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RESEARCH ARTICLE

GC-MS analysis and in vitro Antioxidant, Cytotoxicity study of DCM-ME extract of *Dendrophthoe falcata* (L.F) Ettingsh leave against human lung carcinoma (A-549) and human Chronic Myelogenous leukemia (k-562) cell Line

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ABSTRACT:
The present study explored GC-MS analysis and in vitro antioxidant cytotoxicity study of dichloromethane: methanol (DCM-ME) extract of *Dendrophthoe falcata* plant and fractions (DFDM I-III) against human chronic myelogenous leukemia, bone marrow (k-562) Human lung carcinoma (A-549) cell lines by MTT and SRB cell viability assay method. Phytochemical screening of DCM-ME extract shows the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, sterols and triterpenes. DCM-ME extract shows cell inhibition 84.15±0.12% and 86.11±0.52% at 80µg/ml (IC50 values 20µg/ml, GI50 <20µg/ml) by MTT and SRB assay respect to Cisplatin (IC50<7.5µg/ml), Adriamycin (GI50 <2.5µg/ml). DFDM-I fraction shows significant effect ($p< 0.01$) with maximum cell inhibition activity 43.93±0.88%, and at 20µg/ml shows moderate activity ($p<0.05$) with cell inhibition 60.11±0.33% by MTT assay. SRB assay shows that DFDM-I at (10µg/ml) shows significant effect ($p<0.01$) with 47.72±0.33%, and at 20µg/ml shows moderate effect ($p<0.05$) with cell inhibition 65.15±0.58%. DCM-ME extract shows cell inhibition 70.72±1.15 at 80µg/ml (IC50 values 19.39µg/ml) against A-549 cell lines DFDM-I shows moderate effect ($p<0.05$) 51.56± 1.15 at 20µg/ml by MTT assay. Antioxidant activity evaluated by DPPH free radical scavenging activity found to be 87.27±3.76% for extract and 83.02±1.15, 78.03±2.49 76.03±2.33% for fractions respectively and 98.26±2.56% for ascorbic acid. GC-MS analysis DFDM-I fractions by Agilent 7890A GC coupled with Agilent triple quadrupole mass detector shows that the presence of the 17 phytocompounds. The major bioactive compounds of DFDM-I fractions were separated and identified as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (27.0%), 6,10,14-trimethyl-2-Pentadecanone (52.8%), Hexadecanoic acid, methyl ester (56.4%),1,1-Diphenyl-4-phenylthiobut-3-en-1-ol (40.8%), Hexadecanoic acid butyl ester (57.6%), and 1-Monolinoleoylglycerol trimethylsilyl ether (35.1%). The study showed that the presence phytochemicals in the leaves extracts of *D. falcata* might be responsible for cell inhibitory potential against k-562 cell lines.

KEYWORDS: *Dendrophthoe falcata,* Cytotoxicity, Antioxidant, GC-MS, Bioactive compounds.

INTRODUCTION:
Plant extracted phytochemicals were the rich sources of herbal products, used to treat different diseases because of their pharmacological activities and richness in bioactive compounds. Now days due to low toxicity and less side effects most of phytocompounds were in demand and day by day their use was increasing in the ayurvedic and allopathic medicines. Phytochemical isolation studies reports thousands of new phytomolecule every year. Pharmacological testing, structural modifications, derivatizing on these phytomolecule represent a major approach for discovering and

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developing new drugs[1]. Plant containing secondary metabolites and present chemicals were important for human health. Phytochemicals belonging to the alkaloids, flavonoids and terpenoids were recently used as a drugs or medicines to treat various infections, diseases and few of them were effective in preventing and inhibiting diverse types of tumors infections[2-3]. *Dendrophthoe falcata* (L.f) hemiparasitic mistletoe belonging to loranthaceae family, with smooth gray-brownish bark, leaves were unequal and opposite in direction, thick 1.6-25.4cm long, flowers are in yellow or white in colour, pink-red or scarlet softly, fruit berries were pink-red soft ovoid oblong, 1.3cm in diameter, 2-3cm long, original to India, Srilanka, Thailand, Indo-China and Australia[4]. Leaves part, flowers of plant were used in traditional treatment of wounds, cuts, menstrual problems, breathing problem, psychic disorders, urine infection and pulmonary tuberculosis in India[5]. Leaves paste used in skin disorder, wounds[6]. The plant shows various pharmacological activities such as diuretic, antilithiatic, immunomodulatory and cytotoxic activities[7-8]. In previous research article of *Dendrophthoe falcata* plant reported to contain different cardiac glycosides, triterpenes and flavonoids[9-10]. *Dendrophthoe falcata* plant posses' contraceptive[11], hepatoprotective, wound healing, antimicrobial, antinociceptive, antihyperlipidemic, cardioprotective, antioxidant, antinociceptive, anti-hyperlipidemic, cardioprotective and also antitumor activities[12]. This present study was carried out to find the major bioactive compounds present in DCM-ME extract of *Dendrophthoe falcata* plant leaves using GC–MS analysis and in vitro antioxidant, cytotoxic activity against K-562 and A-549cell lines.

MATERIALS AND METHODS:

Chemicals and reagents:

All the chemicals and reagents used in the research were of analytical grade and purchased from SD-Fine, Research-Lab, Sigma-Aldrich (India), Silica gel 60 F 254 HPTLC aluminum sheets 20×20cm, Merck KGaA, Germany.

Plant material:

Dendrophthoe falcata (L.f) a hemiparasite of *Mangifera indica* plant were collected from local region at flowering stage September - November from Bhor-Kapurhol road, Pune, Maharashtra, India. (Lat.-18012'51" N; Long-73054'35"E), and were taxonomically identified and authenticated by Dr. Rashami Dubey, Scientist Govt. of India, Botanical Survey of India (BSI), Pune, Maharashtra (India). The herbarium of plant specimen has been deposited at B.S.I Pune voucher specimen number-VIBDEF3 - BSI/WRC/TECH/2013.

Extraction:

Shade dried leaves were powdered (1kg), extracted through soxhlet apparatus with dichloromethane: methanol (DCM-ME) (7:3). Extract were vacuum concentrated by rotary evaporated under reduced pressure at $60^0C\pm1^0C$. Extracts were dried in hot air oven at $40\text{-}45^0C$, then extract was stored at- 20^0C till bioevaluation in an air tight container. Phytochemical screenings of extracts were carried out using phytochemical tests as described by Trease, G.E., Evans and Harborne[13-14]. Extracts were analyzed using TLC and HPTLC method.

Chromatographic analysis:

Thin layer chromatography (TLC) study was done by using standard method[14]. 2mg/ml of sample was papered in methanol. Various mobile phase with varying concentration were employed in screening program and selected the one in which maximum spots are separated was n-hexane: toluene: ethyl acetate (2:4:1.3). After drying all plates were visualized directly under UV -TLC viewer. The Rf value of the different spots that were observed was calculated.

High Performance Thin Layer Chromatography (HPTLC):[15]

Fingerprint analysis performed by CAMAG HPTLC equipment consists of automatic and Linomat syringe using the Linomat applicator IV sample applicator, developing in CAMAG twin trough chamber. Evaluated by CAMAG HPTLC densitometer with win CATS planar chromatography manager software was used data collection. 5mg of extract was dissolved in 10ml of methanol. Silica gel 60 F254 and HPTLC aluminum sheets were used as adsorbent. 10µl of sample was applied as a band of 5-6mm and at a separation of 6 mm from each other. Nitrogen gas was flushed on plates for simultaneous drying of bands. Flat bottomed CAMAG Twin chamber saturated with 10ml of n-hexane: toluene: ethyl acetate (2: 4: 1.3) mobile phase before development. Developed plate was scanned using TLC scanner with WinCATS software. TLC plates were visualized and a fingerprint profile was photo documented at 560nm by deuterium and tungsten lamp. Rf values were calculated and data reported.

Column chromatography[15].

N-Hexane: Toluene: Ethyl acetate (2: 4: 1.3) was used as a solvent system for separating the fraction of DCM-ME extract. Fractions DFDM I-III, were collected from DCM-ME extract of *Dendrophthoe falcata* by column chromatography using silica gel particle size (60-120) mesh size. Fractions having similar colour were pooled and combined. All fractions were concentrated and subjected cytotoxicity study. The biological active fraction DFDM-I was subjected to GC-MS analysis.

Gas chromatography mass spectrophotometry (GC-MS) Analysis:[16-17]

GC-MS analysis of active fraction DFDM-I of DCM-ME extract of *D. falcate* (L.f) studied by Agilent 7890A Gas Chromatography, Agilent 7000B Mass spectrophotometer (GC-MS) (USA) coupled with triple quadrupole mass spectrometer detector. The GC-MS system was equipped with a DB-5MS column (30mm× 0.25m 0.2micron film Filter). Carrier gas Helium is used as at a flow rate of 1.0mL/min and a split less. In temperature programming initial temperature is 1100C (hold 2 min ramped at 15°C/min to 150°C (hold 1 min) ramped at 10°C/min to 280^0C (hold 5 min), final experiments total run time 23.5 min. The injector and detector were held at 250°C. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 50-700.amu.

In-vitro Cytotoxicity study:

MTT assay[18-20]

Cell culture:

The Human chronic myelogenous leukemia (K-562) and Human lung carcinoma (A549) cell lines were procured from national center for cell science (NCCS), Pune; India. Cells were grown and maintained as monolayer in DMEM medium supplemented with 10% fetal bovine serum containing 5% of mixture of Gentamycin (10ug), penicillin (100Units/ml) and streptomycin (100µg/ml) in presence of 5% CO_2 at 37ºC. The cells were plated concentration of 1×10^4 cells per well in a 96-well plate and cultured for 24h at 37°C.

Assay:

Cell viability was determined with 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) with minor modifications. This assay measures the conversion of MTT to dark blue formazan precipitation by succinate dehydrogenase of the intact mitochondria of living cells. K-562 and A549 cell lines were seeded into 96-well plates at a density of 1×10^4 cells per well in DMEM (10%FBS) incubated for12h.The DCM–ME extracts and fractions (DFDM I-III) were firstly dissolved in dimethyl sulfoxide (DMSO) and then diluted in culture medium with final DMSO concentration of 0.5% (v/v). After that the cells were exposed 10, 20, 40 and 80 (µg/ml) concentrations appropriately diluted with DMSO and incubation at 37ºC in a humidified atmosphere of 5% CO_2. Medium containing 0.5% DMSO was used as control. After treatment medium was replaced with fresh culture medium without FBS containing MTT at a concentration of 0.5mg/ml and the cells were further incubated for 4h at 37°C. The medium was removed and formazan crystal was dissolved in DMSO. The optical density (OD) was measured at 490nm using a microplate reader. The result were represents the mean of three reading. Cell viability was determined by OD of treated wells divided by OD of vehicle control.

SRB assay[21-22]

For present study cell were inoculated into 96 well microtiter plates in 100µl of individual cell lines by Sulforhodamine B (SRB) method. The Human chronic myelogenous leukemia (K-562) was maintained in DMEM medium supplemented with 10% fetal bovine serum. The cells were consequently expose to 10, 20, 40, and 80µg/ml concentrations of DCM-ME extracts and fraction (DFDM I-III). After extracts incubation add 50 µl TCA (50%) and kept for 1 hour at 4^0C then plate washed with triple distilled water and dry the plate. Then add 100µl SRB dyes in each well and kept for 30min at room temperature. Again wash three times with 1% acetic acid and air dry the plate then add 200µL tris buffer, and absorbance was read at 490nm. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells.

$$\text{Percent cytotoxicity} = \frac{\text{Reading of control} - \text{Reading of treated cells}}{\text{Reading control}} \times 100$$

Antioxidant activity:

DPPH radical scavenging activity[23-24]**:**

The ability of extracts to scavenge DPPH radical (to reduce) was assessed using ascorbic acid as standard method with modification. Aliquots of extracts were prepared in methanol to get the concentration 1mg/ml. Dilution are prepared to get concentration of extracts (20, 50, 75, 100, 200µg/ml). Prepared dilution (1ml each) mixed with 1ml of DPPH in methanol (0.5mmol/L). After 30 min incubation at room temperature absorbance of resulted aliquots measured at wavelength 517nm using UV-Visible spectrophotometer. Repeat the procedures to prepared standard solutions of ascorbic acid with respect to extracts.

The percentage of scavenging activity was derived using the following formula

$$\%\text{ of Inhibition} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

Where

Abs. control - absorbance of DPPH

Abs. sample - absorbance reaction mixture (DPPH with Sample).

All samples were prepared and analyzed in triplicates.

Statistical analysis:

Results were presented as a mean±SEM and analyze using one way analysis of variance (ANVOA). The data was analyzed using students-t test using Graph Pad Prism 8.3.1 (549) software and the difference was considered significant when ($p<0.01$), moderate ($p<0.05$).

RESULTS AND DISCUSSION:

Extraction and Phytochemical screening:

Phytochemical extraction plant was performed by soxhlet apparatus. Around 1kg of air dried leaves powder was used; Percentage yields of DCM-ME extract was 8.40%. Phytochemical study of DCM-ME extract shows presence of flavonoids, alkaloids, phenolic compound, tannins, saponins, phytosterols, Steroids, triterpenoids and diterpenes. Review of literature shows that alkaloids extracted from plants show biological activities such antimicrobial[25], anticancer[26]. According to research, tannins, also shows potent antitumor and antiviral activities[27]. Saponins from plants shows good antioxidant, immunostimulant, anticancer activity and also act on permeability of cell membranes act through cell membrane mediated transport by the pore formation make lysis of cell and also increased intracellular reactive oxygen species (ROS) formation which induced cytosolic Ca2+ mobilization, and decreased mitochondrial membrane potential. Triterpenoids saponins are potent cytotoxic agent act through cell membrane mediated transport[28]. Presence of such phytochemical might be responsible for cytotoxic activity.

Table 1: Preliminary photochemical screening of DCM-ME extract of *Dendrophthoe falcata*.

Phytochemical test	DCM-ME extract
Alkaloids	+
Flavonoids	++
Glycosides	-
Phenolic compound	+
Tannins	++
Saponins	+++
Phytosterols	+
Steroids	+
Diterpenes	+

HPTLC fingerprints study of DCM-ME extract of *Dendrophthoe falcata* (L.f) plant.

The HPTLC fingerprints study of extract showed 9 peaks at 560 nm with R_f values 0.20-0.94. Major peaks are peak no.4 (Rf-0.45, 10.05%), peak no.5 (Rf-0.55, 13.33%), peak no.6 (Rf-0.62, 8.20%) and peak no.7 (Rf-0.69, 24.50%) showed maximum concentration justifies the presence of phytochemicals. (Figure 1)

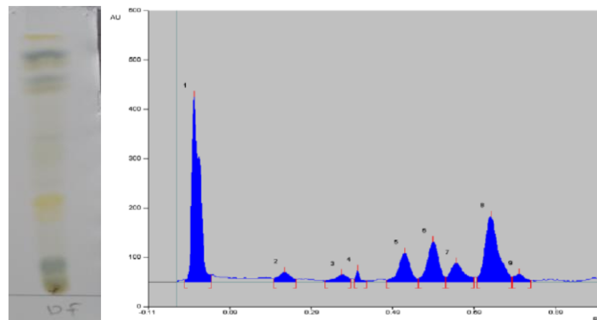


Figure 1 a) TLC b) HPTLC Chromatogram DCM-ME extract of *Dendrophthoe falcata* measured at 560nm.

GC-MS analysis active fraction DFDM-I of DCM-ME extract of *Dendrophthoe falcata*:

GC-MS analysis DFDM-I fraction shows that presence of 17 peaks (Photochemical) (Figure.2) and the compounds were identified using NIST database. Identification of eluted phytocompounds was confirmed on basis of peak area (percentage), retention time, molecular formula, molecular weight detected. The major bioactive components of DFDM-I fraction were identified as 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (27.0%),6,10,14-trimethyl-2-Pentadecanone (52.8%), Hexadecanoic acid, methyl ester (56.4%); 1,1-Diphenyl-4-phenylthiobut-3-en-1-ol (40.8%), Hexadecanoic acid butyl ester (57.6%), Flunixin (60.0%) and 1-Monolinoleoylglycerol trimethylsilyl ether (35.1%) (Table 2, Figure.3). Phytochemical compounds play important roles in against diseases and in general metabolisms. Basically nature of identified bioactive compounds was fatty acids, alcohols, esters, diterpenes, and alkaloid, heterocyclic compounds. Review of research articles reported that phytochemical such as N-hexadecanoic acid act as cytotoxic by inhibiting DNA topoisomerase-I and prevents cell proliferation[29]. Sulfur containing molecules involved in G2/M arrest, and apoptos process of cell and activation of p53 pathway in response to the oxidative DNA damage of cancer cell[30]. Review of research articles and obtained results recommend that the major phytomolecule separated from DFDM-I fraction by GC-MS responsible for anticancer activity against human chronic myelogenous leukemia (K-562).

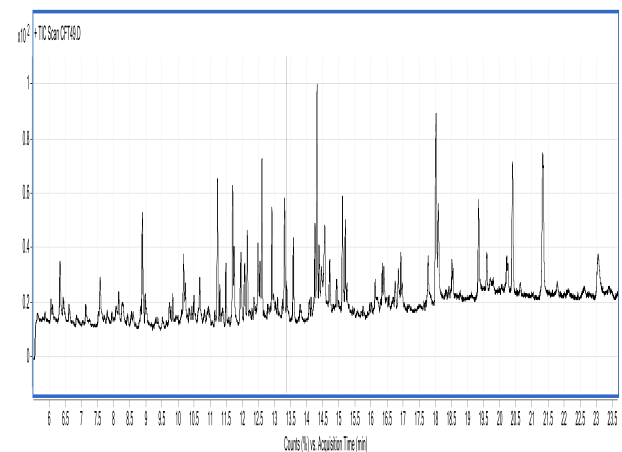
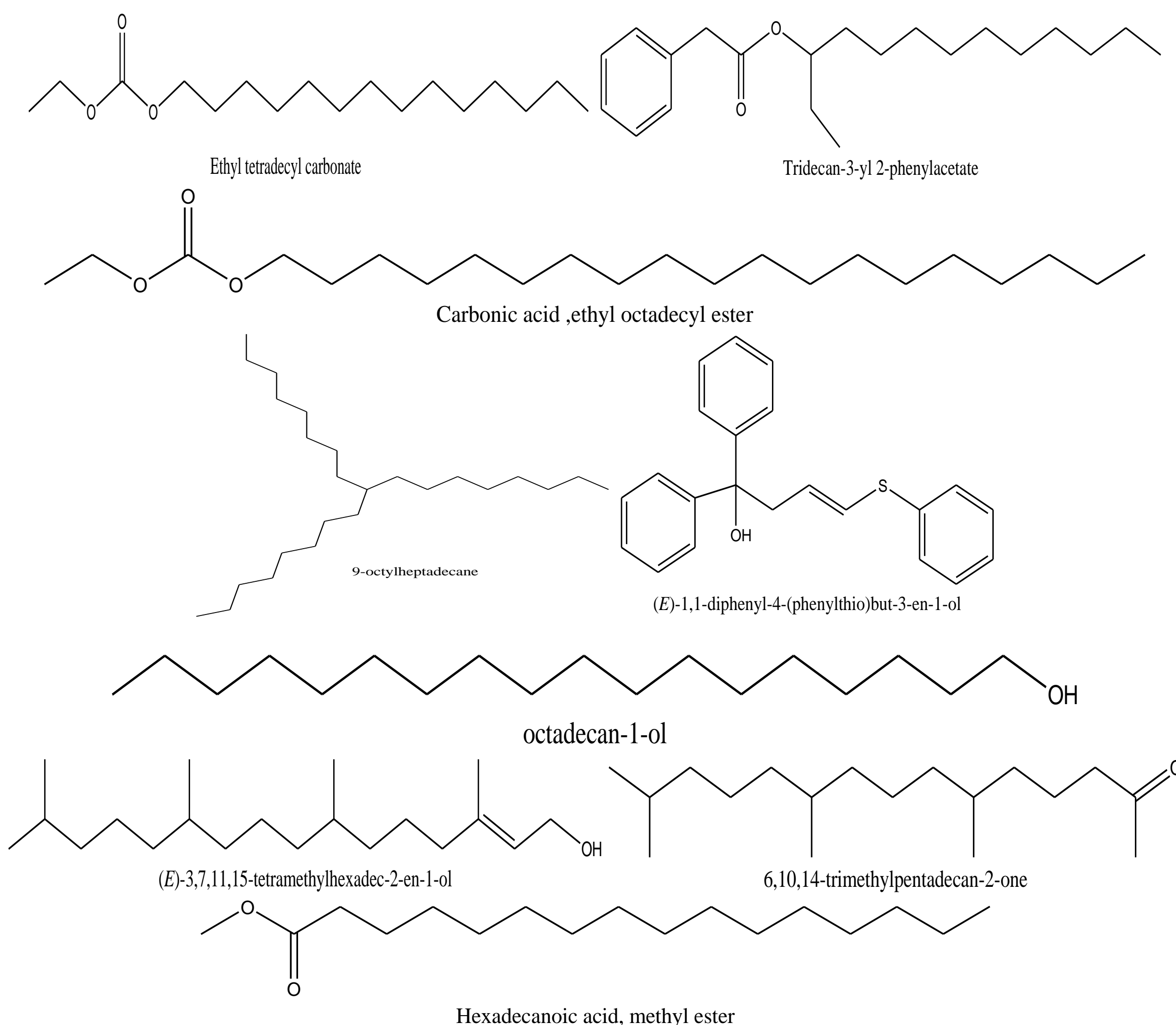


Figure 2. GC-MS profile of DFDM-I fraction DCM-ME extract of *D. falcata* leaves.

Table 2 Phytochemical components separated and identified from DFDM-I fraction of DCM-ME extract of *D. falcata* leaves of using GC–MS

Sr. No	RT (min.)	Name of compounds	MF	MW (g/mol)	PA (%)
1	6.330	Carbonic acid ethyl tetradecyl ester	$C_{17}H_{34}O_3$	286.250	7.89
2	7.576	Benzene acetic acid 3-tetradecyl ester	$C_{22}H_{36}O_2$	332.27	14.6
3	8.885	Carbonic acid ethyl octadecyl ester	$C_{21}H_{42}O_3$	342.31	5.50
4	10.172	9-Octylheptadecane	$C_{25}H_{52}$	352.40	12.6
5	11.215	1-Octadecanol	$C_{18}H_{38}O$	270.292	5.23
6	11.687	(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296.307	27.0
7	11.736	6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	268.276	52.8
8	12.602	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.255	56.4
9	12.911	1,1-Diphenyl-4-phenylthiobut-3-en-1-ol	$C_{22}H_{20}OS$	332.123	40.8
10	14.245	8.11-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294.225	7.31
11	14.307	Cyclopropaneoctanoic acid,2-2-[(2-ethyl cycloproyl) methyl]-methyl ester	$C_{22}H_{38}O_2$	334.28	13.3
12	15.100	Hexadecanoic acid butyl ester	$C_{20}H_{40}O_2$	312.30	57.6
13	16.337	Cis-Oxiraneundecanoic acid, 3-pentyl-methyl ester.	$C_{19}H_{36}O_3$	312.266	17.7
14	18.005	Flunixin	$C_{14}H_{11}F_3N_2O_2$	296.077	60.3
15	18.079	2,3-Diphenyl Quinoxaline	$C_{20}H_{14}N_2$	282.11	10.7
16	19.578	1-Monolinoleoylglycerol trimethylsilyl ether/Linoleic acid ,2,3 bis(O-TMS)propyl esters	$C_{27}H_{54}O_4Si_2$	498.35	35.1
17	20.379	2,2,4-Trimethyl-3(3,8,12,16-tertamethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428.40	12.4

*RT = retention time; MF = molecular formula; MW = molecular weight; PA = peak area.



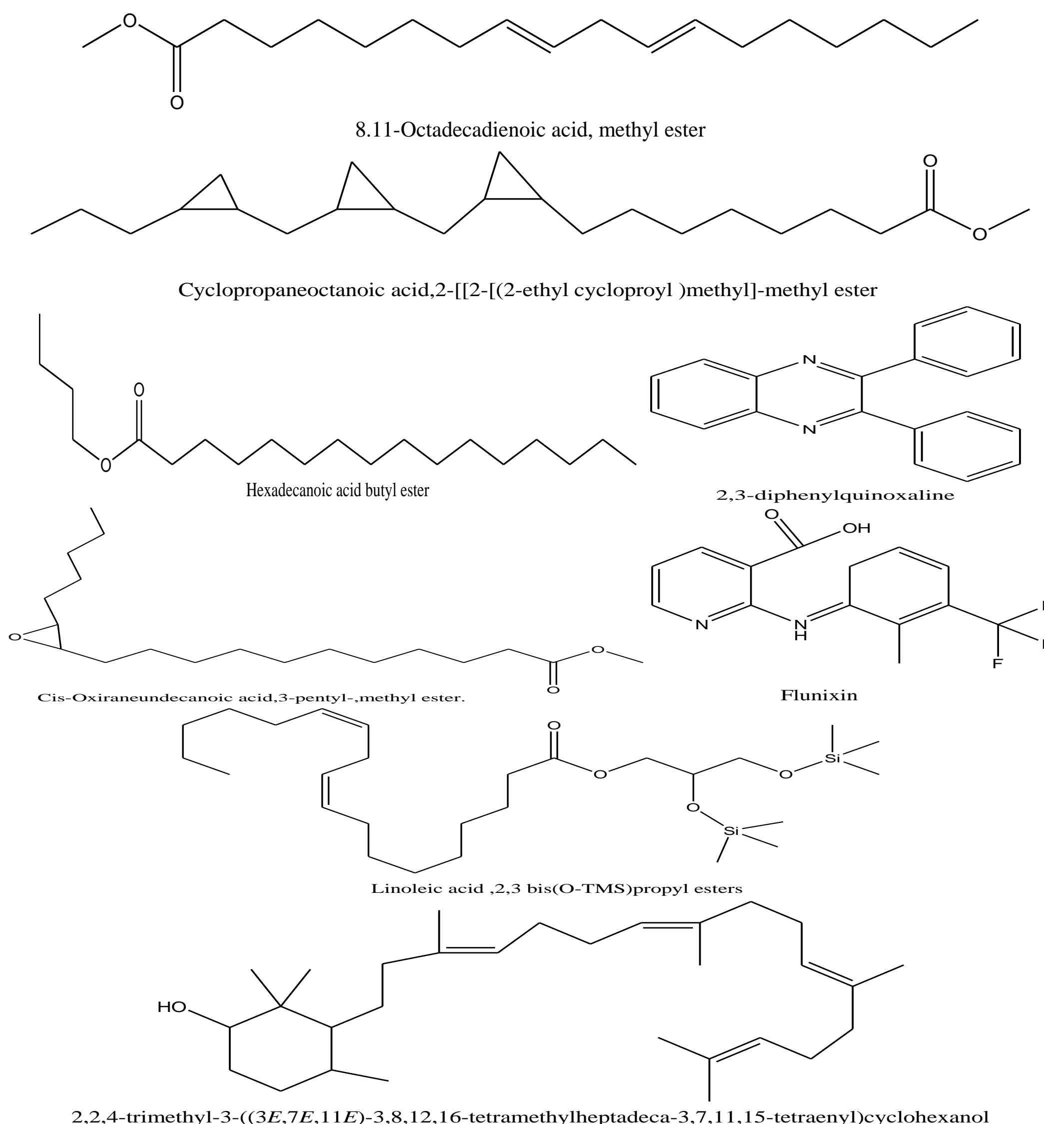


Figure 3 – Phytochemical components separated and identified from DFDM-I fractions of DCM-ME extract of *Dendrophthoe falcata* by GC-MS.

Cytotoxicity assay:

The cytotoxicity effect of DCM-ME extract and their fractions (DFDM I-III) against human chronic myelogenous leukemia, bone marrow (K-562) and human lung carcinoma (A549) cell line with increasing concentrations of (10–80µg/mL) for 24 h, which was confirmed by MTT and SRB assay. The result shows that DCM-ME extract and fractions significantly induce cytotoxicity in a dose dependent manner. DCM-ME extract shows cell inhibition 84.15±0.12% and 86.11±0.52% at 80µg/ml by MTT and SRB assay. Extract shows strong cells inhabitation activity with IC_{50} values 20µg/ml, GI_{50} <20µg/ml, respectively to positive control cisplatin (IC_{50}<7.5µg/ml), Adriamycin (GI_{50}<2.5 µg/ml) given in (Table 3 and 4). The cell viability was gradually decreased, when treated with concentration of10, 20, 40 and 80g/ml. Fractions (DFDM I-III) isolated from the DCM-ME extract by column chromatography tested against K-562 cell lines by MTT and SRB assay. DFDM-I fraction (10µg/ml) exhibited a significant effect ($p<0.01$) with maximum cell inhibitory activity 43.93±0.88%, at 20µg/ml exhibited a moderate effect ($p<0.05$) with cell inhibitory activity 60.11±0.33% by MTT assay (Figure 4). Fractions DFDM-II and DFDM -III show (56.93±0.88% and 55.49±0.48%) moderate effect on cell inhibition at conc. 20µg/ml respect to positive control. SRB growth inhibition assay shows that DFDM-I fraction (10µg/ml) exhibited a significant effect ($p<0.01$) with maximum cell inhibitory activity 47.72±0.33%, at 20µg/ml exhibited a moderate effect ($p<0.05$) with cell inhibitory activity 65.15±0.58% by SRB assay (Figure 5). Fractions DFDM-II and DFDM -III show (52.77±0.33% and 56.81±0.78%) moderate effect on cell inhibition at conc. 20µg/ml respect to

positive control justified by microscopic images (Figure 6).DCM-ME extract shows cell inhibition 70.72±1.15 at 80µg/ml (IC_{50} values 19.39µg/ml) against human lung carcinoma (A-549) cell lines respect to positive control cisplatin (IC_{50} 8.5µg/ml), DFDM-I shows significant effect ($p<0.01$) 72.54± 0.58at 80µg/ml, DFDM-II shows moderate effect ($p<0.05$) 24.69±1.14 at 10µg/ml by MTT assay given in (Table 5, Figure 7) Obtained results recommend that the DFDM-I fractions shows significant anticancer activity against K-562 and A-549. Cytotoxicity of DFDM-I also justified by phytomolecule separated and identified by GC-MS. Presence of major phytochemical were responsible for anticancer activity against human chronic myelogenous leukemia (K-562) and human lung carcinoma.

Table 3. Cytotoxic effect of *Dendrophthoe falcata* (L.f) plant extract and fractions against K-562 cell lines by MTT assay

Sr. No	Compound / Extracts / Fractions	% Cell Lysis				
		MTT Assay				
		10µg/ml	20µg/ml	40µg/ml	80µg/ml	IC_{50} value µg/ml
1	Control	00	00	00	00	-
2	DCM-ME extract	26.88 ± 0.33	52.28 ± 0.57	66.73 ±0.58	84.15 ±0.12	20
3	Positive Control	74.35 ±0.33	100	100	100	7.5
4	DFDM-I fraction	43.93±0.88**	60.11±0.33*	72.25±0.57	86.41±0.38*	>10
5	DFDM-II fraction	28.61±0.66	56.93±0.88*	71.67±0.58	85.57±0.57*	20
6	DFDM-III fraction	22.54±0.33	55.49±0.48	67.34±0.66	79.47±0.78	20

Table 4. Cytotoxic effect of *Dendrophthoe falcata* (L.f) plant extract and fractions against K-562 cell lines by SRB assay

Sr. No	Compound / Extracts / Fractions	% Growth Inhibition				
		SRB Assay				
		10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	GI_{50} value µg/ml
1	Control	00	00	00	00	-
2	DCM-ME extract	21.43±0.48	60.04±0.57*	74.78±0.39	86.11±0.52	< 20
3	Positive Control	98.86±0.34	100	100	100	< 2.5
4	DFDM-I fraction	47.72±0.33**	65.15±0.58*	76.51±0.21	89.39±0.38	>10
5	DFDM-II fraction	32.07±0.78	52.77±0.33	78.28±0.48	87.12±0.57	20
6	DFDM-III fraction	35.85±0.28	56.81±0.78	72.47±0.41	83.83±0.38	20

Data are means ±S.D; n = 3. (** significant effect ($p<0.01$), * moderate effect ($p<0.05$)
IC_{50} –Concentration of drug required 50% inhibition of cell viability.
GI_{50} –Concentration of drug causing 50% inhibition of cell growth.

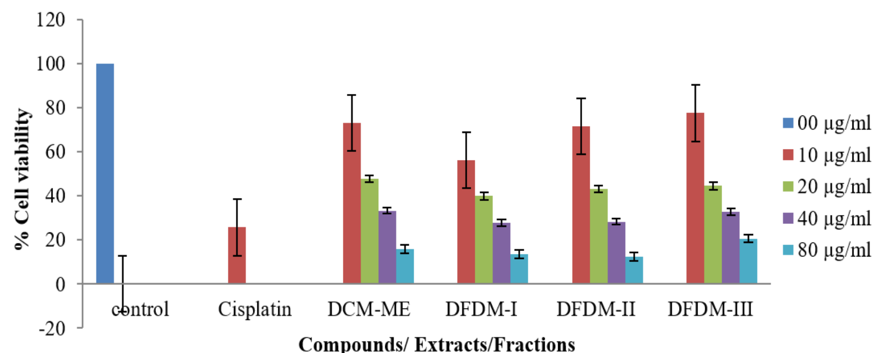


Figure 4. Cytotoxic effect of *Dendrophthoe falcata* (L.f) plant extract and fractions against K-562 cell lines by MTT assay.

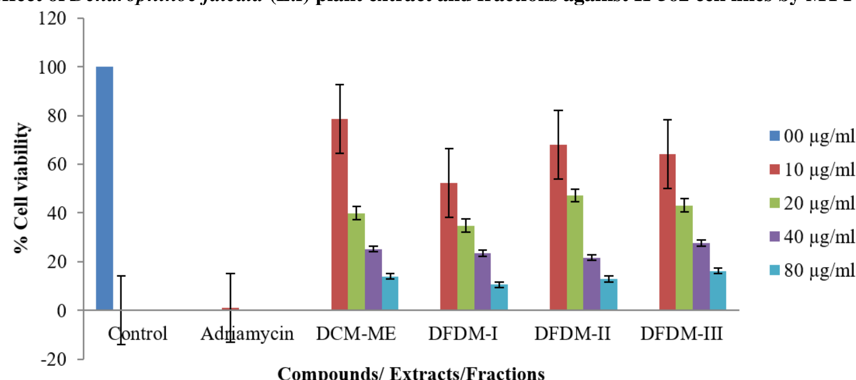


Figure 5. Cytotoxic effect of *Dendrophthoe falcata* (L.f) plant extract and fractions against K-562 cell lines by SRB assay.

