

Original Article

CYTOTOXIC ACTIVITY OF LACTUCA RUNCINATA DC AND GYROCARPUS ASIATICUS WILLD ON CANCER CELL LINES *IN VITRO*

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ABSTRACT

Objective: The present study was designed to evaluate the in-vitro cytotoxic activity of *Lactuca runcinata* methanol extract (LRME) and *Gyrocarpus asiaticus* methanol extract (GAME) on MCF-7, HepG2, HeLa, A-549 cell lines.

Methods: Cytotoxicity was determined using 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay and IC₅₀ was calculated, and cellular morphological alterations were studied using phase contrast light microscopy.

Results: GAME exhibited better values of growth inhibition than LRME and altered the cellular morphology (for 1000 µg/ml concentration) against four cell lines. GAME showed significantly higher cytotoxicity against HeLa cells with an IC₅₀ of 113.33±0.5 µg/ml than in HepG2 cells with an IC₅₀ of 116.67±0.5 µg/ml and lowest cytotoxic effect against A-549 cells with an IC₅₀ of 370 µg/ml than in MCF-7 cells with an IC₅₀ of 380 µg/ml. However, no cytotoxic effect was observed in LRME with an IC₅₀ of >1000 µg/ml against four cell lines.

Conclusion: The cytotoxic activity revealed that *Gyrocarpus asiaticus* could be useful for preparation of neutraceuticals as potent anticancer to treat various cancer diseases.

Keywords: *Lactuca runcinata*, *Gyrocarpus asiaticus*, Cytotoxicity, MCF-7, HepG2, HeLa, A-549.

INTRODUCTION

The investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades due to their potent pharmacological activities, convenience to users, economic viability and low toxicity.[1,2] 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. [3]

Lactuca runcinata DC [*L. runcinata*, Synonym-*Lactuca heyneana* DC.] commonly known as Udirachakam [4] or Atheli is an annual erect herb belonging to the family Compositae (Asteraceae). Traditionally this plant finds its wide applicability as diuretic and in chronic obstruction of liver and bowel. [4] *Lactuca runcinata* DC also has been reported to be a valuable source of essential nutrients, such as carbohydrate, protein, fat etc. and micro-nutrients like calcium, iron, phosphorous etc. Chewing *Lactuca runcinata* with betel leaf is useful to cure the blisters of mouth and tongue. [5]

Gyrocarpus asiaticus Willd commonly known as Taniki or Nalla poliki [6] is a tree belonging to the family Hernandiaceae. *Gyrocarpus asiaticus* Willd is one of the species in the genus *Gyrocarpus* with the class Magnoliopsida. *Gyrocarpus asiaticus* have different kinds of pharmacological behaviors such as antioxidant activity, [7] anthelmintic activity, [4] anti-cancer activity. [8]

The methanolic extract of *Gyrocarpus asiaticus* showed positive results for the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins and terpenoids but methanolic extract of *Lactuca runcinata* showed positive results for alkaloids, cardiac glycosides, flavonoids, phenols, phlobatannin, reducing sugars, saponins, steroids, tannins and terpenoids. [4] Therefore, the present investigation came to evaluate cytotoxic effects of *Lactuca runcinata* DC and

Gyrocarpus asiaticus Willd methanol extracts *in vitro* against MCF-7 (Breast cancer), HepG-2 (Liver cancer), HeLa (Cervical cancer) cancer cell lines using MTT assay.

MATERIALS AND METHODS

Chemicals

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 were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell lines and Culture medium

MCF-7 (Human breast adenocarcinoma), HepG2 (Human liver carcinoma), HeLa (Human cervix carcinoma) and A-549 (Human Lung adenocarcinoma epithelial) 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).

Plant collection and identification

The botanist Dr. V. Chelladurai (Retired research officer-Botany, Central council for research in Ayurveda and Sidda, Govt. of India, Tirunelveli, Tamil Nadu, India) identified the plants *Lactuca runcinata* DC and *Gyrocarpus asiaticus* Willd, which were collected (aerial parts) from the nearby area of Thoothukudi District fields (Tamil Nadu) in December 2011. Herbarium of the plants were prepared and preserved in Department of Pharmacognosy, Koringa college of Pharmacy, Korangi, East Godavari Dist., Andhra Pradesh, India.

