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Phytochemical screening, structural identification and anticancer activity from *Decalepis hemiltonii*

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Abstract

The aim of this studyis to screen the phytochemicals present in the root tuber of *Decalepis hamiltonii*and further analysis to characterize the chemical constituents of *Decalepis hamiltonii*using GC-MS method. GC-MS analysis of ethyl acetate extract of root tuber of *Decalepis hamiltonii*revealed the presence of 10 bioactive compounds namely 4-methyl-quinazoline, Cyclopentaneundecanolic acid, 5-hydroxy-4'methoxy-7-methyl- flavones, Oleic acid, 8,11,14-Eicosatrienoic acid, Isopropyl stearate, Estra-1,3,5(10)-trien-17a-ol, Quinazolin-4(3H)-one, Docosanoic acid and 4-Piperidineacetic acid with their retention times 8.48, 17.05, 17.73, 18.78, 19.43, 20.28, 21.18, 22.35, 23.95 and 28.18 minutes respectively. The results revealed that ethyl acetate extract of *Decalepis hamiltonii*possesses a broad spectrum of many medicinal properties.

Key Words: Decalepis hemiltonii;Phytochemical; GC-MS analysis; Anti-cancer activity

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Saurabh Dixit et al., 2016). About 80% of individual from developed countries use traditional medicine, which has compounds obtained from medicinal plants (Gordon and David, 2001). *D. hemiltonii* is endemic and endangered to peninsular India geographical distribution in southern India and rare in evergreen forests of Western Ghats and commonly called as maredukommulu or barre sugandhi or maraudgaddalu or makaliberu belonging to the family Apocynaceae, (Raju and Ramana, 2009).Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with lesser side effects. The pharmacological action of crude plant extract is determined by the nature of its constituents. Plant drug have been the major resource for treatment of infectious disease for a long time (Mandal et al., 2007).Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Sofowora, 1993). Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs (Mandal et al., 2007). Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, tannins, steroids, glycosides and saponins. Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Cowa, 1999). Hence it is obligatory to screen the secondary metabolites, the inevitable and significant key factors in therapeutics.

GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile (Raju et al., 2009). In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in plant species (Sofowora, 1993).Secondary products from the plants are responsible for its action or pharmacological activity (Patel et al., 2012).

2. Materials and Methods

2.1. Chemicals and Reagents

Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), MTT (3-[4, 5- dimethylthiozol-2-yl]-2-5-diphenyl

tetrazolium bromide) and Phosphate buffer solution (PBS) was purchased from Himedia Laboratories, Mumbai. Hexane, Acetone, Methanol, Aqueous, Ethyl Acetate, Dichloro Methane, Sulphuricacid, Hydrochloric acid,Nitric acid, Sodium hydroxide, Chloroform, Fehling's solution, Acetic acid and Ammonia (NH_3) was procured from Ranbaxy fine chemicals Ltd, Mumbai. All the other fine chemicals were obtained from sigma-Aldrich, Mumbai, India.

2.2. Preparation of extract

The roots of *D.hemiltonii* were collected from Tamil Nadu, India and roots were washed and rinsed with distilled water and then dried.Extraction was made using 30g of sample was extracted with using Soxhlet apparatus. The extracts were concentrated with rotary evaporator and dried to obtain dark brown residues. The obtained residues were subjected to GC-MS analysis and anti-cancer activity.

2.3. Phytochemical screening

Since the *D. hemiltonii* is a medicinal plant, only roots were collected and shade dried. The previous method adopt analysing and extracting the plant required high quantity of raw materials hence, a modified procedure of (Harborne, 1973) was applied for the analysis and extraction of phytochemical screening as well as quantification, structural identification and anticancer activity.

2.3.1. Test for carbohydrates

The extracts were mixed with 2 ml of Benedict's reagent and boiled; a reddish brown precipitate formed which showed the presence of the carbohydrates.

2.3.2. Test for amino acids

The analysis of amino acid, extract (3 ml) was treated with 3 drops of 5% Ninhydrin solution was heated with water bath for 10 min. and the mixture formation for purple or bluish color, the appearance of color indicated that the presence of amino acids.

