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Chemical constituents of the ethyl acetate extract from the leaf of mugwort (*Artemisia vulgaris* L.)

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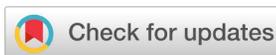
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ABSTRACT

Introduction: Mugwort (*Artemisia vulgaris* L.) is a familiar herbal medicine and also a daily vegetable. It is one of the ingredients in the famous remedy "Cao ích mẫu" specializing in menstrual disorders or the omelet with mugwort that helps save blood flow to the brain to treat headaches. In both traditional medicine and the new drugs, diseases are usually treated by mugwort as diabetes, epilepsy combination for psychoneurosis, depression, irritability, insomnia, anxiety, and stress. To demonstrate the medicinal uses, the chemical constituents of this herbal were continually studied. **Methods**: The leaves of mugwort were collected in Ba Ria - Vung Tau province, Vietnam. The plant was identified by the late pharmacist and botanist Binh Duc Phan. A voucher specimen (AV001) was deposited in the herbarium of the Department of Organic Chemistry, VNUHCM–University of Science. Dried leaf powder of *A. vulgaris* (11 kg) was extracted with methanol and evaporated under reduced pressure to give a methanol extract (910 g), which was dissolved in methanol-water (1:9) and then successively partitioned with petroleum ether, chloroform, and ethyl acetate. From the previously researched ethyl acetate fraction, nine compounds were isolated: six known phenolic compounds (luteolin, 6-methoxyluteolin, eupatilin, *o*-coumaric acid, vanillic acid, and protocatechuic acid), sinapyl alcohol diisovalerate, vulgarin, and one new compound (artanoic acid). **Results**: In this research, ethyl acetate fraction was also studied. From subfraction EA4, six compounds were isolated by three skeletons: phenolic compounds (5,4′ -dihydroxyflavone and 4-hydroxyphenyl acetate), phenyl propanoid (methyl 2-O-β-D-glucopyranosylcoumarate and 2-O-β-D-glucopyranosylcoumaric acid) and uracil (5-methyluracil and uridine). The structure of the isolated compounds was determined to base on 1D, 2D NMR spectra, HR-ESI-MS, and comparison with published data. **Conclusion**: Particularly, four compounds (methyl 2-O-β-D-glucopyranosylcoumarate, 2-O-β-D-glucopyranosylcoumaric acid, 5-methyluracil, and uridine) were known for the first time from this species.

Key words: mugwort, Artemisia vulgaris L., flavone, coumarin, and uracil

INTRODUCTION

Artemisia vulgaris L., a familiar herbal species in Viet Nam, is used in both traditional medicine and new drugs. Diseases are usually treated by mugwort as diabetes, epilepsy combination for psychoneurosis, depression, irritability, insomnia, anxiety, and stress[1]. The primary responsibility for these activities are constituents, such as flavonoids, coumarins, sesquiterpene lactones, volatile oils, inulin, and traces of alkaloids[2]. In the previous research of my group, three flavonoids (luteolin, 6-methoxyluteolin, and eupatilin), four phenolic compounds (o-coumaric acid, vanillic acid, protocatechuic acid, and sinapyl alcohol diisovalerate), and two sesquiterpene lactones (vulgarin and artanoic acid) are isolated from the ethyl acetate fraction[3].

In this study, the ethyl acetate fraction is continuously researched and six compounds are isolated, including methyl 2-O-β-D-glucopyranosylcoumarate (1), 2-O-β-D-glucopyranosylcoumaric acid (2), 5-methyluracil (3), uridine (4), 5,4′-dihydroxyflavone (5), and 4-hydroxyphenyl acetate (6).

METHOD

General experimental procedures

The NMR spectra were acquired on a Bruker Avance III 500 MHz spectrometer with tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in *d* (ppm) values. The HR-ESI-MS were determined with a MicrOTOF QII mass spectrometer (Bruker Daltonics). Analytical and preparative thin-layer chromatography (TLC) were performed on precoated Merck Kieselgel 60 F_{254} or RP-18 F_{254} plates (0.25 mm or 0.5 mm thickness).

