IN VITRO ANTICANCER ACTIVITY AND XANTHINE OXIDASE INHIBITORY PROPERTIES OF Justicia beddomei

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ABSTRACT: The plant Justicia beddomei has numerous therapeutic utility in folk medicine. In the present study the in vitro anticancer and xanthine oxidase inhibitory (XOI) activity was investigated. The dried aerial parts of J. beddomei were successively extracted with methanol. The extract was subjected to MTT colorimetric assay in HeLa and MCF-7 cell lines and its xanthine oxidase inhibitory activity determined by using standard allopurinol. The XOI activity of the methanolic extract increased in a dose dependent manner and the MTT assay showed moderate cytotoxicity against the cell lines. The results obtained in the present study indicate that the methanolic extract of J. beddomei contains phytochemicals which has anticancer and XOI activities.

KEYWORDS: - HeLa, MCF-7, Justicia beddomei, Anticancer, Xanthine oxidase

1. INTRODUCTION

The synthetic anticancer remedies are beyond the reach of common man because of cost factor. Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical (1). A variety of bioactive compounds and their derivatives has been shown to inhibit carcinogenesis in a number of experimental systems involving initiation, promotion and progression (2, 3). Plants contain abundant quantities of these substances and have consistently been shown to be associated with a lower risk of cancers at almost every site (4). Efforts, therefore, are being made to identify naturally occurring anti-carcinogens which would prevent, slow or reverse the cancer induction and its subsequent development (5). It has been estimated that only 5-15% of 250,000 species of higher plants have been screened systematically for natural bioactive compounds. Sixty percent of currently used anticancer agents are derived in one way or another from natural sources. To study new therapeutic approaches, cell lines are used to investigate novel compounds and their effects on the tumor cells (6, 7).

Xanthine oxidase (XO) is a member of a group of enzymes known as molybdenum iron- sulphur flavin hydroxylases and it catalyses the oxidative hydroxylation of purines and subsequent reduction of oxygen at the flavincentre with generation of reactive oxygen species (ROS), either superoxide anion radical or hydrogen peroxide (the oxidative half-reaction)(8). It converts hypoxanthine to xanthine and xanthine to uric acid in our body. Any deficiency or excess of a specific metabolite in the body may cause diseases, or at least symptoms of the diseases (9).

Xanthine oxidase inhibitors (XOI) are agents that directly inhibit the synthesis of uric acid in vivo and thus these drugs should be given prime importance in the therapeutic approach for the treatment of gout and hyperuricemia (10). However, allopurinol a widely used XO inhibitory agent suffers from many side effects such as hypersensitivity reactions and renal toxicity (11). Certain active constituents present in crude plant extracts like flavonoids and polyphenolic compounds have been reported to possess XOI as well as free radical scavenging and inhibition of oxidative enzymes (12, 13). Thus, these findings open up the possibility of developing newer natural compounds from medicinal plants that are free of any unwanted side effects.

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In Cancer therapy, which involves greater protein catabolism leading to an increase in production of an end product uric acid which on accumulation can lead to conditions like gouty arthritis which involves deposition of urate crystals in the synovial joint causing inflammation. Hyperuricemia may be an early manifestation of the carcinogenic process (14). Anti-cancer drugs which are having the ability to inhibit xanthine oxidase can prove to be beneficial in cancer therapy as ultimately the drugs can inhibit xanthine oxidase and their by decrease the deposition of uric acid and prevent gouty arthritis.

The genus Justicia comprises about 300 species world over and nearly 50 species occur in India (15). About 20 species have been chemically investigated and reported to contain lignans, triterpenoidal glycosides and amide (16). The plants of Justicia genus are rich sources of bioactive lignans particularly arylnaphthalides (17, 18). The plant has been found to be having anti-diabetic, anti-inflammatory and anthelmintic properties (19, 20). The plant is distributed in the hilly regions of Kerala. Various parts are widely used in folk medicine as an expectorant, diuretic, antispasmodic, depurative and styptic. Leaves are used in diarrhoeal bleeding and irritative cough. Flowers are used in ophthalmic preparations and the roots along with the leaf juice are used in phthisis, cough, and asthma (21). Along with turmeric it is used in an anti-ageing cream to prevent wrinkles in the skin. However no such reports on XOI and anti-cancer studies are reported till date.

Hence the methanolic extract of the aerial part of Justicia beddomei which exhibited significant antioxidant activity (22) was evaluated for its in vitro XOI and anticancer activities.

2. MATERIALS AND METHODS

2.1. Plant material

The plant material was collected from hilly regions of Kerala, India. The plant was botanically identified by Dr. V. Chelladurai, Research Officer Botany, (Rtd) CCRAS, Government of India. A voucher Specimen has been kept in the department of chemistry (NCP/CH/ PS/JB01), National College of Pharmacy, Calicut.

2.2. Chemicals and Cell lines

All the chemicals of superior grade were purchased from SD Fine Chemicals and cell lines in National Institute of technology was used for anticancer study.

2.3. Extraction

The aerial parts of J. beddomei were dried, grounded into fine powder and sieved through No. 20 mesh sieve. About 100 g of powdered aerial part was defatted and subjected to soxhlet extraction with 500 ml of methanol. The percentage yield of methanol was 8.8% w/w.

