Current Botany 2026, 17: 24-30
https://doi.org/10.25081/cb.2026.v17.9660

Research Article

In vitro antioxidant, cytotoxicity and FTIR analysis of leaf extract of *Ayapana triplinervis*

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(Received: July 23, 2025; Revised: March 24, 2026; Accepted: March 26, 2026; Published: April 10, 2026)

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Abstract

Ayapana triplinervis (Vahl) R.M. King & H. Robinson belongs to the family Asteraceae, is an erect, perennial, ethnomedicinal, ornamental herb and is widely used in various mystical religious rituals of the various traditional communities in Asia, Africa, and South America. The leaves are used as decoctions, teas, antiviral agents, blood coagulants, cardiac stimulants, expectorants, emetic laxatives, antiscorbutic and are widely used in wounds, bleeding stomachs, diarrhoea, etc. The main aim of the proposed work is the preliminary phytochemical screening, *in vitro* antioxidant, cytotoxicity and FTIR analysis of crude methanolic leaf extract of *A. triplinervis.* The preliminary phytochemical analysis reveals the presence of glycosides, alkaloids, terpenoids, phenols, flavonoids, tannins and saponins, whereas steroids were absent. In antioxidant analysis, the DPPH radical scavenging assay and the nitric oxide radical scavenging assay were selected. In both the assays, it was shown that the ability of the methanolic leaf extract had the highest scavenging effect on DPPH radical than that of nitric oxide radical, which was increasing with an increase in the concentration of the sample. In *in vitro* cytotoxic analysis, leaf extract showed potential cytotoxic activity against different cell lines like HCT 116 (Human Colon Cancer Cell Line) and MCF 7 (Human Breast Cancer Cell Line). The cytotoxic effect was expressed as IC_{50}. The IC_{50} value against the HCT 116 cell line was 80.14±3.12 µg/mL whereas 94.02±2.43 µg/mL against the MCF7 cell line, which indicated that the crude leaf extract of *A. triplinervis* showed the highest cytotoxicity against selected cancer cell lines. The functional group identification of the active compounds in the plant samples based on the peak value is mainly done by FTIR analysis. This study revealed that crude leaf extract of *A. triplinervis* exhibits high antioxidant and cytotoxicity potentials and will be helpful for the sustainable utilization of the plant.

Keywords: *Ayapana triplinervis*, *In vitro* antioxidant, *In vitro* cytotoxicity, FTIR analysis

Introduction

Plants are the inevitable source of medicines which play an important role in world health (Salmerón-Manzano *et al.,* 2020). Drug discovery must be a continuing process which leads to the search for numerous medicinal plants with the greatest demand due to its pharmaceutical, cosmetic, nutritional and medicinal applications. Since ancient times, herbal plants acted as good antioxidants, anticancer, antimalarial, anti-inflammatory, and hepatoprotective agents (Rivera *et al.,* 2013). One of such medicinally important herbs is *Ayapana triplinervis* (Vahl) R.M. King & H. Robinson.

A. triplinervis is an erect, perennial, ornamental herb which belongs to the family Asteraceae, is widely used as a folk medicinal plant in the healing processes, used in mystical religious rituals by the traditional communities in Asia, South America and Africa (Rajasekaran *et al.,* 2010). *A. triplinervis*, *E. ayapana* and *E. triplinerve* are the three Latin names of Ayapana and in Ayurvedic literature, it is named as 'Vishalyakarani' (Rodrigues *et al.,* 2022a, b). The plant is used in wounds, haemorrhages, bleeding stomachs, snake bite, nausea and vomiting (Cheriyan *et al.,* 2019). The leaves are widely used as decoctions, teas or baths against viral infections, urinary tract infections, respiratory and gynaecological diseases in Brazil, Bangladesh, Peru, Mauritius, and some European countries (Gauvin-Bialecki & Marodon, 2008; Cheriyan *et al.,* 2019). The plant is thought to be a good cardiac stimulant, antiscorbutic, expectorant, laxative, alternative, antineoplastic, astringent and antidysenteric (Ajeesh Krishna *et al.,* 2024).