Plant material

The leaves of *Artemisia vulgaris* L. were collected at Lang Dai, Dat Do, Ba Ria - Vung Tau province, Vietnam on May 2011. The plant was identified by the late pharmacist and botanist Binh Duc Phan. A voucher specimen (AV001) was deposited in the herbarium of the Department of Organic Chemistry, VNUHCM–University of Science.

Extraction and isolation

From the ethyl acetate fraction in the previous research[3], fraction EA4 (6.14 g) was subjected to silica gel column chromatography eluting with petroleum ether –ethyl acetate, followed by ethyl acetate – methanol with increasing polarity to yield six fractions (EA4.1 – 6). Fraction EA4.3 (300.6 mg) was separated over a silica gel column eluted with chloroform-methanol (from 9.5:0.5 to 0:10), as well as preparative TLC, eluted with petroleum ether– acetone (4:6) to afford **1** (6.0 mg), **2** (5.5 mg), and **6** (8.2 mg). Fraction EA4.4 (207.5 mg) was subjected to Sephadex LH-20 column eluted with methanol, and further fractionated by silica gel column chromatography eluted with chloroform– ethyl acetate (from 9:1 to 3:7) to obtain **3** (6.3 mg), **4** (4.0 mg), and **5** (4.5 mg).

RESULTS AND DISCUSSION

Six compounds (**1 – 6**) were isolated from the ethyl acetate fraction of the leaves of *Artemisia vulgaris* L.

Compound **1** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{16}H_{20}O_8$ by HR-ESI-MS analysis at *m/z* 341.1161 $[M+H]^+$. The ^{1}H-NMR spectrum of **1** showed four signals of four aromatic protons at δ_H 7.02 (1H, *dd*, 7.5, 7.5 Hz, H-5), δ_H 7.19 (1H, *d*, 8.5 Hz, H-3), δ_H 7.37 (1H, *ddd*, 8.5, 7.5, 1.5 Hz, H-4), δ_H 7.71 (1H, *dd*, 7.5, 1.5 Hz, H-6). Furthermore, the ^{13}C-NMR and HSQC spectra showed aromatic carbon signals at δ_C 115.5 (C-3), 122.3 (C-5), 128.7 (C-6), 132.1 (C-4), and two signals of quartet carbon at δ_C 123.4 (C-1), δ_C 156.1 (C-2). It demonstrated that **1** had a 1,2-di substituted benzene. There were two signals of two olefin protons, (E) configuration, at δ_H 7.95 (1H, *d*, 16.0 Hz, H-7), and δ_H 6.64 (1H, *d*, 16.5 Hz, H-8) with carbon signals at δ_C 139.8 (C-7), δ_C 118.7 (C-8); and a carboxyl group at δ_C 167.3 (C-9). HMBC correlations between H-7/C-2, C-8, C-9; H-8/C-9 showed that **1** had the 2-h ydroxycinnamoyl skeleton. The signals of a methoxy group at δ_H 3.70 (3H, *s*, H-10) and δ_C 51.7 (C-10), correlated with C-9 in the HMBC spectrum (**Figure 1**). Therefore, the methoxy group linked to the carboxyl group of the cinnamoyl skeleton.
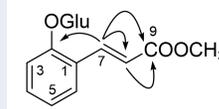
Figure 1: Significant HMBC ($\rightarrow$) correlations of 2-hydroxycinnamoyl skeleton.

^{1}H-NMR and ^{13}C-NMR spectra showed an anomer proton at δ_H 5.00 (1H, *d*, 8.5 Hz, H-1′) and δ_C 100.4 (C-1′). The HMBC correlation between H-1′/C-2, C-3′ showed that the sugar moiety linked to cinnamoyl skeleton at C-2.

The above data compared with the published one indicated that **1** was methyl 2-O-β-D-glucopyranosylcoumarate[4].

Compound **2** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{15}H_{18}O_8$ by HR-ESI-MS analysis at *m/z* 326.1033 $[M]^+$, calcd 326.1002. The ^{1}H-NMR, ^{13}C-NMR spectra of **2** were similar to those of compound **1**. However, the lack of the methoxy group in compound 2 showed that it was a carboxylic acid. The comparison of the above data with the one in the literature[5] assigned 2 as *O*-coumarico glucosidase acid.

