



Anticancer activity of methanolic leaf extracts of *Andrographis paniculata* (Nees) and *Cardiospermum halicacabum* (Linn) against human breast cancer cell line (MCF-7)

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Abstract

Crude methanolic extracts of *Andrographis paniculata* and *Cardiospermum halicacabum* leaves were examined for their anticancer activity. To determine anticancer activity, different concentrations of crude extracts were tested on MCF-7, a human breast adenocarcinoma cell-line by 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. *A. paniculata* extracts showed a significant antiproliferative activity than *C. halicacabum* and a dose dependent effect was observed. Minimum inhibition of 9.47% was shown by extract at concentration 31.25µg/ml and maximum inhibition of 99% was observed at 500µg/ml for *A. paniculata* and Minimum inhibition of 3.18% was shown by extract at concentration 62.5µg/ml and maximum inhibition 79.60% were observed at 500µg/ml for *C. halicacabum*. These results indicate the possible potential use of *A. paniculata* as Antineoplastic agent. Preliminary phytochemical screening revealed the presence of Flavonoids, Steroids, Terpenoids, Alkaloids, Glycosides and Tannins in the extracts of *Andrographis paniculata* and *Cardiospermum halicacabum*. The presence of these chemical constituents may be the reason for the anticancer activity.

Key-Words: *Andrographis paniculata*, *Cardiospermum halicacabum*, MCF-7, Antineoplastic

Introduction

The plants are the natural reservoir of medicinal agents almost free from the side effects normally caused by synthetic chemicals. The World Health Organization (WHO) estimates that herbal medicine is still the main stay of about 75-80% of the world's population, mainly in the developing countries for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side-effects. The over-use of synthetic drugs with impurities resulting in higher incidence of adverse drug reactions has motivated mankind to go back to nature for safer remedies. Due to varied locations where these plants grow, coupled with the problem of different vernacular names, the WHO published standards for herbal safety to minimize adulteration and abuse (2).

Herbal cancer therapy comprises a number of alternative treatments in the fight against cancer. As early as more than 3,000 years ago, Chinese doctors had used herbs in treatment of cancer patients. Ayurveda is an ancient Indian herbal medicine system and is followed till date for anticancer treatment.

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This medicinal system is also proven to be at par with traditional Chinese Medicinal System. Currently, there are numerous herbal databases that provide information on herbal anticancer compounds (9).

Material and Methods

Collection of plant materials

The leaves of *Cardiospermum halicacabum* and *Andrographis paniculata* plants were collected from Coimbatore district and dried in shade. These leaves were then powdered and stored in air tight container at room temperature until further use.

Preparation of plant extract

10g of air dried powder were taken in 100mL of methanol. Plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hours. After 24hours the supernatant were collected and the solvent were evaporated to make the final volume one-fourth of the original volume and stored at 4⁰c in air tight container.

Qualitative determination of phytochemicals

Qualitative analysis of the leaves and roots of *C. halicacabum* was carried out systematically to identify the phytochemicals like Alkaloids (6), Flavonoids (1),

Glycosides (6), Steroids (3), Tannins (8) and Terpenoids (7).

***In vitro* Anti cancer activity**

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x10⁵ cells/mL. one hundredmicrolitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. After 24hrs the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100µl of these different sample dilutions were added to the appropriate wells already containing 100µl of medium, resulted the required final sample concentrations. Following the plates were subjected to different drug concentration and incubated for an additional 48hrs at 37°C, 5% CO₂, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations (5).

MTT assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48hrs of incubation, 15µl of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4hrs. The medium with MTT was then flicked off and formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % of cell inhibition was determined using the following formula (4).

$$\% \text{ cell Inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100.$$

Nonlinear regression graph was plotted between % Cell inhibition and Log₁₀ concentration and IC₅₀ was determined using GraphPad Prism software

Results and Discussion

Phytochemical Analysis

The powdered leaves of *Andrographis paniculata* was extracted using methanol solvent. The extract obtained was subjected to preliminary phytochemical screening for the presence of secondary metabolites such as Alkaloids, Terpenoids, Tannins, Saponins, Flavanoids and Steroids. The phytochemical screening for the Methanolic leaf extracts of *Andrographis paniculata* (Table-1) showed the presence of Alkaloids, Terpenoids, Tannins, Saponins, Flavanoids and Steroids. All the parameters tested were found to be positive and confirmed the presence of all the above compounds.

The methanolic leaf extract of *Cardiospermum halicacabum* was also screened for the presence of various chemical constituents (Table-1). The study confirmed the presence of compounds such as Alkaloids, Terpenoids, Tannins, Saponins, Flavanoids and Steroids. The similar results has been reported by Sagadevan *et.al.* (2012) in *Andrographis paniculata*. Further Suresh *et.al.* (2012) reported the presence of Alkaloids, Flavanoids, Glycosides, Steroids, Tannins and Terpenoids in *Cardiospermum halicacabum*. There are numerous reports available in literature regarding the role of these chemicals (Secondary metabolites) which plays an important role in ailments to various diseases from common cold to life threatening. Innumerable reports have been suggesting the possible cure in various healthcare systems also.