A. triplinervis contains numerous secondary metabolites such as various coumarins like ayapin, ayapanin, daphnetin etc., essential oils like thymoquinone, thymohydroquinone dimethyl ether, β-caryophyllene, β-selinene, dimethyl ether thymoquinone and 2,5 dimethoxy p-cymene, thymohydroquinone dimethyl ether, stigmasterol and carotene (Haddad *et al.,* 2019). The plant possesses various biological activities such as antioxidant, antimicrobial, hepatoprotective, anticancer, anti-inflammatory, anti-nociceptive, antimelanogenic, antidepressant, anxiolytic, antiulcer, hypocholestemic, antidiabetic and gastroprotective effects (Venugopala *et al.,* 2013). A phytoalexin named Ayapin or 6,7 methylendioxycoumarin, used as an antiviral and antimicrobial agent, was isolated from this plant (Balakrishnan *et al.,* 2021). An anticoagulant drug Warfarin is isolated from this plant (Ajeesh Krishna *et al.,* 2024).The essential oils of *A. triplinervis* produced a nanoemulsion which used as a mosquito repellent (Rodrigues *et al.,* 2021). In general, some properties of this plant have been documented previously but still there are lack of knowledge and research on various aspects like cytotoxic activity against cancer cell lines, FTIR analysis etc. So, this is an attempt to estimate the preliminary phytochemical screening, *in vitro* antioxidant, cytotoxicity and FTIR analysis of leaf extract of *A. triplinervis.*

Materials and Methods

Preparation of whole methanol extract

Fresh plant materials (Figure 1) were collected from various places in the Kollam district. The botanical identification was carried out in the Department of Botany. Herbarium of the *Ayapana triplinervis* bearing the voucher number TKMCAS/KOL/I/2025/MSJ/01 was deposited the same at TKM College of Arts & Science, Kollam for future reference. Collected leaves of the plantwere cleaned, weighed, and dried under shade followed by oven drying. 50 g of dried powdered material was defatted with petroleum ether (60 to 85 °C) in a Soxhlet apparatus (hot extraction method) to remove waxy substances. The defatted powder leaf material was further extracted with methanol for 72 h (Sangeetha & Jisha, 2025). Crude methanol extract was used for preliminary phytochemical studies and *in vitro* antioxidant, cytotoxicity and FTIR analysis.

Preliminary phytochemical studies

The preliminary phytochemical studies of crude leaf extract were done for the detection of phytoconstituents, using standard chemical tests (Sangeetha & Jisha, 2025).

In vitro antioxidant activity of A. triplinervis

DPPH radical scavenging Assay (2, 2-diphenyl -1-picryl hydrazyl assay)

According to the method of Rao *et al.* (2014) the radical scavenging activity of the crude methanolic leaf extract against stable DPPH reagent (2,2-diphenyl 1- picryl hydrazyl hydrate) was determined with slight modification. In this assay, standard drug ascorbic acid was used. The ascorbic acid stock solution was prepared in distilled water (1 mg/mL). A 60 µM solution of DPPH in methanol was freshly prepared and a 200 µL of this solution was mixed with 50 µL of leaf extract at various concentrations (3.12, 6.25, 12.5, 25, 50, 100 µg/mL). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank (Sangeetha & Jisha, 2025). DPPH radical scavenging activity was calculated by using the following formula:

Figure 1: *Ayapana triplinervis* (Vahl) R.M. King & H. Robinson

$$\% \text{ inhibition} = (\text{O.D of control - O.D. of Test/O.D. of control}) \times 100$$

The antioxidant activity of the extract was expressed as IC_{50}. IC_{50} value was defined as the concentration (in µg/mL) of extracts that inhibits the formation of DPPH radicals by 50% (Sangeetha & Jisha, 2025).

Nitric oxide scavenging assay

Griess Illosvory reaction was used to estimate thenitric oxide radical inhibition. In this assay, Griess Illosvory reagent was normally modified by using naphthyl ethylene diamine dihydrochloride (0.1%) rather than 1-napthylamine (5%). A total 3 mL of reaction mixture containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL saline phosphate buffer and 0.5 mL of standard solution or samples (6.25-200 µg/mL) were incubated at 25°C for 150 minutes. After incubation, 1 mL of the reaction mixture was mixed with 1 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for the completion of the reaction of diazotization. After this, a further 1 mL of the naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 minutes at 25°C (Sangeetha & Jisha, 2025). The concentration of nitrite was assayed at 546 nm and was calculated with the control absorbance of the standard nitrite solution (without extracts or standards, but under the same condition should be followed). Here buffer was used as a blank solution and ascorbic acid was taken as a standard solution (Sangeetha & Jisha, 2025). The antioxidant activity of the extract was expressed as IC_{50}.

$$\% \text{ inhibition} = (\text{O.D of control - O.D. of Test/O.D. of control}) \times 100$$

