Academic Research Journal of Science and Technology (ARJST)
Volume 5, Issue 04, July 2026
Peer-Reviewed | Open Access | Crossref DOI & Global Indexing | Google Scholar | Multidisciplinary

eISSN:**3048-9644**

Anthocyanin Quantification and In Vitro Anticancer Activity of Alpinia purpurata Flower Extract Against Human Lung Adenocarcinoma (A549) Cells

Sumi P[1*], Nirmala Devi N[2],

[1]*Department of Biochemistry, Sree Narayana Guru College, KG Chavadi, Coimbatore, Tamil Nadu-641105, India*

[2]*Associate Professor and Head of the Department of Biochemistry, Sree Narayana Guru College, KG Chavadi, Coimbatore, Tamil Nadu-641105, India. Email: biochemnirmala@gmail.com.*

**Corresponding Author Email: sumiaryar05@gmail.com*

DOI: https://doi.org/10.63300/arjst0504062026.03

Date of Submission: 01-07-2026 Date of Acceptance: 14-07-2026

Abstract

Anthocyanins are naturally occurring flavonoid pigments recognized for their strong antioxidant and anticancer properties. Alpinia purpurata, an important medicinal plant, is a rich source of bioactive phytochemicals with potential therapeutic applications. The present study investigates the total anthocyanin content of Alpinia purpurata extract and evaluates its in vitro anticancer activity against the human lung adenocarcinoma (A549) cell line using the MTT assay. Anthocyanin content was estimated by the pH differential spectrophotometric method, while cytotoxic activity was assessed at extract concentrations of 25, 50, and 100 µg/mL, with doxorubicin serving as the positive control. The results demonstrated a concentration-dependent increase in cytotoxicity, with values of **37.6 ± 0.75%**, **54.3 ± 1.05%**, and **65.6 ± 0.75%** at 25, 50, and 100 µg/mL, respectively, accompanied by a corresponding decrease in cell viability to **62.3 ± 1.05%**, **45.6 ± 0.75%**, and **34.3 ± 1.05%**. The extract exhibited an **IC_{50} value of 41.6 µg/mL**, indicating significant antiproliferative activity against A549 cells. Microscopic examination further confirmed dose-dependent morphological alterations, including cell shrinkage, loss of normal morphology, and increased numbers of dead cells following treatment. At the highest concentration (100 µg/mL), the extract produced cytotoxicity comparable to that of doxorubicin (**71.9 ± 0.57%**). These findings demonstrate that Alpinia purpurata extract possesses appreciable anthocyanin content and significant cytotoxic potential against lung cancer cells, suggesting that it may serve as a promising natural source of anticancer compounds. Further phytochemical characterization and mechanistic studies are warranted to identify the active constituents and validate their therapeutic potential for lung cancer management.

Keywords: *Alpinia purpurata, anthocyanins, A549 cell line, lung cancer, MTT assay, cytotoxicity, phytochemicals, anticancer activity.*

INTRODUCTION

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, accounting for a significant proportion of cancer deaths due to its high incidence, late-stage diagnosis, and resistance to conventional therapies. Despite advances in chemotherapy, radiotherapy, targeted therapy, and immunotherapy, the development of drug resistance and severe adverse effects continue to limit treatment outcomes. Consequently, there is growing interest in identifying naturally derived bioactive compounds with improved therapeutic efficacy and reduced toxicity.

Medicinal plants have long served as valuable sources of pharmacologically active compounds, including flavonoids, phenolics, alkaloids, and terpenoids, which exhibit antioxidant, anti-inflammatory, and anticancer properties. Among these phytochemicals, anthocyanins are water-soluble flavonoid pigments responsible for the red, purple, and blue coloration of many plants. Numerous studies have demonstrated that anthocyanins possess potent antioxidant activity and can inhibit cancer cell proliferation by inducing apoptosis, suppressing oxidative stress, and modulating signaling pathways involved in tumor progression.