2.3.3. Test for proteins

Protein in the extract (3 ml) was tested by adding 4% NaOH and few drops of 1% $CuSO_4$solution and observed for violet or pink color formation.

2.3.4. Test for tannins

The extracts were tested with using 250 µl of extract solution with 500 µl of distilled water two drops of 5% ferric chloride ($FeCl_3$) solution and observed for deep blue black coloration in the test presence of tannins.

2.3.5. Test for Phenolic compound

The extract was diluted to 5 ml with distilled water. To that a few drop ofneutral 5% ferric chloride solution was added. A dark green color indicates thepresences of phenolic compounds.

2.3.6. Test for flavonoids

The flavonoids in the extracts were tested with few drops of lead acetate solution and the formation of yellow color precipitation confirmed the presence of flavonoids.

2.3.7. Test for alkaloids

Hydrochloric acid (HCl) (1%, 500µl) followed by 2.0 ml of extract was treated with three drops of Mayer's reagent. A white precipitate was indicated the presence of alkaloids.

2.3.8. Test for saponins

Saponins were tested by using 2 ml of extract and were shaken vigorously to observe a stable persistent froth and added two drops of olive oil in the froth allowed for the formation of an emulsion, which showed the presence of saponins.

2.3.9. Test for steroids

Steroids in the extracts were tested by adding 2 ml of extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4were added in the test

tube and formation of chloroform layer for red color and acid color for fluorescence.

2.3.10. Test for glycosides

To 2 ml of plant extract, 1 ml of glacial acetic acid and 5 % ferric chloride wasadded then a few drops of concentrated sulphuric acid were added. Presence ofgreenish blue colour indicates glycosides.

2.4. Determination of total phenolic and flavonoids content

The total phenolic in extract was performed with Folin-Ciocalteu phenol reagent according to the method of (Jayaseelan et al, 2014). 10µL of extract (10mg/2mL) was added into test tubes with 1ml of distilled water. Added 0.5mL of Folin-Ciocalteu reagent and content of the flask was mixed well. After 1- 5min, 2.5mL of Na_2CO_3 (20%) was added in each tube. The mixture was allowed to stand for 40min with intermittent shaking. Absorbance was measured at 725nm using UV-Spectrophotometer. The total phenolic contents were estimated as mg of tannic acid equivalents.

Flavonoids contents were determined by aluminium chloride colorimetric method (Siddhuraju and Manian, 2007). 0.5mL of extracts of different concentration solution was mixed with 2mL of distilled water and added 0.15mL of 5% $NaNO_2$ solution. After 6min, 0.15mL of $AlCl_3$ solution was added and stands for 6min and the 2mL of 4% NaOH solution was added to the mixture and the volume was made up to 5ml using distilled water. The mixture was allowed for 15min; the absorbance of the reaction mixture was measured at 510nm. The total content of flavonoids compound in this extract in Rutin equivalents (RE) was calculated.

2.5. Gas Chromatography-Mass Spectrometry

Ethyl acetate extracts of *D.hamiltonii* was performed GC-MS analysis previously developed method with minor modification (Mustapha et al., 2016) using Agilent Technologies (GC) 5975C Agilent with mass selective detector (MS), HP-5MS (5% phenyl methyl siloxane) capillary column of dimension 30m x 250µm x 0.25 µm and used helium carrier gas 1 mL /min. and the column temperature was programmed initially at 500C for 10 min, followed by an increase of 30C/ min to 2400C and the it was kept isothermally for 5 min. The mass spectrometry was working with 70eV. The phytochemical identification of the compounds was confirmed based on theirpeak area, retention time, molecular formula and molecular weight.The active components were identified with comparison of their mass spectral data analysis by those from NIST 0.5 spectral library matches.

2.6. *In vitro* assay for anti-cancer activity

2.6.1. Cell Culture

MCF-7 and 3T3 cells were obtained from National Centre for Cell Science (NCCS), Pune and it was cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10%Fetal Bovine Serum (FBS), Streptomycin (250 U/mL). All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO^2 incubator. Cells were allowed to grow to confluence (85-90%) over 24h before use.

