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GC/MS Volatile Constituents Analysis and Anticancer Activity of *Moltkiopsis ciliata* (Forssk.)

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ABSTRACT

Methanolic extract of the aerial part of *Moltkiopsis ciliata* (Forssk.) (Boraginaceae) was obtained and successively fractionated into two main fractions (hexane fraction and methylene chloride fraction), which were phytochemically investigated using GC/MS technique. The whole of thirty-five compounds were identified and quantified. They are belonging to different classes; twenty-one acetogenins (Fat derivatives), six shikimates, five terpenes and one sterol. Beside the characterization of the volatile compounds profile, *β*-amyrin was isolated and identified using chromatographic and spectral techniques. The methanolic extract and two fractions were screened for their cytotoxic activity against breast cancer cell line MCF7 and hepatocellular carcinoma cell line HepG2. The methylene chloride fraction was proved to be the most potent cytotoxic agent. The major phyto-components of the two fractions were known by their various biological activity as antimicrobial, antiprofilative, antioxidant, Hypocholesterolemic, Antiandrogenic flavor, Nematicide, pesticides, anti-inflammatory, Hemolytic and have significant pharmaceutical importance especially as anticancer agents. The bioactive volatile constituents recommended phytopharmaceutical importance of the plant.

Key Words:
Moltkiopsis ciliata, Boraginaceae, GC/MS technique, volatile constituents, cytotoxic activity

1. INTRODUCTION

Moltkiopsis ciliata (Forssk.) (Boraginaceae), is a perennial flowering shrub with a woody base, up to 25cm high, and has white beginning branches, the plant shielded with stiff white hairs. Flower varies in color significantly. Blue-violet, red, bluish limb with a red tube, white and pure yellow [1].

M. ciliata exposed carbohydrates, flavonoids, terpenes, sterols, nitrogenous bases, condensed tannins, and saponins [2]. Phenolics have attracted great consideration and get high importance due to their antioxidant activity. M. ciliata contains protocatechuic acid, caffeic acid, vanillic acid, p-coumaric acid, iso-ferulic acid, ellagic acid, salycilic acid, o-coumaric acid, e-vanillic acid, and p-OH-benzoic acid, apigenin 6-C-arabinoside-8-C-glactoside, apigenin 6-C-rhamnoside-8-C-glucoside, apigenin 6-C-glucoside 8-C-rhamnoside, luteolin 6-C-arabinoside-8-C-glucoside, luteolin 6-C-glucoside 8-C-arabinoside, apigenin, apigenin 7-O-β-glucoside, acacetin, luteolin 7-O-β-glucoside, kaempferol,

kaempferol 3,7-di-O-rhamnoside, quercetin, rutin, hesperidin, and naringenin were detected by using HPLC technique and compared with standards [3, 4].

In Veterinary medicine, *M. ciliata* used to improve lactation of camels [5]. Also, aqueous ethanolic extract of *M. ciliata* demonstrated moderate antibacterial activity against the Gram-negative *Moraxella catarrhalis* [2].

The Gas Chromatography-Mass Spectrum technique is applied for the identification and quantification of volatile constituents since this method provides high resolution, good sensitivity, as well as structural information about the analysis [6].

The main aim of the study was to extract and identify the bioactive volatile natural products of *Moltkiopsis ciliata* by using diverse solvents and then investigate their anticancer activity.

2. EXPERIMENTAL

General: GC/MS analysis of the volatile moieties was carried out on a "Varian GC" connected to the selective mass detector (SMD); "Finnegan SSQ 7000 " with "ICIS V2.0" data module in order to identify the Mass spectra of the GC composites. The used column was DB-5 (J&W Scientific, Folosm, CA) interfaced with a fused silica capillary one (30 m long, 0.25 mm internal diameter) glazed the poly dimethyl-siloxane (0.5µm film thickness). The oven temperature was isothermally set at 50°C for 3 min, and after that was raised by 7°C /mi up to 250°C, then isothermally at the last temperature for 10 min. The temperature of the Injector was 200°C, whereas the injected volume was 0.5 µl. Ionization energy was adjusted at 70 eV. (Agriculture Research Center (NRC), Dokki, Cairo).

