Research Paper

A NEW ANTICANCER FLAVONOID FROM THE LEAVES OF *Andrographis paniculata*

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Abstract

Extract of *Andrographis paniculata* traditionally used as a medicine to treat different diseases in India, China and Southeast Asia. In the present study, we evaluated the anticancer activity of the methanolic extract of *Andrographis paniculata* in human cancer and immune cells. The chloroform extract of the leaves of *Andrographis paniculata* yielded a labdanediterpenoid Andrographolide and a new flavonoid. These compounds were evaluated for their anticancer activity against selected human cancer cell lines. These isolates were significantly inhibited M14 (malonoma) and U251 (CNS) human cancer cell lines. These two compounds can serve as a scaffold for design and synthesis of novel, potent, non-toxic anticancer molecules.

Key words: *Andrographis paniculata*, chloroform extract, Andrographolide, flavonoid, anticancer activity.

INTRODUCTION

*Andrographis paniculata* belongs to the family of Acanthaceae. It is also commonly known as “kalmegh,” and a well-known medicinal plant of Ayurveda and has been used for centuries in Asian countries. Several polyherbal formulations of this plant are stated in Ayurveda as a popular remedy for the treatment of various disorders. *Andrographispaniculata* is an annual shrub grows abundantly in India and cultivated extensively in China and Thailand. The aerial parts of the plant (leaves and stems) are used to extract the active phytochemicals. The plant extract is known to contain labdane, neoclerodane-type of diterpenoids, sesquiterpenes, flavonoids and stigmasterols[45]. Extracts of plants and their major metabolites including labdanediterpenoids, neoclerodanediterpenoids and flavonoids have been reported to exhibit a wide range of biological activities [2-44] of therapeutic importance that include anti-inflammatory, hepatoprotective, antimalarial, antibacterial, antithrombotic, immune stimulant, antidepressive, antiallergic, central nervous system disorders [15, 17, 19, 21, 26-31], anti HIV, and anticancer.

Andrographolide is the major diterpenoid of the *Andrographispaniculata* extract has shown cytotoxic activity against KB (human epidermoid carcinoma) and P388 (lymphocyticleukaemia) [45] cells. The methanol extract of aerial parts of *Andrographis paniculata* and some of the isolated compounds showed growth inhibitory and differentiating activity on M1 (mouse myeloidleukaemia) cells [46]. However, no systematic study has been reported addressing the cytotoxic activity of *Andrographis paniculata* extract in human immune cells. Here we report the isolation of new flavonoid and
labdanediterpenoidandrographoliderfrom chloroform extract of leaves of Andrographis paniculata, and evaluated their anticancer activity against selected human cancer cell lines.

MATERIALS AND METHODS

Plant Material

Fresh leaves of Andrographis paniculata was collected from Chandigarh, Haryana, India in October 2014. Botanical identification was done by Prof. Jnanendra Shukla, taxonomist, Ayurvedic medicinal Plants Division, and voucher specimen HR-102/1A was deposited at the herbarium.

Extraction and isolation

The powdered plant material (750 mg) was extracted with chloroform for 24 h (5 L × 2 times). The chloroform extract was concentrated in vacuo, and 1 g of crude extract was obtained. A portion of the chloroform soluble fraction (1 g) was chromatographed on a column of silica gel eluted with chloroform and methanol mixture in order of increasing polarity. Fractions were collected and combined according to similar TLC pattern. Fractions 5-7 of chloroform–methanol (9:1) were found to be mixture (250 mg) of compounds andrographolide and a new flavonoid.

Cell growth assay

Cells undergoing exponential growth were seeded on a 96-well cell culture plates at a concentration of 10,000 cells per well and incubated at 37°C in a CO₂ incubator. Twenty-four hours later cells were treated with different concentrations of extracts or pure compounds dissolved in DMSO to a final concentration of 0.05% in the culture medium and incubated for 48 h. Cells were fixed by adding ice-cold 50% trichloroacetic acid (TCA) and incubating for 1 h at 4°C. The plates were washed with distilled water, air-dried and stained with SRB solution (0.4%, w/v, in 1% acetic acid) for 10 min at room temperature. Unbound SRB was removed by washing thoroughly with 1% acetic acid and the plates were air-dried. The bound SRB stain was solubilized with 10 mM Tris buffer, and the optical density was read on a spectrophotometric plate reader at a single wavelength of 515 nm. At the time of drug addition, a separate reference plate for cell growth at time 0 h (the time at which drugs were added) was also terminated as described above. The percentage growths were calculated and the GI₅₀ values were calculated from the growth curves.

