



## **A Study on Phytochemical and Anticancer Activities of Epiphytic Orchid *Aerides odorata* Lour.**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author SMK designed the study. Authors JK and VR performed the experimental analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aim:** The present study was carried out to evaluate the phytochemical composition and anticancer activities of leaf extract of *Aerides odorata* Lour., a widely distributed epiphytic herb found in the Eastern Ghats of Vizianagram district.

**Methodology:** The solvents like n-hexane, ethyl acetate and methanol were used to extract dried leaf material of *A. odorata*. These extracts were analysed for phytochemical constituents by GC-MS analysis and *in vitro* anticancer activity was done against two cancer cell lines (*MCF-7* and *HeLa* cell line) by using MTT assay.

**Results:** Preliminary phytochemical analysis revealed the presence of alkaloids, coumarins, flavonoids, glycosides, phenols, and terpenoids. GC-MS analysis determines presence of 15 compounds in ethyl acetate and 14 compounds in methanol extracts respectively. Among two extracts a total 13 compounds have anticancer activity. Both the solvent extracts exhibit

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significant cancer cell growth inhibition with  $IC_{50}$  value ranging between 26.211  $\mu\text{g/mL}$  to 59.061  $\mu\text{g/mL}$ .

**Conclusion:** Methanol about the best solvent and its activity. Our result showed *A. odorata* is a promising source of anticancer drugs.

**Keywords:** GC-MS analysis; anticancer; *Aerides odorata*.

## 1. INTRODUCTION

Orchids are one of the beautiful flowering plants and they are highly confined to ornamentation. In addition to ornamental, orchids have medicinal value in folklore and traditional systems [1,2]. Current ethnobotanical studies on orchids indicate that orchids have immense potential in the treatment of various diseases [3,4] and Chinese first described medicinal uses of orchids [5]. India is a harbour of orchids with 1331 species and 186 genera [6]. Among them 33 genera belonging to 66 species were distributed mainly in the hilly areas of Andhra Pradesh. About 10 species of orchids have been used ethnobotanically by tribals in different regions of Andhra Pradesh to treat various diseases [7,8]. *A. odorata* is widely distributed epiphytic herb found in the Eastern Ghats of Vizianagaram district. Ethno botanically *A. odorata* used to treat various diseases such as chest pain and stomach disorder, skin disorders, tuberculosis, cuts and wounds, boils in ears and nose, pneumonia, inflammations etc. in various regions [2,9,10,11,12,13]. Many pharmacological activities of these ethnomedicinal plants are due to natural phytochemical composition. Phytochemical analysis of *A. odorata* may leads to explore of new bioactive compounds. Hence, the present study was carried out to determine the phytochemical analysis and anticancer efficiency of *A. odorata* leaf extracts.

## 2. METHODOLOGY

In present study fresh leaves of *A. odorata* were collected from Vizianagaram District, Andhra Pradesh. Plant was authenticated with voucher number of ANUBH01211 and preserved at the herbarium of department of Botany, Acharya Nagarjuna University, Guntur. The fresh healthy leaves of *A. odorata* were air-dried under shade at room temperature for fifteen days. The dried material pulverized into a coarse powder by means of electrical grinder. The dried leaf powder of (250g) was extracted with Soxhlet apparatus with n-hexane, ethyl acetate and methanol solvents for about 12-15hr at room temperature of 35-40°C. Finally, crude extracts of different solvents were concentrated in a vacuum

rotary evaporator (Buchi Labortechnik Ag, model I, R-215) under reduced pressure. The concentrates of various solvent extracts were kept in the refrigerator at 4°C until use.

### 2.1 Preliminary Phytochemical Screening

The dried extract of various solvents hexane, chloroform, ethyl acetate and methanol were preliminary screened by using standard procedures/tests [14,15,16,17].

### 2.2 GC-MS Analysis

The GC-MS analysis of methanol and ethyl acetate solvent extracts was injected to Agilent 7890 A, GC system coupled with MS 5975. The operating conditions of GC-MS set for analysis were as follows: oven temperature was programmed from 50-150°C at 3C/min s. An aliquot of 2  $\mu\text{L}$  of the sample was injected and the carrier of inert helium gas at a constant flow rate of 1mL/1 min. The electron ionization of sample components was carried out with ionization energy 70<sup>ev</sup>. The total running time was 55.3 minutes. National Institute of Standard and Technology (NIST) Data Base Library 2.0 version searched to compare structure of the compounds. Compounds were identified based on the retention times and mass spectra of NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

### 2.3 Anticancer Activity by MTT Assay

The two solvent extracts (Ethyl acetate and Methanol) were tested for in vitro cytotoxicity using *MCF-7* and *HeLa* cell lines by MTT (3, 4 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) assay. 100 mL of diluted leaf extract was added to 100 mL of media followed by the addition of cell lines ( $6 \times 10^5$ ) into 96 well microtiter and incubated overnight at 37°C for 48 hrs. MTT was added after the incubation, precipitates were formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density was measured at 570 nm on a microplate reader. Dose response curve was used to calculate  $IC_{50}$  dose values [18].