3. MATERIALS AND METHODS

3.1.Solvents and chemicals

Hexane, methylene chloride, methanol petroleum ether (60–80°C), ethyl acetate, and anhydrous sodium sulphate were purchased from ADWIC Company. PTLC was performed on silica gel (Kieselgel 60, F 254) of 0.25mm thickness.

3.2.Plant material

Moltkiopsis ciliata was collected from the north coastal road before Baltym by half Km. Egypt in April 2017 and was identified by prof. Dr. Ibrahim Mashaly, Department of Botany, Faculty of Science, Mansoura University**.**

3.3.Extraction and Isolation

The dried aerial part plant powder of *M. ciliata* (1.25 Kg) was extracted with methanol (10L x 5), then filtrated and evaporated using a rotary evaporator to its 1/3 volume. Exhaustive liquid–liquid extraction using Hexane (60-80°C) and methylene chloride was performed to yield hexane (16.8g), methylene chloride (2.81g) fractions. The hexane fraction was defatted using cold methanol, and then both fractions were subjected to GC/MS. The methylene chloride fraction was fractionated with the aid of column chromatography (silica gel, hexane: ethyl acetate, 70:30) to yield ten fractions. Fraction 3 (80 mg), was further purified on TLC (silica gel, petroleum ether: ethyl acetate, 8.5:1.5) to give compound 37 (25mg).

3.4.Anti-proliferative activity against cancer cell lines

Cancer cell lines namely; breast cancer cell line MCF7 and hepatocellular carcinoma cell line HepG2 were obtained from" VACSERA", Egypt. The capability of cells in culture was calculated by the MTT assay [4]. As with all cells, the cell counts were adjusted to 3 × 103 cells/well and plated in 100 µL of medium/well in 96-well plates. After incubation all overnight, various concentrations from different extracts were incubated with the cell line; 3 wells were involved in each concentration. After treatment for two days, 20 µL of 5 mg/ml MTT (pH 4.7) was added per well and cultivated for another 4 h, removing the supernatant fluid, and then 100 µL DMSO was added per well and shake well for 15 min. The absorbance measurements was reported at 570 nm with a microplate reader "Bio-Rad, Richmond, CA" using wells without cells as blanks. All experiments were performed in triplicate.

4. RESULTS AND DISCUSSION

The lipophilic volatile compounds of *Moltkiopsis ciliata* were extracted and obtained by the aid of two less polar organic solvents; hexane and methylene chloride.

GC/MS is the most befinicial technique designed for the identification and quantification of bioactive composites in plant extracts. GC/MS has been applied for the analysis of different medicinal herbs and was confirmed to be a valuable method for identifying different volatile oils, hydrocarbons, acids, alkaloids, esters, fatty acids, phytosterols and phenolic compounds [7, 8].

GC/MS analyses of hexane and methylene chloride fractions (Table 1 and 2) revealed the existence of important bioactive products, including acetogenins (fat derivatives), terpenes, sterols and shikimates derivatives (phenolic constituents) having many medicinal, pharmaceutical and antimicrobial properties.

The GC/MS characterization of hexane fraction has identified twenty components based upon a comparison of their EI-MS spectra with those of their analogous reported by the NIST database spectral library. Among the most prevailing matched constituents were oleic acid (22.49 %), (E)-9-octadecenoic acid methyl ester (14.76%), hexadecanoic acid methyl ester (11.47 %), (E,E)-9,12-octadecadienoic acid methyl ester (9.46 %), n-hexadecanoic acid (4.46%), Ar-tumerone (2.61 %), methyl stearate (2.15 %) which are used pharmaceutically as antimicrobial, antibacterial, anti-inflammatory, diuretic, antiasthma, antioxidant, inhibition of certain cancers, heart protection, antimalaria, dermatologic agent against acne, hypocholesterolemia, avoidance and treatment of diabetic retinopathy [7, 9, 10].

