The Journal of Phytopharmacology 2025; 14(2):78-86
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2025; 14(2): 78-86
March- April
Received: 11-02-2025
Accepted: 12-04-2025
Published: 15-05-2025

doi: 10.31254/phyto.2025.14202

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Evaluation of cytotoxic potential of leaves of *Cyclea peltata* in MCF-7 cell lines

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ABSTRACT

It is a well-established fact that breast cancer causes severe mortality among women globally. The invention of a vast number of medications currently used in the treatment of cancer was made possible from studies on natural products. *Cyclea peltata* is a locally available medicinal plant which has been used for the treatment of various diseases including diabetes. The present study reports the antiproliferative and cytotoxic action of alcoholic extract and its fractions of the leaves of *C. peltata* in MCF-7 cell lines. The extract of *C. peltata* and its fractions were subjected to qualitative phytochemical analysis and the cytotoxicity assay was carried out in MCF-7 cells using the MTT dye reduction assay. Morphological changes were screened by AO/EB and JC-1 staining techniques after exposing the cells to the extract and fractions. The methanolic extract of *C. peltata* and its fractions showed dose dependent increase in per cent cell inhibition. The n-hexane fraction with IC_{50} of 42 µg/mL exhibited highest cytotoxicity and induced intrinsic pathway of apoptosis proved by the green fluorescence on JC-1 staining. The analysis of n-hexane fraction by mass spectroscoptic and FTIR techniques exposed the presence of compounds, including deoxycholic acid, 1, 7- Heptanediol, G- strophanthin etc. Methanolic extract and fractions of *C. peltata* were cytotoxic to MCF-7 cells that produced apoptosis and can be further analysed for anticancer compounds.

Keywords: Anticancer agent, Breast cancer, Apoptosis, Cell viability, Padal.

INTRODUCTION

The battle with cancer has been a never-ending process around the world with increase in rate morbidity and mortality as many of the diseases gain resistance [1]. According to the most recent estimates of the global cancer burden, there were 2.26 million new instances of breast cancer worldwide in 2020, and is the leading cause of cancer mortality among women globally. It possesses a significant challenge to modern medicine, especially to ascertain its prognosis and specific treatment, particularly in those with diverse etiology and hormone dependence [2]. The frontier areas of research in cancer now focuses on the protective and/or preventive effects of natural products, as therapy or adjuncts in mitigation of cancer. The National Cancer Institute collected 35,000 plant samples from 20 nations and identified more than 3000 plant species with antitumor activities [3]. However, one of the biggest problems facing modern science is comprehending the mechanism of action and validation of pharmacokinetics and toxicokinetics in cancer treatment and prevention. Sulphoraphane, Paclitaxel (Taxol), Epipodophyllotoxin, Vinca alkaloids, Pomiferin and Noscapine are some of the plant-derived anticancer drugs that are widely used in cancer chemotherapy [4].

Cyclea peltata, locally known as “Padal” or “Pada thalli” is a herb of the family Menispermaceae. The plant is a slender twining shrub and has a wider distribution in India, Srilanka, and in the Andaman and Nicobar Islands [5]. It has simple, alternate, heart-shaped leaves that are 2.5-10 cm long and 2.5-3.75 cm wide, stipules that are 5-10 cm long, and nerves that are7-11cm in length [6]. Different parts of this plant have been used for the treatment of ailments like diabetes, tooth ache, malaria and asthma [7]. Root extract of *C. peltata* has antitoxin activity and the alkaloid extract of root has hepatoprotective activity [8, 9]. Leaf extract of *C. peltata* has antifungal activity against *Aspergillus nigrus* and *Candida albicans* [10]. Tetrandrin from the alkaloid fraction of *C. peltata* possess anti-inflammatory activity [11]. Furthermore, it exhibits anticancer activity against human colon carcinoma cell line and Daltons ascites lymphoma (DLA) cells [12,13]. However, there is only limited literature to consolidate the cytotoxic potential of *C. peltata* leaves on breast cancer cell line.

The present study evaluated the antiproliferative and cytotoxic potential of extract of *C. peltata* leaves and its fractions in MCF-7 cell lines by considering its ability to induce apoptosis in the cells. Identification of most potent extract and phytochemical analysis of the potent extract by GC-MS and FTIR analysis was also carried out in this study.

MATERIALS AND METHODS

Plant materials

Whole plant of *C. peltata* was collected locally, from Mannuthy, Thrissur, Kerala during March, 2022. The collected plants were identified and authenticated at the Department of Botony, St. Thomas College, Thrissur. Herbarium of the plant was prepared and a voucher specimen was deposited in the Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy. Leaves of this plant were used for the study.