2.4. MTT assay

Sensitivity of MCF-7 and HeLa cells to methanolic extract of J. beddomei was determined individually by the MTT colorimetric assay. Cells were seeded in a flat-bottomed 96-well plate and incubated for 24 h at 37°C and in 5% CO₂. Both cell lines were exposed to the extract. Medium was changed at 48 hrs intervals. After 24 hours, when the monolayer formed, the supernatant was flicked off and added previously diluted with media of 100µl of different concentrations of test extract in microtitre plates and kept for incubation at 37°C in 5% CO₂ incubator for 72 hour and cells were periodically checked for granularity. The solvent DMSO treated cells served as control. Cells were then treated with MTT reagent (20 µl/well) for 4 h at 37°C and then DMSO (200 µl) was added to each well to dissolve the formazan crystals. The optical density (OD) was recorded at 492 nm in a microplate reader.

Percentage of residual cell viability was determined as

\[ \frac{[1 - (OD \text{ of treated cells/OD of control cells})] \times 100}{\} \]

2.5. In vitro xanthine oxidase inhibitory activity

The xanthine oxidase inhibitory activity was assayed spectrophotometrically under aerobic conditions.
using xanthine as the substrate (26). The extract and the standard drug allopurinol (1 mg/ml) were prepared by dissolving in DMSO initially and then made up to the required volume with potassium dihydrogen phosphate buffer, pH 7.5. The assay mixture (250 μl) of extracts at different concentrations (2.5-40 μg/ml) consisted of 175 μl of potassium dihydrogen phosphate buffer, pH 7.5. The assay mixture (250 μl) of extracts at different concentrations (2.5-40 μg/ml) consisted of 175 μl of potassium dihydrogen phosphate buffer and 75 μl of xanthine oxidase enzyme (1.33U/ml) solution. After pre-incubation at 37°C for 10 min 500 μl of 0.15mM substrate (xanthine) was added, mixed and incubated at 37°C for 30 min. Then 200 μl of 1N HCl was added to arrest the reaction and the absorbance was measured at 290 nm against blank (buffer solution) and a control reaction was carried out without the test sample (27). The percentage inhibition was calculated using the following formula,

\[ I\% = \frac{A_c - A_t}{A_c} \times 100 \]

Where \( A_c \) is the absorbance of control reaction and \( A_t \) is the absorbance of test reaction. The assay was done in triplicate for each concentration. Allopurinol (2.5-40 μg/ml) was used as standard.

### 3. RESULTS AND DISCUSSION

#### 3.1. MTT assay

In the present study, the cytotoxic effect of methanolic plant extract on MCF-7 and HeLa cells was evaluated by MTT assay. MTT assay is a well-established *in vitro* method for cytotoxicity against cancer cell lines and non-cancer cell lines. This assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-dimethyl thiazol-2-yl)-2, 5 diphenyl tetrazolium bromide (MTT), which is measured spectrophotometrically (28). This formazan production is inversely proportional to the degree of cytotoxicity (29). Different dilutions of extracts were treated and IC\(_{50}\) values were calculated. Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties for future work. In the present study methanolic extract on both the cell lines showed moderate activity. The results are tabulated in Table 1.

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>% Cell inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEJ</td>
<td>Tamoxifen</td>
</tr>
<tr>
<td>MCF-7</td>
<td>HeLa</td>
</tr>
<tr>
<td>12.5</td>
<td>15.06±0.7</td>
</tr>
<tr>
<td>25</td>
<td>26.10±0.8</td>
</tr>
<tr>
<td>50</td>
<td>39.35±0.2</td>
</tr>
<tr>
<td>100</td>
<td>46.50±0.7</td>
</tr>
<tr>
<td>200</td>
<td>53.21±0.5</td>
</tr>
<tr>
<td>IC(_{50})</td>
<td>152.16*</td>
</tr>
</tbody>
</table>

**Table 1.** MTT assay

#### 3.2. *In vitro* xanthine oxidase inhibitory activity

The methanolic extract was assayed for their XOI activity at different concentrations in the assay mixture. From the results it is evident that the methanolic extract (IC\(_{50}\) = 8.37±0.21) exhibits significant inhibitory activity. These results (Table 2) when compared with the standard (IC\(_{50}\) = 4.18 ± 0.17) allopurinol showed significant (p<0.05) inhibitory activity. Flavonoids are a group of poly phenolic compound which has been proved to possess xanthine oxidase inhibitory activity (30). Thus the presence of flavonoids and phenolic in the crude extract would have contributed towards the inhibitory activity.

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEF</td>
<td>Allopurinol</td>
</tr>
<tr>
<td>2.5</td>
<td>29.18±0.24</td>
</tr>
<tr>
<td>5</td>
<td>39.80±0.18</td>
</tr>
<tr>
<td>10</td>
<td>59.91±0.17</td>
</tr>
<tr>
<td>20</td>
<td>68.50±0.21</td>
</tr>
<tr>
<td>40</td>
<td>81.22±0.18</td>
</tr>
<tr>
<td>IC(_{50})</td>
<td>8.37±0.21*</td>
</tr>
</tbody>
</table>

**Table 2.** *In vitro* xanthine oxidase inhibitory activity

**MEJ:** *J. beddomei* methanolic extract; All values determined were mean±SEM; n= 3; *P < 0.05 when compared with standard.
4. CONCLUSION

The current study has demonstrated that the methanolic extract of aerial parts of *Justicia beddomei* Linn. contained compounds capable of inhibiting XO and inducing cytotoxic effects on cell lines. Furthermore, these compounds might be helpful in treating various cancer conditions and resulting gouty arthritis. However, further investigations such as isolation of active compounds present in the extract and *in vivo* studies are necessary to identify the specific chemical entity for clinical use.

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REFERENCE