Alpinia purpurata (red ginger), a member of the Zingiberaceae family, is an ornamental and medicinal plant traditionally used in folk medicine for treating various ailments. Although several species of *Alpinia* have been reported to possess significant biological activities, the anticancer potential of *Alpinia purpurata* remains relatively unexplored. Therefore, the present study aimed to estimate the total anthocyanin content of *Alpinia purpurata* extract using the pH differential method and to evaluate its in vitro cytotoxic activity against the human lung adenocarcinoma (A549) cell line using the MTT assay. The findings of this study may provide scientific evidence supporting the potential use of *Alpinia purpurata* as a natural source of anticancer agents for future therapeutic applications.

REVIEW OF LITERATURE

Raj et al. (2012) evaluated the *in vitro* antioxidant and anticancer activities of *Alpinia purpurata* ethyl acetate extract using free radical scavenging assays and the MTT assay. The study reported concentration-dependent antioxidant activity and significant cytotoxic effects against OAW42 ovarian cancer cells, suggesting that *A. purpurata* possesses promising anticancer potential. **Chan and Wong (2015)** reviewed the phytochemistry and pharmacological properties of *Hedychium coronarium* and *Alpinia purpurata*. They reported that *A. purpurata* contains flavonoids, phenolic compounds, and other bioactive metabolites exhibiting antioxidant, antibacterial, anti-inflammatory, and cytotoxic activities, highlighting its importance as a medicinal plant with therapeutic potential. **Tsuda (2018)** reviewed the physiological functions of anthocyanins and highlighted their role in reducing oxidative stress and regulating intracellular signaling pathways associated with cancer progression. The study suggested that anthocyanins interfere with cell proliferation, angiogenesis, and metastasis, making them promising candidates for anticancer therapy. **Lin et al. (2016)** reviewed the role of anthocyanins in cancer prevention and treatment. The authors concluded that anthocyanins inhibit cancer cell proliferation through antioxidant activity, induction of apoptosis, cell cycle arrest, and suppression of metastasis, making them promising natural compounds for cancer therapy. **Nascimento et al. (2022)** conducted a systematic review on the *in vitro* anticancer properties of anthocyanins. The review summarized evidence that anthocyanins significantly reduce cancer cell viability, induce apoptosis, regulate the cell cycle, and inhibit tumor progression in various human cancer cell lines, supporting their potential as natural anticancer agents. **Sumi and Nirmala Devi (2023)** reviewed the phytochemical and pharmacological activities of *Alpinia purpurata* and reported the presence of flavonoids, rutin, kaempferol derivatives, and other phenolic compounds. They concluded that the plant possesses antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, emphasizing its potential for pharmaceutical and nutraceutical applications. **Youn et al. (2024)** reviewed the phytochemical and pharmacological properties of the genus *Alpinia* published between 2016 and 2023. The authors highlighted that *Alpinia* species contain diverse bioactive constituents, including flavonoids, diarylheptanoids, terpenoids, and

phenolics, which exhibit significant antioxidant, anti-inflammatory, and anticancer activities, indicating their potential in drug discovery. **Chen et al. (2020)** reviewed the molecular mechanisms underlying the anticancer activities of anthocyanins. The authors reported that anthocyanins induce apoptosis, arrest the cell cycle, inhibit angiogenesis, and suppress metastasis through regulation of NF-κB, PI3K/Akt, and MAPK signaling pathways in different cancer models.**Zhang et al. (2020)** reviewed recent advances in anthocyanin research and reported that anthocyanins exhibit significant cytotoxicity against breast, colon, lung, liver, and gastric cancer cells. They concluded that these compounds exert anticancer effects through antioxidant activity, mitochondrial-mediated apoptosis, and inhibition of tumor invasion.

Jing et al. (2021) reviewed the pharmacological activities of anthocyanins and emphasized their therapeutic potential against chronic diseases, including cancer. The authors reported that anthocyanins reduce oxidative damage, regulate inflammatory mediators, and induce programmed cell death in malignant cells while exhibiting low toxicity toward normal cells. **Luo et al. (2021)** summarized the mechanisms of anthocyanin-mediated cancer prevention and treatment. The review concluded that anthocyanins inhibit tumor growth by modulating oxidative stress, inflammatory responses, autophagy, apoptosis, and cell cycle progression, supporting their application in functional foods and pharmaceuticals. **Teng et al. (2022)** reviewed the anticancer mechanisms of anthocyanins and reported that these compounds suppress tumor initiation, proliferation, angiogenesis, and metastasis through multiple molecular targets. The authors suggested that anthocyanins could serve as complementary agents in cancer therapy due to their multitargeted biological activities. **Li et al. (2023)** reviewed recent developments in anthocyanin-based cancer therapy. The authors highlighted that anthocyanins exhibit selective cytotoxicity against various cancer cell lines, including lung, liver, breast, and colorectal cancers, by inducing apoptosis, regulating mitochondrial function, and reducing oxidative stress. **Prasad et al. (2023)** reviewed medicinal plants belonging to the family Zingiberaceae and reported that species of *Alpinia* contain flavonoids, phenolic acids, diarylheptanoids, and terpenoids with remarkable antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. The review emphasized the importance of *Alpinia* species as promising sources of bioactive compounds for pharmaceutical applications.