In vitro cytotoxic activity of A. triplinervis

MTT cell viability assay

HCT 116-Human Colon Tumor Cell Line and MCF 7-Human Breast Cancer Cell line were purchased from NCCS (National Centre for Cell Sciences) Pune, India, were maintained in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 1% antibiotic cocktail containing Penicillin (100 µg/mL) and 10% heat inactivated Fetal Bovine Serum (FBS), Amphotericin B (2.5 µg/mL) and Streptomycin (100 µg/mL). TC flasks (25 cm^2) containing the cells were incubated at 37°C at 5% CO_2 environment with humidity in a cell culture incubator (Galaxy® 170 Eppendorf, Germany). The cells (500 cells/well) were seeded on 96-well plates and allowed to acclimatize to the culture conditions such as 37°C and 5% CO_2 environment in the incubator for 24 h. Various concentration of leaf extractsuch as 6.25, 12.5, 25, 50 and 100 µg/mL were then diluted in DMEM media and added to the wells containing cultured cells. Untreated wells were

kept as controls. The plates were further incubated for 24 h after treatment. Then the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 h for the development of formazan crystals. The supernatant was removed and 100 µL DMSO was added per well. The absorbance at 570 nm was measured with a microplate reader. Two wells per plate without cells served as a blank. All the experiments were done in triplicate (Ho *et al.,* 2021; Sangeetha & Jisha, 2025). The cell viability was expressed using the following formula:

Percentage viability= (OD of test/OD of control) × 100

Fourier transform infrared spectrophotometeric (FTIR) analysis

A powerful tool used for analysing the functional groups such as chemical bonds present in the sample is FTIR. For this analysis, dried powder leaf methanolic extract was used. A dried extract powder (10 mg) was encapsulated in 100 mg of KBr pellets, to prepare the translucent sample discs. Then it was loaded in the FTIR spectroscope at the scan range 400 to 4000 cm^{-1} s with a resolution of 4 cm^{-1} (Balade *et al.,* 2018; Sangeetha & Jisha, 2025).

Statistical analysis

All the above experiments were performed in triplicate. Results of experimental assays were expressed as Mean±standard deviations were calculated by GraphPad InStat DTCG. According to the Tukey-Kramer Multiple Comparisons Test, analysis of variance was carried out.

Results

Preliminary phytochemical evaluation of A. triplinervis

Phytochemicals are bioactive compounds naturally produced by plants and have gained significant attention due to their potential therapeutic properties, including antioxidant, anti-inflammatory, antimicrobial and anticancer activities (Krishnan *et al.,* 2014). Preliminary phytochemical screening is a crucial step in identifying the presence of these bioactive compounds in plant extract. It bridges traditional knowledge and modern science contributing to the development of new drugs and functional foods (Selvamangai & Bhaskar, 2012). The result of phytochemical screening of *A. triplinervis* is shown in Table 1. The presence of important secondary metabolites like glycosides, terpenoids, flavonoids, alkaloids, phenols, tannins detected by conventional protocols, which might be the reason for the antioxidant and cytotoxicity activity of the plant.

In vitro antioxidant activity of A. triplinervis

DPPH radical scavenging assay and nitric oxide scavenging assays were used to detect the free radical scavenging activity of the leaf methanolic extract of *A. triplinervis.*

Table 1: Preliminary phytochemical evaluation of *A. triplinervis*

Phytochemicals	Standard Chemical test	Leaf methanol extracts of *A. triplinervis*
Glycosides	Keller- Killani Test	+
Alkaloids	Dragendroff's Test	++
Flavonoids	Shinoda Test	++
Terpenoids	Salkowski Test	+++
Steroids	Liebermann-Burchard Test	-
Phenols	Ferric Chloride Test	+++
Tannins	Lead Acetate Test	++
Saponins	Foam Test	+

'+' means 'present', '-' means absent

DPPH radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl assay)

In this assay, it was noticed that the ability of the methanolic leaf extract of *A. triplinervis* had substantial scavenging effect on the DPPH radical which was increased with an increase in the concentration of the sample. Maximum scavenging activity that is 81.23±2.32% was observed at 100 µg/mL concentration (Figure 2) and the IC_{50} value of the leaf extract of *A. triplinervis* and ascorbic acid (standard) were found to be 33.30±1.54 µg/mL and 28.71±2.49 µg/mL, respectively.

Nitric oxide scavenging assay

In this assay the methanolic leaf extract of *A. triplinervis* showed significantly high ability to compete with oxygen and decrease the production of nitrite ions (Figure 3). The IC_{50} value of methanolic leaf extract and ascorbic acid (standard) were found to be 40.31±3.98 µg/mL and 29.25±3.51 µg/mL, respectively.