Compound **3** was obtained as a white amorphous powder and its molecular formula was determined as $C_5H_6N_2O_2$ by HR-ESI-MS analysis at *m/z* 127.0451 $[M+H]^+$. The ^{1}H-NMR spectrum displayed four signals including two amide protons at δ_H 10.56 (*br*), and δ_H 10.97 (*br*), a methyl group at δ_H 1.71 (3H, *d*, 1.0 Hz, H-7), and an olefin proton signal at 7.23 (1H, *s*). The ^{13}C-NMR and HSQC spectra showed two signals of two carbonyl carbons at δ_C 165.2 (C-2) and 151.7 (C-4), two olefin signals at δ_C 108.2 (C-5) and 138.2 (C-6) and a methyl group at δ_C 12.2 (C-7). The above information showed an uracil skeleton in compound 3. The HMBC correlations between H-7/C-5, C-6 confirmed the position of the methyl group on the C-5 of the uracil skeleton (**Figure 2**).

Based on the above discussions and the comparison with the published ones[5], the structure of **3** was 5-methyluracil.

Compound **4** was obtained as a white amorphous powder and its molecular formula was determined as $C_9H_{12}N_2O_6$ by HR-ESI-MS analysis at *m/z* 267.0628 $[M+Na]^+$ and *m/z* 245.0825 $[M+H]^+$. ^{1}H-NMR, ^{13}C -NMR, HSQC spectra showed that compound **4** had the uracil skeleton as in compound 3. The ^{1}H-NMR spectrum showed an amide proton at δ_H 11.29 (*br*), two olefin protons with the (Z) configuration at δ_H 7.88 (1H, *d*, 8.0 Hz, H-6), 5.64 (1H, *d*, 8.0 Hz, H-5).
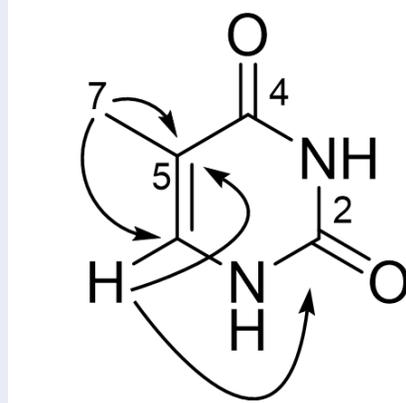
Figure 2: Significant HMBC (→) correlations of uracil skeleton.
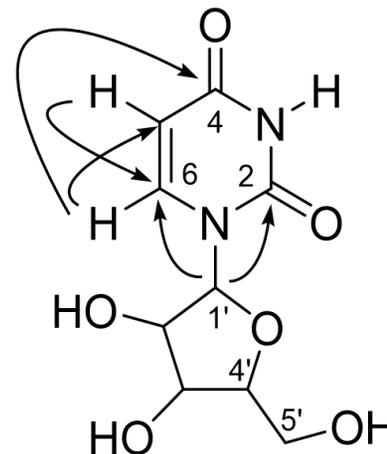
Figure 3: Significant HMBC (→) correlations of compound **4**.

The ^{13}C-NMR spectrum showed two olefin carbons at δ_C 102.2 (C-5) and δ_C 141.2 (C-6); two carbonyl groups at δ_C 163.6 (C-2) and δ_C 151.2 (C-4).

In addition, there was an anomer proton at δ_H 5.77 (1H, *d*, 5.5 Hz, H-1′) and five oxymethine proton of a sugar moiety at δ_H 3.53–5.36 in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum showed an anomer carbon at δ_C 88.1 (C-1′), and four oxynated carbons at δ_C 85.4 (C-4′), 73.8 (C-2′), 70.3 (C-3′) and 61.2 (C-5′). It demonstrated a sugar moiety in compound **4**.

