



Received on 26 November, 2014; received in revised form, 23 January, 2015; accepted, 27 March, 2015; published 01 July, 2015

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

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### Keywords:

Antigonon leptopus flowers, GC-MS profiling, cytotoxic activity, CHOK 1 (Chinese Hamster Ovary cell line), A-549 (Human Lung adenocarcinoma epithelial cell line).

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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 D<sub>2</sub> 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 phytochemicals. 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<sup>1</sup>. 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<sup>2</sup>.

*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<sup>3</sup>. The coral vine is found in coastal 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

<b>QUICK RESPONSE CODE</b> 	<b>DOI:</b> 10.13040/IJPSR.0975-8232.6(7).3083-87
	Article can be accessed online on: <a href="http://www.ijpsr.com">www.ijpsr.com</a>
<b>DOI link:</b> <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.6(7).3083-87">http://dx.doi.org/10.13040/IJPSR.0975-8232.6(7).3083-87</a>	

pain<sup>4</sup>. In Thailand, flour coated leaves and flowers of *A.leptopus*, are served with noodles. The flowers are also used in omelets<sup>5</sup>. Phenols, saponins, aminoacids, steroids, phytosterols, triterpenoids, sapogenis, tannins, xanthoprotein, carboxylic acid and coumarins were present in the methanolic extract of *A. leptopus* flowers. Studies have also shown that *A. leptopus* methanolic flower extract exhibited antibacterial<sup>6</sup>, anti-thrombin<sup>7</sup>, anti inflammatory<sup>8</sup>, anti-diabetic<sup>9</sup> and lipid peroxidation inhibitory activities<sup>10</sup>. But there is no report about the phytochemical analysis by GC-MS and cytotoxic activity on the methanolic flower extract of *A. leptopus*. With this background the present study was aimed to explore the bioactive compounds and cytotoxic activity of methanolic flower extract of *A. 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<sup>0</sup>C) for three days. The extract was filtered through whatman no: 1 filter paper and then concentrated at 45<sup>0</sup>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<sup>0</sup>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% CO<sub>2</sub> at 37<sup>0</sup>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 cm<sup>2</sup> 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  $1.0 \times 10^5$  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% CO<sub>2</sub> 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%CO<sub>2</sub> 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<sup>12</sup>. The percentage growth inhibition was

calculated using the following formula and concentration of test sample needed to inhibit cell growth by 50% (CTC<sub>50</sub>) values is generated from the dose-response curves for each cell line<sup>13</sup>.

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

## 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<sup>11</sup>. 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 D<sub>2</sub>, 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-silacyclononane-9; (1R,3R, 4R,5R)-(-)-Quinicacid; 1,2,3,5-Cyclohexanetetrol, (1à,2á,3â, 5â)-; benzo[b] thiophene-1,4-dione, 5-Propylindolizidin-7-ol; Friedelan-3 one.

