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Study of Peliosanthes micrantha medicinal plant: UHPLC-Q-TOF-MS analysis of phytochemicals and antioxidant activity

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Abstract

Purpose: The medicinal herb *Peliosanthes micrantha* Aver. & N. Tanaka has been used in traditional Vietnamese medicine to treat various diseases such as diuretic, hemorrhoids, jaundice, asthma, diarrhea, blennorrhagia and for its fatigue, detoxification and male wellness effects. Till now, we have not found any research of this herbal plant. This study explores the first chemical information and antioxidant activity of the species.

Methodology: Three procedures were applied to extract *P. micrantha* rhizomes: 1) extraction by ethanol, 2) then sequentially by water, and 3) extraction by water. UHPLC-Q-TOF spectroscopy was used to study three extracts. Antioxidant activity was measured by DPPH assay.

Findings: In alcoholic extract, UHPLC-Q-TOF-MS tentatively identified three known compounds undescribed in the *Peliosanthes* species, namely pumilaside A, pumilaside C, and β–sitosterol together with one known compound of glycoside J-3, previously isolated in the *Peliosanthes sinica*. Not any identified compounds was found in water extracts. All three extracts of *P. micrantha* rhizomes showed mild DPPH antioxidant activity.

Unique contribution to theory, practice and policy: The experimental findings contribute to the chemical literature of Vietnamese natural flora. *Peliosanthes micrantha* should be further studied for pharmaceutical activities.

Keywords: *Peliosanthes micrantha, pumilasides, β–sitosterol, UHPLC-Q-TOF, DPPH*

I. INTRODUCTION

The genus *Peliosanthes* Andrews currently usually referred to Asparagaceae [1], is indigenous to Asia. Many studies on the discovery and ecological characteristics of the *Peliosanthes* species have been reported that the *Peliosanthes* occurs mainly in subtropical to tropical regions within eastern Asia [2] while only a single study was published on the isolation of five compounds including glycoside J-3, glycoside J-4 and three spirostanol glycosides, peliosanthosides A-C from *Peliosanthes sinica* species [3]. Among them, *Peliosanthes micrantha* Aver.et N.Tanaka, sp. nov. (called Sam cau Krông Nô or Hue da la nho in Vietnamese) was found in central highland Vietnam [4]. According to the indigenous people in Krong No district, Dak Nong province, Vietnam, the rhizomes of *P. micrantha* were alcohol extracted and used for tonic, detox, body nourishment, male vitality enhancement. There is no research on phytochemistry and pharmacological effects of the *P. micrantha* have been seen up to date.

The ultra-performance liquid chromatography coupled with quadrupole/time-of-flight mass spectrometry (HPLC-QTOF-MS/MS) is deemed as a powerful tool in both chromatographic separation and mass spectrometric detection of the secondary metabolite compounds in the herbal extracts [5, 6]. The technique is capable of accurately measuring molecular mass by giving the elemental composition of obtained ions and widely used in analyzing complex samples due to the high resolution and sensitivity. In some previous published research, UHPLC-QTOF-MS was applied to characterize chemical constituents and metabolites in medicinal herbs, and obtained considerable results. Zhang L. et al. (2018) has succesfully applied the UPLC-QTOF-MS/MS-guidance strategy including finding, tracking, purity determination and structural elucidation of targeted compounds as well as UPLC chromatographic conditions transplantation to prepare the sulfur-containing ginsenoside Rg1 derivative from sulfur-fumigated ginseng [7]. Xue J. et al. (2023) has developped one integrated data-mining strategy that combines molecular networking (MN), in-house polygonal mass defect filtering (MDF), and diagnostic fragment ion filtering (DFIF) to identify phytochemicals in *Stephania tetrandra* based on LC-MS data; then applied to isolate two new bioflavonoids [8]. In Vietnam, the application of HPLC-QTOF-MS/MS to identify the metabolites in *Hedera helix* leaves and *Hedera heix* leaves was used in some research groups [9, 10].

