CHEMICAL CONSTITUENTS FROM ETHYL ACETATE EXTRACT OF SCOPARIA DULCIS LINN.

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ABSTRACT

This research focuses on the isolation and characterization of eight natural compounds from the ethyl acetate extract of *Scoparia dulcis* L. collected in An Giang province, Vietnam. Six flavonoid compounds: apigenin (1), luteolin (2), baicalein (3), oroxylin A (4), oroxylin A 7-O-β-D-glucoside (5) and vitexin (6) along with two nitrogen-containing compounds: 2,4-dihydroxy-1,4-benzoxazin-3-one (7) and uracil (8) were successfully isolated via the methods of natural product extraction. The structures of these compounds were elucidated base on 1D-NMR and 2D-NMR spectral data as well as MS and compared with those in the authentic reports. This study contributed new results to the phytochemical examination of *Scoparia dulcis* Linn. growing in Vietnam with the presence of the first isolated compounds as (3), (4), (5), (7) and (8).

Keywords: *Scoparia dulcis* Linn., ethyl acetate extract, apigenin, luteolin, baicalein, oroxylin A, vitexin, oroxylin A 7-O-β-D-glucoside, DIBOA, uracil.

1. INTRODUCTION

The medicinal plant *Scoparia dulcis* L., commonly known as sweet broomweed or goatweed, is a species belonging to the Scrophulariaceae family. It is an edible herb mainly found in many tropical regions around the world such as Mexico, India, Thailand, Malaysia, etc. In Vietnam, *Scoparia dulcis* is naturally distributed from north to south; due to the valuable medicinal properties of this herb so that it has been cultivated throughout the country. Traditionally, all parts of the plant are used to treat various diseases. The roots are used by the people in Antilles to treat menorrhagia and gonorrhea [1]. Vietnamese and Chinese use this plant as an antidote. In folk medicine of China and Taiwan, some diseases like hypertension, hepatitis, bronchitis and gastric ulcers could also be cured with *Scoparia dulcis* Linn. [2].

Much research has been carried out to evaluate the bioactivities of *Scoparia dulcis* L. including cytotoxic, β-glucuronidase inhibitory, radical scavenging, anti-inflammatory, antiviral, antimicrobial, antifungal and diuretic activities [2 - 6]. Over 50 compounds, including flavonoids, terpenoids, benzoxazinoids, etc., have been isolated from *Scoparia dulcis* L. around the world.
2. MATERIAL AND METHOD

2.1. Plant material

*Scoparia dulcis* Linn was collected in An Giang province, Vietnam in July 2015. The specimen was identified the scientific name and stored with the No 02-2015 at the Organic Chemistry lab, College of Natural Sciences, CTU. After washing, the air dried parts (leaves, flower and stem), except the root, were ground into fine powder.

2.2. Method

2.2.1. Extraction and isolation

The plant powder (8 kg) was exhaustively extracted with methanol. The crude methanol extract (1.0 kg) was diluted with distilled water then partitioned in turn with petroleum ether, dichloromethane and ethyl acetate. The partitioned ethyl acetate solution was concentrated to afford ethyl acetate extract (75.0 g). This extract (60.0 g) was subjected on dry-column flash, eluted with the solvent system: *n*-hexane:ethyl acetate (30:70) to ethyl acetate:methanol (80:20), to afford 10 fractions (F1–F10). Fraction F1 (2.4 g) was then subjected to an open column, eluted with *n*-hexane:ethyl acetate (5:5) to yield 1 (5 mg), 2 (6 mg) and 7 (5 mg). Fraction F2 (7 g) was also analysed with silica gel, using *n*-hexane:ethyl acetate (1:9) solvent system to afford 6 (4 mg) and 8 (5 mg).

The rest amount of the extract (15.0 g) was subjected to another column, eluted with CH$_2$Cl$_2$:MeOH gradients to yield 4 fractions (P1–P4). Fraction P1 was concentrated to give crystals; 5 (3 mg) was afforded after recrystallizing. Fraction P4 was purified with open-column chromatography, using ethyl acetate: methanol gradients to yield 3 (2 mg) and 4 (3 mg).

2.2.2. Spectroscopic methods

$^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectroscopy were recorded by a 500 MHz Bruker Avance spectrometer using tetramethylsilane (TMS) as the internal standard; ESI-MS was recorded with a VG 7070 Mass spectrometer operating at 70 eV. All spectroscopic methods were carried out at Institute of Chemistry, Vietnam Academy of Science and Technology.