OBJECTIVES OF THE STUDY

1. To prepare the extract of *Alpinia purpurata* using suitable solvents for phytochemical analysis.
2. To estimate the total anthocyanin content of *Alpinia purpurata* extract using the pH differential spectrophotometric method.
3. To assess the cytotoxic activity of *Alpinia purpurata* extract against A549 human lung cancer cells using the MTT assay.
4. To determine the concentration-dependent effect of the extract on cancer cell viability and cytotoxicity at different concentrations (25, 50, and 100 µg/mL).
5. To determine the IC_{50} value of *Alpinia purpurata* extract against the A549 cell line.
6. To compare the anticancer efficacy of *Alpinia purpurata* extract with the standard anticancer drug doxorubicin.
7. To evaluate the potential of anthocyanin-rich *Alpinia purpurata* extract as a promising natural source of anticancer agents for lung cancer therapy.

Academic Research Journal of Science and Technology (ARJST)
Volume 5, Issue 04, July 2026
Peer-Reviewed | Open Access | Crossref DOI & Global Indexing | Google Scholar | Multidisciplinary
eISSN:3048-9644

METHODOLOGY

1. Anthocyanin estimation

To estimate the anthocyanin, 0.5 g of the sample was dissolved in coical flask containing 20ml of a water, ethanol and methanol solvent. Then it was incubated in orbital shaker at 40°C for 24 hours. The mixture was filtered through a Buchner funnel to obtain the supernatant. The anthocyanin concentration was determined using a pH method, with 0.5M of HCl prepared for pH 1 and 0.5M NaOH solution prepared for pH 4.5. For the analysis, 2ml of the supernatant was measured at 520nm and 700nm using a spectrophotometer for pH1 and pH 4.5, to calculate the quantity of anthocyanin.

2. Anti-cancer activity

Determining the anti-cancer activity of ALPINIA PURPURATA extract in A 549 (Lung cancer cell lines) using MTT - Cytotoxicity assay Human lung cancer cell line (A 549) was used in the present study to determine the anticancer activity of the samples (ALPINIA PURPURATA extract) separately. Cytotoxicity activity of the sample on different concentrations (25, 50 and 100µg/mL) of cancer cell line was evaluated in-vitro. It is based on the use of tetrazolium salt 3-[4,5-dimethylthiazolyl-2]-2,5-diphenyl tetrazolium bromide (MTT), which can be converted to an insoluble blue formazan product by mitochondrial enzymes in viable cells. The cell lines were cultivated in 12-well-microtitre plates to reach confluence growth. The provided samples were applied directly to the developed cell line monolayer. Before cell seeding, the specimens were pre-wetted in 70 % aqueous ethanol solution for 48 h, rinsed twice with ultrapure water and immersed in 1ml DMEM medium in 24-well plates for 2h in an incubator at 37°C. The specimens were then seeded with cell line at 10,000 cells per well according to routine cell-culture methods. The plates were incubated at 37°C and 5% CO 2 for fifteen days. At each time point, samples were taken from the 24-well plates and transferred into new plates for the MTT study. The MTT solution was prepared by dissolving the powder in phosphate buffered saline at a concentration 1mg/ml. After 1hr of incubation, the purple crystals were dissolved by adding sodium dodecylsulphate (SDS) in a 1:1 mixture of water and dimethyl formamide (DMF) at a concentration of 20% w/v. After adding 1ml of MTT medium (0.0005mg/ml) to each well, the plates were incubated for 3h, rinsed and desorbed in 100ul of 70% isopropanol. After being agitated rapidly at 400rpm/min for 40min, the dyed medium was transferred to 96-well plate, and read at 550nm using ELISA reader. Cell proliferation and cell viability was calculated and observed under inverted microscopy. The difference between the untreated, sample treated and positive control (Doxorubicin treated) cell line images were presented separately.

