EVALUATION OF ANTICANCER ACTIVITY OF METHANOLIC EXTRACT OF CARALLUMA FIMBRIATA WALL. AGAINST LUNG CANCER CELL LINE

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ABSTRACT
The present study is designed to evaluate the in-vitro anticancer activity of methanolic extract of Caralluma fimbriata Wall. against Lung cancer cell line A-549 for its effects on cell viability, growth inhibition and cell morphology. Cell viability and inhibition was determined by cytotoxicity assay and MTT assay. Cell morphology was observed under inverted microscope. The cytotoxicity effect of Methanol extract carried out using various concentration in L6 muscle cells. The result showed that the methanol extract upto 100µg/ml having lesser toxicity. Hence the doses for antiproliferative activity fixed within that concentration. The antiproliferative results showed decreased cell viability and increased growth inhibition in a dose and duration dependent manner and also altered cell morphology after treatment with methanolic extract of Caralluma fimbriata Wall. The data demonstrated that methanolic extracts of Caralluma fimbriata Wall. has a potential anticancer agent on A-549.

Keywords: Caralluma fimbriata Wall., Anticancer, cytotoxic, 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay, A-549 and L6 cell line.

INTRODUCTION
For centuries, People have been using plants for their therapeutic values. Today 85000 plants have been documented for therapeutic use globally [1]. The World Health Organization
(WHO) estimates that almost 75% of world’s population has therapeutic experience with herbal drugs. Cancer is one of the most dangerous disease in humans and presently there is a considerable scientific discovery of new anti cancer agents from natural products [2]. The potential of using the natural products as anti cancer drugs was recognized in 1950’s by U.S Natural Cancer Institute (NCI) Since 1950 major contributions have taken for the discovery of naturally occurring anti cancer drugs [3]. Phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds [4].

*Caralluma fimbriata* Wall. an edible succulent cactus is a perennial herb growing in dry parts of Tamil Nadu, India. It belongs to the family Apocynaceae is also a well known as Famine Food, Appetite Suppressant & thirst quencher among tribal population. Genus *Caralluma* comprises about 200 genera & 2500 species [5]. It grows wild all over India & is also planted as a roadside shrub & boundary marker in gardens. Several members of the genus *Caralluma* have found medicinal uses in the treatment of Rheumatism, Diabetes, Leprosy, Antiseptics & Disinfectants [6]. The species of *Caralluma* found in India are edible and form part of the traditional medicine system of the country. *Caralluma fimbriata* is listed in The Wealth of India (1992) as medicinal plant used as an appetite suppressant and has also been used to treat diabetic, pain, fever, and inflammation. Native Indian diets over many centuries have included these edible wild succulent cacti, with claims in folklore about its Appetite Suppressant Activity. An investigation was carried out to find out the effect of *Caralluma fimbriata* extract on appetite, food intake and anthrometry in adult Indian men and women [7]. The extract of *Caralluma fimbriata* in the form of capsules, has been released under the trade name GENASLIM for body weight control. The species of this family have significant anti inflammatory and antitumor activity, anticancer, cytoprotective and antiulcer activity, antinociceptive, antioxidant, hypolipidemic, antihyperglycemic, antidiabetic, treating paralysis and joint pains, antipyretic. The phytochemical constituents of the herb are Alkaloids, Phenolic compounds, Flavonoids, Saponins, glycosides and quinone [8]. Due to uniqueness of curing different ailments this whole plant is selected for the study. hence the present investigation was carried out to determine the the *in-vitro* anticancer activity of methanolic extract of *Caralluma fimbriata* Wall. against Lung cancer cell line A-549 for its effects on cell viability, growth inhibition and cell morphology.
MATERIALS AND METHODS

Collection of plant Material
The apocynaceae family members are mainly distributed in the Himalayan, southern and western parts of India. The plants chiefly inhabit arid soil. The plants were collected from Pudukkottai (District), Tamil Nadu, India and authenticated (Specimens No. BSI/SRC/5/23/09-10/Tech-1569) in Botanical Survey of India, Coimbatore. MS media, sucrose and all the chemicals for this study were purchased from HiMedia, Mumbai, India. Glasswares were purchased from Borosil, India.

Preparation for extracts
The plant was collected, washed and dried. Then it was ground in a grinding machine to fine powder and passed through a 24-mesh sieve and the extract is weighted and stored at room temperature.

Extraction of plant material
The powdered sample (20g) of Caralluma fimbriata was successively extracted with 200ml of solvent (ethanol, ethyl acetate and methanol) using magnetic stirrer and stirred for 3hrs. Then it was filtered using whatmann filter paper. Again the residue was dissolved with 200ml solvent and stirred for 2hrs. The solvent containing the extract is dried under reduced pressure. The aqueous extract was prepared with 10g of powder in 100ml of distilled water & stirred for 3 hrs. The supernatant was boiled up to minimum volume.

Maintanence of cancer cells
Lung cancer cell line A-549 and L6 muscle cell line were grown in Dulbecco’s medium with 10% FBS and 2% antibiotics. Stock cultures were sub-cultured every 7th day after harvesting the cells with trypsin-EDTA and then seeding them in tissue culture flasks to maintain in exponential phase.

Cell Culture Media
Dulbecco’s medium with glutamine and without sodium bicarbonate was used for growing the cells. Fetal bovine serum was used for the growth of cells.