Cell line and culture conditions

MCF-7 cells were procured from NCCS, Pune and was received in T25 flasks which was at around 60 % confluency. Cells were adapted to grow in Rosewell Park Memorial Institute (RPMI) -1640 media that was fortified with 10 % FBS and 1% antibiotic solution. They were maintained in a carbon dioxide incubator at 5% level in 37ºC. The cells were sub cultured by digestion using 0.25 per cent trypsin/1mM EDTA as they were approximately 70 to 80 % confluent in the T25 flask and were used for further studies.

Preparation of methanolic extract of *C. peltata* leaves

The leaves of plant were procured locally, washed and dehydrated under shade. The dried leaves were pulverised to rough powder using electrically operated mechanical pulveriser- grinder and used for extraction process in soxhlet extraction apparatus using methanol as a solvent at around 60°C. The thimble containing the plant material was weighed and placed in the soxhlet apparatus and efflux of solvent was done till all the possible phytochemicals were extracted. Solvents were removed using rotary vacuum evaporator at 172 mbar at a temperature of 40°C [14]. The extract was finally collected and the yield of the extract was calculated and kept under refrigeration in an airtight container until further use.

Preparation of different fractionation of methanolic extract of *C. peltata*

About 100g of the methanolic extract was taken in a separating funnel, and mixed thoroughly with hexane (150 mL) and the fraction that got solubilised in the hexane was collected. The leftover solid was mixed with dichloromethane (150 mL) and its soluble fraction was collected. The sediment was mixed with equal volumes of n-butanol and distilled water shaken vigorously for 5 minutes and allowed to separate. the respective fractions were collected and solvents were removed using rotary vacuum evaporator [15].

Phytochemical screening

The presence of various active phytochemicals in the extract and fractions were qualitatively assessed as per the method of Harborne [16].

Cytotoxicity studies: 3-(4, 5- dimethyl thiazol-2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) assay

The cytotoxicity study on the extract of C. *peltata* in MCF-7 cells was conducted by MTT dye reduction test [15]. A stock solution of extract and fractions was prepared in one per cent DMSO at a concentration of 6.4 mg/mL. It was then serially diluted from 320, to 5 µg/mL using RPMI-1640 medium. For the assay conducted in 96 well plates, MCF-7 cells were plated in the wells of the plate and was allowed to grow for 24 h. Then test substances diluted to their desired concentrations was added to the cells, again incubated for 24 h. The entire media were removed and 10 µL of MTT was added to all wells along with 100 µL of serum free medium and incubated at 37°C for 4 h, in CO_2 incubator. Then the media was removed and the formazan crystals formed were dissolved by adding 200 µL of DMSO. The absorbance was measured using microplate reader (Varioskan Flash, Thermofischer Scientific, Finland) at a wavelength of 570 nm.

The per cent cell viability and per cent cell inhibition were calculated using the following formulae:

Per cent cell viability = (Average absorbance of treated cells /Average absorbance of untreated cells) × 100

Per cent cell inhibition = 100 - per cent cell viability

The IC_{50} values of extracts were calculated by plotting the concentration against per cent cell inhibition using Graph pad prism v.8.0 [17].

Acridine orange / Ethidium bromide (AO/EB) staining

A concentration of 3 x 10^5 MCF-7 cells/mL (2mL) were seeded into a six well cell culture plate in RPMI-1640 media supplemented with heat inactivated FBS and allowed to grow for 24 h. Cells were treated with half IC_{50}, IC_{50} and double IC_{50} of methanolic extract and fractions of *C. peltata*, vehicle control and positive control (Doxorubicin) for 24 h at 37°C in CO_2 incubator. After treatment, cells were subjected to trypsinisation, the pellet formed was collected along with the neutralised enzyme and centrifuged at 3000 rpm for three min and supernatant was discarded. The pellet was re-suspended in 100 µL of fresh media. 25 µL of treated or untreated cells was taken and stained to 5 µL of AO/EB solution (10 µg/mL of Ethidium bromide and 10 µg/mL of Acridine orange). The solution was analysed under Trinocular Research fluorescence microscope, DM 2000 LED, Leica with blue excitation (488 nm) and emission (550 nm) filters at 20X magnification [16].

Jc-1 Staining (5, 5', 6, 6' -tetrachloro- 1, 1', 3, 3' –tetra-ethylbenzimidazolocarbo-cyanine iodide)

The cells were treated with the extract as described in the previous paragraph. After 24h post treatment with the test substances, cells were incubated with five millimolar JC- 1 for 30 min at room temperature in the dark [17]. Then, the stain was pipetted out, cells were washed, and new medium was added and analysed directly under the fluorescent microscopewith filters having, emission spectra at 405 and 488 nm and excitation spectra at 530 and 595 nm filters (DM 2000 LED, Leica).