### 3. RESULTS

#### 3.1 Phytochemical Analysis

Preliminary phytochemical screening of the different solvent extracts like hexane, ethyl acetate and methanol extract of leaves in *A. odorata* revealed the presence of various secondary metabolites such as alkaloids, coumarins, flavonoids, glycosides, phenols, steroids and terpenoids (Table 1). Gas chromatography and mass spectroscopy is an important technological tool used to identify phytochemicals in plant species [19,20]. GC-MS analysis carried out based on the results of preliminary phytochemical analysis. Methanolic and ethyl acetate extracts of *A. odorata* used for the identification of bioactive compounds. GC-MS analysis of ethyl acetate leaf fraction of *A. odorata* revealed the presence of 12 bioactive compounds and 6 unknown compounds as shown in Table 2; Fig. 1. From the results of GC-MS spectra compounds found in the ethyl acetate extract are 2-Methyl-5-(1,2,2-Trimethylcyclopentyl)phenol (Fig. 2A), 1,3-Propanediol (Fig. 2B), 1,2,3-Propanetriol, 1-acetate (Fig. 2C), Butanamide (Fig. 2D), Phenyl(piperidin-3-yl)methanone (Fig. 2E), 4-Methyl-2-pentadecyl-1,3-dioxane (Fig. 2F), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Fig. 2G),  $\beta$ -Selinene (Fig. 2H), Longipinocarvone (Fig. 2I), (E)-5-Methylundec-4-ene (Fig. 2J), Methyl heptadecanoate (Fig. 2K), Hexadecan-1-ol (Fig. 2L), Methyl 14-methylpentadecanoate (Fig. 2M) 2-O-(2-Ethylhexyl) 1-O-pentadecyl oxalate (Fig. 2N), Squalene (Fig. 2O), and three Unidentified compounds.

The methanol crude extracts isolated from the leaves of *A. odorata* analyzed by using GC-MS had led to the identification of 14 different organic compounds and 4 unidentified compounds

shown in Table 3; Fig. 3. The compounds in the methanol extract are 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl - (Fig. 4A), (2R-cis)-, 2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl) (Fig. 4B), *m*-Toluyaldehyde (Fig. 4C), Methyl (2E) - 3-phenyl - 2-propeonate (Fig. 4D), 1,2,3-Propanetriol, diacetate (Fig. 4E), 5-Ethyl-2-methyl-2,3-dihydrofuran (Fig. 4F), cis-11-Eicosenoic acid (Fig. 4G), Ethyl  $\alpha$ -D-glucopyranoside (Fig. 4H), 6-Isopropyl-3-methyl-1-cyclohex-2-enone (Fig. 4I), 3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (Fig. 4J), Erucic acid (Fig. 4K), (9Z,12Z)-Octadeca-9,12-dienoyl chloride (Fig. 4L), (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (Fig. 4M) and 9,12,15-Octadecatrienoic acid, methyl ester (Fig. 4n).

#### 3.2 Anticancer Activity

**Anticancer activity** The MTT assay for cytotoxicity of ethyl acetate and methanol extracts of *A. odorata* was carried out at five different concentrations of 5, 10, 25, 50, 75 and 100  $\mu\text{g/mL}$  on two different cell lines *MCF-7* and *HeLa* (Plates 1 and 2; Plates 3 and 4). The results of the cytotoxicity of *A. odorata* two solvent extracts on both the cell lines are shown in Tables 4, 5. The data suggest that the methanolic leaf extract of *A. odorata* showed more cytotoxicity as compared to the ethyl acetate extract on *MCF-7* cell lines. The ethyl acetate extract of the *A. odorata* at the concentration 100  $\mu\text{g/mL}$  showed the highest growth inhibition 61.128% on *MCF-7* cell lines as compared to the methanol extract having 60.69%. The recorded  $\text{IC}_{50}$  (50% of growth inhibition) value for methanol extract was 26.211  $\mu\text{g/mL}$  and 41.094  $\mu\text{g/mL}$  in ethyl acetate extracts. It indicates that the methanol extract exhibit significant cytotoxicity effect on *MCF-7* cell lines.

**Table 1. Preliminary phytochemical screening of leaf extracts of *A. odorata***

Sl. no	Phytochemicals	Test name	Hexane	Ethyl acetate	Methanol
1	Alkaloids	Dragendorff's test	-	+	+
2	Coumarins	Sodium hydroxide test	-	+	+
3	Flavonoids	Ferric chloride test	-	-	+
4	Glycosides	Anthrone test	-	-	+
5	Phenolic compounds	Phenol test	-	+	-
6	Quinones	H <sub>2</sub> SO <sub>4</sub> test	-	+	+
7	Resins	Acetone H <sub>2</sub> O test	-	-	-
8	Saponins	Foam test	-	-	-
9	Tannins	Braemer's test	-	-	-
10	Steroids	Salkowski test	-	+	-
11	Terpenoids	Salkowski test	-	+	-

(+) = positive (present); (-) = negative (absent)