In vitro cytotoxic activity of A. triplinervis

In this study, the methanolic leaf extract of *A. triplinervis* showed potentially high cytotoxic activity against different cell lines such as the HCT 116 cell line (Human Colon Cancer Cell Line) and MCF 7 cell line (Human Breast Cancer Cell Line). Methotrexate was used as the standard. Different concentration of methanolic leaf extract like 6.25, 12.5, 25, 50 and 100 µg/mL were used to pre-treat the cells and the percentage of cancer cell death was increased with increasing concentration of the sample. The maximum cytotoxicity was noticed with 100 µg/mL of the samples (Table 2, Figures 4 & 5). The cytotoxic effect was expressed as IC_{50}. IC_{50} values of a good drug should be significantly low to avoid any possible drastic effects. A substantiate cytotoxic effect of promising anticancer product exhibits an IC_{50} value <30 µg/mL assigned by the American National Cancer Institute (Sangeetha & Jisha, 2025). In the present study, the IC_{50} value of leaf methanol extract of *A. triplinervis* against the HCT 116 cell line was 80.14±3.12 µg/mL whereas 94.02±2.43 µg/mL against the MCF 7 cell lines which indicated that the crude methanolic leaf extract

Table 2: Cytotoxic effect of the standard methotrexate and methanolic leaf extract of *A. triplinervis* in MTT assay

Sample concentration (µg/mL)	% of cell death			
	HCT 116 cell line		MCF 7 cell line	
	Leaf extract of *A. triplinervis*	Standard drug (Methotrexate)	Leaf extract of *A. triplinervis*	Standard drug (Methotrexate)
6.25	2.98±0.54	12.42±2.22	1.82±0.18	7.65±2.05
12.5	6.32±2.31	23.54±3.01	5.53±0.23	13.01±0.83
25	15.04±1.23	40.59±2.30	11.62±1.24	29.25±2.22
50	36.31±2.97	59.51±3.08	27.54±2.11	41.35±1.25
100	60.21±1.01	85.26±3.21	53.36±2.54	82.21±0.187
IC_{50}	80.14±3.12 µg/mL	51.67±3.50 µg/mL	94.02±2.43 µg/mL	59.30±2.98 µg/mL

Table 3: FTIR spectra of the leaf extract of *A. triplinervis*

S. No.	Peak values (Frequency, cm^{-1})	Wave number (cm^{-1})	Functional group assignment
1	3731	3750-3645	O-H stretching of Phenols
2	3308	3200-3550	N-H stretching of amines
3	2359	2700-2250	N-H stretching
4	1627	1600-1580	C=C stretching in aromatic rings or conjugated alkenes
5	1386	1410-1310	C-H bending or O-H bends
6	1038	1030-1150	C-O stretching in alcohols, esters or carboxylic acid
7	436	430-500	Aryl disulfides (S-S stretch)

showed significantly high cytotoxic effect than that of the standard drug methotrexate.

Fourier Transform Infrared Spectrophotometeric (FTIR) analysis

The functional groups/chemical bonds present in the methanolic leaf extract of *A. triplinervis* were predicted using FTIR spectrum shows the % of transmittance versus wavenumber, providing information about the functional groups based on characteristic absorption peaks like Phenolic O-H stretch (3731 cm^{-1}), Amines N-H stretch (3308 cm^{-1}), Aromatic or conjugated double bond (1627 cm^{-1}) (Table 3 & Figure 6).

Discussion

A purple coloured stable free radical is DPPH (1,1-diphenyl-2-picrylhydrazyl) which turns yellow when scavenged. This colour changing character of DPPH was used to show the free radical scavenging activity. When an antioxidant reacted with DPPH, it became reduced and leading to a loss of purple colour and then the absorbency decreased. The degree of discolouration indicates the scavenging potentials of antioxidant compounds or plant extracts in terms of hydrogen donating ability (Krishnan *et al.,* 2014). In the present study, it was noticed that the ability of leaf extracts of *A. triplinervis* had a substantiated scavenging effect on the DPPH radical. Maximum scavenging activity was observed that is 81.23±2.32% at 100 µg/mL concentration of leaf extract. Similarly the highest scavenging activity of the methanolic leaf extract of *A.triplinervis* was already reported by Yunitrianti *et al.* (2020).