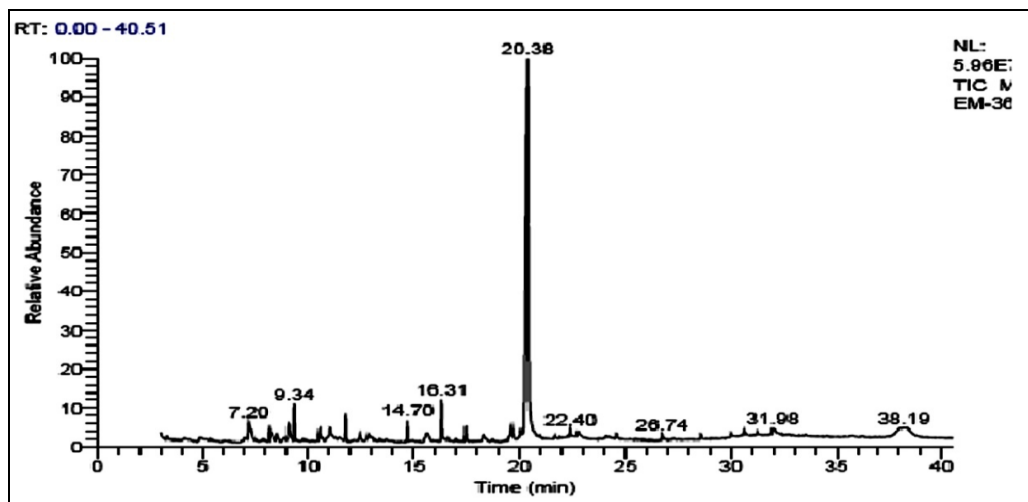


FIG. 1: GC-MS ANALYSIS OF THE n-HEXANE FRACTION FROM METHANOLIC FLOWER EXTRACT OF *A. LEPTOPUS*

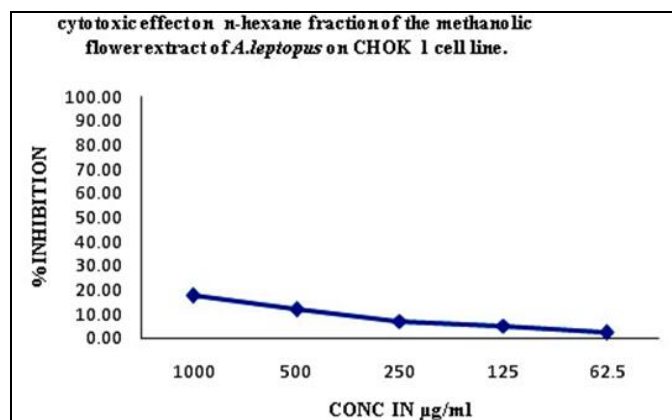
**TABLE 1: CHEMICAL COMPOSITION OF A. LEPTOPUS FLOWERS n-HEXANE FRACTION ANALYSED BY GC/MS**

Sl. no	RT	Compound name	Compound nature	Molecular formula	Molecular weight	Area (%)
1	4.21	o-acetyl-L-serine	amino acid	C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub>	147	0.58
2	4.92	cyclopentasiloxane, decamethyl-	siloxane	C <sub>10</sub> H <sub>30</sub> O <sub>5</sub> Si <sub>5</sub>	370	0.87
3	7.22	1,3-bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazoline	quinazoline	C <sub>26</sub> H <sub>20</sub> C <sub>1.2</sub> N <sub>2</sub>	430	4.62
4	7.89	Tris trimethyl silyl ether derivative of 1,25-dihydroxyvitamin D <sub>2</sub>	vitamin D <sub>2</sub>	C <sub>37</sub> H <sub>68</sub> O <sub>3</sub> Si <sub>3</sub>	644	0.40
5	8.22	alpha-l-rhamnopyranose	carbohydrate	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	164	1.58
6	8.52	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	ketone	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	1.08
7	9.07	1,2-Benzene diol	Alcohol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	110	2.79
8	9.34	Tetradecamethyl cyclohepta siloxane	siloxane	C <sub>14</sub> H <sub>42</sub> O <sub>7</sub> Si <sub>7</sub>	518	2.78
9	11.06	7-[(Methoxy)iminomethyl]-furo[3,2-c]pyridine	heterocyclic	C <sub>9</sub> H <sub>8</sub> N <sub>2</sub> O <sub>2</sub>	176	2.31
10	12.45	Quercetin 7,3',4' trimethoxy	benzopyran	C <sub>18</sub> H <sub>16</sub> O <sub>7</sub>	344	0.40
11	16.31	5,5-Dimethyl-1-oxa-5-silacyclononanone-9	silylcompound	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub> Si	186	2.46
12	19.64	(1R,3R,4R,5R)-(-)-Quinic acid	Carboxylic acid	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	192	1.96
13	20.36	1,2,3,5-Cyclohexanetetrol, (1à,2à,3à,5à)-	alcohol	C <sub>6</sub> H <sub>12</sub> O <sub>4</sub>	148	55.39
14	24.14	benzo[b]thiophene-1,4-dione	heterocyclic	C <sub>8</sub> H <sub>4</sub> O <sub>2</sub> S	164	0.71
15	30.64	5-Propylindolizidin-7-ol	aromatic	C <sub>11</sub> H <sub>21</sub> NO	183	0.71
16	38.17	Friedelan-3-one	triterpene	C <sub>30</sub> H <sub>50</sub> O	426	5.37

RT= Retention time (min).

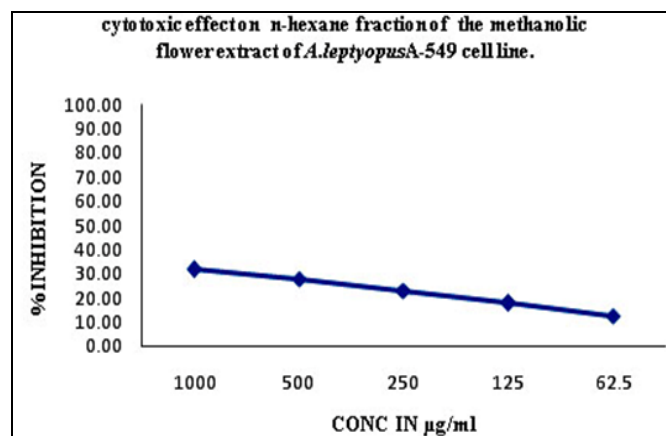
### In-vitro cytotoxic activity:

Through the MTT method, the median cytotoxic concentration (CC<sub>50</sub>) 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 (2.30, 4.95, 7.15, 11.75 and 17.59% for the concentrations 62.5, 125, 250, 500 and 1000 µg/ml respectively) against CHOK 1 cell line (Fig. 2). The mean value of CC<sub>50</sub> observed for CHOK 1 cells was (p) >1000±0.00 and there was no significant activity.



