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Cytotoxicity and Cell Cycle Arresting Potential of *Ficus racemosa* leaves

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ABSTRACT: Millions of individuals worldwide are affected by the serious health problem of cancer. The challenges faced during the treatment that includes chemotherapy and radiotherapy, are known to have adverse health effects. Plants have potential anticancer compounds that can be used in cancer treatment. Phytochemicals have been proved to act as cytotoxic agents against cancerous cell types and prevent oxidative damage. This intends to evaluate the anticancer activities of the leaf extracts obtained from *Ficus racemosa* against the carcinogenic HeLa cells. The study analyses three separate extracts (water, ethanol, and ethyl acetate) that were prepared from the leaves of *Ficus racemose.* Further, evaluating for phytochemical screening, cytotoxic assay, cell cycle analysis assay, and antioxidant assay. The ethyl acetate extract shows significant cytotoxic effects on HeLa cell lines with an IC50 of 80 g/ml. The cell cycle studies revealed that the ethyl acetate extract conferred a minimum proportion of viable HeLa cells at G0/G1 at a concentration of 160 µg/ml. However, the DPPH antioxidant assay, showed that the ethanol extract had highest significance with an IC_{50} of 30.19 µg/ml. The GC-MS analysis of the ethyl acetate extract revealed the presence of useful phytochemicals, such like pentadecanoic acid and lupeol. These compounds have already shown to lower oxidative stress and support metabolism. The study carried out contributes new light on the *Ficus racemosa* leaves' potential medicinal properties, but more thorough study is needed to fully understand the compound's pharmacokinetic characteristics.

Keywords: Cancer, *Ficus racemosa*, phytochemicals, antioxidant, cytotoxic

INTRODUCTION

Cancer has been a predominant health issue affecting millions of people worldwide; a constant battle cry that requires the development of preventative therapies. In cancer, cells lose their potency to terminate beyond the hayflicks limit, resulting in the formation of a tumor and eventually becoming metastatic. The most common forms of cancer that affect people worldwide include cervical cancer (22.86%), lung cancer (12.7%), breast cancer (10.9%), and colorectal cancer (9.7%). A high mortality rate is associated with lung cancer (18.2%), liver cancer (9.2%), and stomach cancer (9.7%). Therefore, these death-causing diseases must be prevented and treated at the earliest (Bray *et al*., 2013). Treatment options for cancer include radiotherapy, chemotherapy, immunotherapy, and targeted therapy at the cost of causing strain, supplementary damage, and side effects in patients. Hence, developing and substituting treatment plans with fewer side effects has been the area of interest among researchers worldwide (Jayakumar *et al*., 2018).

Traditional/Ayurvedic medicine has a major role in today's treatment for chronic and life-threatening diseases. Industries have utilized medicinal plants to design and develop chemo-drugs against cancer (Ochwang'i *et al*., 2014). It has been found that phytochemicals derived from plants possess antioxidant properties that scavenge free radicals and prevent oxidative damage (Bhattacharya *et al*., 1997). Compounds like vinblastine, etoposide, topotecan, irinotecan, vincristine, epipodophyllotoxin derivatives, and paclitaxel (taxol®) have been demonstrated to be clinically useful and function as plant-derived anti-cancer agents (Cragg and Newman, 2005). Plant-derived drugs like vincristine, vindesine, proImmu, etoposide, teniposide, and docetaxel are used as antineoplastic compounds. A study conducted with methanolic extracts of medicinal plants that belonged to groups of endemic species in Anatolia against the human HeLa cervical cancer cell line showed positive results for cytotoxic activity (Artun *et al.,* 2016). A study carried out using 10 Chinese medicinal plants on 4 different cancer cell lines; BALL-1 (acute lymphoblastic leukemia), MCF-7 (breast carcinoma), Huh-7 (hepatocellular carcinoma), and HeLa (cervical carcinoma) exhibited potential cytotoxicity against cancerous cell lines compared to the normal cells. A recent study further showed that the methanol extract of *Solanum nigrum* fruits have inhibitory effects on the HeLa cell line (Patel *et al*., 2009). The above data and studies affirm the hypothesis that phytoconstituents derived from plants hold promising anti-cancer activity. The methanolic fruit extract of *Ficus cordata* ssp. *Salicifolia* showed promising cytotoxic activity of $IC_{50:}$ 21.11 $\mu g \cdot mL^{-1}$ against MCF7 (Omari *et al*., 2021). A study shows that *F.carica* uses multitargeted pathways to prevent the initiation and development of cancer.

They modulate various dysregulated signaling spills that is involved in cell proliferation, cell cycle regulation, apoptosis, invasion, autophagy inflammatory processes, metastasis and angiogenesis (Morovati *et al*., 2022). There are plants that belong to this genus and that have medicinal properties which can be explored.