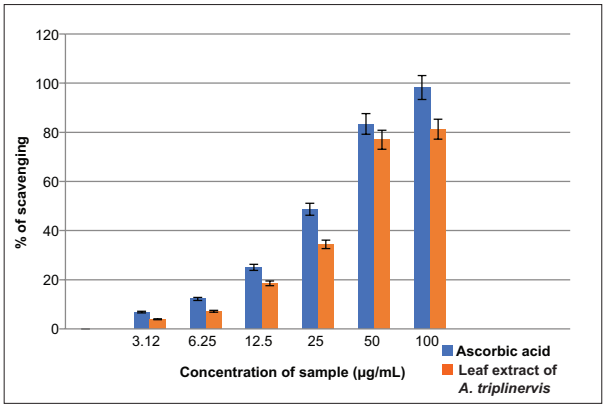

Figure 2: Effect of methanolic leaf extract of *A. triplinervis* in DPPH radical scavenging assay

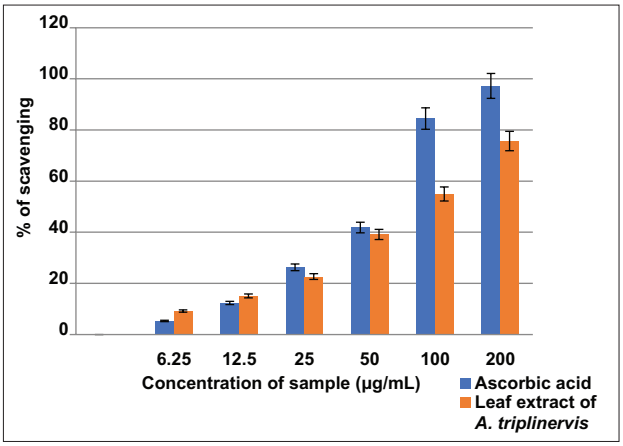

Figure 3: Effect of methanolic leaf extract of *A. triplinervis* in nitric oxide radical scavenging assay

In the nitric oxide scavenging assay, nitric oxidewas spontaneouslygenerated by sodium nitroprusside which mingled with oxygen to produce nitrite ions that can be analyzed using Griess reagent. Nitric oxide scavengers compete with oxygen, leading to reduced production of nitrite ions. Huge amounts of nitric oxide may lead to tissue damage. In this assay, the methanolic leaf extract of *A. triplinervis*showed significantly high capacity to compete with oxygen and reduce the production of nitrite ions. The IC_{50} value of methanolic leaf extract and ascorbic acid (standard) were found to be 40.31±3.98 µg/mL and 29.25±3.51 µg/mL, respectively. A similar type of nitric oxide scavenging activity was noticed in the methanolic

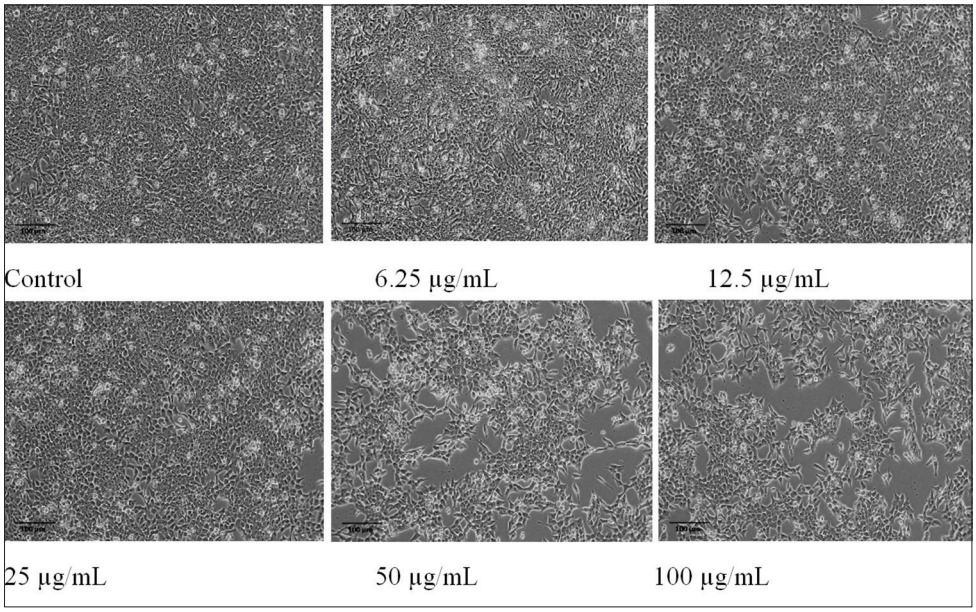

Figure 4: Effect of crude methanolic leaf extract of *A. triplinervis* in MTT assay against HCT 116 cell line