The methylene chloride fraction afforded fifteen phytochemicals and the more predominant constituents were eicosane (53.83%), n-hexadecanoic acid (12.54%) with antioxidant, hypocholesterolemic, antiandrogenic and 5-Alpha reductase inhibitor activities [7], squalene (2.56 %) which has the property of antioxidant and anticancer activities [11], hexadecanoic acid methyl ester (2.27%), 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (1.07%) beside, the phenolic constituents (3.58 %) which are considered to be natural antioxidants due to their capacity to react with free radicals (scavenging) [12].

Table 1. Identified volatile constituents of hexane extract of *M. ciliata* using GC/MS technique.

No.	Compound name	RT (min)	Area %	Mol. formula	Mol. Wt.	m/z (%)
Acetogenins (Fat Derivatives)						
1	**E-15-Heptadecenal**	34.84	0.14	$C_{17}H_{32}O$	252	252 (100%) $[M]^{+\cdot}$, 83 (100%) $[C_5H_7O]^+$, 55 (83%) $[C_3H_3O]^+$, 97 (79%) $[C_6H_9O]^+$, 41 (77%) $[C_3H_5]^+$, 43 (75%)$[C_3H_7]^+$
2	**Tetradecanoic acid methyl ester**	37.82	0.43	$C_{15}H_{30}O_2$	242	242 (7%) $[M]^{+\cdot}$, 74 (100%) $[C_3H_6O_2]^{+\cdot}$ 87 (75%) $[C_4H_7O_2]^{+\cdot}$ 43 (26%) $[C_3H_7]^{+\cdot}$ 41 (23%) $[C_3H_5]^{+\cdot}$ 143 (25%) $[C_8H_{15}O_2]^+$
3	**Tetradecanoic acid**	38.56	0.28	$C_{14}H_{28}O_2$	228	228 (13%) $[M]^{+\cdot}$, 137 (100%) $[C_{10}H_{17}]^+$, 135 (89%) $[C_{10}H_{15}]^+$, 41 (78%) $[C_3H_5]^+$, 55 (73%) $[C_4H_7]^+$, 73 (71%) $[C_3H_5O_2]^+$
4	**1-Octadecene**	39.38	0.30	$C_{18}H_{36}$	252	252 (3%) $[M]^{+\cdot}$, 55 (100%) $[C_4H_7]^+$, 83 (94%) $[C_6H_{11}]^+$, 97 (85%) $[C_7H_{13}]^+$, 43 (75%) $[C_3H_7]^+$, 41 (74%) $[C_3H_5]^+$
5	**6,10,14-trimethyl-2-pentadecanone**	40.40	0.26	$C_{18}H_{36}O$	268	268 (3%) $[M]^{+\cdot}$, 43 (100%) $[C_2H_3O]^+$, 58 (81%) $[C_3H_6O]^+$, 71 (54%) $[C_4H_7O]^+$, 57 (44%) $[C_4H_9]^+$, 55 (35%) $[C_4H_7]^+$
6	**(Z)-9-Hexadecenoic acid methyl ester**	41.68	0.23	$C_{17}H_{32}O_2$	268	268 (3%) $[M]^{+\cdot}$, 55 (100%) $[C_4H_7]^+$, 69 (67%) $[C_5H_9]^+$, 41 (62%) $[C_3H_5]^+$, 43 (59%) $[C_3H_7]^+$, 83 (58%) $[C_6H_{11}]^+$
7	**Hexadecanoic acid**	42.10	11.47	$C_{17}H_{34}O$	270	270 (12%) $[M]^{+\cdot}$, 74 (100%)