Antioxidants have the ability of protecting organisms from damage caused by free radical-induced oxidative stress. Studies for the determination of the antioxidant activity of different plant species could contribute to revealing the value of these species as a source of new antioxidant compounds [11]. There is a large variety of in vitro methods to quantify antioxidant activity such as ferric reducing (FRAP) assay, Trolox equivalent antioxidant capacity (ABTS) assay, copper reduction (CUPRAC) assay, reducing power assay (RP) and 2,2-di-phenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) assay Regarding the antioxidant assays, it has been found that molecules with hydrogen donating capability such as ascorbic acid, tocopherol, carotenoids, and

phenolic compounds can reduce the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical to its non-radical form 2,2-diphenyl-1- picrylhydrazine [12]. Due to its simplicity and efficiency, the DPPH method has become one of the most used in the evaluation of antioxidant activity [13, 14].

The aim of this study was to identify phytochemicals by UHPLC-Q-TOF-MS, then to assess the in vitro antioxidant activity of ethanolic and water extracts of *P. micrantha* rhizomes.

II. METHODOLOGY

2.1. Chemicals and reagents

All reagents were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO., USA). Deionized water for HPLC; HPLC grade acetonitrile, methanol, analytical grade formic acid (≥98%) were obtained from Fisher (USA).

2.2. Plant material and extract preparation

The rhizomes of *P. micrantha* from the wild and cultivation were collected in Dak Nong Province, Vietnam in October 2023 and were identified by Dr. Nguyen Sinh Khang, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). The voucher specimen has been lodged at the CHTD, VAST.

Ethanol extraction and sequential water extraction

Procedure 1. The fresh rhizomes of *P. micrantha* (8.700 kg) are washed, sliced and dried for 5 days in an oven at 40^0C and ground into powder. Dried powder of the rhizomes of *P. micrantha* (2.800 kg) was extracted with 95% ethanol (17.5 L x 3 times) at room temperature in an ultrasonic 20 kHz and 1 kW extractor. The combined extract was concentrated to dryness under reduced pressure, yielding a residue (123,300g, brown solid). The residue of ethanolic extract (PM-ET) was flushed with nitrogen gas and stored at -20^0C for future use.

Procedure 2. After ethanol extraction of dry powder of *P. micrantha* rhizomes as in Procedure 1, the residues were filtered, dried and polysaccharides were extracted. The powder (2.224kg) has been mixed with distilled water (8.96 liters) in an extractor. It was extracted by using boiling distilled water at 80^0C for 4h and then filtered. Residue extraction was repeated 3 times. Filtrates were concentrated under reduced vacuum till 1/5 volume (~ 6 liters), then cooled to room temperature and treated with SEVAG chloroform: methanol mixture (3:1 v/v) to precipitate protein overnight. Then water - methanol phase was separated and organic phase above was discarded. Procedure was repeated three times. Water - methanol phase was gathered, then concentrated under reduced vacuum for methanol removal. Water phase (~ 6.00 liters) was precipitated with four volumes of 95% ethanol (v/v) and left overnight at 4°C. By centrifugation at 4000 rpm for 20 minutes, supernatant was wasted and crude sediment was collected, assigned as PM-ET-W-P2 (1.151g).

Water extraction

Procedure 3. Dry *P. micrantha* rhizomes powder (2,800kg) has been extracted with distilled water at 80^0C for 4h (4:1 v/v) and then filtered. Extraction was repeated three times. Filtrates were concentrated under reduced vacuum till 1/5 volume (~ 5.00 liters), then treated with n-hexane (1:1 v/v) to precipitate fat overnight. Water phase above was separated and then precipitated with 95% ethanol (1:4 v/v) and left overnight at 4°C. Sediment which gained via centrifuge at 4000 rpm for 20 minutes, and then the supernatant was wasted. The crude solid was assigned as PM-W-P34 (10,641g).