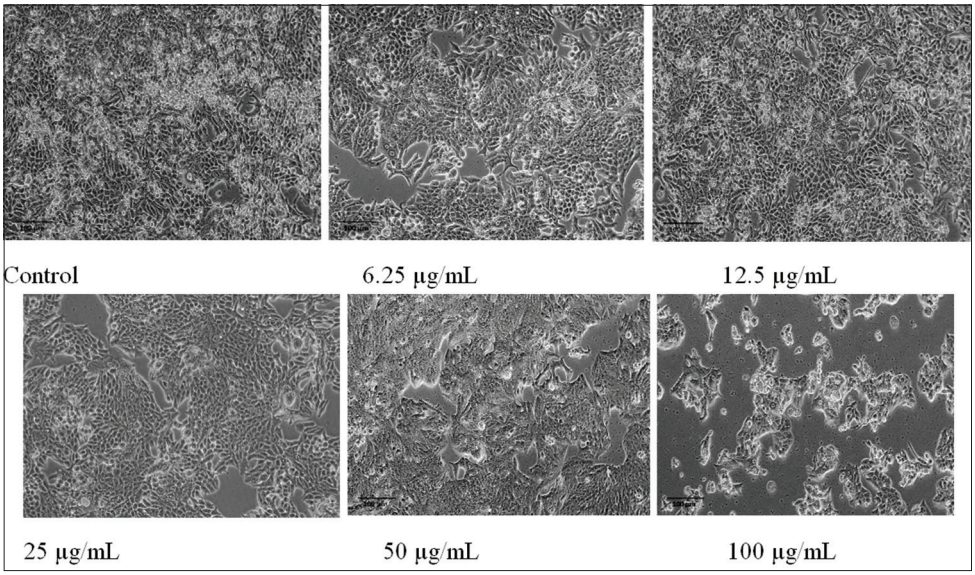

Figure 5: Effect of crude methanolic leaf extract of *A. triplinervis* in MTT assay against MCF7 cell line

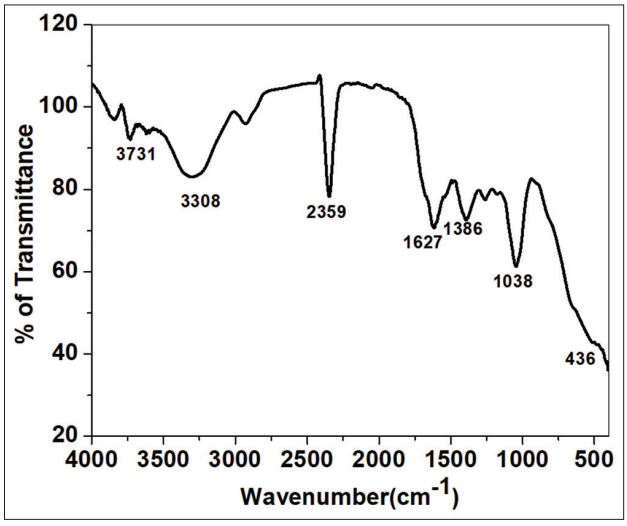

Figure 6: FTIR spectra of the leaf extract of *A. triplinervis*

whole plant extract of *Elephantopus scaber* L. (Sangeetha & Jisha, 2025) which was low as compared to *A. triplinervis*.

A cell or a group of cells constitute the unchecked growth, invasion and metastasis which lead to cancer. A limited number of effective anticancer drugs for the treatment of cancer are currently in use, even though they have higher cases of vomiting, nausea, skin rashes, headache, diarrhoea, etc. (Fernezelian *et al.,* 2023). Therefore, there is a real need for something novel, side effect less, inexpensive and effective anticancer drugs to combat this dangerous disease. Natural plant products are a main source of pharmaceuticals and for the detection of new bioactive compounds (Selvamangai & Bhaskar, 2012). Aqueous and alcoholic leaf extract of *A. triplinervis* against HeLa (Cervical cancer) cell lines was reported by John *et al.* (2021). In the present study, the methanolic leaf

extract of *A. triplinervis* showed potentially high cytotoxic activity against different cell lines, such as the HCT 116 cell line (Human Colon Cancer Cell Line) and MCF 7 cell line (Human Breast Cancer Cell Line). Asha *et al.* (2017) also reported the cytotoxic effect of the leaf of *A. triplinervis*.

