ORIGINAL ARTICLE

Cytotoxic potentiality of *Colocasia esculenta* leaves extract on five different cancer cell lines using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

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Abstract

Background: Medicinal plants have been playing a major role because of their therapeutic importance in terms of combating various diseases. *Colocasia esculenta* is an annual plant known for its medicinal properties. **Aim:** The present investigation was aimed to screen the cytotoxic potentiality of *C. esculenta* leaves extract against five different cancer cell lines. **Materials and Methods:** Ethanol was used as a solvent to extract plant material using hot extraction method. In our investigation, five different cancer cell lines such as human lung cancer (A549), ovarian cancer (Pa-1), prostate cancer (PC3), colon cancer (HCT 116), and acute leukemia (K562) were exposed to dose dependent cytotoxic studies. 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide assay carried out to determine the efficacy of ethanol extract of indigenous medicinal plant *C. esculenta* leaves in hindering the activity of cancerous cells and cisplatin was used as standard anticancer drug. **Results:** The inhibitory concentration (IC_{50}) values recorded for Pa-1, A549, HCT116, K562, and PC-3 are 93.2 µg/mL, 133.6 µg/mL, 172.87 µg/mL, 217.54 µg/mL, and 223.08 µg/mL, respectively. The IC_{50} values ranged 93.2 µg/mL–223.08 µg/mL indicate the presence of variety of active principles present in the ethanolic extract. **Conclusion:** The ethanolic extract showed dose-dependent activity against all five different cancer cell lines. Investigation provides basic evidence to enlist *C. esculenta* leaves as one of the plants with cytotoxic properties, encourages to isolate bioactive compounds, and understands their interaction with cancer cells.

Key words: *Colocasia esculenta*, human lung cancer (A549), ovarian cancer (Pa-1), prostate cancer (PC3), colon cancer (HCT 116), and acute leukemia (K562)

INTRODUCTION

Cancer is one of the foremost causes of mortality across the globe. Usually, type cancer depends on its tissue of origin. More than 200 types of cancers have been reported. Different approaches are being used to combat caner at various levels.[1] Chemotherapy is one of the promising methods of treatment for most of the cancer types. The side effects of the drugs that are used to treat cancer have forced to advancements in the development of safer bioactive compounds.[2] In the modern research, medicinal plants have been playing a major role because of its therapeutic importance in terms of various diseases.[3]

Colocasia esculenta is an annual herbaceous perennial plant from tropical and subtropical regions and a member of the Araceae family is a low cost and widely consumed food in the human diet perennial plant.[4] It is commonly known as "Taro" in English and "Arbi" or "Khuyya" in Hindi. The plant is also known as "elephant ears," due to the shape of the broad leaves, when it is cultivated for decorative purposes.[5] In India, it is locally cultivated and used as vegetable where the leaves, roots, and corms can be used as dietary ingredients, but the plant must

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be cooked. The corms contain calcium oxalate that makes it acrid when eaten raw or partially cooked.[6]

The plant has been investigated for pharmacological activities antidiabetic, anti-inflammatory, antioxidant, anticancer, analgesic, antidiarrheal, and anticancer activities.[7] As flavonoids, glycosides, sterols, etc., are some of the bioactives found in *C. esculenta*, it becomes important to check its efficacy in the therapeutics.[7] The plant is rich in minerals such as calcium, magnesium, potassium, and phosphorus. The starch is abundant in the roots and the tender leaves are rich in Vitamin C. The *in vitro* anticancerous studies on colonic adenocarcinoma cells have showed that the plant possess mechanism of inducing apoptosis in colon cancer cells and also activates lymphocytes to destroy cancerous cells.[8] The anthocyanins in *C. esculenta* have been reported to inhibit human cancer cell growth.[9] The moderate anticancer activity has been reported in the tuber and leaves extract.[10]

There is a need to explore the treasure of herbs and medicinal plants, especially indigenous medicinal plants to search safer, promising, and easily affordable green medicine to treat various hazards diseases. Hence, in the present study, we attempted to screen *C. esculenta* a medicinal plant which is reported for its phytochemicals against five different types of cancer cell lines.