2.3. UHPLC-Q-TOF analysis

Sample analysis by UHPLC-Q-TOF instrument was investigated with procedure described elsewhere [9]. 100.0 (mg) of the extract was accurately weighed into a tube with a cover, and 2.0 (mL) methanol–water (8:2, v/v) solvent was added. The sample was ultrasonicated for 10 min. The sample was filtrated through a 0.45 (μm) filter membrane before injecting for UHPLC-Q-TOF analysis. Sample analysis was performed on an Exion LCTM UHPLC system (AB SCIEX, USA) consisting of Exion LC degasser, AC pumps, AC autosampler, controller, and AC column oven. Samples were analyzed on a Hypersil GOLD C18 column (150 x 2.1 mm, 3μ) (Thermo Fisher Scientific, USA). The mobile phase, water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B), was run at a flow rate of 0.4 (mL/min) at room temperature. The gradient programming was as follows: 0–4 min, 2-20% B; 4-30 min, 20-68% B; 30-32 min, 68-98% B; 32-40min, 98% B. Sample injection volume was 5.0 (μL).

An AB SCIEX X500R QTOF mass spectrometer (AB SCIEX, USA) with a Turbo V ion source was coupled with the UHPLC system. Mass data were acquired in both negative and positive Electrospray Ionization (ESI) modes and Atmospheric Pressure Chemical Ionization (APCI) modes. The ESI-MS conditions were set 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 TOF MS scan, the mass range was set at m/z 70–2000. For TOF MS/MS scan, the mass range was set at m/z 50–1500. 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. All the obtained data were processed by SCIEX OS software version 1.2.0.4122 (AB SCIEX, USA).

2.4. In vitro antioxidant assay

The antioxidant properties of the extracts were investigated by applying DPPH antioxidant assay with some modifications in the procedure adopted by Baliyan et al. and Blois protocols [12, 14]. About 1 mL of test solution of each extract was dissolved in equivalent amount of DPPH solution (0.1 mM). The increase in DPPH absorbance of tested sample was measured after 20 min incubation at room temperature, by taking the absorbance at 517 nm. Standard vitamin C (1 mM) was taken as a reference solution in this DPPH antioxidant assay. The formula used to calculate

the inhibition (%) was:

$$\text{DPPH Inhibition (\%)} = \left(\frac{AB - AA}{AB}\right) x100$$

where, A B denotes the absorbance of DPPH radical + methanol; A A represents the absorbance of DPPH radical + sample extract/standard.

III. RESULTS AND DISCUSSION

3.1. UHPLC-Q-TOF analysis

The total ion chromatograms (TICs) of *P. micrantha*'s ethanolic extract (PM-ET) in both positive and negative modes were showed in Figures 1 and 2.

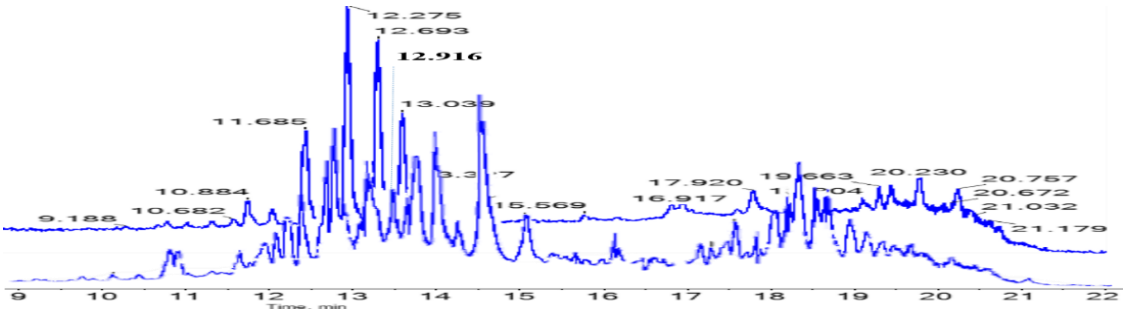

Figure 1: Total ion chromatogram of HPLC-ESI-QTOF negative mode for ethanol extract of P. micrantha rhizomes collected from wild (above) and cultivation (below)