**Table 2. Bioactive compounds present in ethyl acetate extract of *A. odorata* by using GC-MS analysis**

Sl. no	R.T (min)	Name of the compound	Molecular formula	Molecular Mass (gm/mol)	Peak area %	Biological activity
1	4.0167	2-Methyl-5-(1,2,2-Trimethyl cyclopentyl)phenol	C <sub>15</sub> H <sub>22</sub> O	218.34	0.56	Anticancer [21]
2	4.5167	1,3-Propanediol	C <sub>3</sub> H <sub>8</sub> O <sub>2</sub>	76.095	7	-
3	5.8	1,2,3-Propanetriol, 1-acetate	C <sub>5</sub> H <sub>10</sub> O <sub>4</sub>	134.131	1.74	Antibacterial [22]
4	6.1167	Butanamide	C <sub>4</sub> H <sub>9</sub> NO	87.122	6.58	-
5	9.2667	Phenyl(piperidin-3-yl)methanone	C <sub>12</sub> H <sub>15</sub> NO	189.258	4.76	Anticancer [23]
6	16.65	4-Methyl-2-pentadecyl-1,3-dioxane	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312.538	0.64	Antibacterial and Antifungal [24]
7	19.99	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	C <sub>20</sub> H <sub>40</sub> O	296.539	2.72	Anticancer [25], antihelmintic and anti-inflammatory [26]
8	20.0333	β-Selinene	C <sub>15</sub> H <sub>24</sub>	204.357	6.93	Antioxidant and anti-inflammatory [27]
9	22.9833	Longipinocarvone	C <sub>15</sub> H <sub>22</sub> O	218.34	2.03	-
10	31.2167	(E)-5-Methylundec-4-ene	C <sub>12</sub> H <sub>24</sub>	168.324	1.69	Anticancer and Antitumor [26]
11	41.4167	Methyl heptadecanoate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.484	2.8	Catechol-O-Methyl-Transferase Inhibitor [26]
12	41.5003	Hexadecan-1-ol 1-	C <sub>16</sub> H <sub>34</sub> O	242.447	14.72	Skin diseases [28]
13	47.9833	Methyl 14-methylpentadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.457	4.63	Methyl guanidine inhibitor [26]
14	50.0607	2-O-(2-Ethylhexyl) 1-O-pentadecyl oxalate	C <sub>25</sub> H <sub>48</sub> O <sub>4</sub>	412.655	1.55	Anticancer, Antitumour and Inhibit production of tumour necrosis factor [26]
15	58.2667	Squalene	C <sub>30</sub> H <sub>50</sub>	410.73	2.15	Antibacterial, Antioxidant, pesticide, Antitumour, anti-cancer, preventive, Immunostimulent, Chemo preventive, Lipoxigenase-inhibitor [29,30]
16	6.58	Unidentified compound 1	-	297.58	10.9500	-
17	4.76	Unidentified compound 2	-	344.08	14.4167	-
18	14.79	Unidentified compound 3	-	140.46	27.0667	-

**Table 3. Bioactive compounds present in methanolic extract of *A. odorata* by using GC-MS analysis**

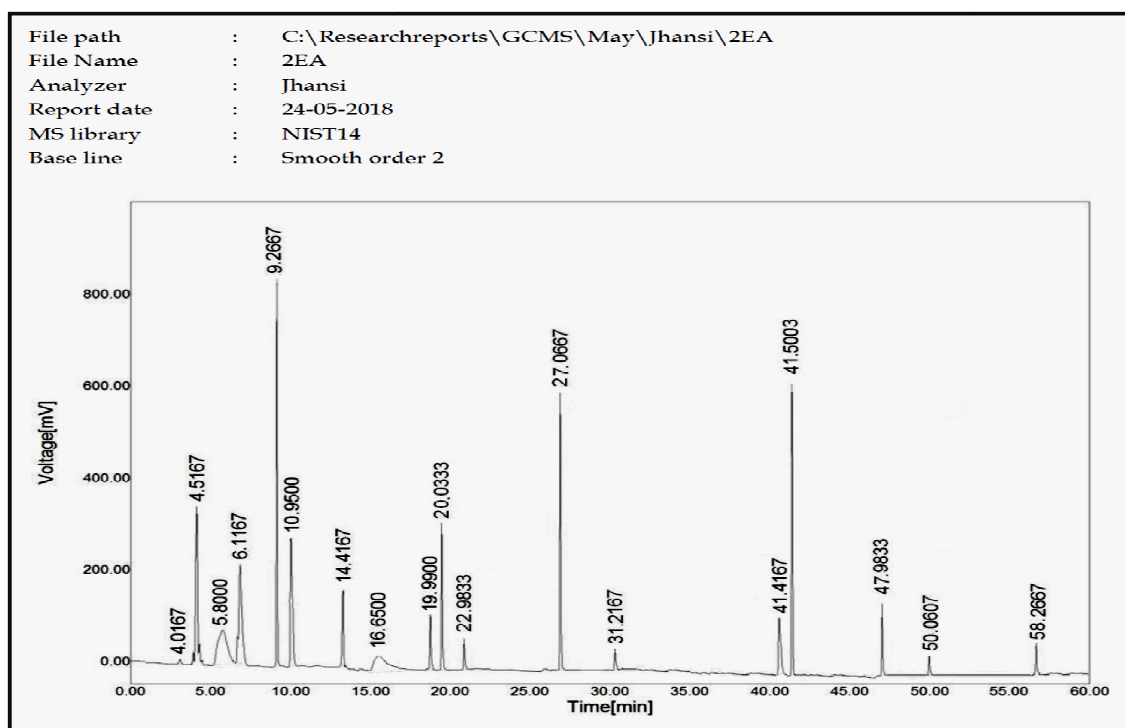
Sl. no	R.T (min)	Name of the compound	Molecular formula	Molecular Mass (gm/mol)	Peak area %	Biological activity
1	1.15	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl-, (2R-cis)-	C <sub>15</sub> H <sub>26</sub> O	222.372	6.9167	Antimicrobial [31]
2	2.41	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	C <sub>12</sub> H <sub>20</sub> O	180.291	8.15	-
3	2.3	m-Toluylaldehyde	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45	12.6667	Anticancer and antidote [26]
4	1.21	Methyl (2E) - 3-phenyl - 2-propeonate	C <sub>10</sub> H <sub>9</sub> DO <sub>2</sub>	162.188	15.4833	Anticancer, antitumour and Cytochrome-P450-2E1-Inhibitor [26]
5	4.44	1,2,3-Propanetriol, diacetate	C <sub>7</sub> H <sub>12</sub> O <sub>5</sub>	176.168	22.6667	Cellular narcotic and fragrance agent [32, 33]
6	17.11	5-Ethyl-2-methyl-2,3-dihydrofuran	C <sub>7</sub> H <sub>12</sub> O	112.172	29.8	Methyl guanidine inhibitor [26]
7	4.17	cis-11-Eicosenoic acid	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310.522	31.4833	Acidifier [26], Antimicrobial [34]
8	4.77	Ethyl $\alpha$ -D-glucopyranoside	C <sub>8</sub> H <sub>16</sub> O <sub>6</sub>	208.21	34.8833	Hepatic and skin moisturizing effect [35]; Anticancer and alcohol dehydrogenase inhibitor [26]
9	4.1	6-Isopropyl-3-methyl-1-cyclohex-2-enone (piperitone)	C <sub>10</sub> H <sub>16</sub> O	152.237	35.3137	Antibacterial [36]
10	6.45	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (Nerolidol)	C <sub>15</sub> H <sub>26</sub> O	222.372	38.75	Antimicrobial, antioxidant, anti-nociceptive, anti-inflammatory and anti-cancer [37]
11	6.53	Erucic acid	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338.576	40.4167	Antibacterial [38]
12	2.48	(9Z,12Z)-Octadeca-9,12-dienoyl chloride (Linoleoyl chloride)	C <sub>18</sub> H <sub>31</sub> OCl	298.895	43.15	Antimicrobial [26]
13	12.32	(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (farnesol)	C <sub>15</sub> H <sub>26</sub> O	222.372	43.4833	Antifungal [39]; Anticancer and antitumour [26]
14	4.47	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292.463	55.9667	Anticancer, Antimicrobial, Antioxidant and Hypercholesterolemic [40,41]
15	3.7500	Unidentified compound 1	-	158.74	6.43	-
16	10.5667	Unidentified compound 2	-	134.18	12.87	-
17	18.4167	Unidentified compound 3	-	276.38	4.47	-
18	25.6533	Unidentified compound 4	-	209.11	2.32	-

