Cytotoxic and Antioxidant Activity of Marrubium vulgare and its Flavonoid Constituents

Nawal, H. Mohamed and Atta, E. M

Abstract— Six flavonoid compounds were isolated from Marrubium vulgare. by chromatographic methods and identified by UV, $^1$H, $^{13}$C NMR spectroscopy as Acacetin, Acacetin-7-rhamnoside, Apigenin, Diosmetin, Diosmetin-7-glucoside and Luteolin-7-rhamnoside, and. In vitro the anticancer activity of Marrubium vulgare total extract and some isolated flavonoid (Acacetin, Apigenin, and Acacetin-7-rhamnoside) compounds were tested against Ehrlich tumor cell lines and Human tumor cell lines U251 and MCF7 (brain tumor and breast carcinoma cell lines respectively). Both total alcoholic, Acacetin, Apigenin, and Acacetin-7-rhamnoside show high anticancer activity against breast carcinoma where ED$_{50}$ < 20µg/ml, whereas all of them have anticancer activity against Ehrlich tumor cell lines. The plant alcoholic extract and isolated flavonoids also have high antioxidant activity in vitro using DPPH scavenging activity method.

Keywords--- Antioxidant, cytotoxic, Flavonoids, Labiatae, Marrubium vulgare.

I. INTRODUCTION

Marrubium vulgare belongs to family Labiatae. Many members of this family are used as medicinal herb and source of volatile oils. It also contains diterpenoids, triterpenoids, saponins, polyphenol, tannins and flavonoids [1]. Marrubium vulgare is used in treatment of jaundice, diabetes, fever and diuretic.

Treatment of diseases by medicinal plants was known by old civilizations. Recently many schools in the scientific societies calling for the use of natural products since most synthetic one proved to exert certain side effects. Among the disease which is widely distributed is cancer, the present work was attempt to study anticancer activity of the plant as well as its isolated flavonoid compounds.

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II. MATERIALS AND METHODS

A. Plant materials

The aerial parts of Marrubium vulgare were collected from Northern Sinai identified by Botany Department, Faculty of Science, Cairo University and by comparison with plant description in the flora of Egypt [2],[3].

B. Extraction, Isolation and purification of flavonoid compounds

Two kg of the air-dried powder of Marrubium vulgare were extracted with 90% alcohol by percolation till exhaustion. Alcoholic extract was evaporated in vacuo. The alcoholic extract then precipitated till free from salts. For isolation of the flavonoid compounds, columns and thin layer chromatography were used. Then sephadex LH20 column using[4],[5] to obtain pure compounds(1-6).

C. Anticancer activity (cytotoxic activity):

Concerning with anticancer activity, both Marrubium vulgare total extract and some isolated compounds were tested for anticancer activity against Ehrlich tumor cell lines and Human tumor cell lines U251 and MCF7 (brain tumor and breast carcinoma cell lines respectively). Potential Cytotoxicity of the plant and its isolated compounds were tested according to[6].

D. Antioxidant activity DPPH scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl free radical) assay in an excellent in vitro method to investigate the free radical scavenging activity of an antioxidant. This is carried out according to[7].

III. RESULTS AND DISCUSSION

A. The isolated flavonoid compounds:

Six flavonoid compounds were isolated from Marrubium vulgare; they were identified by spectral data and by comparing with published data before according to[4],[8]-[10].
TABLE I
UV SPECTRAL DATA OF THE ISOLATED FLAVONOIDS FROM Marrubium vulgare