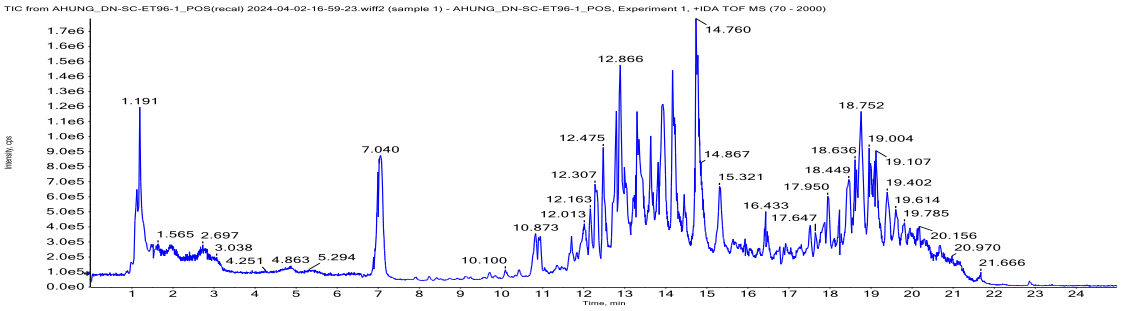

Figure 2: Total ion chromatogram (TIC) of HPLC-ESI-QTOF positive mode for the extract of P. micrantha rhizomes collected from the wild

Some parent and fragment ions were observed in the mass spectra of PM-ET and summarized in Table 1.

Table 1: UPLC-QTOF assignment data of compounds in P. micrantha rhizomes

T_R (min)	Formula	Chemical Name	MS mode	Error (ppm)	Exact mass	Mass found	MS ion
0.14	$C_{29}H_{50}O$	β-sitosterol	APCI-POS	2.3277	397.3834	397.3825	$[M+H-H2O]^+$
10.91	$C_{21}H_{38}O_8$	pumilaside A	ESI-NEG	0.7016	463.2540	463.2525	$[M+HCOO-]^-$
10.92			ESI-POS	0.5416	441.2462	441.2457	$[M+Na-]^+$
11.99	$C_{21}H_{36}O_7$	pumilaside C	ESI-NEG	- 1.4374	445.2438	445.2444	$[M+HCOO-]^-$
12.04			ESI-POS	- 2.6605	423.2359	423.2370	$[M+Na]^+$
17.42	$C_{45}H_{70}O_{20}S$	glycoside J-3	ESI-NEG	0.6133	961.4103	961.4008	$[M-H]^-$

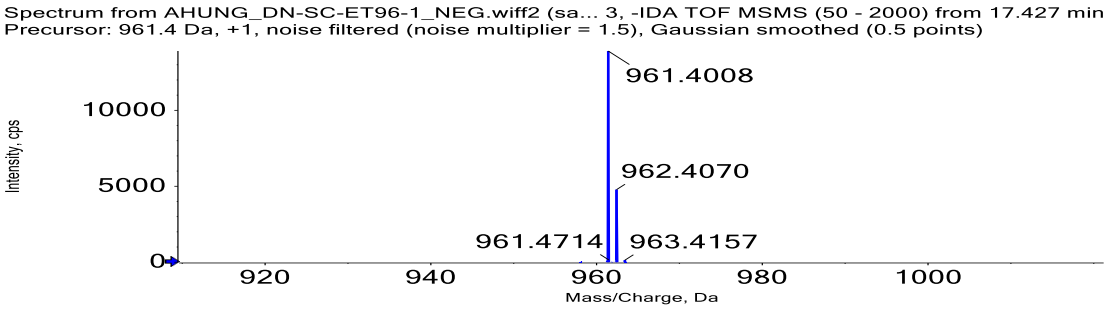

Figure 3: ESI-QTOF negative mode MS spectrum dereplication of a parent ion [M-H]- at m/z 961,4090 from glycoside J-3 (1) (T_R = 17.427 min)