2.6.2. Cell growth inhibition by MTT assay

Cell viability was measured with MTT assay (Mosmann, 1983). In briefly, Normal Fibroblast Cell line (3T3) and MCF-7 cells were seeded at a density of 5 x 10^3 cells/ well in 96-well plate for 24 h, in 200µL of DMEM medium with 10% FBS and culture supernatant was removed. Then DMEM medium containing various concentrations (20 to 200µg/mlL) of test extract was added and incubated for 48 h. After treatment of cells were incubated with MTT reagent (20µL, 5mg/mL in PBS) at 37°C for 4 h and then added with DMSO (200 µL) at room temperature for 1 h. The plates were read at 595nm. The percentage of cell viability was calculated the following formula; % cell viability = A_{595nm} of treated cells A_{595nm} of control cells x 100. The graphs are plotted using the % of cell viability at Y-axis and concentration of the sample in X-axis. All the experiments were performed with triplicate.

3. Results and discussion

3.1. Phytochemical screening

The plant extract was tested for its various phytochemicals. The results were presented in Table 1. The present study performed to know the secondarymetabolites present in the extract of *D. hemiltonii.* The results obtained, clearly evident thatcarbohydrates, tannins, phenols, flavanoids, alkaloids and saponins were present inthe extract of *D. hamiltonii*.Ethanol was found to be one of the best sources for the extraction of alkaloids from plants. Commonly, flavonoids were extracted by methanol, ethanol and acetone since these compounds were highly dissolved with these solvents. According to Wang et al (2012), the plant extract showed very high extractability of the bioactive compound with these solvents.

Table 1 Phytochemical screening of *D. hemiltonii* roots extracts

Extracts / Secondary metabolites	Ethanol	Methanol	Acetone	Petroleum ether	Ethyl acetate
Carbohydrates	+	+	+	+	+
Amino acids	-	-	-	-	-
Proteins	-	-	-	-	+
Tannins	+	+	-	+	+
Phenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	-	+	+	-
Steroids	+	+	+	+	+
Glycosides	+	+	+	-	+

Presence (+); Absence (-)

3.2.Quantitative estimation of total phenolic and flavonoid contents

The total phenolic and flavonoids contents were measured with various extracts shown in Table 2. The present result revealed that the phenolic contents were observed in acetone 0.24 mg/g when compared to ethanol (0.53mg/g) extract. These compounds were comparatively less with methanol (0.23mg/g) and ethyl acetate (0.19 mg/g). Hence, this plant extract has other metabolites; it affects the dissolving of the bioactive compounds to be less the analysis levels. Petroleum ether(0.11mg/g) observed low level of flavonoids and these results were concluded that of Maria John et al (2015), however ethanol extracts showed high yield of flavonoid contents. Hence, the total phenolic contents were high (0.55mg/g) followed by acetone (0.24mg/g) extracts, which might be suggested that the plants are rich in phenolic contents.

Table 2 Total Phenols and flavonoids contents of the *D. hemiltonii* extract

Extracts	Total Phenol	Total Flavonoid
Ethanol	0.55 ± 0.09	0.53 ±0.04
Methanol	0.31± 0.02	0.23 ± 0.03
Acetone	0.41 ± 0.02	0.24 ±0.04
Petroleum ether	0.13 ± 0.10	0.11 ± 0.02
Ethyl acetate	0.19 ± 0.02	1.13 ± 0.06

Each value is expressed as mean ± Standard deviation (SD) of triplicate values

According to Upadhyay et al., (2013) the total phenolic contents were highly extracted by ethanol (Wang et al., 2012). But the present investigation shows very poor yield with petroleum ether and ethyl acetate, these due to solubility of the bioactive compounds were strongly affected by nature of the solvent (Yao et al., 2014). Flavonoids are rich in antioxidant properties compound produced by plant. Variation in

flavonoids and phenolics contents of plants directly affects their antioxidant potential. Hence phenolic and flavonoids contents were highly dissolved in water and higher activity in their anticancer properties (Settharaksa et al., 2012).