	methyl ester			$_2$		$[C_3H_6O_2]^+$, 87 (78%) $[C_4H_7O_2]^+$, 43 (26%) $[C_3H_7]^+$, 143 (25%) $[C_8H_{15}O_2]^+$, 227 (24%) $[C_{14}H_{27}O_2]^+$
8	**n-Hexadecanoic acid**	42.87	4.46	$C_{16}H_{32}O_2$	256	256 (30%) $[M]^{+\cdot}$, 73 (100%) $[C_3H_5O_2]^+$, 60 (74%) $[C_2H_4O_2]^+$, 43 (68%) $[C_3H_7]^+$, 55 (61%) $[C_4H_7]^+$, 57 (61%) $[C_4H_9]^+$
9	**(E)-5-Eicosene**	43.49	0.60	$C_{20}H_{40}$	280	280(3%) $[M]^{+\cdot}$, 83 (100%) $[C_6H_{11}]^+$, 55 (99%) $[C_4H_7]^+$, 43 (99%) $[C_3H_7]^+$, 97 (88%) $[C_7H_{13}]^+$, 57 (88%) $[C_4H_9]^+$
10	**15-methyl-hexadecanoic acid methyl ester**	44.06	0.14	$C_{18}H_{36}O_2$	284	284 (13%) $[M]^{+\cdot}$, 74 (100%) $[C_3H_6O_2]^+$, 87 (76%) $[C_4H_7O_2]^+$, 43 (50%) $[C_3H_7]^+$, 55 (49%) $[C_4H_7]^+$, 41 (39%) $[C_3H_5]^+$
11	**(E,E)-9,12-octadecadienoic acid methyl ester**	45.49	9.46	$C_{19}H_{34}O_2$	294	294 (13%) $[M]^{+\cdot}$, 67 (100%) $[C_5H_7]^+$, 81 (95%) $[C_6H_9]^+$,95 (72%) $[C_7H_{11}]^+$, 55 (60%) $[C_4H_7]^+$, 79 (48%) $[C_6H_7]^+$
12	**(E)-9-octadecenoic acid methyl ester**	45.63	14.76	$C_{19}H_{36}O_2$	296	296 (7%) $[M]^{+\cdot}$, 55 (100%) $[C_4H_7]^+$, 69 (70%) $[C_5H_9]^+$, 74 (68%) $[C_3H_6O_2]^+$, 41 (66%) $[C_3H_5]^+$, 83 (61%) $[C_6H_{11}]^+$
13	**Methyl stearate**	46.12	2.15	$C_{19}H_{38}O_2$	298	298 (17%) $[M]^{+\cdot}$, 74 (100%) $[C_3H_6O_2]^+$, 87 (80%) $[C_4H_7O_2]^+$, 43 (31%) $[C_3H_7]^+$, 143 (29%) $[C_8H_{15}O_2]^+$, 55 (27%) $[C_4H_7]^+$
14	**Oleic acid**	46.65	22.49	$C_{18}H_{34}O_2$	282	282 (3%) $[M]^+$, 55 (100%) $[C_4H_7]^+$, 69 (74%) $[C_5H_9]^+$, 41 (70%) $[C_3H_5]^+$, 67 (60%) $[C_5H_7]^+$, 83 (59%) $[C_6H_{11}]^+$
15	**(E)-9-Eicosene**	47.71	0.90	$C_{20}H_{40}$	280	280 (3%) $[M]^{+\cdot}$, 55 (100%) $[C_4H_7]^+$, 69 (83%) $[C_5H_9]^+$, 41 (80%) $[C_3H_5]^+$, 83 (76%) $[C_6H_{11}]^+$, 97 (76%) $[C_7H_{13}]^+$
16	**trans-13-Octadecenoic acid**	50.53	0.35	$C_{18}H_{34}O_2$	282	282 (3%) $[M]^{+\cdot}$, 55 (100%) $[C_4H_7]^+$, 69 (65%) $[C_5H_9]^+$, 57 (62%) $[C_4H_9]^+$, 41 (56%) $[C_3H_5]^+$, 43 (56%) $[C_3H_7]^+$
17	**Eicosanoic acid methyl ester**	51.26	0.50	$C_{21}H_{42}O_2$	326	326 (20%) $[M]^{+\cdot}$, 74 (100%) $[C_3H_6O_2]^+$, 87 (82%) $[C_4H_7O_2]^+$, 43 (43%) $[C_3H_7]^+$,55 (40%) $[C_4H_7]^+$, 41 (56%) $[C_3H_5]^+$
Sesquiterpenes						
18	**Ar-tumerone**	36.70	2.61	$C_{15}H_{20}O$	216	216 (26%) $[M]^{+\cdot}$, 83 (100%) $[C_5H_7O]^+$, 119 (77%) $[C_9H_{11}]^+$, 201 (24%) $[C_{14}H_{17}O]^+$, 132 (22%) $[C_{10}H_{12}]^+$
19	**Curlone**	37.61	0.90	$C_{15}H_{22}O$	218	218 (3%) $[M]^{+\cdot}$, 120 (100%) $[C_9H_{12}]^+$, 83 (28%) $[C_5H_7O]^+$, 105 (17%) $[C_8H_9]^+$, 91 (15%) $[C_7H_7]^+$, 55 (11%) $[C_4H_7]^+$
Diterpenes						
20	**4,8,12,16-**	52.10	0.32	$C_{21}H_{40}O$	324	324 (2%) $[M]^{+\cdot}$, 99 (100%)