**Table 4. Cytotoxic properties of ethyl acetate extract of *A. odorata* on MCF -7 and HeLa cell lines**

Cell line	Concentration (µg/mL)	Absorbance at 570nm			Average	Average-Blank	% Viability	IC <sub>50</sub> (µg/mL)
MCF-7	100	0.792	0.794	0.796	0.794	0.787	38.241	41.094
	75	0.889	0.891	0.893	0.891	0.884	42.954	
	50	0.993	0.995	0.997	0.995	0.988	48.007	
	25	1.105	1.107	1.109	1.107	1.1	53.45	
	10	1.161	1.163	1.165	1.163	1.156	56.171	
	5	1.185	1.187	1.188	1.186	1.179	57.288	
	Untreated	2.065	2.066	2.065	2.065	2.058	100	
	Blank	0.007	0.008	0.007	0.007	0		
HeLa	100	0.803	0.805	0.807	0.805	0.8	41.928	59.061
	75	0.891	0.893	0.895	0.893	0.888	46.54	
	50	0.975	0.977	0.978	0.976	0.971	50.891	
	25	1.08	1.082	1.084	1.082	1.077	56.446	
	10	1.162	1.164	1.165	1.163	1.158	60.691	
	5	1.196	1.197	1.199	1.197	1.192	62.473	
	Untreated	1.913	1.914	1.913	1.913	1.908	100	
	Blank	0.005	0.006	0.005	0.005	0		

In the present study growth inhibition of *HeLa* cell lines increase with a rise in concentration of *A. odorata* leaf extract. The viability percentage of *HeLa* cell lines of ethyl acetate and methanol leaf extracts at concentration 100 µg/mL reduced from 100% to 41.92% and 41.29% respectively.

The reported IC<sub>50</sub> (50% of growth inhibition) value for methanol extract was 52.167µg/mL and 59.061µg/ml in ethyl acetate extracts. Cytotoxic effect of ethyl acetate and methanol leaf extract on *MCF-7* and *HeLa* cell lines were shown in Figs. 5A and 5B; 6A and 6B.



