



***In-vitro* cytotoxic activity and anthelmintic effect of chloroform extract of *Antigonon leptopus* Hook & Arn**

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ABSTRACT

*The present study was designed to evaluate the in-vitro cytotoxic activity and anthelmintic effect of chloroform extract of *Antigonon leptopus* Hook & Arn. Cytotoxicity was determined using 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay and IC₅₀ (inhibit cell growth by 50%) was calculated against A-549 (Human lung adenocarcinoma epithelial cell line) and CHOK-1 (Chinese Hamster Ovary cell line) cell lines. Different concentrations (25, 50, 100 mg/ml) of chloroform extract were used for anthelmintic activity against *Pheretima posthuma*. Effect of inhibition of cell growth showed significantly cytotoxicity against CHOK 1 cell line (Chinese Hamster ovary cell line) with an IC₅₀ (inhibit cell growth by 50%) of >1000 ± 0.00 µg/ml and against and also A-549 cell line (Human Lung adenocarcinoma epithelial cell line) with an IC₅₀ of >1000 ± 0.00. The results obtained from the study indicate significant anthelmintic activity against *Pheretima posthuma*. In conclusion, the present plant *Antigonon leptopus* can be considered as an important source of natural products that have potent anthelmintic activity, due to presence of various phytochemical components but it's not having significant cytotoxic or anticancer activity on cell lines.*

Keywords: *Antigonon leptopus*, Cytotoxicity, Anthelmintic activity, *Pheretima posthuma*.

INTRODUCTION

Natural products have important roles in protecting humans from different diseases throughout the world. Several classes of anticancer agents have been developed and many of them are from natural origin. However, a major problem in the use of these agents in cancer treatment is the undesirable side effects produced as a result of non-tumour specificity and multiple-drug resistance. Therefore in cancer research, traditional medicine has aroused renewed interest in the search for safe, potent and selective anticancer compounds [1]. The World Health Organization (WHO) has classified cancer with non-communicable diseases, which are responsible for 63% of deaths worldwide [2]. The World Bank income groups predictable that the incidence of 12.7 million new cancer cases in 2008 [3] will rise to 21.4 million by 2030, and low or middle-income countries will be the most affected with nearly two thirds of all cancer diagnoses [4, 5].

From the ancient times, indigenous drugs have been used in the Indian medicinal system to treat different ailments and to provide therapeutic benefits. During the recent years, medicinal chemistry has made great strides, especially in synthetic chemistry but, for the sake of therapeutic effect up to the level and nontoxic treatment for helminthiasis,

the research of plant derived drug therapy is mostly needed [6]. Anthelmintic or antihelminthics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them [7].

Antigonon leptopus, usually known as Mexican Creeper or coral vine, is a species of flowering plant in the buckwheat family, Polygonaceae. Traditionally the leaves of *A. leptopus* have been used to reduce swelling, and a tea from the leaves can be made to treat diabetes and the blossoms are used to treat high blood pressure. It possesses anti-coagulant activity, analgesic, anti-thrombin, anti-inflammatory activity, anti-diabetic and lipid peroxidation inhibitory activities, anti-helminthic activity and anti-convulsant activity [8].

A detailed literature review on the plants in investigation has shown that so far there are no published reports worldwide, related to our research work. Hence, in the present study, we were interested in carrying out an *in-vitro* cytotoxic activity and anthelmintic effect of chloroform extract of *Antigonon leptopus* Hook & Arn.

MATERIALS AND METHODS

Collection of Plant Materials

The whole plant of *Antigonon leptopus* Hook & Arn were collected from local areas of Korangi, Kakinada, Andhra Pradesh. The plant was identified and authenticated by Mr.P.V.Prasanna, Scientist -'E'-incharge, Botanical Survey of India, Deccan regional center, Hyderabad, Telengana, where a voucher specimen has been deposited.