The HMBC correlations between proton H-6/C-2, C-4, C-5 and C-1′, proton H-5/C-6, C-1′, and the anomer proton H-1′/C-2, C-5, C-6, C-2′, C-3′, C-4′ showed that the sugar moiety linked to the first nitrogen of the uracil skeleton (**Figure 3**).

The above data compared with the published one indicated that **4** was uridine[6].

Compound **5** was obtained as a yellow amorphous powder and its molecular formula was determined as $C_{15}H_{10}O_4$ by HR-ESI-MS analysis at *m/z* 254.0593 [M]$^+$, calcd 254.0579. The ^{1}H-NMR spectrum displayed two signals of four aromatic protons of a 1,4-disubstituted benzene at δ_H 7.91 (2 H, *d*, *J*= 8.5 Hz, H-2′ and H-6′) and 6.91 Hz (2H, *d*, *J* = 8.5 Hz, H-3′ and H-5′). Moreover, there were three signals of three aromatic protons of a 1,2,3-trisubstituted benzene at δ_H 7.32 (1H, *s*, *J* = 2.5 Hz, H-8); 7.53 (2H, *d*, *J* = 7.5 Hz, H-6 and H-7) and an olefin proton at δ_H 7.08 (1H, *s*, *J* = 2.5 Hz, H-3). The ^{13}C-NMR spectrum showed fifteen carbons: seven aromatic quaternary carbons (δ_C 182.9, 167.4, 162.4, 158.3, 149.8, 120.7, 109.9), eight aromatic methine carbons (δ_C 115.9 (C-3′, C-5′), 132.7 (C-2′, C-6′), 110.8, 130.2, 107.1 and 102.5).

Based on the above discussions and the comparison with the published one[7], the structure of **5** was 5,4′-dihydroxyflavone.

Compound **6** was obtained as a white amorphous powder, and its molecular formula was determined as $C_8H_8O_3$ by HR-ESI-MS analysis at *m/z* 175.0377 [M+Na]$^+$, calcd 175.0371. The ^{1}H-NMR spectrum showed two signals of four aromatic protons of a 1,4-disubstituted benzene at δ_H 7.44 (2H, *d, J* = 9.0 Hz) and 6.74 (2H, *d, J* = 9.0 Hz), one methyl group at δ_H 2.01 (3H, *s*), and one hydroxyl at δ_H 8.93 (1H, *s*). The ^{13}C-NMR spectrum showed four signals of six aromatic carbons (δ_C 154.2, 121.7, 121.6, 115.8), one methyl group (δ_C 24.0), and one carboxyl group (δ_C 171.4). On the basis of the above discussions and the comparison with the published one[8], the structure of **6** was 4-hydroxyphenyl acetate.

CONCLUSION

From the leaves of *Artemisia vulgaris* L. collected at Ba Ria - Vung Tau province, six compounds were isolated, including methyl 2-O-β-D-glucopyranosylcoumarate (**1**), 2-O-β-D-glucopyranosylcoumaric acid (**2**), 5-methyluracil (**3**), uridine (**4**), 5,4′-dihydroxyflavone (**5**), and 4-hydroxyphenyl acetate (**6**). Compounds **1**, **2**, **3**, **4** were known for the first time from this species.

LIST OF ABBREVIATIONS

1D NMR: One-dimensional nuclear magnetic resonance;

2D NMR: Two-dimensional nuclear magnetic resonance;

^{13} **C-NMR**: Carbon-13 nuclear magnetic resonance;

^{1} **H-NMR**: Proton nuclear magnetic resonance;

HR-ESI-MS: High-resolution electrospray ionization mass;

TMS: Tetramethylsilane;

TLC: Thin-layer chromatography;

EA: Ethyl acetate;

HMBC: Heteronuclear Multiple Quantum Coherence.

COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Truong Van Nguyen Thien, Thien Tai Phan, Tung Thanh Phan, Kim Lien Tran Thi, Nhu Tiet Thi Tran, and Phu Hoang Dang have contributed in conducting experiments, getting hold of data and writing the manuscript.

Linh Phi Nguyen, Quang Ton That have contributed significantly explanation of data and revising the manuscript.

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