Cytotoxic Flavonoid Glycosides from *Rapistrum rugosum* L.

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Abstract

Five flavonoid glycosides were isolated from the *n*-butanol soluble fraction of the ethanolic extract of *Rapistrum rugosum* and their structures were assigned from 1H- and 13C-NMR spectra (DEPT) with 2D NMR as quercetin-3-O-α-L-rhamnopyranoside (1), quercetin-3-O-β-D-xyloside (2), quercetin, 3-O-α-L-arabinopyranoside,7-O-α-L-rhamnopyranoside (3), kaempferol 3-O-α-L-arabinopyranoside, 7-O-α-L-rhamnopyranoside (4) and rutin (5). The SRB cytotoxic assay was used to investigate the antitumor activities of *n*-butanol extract, compound 3 and its hexaacetate 3a, for the first time. Compounds 3 and 3a showed cytotoxic activity against the human cancer cell line, namely, HepG2 (hepatocellular carcinoma cell line) with IC50 (concentration of compound required to reduce cell survival by 50%) 0.86 µg/mL and 3.50 µg/mL, respectively. These results proved that compound 3, the major flavonoid of the *n*-butanol soluble fraction, has significant cytotoxic activity compared with the standard antitumor drug doxorubicin (0.60 µg/mL).

Keywords: SRB cytotoxic assay; *Rapistrum rugosum*; Flavonoid glycosides; *n*-butanol.

Introduction

*Rapistrum rugosum* L. commonly known as turnip weed, wild turnip or bastard cabbage, belongs to the family of Cruciferae. This family comprises about 390 genera and is represented in Saudi Arabia by 49 genera. *R. rugosum* is native to North Africa, Europe, the Middle East and Pakistan and is the only *Rapistrum* species in Saudi Arabia (1). The leaves of *R. rugosum* are externally applied to the heal legs furuncles in Italy (2). *R. rugosum* is boiled and used for culinary purposes as one of the most popular, wild food plants in Sicily (3).

The high demand of the innovative lead structures to develop the novel drugs for the treatment of cancer and other menacing diseases drove us to study the cytotoxic activity of the ethanolic plant extract. The ethanolic extract of *R. rugosum* showed cytotoxic activity, on further biological screening of all fractions, the *n*-BuOH soluble fraction revealed a strong cytotoxic activity. This prompted us to carry out the phytochemical study and try to isolate the constituents of the *n*-BuOH soluble fraction of *R. rugosum*. Our goal was also to try and investigate the effect of acetylation on the cytotoxic activity of the major isolated compound.

Experimental

General experimental procedures

The 1H-, 13C-NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometer operating at 400 and 100 MHz for 1H-NMR
Sephadex LH-20 column using \( n\)-BuOH/iso-pr. OH/H\(_2\)O (BIW, 4 : 1 : 5, organic layer) for elution to afford 3 sub-fractions (I-III). Sub-fraction I contained only one spot and it was then purified on a Sephadex LH-20 column with MeOH: H\(_2\)O (2 : 8) as eluent to give compound 5 (13 mg).

Fraction C (1.5 g) was a binary mixture which was separated on a cellulose column with 80% MeOH/H\(_2\)O to afford 1 (15 mg) and 2 (12 mg).

Acetylation of compound 3

Compound 3 (20 mg) was dissolved in pyridine-acetic anhydride (1:1, 2 mL) and stirred overnight at room temperature. The mixture was evaporated to dryness at rotary evaporator in vacuum under N\(_2\) which gave a hexaacetate of 3 as white amorphous powder. HRFABMS (-ve ion mode): m/z 831.0625 calcd. for C\(_{38}\)H\(_{39}\)O\(_{21}\), 831.0633.

Acid Hydrolysis of compound 3

Compound 3 (5 mg) in MeOH (5 mL) containing 1N HCl (5 mL) was refluxed for 4 h, concentrated under reduced pressure and diluted with H\(_2\)O (10 mL). It was extracted with EtOAc and the residue recovered from the organic phase yielded quercetin as an aglycone. The remaining aqueous solutions were evaporated to dryness, resolved in MeOH and subjected to TLC analysis (eluent: EtOAc-MeOH-H\(_2\)O-HOAc, 6:2:1:1). The chromatogram was sprayed with aniline hydrogen phthalate followed by heating at 100°C. The sugars were identified after the comparison through authentic standards.