The plant that has been used to study for its cytotoxic activity against the HeLa cell line is *Ficus racemosa*. It is regarded as an important plant in the ayurvedic system as it has been broadly used in the treatment of biliary disorders, inflammatory conditions, jaundice, dysentery, diabetes, and diarrhea (Ahmed and Urooj 2010; Jeyaraman and Patki 2012; Kirtikar and Basu 1935). The plant belongs to the family Moraceae and is commonly native to Australasia, South-East Asia, and the Indian subcontinent. The leaves are arranged alternately, are coriaceous, and pointed bluntly at the apex (Valvi *et al.*, 2014). The fruits, stem, leaves, latex and bark of *F. racemosa* have been proven to haveanti-diabetic potential (Amin *et al.*, 2015). The leaf extracts of the plants have been shown to exhibit anticancer and antioxidant activities in experimental studies of DMBA-induced mammary carcinoma in rats (Dhana Rangesh Kumar *et al*., 2013).

The ethanolic leaf extract of *F. racemose* showed cytotoxicity against the Dalton Lymphoma Ascites (DLA) cell line with an IC50 value of 175 µg/ml respectively. The ethanolic extract derived from the bark of *F. racemosa* showed a reduction in the level of serum lipids, lipoproteins, and blood glucose compared to the standard anti-diabetic drug-glibenclamide (Suvarna *et al.*, 2019).

Therefore, the purpose of this experiment is to extend the studies in examining the anti-cancer properties of leaf extracts derived from *Ficus racemosa* against the cancerous HeLa cells.

METHODOLOGY

Plant material: The leaves of *Ficus racemosa were* collected from *(Halasuru, Bangalore)*, processed, and pressed using an Herbarium press. Following identification and authentication at The Foundation for Revitalization of Local Health Traditions (FRLHT) – TDU, Bangalore, the leaves were washed, shade dried, and ground to fine powder needed further for experimental analysis.

Isolation and Sequencing of Genomic DNA: The fresh leaves were homogenized thoroughly to a fine powder and were subjected to DNA isolation using the Exposure Plant DNA isolation kit [Bogar Bio Bee]. The isolated genomic DNA was put forward for a PCR reaction using highly conserved rbcL primer sequences (F 5'ATGTCACCACAAACAGAGACTAAAGC 3' and R-5' GTAAAATCAAGTCCACCRCG 3'). The reaction conditions were as follows: Initial Denaturation at 95°C for 2 min; Denaturation at 95°C for 30 sec for 25 cycles; Annealing at 55°C for 30 sec; Extension at 72°C for 2 min; Final extension for 10 min; and Hold at 4°C ∞.

Single-pass sequencing was performed on each template using *RBCL gene* universal primers. The fluorescent-labeled fragments were purified from the unincorporated terminators through ethanol precipitation protocol. The samples were suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

Phylogenetic Analysis: The phylogeny and multiple sequence alignment of the query sequence were analyzed using BLASTn in NCBI. The software MUSCLE 3.7 was applied for multiple sequence alignments (Edgar 2004, MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*) whereas the Gblocks0.91b was used to eliminate alignment noises present in the form of poorly aligned positions and divergent regions (Talavera and Jose 2007). The program, PhyML 3.0 art exhibits similar precision to other phylogeny programs but at a faster magnitude and was used for phylogenetic analysis alongside using HKY85 as a substitution model. Lastly, the phylogenetic tree was rendered using the software Tree Dyn (Dereeper *et al*., 2008).

Plant extraction: 20g of dried sample powder was weighed and thoroughly dissolved in 100ml of water, ethanol, and ethyl acetate in a beaker covered with aluminum foil. The beaker was kept in a hot water bath at 50º C for 4 hours. Following incubation, the extract was filtered with Whatman filter paper and was collected in a clean 50ml beaker. The filtrate/extract was dried at 50ºC and turned into a semisolid form. This semi-solid sample was further weighed and assessed for the phytochemical yield.

Phytochemical screening - Qualitative analysis:

The phytochemical tests analysis was performed according to the standard protocol. The three solvent extracts: aqueous**,** ethanol, and ethyl acetate were used for qualitative analysis of the phytoconstituents.

1. Tannins. The lead acetate test was implemented to analyze the existence of Tannins. 2ml of the three solvent extracts were processed with 3-4 drops of lead acetate solution. Yellow color precipitation characterized the existence of flavonoids.

2. Saponins. 5 ml of three polar extracts were added to 20ml distilled water. The mixtures were perturbed in a graduated cylinder for about 15 - 20 minutes. The formation of foam positively confirmed the existence of saponins.