The confirmation of the functional group of the active compounds in the plant samples based on the peak value is mainly done by FTIR analysis. In the present study, the functional groups/chemical bonds present in the leaf extract of *A. triplinervis* were predicted using the FTIR spectrum, which shows the % of transmittance versus wavenumber, providing information about the functional groups based on characteristic absorption peaks like Phenolic O-H stretch (3731 cm^{-1}), Amines N-H stretch (3308 cm^{-1}), Aromatic or conjugated double bond (1627 cm^{-1}). Phenolics and Aromatic or conjugated compounds might be the reason for the antioxidant and cytotoxic properties of the plant.

Conclusion

The present study thus concluded that the methanolic leaf extract of *A. triplinervis* acts as a promising antioxidant and anticancer agent. FTIR analysis revealed that phenolics and aromatic or conjugated compounds might be the reason for the antioxidant and cytotoxic properties of the plant. This will be helpful for the maximum utilization of the plant to isolate their active compound to develop novel antioxidant and cytotoxic drugs.

Author contributions

M. S. Jisha: Conceptualization, Data curation, Investigation, Formal analysis, writing- orginal draft, Funding acquisition.
G. Sangeetha: Supervision, Writing-review & editing.

Acknowledgements

The authors appreciate the Department of Botany, T.K.M College of Arts and Science, Kollam, Kerala for providing the laboratory support for this study.

References

Ajeesh Krishna, T. P., Krishnakumar, N. M., Maharajan, T., & Cesar, S. A. (2024). Rapid *in vitro* regeneration and genetic fidelity assessment of regenerated plants in *Ayapana triplinervis* (Vahl) R.M. King & H. Robinson, An ethnomedicinal and ornamental herb. *The Open Biotechnology Journal, 18*, e18740707192866. https://doi.org/10.2174/0118740707192866240116112512

Asha, I. Z., Khan, M. I. H., & Mukharjee, S. K., & Rony, S. R. (2017). Total phenolic content, Cytotoxic, Antioxidant, Thrombolytic Membrane Stabilizing and Antimicrobial Activities of *Eupatorium ayapana* Vent. *International Journal of Pharmaceutical Science Invention, 6*(8), 23-28.

Balade, O. P., Akinsiku, A. A., Adeyemi, A. O., Willims, A. B., & Benson, N. U. (2018). Dataset on phytochemical screening, FTIR and GC-MS characterisation of *Azadirachta indica* and *Cymbopogon citratus* as reducing and stabilising agents for nanoparticles synthesis. *Data in Brief, 20*, 917-926. https://doi.org/10.1016/j.dib.2018.08.133

Balakrishnan, A., Aswindas, T. P., Sebastin, D. P., & George, S. (2021). *In vitro* axillary bud multiplication of an important medicinal plant – *Ayapana triplinervis* Vahl. *International Journal of Botany Studies, 6*(4), 329-332.

Cheriyan, B. V., Scarlet, S., Priyadarshini, Joshi, S., Santhseelan, & Mohamed, S. (2019). *Eupatorium triplinerve* (Vahl): An ethnobotanical review. *Asian Journal of Pharmaceutical Research, 9*(3), 200-202. https://doi.org/10.5958/2231-5691.2019.00032.7

Fernezelian, D., Gence, L., Bringart, M., Veeren, B., Bedoui, Y., Meilhac, O., Bascands, J.-L., & Doitel, N. (2023). *Ayapana triplinervis* Vahl: Potential toxicity and therapeutic effects assessed in a zebrafish model. *Phytomedicine Plus, 3*(1), 100384. https://doi.org/10.1016/j.phyplu.2022.100384

Gauvin-Bialecki, A., & Marodon, C. (2008). Essential oil of *Ayapana triplinervis* from Reunion Island: A good natural source of thymohydroquinone dimethyl ether. *Biochemical Systematics and Ecology, 36*(11), 853-858. https://doi.org/10.1016/j.bse.2008.09.006

Haddad, J. G., Picard, M., Bénard, S., Desvignes, C., Desprès, P., Diotel, N., & El Kalamouni, C. (2019). *Ayapana triplinervis* essential oil and its main component thymohydroquinone dimethyl ether inhibit zika virus at doses devoid of toxicity in zebrafish. *Molecules, 24*(19), 3447. https://doi.org/10.3390/molecules24193447

Ho, W. Y., Liew, S. S., Yeap, S. K., & Alitheen, N. B. (2021). Synergistic cytotoxicity between *Elephantopusscaber* and Tamoxifen on MCF-7 Derived multicellular tumorsperoid. *Evidence-Based Complementary and Alternative Medicine, 2021*(1), 355236. https://doi.org/10.1155/2021/6355236