	tetramethylheptadecan-4-olide			2		$[C_5H_7O_2]^+$, 55 (38%) $[C_4H_7]^+$, 43 (38%) $[C_3H_7]^+$, 69 (30%) $[C_5H_9]^+$, 57 (25%) $[C_4H_9]^+$

Table 2. Identified volatile constituents of CH_2Cl_2 extract of *M. ciliata* using GC/MS technique.

No.	Compound name	RT (min)	Area %	Mol. formula	Mol. Wt.	m/z (%)
Shikimates						
22	**2-Methoxy-4-vinylphenol**	16.34	0.25	$C_9H_{10}O_2$	150	150 (100%) $[M]^{+\cdot}$, 135 (82%) $[C_8H_7O_2]^+$, 107 (28%) $[C_7H_7O]^+$, 77 (22%) $[C_6H_5]^+$, 51 (10%) $[C_4H_3]^+$
23	***trans*-Isoeugenol**	19.85	0.26	$C_{10}H_{12}O_2$	164	164 (100%) $[M]^{+\cdot}$, 149 (30%) $[C_9H_9O_2]^+$, 91 (22%) $[C_6H_3O]^+$, 77 (19%) $[C_6H_5]^+$, 131 (18%) $[C_9H_7O]^+$
24	**4-Vinyl-syringol**	22.51	0.41	$C_{10}H_{12}O_3$	180	180 (100%) $[M]^{+\cdot}$, 165 (38%) $[C_9H_9O_3]^+$, 137 (24%) $[C_8H_9O_2]^+$, 122 (12%) $[C_7H_6O_2]^+$, 181 (11%) $[C_{10}H_{13}O_3]^+$
25	**Phenol, 2,6-dimethoxy-4-(2-propenyl)-**	25.53	0.29	$C_{11}H_{14}O_3$	194	194 (100%) $[M]^{+\cdot}$, 131 (82%) $[C_9H_7O]^+$, 167 (34%) $[C_9H_{11}O_3]^+$, 163 (33%) $[C_9H_7O_3]^+$, 103 (31%) $[C_7H_3O]^+$
26	**(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol**	25.62	0.88	$C_{11}H_{14}O_3$	194	194 (100%) $[M]^{+\cdot}$, 179 (16%) $[C_{11}H_{15}O_2]^+$, 91 (16%) $[C_6H_3O]^+$, 131 (14%) $[C_9H_7O]^+$, 119 (13%) $[C_9H_{11}]^+$
27	**4-((1E)-3-Hydroxy-1-propyl)-2-methoxyphenol**	26.44	0.42	$C_{10}H_{12}O_3$	180	180 (72%) $[M]^{+\cdot}$, 137 (100%) $[C_8H_9O_2]^+$, 124 (47%) $[C_7H_8O_2]^+$, 91 (32%) $[C_6H_3O]^+$, 119 (23%) $[C_8H_7O]^+$
28	**6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one**	27.06	1.07	$C_{11}H_{16}O_3$	196	196 (10%) $[M]^{+\cdot}$, 111 (100%) $[C_6H_7O_2]^+$, 178 (61%) $[C_{11}H_{14}O_2]^+$, 140 (43%) $[C_9H_{16}O]^+$, 135 (35%) $[C_8H_7O_2]^+$, 181 (34%) $[C_{10}H_{13}O_3]^+$