Preparation of extracts

35 gms of powdered *Lactuca runcinata* DC and *Gyrocarpus asiaticus* Willd were separately added to 350 ml of methanol and the extracts were obtained using soxhlet apparatus for 8 hrs. The methanolic extracts were then filtered and the filtrate was concentrated by rotary evaporator at 45-50 °C. [9] The extracts were stored in a refrigerator at 4°C for further use.

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 and Morphological analysis

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, 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 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 24 h interval. After 72 h, 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 3 h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100 µl 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 540 nm. [10] The percentage growth inhibition was calculated 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. [11]

$$\% \text{ Growth Inhibition} = 100 - \left\{ \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right\} \text{ss}$$

The morphological changes in MCF-7, H(2-22)epG-2, HeLa and A-549 cancer cells exposed to increasing concentrations (62.5 to 1000 µg/ml) of *Lactuca runcinata* DC and *Gyrocarpus asiaticus* Willd extracts were taken using an inverted phase contrast microscope (MOTIC, Singapore, AE 3L) at 20X magnification.

Statistical analysis

All data are expressed as means±SD. Each data point was obtained by making at least 3 independent measurements.

RESULTS AND DISCUSSION

In-vitro cytotoxic activity of *Lactuca runcinata* DC and *Gyrocarpus asiaticus* Willd methanol extracts at various concentrations against MCF-7, HepG2, HeLa and A-549 cancer cell lines were studied using MTT assay.

Anticancer activity of *Lactuca runcinata* methanol extract at various concentrations against MCF-7, HepG2, HeLa and A-549 cancer cell lines were represented in Figure 1. There was a gradual less increase in the value of percentage of growth inhibition (PGI) as the concentration of the *Lactuca runcinata* extract was increased against MCF-7 cells (17.25, 18.79, 21.62, 24.26 and 26.76 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively), HepG2 cells (6.34, 14.69, 18.64, 21.14 and 23.69 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively), HeLa cells (5.08, 7.88, 15.85, 31.63 and 38.07 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively) and A-549 cells (12.38, 17.65, 22.84, 27.67 and 31.68 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively).

The cytotoxic effect of *Gyrocarpus asiaticus* methanol extract at different concentrations against MCF-7, HepG2, HeLa and A-549 cancer cell lines were represented in Figure 2. There was a gradual

increase in the value of PGI as the concentration of the *Gyrocarpus asiaticus* extract was increased against MCF-7 cells (17.81, 21.62, 24.0, 73.21 and 75.56 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively), HepG2 cells (43.36, 53.29, 64.55, 69.21 and 79.39 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively), HeLa cells (39.82, 57.0, 67.12, 85.35 and 93.31 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively) and A-549 cells (22.24, 26.4, 28.61, 72.02, 74.10 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively).

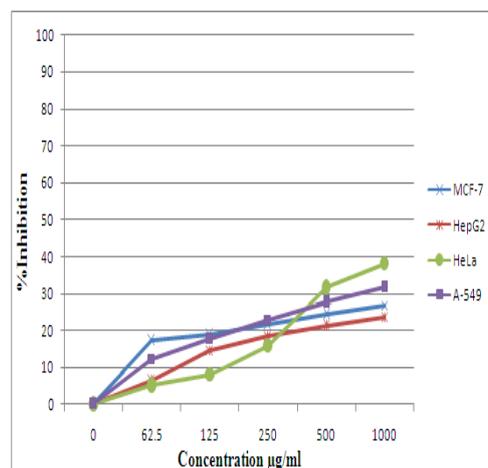


Fig. 1: Cytotoxic Effect of *Lactuca runcinata* Extract Against Different Cancer Cell lines.

