GC–MS ANALYSIS AND IN-VITRO CYTOTOXIC ACTIVITY OF METHANOLIC EXTRACT OF ANTIGONON LEPTOPUS FLOWERS

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ABSTRACT: The present study has been designed to evaluate the bioactive compounds and cytotoxic activity from the n-hexane fraction of methanolic extract of Antigonon leptopus flowers. The n-hexane fraction was analysed by GC–MS (Thermo GC–TRACE ultra ver.: 5.0, Thermo MS DSQ II) which revealed the presence of many diversified compounds including flavonoids, Vitamin D2 derivative, carbohydrates, amino acids, quinazolines etc. All the compounds were identified from Wiley spectral library. Mass spectral data of 16 compounds is presented. The in vitro cytotoxic activity of n-hexane fraction of the methanolic extract was performed by MTT assay method against CHOK 1 cell line (Chinese Hamster Ovary cell line) and A-549 cell line (Human Lung adenocarcinoma epithelial cell line). Under the experimental conditions the cytotoxic property was not observed for n-hexane fraction. From the results it is evident that Antigonon leptopus flowers contain various medicinally active phytocomponents . The absence of cytotoxic activity against CHOK 1 cell line and A-549 cell line recommends the safety of the Antigonon leptopus flowers

INTRODUCTION: The value of natural products in the treatment of ailments is well-known. Amongst the various natural sources, plants are an important source of bioactive constituents, including anticancer, antifungal and antimicrobial drugs. More than 1000 plant species are known for their anticancer potential. The use of plant compounds as prototypes of new drugs has a historical and economic importance.

Some plants extracts were defined as effective in treating cancer, whose action is attributed to additional or synergistic effect of compounds present in the extract 1. In consequence, the cytostatic effect of the extract observed in tumor cells seems to be more effective than the effect of isolated and biologically active compounds 2.

Antigonon leptopus Hook (polygonaceae) is well known in India as garden creeper, picchi batani in Telugu. A. leptopus is a tender perennial vine which can easily grow to 30-40 ft. in length. The coral vine has attractive green heart shaped leaves 3. The coral vine is found in costal and Indian Ocean areas. The hot tea prepared from the aerial portion of this plant is used traditionally for the prevention and treatment of cough and flu-related...
pain. In Thailand, flour coated leaves and flowers of Antigonon leptopus, are served with noodles. The flowers are also used in omelets. Phenols, saponins, aminoacids, steroids, phytosterols, triterpenoids, sapogenis, tannins, xanthoprotein, carboxylic acid and coumarins were present in the methanolic extract of Antigonon leptopus flowers. Studies have also shown that Antigonon leptopus methanolic flower extract exhibited antibacterial, anti-thrombin, anti-inflammatory, anti-diabetic and lipid peroxidation inhibitory activities. But there is no report about the phytochemical analysis by GC-MS and cytotoxic activity on the methanolic flower extract of Antigonon leptopus. With this background the present study was aimed to explore the bioactive compounds and cytotoxic activity of methanolic flower extract of Antigonon leptopus.

Material and methods:
Plant material:
The fresh flowers of plant Antigonon leptopus were collected in November 2012 from local area of cherukupally, Bhogapuram, Vizianagaram Dist., Andhra Pradesh. The plant was identified and authenticated (Specimen Number BSI/DRC/2012-13/Tech/513) by Mr. P. V. Prasanna, Scientist ‘E’- In-charge, Botanical Survey of India, Deccan Regional Center, Hyderabad-500048, where a voucher specimen has been deposited.

Extraction of plant Material:
450g fresh flowers of the plant Antigonon leptopus were washed with distilled water to remove dust particles. The shade dried flowers were powdered. The ground fine powder (135g) of the flowers was extracted with absolute methanol (1liter) at room temperature (30°C) for three days. The extract was filtered through whatman no: 1 filter paper and then concentrated at 45°C using a rotary vacuum evaporator.

This process was repeated thrice to obtain sufficient quantity of absolute methanol extract. The methanol extract (10g) was dissolved in distilled water and then fractionation was performed by using different polarity based solvents and n-hexane (3g), chloroform (3g), ethyl acetate (2g) and n-butanol (2g) fractions were obtained. All these obtained extracts and fractions were stored at -4°C till further analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis:
The GC/MS analysis of n-hexane fraction of methanolic flower extract was performed on a GC-MS equipment (Thermo Scientific Co. Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II). Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 75°C raised to 250°C at a rise of 5°C/min, and held for 30min. The injection volume was 1 µl and sample was injected in split less mode. Finally the sample was run fully at a range of 50–650 m/z and the results were compared by using Wiley Spectral library search programme.