RESULTS

Table No 1

Total Anthocyanin content

Sample	Total content of anthocyanin (mg/g)
Water	58.44
Methanol	31.72
Ethanol	20.03

The total anthocyanin content of *Alpinia purpurata* extracts prepared using different solvents was determined by the pH differential spectrophotometric method. The anthocyanin content varied significantly depending on the extraction solvent (Table 1). Among the solvents tested, the water extract exhibited the highest total anthocyanin content (58.44 mg/g), followed by the methanol extract (31.72 mg/g) and the ethanol extract (20.03 mg/g).

The results indicate that water was the most effective solvent for extracting anthocyanins from *Alpinia purpurata*. The higher anthocyanin content observed in the aqueous extract suggests that these pigments are highly soluble in polar solvents. Since anthocyanins are well known for their antioxidant and anticancer properties, the higher anthocyanin concentration in the water extract may contribute to its biological activity. These findings demonstrate that solvent selection plays a crucial role in maximizing anthocyanin extraction and may influence the therapeutic potential of *Alpinia purpurata* extracts.

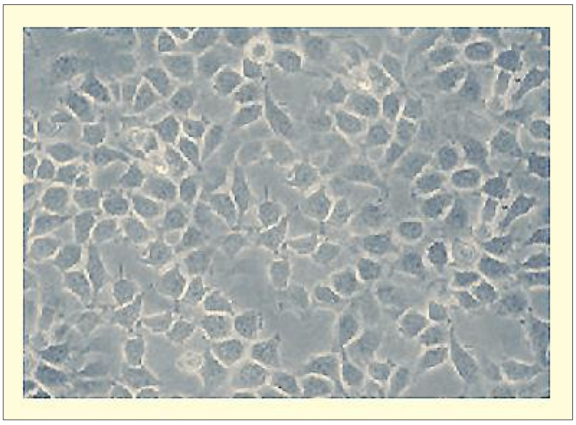
Table No. 2: Cytotoxicity assay of Sample - ALPINIA PURPURATA EXTRACT in A 549 cell lines

A_{549} Cells (Untreated and Treated with sample and Doxorubicin)	**A_{549} cell lines – Cytotoxicity Assay**			**IC_{50}**
	Concentration (µg/ml)	**Cytotoxicity (%)**	**Cell viability (%)**	
Untreated (A)	Control	0	> 99	No cytotoxicity
Cancer (B)	25	37.6 ± 0.75	62.3 ± 1.05	IC_{50} = 41.6µg/ml
Cancer (C)	50	54.3 ± 1.05	45.6 ± 0.75	
Cancer (D)	100	65.6 ± 0.75	34.3 ± 1.05	
Control (Doxorubicin- E)	10	71.9 ± 0.57	28.3 ± 1.05	Cytotoxicity

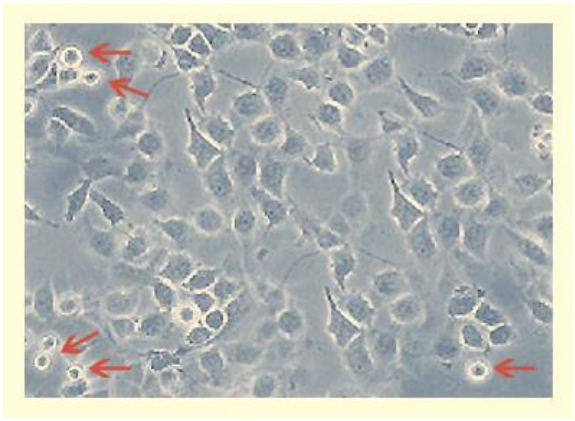
Inference

- Increase in concentration of Samples increased the cancer cell cytotoxicity. This is evident from Table-1 and Fig. 1.
- IC_{50} value for the given Sample (*ALPINIA PURPURATA* EXTRACT) is 41.6µg/ml.
- When compared to positive control, Doxorubicin (71.9 ± 0.57%) almost similar cytotoxicity percentage (65.6 ± 0.75 %) was found evident for the higher concentration (100µg/ml) of Sample – *ALPINIA PURPURATA* EXTRACT.