3. Steroids. The presence of steroids was confirmed through the Salkowski test wherein 2ml of the plant extracts was processed with 2ml chloroform and 2ml conc.sulphuric acid. The formation of a reddish-brown ring at the junction of the solution confirmed the presence of steroids.

4. Terpenoids. 2 mL of the sample extracts were processed with 2 mL conc. sulphuric acid and 2 ml acetic anhydride. A Blue-green ring formation confirmed the presence of terpenoids

5. Carbohydrates. The presence of carbohydrates in the extract was confirmed through Molischs where 2 mL of sample extracts were processed with 2 drops of alcoholic α-naphthol solution. The violet ring configuration at the junction characterized the existence of carbohydrates.

6. Flavonoids. The Shinoda test was performed to analyze flavonoid availability in the extracts. 1 mL sample extracts were processed with 8-10 drops of Conc. Hydrochloric acid and a pinch of magnesium powder and heated. A reddish coloration positively confirmed the presence of flavonoids.

Antioxidant – DPPH assay. DPH assay was performed to analyze the antioxidant properties of the three polar extracts following the protocol laid by (Guha *et al*., 2011). 240 µl of the sample extracts were prepared in six different concentrations of 5µg/ml to 1280µg/ml] was thoroughly mixed with 80µl of DPPH solution and processed with HPLC grade methanol. The reaction mixtures were thoroughly amalgamated and incubated at 25°C for 10 minutes. The mixtures were thereafter assessed for their absorbance at 517 nm using a spectrophotometer wherein a decrease in absorbance indicates an increase in radical scavenging activity. The percentages of the radical scavenging activity of the three extracts were calculated using the formula

% Inhibition = ((OD of Control – OD of sample)/OD of Control) × 100

In vitro cytotoxicity assay:

Cell lines and culture medium: HeLa cells were obtained from American Type Culture Collection (ATCC) and supplemented with DMEM containing 10% inactivated Fetal Bovine Serum (FBS), streptomycin (100µg/ml), and penicillin (100 IU/ml). The cells were cultured until confluent at 5% CO_2 and 37C. Further the confluent cells were trypsinized using 0.2 % trypsin and centrifuged at 1000rpm for 3-2mins. Following centrifugation, 50,000 cells /well was seeded in a 96-well plate (50,000 cells/well).

Cytotoxicity Studies: Following trypsinization, centrifugation, and suspension of the cell precipitate in DMEM medium, 100µl of the diluted cell suspension was added to 96 well microtitre plates. (50,000cells/well). The plate was incubated for 24 hrs and the monolayer was thoroughly washed with DMEM medium.100 µl of test concentrations of the reference drug and the three solvent extracts of the sample were added to the plates and incubated at 37^{o}C for 24hrs in 5% CO_2. The test solution was discarded after incubation and 0.05mg MTT was added to each well and incubated for 4 h at 37^{o}C in 5% CO_2. 100 µl of DMSO was added to the 96 well plates to solubilize formazan formed as a result of the MTT reaction. The absorbance was measured at 590nm, and the percentage of inhibition was calculated. A dose-dependent curve for the cell lines was further used to determine the (IC_{50}) of the test drug respectively.

Calculating Inhibition:

% Inhibition = ((OD of Control – OD of sample)/OD of Control) × 100.

Cell Cycle analysis: $1x10^6$ cells were seeded and cultured for 24hrs in a 6-well plate containing 2 ml of DMEM media supplemented with 10% FBS. Following incubation, the varied concentration of the reference drug and the three solvent extracts of the sample were added and incubated for 24 hrs. The cells were harvested and centrifuged at 2000 rpm for 5 minutes at room temperature and washed thoroughly by resuspension in 2mL of 1X PBS. The cells were fixed by suspending them in 300 µl of sheath fluid. The suspension was shaken gently followed by the addition of 1mL of chilled 70% EtOH and was further stored overnight at 4°C. Post-fixation the cells were centrifuged at 2000rpm for 5mins and the supernatant was discarded. The cell pellet was thoroughly washed twice with 2 ml of cold 1X PBS and was suspended in 450µl of sheath fluid containing 0.05 mg/ml PI and 0.05 mg/ml RNaseA and was incubated in dark for 5 min. The percentage of cells in various stages of the cell cycle in treated and control samples was determined using FACS Caliber (BD Biosciences, San Jose, CA).