Gas Chromatography- Mass Spectrometry (GC-MS) analysis of potent fraction of *C. peltata*

Recognition of compounds in the most potent extract were carried out by a Shimadzu GC-MS QP2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) available in the Central Instruments Laboratory, CVAS, Mannuthy. The column used for the analysis was a 30 mm × 0.25 µm × 0.25 mm i.d. RX1 SILMS (Restek, USA). One milligram of active hexane fraction was dissolved in one millilitre of hexane and filtered, from this one micro litre was injected into the GC-MS system in the split mode (split ratio 1:5). Helium was used as the carrier gas with a flow rate of 1.4 mL/minute [18]. The column temperature was maintained at 60°C for five minutes. Then, it was programmed to 240°C at a rate of 4°C /minute and the final temperature 280°C was held for one minute. Injector temperature and detector temperature were optimized at 220 and 290°C, respectively. The MS operating parameters were as follows: ionization energy, 70eV; ion source temperature, 200°C; solvent delay, 5.0 min; scan range, 40 to 700 µ. The components were identified on the basis of matching of their retention indices and mass spectral library (Wiley 08, NIST 11).

Fourier transform infrared spectroscopy (FTIR) analysis of the potent fraction of *C. peltata*

ATR-FTIR (Attenuated Total Reflectance- Fourier transform infrared) analysis was performed in the Central Instruments Laboratory, CVAS, Mannuthy on most potent fraction of *C. peltata*. Molecular groups were identified using FTIR spectrometer. A Perkin- Elmer spectrum two FTIR spectrometer with attenuated total reflectance was used for FTIR analysis. The sampling station was equipped with an overhead ATR accessory. The ATR diamond crystal was carefully cleaned with pure isopropanol between the measurements. A small quantity of the sample was carefully placed on the diamond crystal surface to cover the ATR diamond window in order to focus the laser beam. Each spectrum was recorded as absorbance under 60 N [19].

Statistical analysis

All results were expressed as Mean± SE with 'n' equal to the number of replicates. The IC_{50} values of extract was calculated using Graph pad prism 5.0.

RESULTS

Extraction of *C. peltata* leaves

In the present study, the yield obtained from methanolic extract of *C. peltata* was 14 per cent. The per cent yield derived from n-hexane, dichloromethane, n-butanol and water fractions were 21.2, 18, 16.8 and 18.3 per cent respectively.

Phytochemical analysis of methanolic extract and fractions of *C. peltata*

The results of phytochemical analysis of methanolic extract and fractions provided under Table 1. Qualitative phytochemical analysis showed the presence of alkaloids, flavonoids, phenols, diterpenes, triterpenes and saponins.

Cytotoxicity studies: 3-(4, 5- dimethyl thiazol-2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) assay

MTT assay was used to assess the *in vitro* cytotoxic effect of methanolic extract and fractions of *C. peltata* leaves in MCF-7 cell line. The per cent inhibition of cell proliferation in MCF-7 cell line was studied by MTT assay after 24 h treatment with methanolic extract and fractions is presented in Table 2.

The per cent cell inhibition obtained from MTT assay were used to find the IC_{50} of methanolic extract and fractions of *C. peltata* leaves. Curves were plotted using the values in Graph pad prism V.5.0 and the graph obtained is represented in the Figure 1. The IC_{50} values of methanolic extract, n-hexane, dichloromethane, butanol and aqueous fractions were identified to be 117 µg/mL, 42 µg/mL, 83 µg/mL, 88 µg/mL and 108 µg/mL respectively.

Acridine orange/ethidium bromide staining (AO/EB)

In the present study cells were treated with half IC_{50}, IC_{50} and double IC_{50} concentrations of extract and fractions and showed dose dependent changes in the morphology as well as fluorescence. The cells treated with half IC_{50} gave green fluorescence with prominent green colour spot in the nucleus (Figure 2). On the other hand, cells treated with IC_{50} and double IC_{50} showed orange and red fluorescence which indicates late apoptotic phase and necrosis respectively.

Analysis of mitochondrial transmembrane potential (MMP)

When cells treated with half IC_{50}, IC_{50} and double IC_{50} concentration of extract and fractions of *C. peltata* leaves the fluorescence changed from red to green in a dose dependent manner, which reflected the lowering of mitochondrial membrane potential (Figure 3). These findings suggested that the plant extracts induced apoptosis by mitochondria dependent intrinsic cascade.

