Anticancer activity of biflavonoids from Lonicera japonica and Benincasa hispida on human cancer cell lines

D. Pradhan¹, P.K.Panda, G.Tripathy, J. R.Nayak and A.Pattanayak

¹University Department of Pharmaceutical Sciences, Utkal University, Vanivihar, Bhubaneswar-751004.

Received on: 12-09-2008; Accepted on: 04-01-2009

ABSTRACT

The biflavones 7,7”-dimethyllanaraflavone (1), agathisflavone (2), and 7”-methylagathisflavone (3) isolated from the stems of Lonicera japonica and flavonoids (4) isolated from the stems and branches of Benincasa hispida, as well as a mixture of 7,7”-dimethyllanaraflavone and 7”-methylagathisflavone, were assayed against HT-29 colon adenocarcinoma, NCI-H460 non-small cell lung carcinoma, MCF-7 breast cancer cell, OVCAR-3 ovarian adenocarcinoma cells, and RXF-393 renal cell carcinoma. The results show significant activities, particularly for 7,7”-dimethyllanaraflavone (IC₅₀ 1.77 ± 0.08, 3.42 ± 0.22, and 3.59 ± 0.32 mg/ml for NCI-H460, MCF-7, and OVCAR-3, respectively), and for 7”-methylagathisflavone (IC₅₀ values of 4 mg/ml). Flavonoids revealed significant cytotoxicity on the five cell lines tested.

Keywords: Anticancer activity; biflavonoids; Lonicera japonica and Benincasa hispida

INTRODUCTION

Anticancer agents may be derived from nature through isolation of active lead compounds. There are examples of successful drugs obtained from plant sources which have had a profound impact in the field of cancer. Indeed, the medical armamentarium is rich in examples of important agents that were isolated from plants and which continue to be used in current, routine clinical practice. Worldwide efforts are on to discover new anticancer agents from plants worldwide efforts are on to discover new anticancer agents from plants. The Japanese Honeysuckle; Lonicera japonica is a species of honeysuckle native to eastern Asia including Japan, Korea, India, northern and eastern China, and Taiwan, which is a major invasive species in North America. It is a twining vine able to climb up to 10 m high or more in trees, with opposite, simple oval leaves 3–8 cm long and 2–3 cm broad. The flowers are double-tongued, opening white and fading to yellow, and sweetly scented. The fruit is a globose dark blue berry 5–8 mm diameter containing numerous seeds. The Japanese Honeysuckle flower is of high medicinal value in traditional Chinese medicine, where it is called ren dong teng; literally “gold silver flower”. It has antibacterial and anti-inflammatory properties, and is used (often in combination with Forsythia suspensa) to dispel heat and remove toxins, including carbuncles, fevers, influenza and ulcers. It is, however, of cold and yin nature, and should not be taken by anyone with a weak and “cold” digestive system. Benincasa hispida (Fam-Cucurbitaceae) fruit is widely used as a vegetable in India and tropical countries. Many empirical applications have been used in India centuries for various ailments such as G.I.T. problem (dyspepsia), burning sensation, heat disease, vermi-fuge, Diabetes and urinary disease, anti-inflammatory activity, diuretic activity, anticancer activity. However the Lehman or traditional people are using this plant for various rigorous disorder. Triterpenoids, flavonoids, glycosides, saccharides & carotenes, vitamins, sitosterin & uromic acid are the major constituents reported & isolated earlier. We have earlier reported the isolation and the structure determination of four biflavonoids: 7”, 7”-dimethyllanaraflavone, agathisflavone, and 7”-ethylagathisflavone from Lonicera japonica (stems) and flavonoids from Benincasa hispida (stems). In the present study we investigated the antiproliferative activity of these compounds against five human cancer cell lines (HT-29, NCI-H460, RXF-393, MCF-7 and OVCAR-3).

MATERIAL AND METHODS

Plant material:

The stems of Lonicera japonica (Caprifoliaceae) were collected at Forest park, Bhubaneswar in October 2006. A voucher specimen (B-145) was deposited at the herbarium of Botany Department, Utkal University, Bhubaneswar. The stems of Benincasa hispida were collected at Bhubaneswar, Orissa. A voucher specimen (156) was deposited at the herbarium, Botany Department, Utkal University, Orissa.

Extraction and isolation:

Air-dried stems of L. japonica (590.0 g) were extracted exhaustively with dichloromethane and methanol. The solvents were removed under vacuum to yield the extracts residues Stems-Dichloromethane (LD, 12.5 g) and Stems-Methanol (LM, 135.0 g). The LD residue (113.8 g) was filtered on a silica gel column, and the fraction eluted with ethyl acetate was crystallized from MeOH to afford 7, 7”-dimethyllanaraflavone (1). The methanolic extract (LM) was partitioned.
Table 1: Evaluation of anticancer activity of biflavonoids from *Lonicera japonica* and *Benincasa hispida* on human cancer cell lines