3. RESULTS AND DISCUSSION

3.1. Compound 1 to 6

The $^1$H NMR and $^{13}$C NMR data of compound 1 to 6 suggest that all these six compounds have the flavone skeleton with six specific carbon signals (C-2, C-3, C-4, C-9, C-10 and C-1') as well as the proton signals of H-3 (1H; s) and aromatic protons. With the number of protons and carbons displayed in the spectral data, the coupling constants calculated and the ESI-MS results, compound 1, 2, 3 and 4 were identified as apigenin, luteolin, baicalein and oroxylin A, respectively (Fig.1). Because of the presence of five oxymethine and one oxymethylene carbon
signals as well as the proton signals from $\delta_{11}$ (ppm) 3.00-4.00, compound 5 and 6 were determined as glucosides. However, both the anomic carbon (73.3 ppm; C-1") and the anomic proton signal (4.71 ppm; H-1") of 6 are upfield shifted, compared to those of 5 (100.2 ppm; C-1") and (5.13 ppm; H-1"); thus, 6 was recommended as a C-glucoside, and 5 was identified as a O-glucoside. The aglycone spectra of compound 5 are very similar to those of oroxylin A, while the genin part spectra of compound 6 are analogous with those of apigenin; moreover, the HMBC spectroscopy of 6 shows the correlation between H-1" and C-8. Therefore, compound 5 was recognized as oroxylin A 7-O-\beta-D-glucoside, and apigenin 8-C-\beta-D-glucoside (vitexin) was assigned to compound 6 (Fig.1). The spectral data of all these six compounds were also in agreement with those in published documents [7 - 10].

**Figure 1.** Chemical structures of compound 1 to 6.

**Apigenin (1):** Yellow powder. ESI-MS: $m/z$ 268.9 [M + H] (calcd. for C_{15}H_{10}O_{6} 269.9).

$^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 12.96 (1H; s; 5-OH); 6.77 (1H; s; H-3); 6.20 (1H; s; H-6); 6.49 (1H; s; H-8); 7.93 (2H; $d$; $J = 8.5$ Hz; H-2', 6'); 6.94 (2H; $d$; $J = 8.0$ Hz; H-3', 5'). $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 163.7 (C-2); 102.8 (C-3); 181.7 (C-4); 161.4 (C-5); 98.8 (C-6); 164.5 (C-7); 94.0 (C-8); 157.3 (C-9); 103.5 (C-10); 121.0 (C-1'); 128.6 (C-2', 6'); 116.0 (C-3', 5'); 161.2 (C-4').

**Luteolin (2):** Yellow powder. ESI-MS: $m/z$ 284.9 [M + H] (calcd. for C_{15}H_{10}O_{6} 285.9).

$^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 12.96 (1H; s; 5-OH); 6.66 (1H; s; H-3); 6.18 (1H; s; H-6); 6.94 (1H; s; H-8); 7.39 (1H; brs; H-2'); 6.88 (1H; $d$; $J = 8.0$ Hz; H-5'); 7.40 (1H; $dd$; $J_1 = 8.0$ Hz, $J_2 = 1.5$ Hz; H-6'). $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 163.9 (C-2); 102.9 (C-3); 181.6 (C-4); 161.5 (C-5); 98.8 (C-6); 164.1 (C-7); 93.8 (C-8); 157.3 (C-9); 103.7 (C-10); 121.5 (C-1'); 113.4 (C-2'); 145.7 (C-3'); 149.7 (C-4'); 116.0 (C-5'); 119.0 (C-6').

**Baicalein (3):** Yellow needles. $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 12.61 (1H; s; 5-OH); 6.92 (1H; s; H-3); 6.63 (1H; s; H-8); 8.05 (2H; $d$; $J = 8.0$ Hz; H-2', 6'); 7.57 (3H; $m$; H-3', 4', 5'). $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 162.9 (C-2); 104.3 (C-3); 182.1 (C-4); 147.0 (C-5); 129.3 (C-6); 153.6 (C-7); 94.0 (C-8); 149.8 (C-9); 104.5 (C-10); 131.0 (C-1'); 126.3 (C-2', 6'); 129.1 (C-3', 5'); 131.8 (C-4').