3.3. GC-MS Analysis

Analysis of ethyl acetate root extracts of *D.hamiltonii*by GC-MS showed the presence of 10 compounds as the medicinal properties of the plant.The phytochemical identification of the compounds was confirmed based on theirpeak area, retention time, molecular formula and molecular weight. Activecompounds of ethyl acetate extracts of *D.hamiltonii*with their retentiontime (RT), percentage of peak area, molecular formula and molecular weight are presented in Table 3 and Fig.1.

Table 3 Bioactive compounds identified in the extract of *D. hemiltonii* using GC-MS

Peak %	RT	Name of the compound	Molecular formula	Molecular weight
7.52	08.48	4-methyl-quinazoline	$C_9H_8N_2$	144.17
4.07	17.05	Cyclopentaneundecanolicacid	$C_{16}H_{30}O_2$	254.44
44.35	17.73	Flavone	$C_{15}H_{10}O_2$	222.24
13.15	18.78	Oleic acid	$C_{18}H_{34}O_2$	282.46
75.02	19.43	Eicosatrienoic acid	$C_{20}H_{34}O_2$	306.48
32.28	20.28	Isopropyl stearate	$C_{21}H_{42}O_2$	326.56
20.87	21.18	3-methoxy-17-(2-methyallyl)	$C_{23}H_{32}O_2$	340.24
14.22	22.35	3-(3-methoxyphenyl)-2-(2-phenylethyl)	$C_{18}H_{16}N_2O_2$	292.33
7.89	23.95	Docosanoic acid	$C_{22}H_{44}O_2$	340.59
6.39	26.18	4-Piperidineacetic acid	$C_{23}H_{32}N_2O_4$	400.51

Analysis of ethyl acetate extracts of *Decalepis hamiltonii*showed thepresence of 10 different compounds namely 4-methyl-quinazoline (7.52 %),Cyclopentaneundecanolic acid (4.07 %), 5-hydroxy-4'methoxy-7-methyl- flavones(44.35 %), Oleic acid (13.15 %), 8,11,14-Eicosatrienoic acid (75.02 %), Isopropylstearate (32.28 %), Estra-1,3,5(10)-trien-17a-ol (20.87 %), Quinazolin-4(3H)-one(14.22 %), Docosanoic acid (7.89 %) and 4-Piperidineacetic acid (6.39 %) with theirretention times 8.48, 17.05, 17.73, 18.78, 19.43, 20.28, 21.18, 22.35, 23.95 and 28.18minutes respectively. The structures of components are shown in Fig 2.

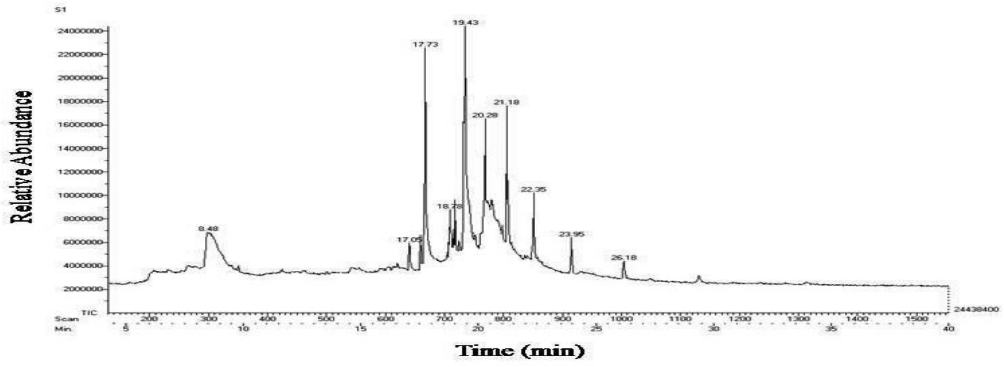

Fig. 1 GC-MS Chromatogram of D. hemiltonii ethyl acetate extract of *D. hamiltonii*