Cytotoxicity Assay on L6 muscle cell line
The initial cytotoxicity assay on the methanolic extract of Caralluma fimbriata was carried out in Indian Institute of Technology, Chennai. L6 muscle cell line was used for knowing the
cytotoxic effect on normal cell towards the different concentration (50µg/ml, 100µg/ml, 200µg/ml, 500µg/ml and 1000µg/ml) of methanolic extract of *Caralluma fimbriata*. And the dosage was fixed based on the viability of normal cells.

Percentage of cell viability = (OD of treated cells / OD of control cells) × 100

**Anticancer assay on A-549 Lung cancer cell line (MTT Assay)**

The anticancer activity on the methanolic extract of *Caralluma fimbriata* was done at Biozone Research Technologies, Chennai. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the antiproliferative activities of the tested extracts against the cancer cell lines. The assay depends on the cleavage of the tetrazolium salt (MTT) into formazan blue by the mitochondrial enzyme succinate dehydrogenase. The conversion takes place only in living cells and the amount of formazan produced is proportional to the number of viable cells present. Thus, the MTT assay is potentially useful for assaying antiproliferative activities of materials.

For this purpose the cancer cells were seeded in complete medium in a 96-well plate at a density of 1x10^5 cells/ml. Each well contains DMEM with 10% FBS (fetal bovine serum) and 5% carbon dioxide. The medium aspirated and different concentrations (6.25, 12.5, 25, 50, 75µg/ml) of methanolic extracts of *Caralluma fimbriata* was added. The cells are incubated in the carbon dioxide incubator for 24hrs at 37 ºC. After incubation remove the supernatant and the fresh medium containing extract of same concentration was added and incubated for 24hrs in the same condition. To this 5mg/ml MTT was added and incubated for 3hrs. The media is removed after the incubation and 100% DMSO was added to each well to solubilize the formazan crystals produced by viable cells. After complete dissolving of formazan blue, the absorbance was measured at 540 nm using microplate reader and viewed under inverted microscope. The percentage of cell viability was calculated [9]. The samples without extract (viable cells with MTT) was used as control. Cyclophosphamide is used as positive control. Cultures were viewed using an inverted phase contrast microscope.

Percentage Growth inhibition =100 – (Mean OD of individual test group/ Mean OD of control group x 100)
RESULTS AND DISCUSSION

The anticancer activity of methanolic extracts of *Caralluma fimbriata* (test) and cyclophosphamide (control) on A549 lung cancer cell line were examined by the MTT assay. The dosage level was fixed as 50µg/ml of methanolic extract from the cytotoxicity assay on L6 muscle cell line because of its lesser toxicity (Fig.1). The methanolic extracts of *Caralluma fimbriata* exhibits an increasing percentage of growth inhibition in the cancer cells with increasing concentration of extract such as 6.25, 12.5, 25, 50, 75 µg/ml (Fig.2). At the concentration of 50 µg/mL, the methanolic extract of *Caralluma fimbriata* possess high anticancer activity against A-549 cancer cell lines, with the corresponding inhibitory activity of 50.32%.

Results indicate that the cytotoxic effect strengthens with increase in the concentration of extract. Due to the mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan and the amount of formazan produced is directly proportional to the number of viable cells [10]. Polyphenol compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells [11]. The mechanism of action of anticancer activity of phenolics could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenolics reduced the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells. The presence of a 4-carbonyl group of the flavonoid molecule also contributes to anticancer activity. In addition, the presence of 2,3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death [12]. The main objective of this assay is to check the cytotoxicity brought about by the compounds and find the toxicity levels in terms of IC$_{50}$ dose when live and dead cell percentages are equal, which is considered as the optimum dose for the various assays. It seen us that the methanolic extract possess cytotoxicity at lower concentration .From graph it could be concluded that the cytotoxicity was observed both in dose- and duration-dependent manner.

The Methanolic extract of *Caralluma fimbriata* was cytotoxic to A549 cells, which was clearly observed when viewed under inverted microscope (Fig.3). MTT assay was used to evaluate cytotoxicity based on metabolic reduction of MTT. Thus, the methanolic extract of *Caralluma fimbriata* is non-toxic to the normal cells and also has both anticancer and anti-
proliferative activities against the cancerous cells.

**Cell Morphology**

Based on MTT assay results, Changes in cell structures and morphological alterations were confirmed via inverted microscope. As shown in Figure 3 after 24 h of incubation with various concentrations many of the cells showed cytoplasmic shrinkage and loss of normal nuclear architecture, became detached and found floating in the medium. As a result, the number of cytotoxic cells increased with concentration, with the highest having the most pronounced inhibitory effect on cell proliferation than the control.

![MTT Assay on L6 cell line](image1)

**Concentration of methanolic extract (µg/ml)**

**Figure 1: MTT Assay on L6 cell line**

![MTT Assay on A-549 cancer cell line](image2)

**Concentration of methanolic extract (µg/ml)**

**Figure 2: MTT Assay on A-549 cancer cell line**
Figure 3: MTT ASSAY

Control

Positive control

-549 treated with 6.25µg/ml of extract

A-549 treated with 12.5µg/ml of extract

A-549 treated with 25µg/ml of extract

A-549 treated with 50µg/ml of extract
CONCLUSION
It is concluded that the methanolic extract of Caralluma fimbriata possess anticancer properties against A549 lung cancer cell line. Further experimental analysis on these plants would definitely reveal the important chemical constituents responsible for cancer cell death because the probable inhibitions with active principles/chemical constituents would be higher than the extracts (due to the presence of mixture of varied constituents). Thus the current work on identification and evaluation of anticancer activity in edible Caralluma fimbriata may prove its importance in improving human health.

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