Compound (1), at T_R = 17.427, in the ESI-QTOF negative mode (Figure 3), yielded a parent ion $[M-H]^-$ at m/z 961.4090, and provided no fragment ions. Watanabe et al. (1984) found the same compound in the underground parts of *Ruscus aculeatus* and determined its structure as glycoside J-3 [15]. Thus, compound (1) was tentatively defined as glycoside J-3 (1) which was already found in *P. sinica* [3].

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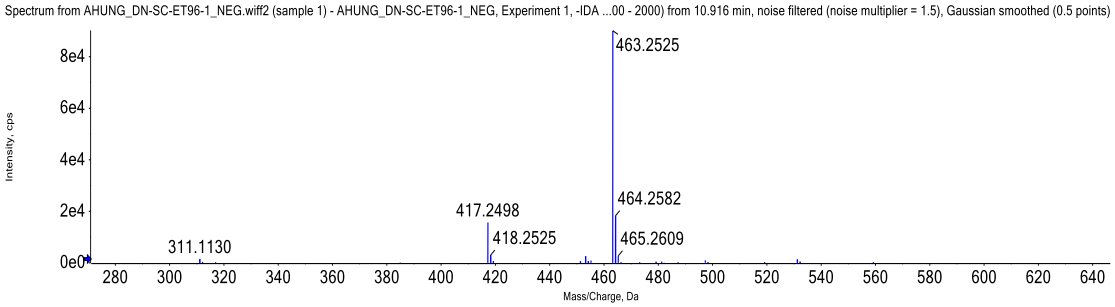

Figure 4: ESI-QTOF negative mode MS spectrum dereplication of a parent ion $[M+HCOO]^-$ at m/z 463.2525 from pumilaside A (2) (T_R = 10.916 min)

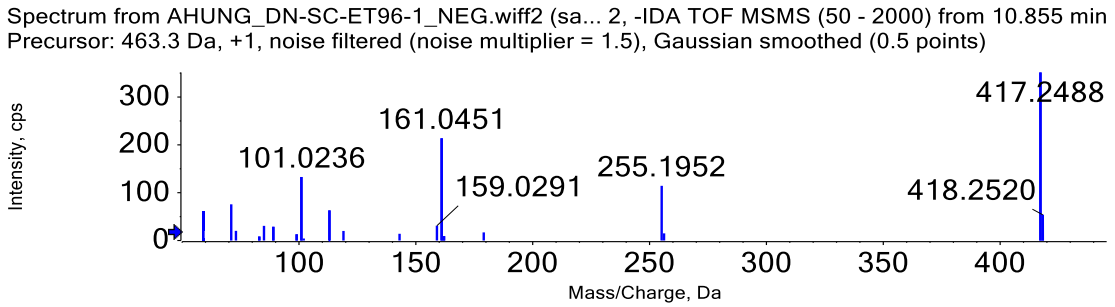

Figure 5: ESI-QTOF negative mode MS^2 spectrum dereplication of a parent ion $[M+HCOO]^-$ at m/z 463.2525 from pumilaside A (2) (T_R = 10.855 min)

