



# IN VITRO ANTICANCER ACTIVITY OF *CANTHIUM DICOCCUM* (GAERTN.) AGAINST LUNG CANCER CELL LINE

Meghashree K.S. and K.P. Latha\*

Department of P.G. Studies and Research in Chemistry, Sahyadri Science College, Kuvempu University, Shivamogga- 577203 (Karnataka), India.

## Abstract

*Canthium dicoccum* (Gaertn.) the Ceylon boxwood also known as Bellachi in Kannada belongs to the family *Rubiaceae*. The crushed leaves were subjected to the hot method of extraction using soxhlet extractor. The extraction method was carried out using numerous solvents viz., pet ether, ethyl acetate and ethanol per their increasing polarity. The ethanol extracts were tested against A549 cell line with maximum reduction in cell viability were chosen for further staining experimentation. Cytotoxic activity was studied using MTT assay the isolated compound have  $IC_{50}$  values lower than that standard Doxorubicin. At 50  $\mu$ g/mL concentration there is a lesser values, i.e., 21.17 %. The results clearly revealed that the isolated pure compound from crude ethanol extract from column chromatography of *Canthium dicoccum* was more potent on A549 cell line..

**Key words :** A549 Cell line, Ethanol Extract, Anticancer activity, *Canthium dicoccum*.

## Introduction

*Canthium dicoccum* (Gaertn.) the Ceylon boxwood also known as Bellachi in Kannada belongs to the family *Rubiaceae* (Raja Rajeswari N *et al.*, 2011). In India its bark is used for fever and decoction of the root is used internally for diarrhea. Bark powder with sesame oil is used in rheumatic pain (Neelima M *et al.*, 2011; Santhan S *et al.*, 2013). The plant is proved for its antidiabetic and nephroprotective activity (Vidyarthi RD *et al.*, 2005). The use of plant extracts and phytochemicals, both are very important properties for antimicrobial activity and great significance in therapeutic treatments. A number of studies have been conducted to show such efficiency (Abtele and Erdourul 2003; Reddy *et al.*, 2001; Syed Nyamath and Karthikeyan 2018 and Pinkusatnami *et al.*, 2016).

## Materials and Methods

### Collection and identification plant material

The *Canthium dicoccum* (Gaertn.) leaves were collected in the month of June-July in Hosnagar (T), Shimoga district, Karnataka. The plant were authenticated

\**Author for correspondence* : E-mail: lathakp337@gmail.com

and deposited in the department of Botany Kuvempu University, Shanakaragatta, with voucher number KUAB4688. A voucher specimen has been preserved in our laboratory for future reference.

### Processing and extraction

The collected leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was subjected to the hot method of extraction using soxhlet extractor with petroleum ether, ethyl acetate and ethanol per their increasing polarity. The obtained extract was filtered and evaporated to dryness under reduced pressure in a rotary vacuum evaporator.

### Anticancer activity

#### Procedure

A549 cells were obtained from NCCS (Pune, India). A549 cells were cultured as monolayer in DMEM containing high glucose - 4.5 g/L, 4 mM glutamine, 3.7 g/L sodium bicarbonate, 25 mM sodium pyruvate and supplemented with 10% heat inactivated bovine serum, 100 U penicillin and 100 ig/mL streptomycin. Cell lines were maintained in a humidified atmosphere of 95% air

and 5% CO<sub>2</sub> at 37°C. Cultured cells were passed 3 days once by trypsinisation (0.25% trypsin containing 0.02% EDTA) and between passages 4 to 20 were considered for experiments.

The cellular viability was evaluated using an assay based on the cleavage of the yellow dye MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide) to purple formazan crystals by dehydrogenase activity in mitochondria. Briefly, 5×10<sup>3</sup> of A549 cells were seeded in a 96-well plate and after 24 hours, cells were treated with separately either with compound (5µg, 10µg, 15µg, 20µg, 25µg, 30µg, 35µg, 40µg, 45µg, 50µg) and without compound (control) were incubated for 24 h. After 24 hours, cells were rinsed with media and then they received MTT diluted in media for 3 hours and DMSO was used to dissolve the purple formazan crystals, and the optical density of the solution was measured at 570 and 690 nm.

Based on the effect of compound A549 cell lines with maximum reduction in cell viability were chosen for further staining experimentation. Apart from MTT assay, portion of cells were subjected for tryphan blue staining (0.4%) using haemocytometer to count the number of viable cells and dead cells.

Mean ± SE IC<sub>50</sub> values were determined by MTT assays following 24 h exposure of cells to test compounds (n = 3) and expressed as a mean and standard error of 3 independent trials.