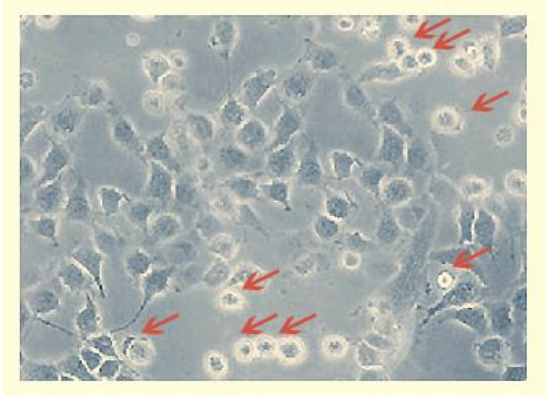
Academic Research Journal of Science and Technology (ARJST)
Volume 5, Issue 04, July 2026
Peer-Reviewed | Open Access | Crossref DOI & Global Indexing | Google Scholar | Multidisciplinary
eISSN:3048-9644



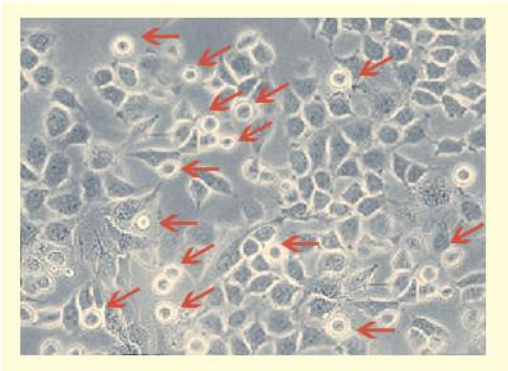
A. Untreated A_{549} cells



B. Treated A_{549} cells with 25µg/ml of Alpinia purpurata Extract

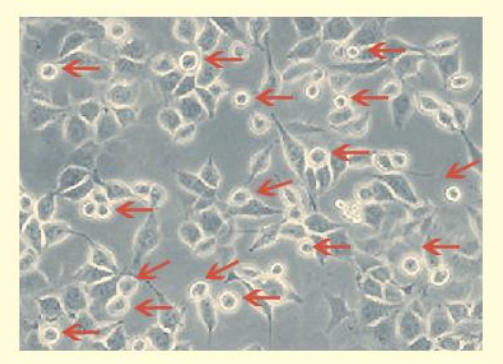


C. Treated A_{549} cells with 50µg/ml of Alpinia purpurata extract



Academic Research Journal of Science and Technology (ARJST)
Volume 5, Issue 04, July 2026
Peer-Reviewed | Open Access | Crossref DOI & Global Indexing | Google Scholar | Multidisciplinary
eISSN:3048-9644

D. Treated A_{549} cells with 100µg/ml Alpinia purpurata extract



E. Treated A_{549} cells with Doxorubicin (Positive control)

Figure 1: Microscopic images of A_{549} cell lines

(Untreated, Sample – *ALPINIA PURPURATA* EXTRACT and Doxorubicin treated cells)

- Microscopic images revealed morphology of untreated, doxorubicin-treated and Sample-Alpinia purpurata extract-treated lung cancer cells after treatment with three different concentrations (25µg/ml, 50µg/ml and 100µg/ml).
- Untreated control cells were found to be in fusiform shape (Fig. 1A); whereas, the cells treated with Sample-Alpinia purpurata extract produced many white circular dead cells under microscope.
- The number of dead cells found increasing as the concentration of Sample-Alpinia purpurata extract increased. This was found evident from the images presented in Fig. 1B, 2C and 2D.

CONCLUSION

The present study demonstrates that *Alpinia purpurata* is a promising medicinal plant with significant anthocyanin content and notable **in vitro** anticancer activity against the human lung adenocarcinoma (A549) cell line. Anthocyanin estimation revealed that the extraction efficiency varied with the solvent used, with the aqueous extract showing the highest anthocyanin content (58.44 mg/g), followed by methanol (31.72 mg/g) and ethanol (20.03 mg/g). These findings indicate that water is the most effective solvent for extracting anthocyanins from *A. purpurata*.