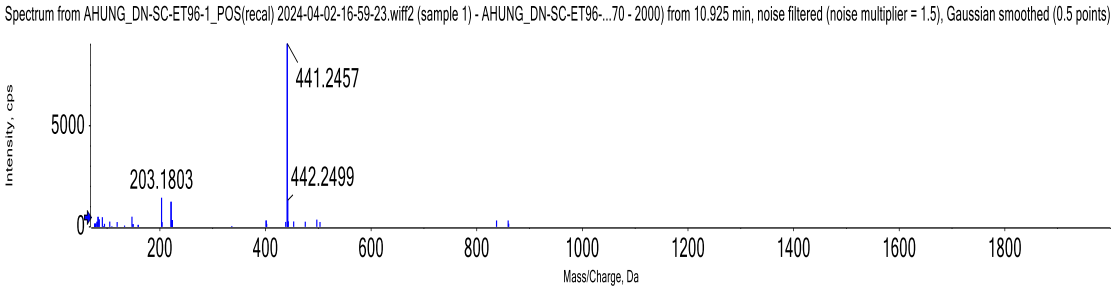

Figure 6: ESI-QTOF positive mode MS spectrum dereplication of a parent ion $[M+Na]^+$ at m/z 441.2457 from pumilaside A (2) (T_R = 10.925)

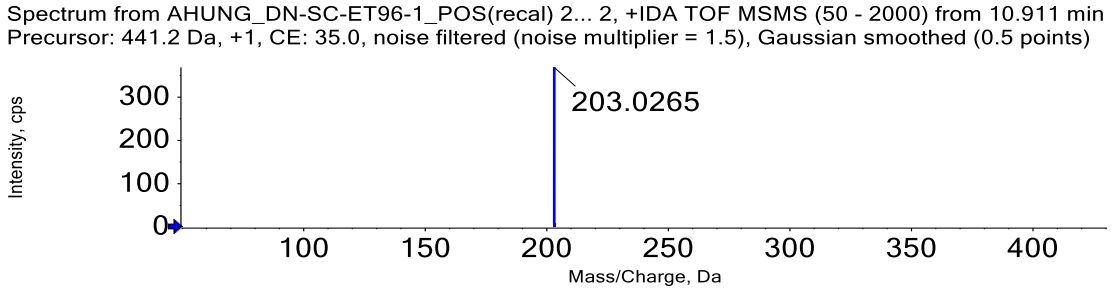

Figure 7: ESI-QTOF positive mode MS/MS spectrum dereplication of a parent ion $[M+Na]^+$ at

m/z 441.2457 from pumilaside A (2) (T_R = 10.911)

Compound (2), at T_R = 10.916, in the ESI-QTOF negative mode (Figures 4 and 5), yielded an solvent adducts ion $[M+HCOO]^-$ at m/z 463.2525, and provided major fragment ions at m/z 101.0236, m/z 161.0451, m/z 255.1952: m/z 417.2488 among them m/z 161.0451, and m/z 255.1952 corresponding to the loss of 1 glucopyranosyl, and 1 aglycone unit. At T_R = 10.925, in the ESI-QTOF positive mode (Figures 6 and 7), it yielded a parent ion $[M+Na]^+$ at m/z 441.2457, and provided only one fragment ion at m/z 203.0265. Kitajima et al. (2000) found the same compound in *Ficus pumila* fruit and determined its structure as pumilaside A [16]. Hence, compound (2) was tentatively assigned as pumilaside A. Pumilaside A was also identified in Litchi semen and *Artemisia vulgaris* [17, 18].

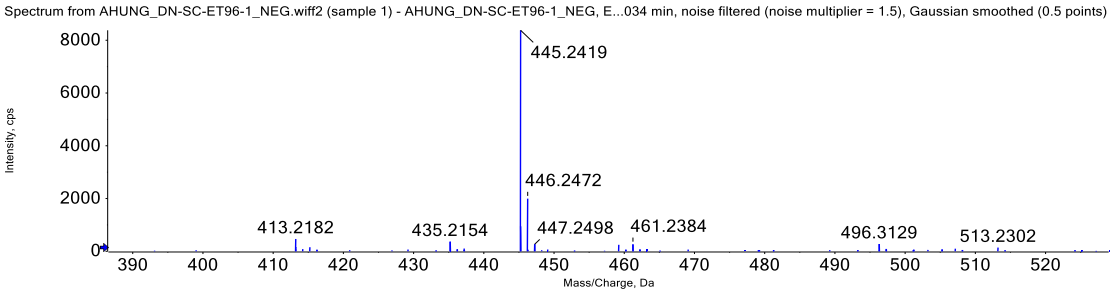

Figure 8: ESI-QTOF negative mode MS spectrum dereplication of a parent ion $[M+HCOO]^-$ at m/z 445.2419 from pumilaside C (3) (T_R = 11.993 min)