Extraction of Plant Material

350g whole plant parts of *Antigonon leptopus* were shade dried at room temperature, powdered and passed through 60 mesh size sieves. 200gms of powdered plant parts were weighed accurately and extracted with 1320 ml chloroform solvent using cold maceration method. Thus obtained extract were filtered through Whatman No.1 filter paper was concentrated. The extract (1.8 g) were transferred to sterile screw cap bottles, labeled and stored in refrigerator until use.

Phytochemical Evaluation

The chloroform extract was tested for carbohydrates, amino acids, proteins, fixed oils, alkaloids, glycosides, flavonoids, tannins, saponins, volatile oils, steroids [9-15].

Cytotoxic activity

Chemicals

The chemicals used in *in-vitro* cytotoxic activity are 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin which were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol obtained from E.Merck Ltd., Mumbai, India.

Cell lines and Culture medium

The two selected cancer cell lines used in this research were derived from 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 µg/ml) and amphotericin B (5 µg/ml) in a 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 1 mg/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 *in-vitro* cytotoxic activity was determined using MTT (3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay to measure cell viability.

The monolayer cell culture was trypsinized and the cell count was attuned to 1.0×10^5 cells/ml using DMEM containing 10% FBS. Every well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hrs, when a partial monolayer was produced, the supernatant was flicked off, the monolayer was washed once with medium and 100 μ l of different test concentrations of extracts were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 $^\circ$ C for 3 days in 5% CO₂ atmosphere, and microscopic examination was carried out and observations were noted in every 24 h interval. After 72 h, the sample solutions in the wells were discarded and 50 μ l of MTT in PBS was added to each well. The plates were lightly shaken and incubated for 3 h at 37 $^\circ$ C in 5% CO₂ atmosphere. The supernatant was removed and 100 μ l of propanol was added and the plates were smoothly shaken to solubilize the formed formazan. The absorbance was calculated using a microplate reader at a wavelength of 540 nm [16]. The percentage growth inhibition was calculated by using the following formula and concentration of test sample needed to inhibit cell growth by 50% (IC₅₀) was generated from the dose-response curves for each cell line [17].

$$\% \text{ Growth Inhibition} = 100 - \{(\text{Mean OD of individual test group} / \text{Mean OD of control group}) \times 100\}$$

Anthelmintic activity

The anthelmintic effect was performed according to the standard methods on the adult Indian earthworm *Pheritima posthuma* [18, 19]. The standard drug albendazole was diluted with normal saline solution and prepared the four concentrations of standard drug sample 25, 50 and 100 mg/ml concentrations which were poured into the petridishes. The chloroform extract of *Antigonon leptopus* was diluted with normal saline solution to achieve 25, 50 and 100 mg/ml concentrations. Normal saline solution (0.9% NaCl) alone used as the negative control. All these dilutions were poured into the petridishes consequently. The equal size of ten petridishes were taken & numbered. After that, similar size (about 8 cm) of six earthworms (n=6) were placed in every petridish at room temperature. Then the paralysis and death (lethal) time observed and noted down from all petridishes. The paralysis time and lethal time were recorded in terms of minutes. The experiments were performed in triplicate.

The anthelmintic screening was followed by the investigation time of paralysis and death occurs in earthworm which observed the time taken for paralysis was noted when no movement or loss of movement (Not retrieve even in normal saline) of earthworms and death time was recorded if the earthworms not having any movement after shaking forcefully and also dipped in 50 $^\circ$ C warm water and also fading away the colour of worm.

RESULT AND DISCUSSION

Phytochemical evaluation

The preliminary phytochemical study revealed that chloroform extract of *Antigonon leptopus* contains carbohydrates, amino acids, proteins, fixed oils, alkaloids, glycosides, flavonoids, tannins, saponins, steroids, and volatile oils.