MATERIALS AND METHODS

Sample Collection and Authentication

The selected plant leaves of *C. esculenta* were collected during the period of August–December 2019 from the Hassan district of Karnataka, India, and the plant sample was authenticated by Dr. Shiddamallayya Mathapathi, Research Officer (Botany), at Regional Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH and Government of India.

Processing of Plant Material

The healthy leaves were separated and washed thoroughly under tap running water followed by 0.1% NaCl to avoid fungal infections and shade dried at room temperature. Fine powder was prepared from the dried leaves of mesh size 20 mm.[11]

Preparation of Extracts

Leaf powder was extracted with ethanol using Soxhlet extractor for 3–4 h. Three hundred grams of leaf powder were subjected to Soxhlet extraction with 900 ml ethanol in the ratio of 1:3. The extract was collected and the solvent was evaporated under vacuum. The dried sample was stored at 4°C for the further experimental purpose.[12]

Cell Line Procurement and Maintenance

Cell lines were obtained from National Centre for Cell Sciences, Pune, and Maharashtra, India, corresponding to human lung (A549), ovary (Pa-1), prostate (PC3), and colon (HCT 116) cancer and acute leukemia (K562) cell lines. All five carcinoma cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with high glucose supplemented with 10% fetal bovine serum. Incubation was carried out at 37°C with an atmosphere of 5% CO_2 maintained the cultures until they reach to 70% confluence growth and avoided the cross contamination.[13]

3-(4,5-dimethylthiazol-2yl)-2,5-Diphenyltetrazolium Bromide (MTT) Cytotoxicity Assay

The assay was carried out on human lung (A549), ovary (Pa-1), prostate (PC3), and colon (HCT 116) cancer, and acute leukemia (K562) cells lines were determined by the MTT. Cell density (20,000 cell/well) was plated in 0.0 2 ml of medium/well in 96-well plates. All these cell lines were culture in required medium and to allow them to grow for 24 hour. Appropriate concentrations of ethanolic crude extract (31.25, 62.5, 125, 250, and 500 µg/ml) dissolved in DMEM media high glucose (Cat No. AL111, HiMedia) were added and incubated for 24 h at 37°C with an atmosphere of 5% CO_2. Cisplatin with the concentration of 15 µM was used as a positive control for the study. After the incubation period, discard the used media and 100 µl of MTT reagent (Cat No: 4060, HiMedia) was added then incubated for 3 h at 37°C. After incubation period, the formed formazan crystals were dissolved with 100 µl of DMSO (Cat No. 1309, Sigma) and the absorbance readings were taken by ELISA Reader (ELX 800, Biotek) at 570 nm and the inhibitory concentration (IC_{50}) value is calculated using linear regression equation, that is, Y =Mx + C derived from the cell viability graph.[14]

The cells viability was determined by the following formula:

% of viability = (OD of test compound treated cells/OD of untreated cells) x 100

RESULTS

Ability of *C. esculenta* leaves ethanolic extract to reduce the cell viability capacity of cancer cell lines was assessed using the MTT assay. The ethanolic extract exhibited good cytotoxic potential against all the cell lines in MTT cytotoxicity assay. A dose-dependent action was shown by ethanolic extract of *C. esculenta* leaves in reducing the number of viable cells [Table 1 and Figure 1]. By increasing the concentration of the plant extract, the cell viability decreased gradually. The cytotoxicity effect of the extract was very high against the human ovarian cancer cell lines (Pa-1) by showing the IC_{50} concentration at 93.2 µg/mL which is comparatively better

than the IC_{50} concentration of other four different cell lines. IC_{50} values were found to be 133.6 µg/mL for human lung cancer cell lines, 172.87 µg/mL for colon cancer cell lines, 217 µg/mL for acute leukemia cancer cell lines, and 223.08 µg/mL for prostate cancer cell lines. *C. esculenta* leaves extract was least effective on prostate cancer cell lines (PC-3) compared to other cell lines. The moderate action was recorded against colon cancer cell lines (HCT-116). All five different cancer cell lines after treating with different concentrations were observed under microscope to evaluate to their response to varying concentrations of plant extracts and photographed.