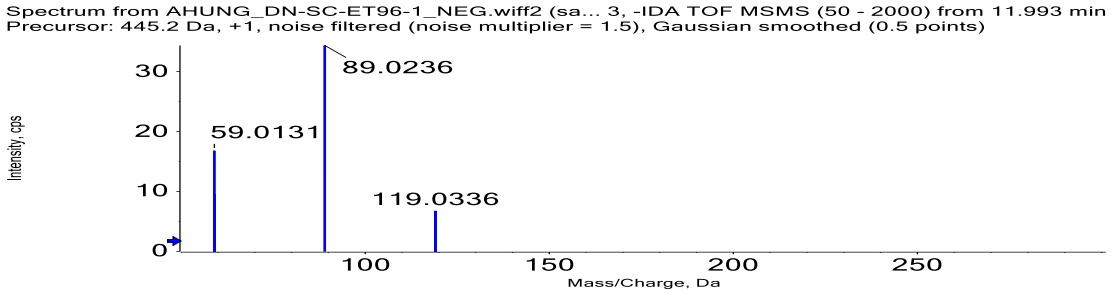

Figure 9: ESI-QTOF negative mode MS^2 spectrum dereplication of a parent ion $[M+HCOO]^-$ at m/z 445.2419 from pumilaside C (3) (T_R = 11.993 min)

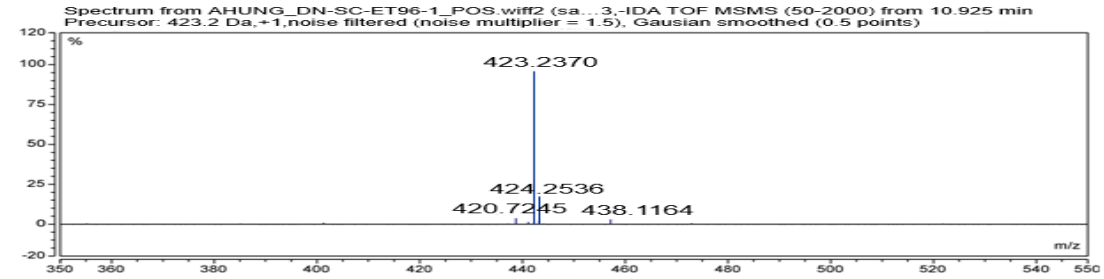

Figure 10: ESI-QTOF positive mode MS spectrum dereplication of a parent ion $[M+Na]^+$ at m/z

423.2370 from pumilaside C (3) (T_R = 10.925)

The MS spectra of compound (3) (T_R = 11.993 min) (Figures 8 and 9), yielded a parent ion $[M+HCOO]^-$ at m/z 445.2444 in the negative mode, primarily fragmented into ion at m/z 59.0131, m/z 89.0236, and m/z 119.0336. At T_R = 12.04, in the ESI-QTOF positive mode (Figure 10), compound (3) yielded a parent ion $[M+Na]^+$ at m/z 423.2370. Compound (3) was determined as pumilaside C. Kitajima et al. (2000) found pumilaside C in *Ficus pumila* fruit showing the $[M+Na]^+$ ion peak at m/z 423 in the positive FABMS [16].