The MTT assay demonstrated a concentration-dependent increase in cytotoxicity, with the extract exhibiting an IC_{50} value of 41.6 µg/mL. At the highest tested concentration (100 µg/mL), the extract produced 65.6% cytotoxicity, which was comparable to the positive control, doxorubicin. Microscopic observations further confirmed the induction of cell death through morphological changes, including cell shrinkage and disruption of normal cellular architecture.

The observed cytotoxic effects may be attributed to the presence of anthocyanins and other bioactive phytochemicals with known antioxidant and antiproliferative properties. Overall, the findings suggest that *Alpinia purpurata* possesses considerable potential as a natural source of anticancer compounds. Further studies involving phytochemical characterization, molecular mechanism analysis, toxicity assessment, and **in vivo** evaluation are required to validate its therapeutic efficacy and support its development as a potential plant-based treatment for lung cancer.

REFERENCES:

[1]. Raj, C. A., Ragavendran, P., Sophia, D., Rathi, M. A., & Gopalakrishnan, V. K. (2012). Evaluation of *in vitro* antioxidant and anticancer activity of *Alpinia purpurata*. *Chinese Journal of Natural Medicines, 10*(4), 263–268.

[2]. Chan, E. W. C., & Wong, S. K. (2015). Phytochemistry and pharmacology of ornamental gingers, *Hedychium coronarium* and *Alpinia purpurata*: A review. *Journal of Integrative Medicine, 13*(6), 368–379.

[3]. Tsuda, T. (2018). Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies. *Molecular Nutrition & Food Research, 62*, 1700914.

[4]. Lin, B.-W., Gong, C.-C., Song, H.-F., & Cui, Y.-Y. (2017). Effects of anthocyanins on the prevention and treatment of cancer. *British Journal of Pharmacology, 174*(11), 1226–1243.

[5]. Nascimento, E. A., Coutinho, L. L., Silva, C. J., Lima, V. L. A. G., & Aguiar, J. S. (2022). *In vitro* anticancer properties of anthocyanins: A systematic review. *Biochimica et Biophysica Acta (BBA) – Reviews on Cancer, 1877*(4), 188748.

[6]. Sumi, P., & Nirmala Devi, N. (2023). Review article on phytochemical and pharmacological activities of *Alpinia purpurata* and *Erythrina variegata*. *Research Journal of Pharmacognosy and Phytochemistry, 15*(4), 298–304.

[7]. Youn, I., Han, A.-R., Piao, D., Lee, H., Kwak, H., Lee, Y., Nam, J.-W., & Seo, E. K. (2024). Phytochemical and pharmacological properties of the genus *Alpinia* from 2016 to 2023. *Natural Product Reports, 41*, 1346–1367.

[8]. Chen, X., et al. (2020). Anthocyanins as potential anticancer agents: Molecular mechanisms and therapeutic perspectives. *Food & Function, 11*, 6415–6430.

[9]. Zhang, Y., et al. (2020). Recent advances in anthocyanin research for cancer prevention and treatment. *Journal of Agricultural and Food Chemistry, 68*, 12325–12342.

[10]. Jing, P., et al. (2021). Anthocyanins in cancer prevention and therapy: Molecular mechanisms and pharmacological effects. *Critical Reviews in Food Science and Nutrition, 61*, 2473–2491.

[11]. Luo, C., et al. (2021). Anthocyanins in cancer prevention: Molecular mechanisms and therapeutic applications. *Nutrients, 13*, 2386.

[12]. Teng, H., Fang, T., Lin, Q., Song, H., Liu, B., & Chen, L. (2022). Red raspberry and other anthocyanin-rich foods in cancer prevention and treatment: A review. *Critical Reviews in Food Science and Nutrition, 62*, 1185–1208.

[13]. Li, Y., et al. (2023). Anthocyanins in cancer prevention and therapy: Recent advances and future perspectives. *International Journal of Molecular Sciences, 24*, 4502.

[14]. Prasad, K. N., et al. (2023). Pharmacological importance of Zingiberaceae plants: A comprehensive review. *Journal of Herbal Medicine, 39*, 100651.