<table>
<thead>
<tr>
<th>AGENTS</th>
<th>HT-29</th>
<th>NCI-H460</th>
<th>RXF-393</th>
<th>MCF-7</th>
<th>OVCAR-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,7&quot; dimethyl lanaraflavone(1)</td>
<td>&gt;50&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>1.77 ± 0.08&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
<td>41.61 ± 1.44&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>3.42 ± 0.22&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
<td>3.59 ± 0.32&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Agathisflavone(2)</td>
<td>&gt;50&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>&gt;50&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>45.89 ± 1.84&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>&gt;50&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
<td>&gt;50&lt;sup&gt;b,c,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>7&quot;-methyl agathisflavone(3)</td>
<td>4.38+0.42&lt;sup&gt;x,c,d,e&lt;/sup&gt;</td>
<td>5.36 ± 0.35&lt;sup&gt;x,c,d,e&lt;/sup&gt;</td>
<td>4.86 ± 0.64&lt;sup&gt;x,c,d,e&lt;/sup&gt;</td>
<td>5.58 ± 0.22&lt;sup&gt;x,c,d,e&lt;/sup&gt;</td>
<td>5.18 ± 0.82&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>13.01 ± 1.59&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>7.85 ± 0.73&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>7.46 ± 0.72&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>8.76 ± 0.47&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>7.43 ± 1.24&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>OFMHE-6</td>
<td>9.19 ± 1.19&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>10.88 ± 1.57&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>9.39 ± 1.46&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>10.02 ± 1.20&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>11.45 ± 2.56&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Etoposide</td>
<td>2.22 ± 0.09&lt;sup&gt;x,c,d,e&lt;/sup&gt;</td>
<td>1.27 ± 0.02&lt;sup&gt;x,c,d,e&lt;/sup&gt;</td>
<td>14.77 ± 2.67&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
<td>4.42 ± 1.0&lt;sup&gt;c,d,e&lt;/sup&gt;</td>
<td>10.42 ± 1.62&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>One-way ANOVA</td>
<td>F 1806.7</td>
<td>1326.4</td>
<td>515.99</td>
<td>1318.0</td>
<td>523.58</td>
</tr>
<tr>
<td>df 5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>P 0.0079</td>
<td>&lt;0.0001</td>
<td>0.2409</td>
<td>0.0061</td>
<td>0.0862</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Different from 7, 7'-dimethyllanaraflavone; <sup>b</sup>Different from 7"-methylosaflavone; <sup>c</sup>Different from Flavonoids; <sup>d</sup>Different from Agathisoflavone; <sup>e</sup>Different from OFMHE-6.

Results of the present study indicated that the biflavonoids 1-3 were more active than the single flavonoids as measured by their IC<sub>50</sub> values. The biflavonoids and flavonoid mixtures were tested at concentrations ranging from 8-10 µg/ml [Table-1]. The 7, 7"-dimethyllanaraflavone (1) and 7"-methylagathisflavone (3) had the lowest IC<sub>50</sub> values with the highest activity on all cell lines. The IC<sub>50</sub> values of the 7, 7"-dimethyllanaraflavone (1) and 7"-methylagathisflavone (3) were around 4 µg/ml for all the five cell lines tested [Table-1]. Flavonoids (4), showed similar IC<sub>50</sub> values on NCI-H460, RXF-393, MCF-7, and OVCAR-3 cell lines [Table-1]. On the other hand, in HT-29 cells, the IC<sub>50</sub> increased by 1.7 fold compared to that of other cell lines. Agathisflavone (2) did not have a major impact on the cell growth in any of the cell lines tested [Table-1]. Considering the better antitumor activity (P<0.05) observed with 7, 7"-dimethyllanaraflavone and 7"-methylagathisflavone than other biflavones, we decided to test the effect of the mixture (OFMHE-6) of those compounds. The OFMHE-6 showed growth inhibitory activity (<25% of control cell growth) among the cell lines tested at 18-20 µg/ml and the IC<sub>50</sub> values ranged from 8-10 µg/ml [Table-1].
DISCUSSION:

In order to evaluate the cytotoxic activity of four biflavonoids, antiproliferative assay with five human cancer cell lines were performed. As observed with the anticancer agent etoposide, the biflavonoids demonstrated different patterns of growth inhibition among the cell lines tested. Our results indicated that 7, 7"-dimethyllanaraflavone (1) had more selective activity among the cell lines tested. HT-29 and RXF-393 cell lines seem to be resistant, while NCI-H460 was the most sensitive cell line for this biflavone. The results are similar to a previous report of concentration-dependent growth inhibitory activity of another biflavonoid (4', 5, 7-trihydroxyflavone (3''O''4''')-5', 7''-dihydroxyflavone) isolated from Benincasa hispida on murine Ehrlich carcinoma cells but not on human K562 leukemia cells.16,17

The result showed significant growth inhibition induced by 7''-methylagathisflavone (3) on all the cell lines tested is in agreement with the observed antiproliferative activity of this biflavonoid against human K562 leukemia cells.16 The effect demonstrated with 7, 7"-dimethyllanaraflavone, 7''-methylagathisflavone, and OFMHE-6 (a mixture of 7,7"-dimethyllanaraflavone and 7''-methylagathisflavone) could be explained by the presence of a methoxy group in carbon 7. This group confers more lipophilicity to the substance, allowing better incorporation of these molecules into the cells. In contrast; agathisflavone does not have a methoxy group. This could probably explain the absence of antiproliferative activity with this compound. The antitumor activity observed with flavonoids (4) is in accordance with several studies demonstrating that chalcones are cytotoxic in different tumor cell lines.18,19,20,21 Our findings suggest that, although the three bioflavonoid demonstrated cytotoxicity on the five cell lines tested, the antiproliferative effect appears to vary depending upon tumor cell type; this should be further investigated to study the mechanism apart from possible toxicity.

ACKNOWLEDGEMENT

We specially thank H.O.D, University Department of Pharmaceutical Sciences for his valuable moral support in time to time. This work was supported by the grant obtained from A.I.C.T.E, New-Delhi.

REFERENCES


Source of support: Nil, Conflict of interest: None Declared