Gas Chromatography Mass Spectroscopy (GC-MS) analysis of n-Hexane fraction of *C. peltata*

The GC-MS analysis of n-hexane fraction revealed the presence of Cycloheptasiloxane, tetradecamethyl-, Phenol, 2,4-bis(1,1-dimethylethyl)-, Cyclooctasiloxane, hexadecamethyl-, Tetradecanoic acid, Cyclononasiloxane, octadecamethyl-, 2-Pentadecanone, 6,10,14-trimethyl-, Pentadecanoic acid, 7,9-Di- tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Hexadecanoic acid, methyl ester, Benzenepropanoic acid, 3, 5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester, n-hexadecanoic acid, Phytol, Methyl stearate and Octadecanoic acid. Chromatogram obtained on phytochemical analysis of n-hexane fraction of *C. peltata* using GC-MS is given in Figure 4. Phytoconstituents obtained on GC-MS analysis of n- hexane fraction have been listed in Table-3.

Fourier transform infrared spectroscopy (FTIR) analysis of n-hexane fraction of *C. peltata*

The most likely compounds identified by comparing the spectra of the extract using FTIR and the FLUKA library were tabulated in Table-4. The spectrum of n-hexane fraction of *C. peltata* is shown in Figure 5. FTIR spectra showed intense peak at the wavelength of 2926, 2848, 1717, 1603, 1469, 1186, 1079 and 727 cm-1. The structurally similar compounds to n- hexane fraction of *C. peltata* were Methyl linoleate, deoxycholic acid, ethyl palmitate, ethyl decoanate, 1, 7- Heptanediol, G- strophanthin, octyl-B-D- glucopyranoside, butyl stearate, monostearin, cis- androsterone etc.

Table 1: Phytochemical analysis of methanolic extract and fractions of methanolic extract of *C. peltata*

Phytochemical Test	Methanolic extract	Hexane fraction	Dichloromethane fraction	n-Butanol fraction	Water fraction
Steroids	-	-	-	-	-
Alkaloids	+	+	-	+	+
Glycosides	-	-	-	-	-
Phenolic compounds	+	+	-	+	-
Tannins	-	-	+	+	-
Flavonoids	+	+	+	+	+
Diterpenes	+	+	+	+	+
Triterpenes	+	+	-	+	+
Saponins	+	+	-	+	-

Table 2: The per cent cell inhibition of MCF-7 cells after 24 h treatment with methanolic extract and fractions of *C. peltata* leaves

Concentrations (µg/mL)	**Per cent inhibition (Mean ± SEM)**				
	Methanolic extract	n-Hexane	Dichloromethane	Butanol	Water
5	39.59±0.02	10.24±0.04	38.65±0.02	41.05±0.03	28.74±0.02
10	41.24±0.04	20.72±0.05	41.77±0.05	42.43±0.05	31.72±0.03
20	45.46±0.07	43.64±0.03	45.60±0.04	43.77±0.03	40.87±0.01
40	46.31±0.09	50.81±0.05	47.58±0.04	45.77±0.03	48.73±0.02
80	44.49±0.07	66.52±0.02	52.86±0.00	50.53±0.06	48.85±0.01
160	47.41±0.09	75.56±0.02	53.43±0.01	53.41±0.01	44.13±0.04
320	53.34±0.12	77.17±0.01	53.11±0.05	54.83±0.04	58.60±0.01

Table 3: Gas chromatography- mass spectroscopy analysis of phytochemicals in n-hexane fraction of *C. peltate*

Selected compounds	**Retention time**	**Probability**	**Peak area (%)**
Cycloheptasiloxane, tetradecamethyl-	9.69	70.45	0.31
Phenol, 2,4-bis(1,1-dimethylethyl)-	10.36	64.69	1.56
Cyclooctasiloxane, hexadecamethyl-	11.73	93.65	0.52
1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2-enyl}ethanone	12.66	41.27	0.41
4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	12.66	27.48	0.41
Tetradecanoic acid	13.34	71.99	1.33
Cyclononasiloxane, octadecamethyl-	13.48	84.62	0.81
2-Pentadecanone, 6,10,14-trimethyl-	14.28	79.02	0.86
Pentadecanoic acid	14.44	62.26	0.50
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene- 2,8-dione	15.06	96.30	2.63
Hexadecanoic acid, methyl ester	15.14	74.45	4.76
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methylester	15.26	97.31	3.02
n-Hexadecanoic acid	15.55	54.03	15.01
l-(+)-Ascorbic acid 2,6-dihexadecanoate	15.65	21.00	1.27
Heptadecanoic acid, methyl ester	16.15	37.81	0.53
Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	16.43	43.75	0.44
Heptadecanoic acid	16.51	26.42	1.13
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.88	19.37	5.35
Phytol	17.09	62.22	2.78

