

# Anti-Cancer Activity of *Cayratia Auriculata* Ethanolic Extracts Against Cancer Cell Line A549 - An *In Vitro* Analysis

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## ABSTRACT

**Background:** The purpose of this study was to evaluate the anticancer activity of ethanolic *cayratia auriculata* extracts using the A549 cell line MTT assay. **Materials and Methods:** Using Soxhlet apparatus, ethanolic extracts from *cayratia auriculata* were prepared. The cancer cells were exposed to 12.5, 25, 50, 100, 150, 200 µg / mL and incubated for 24 h at different concentrations. Compared with control, *C. auriculata* exhibited a cytotoxic effect. **Results:** At 150 and 200µg / ml concentrations, with 61 percent and 73.7 percent respectively, the highest cytotoxicity was identified. The findings show that cytotoxicity is directly proportionate to the concentration of the extract. IC50 of the ethanolic extract value of *C. auriculata* was found to be 102.9µg / ml against the A549 cell line. **Conclusion:** In the present analysis, *C.auriculata* ethanolic extract was shown to be a strong suppressant for cell division and proliferation. As for anti-tumor medicine, it can be a new source and can be effectively used as an immunological anti-malignant compound.

**Key words:** Activity against cancer, Cell line cancer, Ethanol extract, MTT assay.

## BACKGROUND

Lung cancer is the world's leading cause of death and it has been noted that 7.6 million deaths were recorded in 2008. Death rates have been forecast to rise by more than 11 million in 2030<sup>1</sup>. Usually, 80 percent of cases of histologically non-small cell lung cancer are a big health issue<sup>2</sup>. Due to the high incidence rate, rapid development and poor prognosis, lung cancer-related deaths have been the leading cause<sup>3</sup>. With an average survival rate of around 15 percent, it has become a burden worldwide<sup>4</sup>. There are more medications available today to manage lung cancer. Chemotherapy is the routine treatment for lung cancer, but it does have several side effects that also affect the quality of life of patients<sup>5</sup>. It is still deficient, considering the numerous treatment methods available to avoid these side effects<sup>6,7</sup>. Therefore, new drugs or innovative ways to cure or avoid this global warming disease are being pursued. Several medicinal plants have been used globally for the treatment of different forms of cancer<sup>8</sup>. Conventional medicine with its low toxicity and side effects has the ability to treat anticancer activity. According to the WHO study, in contrast to modern medicine, 80 % of the world's population relies on conventional medicine because of its less side effects, cost effectiveness, protection, and effective prognosis<sup>9</sup>. Even today, several tribal groups are practicing herbal medicine to cure various diseases<sup>10</sup>. The medicinal plant belonging to the *Vitaceae* family is *Cayratia auriculata*. *Cayratia auriculata* (Roxb.) gamble is a synonym for *Cyphostemma auriculatum* (Roxb), a climber well cultivated in dry evergreen to dry deciduous forests and well distributed in Andhra Pradesh, Tamilnadu, and Maharashtra<sup>11,12</sup>. For several diseases, *cayratia auriculata* has been used

as herbal medicine. The leaf is the most important part of the plant as it has potent ethno-medicinal properties. It is used in the treatment of snake bite, dog bite, abscess, rheumatism, cardiovascular disease, tumours, and also in the treatment of cold, cough, worm infestation<sup>13</sup>. The genus is also used for bloody dysentery and diarrhoea in veterinary medicine. Based on previous studies, a study on the cytotoxic activity of *C. auriculata* extract is still limited to lung cancer. The present study was therefore conducted to determine the anticancer and cytotoxicity potential of *Cayratia auriculata* unrefined ethanolic leaf extract.

## MATERIALS AND METHODS

### Plant content processing and authentication

*Cayratia auriculata* was collected in November 2018 from the botanical garden of Andhra University, Visakhapatnam, Andhra Pradesh. The plant content has been identified and approved by Dr. S.B. Padal, Associate Professor, Department of Botany, Visakhapatnam, Andhra University.