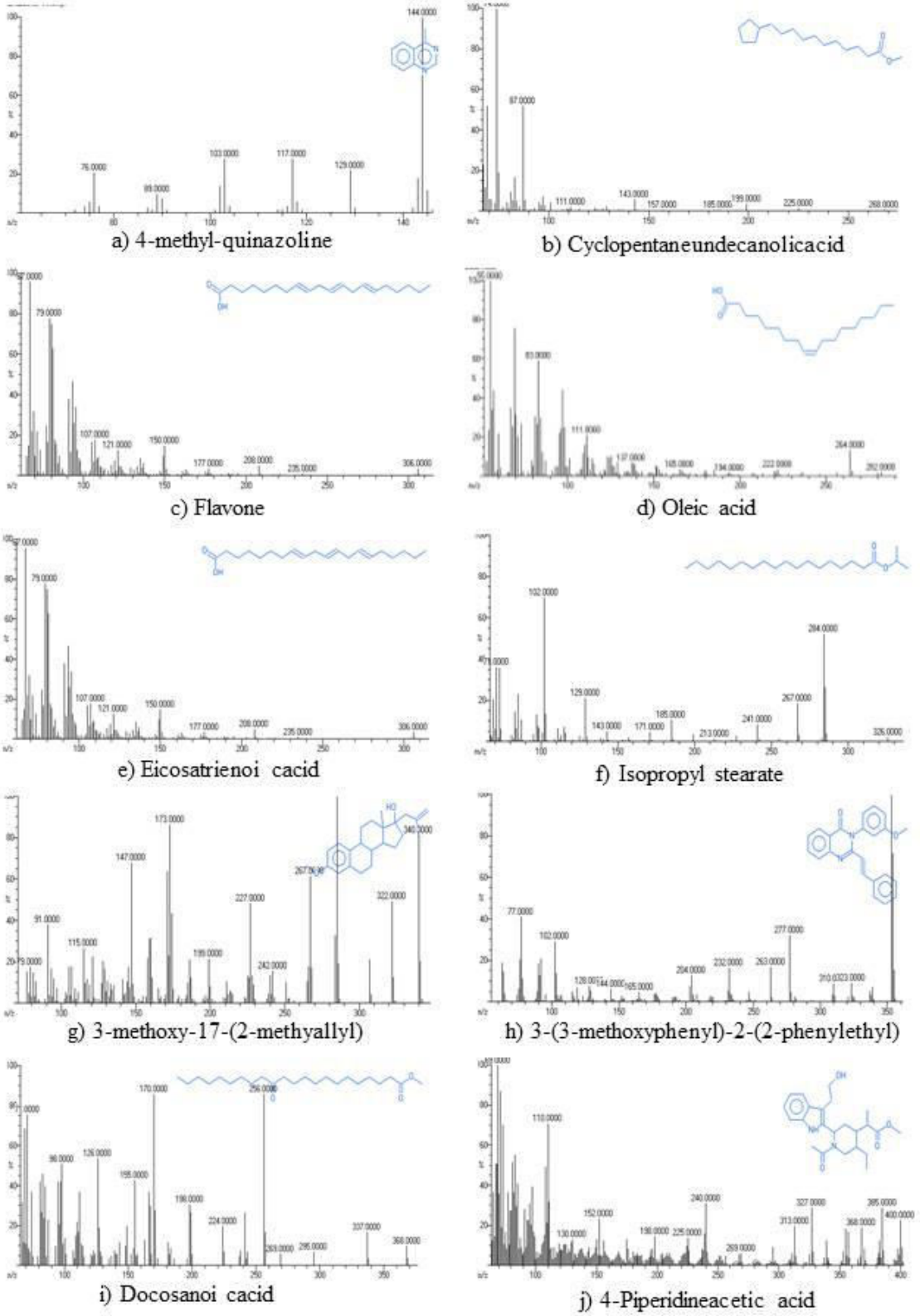

Fig. 2.Bioactive compounds identified in the extract of *D. hemiltonii* using GC-MS

Among the ten bioactive compounds isolated,Cyclopentaneundecanolic acid has significant anticancer and antioxidant properties Mustapha et al., (2016); Arumugam et al., (2018). The Docosanoic acid compounds have been proved clinically as cholesterol reducer was studied by Jayaseelan et al., (2016) and it was also proved as novel metabolites against rheumatoid arthritis and chronic inflammatory disease was reported by Ogunlesi et al., (2009).