Evaluation of cytotoxic activity:
Chemicals:
3-(4, 5–dimethyl thiazol – 2 – yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco’s Modified Eagle’s Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics were obtained from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol were obtained from E.Merck Ltd., Mumbai, India.

Cell lines and Culture medium:
A-549 (Human Lung adenocarcinoma epithelial cell line) and CHOK 1 (Chinese Hamster Ovary cell line) cell lines were procured from National Centre for Cell Sciences (NCCS) Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 g/ml) and amphotericin B (5 g/ml) in humidified atmosphere of 5% CO2 at 37°C until confluent.

The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).
Preparation of test solution:
For Cytotoxicity studies, weighed test extracts were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay:
The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100µl of different test concentrations of test extracts were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, and microscopic examination was carried out and observations were noted in every 24h interval. After 72h, the sample solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well.

The plates were gently shaken and incubated for 3h at 37°C in 5%CO₂ atmosphere. The supernatant was removed and 100µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm. The percentage growth inhibition was calculated using the following formula and concentration of test sample needed to inhibit cell growth by 50% (CTC₅₀) values is generated from the dose-response curves for each cell line.

\[
\text{Growth inhibition(\%)} = \left( \frac{\text{Control Absorbance} - \text{Treated Absorbance}}{\text{Control Absorbance}} \right) \times 100
\]

RESULTS AND DISCUSSIONS:
Chemical composition of n-hexane fraction:
The results pertaining to GC-MS analysis of the n-hexane fraction from methanolic flower extract of A. leptopus lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC (Fig.1). The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The major components present in the n-hexane fraction of A. leptopus detected by the GC-MS (shown in Table 1) are O acetyl-L-serine, cyclopenta siloxane decamethyl, 1,3-bis (4 - chlorobenzyl) – 5 ,6 dihydro benzo [f] quinazoline, tris trimethyl silyl ether derivative of 1, 25 - dihydroxy vitamin D₂, alpha – 1 - rhamno pyranose, 4H-pyran – 4 – one, 2, 3 – dihydro – 3, 5 – dihydroxy – 6 – methyl, 1,2-Benzenediol, tetradecamethyl cyclo heptasiloxane; 7- [(Methoxy)iminomethyl] –furo [3,2-c] pyridine; Quercetin7, 3’4’trimethoxy; 5,5- Dimethyl-1-oxa-5-silacyclononanone-9; (1R,3R, 4R,5R) - Quinicacid; 1,2,3,5-Cyclohexanetetrol, (1à,2à,3à, 5à)-; benzo[b] thiophene-1,4-dione, 5-Propylindo lizidin-7-ol; Friedelan-3 one.

**FIG. 1: GC-MS ANALYSIS OF THE n-HEXANE FRACTION FROM METHANOLIC FLOWER EXTRACT OF A. LEPTOPUS**
TABLE 1: CHEMICAL COMPOSITION OF A. LEPTOPUS FLOWERS n-Hexane Fraction Analysed by GC/MS