**Fig. 1. GC-MS chromatogram of ethyl acetate leaf extract of *A. odorata***

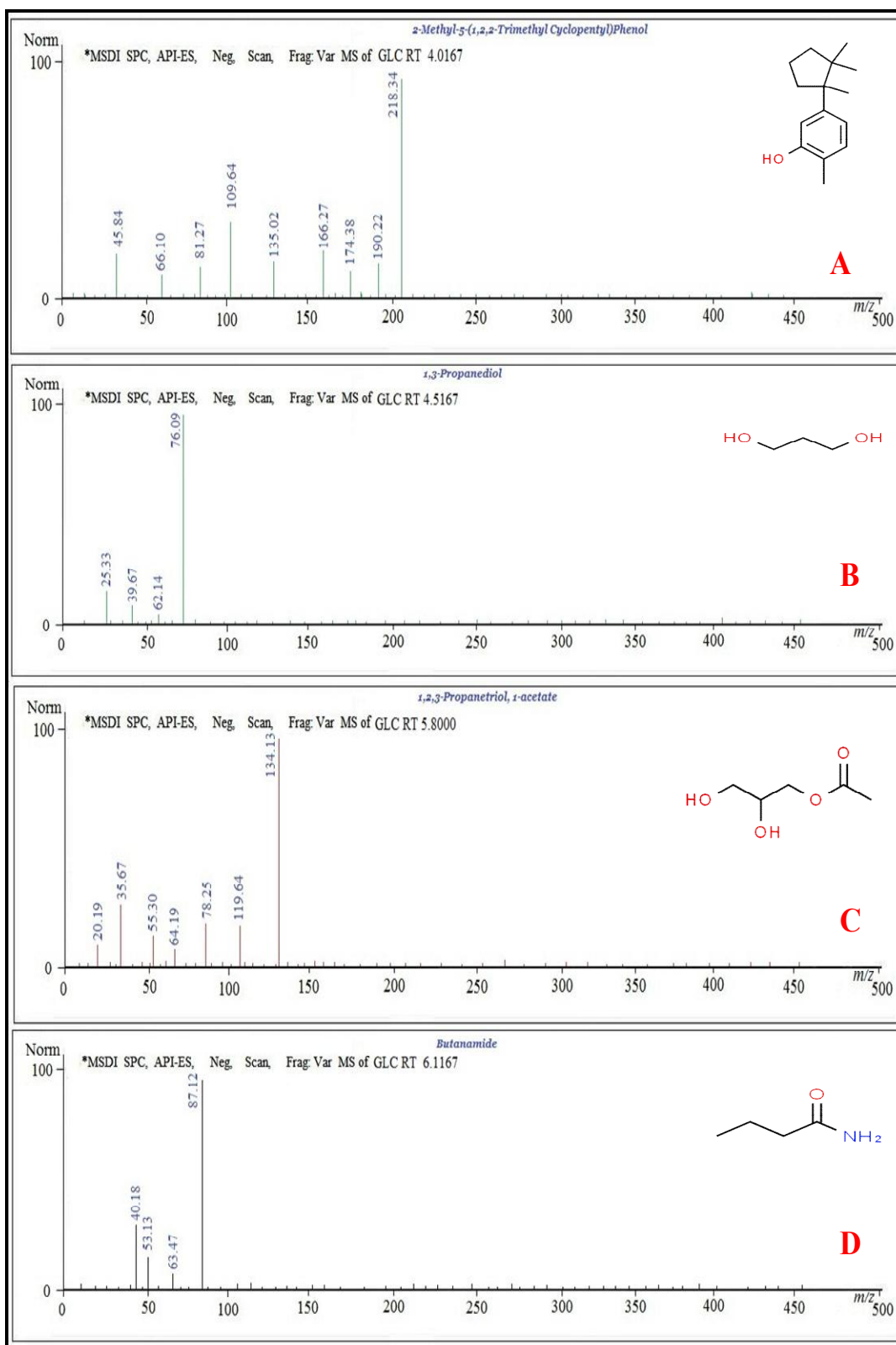


Fig. 2(A-D). Phytochemicals identified in ethyl acetate leaf extract of *A. odorata*