Table 1: IC_{50} concentration of the ethanolic extract of *Colocasia esculenta* leaves on cell lines

S. No.	Cell lines	IC_{50} (µg/mL)
1.	Pa-1	93.2
2.	A549	133.6
3.	HCT116	172.87
4.	K562	217.54
5.	PC-3	223.08

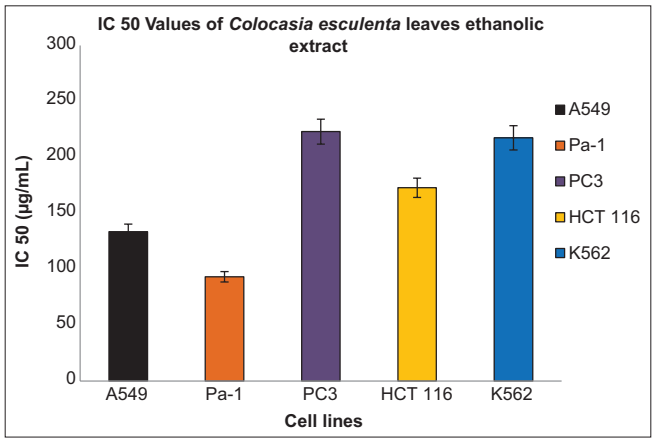

Figure 1: Effect *Colocasia esculenta* extract on A549, Pa-1, PC-3, HCT 116, and K562 cell lines

Figures 2-6 show microscopic images of ethanol extract of *C. esculenta* leaves induced morphological changes of the human lung (A549), ovary (Pa-1), prostate (PC3), and colon (HCT 116) cancer and acute leukemia (K562) cells lines to determine the MTT assay. The images exhibited control group as untreated and graded concentration of the ethanol extract treated group. All the images magnification took at 40×.

DISCUSSION

Scientific interest has been increasing into plant-based phytochemicals to prevent and treat cancer due to negligible toxicity and side effects, availability, low cost, and reliability.[15] Studies conducted by Pritha *et al*. who reported the presence of various phytochemicals in different parts of *C. esculenta*[16] their study reported the antimicrobial and antioxidant potentiality of *C. esculenta*. The presence of tannins, flavonoids, and proteins in considerable amounts was studied by Manjulika *et al*.[14] Phytochemicals exhibit different mode of action while interacting with any pathogens or diseases. Most of the metabolites found to be useful for the human body, hence, there is lesser chance of negative impact when treated with the dose dependent biological activities and for the any kind of diseases.[17-19] In our present investigation, ethanolic extract of *C. esculenta* leaves shown. Studies reported by Jayashree *et al*. and Alcantara *et al*. who also shown that the flavonoids, tannins, and other phytochemicals produce cytotoxic effect on tumor cells line.[20,21] It is also suggested that herbal drugs play anticancer role by enhancing immune system as well as

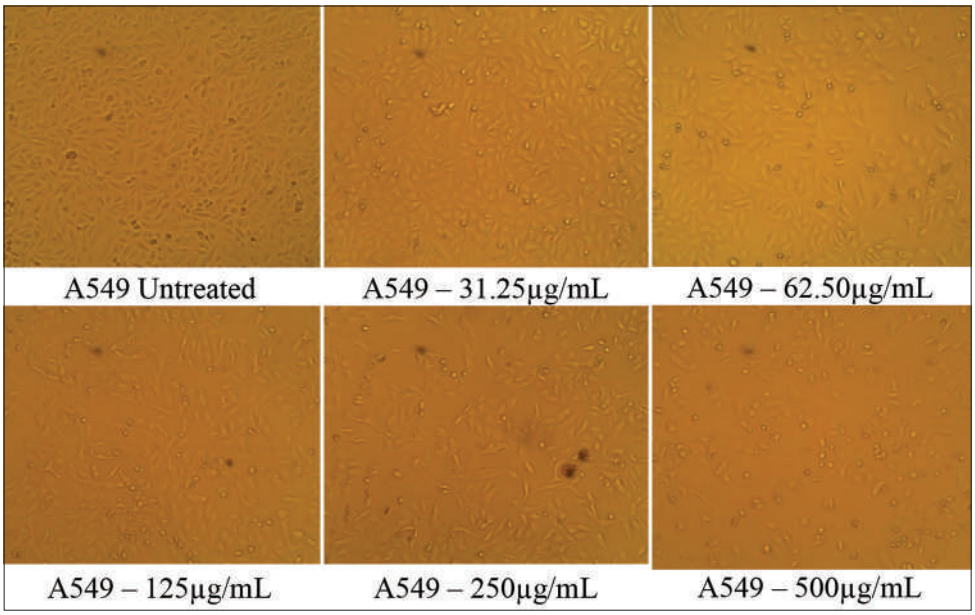

Figure 2: Effect of *Colocasia esculenta* extract on A549 cell line at different concentrations