Cytotoxicity assay

In-vitro SRB cytotoxic assay against Human liver cancer cell line (HepG2 cells)

Potential cytotoxicity of \( n\)-butanol extract, compounds 3 and 3a were tested using the method of Skehan and Storeng (4). The sensitivity of the human tumor cell lines to thymoquinone was determined through the SRB assay. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. Hepatocellular cell line (HepG2) was obtained frozen in liquid nitrogen at -180°C from the American Type...
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Table 1. Effect of n-butanol extract, compounds 3 and 3a on liver carcinoma cell line (HepG2). Mean of surviving fraction ± SD, n = 6.

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Extract and compounds conc. µg/mL</th>
<th>n-butanol extract</th>
<th>Compound 3</th>
<th>Compound 3a</th>
<th>Doxorubicin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG2</td>
<td>0.000</td>
<td>1.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
</tr>
<tr>
<td></td>
<td>1.000</td>
<td>0.973 ± 0.180± 0.087</td>
<td>0.485 ± 0.186</td>
<td>0.847 ± 0.115</td>
<td>0.347 ± 0.117</td>
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<tr>
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<td>2.500</td>
<td>0.678 ± 0.172± 0.159</td>
<td>0.477 ± 0.161</td>
<td>0.597 ± 0.149</td>
<td>0.350 ± 0.136</td>
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<tr>
<td></td>
<td>5.000</td>
<td>0.479 ± 0.227± 0.124</td>
<td>0.291 ± 0.070</td>
<td>0.358 ± 0.117</td>
<td>0.359 ± 0.124</td>
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<tr>
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<td>10.000</td>
<td>0.430 ± 0.159± 0.117</td>
<td>0.249 ± 0.089</td>
<td>0.360 ± 0.136</td>
<td>0.345 ± 0.115</td>
</tr>
</tbody>
</table>

*IC₅₀

<table>
<thead>
<tr>
<th>Extract and compounds conc. µg/mL</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-butanol extract</td>
<td>4.78 µg/mL</td>
</tr>
<tr>
<td>Compound 3</td>
<td>0.350 ± 0.136 µg/mL</td>
</tr>
<tr>
<td>Compound 3a</td>
<td>0.358 ± 0.117 µg/mL</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>1.000 ± 0.000 µg/mL</td>
</tr>
</tbody>
</table>

**Results and Discussion**

The mixture of flavonoid glycosides obtained from the n-BuOH fraction of the ethanolic extract of R. rugosum was subjected to a series of column chromatographic separations to isolate compounds 1-5, namely; quercetin-3-O-α-L-rhamnopyranoside (1), quercetin-3-O-β-D-xiloside (2), quercetin, 3-O-α-L-arabinopyranoside, 7-O-α-L-rhamnopyranoside (3), kaempferol 3-O-α-L-arabinopyranoside, 7-O-α-L-rhamnopyranoside (4) and rutin (5). Their structures were established via mass techniques and through comparison with the reported data in the literature (6-13).

**Quercetin; 3-O-α-L-arabinopyranoside, 7-O-α-L-rhamnopyranoside (3)**

Yellow crystalline solid (75 mg). M.p. 248-250°C: EIMS m/z (rel. int.): 302 (100), 270 (10), 152 (27), 134 (28). HRFABMS (ve ion mode): m/z: 579.0625 calcd. for C₁₃H₁₂O₁₃, 579.0633. ¹H-NMR (400 MHz, DMSO-d₆) δ: 6.44 (1H, d, J = 2.0 Hz, H-6), 6.78 (1H, d, J = 2.0 Hz, H-8), 6.82 (1H, d, J = 8.0 Hz, H-5′), 7.56 (1H, d, J = 2.2 Hz, H-2′), 7.70 (1H, dd, J = 8.0, 2.2 Hz, H-6′), 5.56 (1H, brs, H-1″), 5.30 (1H, d, J = 4.5 Hz, H-1″), 3.76 (1H, dd, J = 8.4, 4.7 Hz, H-2″), 3.53 (1H, m, H-3″), 3.63 (1H, m, H-4″), 3.24 (1H, m, H-5a″), 3.62 (1H, m, H-5b″), 3.5 (1H, m, H-2‴), 3.3 (1H, m, H-3‴), 3.1 (1H, m, H-4‴), 1.12 (3H, d, J = 5.5 Hz, H-6‴). ¹³C-NMR (100 MHz, DMSO-d₆) δ: 156.7 (C-2), 133.8 (C-3), 177.6 (C-4), 160.8 (C-...
The major compound 3 was derivatized into its hexaacetate derivative (3a) and analyzed through HRFABMS and NMR. The major flavonoid diglycoside (3) and its hexaacetate (3a) were screened for cytotoxicity against the human cancer cell line, namely, HepG2 (hepatocellular carcinoma cell line). From the results shown in Table 1 and Figures 1 and 2, it could be seen that compound 3 shows a significant cytotoxic activity against the liver carcinoma cell line (IC$_{50}$ = 0.86 µg/mL), while the acetylated compound 3a shows a lower cytotoxic activity (IC$_{50}$ = 3.50 µg/mL) compared to the standard drug doxorubicin (IC$_{50}$ = 0.60 µg/mL).