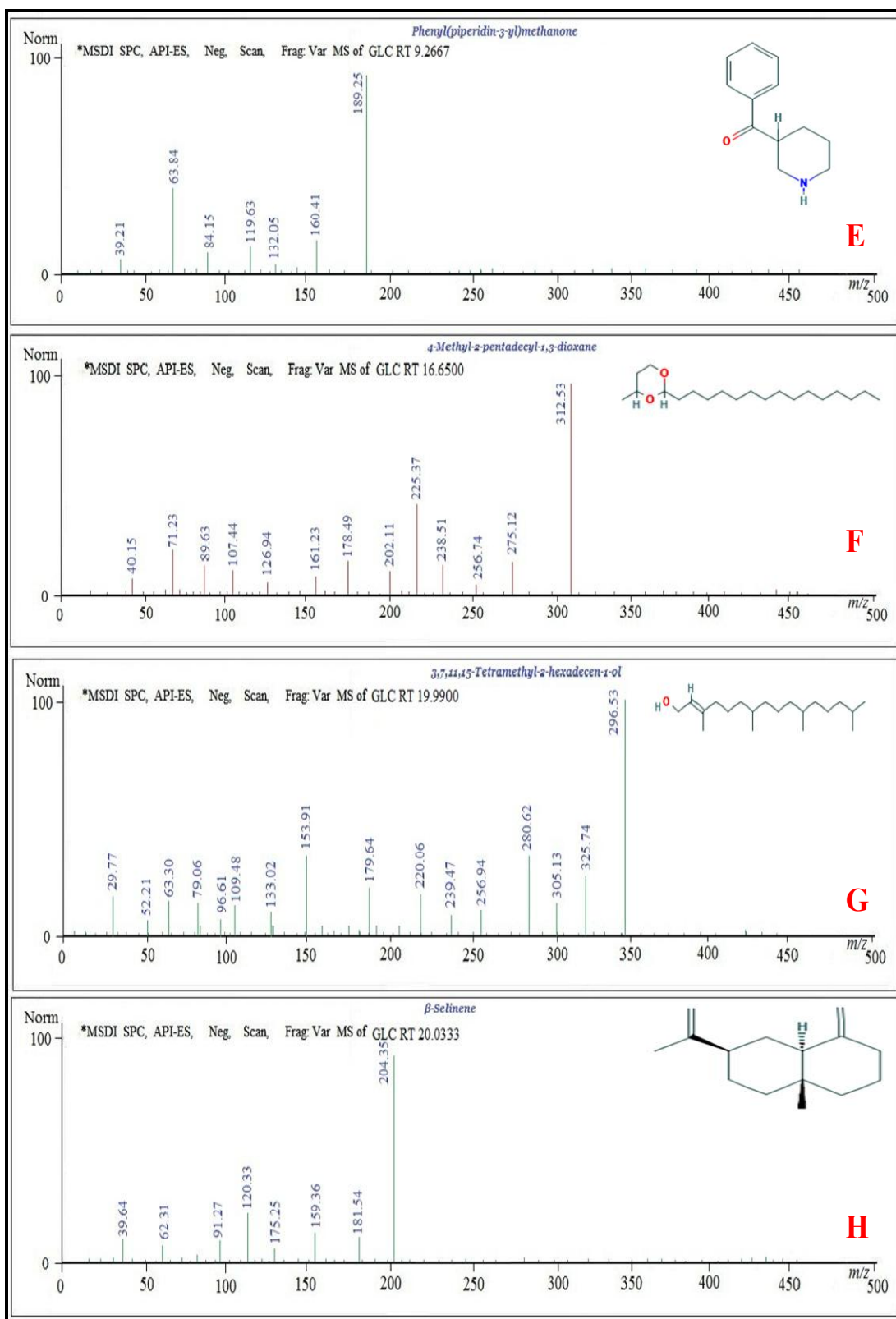


Fig. 2(E-H). Phytochemicals identified in ethyl acetate leaf extract of *A. odorata*



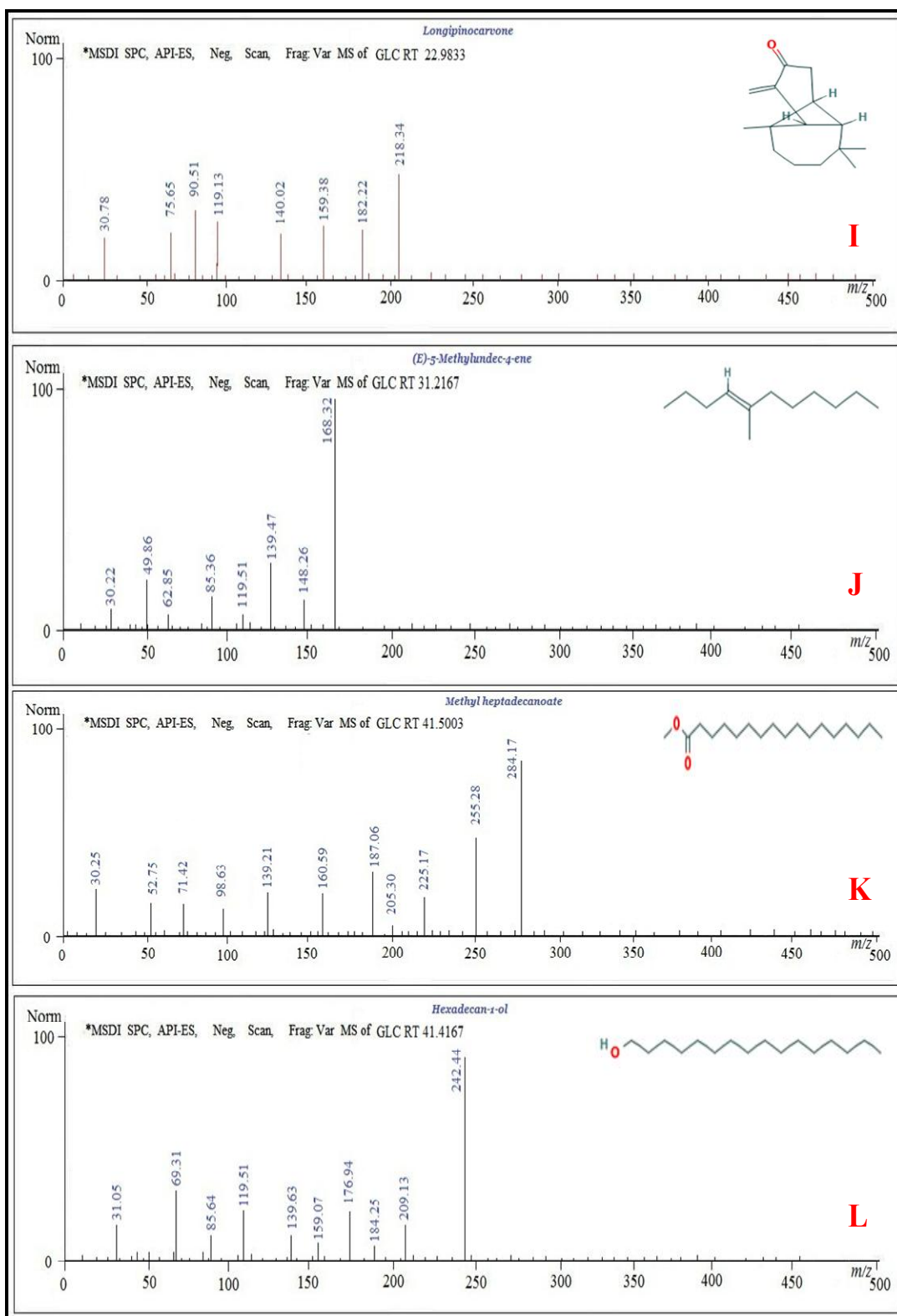


Fig. 2(I-L). Phytocompounds identified in ethyl acetate leaf extract of *A. odorata*