GCMS - Gas Chromatography-Mass Spectrometry Analysis: The ethyl acetate extract that exhibited maximum cytotoxicity against HeLa cell lines was subjected to GC-MS analysis. 1 µl of 10mg/ml stock was injected and was analyzed in GC/MS Clarus 500 (Perkin Elmer) instrument equipped with RestekRtx^{R}–5, (30-meter × 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane) column. The GC oven was maintained with the OVEN TEMP - 40 ^{0}c for 5 min, Ramp 1: - 6°C / min to 280°C, - 280 ^{o}C for 15 min. The phytochemical components present in the ethyl acetate extract were identified through a comparison of retention times and mass spectral pattern database so present in the National Institute of Standards and Technology (NIST) library.

RESULTS

Herbarium Authentication: The morphological features of the collected plant specimen (RRBD01) were pressed, dried, and mounted with care on a herbarium sheet. The bark was coarse, yellowish-brown, 8-10mm thick with young shoots. The leaves were simple with an alternate arrangement present on young shoots, whereas the petioles appeared slender, obtuse, 10-15mm long blistered membranous structure. The flowers were unisexual with syconia inflorescence, and the peduncles were short, stout, and closed by 5-6 apical bracts.

The specimen was identified as *Ficus racemosa* of phylum Tracheophyta, class Magnoliopsida, order Rosales, and family Moraceae. They are deciduous and considered sacred under Indian rituals. The identification was further authenticated and verified at FRLHT-TDU, Bangalore, with an authentication number 124381.

Molecular sequencing and Phylogenetic Analysis: The rbcL gene from the specimen (RRRBD01)) was PCR amplified and sequenced. The sequence length of 471 bp was aligned to the rbcL gene sequence of plants present in the NCB1 Gene Bank database using BLASTn. The nucleotide alignment gave a similarity index of 100% with *Ficus racemosa*. Further, the data obtained from sequence alignment studies were submitted to the NCBI GenBank database with accession ID ON157663.

MEGA-X software was applied to render the phylogenetic tree as shown in Fig. 2. The specimen RRBD01 showed phylogenetic similarities to *Ficus*

Racemosa voucher (FR01) and was identified and confirmed to be *Ficus racemosa* respectively.

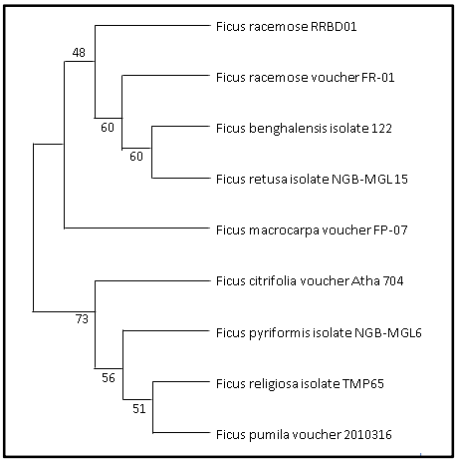


Fig. 1. Phylogenetic Tree highlighting the position of *Ficus racemosa* (RRBD01).

Phytochemical Extraction: Plants are composed of varied constituents with varied properties such as saponins, flavonoids, and glycosides which require different solvents for extraction. Owing to this concept, 20g of dried leaf powder of *Ficus racemosa*was dissolved in 100 ml of water, ethanol, and ethyl acetate. The mixtures were incubated, and filtered, to procure phytoconstituents in their respective solvents as illustrated in Table 1. The amount of sample taken and its percentage yield is calculated using the formula

% Yield of extract = weight of crude × 100/weight of the sample

The aqueous extract procured a maximum yield of (4.27%) due to the presence of primary and secondary metabolites. Whereas the ethanol and ethyl acetate extract composed of primary metabolites only procured a lesser yield of 2.54% and 1.58%.

Table 1: Phytochemical Yield of the solvents.

Sample	Sample Taken for extraction	Yield
Aqueous Extract	20g	4.27%
Ethanol Extract	20g	2.54%
Ethyl Acetate Extract	20g	1.58%

Cytotoxicity Assay (MTT Assay): The cytotoxic potential of all three extracts (aqueous, ethanol, and ethyl acetate) was assessed through an MTT assay using HeLa cell lines. Results of the MTT assay are tabulated in Table 2. The extracts were dissolved in a DMEM medium in different proportions to obtain six different concentrations of 10, 20, 40, 80, 160, 320 µg/ml respectively. The standard drug Vincristine was used as a reference to compare and determine the cytotoxic effects of the three extracts against HeLa cell lines. Co-relational regression analysis showed ethyl acetate extract to have a lower IC50 value of 82.57 µg/ml close to the reference molecule Vincristine.