Acetogenins (Fat Derivatives)						
29	**Eicosane**	3.13	53.83	$C_{20}H_{42}$	282	282 (7%) $[M]^+$, 57 (100%) $[C_4H_9]^+$, 71 (82%) $[C_5H_{11}]^+$, 85 (69%) $[C_6H_{13}]^+$, 97 (40%) $[C_7H_{13}]^+$, 55 (37%) $[C_4H_7]^+$
30	**Hexadecanoic acid, methyl ester**	30.30	2.27	$C_{17}H_{34}O_2$	270	270 (13%) $[M]^{+\cdot}$, 74 (100%) $[C_3H_6O_2]^+$, 87 (79%) $[C_4H_7O_2]^+$, 143 (24%) $[C_{10}H_{23}]^+$, 227 (24%) $[C_{14}H_{27}O_2]^+$, 55 (20%) $[C_4H_7]^+$
31	**n-Hexadecanoic acid**	31.15	12.54	$C_{16}H_{32}O_2$	256	256 (33%) $[M]^{+\cdot}$, 73 (100%) $[C_3H_5O_2]^+$, 60 (73%) $[C_2H_4O_2]^+$, 129 (59%) $[C_7H_{13}O_2]^+$, 57 (57%) $[C_4H_9]^+$, 55 (51%) $[C_4H_7]^+$

32	**10-Octadecenoic acid, methyl ester**	33.57	0.32	$C_{19}H_{36}O_2$	296	296 (10%) $[M]^{+\cdot}$, 55 (100%) $[C_4H_7]^+$, 69 (78%) $[C_5H_9]^+$, 83 (73%) $[C_6H_{11}]^+$, 84 (60%) $[C_6H_{12}]^+$, 97 (55%) $[C_7H_{13}]^+$
33	**Heptadecanoic acid, 16-methyl-, methyl ester**	34.06	0.50	$C_{19}H_{38}O_2$	298	298 (17%) $[M]^{+\cdot}$, 74 (100%) $[C_3H_6O_2]^+$, 87(80%) $[C_4H_7O_2]^+$, 143 (31%) $[C_8H_{15}O_2]^+$, 255 (27%) $[C_{16}H_{31}O_2]^+$, 55 (26%) $[C_4H_7]^+$
Phenolic terpene						
34	**Alliodorin**	37.76	0.35	$C_{16}H_{20}O_3$	260	260 (85%) $[M]^{+\cdot}$, 177 (100%) $[C_{11}H_{13}O_2]^+$, 161 (90%) $[C_{10}H_9O_2]^+$, 123 (70%) $[C_7H_7O_2]^+$, 55 (58%) $[C_3H_3O]^+$
Triterpenes						
35	**Squalene**	44.85	2.56	$C_{30}H_{50}$	410	410 (3%) $[M]^{+\cdot}$, 69 (100%) $[C_5H_9]^+$, 81 (60%) $[C_6H_9]^+$, 149 (19%) $[C_{11}H_{17}]^+$, 137 (18%) $[C_{10}H_{17}]^+$, 95 (18%) $[C_7H_{11}]^+$
Steroids						
36	**Cholesta-3,5-diene**	46.07	0.97	$C_{27}H_{44}$	368	368 (100%) $[M]^{+\cdot}$, 147 (81%) $[C_{11}H_{15}]^+$, 353 (46%) $[C_{26}H_{41}]^+$, 145 (45%) $[C_{11}H_{13}]^+$, 105 (45%) $[C_8H_9]^+$