<table>
<thead>
<tr>
<th>Sl no</th>
<th>RT</th>
<th>Compound name</th>
<th>Compound nature</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.21</td>
<td>o-acetyl-L-serine</td>
<td>amino acid</td>
<td>C₆H₁₂NO₂</td>
<td>147</td>
<td>0.58</td>
</tr>
<tr>
<td>2</td>
<td>4.92</td>
<td>cyclopentasiloxane, decamethyl-1,6-</td>
<td>siloxane</td>
<td>C₁₀H₃₁O₂Si₅</td>
<td>370</td>
<td>0.87</td>
</tr>
<tr>
<td>3</td>
<td>7.22</td>
<td>1,3-bis(4-chlorobenzyl)-5,6-</td>
<td>quinazoline</td>
<td>C₂₆H₂₀C₁₃N₂</td>
<td>430</td>
<td>4.62</td>
</tr>
<tr>
<td>4</td>
<td>7.89</td>
<td>Tris trimethyl silyl ether derivative</td>
<td>vitamin D₂</td>
<td>C₃₇H₆₀O₂Si₃</td>
<td>644</td>
<td>0.40</td>
</tr>
<tr>
<td>5</td>
<td>8.22</td>
<td>alpha-L-rhamnopyranose</td>
<td>carbohydrate</td>
<td>C₆H₁₂O₃</td>
<td>164</td>
<td>1.58</td>
</tr>
<tr>
<td>6</td>
<td>8.52</td>
<td>4H-pyran-4-one, 2,3-dihydroxy-3,5-</td>
<td>ketone</td>
<td>C₆H₄O₄</td>
<td>144</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-methyl-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9.07</td>
<td>1,2-Benzene diol</td>
<td>Alcohol</td>
<td>C₆H₁₂O₂</td>
<td>110</td>
<td>2.79</td>
</tr>
<tr>
<td>8</td>
<td>9.34</td>
<td>Tetradecamethyl cyclohexa siloxane</td>
<td>siloxane</td>
<td>C₁₄H₂₆O₂Si₇</td>
<td>518</td>
<td>2.78</td>
</tr>
<tr>
<td>9</td>
<td>11.06</td>
<td>7-(Methoxy)iminomethyl]-furo[3,2-c]pyridine</td>
<td>heterocyclic</td>
<td>C₆H₈N₂O₂</td>
<td>176</td>
<td>2.31</td>
</tr>
<tr>
<td>10</td>
<td>12.45</td>
<td>Quercetin 7,3',4'-trimethoxy</td>
<td>benzyopyran</td>
<td>C₁₇H₁₆O₇</td>
<td>344</td>
<td>0.40</td>
</tr>
<tr>
<td>11</td>
<td>16.31</td>
<td>5,5-Dimethyl-1-oxa-5-silacyclonanone-9</td>
<td>silyl compound</td>
<td>C₃₀H₂₀Si</td>
<td>186</td>
<td>2.46</td>
</tr>
<tr>
<td>12</td>
<td>19.64</td>
<td>(1R,3R,4R,5R)-(-)-Quinic acid</td>
<td>Carboxylic acid</td>
<td>C₃₃H₂₂O₄</td>
<td>192</td>
<td>1.96</td>
</tr>
<tr>
<td>13</td>
<td>20.36</td>
<td>1,2,3,5-Cyclohexatetrol, (1,2,3,5a)-</td>
<td>alcohol</td>
<td>C₃₂H₂₂O₄</td>
<td>148</td>
<td>55.39</td>
</tr>
<tr>
<td>14</td>
<td>24.14</td>
<td>benzo[b]thiophene-1,4-dione</td>
<td>hydro cyclic</td>
<td>C₃₂H₂₂O₂S</td>
<td>164</td>
<td>0.71</td>
</tr>
<tr>
<td>15</td>
<td>30.64</td>
<td>5-Propylindolizinid-7-ol</td>
<td>aromatic</td>
<td>C₁₈H₁₇NO</td>
<td>183</td>
<td>0.71</td>
</tr>
<tr>
<td>16</td>
<td>38.17</td>
<td>Friedelan-3-one</td>
<td>triterpene</td>
<td>C₃₀H₅₀O</td>
<td>426</td>
<td>5.37</td>
</tr>
</tbody>
</table>

RT= Retention time (min).

**In-vitro cytotoxic activity:**
Through the MTT method, the median cytotoxic concentration (CC₅₀) on CHOK 1 cell line (Chinese Hamster Ovary cell line), and A-549 cell line (Human Lung adenocarcinoma epithelial cell line) were established for n-hexane fraction of methanolic extract of A. leptopus flowers. There was a gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (12.38, 17.65, 22.84, 27.67 and 31.68 % for the concentrations 62.5, 125, 250, 500 and 1000µg/ml respectively) against A-549 cell line (Fig. 3). The mean value of CC₅₀ observed for A-549 cells was >1000±0.00 and there was no significant activity.

A similar observation was made on A-549 cell line (Human Lung adenocarcinoma epithelial cell line). There was gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (12.38, 17.65, 22.84, 27.67 and 31.68 % for the concentrations 62.5, 125, 250, 500 and 1000µg/ml respectively) against A-549 cell line (Fig. 3). The mean value of CC₅₀ observed for A-549 cells was >1000±0.00 and there was no significant activity.

**CONCLUSION:** The GC MS analysis report has shown that A.leptopus flowers contain various bioactive compounds like flavonoids, amino acids,
carbohydrates, siloxanes, pyrones, terpenoids, Quinazolines, Vitamin D₂ etc. O-acetyl-L-serine is a cysteine precursor. Siloxanes are useful in cosmetic preparations. Rhamnose is a common glycone component of glycosides from many plants. Friedelan-3 one exhibits antihypertensive and antihyperlipidemic activity. The isolation of pure phytochemical constituents and subjecting them to the screening of biological activity will be definitely giving fruitful results and will open a new area of investigation of individual components and their pharmacological potency.

Due to the presence of various phytochemical components, and the established medicinal uses of the flowers, we thought of screening the extract for cytotoxic properties. The n-hexane fraction of methanolic extract of A. leptopus flowers showed no cytotoxicity against Chinese Hamster Ovary cell line and Human Lung adenocarcinoma epithelial cell line. On the basis of the present study, it might be suggested that the flowers of Antigonon leptopus have no significant cytotoxic activity giving a preliminary indication of safety of the flowers for their intake in food. Evaluation of other pharmacological activities is under progress.

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REFERENCES: