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Journal of Drug Delivery and Therapeutics

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Research Article

Analytical evaluation of *C. pictus* for its therapeutic properties and antioxidant activity in relation to anticancer potential against selected cancer cell lines

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Article Info:

Article History:

Received 02 June 2025
Reviewed 07 July 2025
Accepted 29 July 2025
Published 15 August 2025

Cite this article as:

Ashwini, Ramachandra YL, Namdeo PS, Dange PP, Rai PS, Analytical evaluation of *C. pictus* for its therapeutic properties and antioxidant activity in relation to anticancer potential against selected cancer cell lines, Journal of Drug Delivery and Therapeutics. 2025; 15(8):112-123 *DOI: http://dx.doi.org/10.22270/jddt.v15i8.7338*

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Abstract

Medicinal plants play an important role in medical care throughout history, including the modern period. *Costus pictus*, also known as the 'insulin plant,' exhibits numerous bioactivities, including anti-diabetic, antioxidant, anticancer, anti-inflammatory, and hepatoprotective properties. In the present investigation, *C. pictus* leaves were extracted using ethanol and petroleum ether, and GC-MS analysis revealed 26 and 24 chemical components, respectively. These compounds are used significantly in pharmacology. The antioxidant capabilities of medicinal plants have been studied since they may be in possession of a number of bioactive molecules. It has been demonstrated that antioxidants revert the development of cancer through micromanagement of tumours, and medicinal plants are a rich source of new and powerful antioxidants and anticancer bioactive molecules. In the present study, the anticancer potential of *C. pictus* leaf extracts in water, ethanol, and petroleum ether against MCF-7 cancer cell lines was evaluated. The MCF-7 cell line was inhibited by all three extracts in a dose-dependent manner.

Key words: anti-cancer, anti-inflammatory, pharmacology, GC-MS, MCF-7 cancer cell lines,

INTRODUCTION:

Nature has provided us with numerous herbs and plants that can be utilized to create medications for maintaining human health. The term "herbs" derives from the Latin 'herba'. It denotes a medicinal herb. Plants have been the primary tools of traditional medicine since time immemorial; however, only a small percentage of botanical wealth is utilized in traditional remedies. Plants produce a variety of chemical substances that are utilized to conduct biological tasks and defend against predators such as insects, fungi, and herbivorous animals. The plant's therapeutic effects are due to the bioactive chemicals found inside it[1].

MATERIALS AND METHODS:

1. **Collection of plant material and Preparation of plant extract:** Fresh and healthy *C. pictus* leaves were taken from Palakkad (Kerala, India), *C. pictus* leaves were thoroughly cleaned, shade-dried at room temperature, and milled into fine powder. The powdered material is then stored in airtight vials for later analysis. The homogenized samples were extracted using ethanol, petroleum ether, and water. 20 g of materials were dissolved separately in three conical flasks containing 150 ml of ethanol, petroleum ether, and water. For further test performed by GC-MS analysis

A

B

Figure 1: A. The study plant – *C. pictus* D. Don, B. *C. pictus* leaves powder

2. **Analysis of Gas Chromatography-Mass Spectrometry:** Thermo GC Trace Ultra Ver-5.0, Thermo MS DSQ II system, and gas chromatograph interfaced to a mass spectrometer (GC-MS) were used for GC-MS analysis under the following circumstances: DB 35-MS Helium (99.999%) was utilized as the carrier gas in a capillary standard column (30 x 0.25 mm ID x 1µM df) running in electron impact mode at 70 eV. The injection volume was 1 µl, and the flow rate was maintained at 1 ml/min. The oven's temperature was set to rise from 70°C (isothermal for two minutes) to 260°C at a rate of 6°C per minute. The GC ran for a total of 37.53 minutes. Using a calibrated microsyringe, 1 µL of the sample was added to the sample port. Helium, the carrier gas, then transported the sample to the column. The chemicals separate as the sample-carrying mobile phase moves through the stationary phase. After that, the sample passes through the mass spectrometer detector, which separates it according to the mass-to-charge ratio. The computer, which has a sample library, then receives the data. To determine which components were eluted at a given retention period, the data is shown in the mass spectrum at that specific moment in the chromatogram.

3. ***In vitro* Antioxidant Properties of Leaves of *C. pictus*:** The DPPH Radical Scavenging activity, Ferric Reducing Antioxidant Potential (FRAP), and Total Antioxidant Activity were used to assess the plant leaf extracts' capacity to scavenge free radicals.

3.1 DPPH Radical Scavenging ActivityReagents

- Diphenyl- 2- picrylhydrazyl (DPPH)
- Methanol

Principle: The reduction of DPPH, a stable free radical, is the foundation of the DPPH test technique. At 517 nm, the odd-electron free radical DPPH exhibits the most absorption, giving it a purple hue. Decolorization (yellow color), about the number of electrons captured, occurs when antioxidants react with DPPH, a stable free radical, which is paired off in the presence of a hydrogen donor (such as a free radical-scavenging antioxidant) and reduced to DPPH. This causes the absorbance to decrease from the DPPH radical to the DPPH form. The more decolorization, the greater the reduction power. Mixing a DPPH solution with one of the substances that can donate a hydrogen atom results in the reduced form (Diphenylpicrylhydrazine; non-radical), which loses its violet color and thus has a lower absorbance. In terms of hydrogen-donating capacity, the degree of discolouration reveals the antioxidant compounds' or extracts' scavenging activity.

Procedure: In the presence of the DPPH stable radical, the hydrogen-donating capacity was investigated. 1 ml of a 0.3 mM DPPH methanol solution was mixed with 1 ml of plant extracts (1000 µg/ml) at several concentrations, and the mixture was left to react at room temperature. The absorbance measurements were taken at 517 nm after 30 minutes. A DPPH solution (1.0 ml, 0.3 mM) with 1 ml of methanol was utilized as a negative control, whereas a methanol solution was utilized as a blank. The positive control was ascorbic acid (1000 µg/ml). The following formula was utilized to determine the DPPH radical's scavenging capability.

$$\% \text{ of inhibition} = \frac{\text{A control} - \text{A test}}{\text{A control}} \text{ X } 100$$

where "A test" was the absorbance while the extract or standard was present, and "A control" was the absorbance of the control reaction. Triplicate analysis was used to get the mean values. IC_{50} was used to express the extract's antioxidant activity.

3.2 Ferric Reducing Antioxidant Potential (FRAP) Reagents

- Acetate buffer (500 mM/l)
- Tripyridyltriazine (TPTZ) (10 mM/l)
- Ferric chloride (20 mM/l)

Procedure: The FRAP agent was made by combining 2.5 ml of tripyridyl triazine (TPTZ) (10 mM/l), 2.5 ml of ferric chloride (20 mM/l) solution, and 25 ml of acetate buffer (500 mM/l). 300 µl of freshly made FRAP reagent that had been warmed to 37°C was added to 10 µl of plant extracts at varying concentrations, as well as 30 µl of water, to create the reaction mixture. Four minutes after the FRAP reagent was added, the absorbance of this solution was measured at 593 nm. It is a rise in absorbance signified improved reducing potential. Using an equation derived from the Fe^{++}-TPTZ standard curve, quantitative calculations were performed for every sample.

Absorbance = 0.274 x µM of Fe^{++} + 0.114 [R^2 = 0.974]

4. Total Antioxidant ActivityReagents:

Phosphomolybdenum reagent: 0.6 M sulphuric acid with 4 nM ammonium molybdate and 28 mM sodium phosphate. The total antioxidant activity of *C. pictus* extracts was measured spectrophotometrically using the phosphomolybdenum test. In sealed test tubes, 0.3 mL of 1 mg/mL extract solution in methanol was combined with 2.7 mL of phosphomolybdenum reagent. The test tubes are incubated in a water bath at 95°C for 90 minutes. After cooling to ambient temperature, the solution's absorbance at 695 nm was measured with a visual spectrophotometer against a blank (0.3 ml methanol without plant extract). The results were represented as trolox equivalents (mg TE/g dry material). Quercetin served as a reference control.

5. *C. pictus* has anticancer activity against the MCF-7 cancer cell line.

The National Centre for Cell Science (NCCS), located in Pune, provided the human breast cancer cell line (MCF-7), which was cultivated on Eagle's Minimum Essential Medium supplemented with 10% Fetal Bovine Serum (FBS). At 37°C, 95% air, 5% CO_2, and 100% relative humidity, the cells were kept. The culture medium was replaced twice a week, and maintenance cultures were passaged once a week.

5.1 Cell Treatment Procedure:

To create single-cell suspensions, the monolayer cells were separated using trypsin-ethylene diamine tetraacetic acid (EDTA). Viable cells were then counted using a hemocytometer and diluted with media containing 5% FBS to achieve a final density of $1x10^5$ cells/ml. 96-well plates were seeded with 100 mL of cell suspension per well at a plating density of 10,000 cells/well. The plates were then incubated at 37°C, 5% CO_2, 95% air, and 100% relative humidity to facilitate cell adhesion. The cells were exposed to repeated concentrations of the test substances after 24 hours.

First, they were dissolved in neat dimethyl sulfoxide (DMSO). Then, using serum-free medium, an aliquot of the sample solution was diluted to twice the final maximum test concentration. In order to obtain five sample concentrations, four more serial dilutions were made. By adding aliquots of 100 µl of these various sample dilutions to the corresponding wells that already contained 100 µl of medium, the necessary final sample concentrations were achieved.

The plates were incubated at 37°C, 5% CO_2, 95% air, and 100% relative humidity for an additional 48 hours after the sample was added. Triplicate samples were kept for every concentration, and the medium devoid of samples was used as a control.

5.2 MTT Assay:

It is a yellow tetrazolium salt that dissolves in water, and it also has another name that is [4,5-dimethylthiazol-2-yl] 3-MTT, or 2,5-diphenyltetrazolium bromide. The tetrazolium ring is cleaved by the mitochondrial enzyme succinate dehydrogenase in live cells, converting the MTT into an insoluble purple formazan. As a result, the number of viable cells directly correlates with the amount of formazan generated. Each well received 15 µl of MTT (5 mg/ml) in phosphate-buffered saline (PBS) after 48 hours of incubation, and the wells were then incubated for 4 hours at 37°C. A microplate reader was used to detect the absorbance at 570 nm after the MTT-containing media was turned off and the formazan crystals that had formed were dissolved in 100 µl of DMSO. Then, using the following formula, the percentage of cell viability was determined to control:

% Cell viability = [A] Test / [A]control x 100

% Cell inhibition = 100 - [A] Test / [A]control x 100

Where [A] is the absorbance

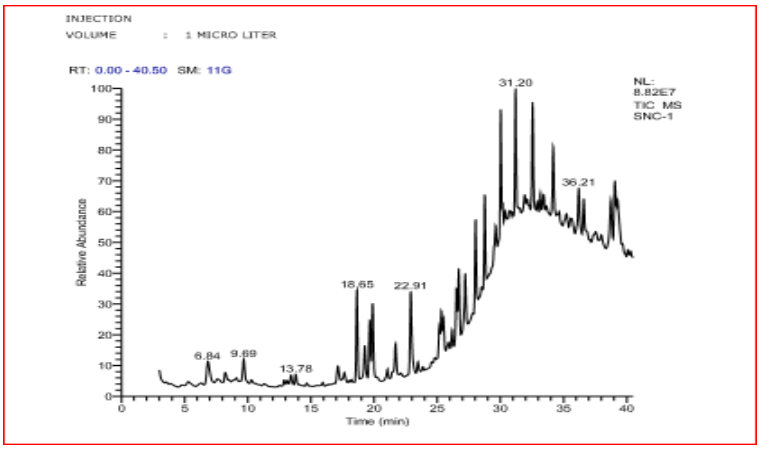
RESULTS:

The GC-MS analysis of *C. pictus* leaves extract was performed by using Thermo GC-MS Trace Ultra Ver: 5.0, with a split injector and a Thermo MS DSQ by using two solvents, ethanol and petroleum ether. The ethanol extract of *C. pictus* leaves indicated the presence of 26 chemical compounds. The active principle is reported in Table 3.1 along with its molecular weight (MW), retention time (RT), molecular formula, and % composition in the extracts. Graph 1 shows a graphical depiction of this information. Major constituents identified are Neophytadiene (4.79%), Phytol, acetate (4.79%), 3,7,11,15- Tetramethyl-2-hexadecen-1- ol (2.05%), 2-Pentadecanone, 6,10,14-trimethyl (6.86%), Hexadecanoic acid, ethyl ester (5.43%), Octadecanoic acid, ethyl ester (6.15%), Linoleic acid ethyl ester (6.15%), Pentacosane (8.36%), Octacosane (6.11%), Docosane (5.32%), Heptacosane (5.18%), Neophytadiene (4.79%), (S)-4-Hydroxymethyl-2-phenyloxazoline (4.40%), Tricosane (3.29%), Squalene (2.38%), Pyranthrene (1.75%), and Methyl 2,4-dimethyltetradecanoate (1.21%) and 13-Docosenamide (6.87%).

Table 3.1: The GC-MS analysis of ethanol extract of *C. pictus* leaves

S. No	RT	Compound name	Molecular formula	Molecular weight	Peakarea(%)
1.	6.84	1-Dodecene	$C_{12}H_{24}$	168	2.28
2.	8.22	Tridecane	$C_{13}H_{28}$	184	0.82
3.	9.69	1-Hexadecene	$C_{16}H_{32}$	224	1.68
4.	17.12	2(4H)Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	$C_{11}H_{16}O_2$	180	1.43
5.	17.64	10-Heneicosene	$C_{21}H_{42}$	294	0.88
6.	18.65	Neophytadiene	$C_{20}H_{38}$	278	4.79
7	18.65	Phytol, acetate	$C_{22}H_{42}O_2$	338	4.79
8.	19.27	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.05
9.	19.89	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268	6.86
10.	22.91	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	5.43
11.	25.31	Docosane	$C_{22}H_{46}$	310	5.32
12.	26.69	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	6.15

13.	26.69	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	6.15
14.	27.27	Tricosane	$C_{23}H_{48}$	324	3.29
15.	28.04	(S)-4-Hydroxymethyl-2-phenyloxazoline	$C_{10}H_{11}NO_2$	177	4.40
16.	29.61	Butyl 9.cis.,11. trans.-octadecadienoate	$C_{22}H_{40}O_2$	336	1.88
17.	30.02	Pentacosane	$C_{25}H_{52}$	352	8.36
18.	30.67	Nonacosane	$C_{29}H_{60}$	408	0.84
19.	31.20	Octacosane	$C_{28}H_{58}$	394	6.11
20	31.91	Pyranthrene	$C_{30}H_{16}$	376	1.75
21.	32.55	Heptacosane	$C_{27}H_{56}$	380	5.18
22.	35.23	Benzenamine, 4,4',4''- methylidynetris[N,N-dimethyl	$C_{25}H_{31}N_3$	373	1.12
23.	35.66	Ethyl tetracosanoate	$C_{26}H_{52}O_2$	396	1.21
24.	35.66	Methyl 2,4-dimethyltetradecanoate	$C_{17}H_{34}O_2$	270	1.21
25.	36.59	Squalene	$C_{30}H_{50}$	410	2.38
26.	39.06	13-Docosenamide	$C_{22}H_{43}NO$	337	6.87



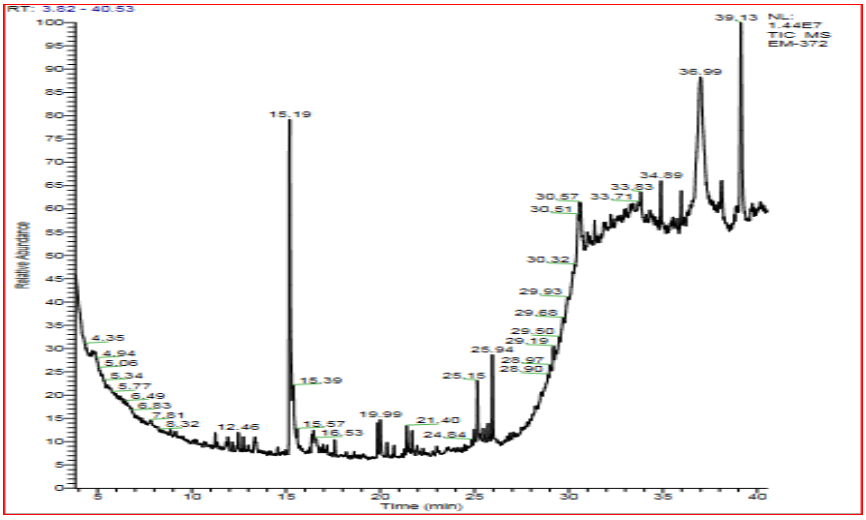
Graph 1: The GC-MS analysis of the ethanol extract of *C. pictus* leaves

GC-MS analysis of petroleum ether extracts of *C. pictus* leaves indicated the presence of 24 chemical compounds (Table 3.2 & Graph 2). Pentane, 3-ethyl-2, 2-dimethyl (82.41%), cis-Asarone (3.76%), á-Tumerone (0.42%), Tetratetracontane(3.42%), 3, 5-Bis (p-Dimethylaminostryl)-2, 2-dimethyl-2H-pyr role 1 – Oxide (2.47%), Phytol (0.64%), Quercetin 7, 3', 4'-trimethoxy (1.65%) and 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane/ Stigmasterol (0.60%).

Table 3.2: The GC-MS analysis of the petroleum ether extract of *C. pictus* leaves

S. No	RT	Compound name	Molecular formula	Molecular weight	Peakarea(%)
1.	3.08	Pentane, 3-ethyl-2,2-dimethyl-	C_9H_{20}	128	82.41
2.	4.81	Tetradecane,1-chloro-	$C_{14}H_{29}Cl$	232	0.15
3.	11.25	9-Octadecen-12-ynoic acid, methyl ester	$C_{19}H_{32}0_2$	292	0.11
4.	11.25	d-Nerolidol	$C_{15}H_{26}O$	222	0.11
5.	11.25	Junipene	$C_{15}H_{24}$	204	0.11
6.	11.91	Stearic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{78}O_3$	594	0.12
7.	11.91	Oleic acid, 3-(octadecyloxy)propyl ester (CAS)	$C_{39}H_{76}O_3$	592	0.12

8.	12.46	Docosane	$C_{22}H_{46}$	310	0.12
9.	13.35	á –sesquiphellandrene	$C_{15}H_{24}$	204	0.17
10.	15.19	cis-Asarone	$C_{12}H_{16}O_3$	208	3.76
11.	16.47	Tumerone	$C_{15}H_{22}O$	218	0.42
12.	19.99	2-Pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	268	0.40
13.	21.42	(E, E)-Farnesyl acetone	$C_{18}H_{30}O$	262	0.25
14.	21.68	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.16
15.	25.17	Phytol	$C_{20}H_{40}O$	296	0.64
16.	25.94	2,2-Dimethyl-3-(3,7,16,20-tetramethyl- heneicosa-3,7,11,15,19-pentaenyl)-oxirane	$C_{29}H_{48}O$	412	0.60
17.	25.94	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethy 3,7,11,15 ,19- heneicosapentaenyl)-	$C_{30}H_{50}O$	426	0.60
18.	30.59	Quercetin 7,3',4'-Trimethoxy	$C_{18}H_{16}O_7$	344	1.65
19.	31.00	Cyclohexane, (1-hexadecylheptadecyl)-	$C_{39}H_{78}$	546	0.13
20.	33.81	Nonacosane	$C_{29}H_{60}$	408	0.45
21.	34.34	1HPuri6 amine, [(2-fluorophenyl) methyl]-	$C_{12}H_{10}FN_5$	243	0.19
22.	34.89	2-Iodo-3',4',4,5-tetramethoxybiphenyl	$C_{16}H_{17}IO_4$	400	0.31
23.	36.99	Tetratetracontane	$C_{44}H_{90}$	618	3.42
24.	39.13	3,5-Bis(p-Dimethylaminostryl)-2,2-dimethyl-2H-p role 1-Oxide	$C_{26}H_{33}N_3O$	403	2.47



Graph 2: The GC-MS analysis of the petroleum ether extract of *C. pictus* leaves

3.1 Antioxidant Properties of Leaf Extracts from *C. pictus*

Medicinal plants' antioxidant properties are investigated since they may be in charge of a number of bioactivities.[2,3] Moreover, atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, and degenerative eye disease might result from excessive free radical production. Cell damage from unstable free radicals is prevented by antioxidants. The antioxidant properties of *C. pictus* leaf extracts in ethanol, water, and petroleum ether were assessed in this investigation. For the experiment, three techniques were employed: DPPH, FRAP, and total antioxidant activity.

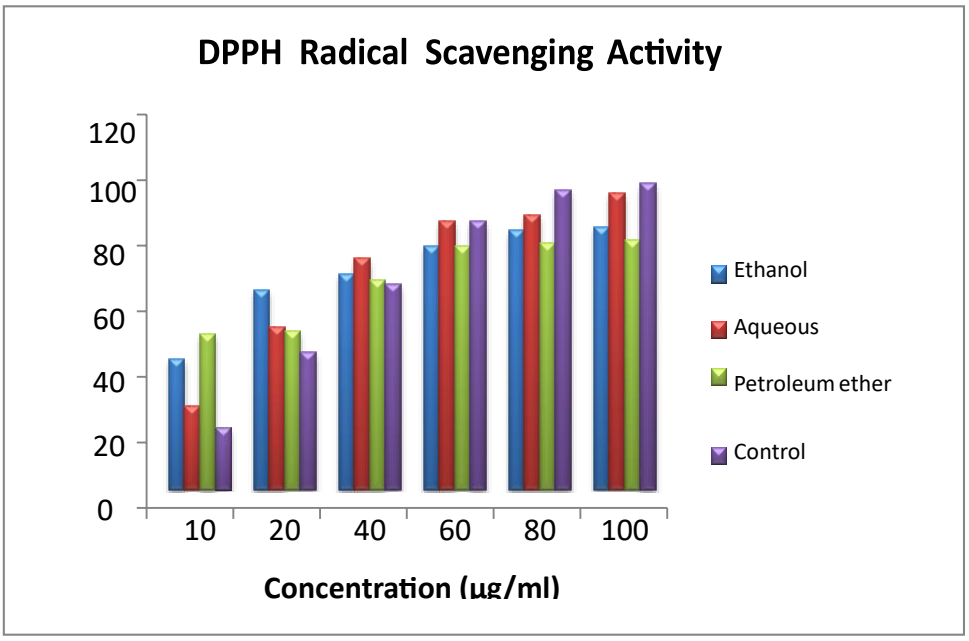
3.1.1 DPPH Radical Scavenging Assay

The DPPH free radical technique, which measures the reduction of DPPH, a stable free radical, has been frequently used to assess the antioxidant activity of plant extracts. As indicated in Table 3.3 and Graph 3, the DPPH scavenging capacity of petroleum ether, ethanol, and aqueous extracts of *C. pictus* leaves was assessed. The outcomes are contrasted with ascorbic acid as a standard. The IC_{50} value was calculated for each extract and the

standard. Ethanol extract (26 µg/ml) and aqueous extract (22 µg/ml) had considerably lower IC_{50} values for DPPH scavenging than ascorbic acid. Ascorbic acid, the standard reference chemical, had a higher DPPH scavenging capacity than the petroleum ether extract (50 µg/ml), with an IC_{50} of 39 µg/ml. The scavenging activity of extracts increased with concentration.

Table 3.3: The percentage inhibition of DPPH Radical Scavenging activity of *C. pictus* leaf extracts

S. No	Concentration(µg/ml)	% of Inhibition			
		Ethanol	Aqueous	Petroleum ether	Standard
1	10	42	27	50	20
2	20	64	52	51	44
3	40	69	74	67	66
4	60	78	86	78	86
5	80	83	88	79	96
6	100	84	95	80	98
IC50 Value (µg/ml)		26.28	22	50	39.66



Graph 3: The DPPH radical scavenging activity of *C. pictus* leaf extracts

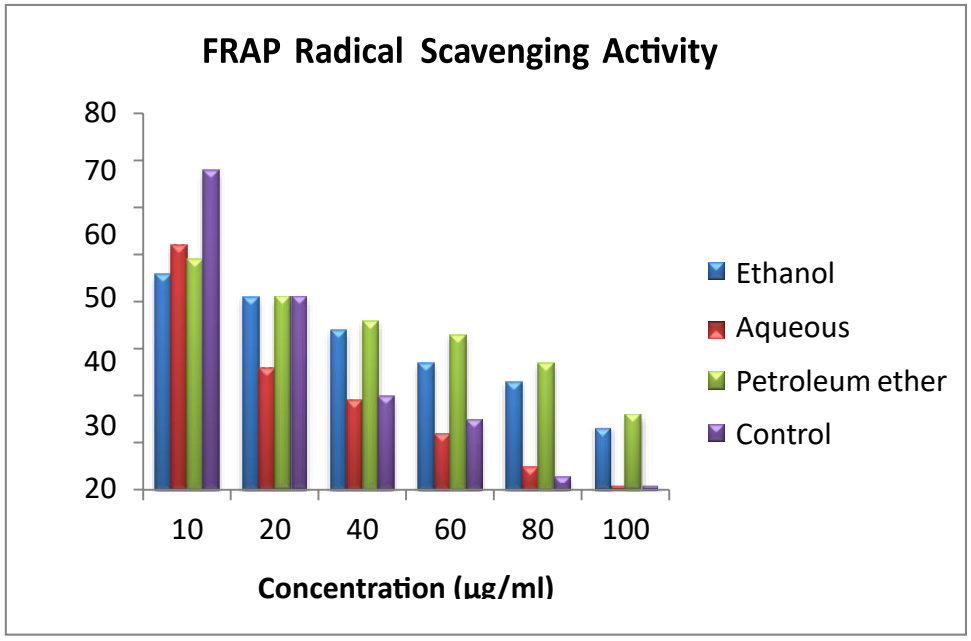
3.1.2 The ferric-reducing antioxidant potential (FRAP) of *C. Pictus* leaf extracts

The ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves' ferrous ion chelating properties are compiled in Table 3.4 and Graph 4. The IC_{50} value of each extract was contrasted with that of ascorbic acid, the standard. The extracts' Fe^{2+} chelating action was higher in the aqueous extract (12.54 µg/ml) than in the ethanol and petroleum ether extracts (IC_{50} 41.90 µg/ml and 62.50 µg/ml, respectively). The IC_{50} for ferrous ion chelating was 14.13 µg/ml for the typical ascorbic acid.

Table 3.4: The percentage inhibition of FRAP Radical Scavenging activity of *C. pictus* leaf extracts.

S. No	Concentration (µg/ml)	% of Inhibition			
		Aqueous	Ethanol	Petroleum ether	Standard (Ascorbic acid)
1	10	52	46	49	68
2	20	26	41	41	41

3	40	19	34	36	20
4	60	12	27	33	15
5	80	5	23	27	3
6	100	1	13	16	1
IC50 Value (µg/ml)		12.54	41.90	62.50	14.13



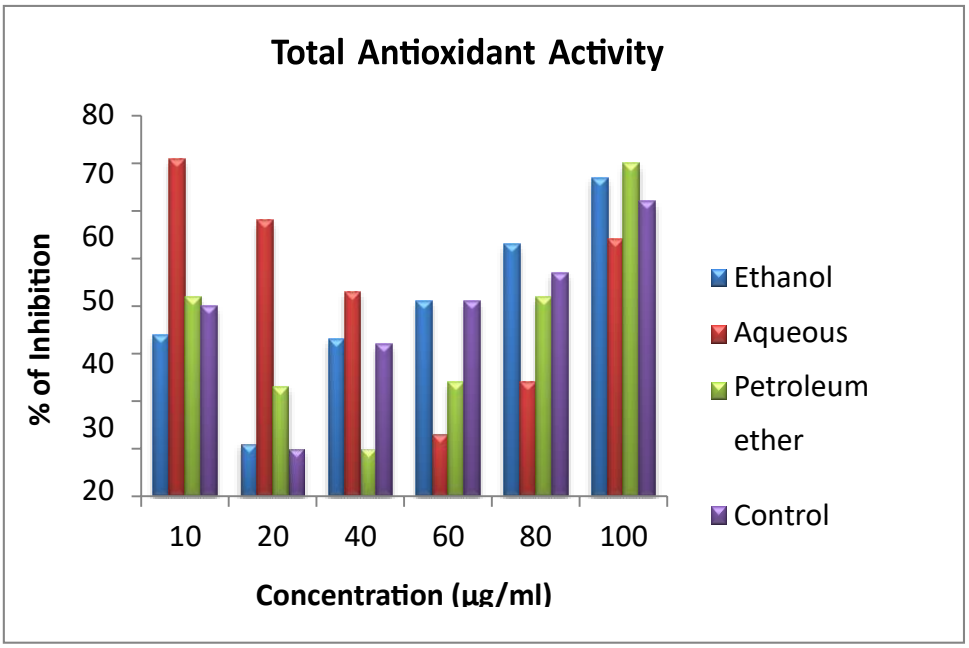
Graph 4: The FRAP radical scavenging activity of *C. pictus* leaf extracts

3.1.3 Total Antioxidant Activity of Leaf Extracts from *C. pictus*

The total antioxidant activity of ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves is presented in Table 3.4 and Graph 5. The IC_{50} value was calculated for each extract as well as the standard. The aqueous extract had a total antioxidant activity of 31.89 µg/ml, whereas the ethanol and petroleum ether extracts had IC_{50} values of 72.75 µg/ml and 92.84 µg/ml, respectively. Quercetin's antioxidant capability of 44.31 µg/ml was chosen as a reference standard.

Table 3.5: The Total antioxidant activity of *C. pictus* leaf extracts

S. No	Concentration (µg/ml)	% of Inhibition			
		Ethanol	Aqueous	Petroleum ether	Standard (Quercetin)
1.	10	34	71	42	40
2.	20	11	58	23	10
3.	40	33	43	10	32
4.	60	41	13	24	41
5.	80	53	24	42	47
6.	100	67	54	70	62
IC50 Value (µg/ml)		72.5	31.89	92.84	44.31



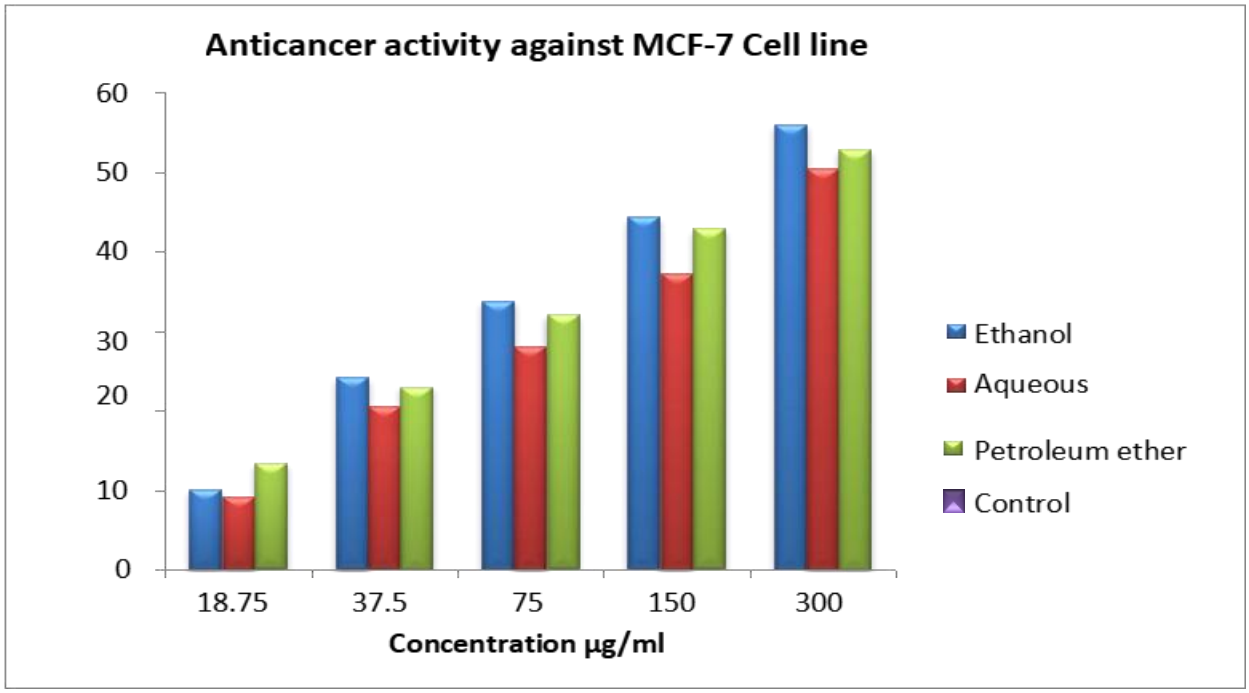
Graph 5: The Total antioxidant activity of *C. pictus* leaf extracts

3.2 *In vitro* Anticancer Activity of *C. pictus* Leaf Extracts against MCF-7 Cell Line

Plant extracts contain a vast array of bioactive compounds that may possess anticancer properties, which excites researchers seeking new and novel therapeutic medications[4].Various quantities of ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves were evaluated against the MCF-7 breast cancer cell line using the MTT assay. The extracts demonstrated greater inhibition with increasing concentration (Table 3.6 and Graph 6). The IC_{50} value was calculated for each extract. All of the extracts reduced the cancer cell lines' growth rate and survival. The study used a maximum concentration of 300 µg/ml, with IC_{50} values of 222.46 µg/ml, 293.94 µg/ml, and 255.24 µg/ml for ethanol, aqueous, and petroleum ether extracts.

Table 3.6: The anticancer activity of *C. pictus* leaf extracts

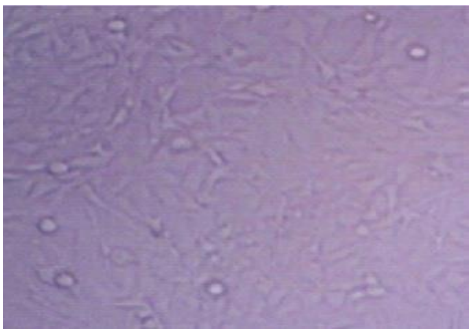
S. No	Concentration(µg/ml)	% of Inhibition		
		Ethanol	Aqueous	Petroleum ether
1	18.75	10.146	9.238	13.344
2	37.5	24.279	20.529	23.056
3	75	33.833	28.109	32.136
4	150	44.374	37.347	42.993
5	300	56.021	50.533	52.981
IC50 Value (µg/ml)		222.46	293.94	255.24



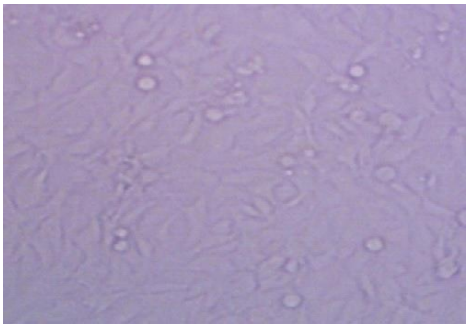
Graph 7: The anti-cancer activity of *C. pictus* leaf extracts against the MCF-7 Cell line

Figure 5: The anticancer activity of *C. pictus* leaf extracts against MCF-7 breast cancer cell line

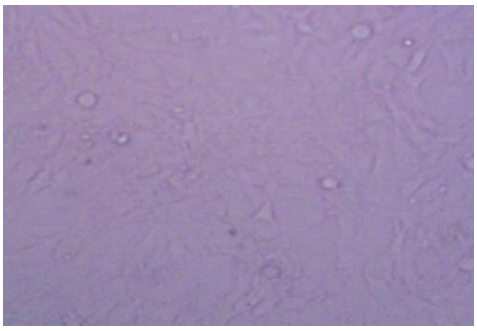
a) Control



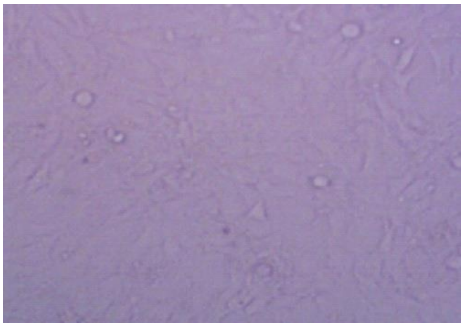
b) Ethanol extract of *C. pictus* leaves



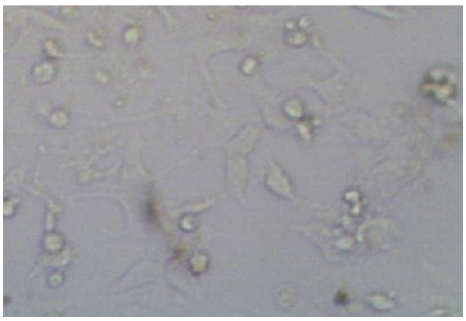
18.75 µg



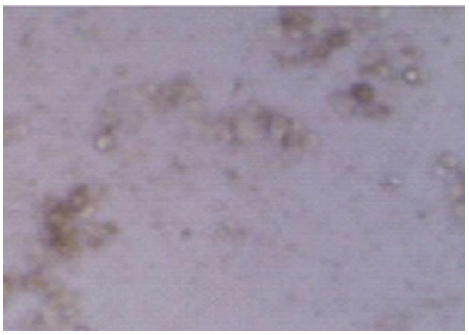
37.5µg



75 µg

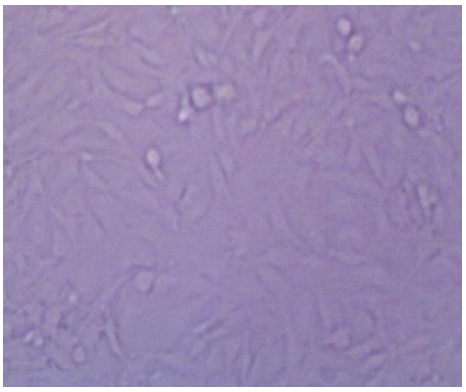


150 µg

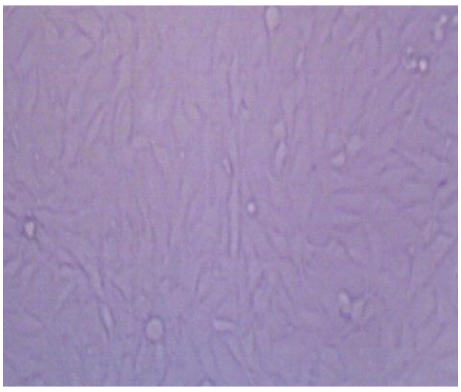


300 µg

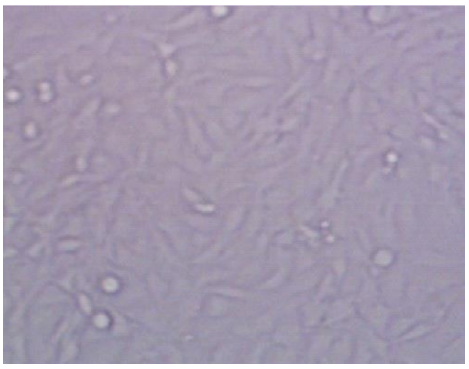
c)Aqueous extract of *C. pictus* leaves



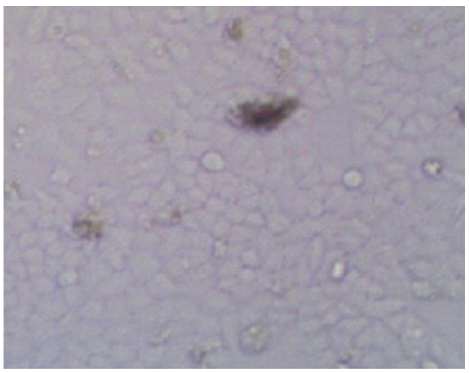
18.75 µg



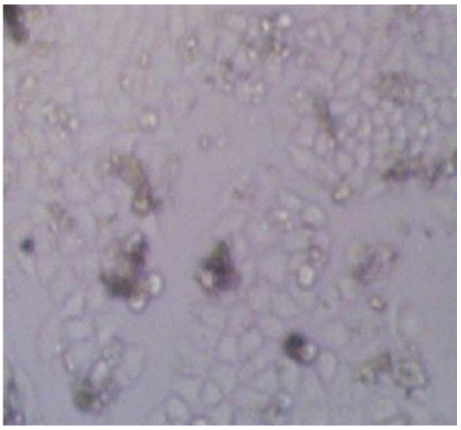
37.5 µg



75µg

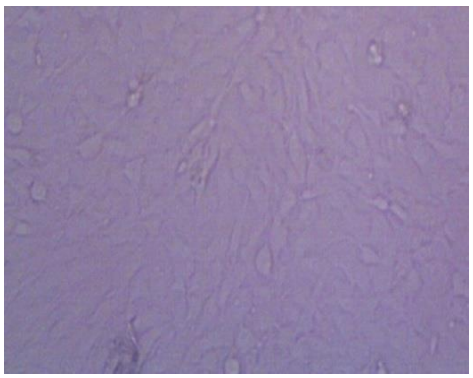


150µg

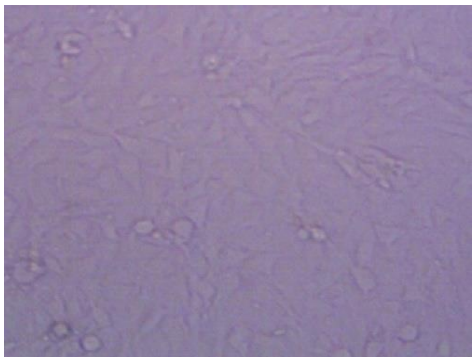


300µg

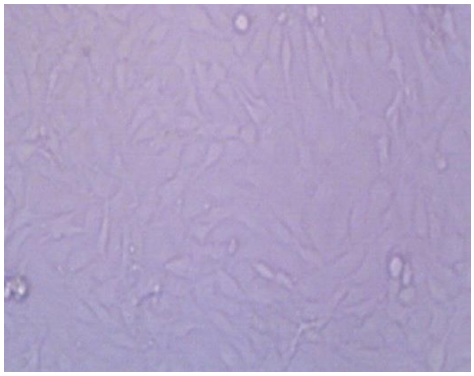
d) Petroleum ether extract of *C. pictus* leaves



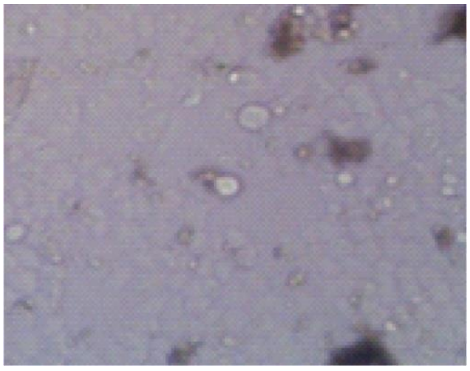
18.75 µg



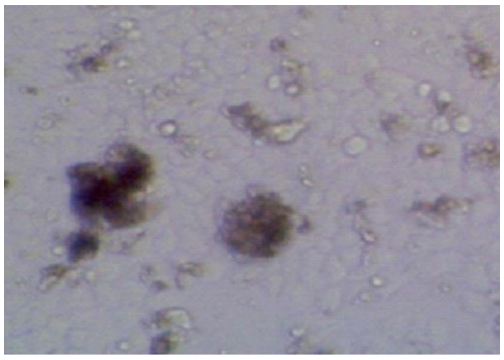
37.5 µg



75µg



150µg



300µg

DISCUSSION

Medicinal plants have traditionally played a significant role in rural and tribal life in India, contributing to social, cultural, spiritual, and medicinal benefits. Traditional medical systems, including Ayurveda, Siddha, Unani, and folklore cures, are complementary and competitive in treating many ailments. Herb research has been done for ethnobotanical purposes, to develop alternative pharmaceuticals to synthetic treatments for treating ailments. The ability to identify the building blocks required to manufacture complex compounds is made possible by an understanding of the biocomponents of plants. For identification and quantification, gas chromatography-mass spectrometry (GC-MS) is the most often used technique. GC-MS investigation of *C. pictus* leaf extracts in petroleum ether and ethanol revealed 26 and 24 chemical components, respectively. *C. pictus* ethanol extract contains terpenes (Neophytadiene), diterpenes (Phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol), fatty acids (Hexadecanoic acid, Octadecanoic acid/Stearic acid, Linoleic acid ethyl ester, 1-Tridecanol, Methyl 2,4-dimethyltetradecanoate), essential oils (Pentacosane), and acyclic alkaloids. Sesquiterpene (d-Nerolidol), terpene (Farnesylacetone, Junipene), fatty acids (Hexadecanoic acid, methyl ester / Palmitic acid, Stearic acid, 3-(octadecyloxy) propyl ester, Oleic acid, 3-(octadecyloxy) propyl ester), essential oil (2-Pentadecanone,6,10,14-trimethyl, à-Sesquiphellandrene), and volatile oil (á-Tumerone). Free radicals and antioxidants work together to prevent the onset and progression of several illnesses, including cancer[5]. It has been demonstrated that antioxidants stop the development of cancer, and medicinal plants are a rich source of new and powerful antioxidants and anticancer chemicals [6].

The study employed three assays: Total Antioxidant Activity, FRAP, and DPPH. Since DPPH is a stable radical that can accept an electron or hydrogen radical to form a

stable diamagnetic molecule[7], it is frequently employed to assess the radical scavenging capacity of natural substances. Phytochemicals high in antioxidants can stop cancer from developing[8]. It has been demonstrated that MCF-7 breast cancer cell lines are useful *in vitro* models for studying the molecular mechanisms causing cancer, tumour cell lines are helpful because they make it possible to study tumour cells in a simple and controlled environment[9]. The MTT assay is the most popular *in vitro* method for assessing anticancer activity.

The present study assessed the anticancer potential of *C. pictus* leaf extracts in petroleum ether, ethanol, and water against MCF-7 cancer cell lines. Each of the three extracts had a dose-dependent inhibitory effect on the MCF-7 cell line. A related study found that the Methanol extract of *Curcuma amada* leaves and rhizomes killed cells in MCF-7 and MDA MB 231 cell lines[10].

CONCLUSION

C. pictus D. Don possesses antibacterial, digestive, stimulant, and tonic properties. *C. pictus* leaves are used to control blood sugar levels. *C. pictus* is used in traditional medicine to alleviate asthma, lower fever, and increase longevity. When *C. pictus* leaves were extracted using ethanol and petroleum ether, GC-MS analysis showed 26 and 24 chemical components, respectively.

The antioxidant and free radical scavenging properties of *C. pictus* leaf extracts in ethanol, water, and petroleum ether were evaluated using the DPPH, FRAP, and Total antioxidant assays. With increasing concentration, the extracts demonstrated enhanced scavenging ability and antioxidant activity. Ethanol and aqueous extracts showed elevated DPPH activity. The FRAP and total antioxidant assays revealed the highest activity in the aqueous extract.

MCF-7 cell lines are crucial for studying breast cancer's genetic makeup. The MTT assay was used to evaluate the anticancer potential of *C. pictus* leaf extracts (ethanol, petroleum ether, and water) against MCF-7 cancer cell lines. The MCF-7 cell line was suppressed by all three extracts in a dose-dependent manner.

Funding Source: No funding source.

Authors' Contribution: Both authors made an equal contribution to the research, data analysis, and manuscript production process. The final version of the manuscript was approved by both authors.

Conflict of Interest: Authors declare there is no conflict of interest with the present publication.

Acknowledgement: The authors express their gratitude to the Department of Biotechnology and Bioinformatics at Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shivamogga, 577 451, Karnataka, India, for providing research facilities.

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