**Oroxylin A (4):** Yellow needles. $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 12.92 (1H; s; 5-OH); 6.96 (1H; s; H-3); 6.63 (1H; s; H-8); 8.06 (2H; $m$; H-2', 6'); 7.59 (3H; $m$; H-3', 4', 5'); 3.76 (3H; s; 6 OCH$_3$). $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 163.2 (C-2); 104.6 (C-3); 182.2 (C-4); 152.6 (C-5); 130.7 (C-6); 157.8 (C-7); 94.4 (C-8); 152.7 (C-9); 104.6 (C-10); 131.5 (C-1'); 126.4 (C-2', 6'); 129.1 (C-3', 5'); 132.0 (C-4'); 59.9 (6 OCH$_3$).

**Oroxylin A 7-O-\beta-D-glucoside (5):** Yellow crystal. ESI-MS: $m/z$ 446.9 [M + H]$^+$ (calcd. for C$_{22}$H$_{20}$O$_{16}$ 445.9). $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 12.79 (1H; s; 5-OH); 7.05 (1H; s; H-3); 7.07 (1H; s; H-8); 8.10 (2H; $d$; $J = 7.0$ Hz; H-2', 6'); 7.62 (3H; $m$; H-3', 4', 5'); 3.78 (3H; s; 6 OCH$_3$). $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 163.7 (C-2); 105.0 (C-3); 182.5 (C-4); 152.3 (C-5); 132.2 (C-6); 156.7 (C-7); 94.5 (C-8); 152.4 (C-9); 106.0 (C-10); 130.6 (C-1'); 126.5 (C-2', 6'); 129.2 (C-3', 5'); 132.6 (C-4'); 60.3 (6 OCH$_3$); 100.2 (C-1''); 73.2 (C-2''); 77.3 (C-3''); 69.6 (C-4''); 76.7 (C-5''); 60.6 (C-6'').
Vitexin (6): Yellow powder. ESI-MS: m/z 430.9 [M – H] (calcd. for C_{21}H_{20}O_{10} 431.9). \(^1\)H NMR (DMSO-\text{d}_6) \(\delta\) ppm: 13.15 (1H; s; 5-OH); 8.01 (2H; s; H-2’, 6’); 6.89 (2H; d; \(J = 8.5\) Hz; H-3’, 5’); 6.75 (1H; s; H-3); 6.24 (1H; s; H-8); 4.71 (1H; d; \(J = 8.5\) Hz; H-1’); 3.00 4.00 (6H; m; H-2” 6”). \(^{13}\)C NMR \(\delta\) ppm: 163.8 (C-2); 102.4 (C-3); 181.9 (C-4); 160.4 (C-5); 98.1 (C-6); 162.5 (C-7); 104.6 (C-8); 160.4 (C-9); 104.0 (C-10); 121.6 (C-1’); 128.9 (C-2’, 6’); 115.8 (C-3’,5’); 161.1 (C-4’); 73.3 (C-1’); 70.9 (C-2”); 78.7 (C-3”); 70.5 (C-4”); 81.3 (C-5”); 61.3 (C-6”).

3.2. Compound 7

Compound 7 was obtained as brown yellow powder. The ESI-MS of 7 shows the pseudomolecular ion peak at \(m/z\) 163.9 [M – H_2O + H]^+; this suggests the molecular formula would be C_8H_7O_4N (181 amu). The \(^1\)H NMR (CD_3OD) spectra displays four aromatic proton signals at \(\delta\) ppm: 7.38 and 7.05 (1H; \(dd\); \(J = 1.5\) Hz and 8.0 Hz); 7.12 and 7.09 (1H; \(dddd\); \(J = 1.5\) Hz; 7.5 Hz and 8.0 Hz). The coupling patterns and coupling constants of these signals indicate the presence of a 1,2-disubstituted benzene ring. Two quaternary carbon signals at \(\delta_c\) 142.5 and 129.7 ppm, together with the correlation between four aromatic protons above with these two carbons in HMBC show that the two atoms attaching to the benzene ring are oxygen and nitrogen. Moreover, with the degree of unsaturation calculated from the molecular formula (\(\Delta = 6\)) as well as other carbon and proton signals, compound 7 was identified as 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA). The result was enhanced by the correspondence of NMR data of compound 7 and those of 2-O-\(\beta\)-D-glucopyranosyl-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-glc) in published data [10] (Table 1).

Table 1. NMR spectral data of compound 7 in comparison with those of DIBOA-glc (D_2O).