Quercetin-3-O-α-L-rhamnopyranoside (1), quercetin-3-O-β-D-xyloside (2), quercetin, 3-O-α-L-arabinopyranoside, 7-O-α-L-

Figure 1. The effects of different concentrations of the n-butanol extract, compounds 3 and 3a on HepG2 cell survival as assessed through the SRB Cytotoxic Assay.

Figure 2. The IC$_{50}$ values of n-butanol extract, compounds 3 and 3a.
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rhamnopyranoside (3), kaempferol 3-O-α-L-arabinopyranoside, 7-O-α-L-rhamnopyranoside (4) and rutin (5) have been isolated for the first time from Rapistrum. By means of chemical methods and spectroscopic analyses, the structures of these compounds (1-5) were established. There were no previous published reports dealing with the NMR data of the flavonoid diglycoside 3.

Compound 3 was obtained as a yellow crystalline solid. The HRFABMS of 3 exhibited a pseudomolecular ion peak [M-H] at m/z 579.0625 (calcd. for C_{26}H_{27}O_{15}, 579.0633) consistent with the molecular formula of C_{26}H_{27}O_{15}. The EIMS spectrum showed different peaks of aglycone at m/z 302, 270, 154 and 150. The UV spectrum exhibited characteristic absorption maxima for a flavonoid glycoside at λ_{max} (nm), (MeOH): 260, 300 (sh). The EIMS gave a peak at m/z 302 due to successive losses of sugar moieties. The 1H NMR spectrum showed a signal at δ 6.82 (d, J = 8.0 Hz, H-5), δ 7.56 (d, J = 2.2 Hz, H-2) and δ 7.70 (dd, J = 8.0, 2.2 Hz, H-6'). In the aliphatic region, an anomeric proton signal at δ 5.30 (d, J = 4.5 Hz), together with two oxymethylene protons observed at δ 3.24 (m) and δ 3.62 (m) were indicative to the presence of α-L-arabinopyranoside moiety. The second anomeric proton was observed at δ 5.56 as (brs). The 1H NMR spectrum further showed signals of oxymethylene protons in the range of δ 3.76-3.10 and the methyl protons resonated at δ 1.12 (d, J = 5.5 Hz) which was characteristic for α-L-rhamnopyranoside moiety.

The 13C NMR and DEPT spectra showed twenty-six signals comprising one methyl, one methylene, fourteen methine and ten quaternary carbons. The signals at δ 156.7, 133.8, 177.6 and 105.5 were typical of C-2, C-3, C-4 and C-10 of a flavonol moiety. The signals of two anomeric carbons of the sugar moieties appeared at δ 101.2 and 99.4. Assignment of all 1H and 13C resonances was proved through their comparison with the reported data in the literature (6-13). Acid hydrolysis of 3 provided L-arabinose and
L-rhamnose and it was confirmed through the TLC of sugars with their standards.

In addition, the major flavonoid diglycoside (3) and its acetylated form (3a) were screened for cytotoxicity against the human cancer cell line, namely, HepG2 (hepatocellular carcinoma cell line).

Previous studies, however, proved the antitumor activity of flavonoids and even aimed at elucidating the structure-activity relationships in order to develop new anticancer drugs (14). This is the first report for the cytotoxic activity of these compounds. This finding may help to show the structural requirements implicated in the anticancer activity of flavonoids, with the goal of rationalizing their development as antitumor agents.

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References