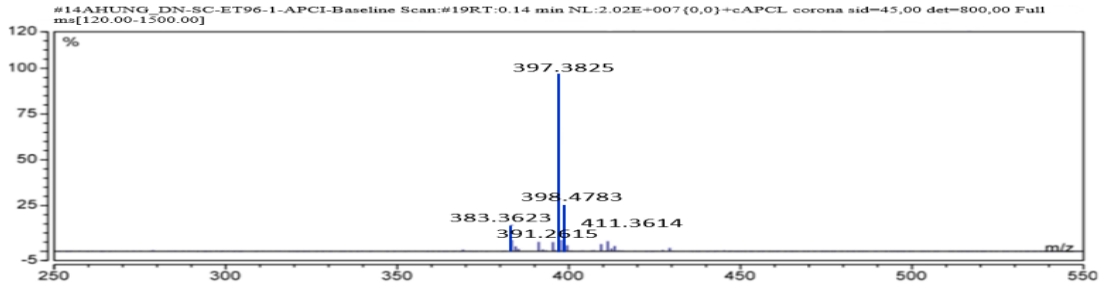

Figure 11: APCI-QTOF positive mode MS spectrum dereplication of a parent ion $[M+H-H2O]^+$ at m/z 397.3825 from β-sitosterol (4) (T_R = 0.14)

Compound 4 (T_R = 0.14 min), exhibited pseudomolecular ion $[M+H-H2O]^+$ at m/z 397.3825 in the APCI-QTOF positive mode as shown in Figure 11. Its ESI-QTOF positive mode spectrum showed unclear deprotonated molecular ions $[M-H]^-$. Chaturvedula et al. (2012) have discovered one molecular ion (M^+) at m/z 414 and several fragment ions at m/z 396, 339, 325, 310, 298, 257, 227, 140, 139, 125, 97, 71, 57; all were assigned to β-sitosterol (4) [19]. β-sitosterol was also isolated from *Aristolochia indica* species [20].

In conclusion, from the alcohol extract of *P. micrantha's* rhizomes, analyzed by ESI-QTOF negative and positive modes, and APCI-QTOF positive mode, one known compound of glycoside J-3 isolated in the *Peliosanthes sinica* and three known compounds, namely pumilaside A, pumilaside C, and β–sitosterol previously undescribed in the *Peliosanthes* genus were tentatively identified.

Not any of the above identified compounds was found in both crude water extracts of *P. micrantha* rhizomes, PM-ET-W-P2 and PM-W-P34 by UHPLC-Q-TOF analysis of any mode.

3.2. DPPH antioxidant activity

The radical scavenging ability measured by 2,2-di-phenyl-1-picrylhydrazyl (DPPH) method in three extracts of *P. micrantha* rhizomes is given in Table 2. A strong antioxidant, vitamin C, was used as reference. In the serial dilution of 0-100 ng/ml, vitamin C gave the regression equation of $y = 27.437\ln(x) - 36.595$ with correlation coefficient $R^2 = 0.8472$.

Table 2: Antioxidant activity by 2,2-di-phenyl-1-picrylhydrazyl (DPPH) method in ethanol extract of P. micrantha rhizomes

Sample (100 ng/ml)	PM-ET	PM-ET-W-P2	PM-W-P34	Vitamin C
DPPH inhibition (%)	9.773	8.024	10.631	90.02

The results showed that three extracts of the rhizome exhibited same antioxidant activity (~10%) and much lower in comparison to vitamin C (~90%) at the same concentration of 100 ng/ml. Karan et al. (2012) have isolated β- sitosterol from *Aristolochia indica* and showed its *in vitro* antioxidant activity as IC50 value being 7.325 µg/ml [22]. IC50 values of three extracts of *P. micrantha* rhizomes were not yet determined. It concluded that the alcohol and water extracts of *P. micrantha* rhizomes showed mild DPPH antioxidant activity.

IV. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

This study contributes to the chemical investigation of the Vietnamese plant *P. micrantha* by UHPLC-Q-TOF spectroscopy with the identification of three known compounds undescribed in the *Peliosanthes* species and one known compound, previously isolated in the *Peliosanthes sinica*. Mild antioxidant activities by DPPH assay were established for all the extracts of *P. micrantha* rhizomes.

4.2. Recommendation

It might be of interest to further investigate the *P. micrantha* rhizomes on its chemical constituents and pharmaceutical activities.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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