### Preparation of extracts for plants

Fresh leaves of *Cayratia auriculata* were washed in flowing tap water, dried in the shade and powdered. Approximately 75 g of coarsely powdered plant materials (75g/250mL) were extracted for 10 to 12 hours in a soxhlet extractor. The desired compounds were condensed in the distillation flask after several cycles, the extracts were finally collected, then condensed and finally dried to a constant weight. Before usage, dried extracts were stored at 20 ° C. In an in vitro trial, this extract was used.

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## Reagents

The Minimum Critical Medium reagents were supplied by HiMedia Lab systems. Cistrion Labs supplied foetal bovine serum (FBS). Reagents like 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), trypsin-EDTA, phosphate buffered saline (PBS) were supplied by Sisco Research Laboratory Chemicals, Mumbai. From Sigma-Aldrich, Mumbai, all the other chemicals and reagents were purchased. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) stock solution 5 mg / ml was prepared and wrapped with aluminium foil as it is light-sensitive. 5 mg of extract was taken and suspended in 1 ml of water. Solutions with various concentrations (200µg / ml, 150µg / ml, 100µg / ml, 50µg / ml, 25 µg / ml and 12.5µg / ml) were prepared from the stock solution<sup>14</sup>.

## Procedure of MTT assay proceedings

In lung cancer, the effect of the drug was tested against the cell line (A549) by MTT assay according to the standard procedure<sup>15</sup>. The cells were seeded in 96-well micro plates (1x10<sup>6</sup> cells / well) and incubated in a 5% CO<sub>2</sub> incubator at 37 ° C for 24 hours and allowed 80% confluence to expand. The medium was then substituted and the cells were treated with drugs at varying concentrations, such as 12.5, 25, 50, 100, 150, 200 µg / mL and were incubated for 24 hours. The cells were then washed and applied to each well with phosphate-buffer saline (PBS, pH-7.4) and 20 µL (MTT) solution (5 mg / mL in PBS). The plates were then placed for an additional 2-4 h at 37°C in the dark. In 100µL DMSO the formazan crystals were dissolved and the absorbance at 570 nm was spectrometrically read. The morphological changes of the untreated (control) and treated cells were observed and photographed after 24 h under a bright field microscope. The concentration required for 50% inhibition (IC<sub>50</sub>) was calculated and graphically determined. The cell viability percentage was expressed as a formula.

## Calculation:

$$\% \text{ cell viability} = \text{O.D value of treated cells} \times 100$$

$$\text{O.D value of cell control}$$

$$\% \text{ inhibition} = 100 - \% \text{viability}$$

Graphs are plotted using the X-axis sample concentration and the percentage of Y-axis cell viability. In each experiment, sample control and cell control were used to compare the full assessments of cell viability. To understand the biological pharmacological characteristics of a chemotherapeutic agent, the determination of a half maximum inhibitory concentration (IC<sub>50</sub>) is essential<sup>16</sup>.

## STATISTICAL ANALYSIS

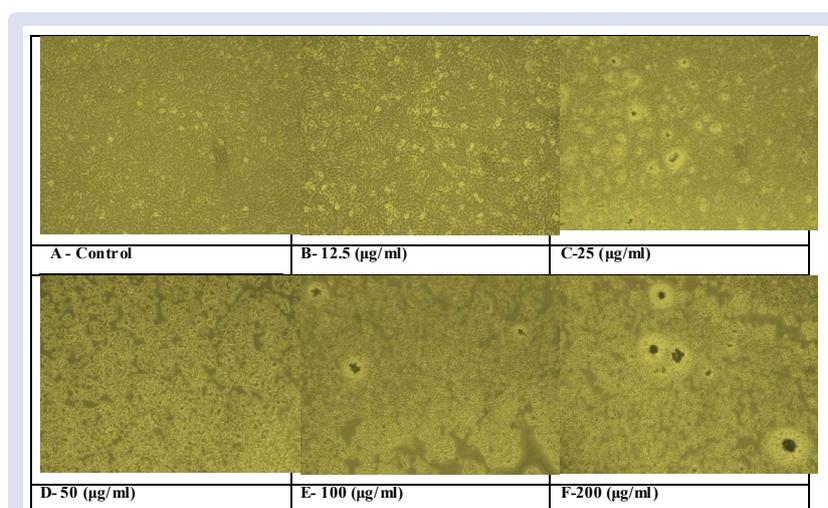
Cell viability data were analysed using one-way ANOVA followed by multiple comparison tests by Dennett with the same sample size using SPSS 17.0.0. The difference was significant when  $P < 0.005$  was assessed.