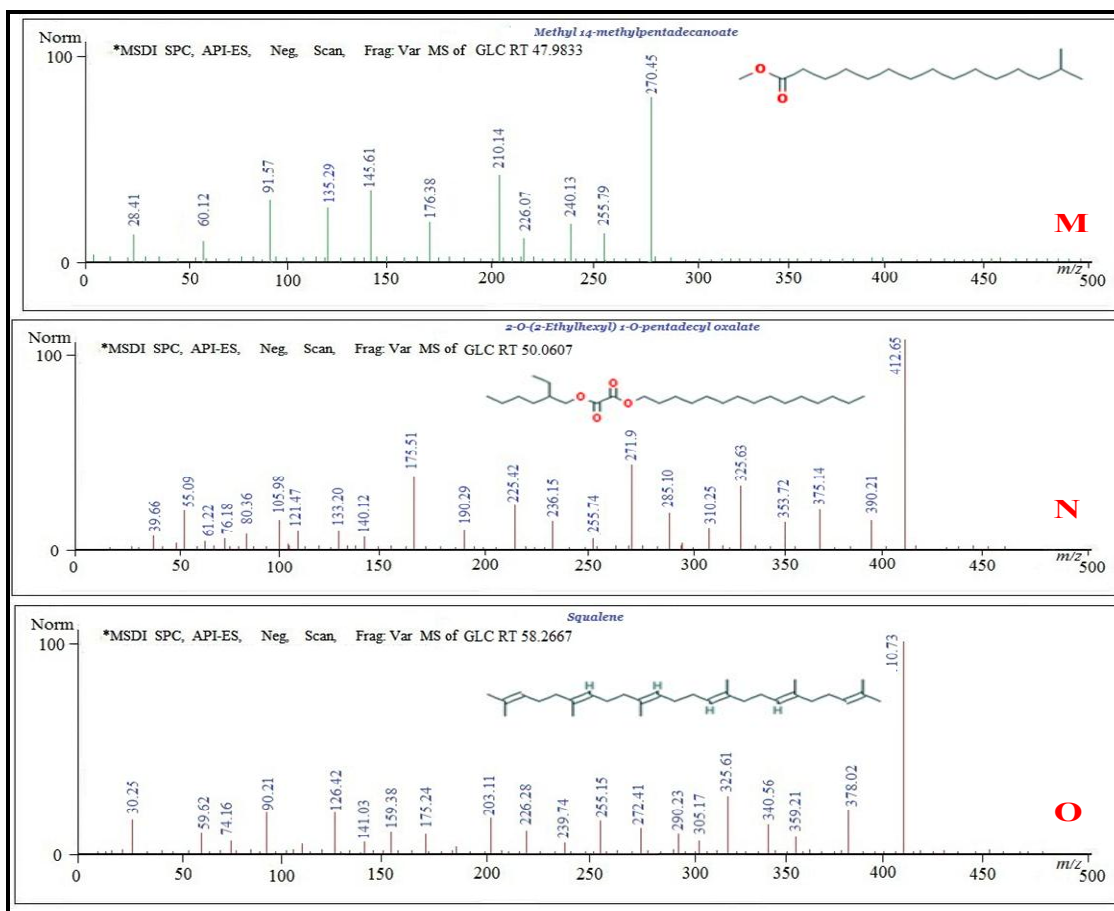


Fig. 2(M-O). Phytochemicals identified in ethyl acetate leaf extract of *A. odorata*

Table 5. Cytotoxic properties of methanolic leaf extract of *A. odorata* on MCF -7and HeLa cell lines

Cell line	Concentration (µg/mL)	Absorbance at 570 nm	Average	Average-Blank	% Viability	IC <sub>50</sub> (µg /mL)	
MCF-7	100	0.814	0.816	0.818	0.816	0.809	26.211
	75	0.871	0.873	0.875	0.873	0.866	
	50	0.922	0.924	0.925	0.923	0.916	
	25	0.995	0.997	0.998	0.996	0.989	
	10	1.068	1.07	1.072	1.07	1.063	
	5	1.176	1.178	1.179	1.177	1.17	
	Untreated	2.065	2.066	2.065	2.065	2.058	
	Blank	0.007	0.008	0.007	0.007	0	
HeLa	100	0.791	0.793	0.795	0.793	0.788	52.167
	75	0.85	0.852	0.854	0.852	0.847	
	50	0.963	0.965	0.967	0.965	0.96	
	25	1.036	1.038	1.039	1.037	1.032	
	10	1.105	1.107	1.109	1.107	1.102	
	5	1.181	1.183	1.185	1.183	1.178	
	Untreated	1.913	1.914	1.913	1.913	1.908	
	Blank	0.005	0.006	0.005	0.005	0	