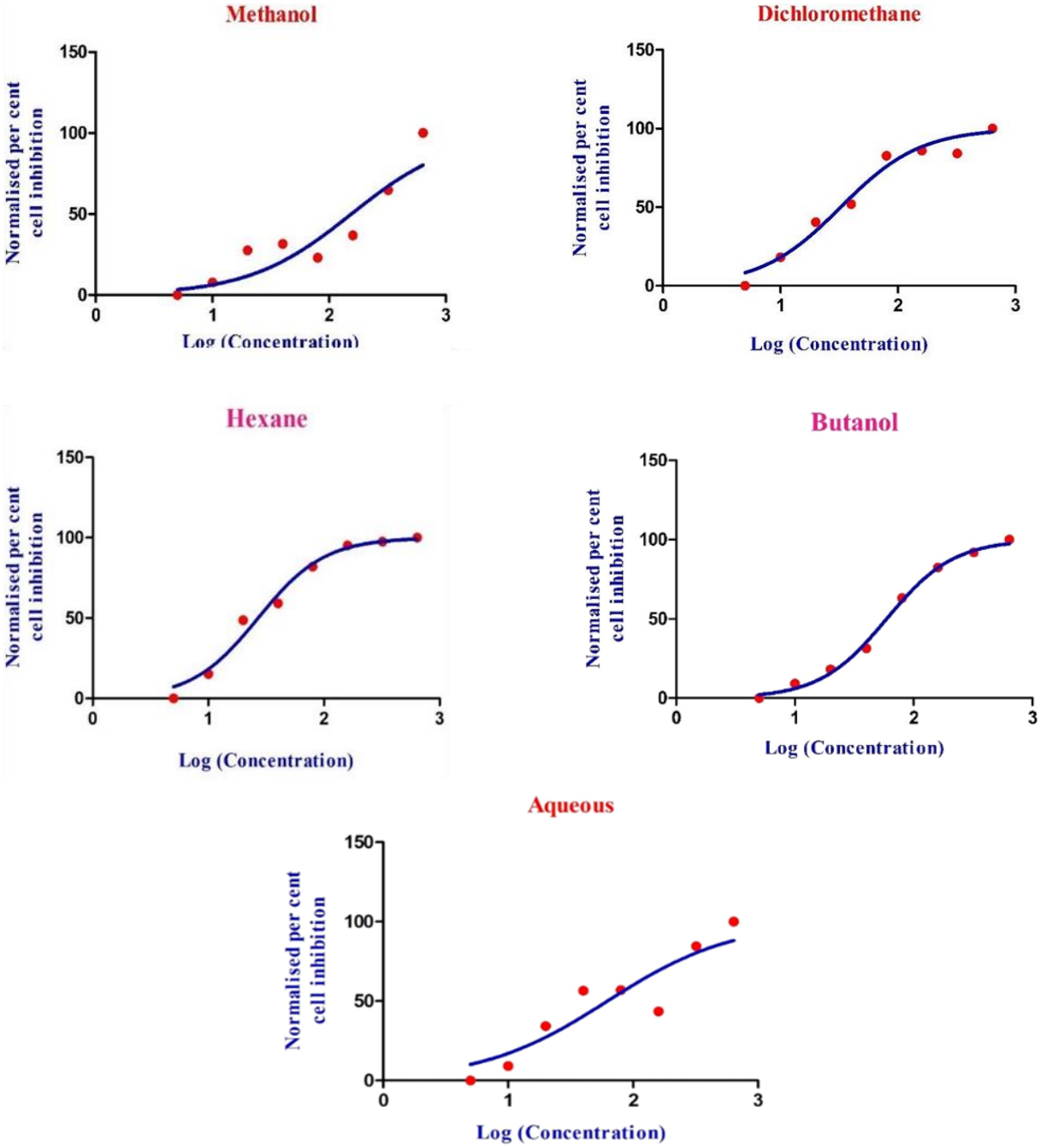

Figure 1: Log Concentration- response curve obtained for calculation of IC_{50} values