<table>
<thead>
<tr>
<th>Position</th>
<th>(^1)H NMR (\delta) ppm, ((J,\ Hz))</th>
<th>(^{13})C NMR (\delta) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 (CD_3OD)</td>
<td>DIBOA-glc 7 (CD_3OD)</td>
</tr>
<tr>
<td>1, 4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5.72 (1H; s)</td>
<td>5.89 (1H; s)</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>7.38 (1H; (dd); (J = 1.5); 8.0)</td>
<td>7.09-7.48 ((m))</td>
</tr>
<tr>
<td>6</td>
<td>7.12 (1H; (dddd); (J = 1.5); 7.5; 8.0)</td>
<td>7.09-7.48 ((m))</td>
</tr>
<tr>
<td>7</td>
<td>7.09 (1H; (dddd); (J = 1.5); 7.5; 8.0)</td>
<td>7.09-7.48 ((m))</td>
</tr>
<tr>
<td>8</td>
<td>7.05 (1H; (dd); (J = 1.5); 7.5)</td>
<td>7.09-7.48 ((m))</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
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</tbody>
</table>

Compound 7 (2,4-dihydroxy-1,4-benzoxazin-3-one)  Compound 8 (pyrimidine-2,4(1\(H\),3\(H\))-dione)
3.3. Compound 8

Compound 8 was obtained as yellow powder. The $^1$H NMR (DMSO-$d_6$) spectra shows two secondary amine protons at 10.98 ppm and 10.97 ppm, along with two olefinic protons at 5.44 ppm and 7.38 ppm with the coupling constants of 7.5 Hz and 8.0 Hz, respectively. The $^{13}$C NMR and DEPT spectra indicates two quaternary and two tertiary carbons. Because of the chemical shifts of these carbons, 8 was assumed to be a heterocyclic compound. By comparing the spectral data of 8 with those of published literature [11] (Table 2), compound 8 was identified as pyrimidine-2,4(1$H$,3$H$)-dione (Uracil).

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H NMR (ppm, Hz)</th>
<th>$^{13}$C NMR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10.98 (1H; s; 1-NH)</td>
<td>10.98 (1H; s; 1-NH)</td>
</tr>
<tr>
<td>8</td>
<td>5.44 (d; $J = 7.5$)</td>
<td>5.44 (d; $J = 7.5$)</td>
</tr>
<tr>
<td>8</td>
<td>7.38 (d; $J = 8.0$)</td>
<td>7.39 (d; $J = 8.0$)</td>
</tr>
</tbody>
</table>

4. CONCLUSION

Eight compounds were successfully isolated from the ethyl acetate extract of *Scoparia dulcis* L. and identified as apigenin, luteolin, baicalein, oroxylin A, oroxylin A 7-$O$-$\beta$-$D$-glucoside, vitexin, 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and uracil. In this study, the presence of five compound as baicalein, oroxylin A, oroxylin A 7-$O$-$\beta$-$D$-glucoside, DIBOA and uracil were first report after isolating. The results have contributed to the chemical constituents of *Scoparia dulcis* L. growing in An Giang province, Vietnam.

REFERENCES


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Nghiên cứu này tập trung vào việc phân lập và nhận danh tám hợp chất từ nhân từ cao ethyl acetate của cây Cam Thảo Đất Scoparia dulcis Linn. thu hoạch ở tỉnh An Giang, Việt Nam. Với các phương pháp tách chiết hợp chất thiên nhiên thường quen, sau hợp chất flavonoid là: apigenin (1), luteolin (2), baicalein (3), oroxylin A (4), oroxylin A 7-O-β-D-glucoside (5) và vitexin (6) cùng với hai hợp chất có nitrogen là: 2,4-dihydroxy-1,4-benzoxazin-3-one (7) và uracil (8) đã được cơ lập thành công. Cấu trúc hóa học của các hợp chất được giải đoạn cần curse vào phổ NMR, MS cùng với đổi chiều dử liệu trong tài liệu đăng tin cậy. Nghiên cứu đã đóng góp vào việc khảo sát hóa thực vật của loài Scoparia dulcis L. sinh trưởng tại Việt Nam với sự hiện diện của các chất lần đầu cơ lập được từ nguyên liệu như (3), (4), (5), (7) và (8).

Từ khóa: Scoparia dulcis Linn., ethyl acetate extract, apigenin, luteolin, baicalein, oroxylin A, vitexin, oroxylin A 7-O-β-D-glucoside, DIBOA, uracil.