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>MeOH</th>
<th>NaOMe</th>
<th>NaOAc</th>
<th>NaOAc/H2BO3</th>
<th>AlCl3</th>
<th>AlCl3/HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cpd 1</td>
<td>268,301</td>
<td>275,323</td>
<td>270</td>
<td>268</td>
<td>277</td>
<td>300</td>
</tr>
<tr>
<td>Cpd 2</td>
<td>266,301</td>
<td>275,323</td>
<td>268</td>
<td>268</td>
<td>275</td>
<td>300</td>
</tr>
<tr>
<td>Cpd 3</td>
<td>250, 265, 275</td>
<td>275</td>
<td>265</td>
<td>275</td>
<td>300</td>
<td>300, 345</td>
</tr>
<tr>
<td>Cpd 4</td>
<td>250, 250, 303</td>
<td>250</td>
<td>250</td>
<td>274</td>
<td>270, 300</td>
<td>350, 400</td>
</tr>
<tr>
<td>Cpd 5</td>
<td>250, 250, 303</td>
<td>250</td>
<td>250</td>
<td>274</td>
<td>270, 300</td>
<td>350, 400</td>
</tr>
<tr>
<td>Cpd 6</td>
<td>253, 280, 400</td>
<td>265</td>
<td>253</td>
<td>253</td>
<td>253, 340</td>
<td></td>
</tr>
</tbody>
</table>

Compound 1: Acacetin (4'-methoxy apigenin)

\[ \text{\^{1}H NMR (DMSO- d$_6$): } \delta 7.8 (2H, d, J= 8 Hz, H2', H-6'); \delta 6.7 (2H, d, J=8 Hz, H-3', H-5'); \delta 6.41 (1H, d, J=2.5Hz H8); \delta 6.4 (1H,d, J=2Hz H3'; H-5'); \delta 6.6 (1H, d, J=7Hz H5); \delta 6.6 (1H, d, J=2Hz H6); \delta 6.6 (1H, H3' and H5'), 17.4 (C-6''). \]

Compound 2: Acacetin-7-rhamnoside

\[ \text{\^{1}H NMR (DMSO- d$_6$): } \delta 7.8 (2H, d, J= 8 Hz, H2', H-6'); \delta 6.7 (2H, d, J=8 Hz, H-3', H-5'); \delta 6.41 (1H, d, J=2.5Hz H8); \delta 6.4 (1H,d, J=2Hz H3'; H-5'); \delta 6.6 (1H, d, J=7Hz H5); \delta 6.6 (1H, d, J=2Hz H6); \delta 6.6 (1H, H3' and H5'), 17.4 (C-6''). \]

Compound 3: Apigenin

\[ \text{\^{1}H-NMR (DMSO-d$_6$): } \delta 7.39 (1H, Q, J = 2, 7.6,H-6'), \delta 7.3 (1H, d, J = 2.5, H-2'), \delta 6.8 (1H, d, J = 8.4, H- 5'), \delta 6.6 (1H, s, H-3), \delta 6.7 (1H, d, J = 2.0, H-6), \delta 5.0 (1H, d, J = 7.6, H-1''), \delta 3.4-4.0 (remaining sugar protons, m). \]

**Fig 1 General scheme of the isolated compounds**

Compound 4: Diosmetin (4'-methoxy luteolin)

\[ \text{\^{1}H NMR (DMSO- d$_6$): } \delta 7.6 (1H, d, J = 2.5 Hz, H2'); \delta 7.4 (1H, d, J=7.5, 2.5Hz, H-6'); \delta 6.9 (1H, d, J=7.5Hz, H-5'); \delta 6.61 (1H, d, J=2.5Hz, H-8); \delta 6.4 (1H,d, J=2Hz H6); \delta 6.6 (1H, H3' and H5'), 17.4 (C-6''). \]

Compound 5: Diosmetin-7-glucoside

\[ \text{\^{1}H NMR (DMSO- d$_6$): } \delta 7.6 (1H, d, J= 2.5 Hz, H2'); \delta 7.4 (1H, d, J=7.5, 2.5Hz, H-6'); \delta 6.9 (1H, d, J=7.5Hz, H-5'); \delta 6.61 (1H, d, J=2.5Hz, H-8); \delta 6.4 (1H,d, J=2Hz H6); \delta 6.6 (1H, H3' and H5'), 17.4 (C-6''). \]