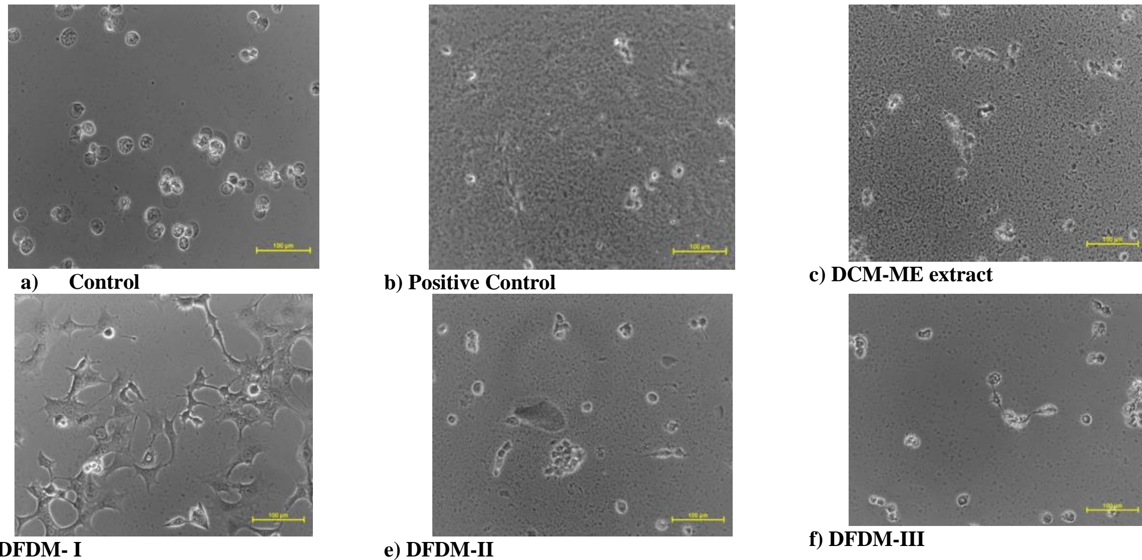


Figure 6. Microscopic images of K-562 cells treated with of extract and fractions of *D. falcata* leaves by SRB assay method.

Table 5. Cytotoxic effect of *Dendrophthoe falcata* (L.f) plant extract and fractions against Human lung carcinoma (A549) cell lines by MTT assay

Sr. No	Compound / Extracts / Fractions	% Cell Lysis				
		MTT Assay				
		10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	IC_{50} value µg/ml
1	Control	00	00	00	00	00
2	Positive Control	74.76± 0.57	100	100	100	8.5
3	DCM-ME extract	11.20± 1.73	51.56± 1.15	64.21± 1.45	70.72± 1.15*	19.39
3	DFDM-I fraction	21.91± 0.57	41.66± 1.15	57.40± 0.66	72.54± 0.58**	34.84
4	DFDM-II fraction	24.69± 1.14*	40.43± 2.56	51.54± 1.67	68.51± 0.45	38.80
5	DFDM-III fraction	23.48± 0.78	42.28± 1.40	58.64± 1.37	65.74± 2.67	34.10

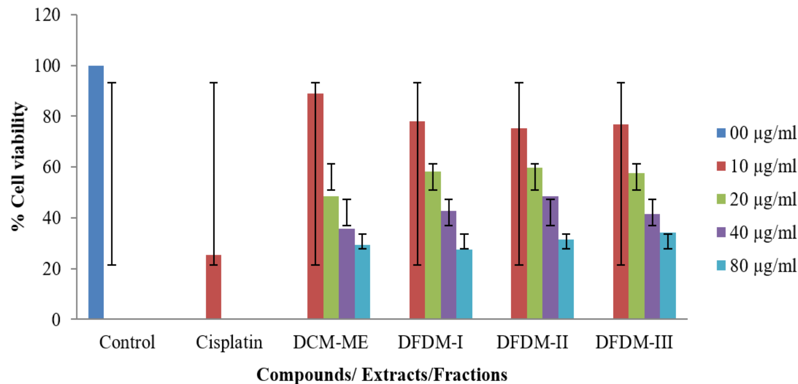


Figure 7. Cytotoxicity of *Dendrophthoe falcata* (L.f) plant extracts against Cell line- A549 - Human lung carcinoma by MTT assay.

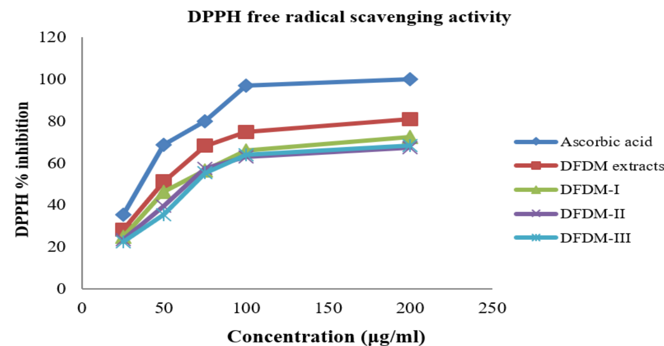


Figure 8. DPPH free radical scavenging activity of *Dendrophthoe falcata* (L.f) plant extract and fractions

DPPH free radical scavenging activity:

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a-diphenyl-bpicrylhydrazyl, DPPH) is characterized as a stable free radical. The delocalization of electron gives the deep violet color, characterized by absorption in ethanol solution centered at about 517nm. When a solution of DPPH is mixed with that of a substrate that donate a hydrogen atom, then shows that reduced form with the loss of this violet colour. The percentage DPPH inhibition activity of DFDM extracts and the ascorbic acid are measured at different concentrations between 25-200µg/ml and at higher DPPH free radical scavenging activity found to be 81.02±3.76% for extract and 72.52±1.15, 67.41±2.49 68.34±2.33% for fractions respectively and 99.89±2.56% for ascorbic acid.

CONCLUSION:

The present study shows that DCM-ME extract and DFDM-I fraction exhibits strong cell inhibition effects against human chronic myelogenous leukemia (K-562) and Human lung carcinoma (A-549) cell lines. Above study shows that anticancer properties might be due to the presence of some phenolic, esters, saponins, alcoholic compounds and presence of hexadecanoic acid methyl ester, hexadecanoic acid butyl ester, sulfur contenting 1,1-Diphenyl-4-phenylthiobut-3-en-1-ol may be inhibits cells growths through different mechanisms. Thus, it was concluded that the DCM-ME extract fractions shows potent anticancer properties against K-562 and A-549 cells line.

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