John, A. M., Thomas, S., Anil, E., Jabbar, M. K., Antony, T., & Remya Gayathri, S. (2021). Evaluation of antioxidant and anticancer activity of *Ayapana triplinervis. World Journal of Pharmaceutical Research, 10*(2), 1329-1338. https://doi.org/10.17605/OSF.IO/PDS2C

Krishnan, M., Jayaraj, R. L., Megala, J., & Elangovan, N. (2014). Antioxidant mediated antiulcer effect of *Eupatorium triplinerve* Vahl against acetic acid induced ulcerative colitis in mice. *Biomedicine & Aging Pathology, 4*(2), 153-160. https://doi.org/10.1016/j.biomag.2013.12.002

Rajasekaran, A., Kalaivani, M., & Ariharasivakumar, G. (2010). Haemostatic effect of fresh juice and methanolic extract of *Eupatorium ayapana* leaves in rat model. *International Journal of Biological and Medical Research, 1*(3), 85-87.

Rao, B. G., Rao, Y. V., Pavani, S., & Dasari, V. S. P. (2012). Qualitative and quantitative phytochemical screening and *in vitro* antioxidant and antimicrobial activities of *Elephantopus scaber* Linn. *Recent Research in Science and Technology, 4*(4), 15-20.

Rivera, J. O., Loya, A. M., & Ceballos, R. (2013). Use of herbal medicines and implications for conventional drug therapy medical sciences. *Alternative & Integrative Medicine, 2*(6), 1-6.

Rodrigues, A. B. L., de Matos, J. L., Martins, R. L., de Menezes Rabelo, E., Brandão, L. B., Santos, L. L., Faustino, C. G., de Oliveira, F. R., & da Silva de Almeida, S. S. M. (2022). Ethnopharmacological use, secondary metabolites and biological activity of *Ayapana triplinervis* (VAHL) R. M. King and H. Rob: A systematic review. *Pharmacognosy Reviews, 16*(32), 70-73. https://doi.org/10.5530/phrev.2022.16.10

Rodrigues, A. B. L., Martins, R. L., de Menezes Rabelo, E., de Matos, J. L., Santos, L. L., Brandão, L. B., do Socorro Barbosa Chaves, R., da Costa, A. L. P., Faustino, C. G., da Cunha Sá, D. M., Farias, A. L. F., de Castrio Cantuaria, P., & da Silva de Almeida, S. S. M. (2022). *In silico* and *in vivo* study of adulticidal activity from *Ayapana triplinervis* essential oil nano-emusion agent *Aedes asgypti*. *Arabian Journal of Chemistry, 15*, 104033. https://doi.org/10.1016/j.

arabjc.2022.104033

Rodrigues, A. B. L., Martins, R. L., de Menezes Rabelo, É., Tomazi, R., Santos, L. L., Brandão, L. B., Faustino, C. G., Farias, A. L. F., dos Santos, C. B. R., de Castro Cantuária, P., Galardo, A. K. R., da Silva de Almeida, S. S. M. (2021). Development of nano-emulsions based on *Ayapanna triplinervis* essential oil for the control of *Aedes aegypti* larvae. *Plos One, 16*(7), 225. https://doi.org/10.1371/journal.pone.0254225

Salmerón-Manzano, E., Garrido-Cardenas, J. A., & Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health, 17*(10), 3376. https://doi.org/10.3390/ijerph17103376

Sangeetha, G., & Jisha, M. S. (2025). Evaluation of *in vitro* antioxidant, cytotoxicity and FTIR analysis of whole plant extract of *Elephantopusscaber* L. (Asteraceae). *Plant Science Today, 12*(1), 1-6. https://doi.org/10.14719/pst.3650

Selvamangai, G., & Bhaska, A. (2012). GC-MS analysis of phytocomponents in the methanolic extract of *Eupatorium triplinerve*. *Asian Pacific Journal of Tropical Biomedicine, 2*(3), S1329-S1332. https://doi.org/10.1016/S2221-1691(12)60419-9

Venugopala, K. N., Rashmi, V., & Odhav, B. (2013). Review on natural coumarin lead compounds for their pharmacological activity. *BioMed Research International, 2013*, 963248. https://doi.org/10.1155/2013/963248

Yunitrianti, Elya, B., & Noviani, A. (2020) Determination of the antioxidant activity of prasman leaf extracts *Ayapana triplinervis* (Vahl) and the total flavonoid and Phenol contents of the most active extracts. *International Journal of Applied Pharmaceutics, 12*(1), 107-111. https://doi.org/10.22159/ijap.2020.v12s1.FF021