**Compound 6: Luteolin-7-rhamnoside**

\[ \text{\^{1}H-NMR (DMSO- d$_6$): } \delta 7.39 (1H, Q, J = 2, 7.6,H-6'), \delta 7.3 (1H, d, J = 2.5, H-2'), \delta 6.8 (1H, d, J = 8.4, H- 5'), \delta 6.6 (1H, s, H-3), \delta 6.7 (1H, d, J = 2.0, H-6), \delta 5.02 (1H, d, J = 7.6, H-1''), \delta 3.4-4.0 (remaining sugar protons, m). \]

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B. Anticancer activity (cytotoxic activity)

Results are expressed as the dose that inhibits 50% control growth after incubation period. Compounds having ED$_{50}$ < 20µg/ml are considered active.

Total alcoholic extract and compounds Acacetine, Apigenin and Acacetine-7-rhamnoside from *Marrubium vulgare* show significant anticancer activity against Ehrlich tumor cell lines and Human tumor cell lines U251 and MCF7 (brain tumor and breast carcinoma cell lines). From Table II it can be concluded that:

Total alcoholic, Acacetine, Apigenin and Acacetine-7-rhamnoside show high anticancer activity against Brain carcinoma U251 where ED$_{50}$ < 20 µg/ml, whereas total extract and Acacetine show moderate activity against breast carcinoma MCF7 where ED$_{50}$>20 µg/ml.

C. Antioxidant activity DPPH scavenging activity

The alcoholic extract with concentrations (8, 10 mg/ml) and compounds (1, 2 and 3) show high antioxidant activity towards scavenging of DPPH radical. The activity was corresponding to 88.2 standard ascorbic acid at concentration 100 µM.

<table>
<thead>
<tr>
<th>Type of extract and concentration µg/ml</th>
<th>Percentage Inhibition of cell viability</th>
<th>EAC %</th>
<th>Breast carcinoma ED$_{50}$µg/ml</th>
<th>Brain carcinoma ED$_{50}$µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>25</td>
<td>85%</td>
<td>31.4</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>90%</td>
<td>28.4</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100%</td>
<td>22.1</td>
<td>15.8</td>
</tr>
<tr>
<td>Acacetin</td>
<td>50</td>
<td>75%</td>
<td>20.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Apigenin</td>
<td>50</td>
<td>85%</td>
<td>14.5</td>
<td>14.8</td>
</tr>
<tr>
<td>Acacetin-7-rh</td>
<td>50</td>
<td>80%</td>
<td>12.1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extract concentration µg/ml</th>
<th>Radical Scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>36.8 ± 0.98</td>
</tr>
<tr>
<td>50</td>
<td>66.5 ± 1.01</td>
</tr>
<tr>
<td>100</td>
<td>85.0 ±0.99</td>
</tr>
<tr>
<td>Acacetin</td>
<td>80.0 +1.0</td>
</tr>
<tr>
<td>Apigenin</td>
<td>85.0 + 0.98</td>
</tr>
<tr>
<td>Acacetin-7-rh</td>
<td>87.5 ± 0.99</td>
</tr>
</tbody>
</table>

IV. Conclusion

Cancer arises from cells harboring mutation that relinquish the need for exogenous growth factor. Deregulation of growth control lead to selection of clonal cells that replicate at embryonic stage and fail to respond to differentiation and maturation signals[11]. Flavonoids are non toxic polyphenolic compounds display a remarkable spectrum of biological activities including those able to influence process that are deregulated during cancer development. These include antioxidant and anticarcinogenic So flavonoid can be considered as possible chemo preventive and therapeutic agents against cancer[12]. *Marrubium vulgare* plant has anticancer and antioxidant activities due to its high content of flavonoid.

REFERENCES

[3] V.Täckholm. Student’s flora of Egypt. 2nd ed. Published by Cairo Univ. printed by Cooperative printing Co. Beirut, 1974