### Results and Discussion

The anticancer activity was determined by MTT assay (Mossman T., 1983). The ethanol extracts of the plant *Canthium dicoccum* leaves were found to have maximum anticancer activity.. Ethanol extract result presented in table 1 and Fig. 3 revealed that the isolated compound have IC<sub>50</sub> values lesser than that of positive



Fig. 1: *Canthium dicoccum* leaves.

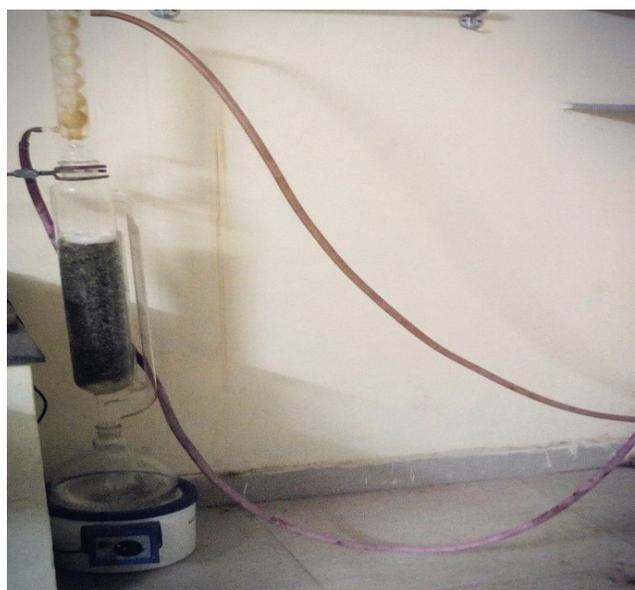


Fig. 2: Overall view of extraction using Soxhlet apparatus.

**Table 1:** Evolution of anticancer activity of *Canthium dicoccum* against A549 cells.

Concentration/µg	Cell Viability (%)
5	86.82±0.156
10	76.92±0.023
15	73.04±0.015
20	70.95±0.017
25	70.30±0.015
30	55.93±0.015
35	55.81±0.015
40	37.87±0.015
45	34.92±0.015
50	21.17±0.156
DOX	39.18±0.015

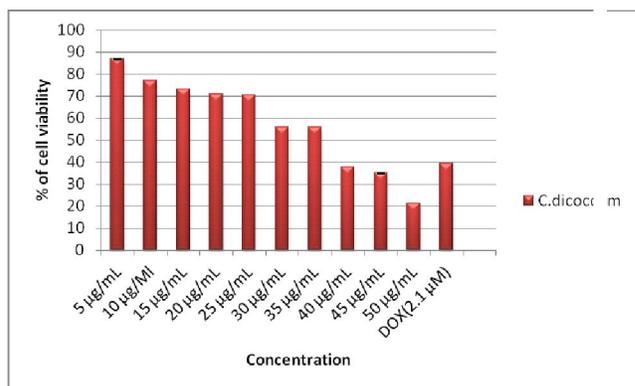


Fig. 3: Anticancer activity of *Canthium dicoccum* at different concentrations against Lung Cancer cell (A549).

control against A549 cell line with the best anticancer activity attributed  $21.17 \pm 0.156$  of cell inhibition at concentration  $50 \mu\text{g/mL}$ . Similar results has been carried out in many research work (Fakri Mustafa *et al.*, 2018). The phytochemical and antioxidant assay which showed the expression of phytophenols, hence lung cancer cell line was chosen for anticancer activity (Raja Rajeswari. N *et al.*, 2011). Presence of phenolic compounds has shown to induce a cascade based apoptosis in cancer cells, thus inducing cytotoxicity (Owen *et al.*, 2000). The cell viability of the lung cancer cell line decreased with increase in concentration of the plant extract and it was found to be the highest in  $50 \mu\text{g/mL}$  concentration. The decrease in cell viability with increased concentration of the plant extract of *canthium dicoccum* suggests the ability of the extract as an effective anti-cancer medicine.

### Conclusion

The present investigation revealed that *Canthium dicoccum* can act as a potential alternative remedy for lung cancer. The extract of *Canthium dicoccum* can be used as an effective ingredient in drug recipe cancer. Further investigation is undertaken to identify the active compound behind the anticancer activity of the plant. The study in the future is to be extended to other cancer cell lines and there is a need to carry out in vivo studies. Therefore, there is no doubt that this plant is a reservoir of potentially useful chemical compounds, which serve as drugs and provide modern drug discovery.

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