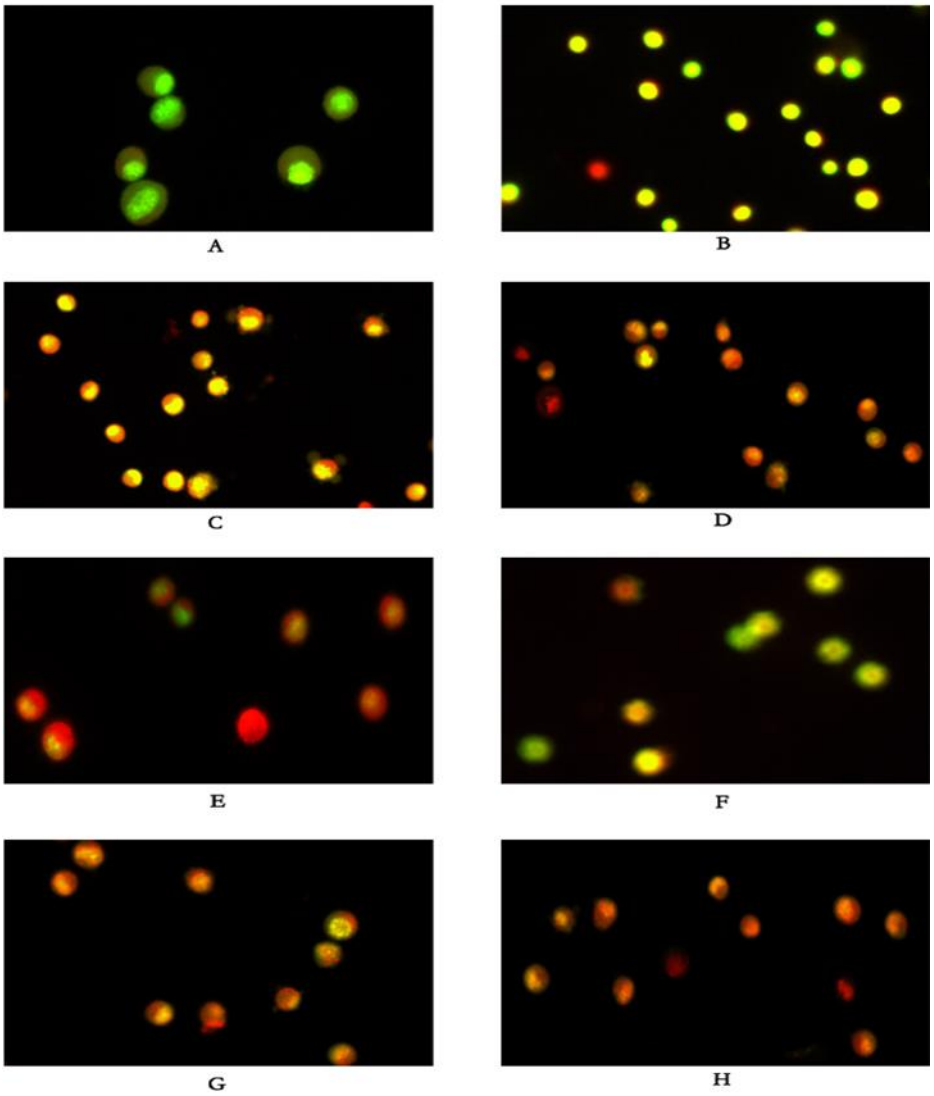

Figure 2: Representative pictures of AO/EB staining 20X (A) Normal cells, (B-D) n-hexane at half IC_{50}, IC_{50} and double IC_{50} respectively, (E)-Doxorubicin IC_{50}, (F-H) dichloromethane at half IC_{50}, IC_{50} and double IC_{50}