3.4. *In vitro* anticancer proliferation assay

In the normal fibroblast cell line (3T3) ethyl acetate root extract of *D.hamiltonii* was found without any damage upto120 µg/ml concentration (Fig.3).

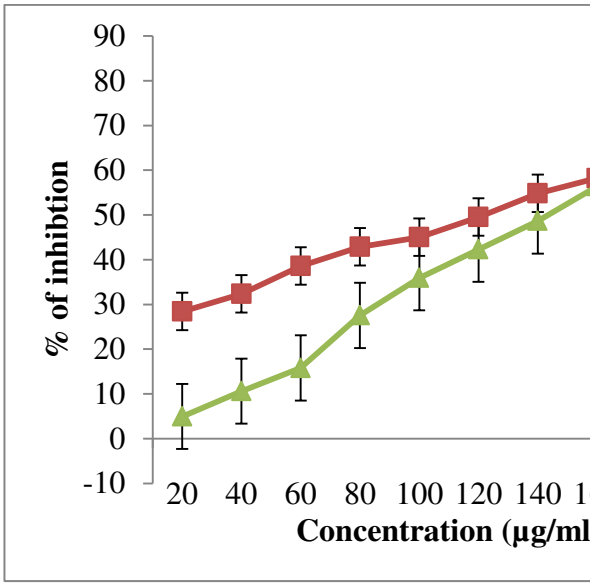

Fig.3. Ant-proliferative effect of *D. hemiltonii* on fibroblast cell line (3T3) and breast cancer cell line (MCF-7) cancer cell lines

The normal cell line was evaluated upto 200 µg/ml concentration of *D. hamiltonii* ethyl acetate extract and its IC_{50} range was obtained at 127.66µg/ml. The normal fibroblast cell viability was shown in Table 4. Ethyl acetate extract on breast cancer cell line (MCF-7) was investigated at dose dependent manner and it is clearly depicted in Table 4. The inhibition range of breast cancer cells was monitored at different concentrations from 10-100 µg/ml. The IC_{50} value of (70.94 µg/ml) the breast cancer cell viability was showed. The morphological changes in ethyl acetate extraction in normal fibroblast cell line (3T3) and in breast cancer cell line (MCF-7) has been exhibited in Fig.3 with respective to IC50 values after 24 hrincubation.

Table 4 *In vitro* anticancer effect of *D. hemiltonii* on 3T3 and MCF-7 cell line

Normal fibroblast cell line (3T3)		Breast cancer cell line (MCF-7)	
Concentration (µg/ml)	Cell Viability (%)	Concentration (µg/ml)	Cell Viability (%)
20	28.40 ± 0.63	10	4.96 ± 3.37
40	32.35 ± 1.08	20	10.61 ± 3.95
60	38.59 ± 1.10	30	15.79 ± 1.39
80	42.86 ± 0.63	40	27.54 ± 4.42
100	45.03 ± 0.56	50	35.94 ± 1.15
120	49.53 ± 0.71	60	42.31 ± 3.12
140	54.83 ± 0.73	70	48.65 ± 3.30
160	58.23 ± 0.82	80	56.38 ± 3.26
180	63.11 ± 0.84	90	62.75 ± 5.48
200	69.01 ± 0.69	100	73.29 ± 3.64
IC_{50}	127.66	IC_{50}	70.94

The values was determined with standard errors (mean ± SD; n=3). The IC_{50} values were determined using ANNOVA

Cell viability of *D. hemiltonii* extract against tested with fibroblast cell line (3T3) and breast cancer cell line (MCF-7) cancer cell lines were determined with MTT assay and examined in viability studies to determine drug efficiency (Chan et al., 2015). In the present study in vitro cytotoxicity activity suggested that the presence of biologically active compound in the extract of *D. hemiltonii*.

4. Conclusion

The present study, the different extracts of *D.hamiltonii* were found in promising anticancer activity. Methanolic extract showed significant anticancer properties and it was subjected to GC-MS analysis, that can evidenced for the presence of anticancer compound. From the above study will be useful for further investigation in the discovery of novel herbal drugs against infectious diseases.

Conflicts of interest

Authors have declared that no conflicts of interest.

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