-Caffeoylquinic acid	$C_{16}H_{18}O_9$	354.0951	353.0873	M-H	3.51	191.0564 $[M-H-C_9H_6O_3]^-$; 179.0340 $[M-H-C_7H_{10}O_5]^-$; 173.0458 $[M-H-C_9H_8O_4]^-$; 135.0456 $[M-H-C_8H_{10}O_7]^-$
11	10.03	515.1202	Isochlorogenic acid B	$C_{25}H_{24}O_{12}$	516.1268	515.1190	M-H	2.42	353.0869 $[M-H-C_9H_6O_3]^-$; 191.0556 $[M-H-C_{18}H_{12}O_6]^-$; 179.0346 $[M-H-C_{16}H_{16}O_8]^-$; 173.0451 $[M-H-C_{18}H_{14}O_7]^-$; 161.0242 $[M-H-C_{15}H_{14}O_{10}]^-$; 135.045 $[M-H-C_{17}H_{16}O_{10}]^-$; 93.0344 $[M-H-C_{19}H_{18}O_{11}]^-$

3.1.2. Phenolic acids

Phenolic acids are bioactive molecules that have been reported to present in *Hedera* species[7, 26-27]. Mass fragmentation for most phenolic acids in the negative mode is generally characterized by loss of water, CO_2, or loss of methyl group from deprotonated molecular ion[28-29]. Several phenolic acids conjugate with glycosides also produce common fragmentation ions by loss of sugar moiety during MS/MS analysis[28]. In this study, six phenolic acids **(Figure 2)** including **compounds 1, 2, 8, 9, 10,** and **11**were determined as chlorogenic acid, quinic acid, 1,5-dicaffeoylquinic acid, neochlorogenic acid, 4-*O*-caffeoylquinic acid, and isochlorogenic acid B, respectively, by comparing the MS/MS fragments with the reference data and NIST 2017 library.

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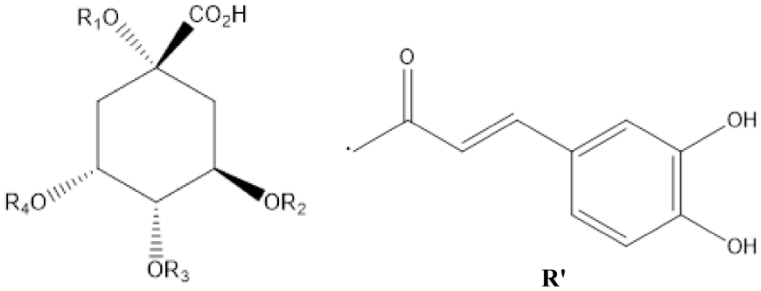

R'

1. $R_1=R_3=R_4=H$, $R_2=R'$ **9.** $R_1=R_2=R_3=H$, $R_4=R'$
2. $R_1=R_2=R_3=R_4=H$ **10.** $R_1=R_2=R_4=H$, $R_3=R'$
8. $R_1=R_4=R'$, $R_2=R_3=H$ **11.** $R_1=R_2=H$, $R_3=R_4=R'$

Figure 2: Chemical structures of identified phenolic acids in *H. nepalensis*samples.

3.2. α-Glucosidase inhibition activity

Antidiabetic potential of *Hedera napalensis* samples was shown in Table 2. The results informed that *H. nepalensis* crude extract from Lao Cai showed the strongest α-glucosidase inhibition activity(27.50%), while this index of the sample planted in Lang Son was the lowest (15.06%).The differences in the α-glucosidase inhibitory capacityof *H. nepalensis* samples in different regions may come from the diversityin the bioactive chemical compositions, which may be influenced by differences in environmental and climatic conditions.To identify the bioactive compounds with antidiabetic activity from *H. nepalensis* species, more research is needed to separate the crude extracts into fractions, isolate and purify the chemical constituents, and assess the isolated phytochemicals' ability to inhibit the α-glucosidase enzyme.

Table 2: α-glucosidase inhibitory activity of *H. nepalensis* at tested concentration.

Sample	Inhibitory activity (%)
Ha Giang	18.86 ± 0.90
Lao Cai	27.50 ± 1.09[a]
Lai Chau	26.33 ± 0.98[a]
Lang Son	15.06 ± 0.60
Acarbose	82.29± 0.30

(Mean values followed by the same uppercase letter are not significantly different from each other at $p \leq 0.05$ according to Tukey's HSD test).

4. Conclusion

The main objectives of this study were to characterize phenolic acids and flavonoids composition by high-resolution mass spectrometry and assess the ability to inhibit α-glucosidase of *H. nepalensis* in four mountainous provinces in northern Vietnam: Ha Giang, Lao Cai, Lai Chau, and Lang Son. The UHPLC-Q-TOF-MS/MS method was utilized to detect 5 flavonoids and 6 phenolic acids from all crude extracts of *H. nepalensis* in both the negative and positive electrospray ionization modes. At the measured concentration, these extracts showed moderate α-glucosidase inhibitory activity. Further research is required to fractionate the crude extracts, isolate, and purify the phytochemicals, and assess the α-glucosidase enzyme inhibitory activity of the compounds from *H. nepalensis* species, in order to determine the bioactive compounds with antidiabetic activity from this medicinal plant.

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Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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