The major phyto-components of *M. ciliata* and its various biological activities obtained through the GC/MS study of hexane and methylene chloride fractions are presented in **Table 3**. The represented data revealed that both fractions are rich with bioactive volatile constituents which recommended phytopharmaceutical importance of the plant.

Table 3. Reported biological activities of the main phyto-constituents of hexane and CH_2Cl_2 extracts

Predominant component	**Biological activity**
Acetogenins (Fat Derivatives)	
Hexadecanoic acid, methyl ester	- Antioxidant, Nematicide, Pesticide, Hypocholesterolemic, Antiandrogenic flavor, Hemolytic, 5-Alpha reductase inhibitor [13]. - Antimicrobial activity [14]. - Antifungal activity [15]. - Antibacterial, antitumor, immunostimulant, chemopreventive and lipoxygenase inhibitor [16].
n-Hexadecanoic acid	- Antioxidant, Hemolytic, Nematicide, Pesticide, Hypocholesterolemic, Lubricant [17] - Alpha reductase and antipsychotic inhibitor [18]. - larvicidal activity against mosquitoes [19]. - Antifungal, flavor, potent antimicrobial agent, antimalarial pesticide and antipsychotic [20]. - Cytotoxicity against human colorectal carcinoma cells (HCT-116) [21] . - Antifouling property [22]. - Control of human pathogens, pests, termites and maggots **[**23]. - Increasing proliferation of MSCs [24].

	- Considered as larvicide [25]. - The leaf ethanolic extract of Centella asiatica showed significant inhibitory activity against *Mycobacterium tuberculosis* due to presence of major bioactive n-Hexadecanoic [26]. - Repellent against Anopheles species and thus useful for malaria control [27]. - Antiandrogenic [28]. - Echo enhancement in sonographic doppler B-mode imaging [29].
9,12-Octadecadienoic acid, methyl ester, (E,E)	-anti-inflammatory, antibacterial, hypocholesterolemic and hepatoprotective activities, 11-octadecenoic acid, methyl ester for having antioxidant and antimicrobial properties [30].
9-Octadecenoic acid, methyl ester, (E)-	-antimicrobial, anti-inflammatory, antioxidant -hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic [31].
Methyl stearate	-anti-inflammatory, intestinal lipid metabolism regulator, antinociceptive, nematicidal, antioxidant, antimicrobial activity [32].
Oleic Acid	- Mediterranean diet (rich in OA) consumption [33]. - it has useful effects on cardiovascular disease, serum lipids and special protecting effects against cancer [34]. - *in vivo* antioxidative [35].
Eicosane	-it mimics the effect of indomethacin, reduces the secretion of histamine, bradykinin, TXs, PGs, and LTs, inhibits the stimulation of cytokines such as IL-1b, TNF, IL-6, and interferon-α , interrelated with the cox enzyme [36].
Sesquiterpenes	
Ar-tumerone	-antioxidant, anti-inflammatory, anticancer, antimicrobial, neuroprotective, cardioprotective and radioprotective effects [37].
Shikimates	
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	-cytotoxic against MCF-7, antimicrobial, antiprofilative, anti-acetyl cholinesterase activity, anti-inflammatory [38].
Triterpenes	
Squalene	critical for reducing free radical oxidative damage to the skin. Serum squalene originates partly from endogenous cholesterol synthesis and partly from dietary sources, especially in populations consuming large amounts of olive oil or shark liver [2]. The endogenous synthesis of squalene begins with the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA). The initial reduction of HMG CoA (a niacin-dependent reaction) results in the formation of mevalonate [4]. critical for reducing free radical oxidative damage to the skin. Serum squalene originates partly from endogenous cholesterol synthesis and partly from dietary sources, especially in populations consuming large amounts of olive oil or shark liver [2]. The endogenous synthesis of squalene begins with the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG

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Repeated chromatographic analysis of methylene chloride fraction using column chromatography and PTLC (silica gel, petroleum ether: ethyl acetate, 17:3) yielded compound (37, R_f = 0.33). ^{1}H NMR and MS spectra proved that compound **37** is *β*-amyrin which was in agreement with those previously those established from *Aster yomena* [41].