A low IC50 value refers to high toxicity at lower concentrations, and it is evident from the data that the crude ethyl acetate extract confers the maximum toxicity against the cancerous cell lines, thus inhibiting its further differentiation. In comparison to the ethyl acetate extract, the aqueous and ethanol extract exhibited high IC50 values of 188.2 ug/ml and 139.8 ug/ml, thereby conferring low cytotoxicity against the cancerous cell lines.

Table 2: Cytotoxicity potential of ethyl acetate leaf extract of *Ficus racemose* against HeLa cell lines.

Compound name	Conc. µg/ml	OD at 590nm	% Inhibition	IC50 µg/mL
Control	0	0.569	0	
Aqueous Extract	10	0.534	6.15	188.2
	20	0.501	11.95	
	40	0.462	18.80	
	80	0.413	27.42	
	160	0.351	38.31	
	320	0.261	54.13	
Ethanol Extract	10	0.510	10.37	139.8
	20	0.468	17.75	
	40	0.423	25.66	
	80	0.366	35.68	
	160	0.256	55.01	
	320	0.187	67.14	
Ethyl Acetate Extract	10	0.489	14.06	82.57
	20	0.421	26.01	
	40	0.351	38.31	
	80	0.309	45.69	
	160	0.231	59.40	
	320	0.142	75.04	
Vincristine	8.2	0.512	10.018	66.0
	16.5	0.462	18.805	
	33.0	0.368	35.325	
	66.0	0.214	62.390	
	132.0	0.162	71.529	
	264.0	0.098	82.777	

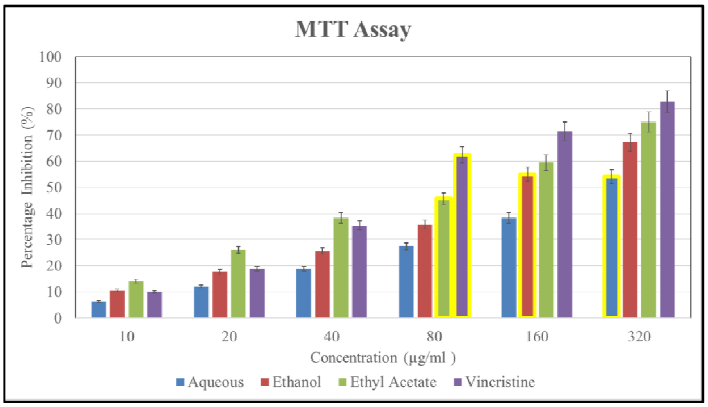


Fig. 2. Percentage inhibition of leaf extracts of *Ficus racemose* against HeLa cell lines.

The graphical data also confirms the above observations wherein the ethyl acetate extract of the sample exhibited an IC50 value at 80ug/ml and confers the maximum cytotoxicity regarding the standard drug Vincristine. The aqueous and the ethanol extract showed IC50 at higher concentrations of 320 ug. /ml and 160 ug/ml, thus exhibiting lesser cytotoxicity and a greater probability of survival for cancer cells.

Therefore, both the observed and experimental data confirm ethyl acetate extract to be a potent cytotoxic compound against the HeLa cell lines.

Flow cytometry Cell Cycle Analysis: Flow cytometry for cell cycle analysis assesses the percentage of viable cell population in different phases of the cell cycle. The three crude extracts were taken at two different concentrations (80 ug/ml and 160 ug/ml) and compared for cell viability against the reference compound Colchicine in HeLa cell lines. The results from the Table 3 showed ethyl acetate extract at 160ug/ml showed the minimum percentage of viable cells at G_0/G_{1phase} when compared to aqueous and ethanol extracts. This confirms that ethyl acetate extract functions as a cytotoxic compound similar to that of Colchicine; that prevents the formation of spindle fibers with an ultimatum of cell death. This cytotoxicity property arrests the division of cancerous HeLa cells at G0/G1 phase, preventing further metastasis and uncontrolled cell division.

DPPH Antioxidant Assay: The antioxidant capacity of the three extracts was assessed using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) antioxidant assay that scavenges upon free hydrogen radicals. The three extracts were taken at 6 different concentrations 5µg/ml to 1280 µg/ml and checked for their anti-oxidant assay against HeLa cell lines. The reference molecule Quercetin was used to determine and compare the antioxidant capacity of the plant extracts. Experimental data as illustrated in Table 4 showed ethanol extract of the sample has the lowest IC50 of 30.19 ug/ml. A low IC50 for DPPH assay confirms the greater anti-oxidant property of the compound. The ethyl acetate extracts exhibited an IC50 value of 266.5 ug/ml while the aqueous extract showed an IC50 value of 820.6 ug/ml.

The above observation confirms ethanol extracts of the sample possess the maximum anti-oxidant capacity and its high value may be justified due to the presence of phenolic contents like tannins that are good scavengers of free radicals. However contrary to the results in the cytotoxic assay, the ethyl acetate extract exhibited a low antioxidant capacity.