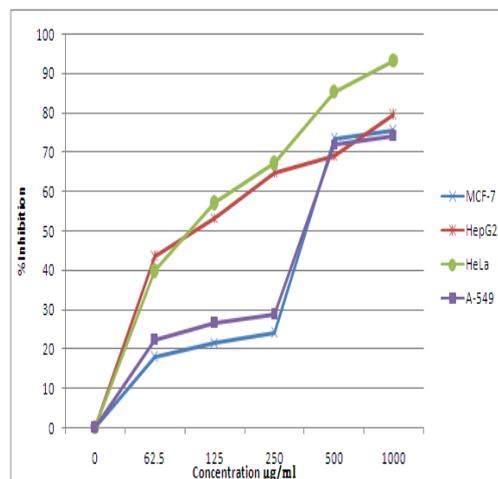


Fig. 2: Cytotoxic Effect of *Gyrocarpus asiaticus* Extract Against Different Cancer Cell lines.

IC₅₀ value were calculated from the extract treated OD (optical density) value of *Lactuca runcinata* extract against MCF-7, HepG2, HeLa and A-549 cancer cell lines were greater than 1000 µg/ml (Table 1) and hence considered non cytotoxic. IC₅₀ value of 380µg/ml, 116.67±0.5 µg/ml, 113.33±0.5 µg/ml and 370 µg/ml were obtained for *Gyrocarpus asiaticus* against MCF-7, HepG2, HeLa and A-549 cancer cell lines respectively (Table 1). The methanol extract of *Gyrocarpus asiaticus* exhibited significant cytotoxic effect against all cancer cell lines. The percentage of inhibition ability of the *Gyrocarpus asiaticus* extract was in the order: HeLa > HepG2 > MCF-7 > A-549 but cytotoxic effect of *Gyrocarpus asiaticus* extract was in the order: HeLa > HepG2 > A-549 > MCF-7.

The morphological changes were observed in various cancer cells. Morphological alteration of MCF-7, HepG2, HeLa and A-549 cancer cell

lines after the exposure of two plants extracts were observed under phase contrast microscope. In case of *Lactuca runcinata*, the morphology of all cells at >1000 µg/ml have shown to little reduce the normal morphology and hence considered non cytotoxic. With 1000 µg/ml of *Gyrocarpus asiaticus* extract, the cell lines lost typical morphology and appeared smaller in size, shrunken and rounded (Figure 3).

In discussion, the discovery of anticancer drugs that must kill or disable tumor cells in the presence of normal cells without

undue toxicity is potential challenge for therapeutic care. [12] Available literatures on medicinal plants indicate that promising photochemicals can be developed for many health problems. [13-15] Plant extracts as traditional remedies are already being used to treat a variety of diseases including cancer. [16-20] The cytotoxic effect of plants is principally contributed by the presence of secondary metabolites like alkaloid, glycoside, steroid, tannin, phlobatannin, terpenoid and flavonoid in their extract. [21]

Table 1: Cytotoxic Activity of *Lactuca runcinata* and *Gyrocarpus asiaticus* Extracts.

Treatment	MCF-7 cell line IC ₅₀ (µg/ml)	HepG2 cell line IC ₅₀ (µg/ml)	HeLa cell line IC ₅₀ (µg/ml)	A-549 cell line IC ₅₀ (µg/ml)
<i>Lactuca runcinata</i> extract	>1000±0.00	>1000±0.00	>1000±0.00	>1000±0.00
<i>Gyrocarpus asiaticus</i> extract	380.00±0.00	116.67±0.5	113.33±0.5	370.00±0.00