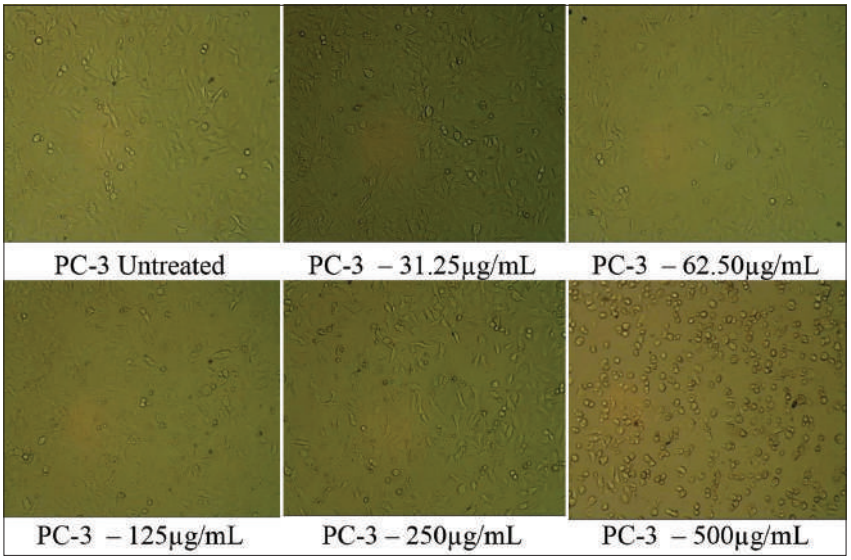

Figure 3: Effect of *Colocasia esculenta* extract on PC3 cell line at different concentrations

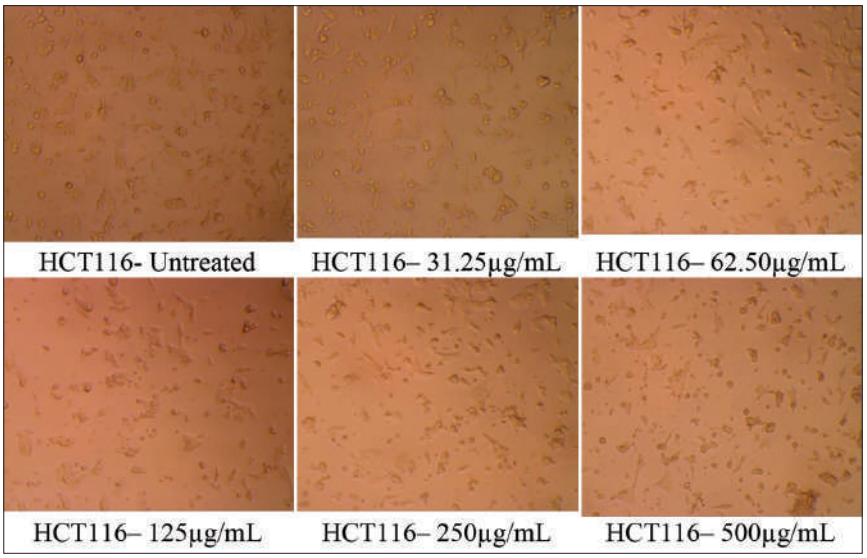

Figure 4: Effect of *Colocasia esculenta* extract on HCT116 cell line at different concentrations