Qualitative Phytochemical Screening: A qualitative analysis was performed for the three crude extracts via varied phytochemical tests, the results of which are illustrated in Table 5. The results show that ethyl acetate extract tested positively only for terpenoids, tannins, and carbohydrates. Whereas the aqueous and ethanol extract tested positive for all six phytoconstituents respectively.

GCMS Analysis: The GC-MS chromatogram was carried out for the ethyl acetate extract which revealed a total of 18 peaks. On syncing, the results with the data present in the mass-spectral databases like FFNSC, NIST, and Wiley, seven major compounds were identified as tabulated in Table 6.

Table 3: Flow Cytometry Cell cycle analysis of the leaf extracts of *Ficus racemosa* against the HeLa cell line.

FACS analysis of Cell cycle arrest in HeLa cells				
Samples	**SUBG$_0$**	**G$_0$/G$_1$**	**S**	**G$_2$M**
Control	0.05	91.62	2.24	5.92
Aqueous Extract_80 µg/ml	3.03	83.73	5.98	7.74
Aqueous Extract_160µg/ml	0.05	75.40	14.70	10.23
Ethanol Extract_80µg/ml	1.73	79.88	9.38	8.28
Ethanol Extract_160µg/ml	0.90	64.74	19.85	14.94
Ethyl Acetate Extract_80µg/ml	0.10	75.89	7.02	17.20
Ethyl Acetate Extract_160µg/ml	0.30	57.69	19.06	22.96
Colchicine_25µM	0.59	40.84	9.20	49.78

Table 4: DPPH anti-oxidant assay of ethyl acetate leaf extract of *Ficus racemose* against HeLa cell lines.

Sample	Conc. (µg/ml)	OD@510 nm	% inhibition	IC50 (µg/ml)
Ethanol Extract	20	0.5128	36.39	30.19
	40	0.3847	52.28	
	80	0.21	73.95	
	160	0.152	81.15	
	320	0.08	90.08	
Ethyl Acetate Extract	20	0.701	13.05	266.5
	40	0.621	22.97	
	80	0.555	31.22	
	160	0.495	38.6	
	320	0.352	56.34	
	640	0.208	74.2	
Water Extract	20	0.729	9.58	820.6
	40	0.7117	11.72	
	80	0.652	19.13	
	160	0.601	25.45	
	320	0.552	31.53	
	640	0.472	41.45	
	1280	0.326	59.56	
Quercetin	0	0.854		1.306
	0.15625	0.815	4.67	
	0.3125	0.796	6.81	
	0.625	0.676	20.87	
	1.25	0.439	48.67	
	2.5	0.252	70.51	
	5	0.121	85.803	
	10	0.081	90.52	

Table 5: List of phytochemicals present in the extract.

Phytochemical Tests	Ethanol	Aqueous	Ethyl acetate
Steroids	+	+	-
Flavonoids	+	+	-
Terpenoids	+	+	+
Saponins	+	+	-
Tannins	+	+	+
Carbohydrate	+	+	+

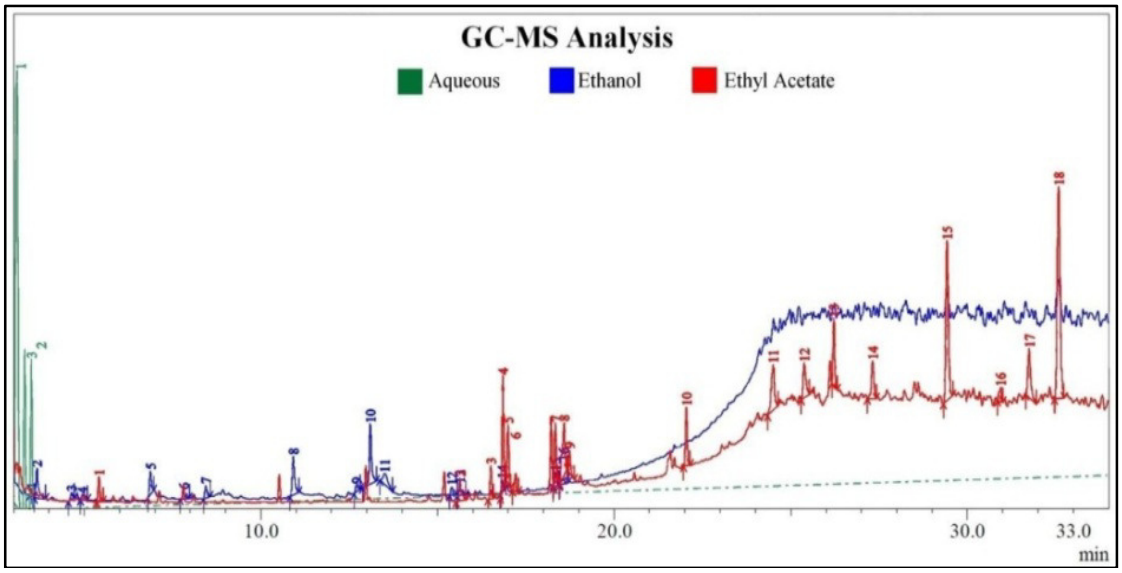
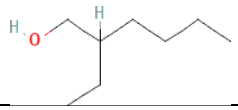
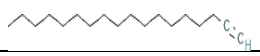
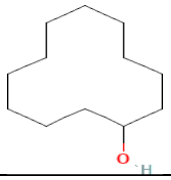
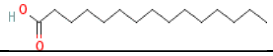
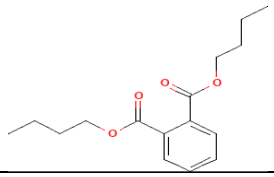
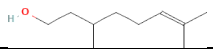
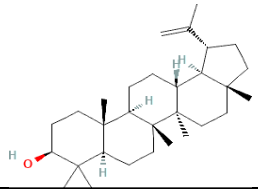