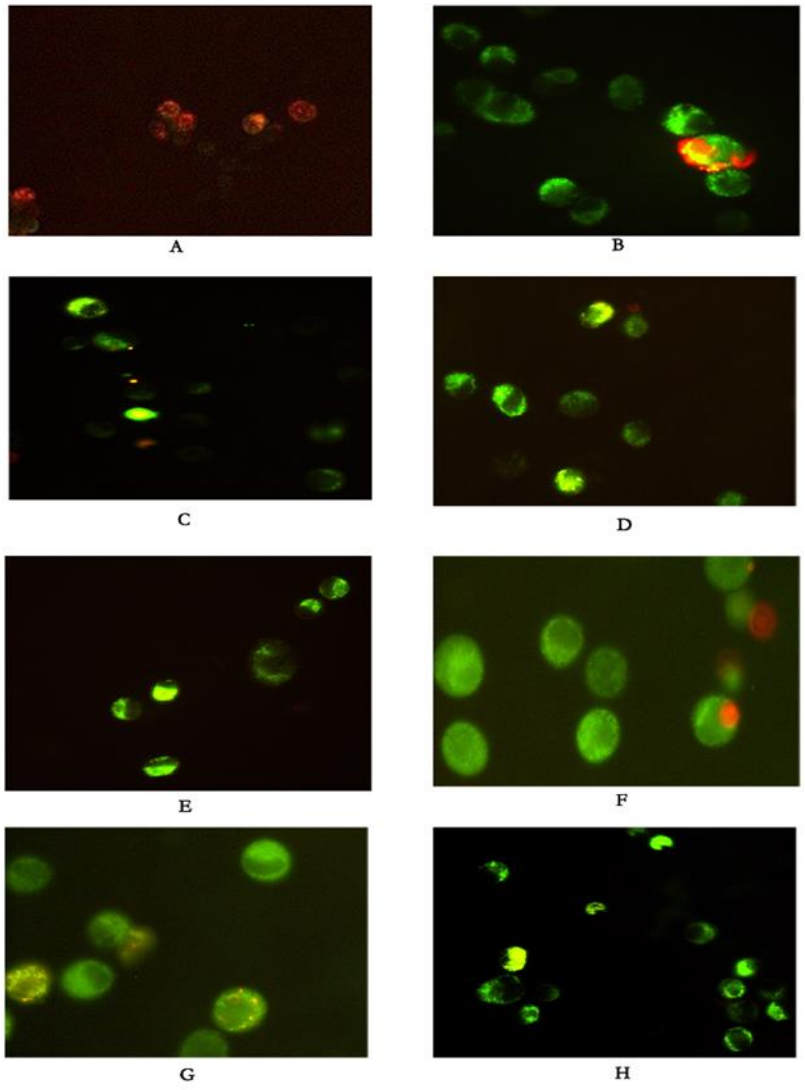

Figure 3: Representative pictures of JC-1 staining 20X (A) Normal cells, (B-D) n-hexane at half IC_{50}, IC_{50} and double IC_{50} respectively, (E)-Doxorubicin IC_{50}, (F-H) dichloromethane at half IC_{50}, IC_{50} and double IC_{50}

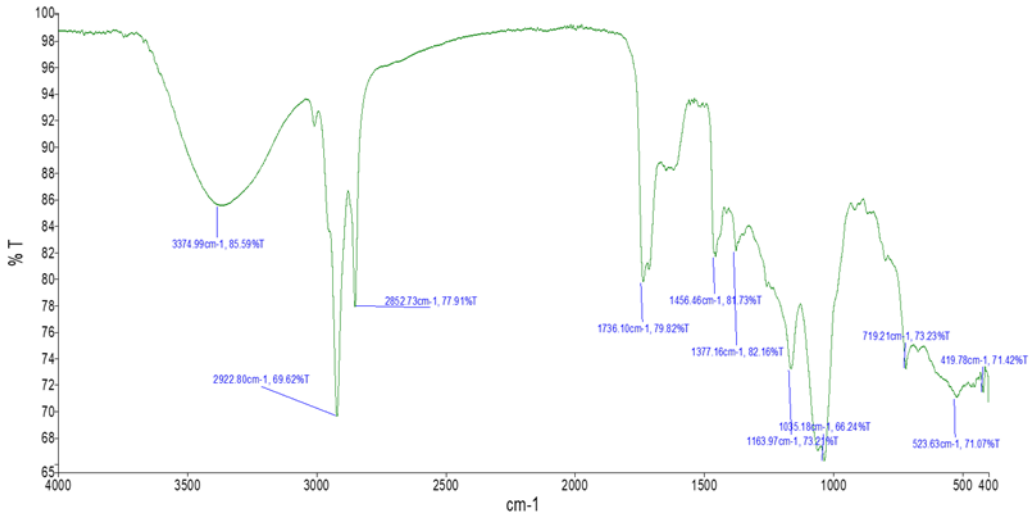

Figure 5: FTIR spectrum of n-Hexane fraction of *C. peltata*

DISCUSSION

This study revealed the cytotoxic effect of methanolic extract of *C. peltata* leaves against MCF-7 cell lines. MCF-7 is a commonly used breast cancer cell line that has produced more data of practical knowledge for patient care than any other breast cancer cell line. It belongs to the luminal A molecular subtype and was estrogen receptor (ER) and progesterone receptor (PR) positive. The growth of breast cancer cells was controlled not only by ER and PR but also by plasma membrane-associated growth factor particularly, the epidermal growth factor receptor (EGFR), and the human epidermal growth factor receptor-2 (HER2) [20]. Methanolic extract of *Mimosa pudica* plant showed antiproliferative effect on MCF-7 cell lines by inducing downregulation of the antiapoptotic gene and protein Bcl-2 [21]. The anticancer activity of *C. peltata* was demonstrated in human colon cancer cell line HCT-116 [11]. Tetradrine isolated from *C. peltata* have cytotoxic effect on pancreatic (PANC-1) and breast (MDA-MB-231) cancer cells [22].

There were only few reports on cytotoxic effect of *C. peltata* on breast cancer cell lines. For that reason, this is a detailed study about the phytochemical properties and cytotoxic effect of methanolic extract and its fractions. Presence of phytochemicals alkaloids, flavonoids, phenols, diterpenes, triterpenes and saponins detected in crude methanolic extract. Whereas, the n-hexane fraction showed the presence of alkaloids, diterpenes, triterpenes and saponins. Although only tannins and diterpenes were present in dichloromethane fraction, alkaloids, phenols, tannins, flavonoids, diterpenes, triterpenes and saponins were present in the n-butanol fraction. These findings unswerving with the previous reports [6, 12].