4.1. Cytotoxic activity

The herbal medicines exert their multiple therapeutic and anticancer properties through inhibition of cancer activating enzymes and hormones, stimulation of DNA repair mechanism, promotion of protective enzymes production, induction of the antioxidant system, and enhancing the immune system [42].

The methanolic extract of *M. ciliata* as well as both fractions; hexane and CH_2Cl_2 were screened for cytotoxic activity against HepG2 and MCF-7 cell lines using MTT-based cytotoxicity Inspect. After 48 h incubation period of the extracts, it was found that methylene chloride showed potent cytotoxicity against all cancer cell lines (HepG2, and MCF7) followed by the methanolic extract and the least one hexane fraction. The viability of cancer cell lines was considerably affected. The cell layer partially condensed, forming cell-free areas, and finally separated from the culture plate [43, 44].

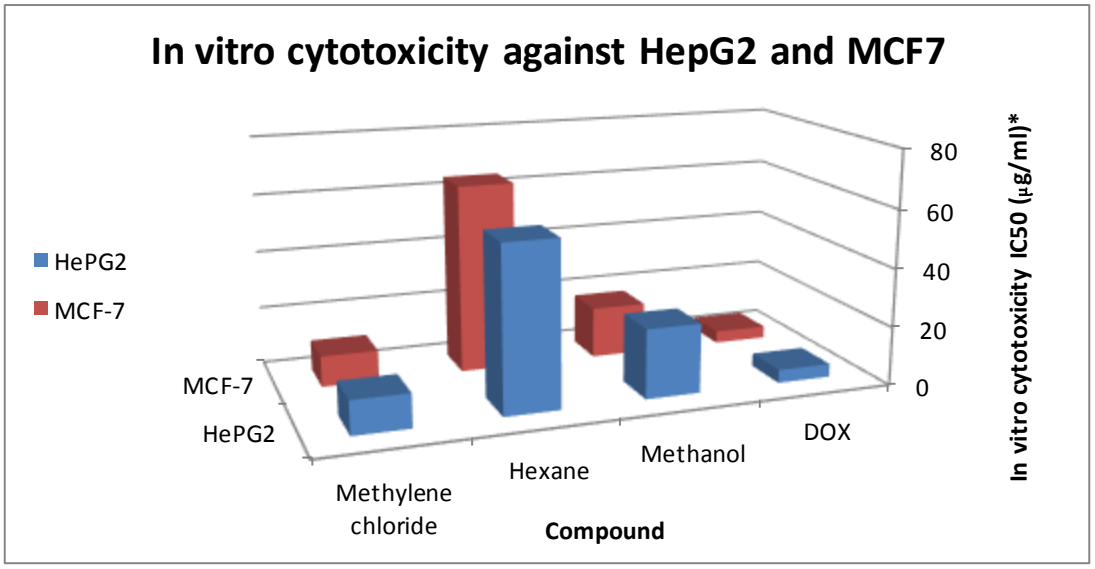

Fig (1): Cytotoxic activity against human cell lines of *Moltkiopsis ciliata* extracts

5. CONCLUSION

The study revealed that *Moltkiopsis ciliata* lipophilic extract contains various volatile constituents belonging to different classes, GC/MS analysis of both hexane and methylene chloride fractions has identified thirty-five compounds, the most predominant constituents have significant pharmaceutical importance, utilized as anticancer agents.

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