Fig. 3. GC-MS chromatogram of all three extracts of *Ficus racemosa* leaves.

Table 6: Compounds identified in the ethyl acetate extract.

Sr. No.	Compound	Structure	Molecular Formula	Molecular Weight
1.	1-Hexanol, 2-ethyl-		C8H18O	130
2.	1-Octadecyne		C18H34	250
3.	Cyclododecanol		C12H24O	184
4.	Pentadecanoic acid		C15H30O2	242
5.	Dibutyl phthalate		C16H22O4	278
6.	DL-Citronellol		C10H20O	156
7.	Lupeol		C30H50O	426

DISCUSSION

As per the classification brought about by National Cancer Institute, chemicals having IC50 21–200 μg/mL are considered moderately cytotoxic. The ethyl acetate extract of leaves of *Ficus racemosa* exhibited the highest cell toxicity with an IC50 value of 82.57 μg/mL against the cancerous HeLa cell lines. The cytotoxicity of the extract was further confirmed through cell cycle analysis where the viability of the cancerous cell at the G0/G1 phase was reduced to an extent comparable to that of the reference drug molecule colchicine. However, the ethyl acetate extracts showed a weak radical scavenging activity when compared to the aqueous and ethanol extracts. The reason could be due to its indirect blocking effect on molecules or enzymes responsible for the formation of free radicals. Studies have shown through invitro method that the methanolic extract of the fruit of *F. racemosa* showed promising anticancer properties against HepG-2 cells (Sivakumar *et al.*, 2019). This shows that the plant has cytotoxic activity.

The GC-MS chromatogram studies of the ethyl acetate extract identified seven significant secondary metabolites that constitute an important role in the defense system of plants. A few of these secondary metabolites show up to improvise human health. Pentadecanoic acid helps in balancing oxidative stress by maintaining a correct steadiness between the free radicals and antioxidants in the body, it also plays an important role in maintaining cardiovascular health and blood sugar level. Lupeol has been shown to possess anti-inflammatory properties; targeting molecular pathways like phosphatidylinositol-3-kinase (PI3K), Wnt signaling pathways, and nuclear factor kappa B (NFκB) respectively. It poses no toxicity to normal cells and tissues, so it can be used as a therapeutic intervention against inflammation and cancer (Saleem, 2009). However, the NIST library lacked certain chemical compounds and hence all compounds obtained through GC-MS chromatogram studies couldn't be.

Therefore, this study partly supports the hypothesis that the ethyl acetate extracts of the leaves of *Ficus racemosa* contain compounds that possess cytotoxic properties against HeLa cell lines and holds promising therapeutic potential against cancer. However, for its usage as a drug molecule against cancer, comprehensive studies need to be done to understand the pharmacokinetic properties of the compounds present in the extract.

CONCLUSIONS

The study shows that ethyl acetate extracts of leaves of *Ficus racemosa* confer cytotoxicity against HeLa cell lines and can be used in therapeutic applications against cancer. However comprehensive studies need to be done to check for its feasibility, usage, and applicability in the drug discovery process.

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Conflict of interests. Nill.

REFERENCES

Ahmed, F. and Urooj, A. (2010). Traditional uses, medicinal properties, and phytopharmacology of *Ficus racemosa*: A review. *Pharmaceutical. Biology*, *48*(6), 672–681.