**FIG. 2: CYTOTOXIC EFFECT ON N- HEXANE FRACTION OF THE METHANOLIC FLOWER EXTRACT OF A. LEPTYOPUS CHOK 1 CELL LINE**

A similar observation was made on A-549 cell line (Human Lung adenocarcinoma epithelial cell line). 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 1000 µg/ml respectively) against A-549 cell line (Fig. 3). The mean value of CC<sub>50</sub> observed for A-549 cells was >1000±0.00 and there was no significant activity.



**FIG. 3: CYTOTOXIC EFFECT ON N- HEXANE FRACTION OF THE METHANOLIC FLOWER EXTRACT OF A. LEPTYOPUS A-549 CELL LINE**

**CONCLUSION:** The GC MS analysis report has shown that *A. leptopus* flowers contain various bio-active compounds like flavonoids, amino acids,

carbohydrates, siloxanes, pyrones, terpenoids, Quinazolines, Vitamin D<sub>2</sub> 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.

**ACKNOWLEDGEMENT:** The authors are thankful to the Management of Avanthi institute of pharmaceutical sciences, Cherukupally, Vizianagaram Dist, Andhra Pradesh, India for availing all the facilities and are also thankful to Radiant Research Services Pvt. Ltd, Srinagar, Bangalore-560 050, India, for carrying out GC-MS analysis of the sample.

## REFERENCES:

1. Li, X.K., Motwani M., Tong, W., Bornmann, W., Schwartz, G.K.: Huanglian, a Chinese herbal extract, inhibits cell growth by suppressing the expression of

- cyclin B1 and inhibiting CDC2 kinase activity in human cancer cells. *Molecular Pharmacology*. 2000; 58, 1287–1293
2. Vickers, A.: Botanical medicines for the treatment of cancer: rationale, overview of current data, and methodological considerations for phase I and II trials. *Cancer Investigation*. 2002; 20, 1069–1079.
3. Ram P. Rastogi and Mehrotra, BN.: *Compendium of Indian Medicinal Plants*, CDRI and NISC, New Delhi, 1998: 52.
4. Mitchell. S. A. and M. H. Ahmad.: A review of medicinal plant research at the University of the West Indies, Jamaica, *West Indian Medicinal Journal*. 2006 55 2: 243–253.
5. Charmaine S.: *Asia food*. Encyclopedia of Asian food (Periplus editions) Australia: New Holland Publishers Pty Ltd. 1998.
6. Praveen Kumar Bhogavalli, Rama Rao. Bandlamuri, Jyothirmai. Jagarlamu, Gupta A.V.N.: Studies on antimicrobial activity of flower extracts of *Antigonon leptopus* against common dental pathogens, *Scholars Research Library ,Annals of Biological Research*. 2011; 2(2) : 99-103.
7. Chistokhodova N, C. Nguyen, T. Calvino, I. Kachirskaia, G.Cunningham, D. H. Miles.: Antithrombin activity of medicinal plants from central Florida, *Journal of Ethnopharmacology*. 2002; 81 2: 277–280.
8. W. C. Mamidipalli, V. R. Nimmagadda, R. K. Bobbala, K. M. Gottumukkala.: Preliminary studies of analgesic and anti-inflammatory properties of *Antigonon leptopus* Hook. et Arn roots in experimental models, *Journal of Health Science*. 2008; 54 3: 281–286.
9. Sujatha S, Swaroopa Rani V, Ravikumar B.: Antidiabetic Effect of Flower Extract of *Antigonon leptopus* Hook & Arn in Alloxan-induced Diabetic Rats, *Indian Journal of Pharmaceutical Education and Research*. 2011; 461: 9-16.
10. Vanisree M, Ruby L., Alexander-Lindo, D. L. DeWitt, M.G. Nair.: Functional food components of *Antigonon leptopus* tea, *Food Chemistry*. 2008; 106, 487–492.
11. Harborne JB.: *Phytochemical methods-A guide to modern techniques of plant analysis*. Chapman and Hall, London 1998.
12. Francis D, Rita L.: Rapid colorimetric assay for cell growth & survival: modification to the tetrazolium dye procedure giving improved sensitivity and reliability, *Journal of immunological methods*. 1986 89,271-277.
13. Ali H. Ad'hiah, Orooba N.H.AL-Bederi, Khulood W.AL-Sammarrae.: Cytotoxic effects of *Agrimonia eupatoria* L. against cancer cell lines in vitro. *Journal of the association of Arab Universities for basic and applied sciences*. 2013 14, 87-92.

### How to cite this article:

Prajna SS, Priya KS, Swarnalatha S, Bhojaraju P, Kanthal LK and Satyavathi K: GC-MS Analysis and *In-Vitro* Cytotoxic Activity of Methanolic Extract of *Antigonon Leptopus* Flowers . *Int J Pharm Sci Res* 2015; 6(7): 3083-87.doi: 10.13040/IJPSR.0975-8232.6(7).3083-87.

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