In living cells, a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzyme transforms the yellow tetrazolium dye MTT into a purple formazan crystal that is soluble in DMSO and absorbance was measured at 570 nm using an ELISA plate reader [23]. In the present study n-hexane fraction exhibited potent cytotoxic activity with a dose dependent decrease in cell viability. Minimum cell viability of 22.82 per cent was observed after 24 h treatment with 320 µg/mL of n-hexane fraction. MTT assay cannot distinguish between necrosis and apoptosis as the cause of the reduction of cell growth. Since apoptosis removes cells with genetic flaws, it is a crucial preventive measure against the development of cancer. Therefore, one highly desired approach for managing cancer is the induction of apoptosis [24]. Acridine orange/ethidium bromide staining was used to identify various apoptotic stages and morphological alterations in the cells. Acridine orange penetrated living and early apoptotic cells and attaches to the DNA, producing green fluorescence; ethidium bromide, on the other hand, only stain late apoptotic and necrotic cells with enlarged nuclei that lost plasma membrane integrity, causing the cells to glow from orange to red. The result of the present study concurs with the early apoptotic stages of MCF-7 cell line after treatment with methanolic extract of *M. pudica* which showed granular yellow green stained nucleus on AO/EB staining [21].

The intrinsic pathway of apoptosis is achieved by raising the permeability of mitochondria, which triggers the release of apoptotic factors by a decrease in the mitochondrial meambrane potential (MMP) [25]. JC-1 is a cationic dye that is used to detect the mitochondrial potential of cells. JC-1 stain has the capability of penetrating into the mitochondria of normal cells due to their high membrane potential and formed bright red fluorescence due to the formation of j- aggregates. Alteration in electron transport chain of apoptotic cells causes reduction in MMP, which leads to the leakage of JC-1 dye from the mitochondria into the cytosol, where it does not form aggregates and hence can emit green fluorescence only [26]. In the present study, methanolic extract and its fractions reduced MMP in a dose dependent manner.

GC-MS analysis of n-Hexane fraction of *C. peltata* extract was found to be constituted with compounds like Cycloheptasiloxane, tetradecamethyl-, and Hexadecanoic acid, methyl ester which has proven to be contribute to the anticancer activity of crude extract and different fractions of *Chlorella vulgaris* on HeLa cell line [27]. Another cytotoxic compound present in the fraction is Phenol, 2, 4-bis (1, 1-dimethylethyl)-, which has proven positive affinity towards caspase-3 and EGFR kinase domain receptors, thus suggesting it as a strong anticancer and antioxidant agent [28].

FTIR spectra of the potent fraction suggested the presence of few active compounds. Among them Stophanthin induces cell death by arresting the expression of key proteins in MAPK signaling pathway [29]. 1,7-Heptanediol disulfamate was found to be cytotoxic against human leukemia cell lines. 12 h of exposure to this drug causes DNA interstrand and DNA-protein cross-links and act as a potent cytotoxic agent [30].

Cancerous cells with dysregulated apoptosis proliferate uncontrollably, which is linked to the formation of tumours, their advancement, and the emergence of chemotherapy resistance [31]. Drug candidates that either trigger apoptosis or restore malignant cells susceptibility to it could be extremely promising for the treatment of cancer [32]. The results of this study support the cytotoxicity and potential for inducing apoptosis of *C. peltata* extract and fractions, and they may also suggest a possible source of bioactive compounds.

CONCLUSION

In the present study it was found that methanolic extract as well as fractions were cytotoxic and among different fractions of the extract, n-hexane fraction with IC_{50} value 42 µg/mL displayed remarkable cytotoxicity through induction of apoptosis. Presence of compounds like, Cycloheptasiloxane, tetradecamethyl-, and Hexadecanoic acid, methyl ester, Stophanthin and 1,7-Heptanediol disulfamate in the n-Hexane fraction could be responsible for the cytotoxic activity. As a result, it can be further used for the characterization of its cytotoxic compounds and used as a lead for the creation of potential anticancer drug.

Acknowledgements

The authors want to acknowledge the support provided by the Kerala Veterinary and Animal Sciences University for the conduct of this research work.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

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HOW TO CITE THIS ARTICLE
Roshni SS, Geevarghese S, Sujith S, John P, Uma R, Nisha AR. Evaluation of cytotoxic potential of leaves of *Cyclea peltata* in MCF-7 cell lines. J Phytopharmacol 2025; 14(2):78-86. doi: 10.31254/phyto.2025.14202