RESULTS AND DISCUSSION

The portion of the chloroform soluble fraction (1 g) was chromatographed on a column of silica gel eluted with chloroform and methanol mixture in order of increasing polarity. Fractions were collected and combined according to similar TLC pattern. Fractions 5-7 of chloroform–methanol (9:1) were found to be mixture (250 mg) of compounds andrographolide and a new flavonoid (Fig. 1).

Compound 1 structure was confirmed as andrographolide by its spectral data (NMR and MS data).

Fig 1: Structures of isolated compounds

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Compound 2 was obtained as white amorphous solid. Its molecular formula $C_{18}H_{14}O_7 [M+H]^+$ at m/z 343.1, and gave a positive flavonoid test with magnesium-hydrochloric acid and exhibited UV absorption at $\lambda_{max}$ (MeOH) nm (log e) 283 (4.27) and 329 (3.76). Its IR spectrum showed absorption bands at $\mu_{max}$(KBr) 3380 (bonded hydroxyl), 1635, 1610, 1510 cm$^{-1}$ (chelated $\alpha$, $\beta$-unsaturated carbonyl). The $^1$HNMR spectrum (400 MHz, CDCl$_3$) of 2 displayed resonances at $\delta$ 3.9 (6H, s, two Ar-OCH$_3$ groups), 6.1 (2H, s, -O-CH2-O-), 6.4 (1H, s, H-7), 6.72 (1H, s C$_3$-H), 6.9 (1H, d, $J = 8$ Hz, H-5'), 7.3 (1H, d, $J = 2$ Hz, H-2'), 7.5 (1H, dd, $J = 2$, 8 Hz, H-6') and $\delta$ 13 (phenolic OH). The EIMS exhibited significant mass peaks at m/z (% rel. int.) 343.1 (M+H, 60.2), 327 (M+H-CH$_3$, 100), 314 (M+H - CO, 7.9), 313 (M+H - CO- H, 18.2), 299 (M+ H-CH$_2$CO, 20.7), 196 and 146 (R.D.A. of 1; 21.6, 14.7), 181 and 146 (R.D.A. of mass fragment 327; 15.2, 14.7), 149 (4.3) and 132 (146 - CH$_3$, 50.6). This characteristic MS fragmentation suggests the presence of two methoxyls and one hydroxyl in ring-A, while the $^1$HNMR spectral analysis indicated one –O-CH$_2$-O- chain at the 3',4'-position in ring B.

The bathochromic shift of UV band I by 20 (283-303) nm in the presence of AlCl$_3$, which remained unchanged on addition of hydrochloric acid suggested the presence of a hydroxyl function at C$_5$ and one of the methoxyl groups at the C$_6$ position. Thus, the other methoxyl must be either at C$_7$ or C$_8$ in ring-A. The presence of an $^1$HNMR signal at $\delta$ 6.4 (1H, s, H-7) and failure of the compound 2 to respond to the gossypetone test confirmed the presence of the second methoxyl group at C$_8$. The above data led us to formulatethe new flavone as 5-hydroxy-6,8-dimethoxy-3',4'-methylenedioxyflavone 2 and the structure was confirmed by $^{13}$CNMR spectral analysis.

$^{13}$CNMR data (100 MHz, CDCl$_3$): $\delta$ 183.7, 163.9, 152.8, 149.7, 147.2, 132.1, 129.2, 122.9, 119.5, 118.6, 110.2, 104.2, 104.1, 101.1, 61.9, 60.8.

Table: Effect of isolated compounds on growth of human cell lines: Growth Inhibition (GI$_{50}$):

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Andrographolide</th>
<th>flavonoid</th>
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<tbody>
<tr>
<td>M14 (malonoma)</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>U251 (CNS)</td>
<td>8</td>
<td>7</td>
</tr>
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</table>

Human cancer cell lines representing two types of cancers were incubated in complete medium with and without test compounds. Percentage growth of the treated cells was calculated compared to the control untreated cells and the concentration required inhibiting the 50% growth (GI50 concentration). The numbers represent the GI50 values in micromolar concentration.

From these two results it is evident that the major constituent andrographolide shows anticancer activity. The in vivo results from hollow fiber assay conducted in immunocompetent Swiss albino mice, demonstrated that andrographolide and new flavonoid significantly inhibits the cancer cell proliferation without showing any signsof toxicity in mice even at high doses.

We conclude that, owing to its potent anticancer activity, the diterpenoid andrographolide and flavonoid can serve as a scaffold for design and synthesis of novel, potent, non-toxic anticancer molecules.

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