Cytotoxic activity

Through the MTT method, the median cytotoxic concentration (IC₅₀) on CHOK 1 cell line (Chinese Hamster ovary cell line) and A-549 cell line (Human Lung adenocarcinoma epithelial cell line) were established for chloroform extract of *Antigonon leptopus*. There was 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, 17.59 % for the concentrations 62.5, 125, 250, 500, 1000 μ g/ml, respectively) against the CHOK 1 cell line (Figure 1). The median value of IC₅₀ observed for CHOK 1 cell was $>1000 \pm 0.00$. 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, 31.68, % for the concentrations 62.5, 125, 250, 500, 1000 μ g/ml, respectively) against A-549 cell line (Figure 2). The median value of IC₅₀ observed for A-549 cells was $>1000 \pm 0.00$.

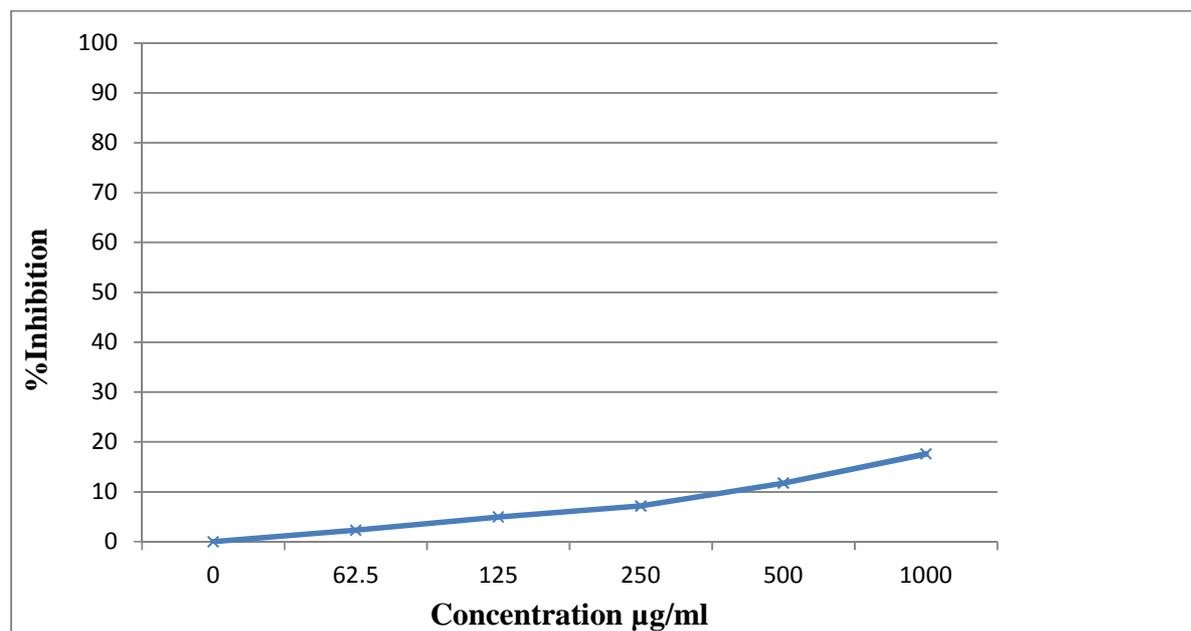


Figure 1: Cytotoxic activity of *Antigonon leptopus* on CHOK-1 cell line

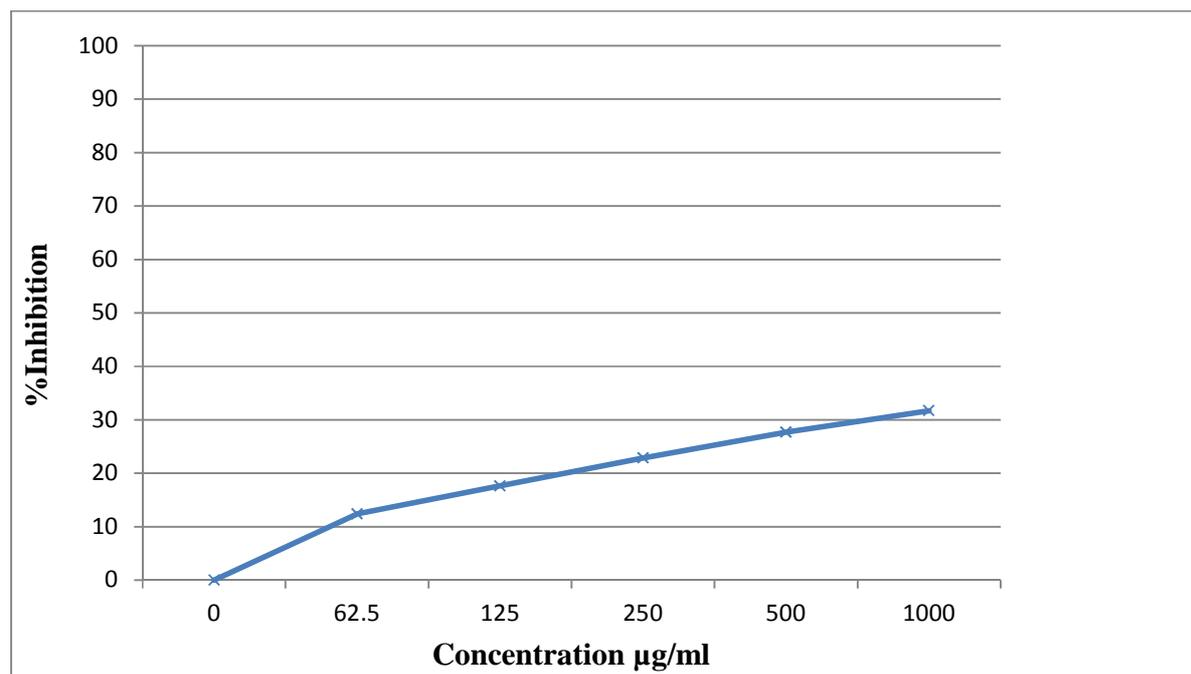


Figure 2: Cytotoxic activity of *Antigonon leptopus* on A-549 cell line

Anthelmintic Activity

The result show that for the 25mg/ml concentration, Mebendazole showed the best activity for death time (96.5 ± 2.29 min) and the chloroform extract of *Antigonon leptopus* showed a death time of (75.33 ± 2.08 min) Also, for the 50mg/ml concentration, Mebendazole showed the highest activity against the worms (76.16 ± 2.3 min) and the chloroform extract of *Antigonon leptopus* showed a death time of (63.0 ± 2.0 min). For the 100 mg/ml concentration, Mebendazole showed the least death time (64.83 ± 2.2 min) and the chloroform extract of *Antigonon leptopus* showed a death time of (45 ± 2.0 min). The paralysis and death times of the plant along with the standard is given in