Amin, M. M., Bhakta, S. and Das, S. K. (2015). Anti-diabetic potential of *Ficus racemosa*: current state and prospect especially in the developing countries. *Journal of Bioscience and Agriculture*, 5(2), 65-72.

Artun, F. T., Karagoz, A., Ozcan, G., Melikoglu, G., Anil, S., Kultur, S. and Sutlupinar, N. (2016). In vitro anticancer and cytotoxic activities of some plant extracts on HeLa and Vero cell lines. *Journal of B.U.ON*, *21*(3), 720–725.

Bhattacharya, S. K., Satyan, K. S. and Ghosal, S. (1997). Antioxidant activity of glycowithanolides from Withania somnifera. *Indian Journal of Experimental Biology*, *35*(3), 236–239.

Bray, F., Ren, J., Masuyer, E. and Ferlay, J. (2013). Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *The International Journal of Cancer*, *132*(5), 1133–1145.

Cragg, G. M. and Newman, D. J. (2005). Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology*, *100*(1–2), 72–79.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V. and Lescot, M. (2008). Phylogeny. fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, *36*(2), W465–W469.

Dhana Rangesh Kumar, V., Jeyanthi, G. and Gurusamy, K. (2013). Evaluation of the anticytotoxic activity of *Ficus glomerata* Roxb. leaf extract against DMBA induced mammary carcinoma in rats. *International Journal of Pharmaceutical and Biological Sciences, 4*, B142–B149.

Guha, G., Rajkumar, V., Kumar, R. A. and Mathew, L. (2011). Antioxidant activity of Lawsonia inermis extracts inhibits chromium (VI)-induced cellular and DNA toxicity. *Evidence-Based Complementary and Alternative Medicine*.

Jayakumar, F. A., Simon, S. E., Yan, Y. W. and Yap, P. W. (2018). Characteristic And Optimized Use Of Bioactive Compounds From Gloriosa Superba And Albizia Amara With Apoptotic Effect On Hepatic And Squamous Skin Carcinoma. *InternationalJournal of Pharmceutical Sciences Research*, *9*(5), 1769–1778.

Jeyaraman, R., and Patki, P. S. (2012). Clinical evaluation of efficacy and safety of a herbal formulation in benign prostatic hyperplasia: A single blind, randomized, placebo-controlled study. *Open Journal of Urology*, *2*(03), 157.

Kirtikar, K. R. and Basu, B. D. (1935). Indian medicinal plants. *Indian Medicinal Plants.*Vol 2, 1347-1348.

Ochwang'iDO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM and Kiama SG (2014). Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *Journal of. Ethnopharmacologyl*, *151*(3), 1040–1055.

Omari, A., Fatima and Ahmed, Abdel Azim and Al-Robai, Sami and Mohamed, Haidar and Elzubier, Mohamed and Abdalla, Ashraf (2021). Total phenol, flavonoid, individual phenolic acid contents, and antioxidant and cytotoxic activities of methanol extracts from Ficus cordata ssp. salicifolia. *Canadian Journal of Plant Science, 101*(4), 517-524.

Morovati , M. R., Ghanbari-Movahed, M., Barton, E. M., Farzaei, M. H. and Bishayee, A. (2022). A systematic review on potential anticancer activities of Ficus carica L. with focus on cellular and molecular mechanisms. *Phytomedicine: International journal of phytotherapy and phytopharmacology*, *105*, 154333.

Patel, S., Gheewala, N., Suthar, A. and Shah, A. (2009). In-vitro cytotoxicity activity of Solanum nigrum extract against Hela cell line and Vero cell line. *International Journal of Pharmacy and Pharmaceutical Science.*, *1*(1), 38–46.

Saleem, M. (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Letters*, *285*(2), 109–115.

Sivakumar, P., Manimekalai, I., Sivakumari, K. and Ashok, K. (2019). Anticancer effect of fig fruit ficus racemosa extract against human heptocellar carcinoma (HepG-2) CELL LINE.

Suvarna, S., Rao Priya, S., Jadhav, R. S. and Dukare, T. P. (2019). A pharmacological review on *Ficus racemosa*. *World Journal of Pharmaceutical Research*, *8*(7), 627-634.

Talavera, G. and Jose, C. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, *56*, 564–577.

Valvi, S. R., Jadhav, V. D., Gadekar, S. S. and Yesane, D. P. (2014). Assessment of bioactive compounds from five wild edible fruits, *Ficus racemosa*, Elaegnus conferta, Grewia tillifolia, Scleichera oleosa and Antidesma ghasembilla. *Association for the Advancement of Biodiversity Science*, *3*(1), 549–555.

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