Table 1: The phytochemical analysis of methanolic leaf extract of *Andrographis paniculata* and *Cardiospermum halicacabum*

Test	<i>Andrographis paniculata</i>	<i>Cardiospermum halicacabum</i>
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Steroids	+	+
Tannins	+	+
Terpenoids	+	+

+ = Present, - = Absent

Anticancer studies

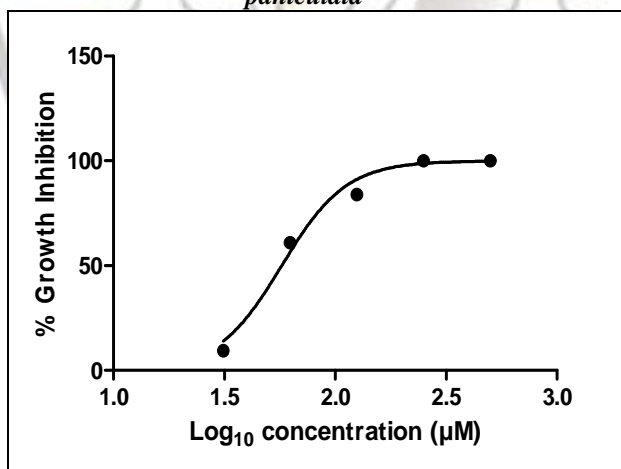
The methanolic leaf extracts of *Andrographis paniculata* and *Cardiospermum halicacabum* were tested for their anticancer potential against MCF-7, breast cancer cell line. The extracts of *Andrographis paniculata* and *Cardiospermum halicacabum* were found to inhibit the growth of MCF-7, breast cancer cell lines (Table-2 & 3, Figure- 1 & 2). The methanolic leaf extract of *Andrographis paniculata* showed a remarkable inhibition in the maximum concentrations of 250 & 500µg/mL to an extent of 99% of cell growth. The lower concentration of the extract 31.25µg/mL showed 9.46%, 62.5µg/mL is 60.92% while 125µg/mL inhibited 83.9% of the cell growth. The IC_{50} value for the methanolic leaf extracts of *Andrographis paniculata* is 57.33µg/mL. The regression value is 0.9837µg/mL.

Table 2: The anticancer activity of methanolic leaf extract of *Andrographis paniculata* using MCF-7 cell line

Plant extract conc (µg/mL)	% inhibition (µg/mL)	IC_{50} (µg/mL)	R^2 (µg/mL)
31.25	9.466437	57.33	0.9837
62.5	60.92943		
125	83.9912		
250	99.00		
500	99.00		

IC_{50} - 50% inhibition concentration, R^2 -Linier regression value

Fig. 1: The % of growth inhibition of cancer cell line against methanolic leaf extract of *Andrographis paniculata*



The methanolic leaf extract of *Cardiospermum halicacabum* was tested for its anticancer potential against MCF-7, breast cancer cell line. The maximum activity (79.60%) was observed under the 500 µg/mL followed by 42.51% in 250 µg/mL and 30.20%,

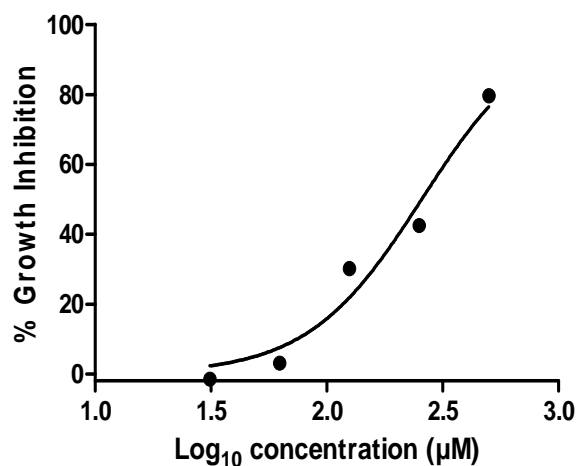
3.184% and -1.46% in 125, 62.5 and 31.25 µg/mL respectively. The IC_{50} value for the methanolic leaf extracts of *Cardiospermum halicacabum* was 256.6 and the regression value is 0.9646.

Table 3: The anticancer activity of methanolic leaf extract of *Cardiospermum halicacabum* using MCF-7 cell line

Plant extract conc (µg/mL)	% inhibition (µg/mL)	IC_{50} (µg/mL)	R^2 (µg/mL)
31.25	-1.46299	256.6	0.9646
62.5	3.184165		
125	30.20654		
250	42.51291		
500	79.60413		

IC_{50} - 50% inhibition concentration, R^2 -Linier regression value

Fig. 2: Growth inhibition of cancer cell line against methanolic leaf extract of *Cardiospermum halicacabum*



Conclusion

The phytochemical screening of methanolic leaf extracts *Andrographis paniculata* and *Cardiospermum halicacabum* showed the presence secondary metabolites such as Alkaloids, Terpenoids, Tannins, Saponins, Flavanoids and Steroids. There is a remarkable anticancer potential was observed against the breast cancer cell lines in methanolic leaf extract of *Andrographis paniculata* and *Cardiospermum halicacabum*. There is a correlation was observed in the concentration and % of growth inhibition against the extract and breast cancer cells. The present study confirms the presence valuable chemicals present in the plants further thorough studies may bring out the real potential of these widely used medicinal plants in the

preparation of antibiotic, antioxidant and anticancer drugs.

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