Table 1. The study revealed that the chloroform extract of *Antigonon leptopus* had significant anthelmintic activity (moderate) at the higher concentration (100mg/ml).

Table 1: *In-Vitro* Anthelmintic Effect of *Antigonon leptopus* against *Pheritima Posthuma*

Treatment	Concentration (mg/ml)	Paralysis time (min)	Deathtime (min)
Mebendazole (standard)	25	76.8 ± 2.2	96.5 ± 2.29
	50	64.6 ± 2.5	76.1 ± 2.30
	100	46.0 ± 2.0	64.8 ± 2.20
Chloroform extract of <i>Antigonon leptopus</i>	25	58.0 ± 2.0	75.3 ± 2.08
	50	45.0 ± 2.0	63.0 ± 2.00
	100	32.3 ± 3.0	45.0 ± 2.00
Control	-	-	-

±SD value, n=3.

CONCLUSION

In conclusion, the present plant *Antigonon leptopus* can be considered as an important source of natural products that have potent anthelmintic activity, due to presence of various phytochemical components but it's not having significant cytotoxic or anticancer activity on cell lines and it is too early to reach a final conclusion and further investigations are required to include further cell lines and worms, respectively.

Future scope demands that there is a need for the isolation of the constituents responsible for the pharmacological action and to screen the exact mechanism of action for the curative purpose.

Acknowledgement

The authors are grateful to the management of Koringa College of Pharmacy, Korangi, East Godavari Dist., Andhra Pradesh, India for making available all the facilities.

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