## RESULTS

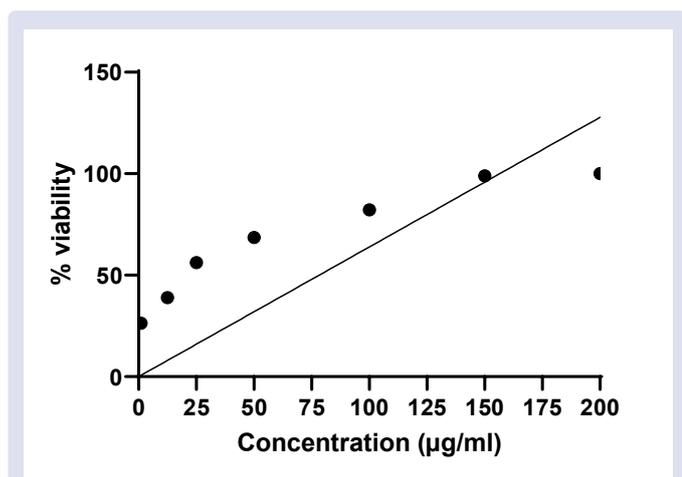
Table 1 tabulates the test concentration and the corresponding percentage of cell growth inhibition. C. Compared with control, *auriculata* exhibited a cytotoxic effect. At 150 and 200µg / ml concentrations, with 61 percent and 73.7 percent, respectively, the highest cytotoxicity was observed. The findings show that cytotoxicity is directly proportionate to the concentration of the extract. Price IC<sub>50</sub> of methanolic extract *C. auriculata* was found to be 102.9µg / ml versus the A549 cell line. Morphological differences were observed in the untreated (control) and treated cells after 24 h under a bright field microscope (Figure 1).

**Table 1: Cytotoxic activity of *C. auriculata* leaf extracts against A549 cell line.**

Concentration (µg/ml)	OD 1	OD 2	OD 3	Mean	% viability	% inhibition
Control	0.943	0.941	0.938	0.941	100.000	0.000
12.5	0.931	0.928	0.934	0.931	98.972	1.028
25	0.773	0.776	0.771	0.773	82.211	17.789
50	0.645	0.642	0.647	0.645	68.533	31.467
100	0.526	0.530	0.529	0.528	56.166	43.834
150	0.367	0.364	0.369	0.367	38.979	61.021
200	0.244	0.248	0.251	0.248	26.329	73.671



**Figure 1:** Morphological changes in cells of A549 after treatment with ethanol extracts of *C. auriculata* leaf extracts.



**Figure 2:** Cell viability after treatment with plant extract on A549 malignant cells.

## DISCUSSION

The key origins of medicines are plant-based herbal extracts. Many herbal extracts and distilled flavonoids have been developed to become medicinal drugs in clinical practice and are used therapeutically in medicine. To evaluate the cytotoxic activity of *C. auriculata* ethanol extracts, the MTT (3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) assay method was used. This technique has been widely used to assess the extract's cytotoxic activity at various concentrations<sup>17</sup>. The basic concept of the method is that every living cell has mitochondrial activity, so the greater the activity, the greater the number of living cells<sup>18</sup>. A cellular reductase enzyme exists in mitochondria that can transform the tetrazolium compound in the MTT reagent into the crystal of purple formazan. The effect of the extract's cytotoxic activity is apparent when the tetrazolium compound's original yellow colour does not change to purple, suggesting low mitochondrial activity. The lower the activity of the mitochondria, the lower the volume of living cells. Usage of the ELISA reader to read the absorption of the sample at 595 nm<sup>17</sup>. The MTT assay uses the IC50-value as a parameter for the interpretation of cytotoxic activity. IC50 is the amount of concentration of the extract that can suppress the cancer cells' 50 percent activity. The relationship between cytotoxic activity and the IC50 value is inversely proportional, so the higher cytotoxic activity is indicated by the lower IC50 value.