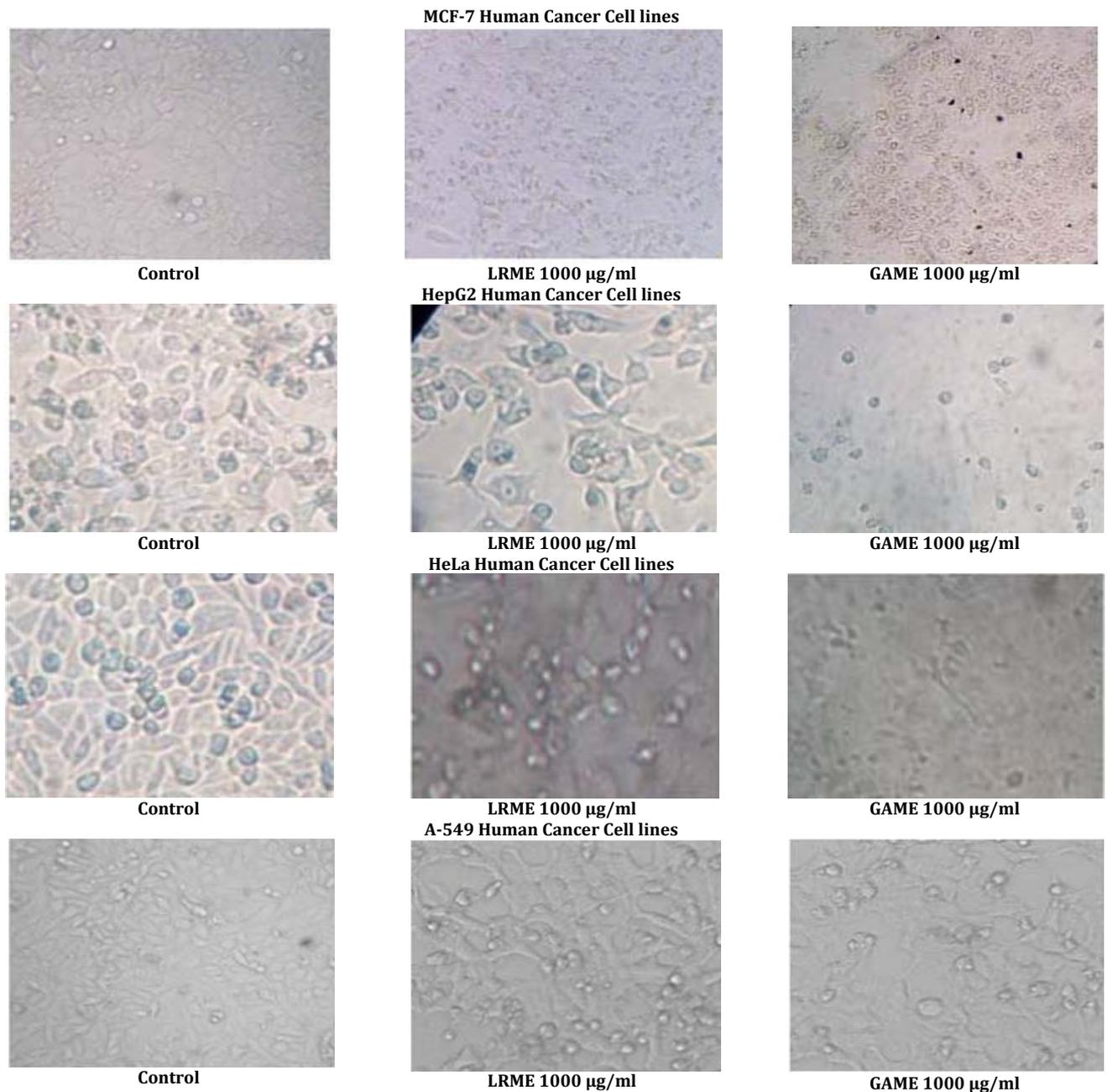


Fig. 3: Morphological Changes in Four Cells exposed to 1000 µg/ml Concentration of *Lactuca runcinata* methanol Extract (LRME) and *Gyrocarpus asiaticus* methanol Extract (GAME).

CONCLUSION

The present study was aimed to evaluate the cytotoxic effects of *Lactuca runcinata* and *Gyrocarpus asiaticus* methanol extracts against MCF-7, HepG2, HeLa, A-549 cancer cell lines by MTT assay. It is interesting that *Gyrocarpus asiaticus* extract exhibits effective antitumor activity and seems to have no side effects. So, it can be useful to the patients as an effective therapeutic tool which is due to copiously presence of secondary metabolites like alkaloids, glycosides, steroids, tannins, terpenoids and flavonoids in *Gyrocarpus asiaticus* extract. Further investigations are required to study the bioactive compounds which are responsible for showing the cytotoxic effect of *Gyrocarpus asiaticus* extract. *Lactuca runcinata* extract has no significant anticancer activity due to little presence of secondary metabolites like alkaloids, glycosides, steroids, tannins, phlobatannins, terpenoids and flavonoids. So, for the *Lactuca runcinata* plant there is a preliminary indication of safety for their intake for the absence of cytotoxic activity.

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