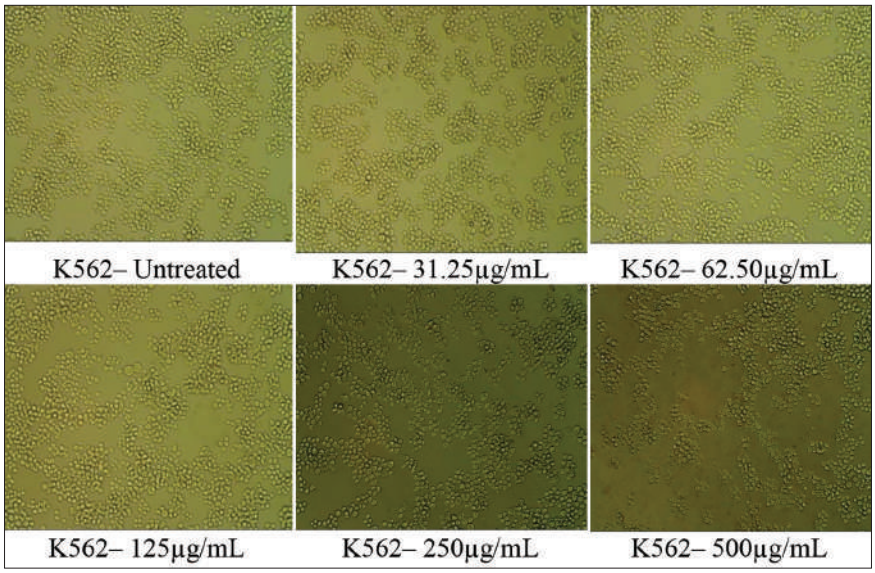

Figure 5: Effect of *Colocasia esculenta* extract on K562 cell line at different concentrations

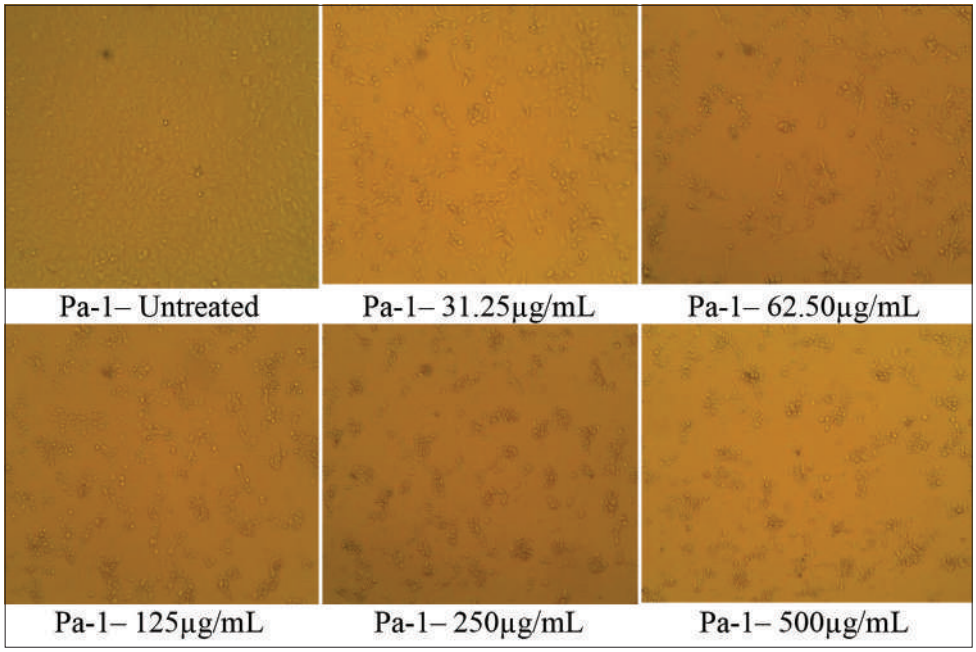

Figure 6: Effect of *Colocasia esculenta* extract on Pa-1 cell line at different concentrations

detoxifying body, inhibition of angiogenesis, and also cell differentiation.[22] Hence, the present experimental approach proves the potential use of ethanolic extract of *C. esculenta* as a source of anticancer drug.

CONCLUSION

The cytotoxic effect of the ethanolic extract of the against the human lung, ovary, prostate, carcinoma cell lines, and leukemia cell lines such as A549, Pa-1, PC3, HCT 116, and K562 with controls was investigated by the MTT assay compared with cisplatin as a reference standard drug. It had been observed that cytotoxic activity of the extract was high against PA-1 cell lines followed by other cancer and leukemia cell lines. Thus, the initial investigation suggests us that the sample may have possible therapeutic potential against human ovarian cancer (PA-1) derived diseases and has made the way for further investigations using advanced techniques so it should bring a hope on the treatment of human ovarian cancer (PA-1) using arum.

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