According to the IC50 value, anticancer activity can be categorised into four groups: IC50 < 20 µg / mL is categorised as active; IC50: 20-100 µg / mL is classified as moderately active; IC50: 100-1000 µg / mL is classified as weakly active; and IC50 > 1000 µg / mL is classified as inactive<sup>19</sup>. In present study, we have found that value of methanol extract of *C. auriculata* showed IC50 around 102 µg/mL range, which means that *C. auriculata* extracts are weakly active in inhibiting A549 lung cancer cells activity. Subsequently, *C. auriculata* extracts, as a natural anticancer agent, may be further developed. As for anti-tumor medicine, it can be a new source and can be effectively used as an immunological anti-malignant compound. The preliminary phytochemical screening using chemical methods conducted on the ethanolic extract revealed the presence of various phytochemicals like flavonoids, phenols, coumarin, saponins, tannins, terpenoids, steroids and glycosides. The existence of flavonoids can be responsible for the anticancer property.

## CONCLUSION

In the current research, *C. auriculata* ethanolic extract was shown to be a strong suppressant for cell division and proliferation. This anticancer

activity may be due to the presence of glycosides and flavonoids. Further studies are required to conclude the actual phytoconstituents and underlying mechanisms responsible for the anticancer property.

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## CONFLICTS OF INTEREST

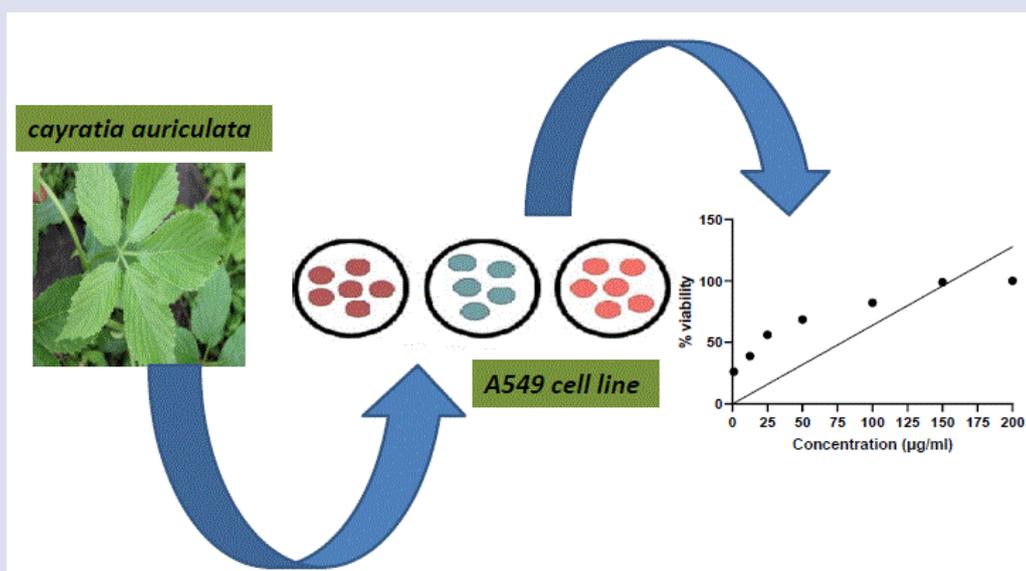
The author declares no competing interest.

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## GRAPHICAL ABSTRACT



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