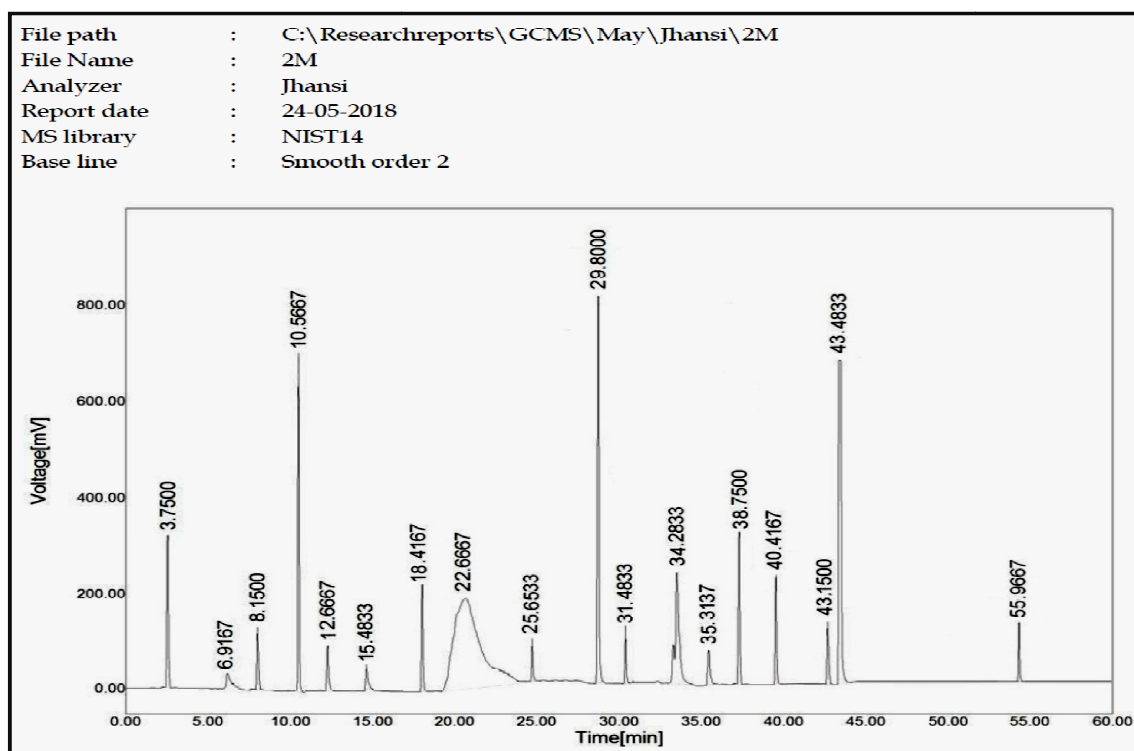


Fig. 3. GC-MS chromatogram of methanol leaf extract of *A. odorata*

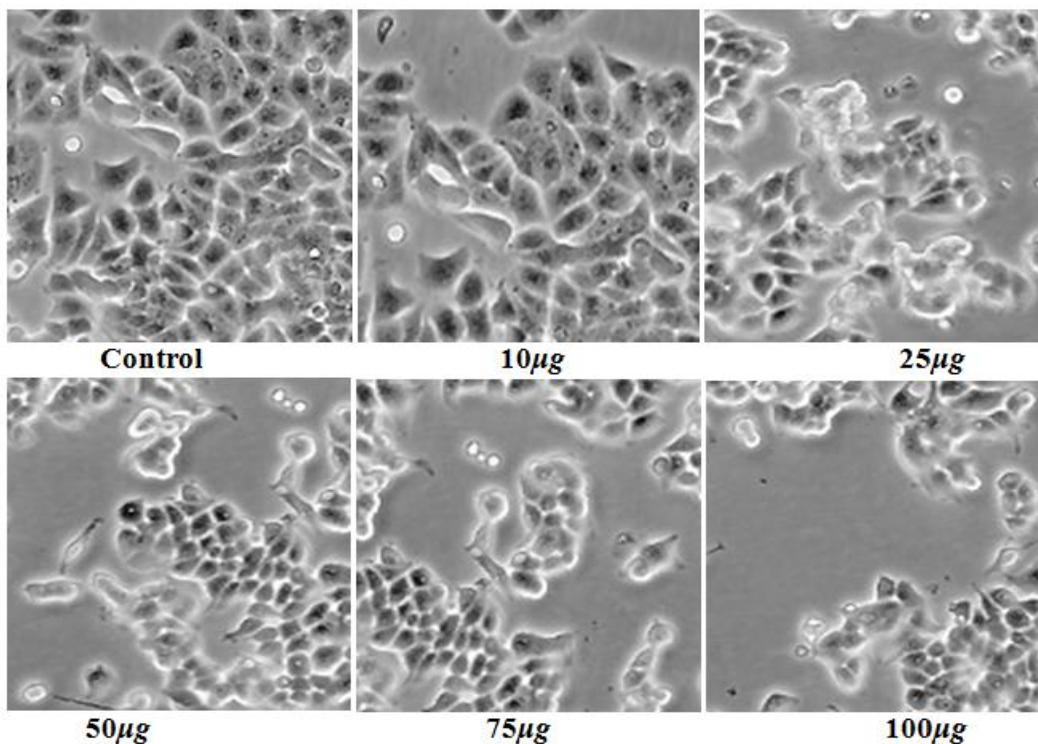


Plate 1. Cytotoxic Properties of ethyl acetate extract on *HeLa* Cell Line

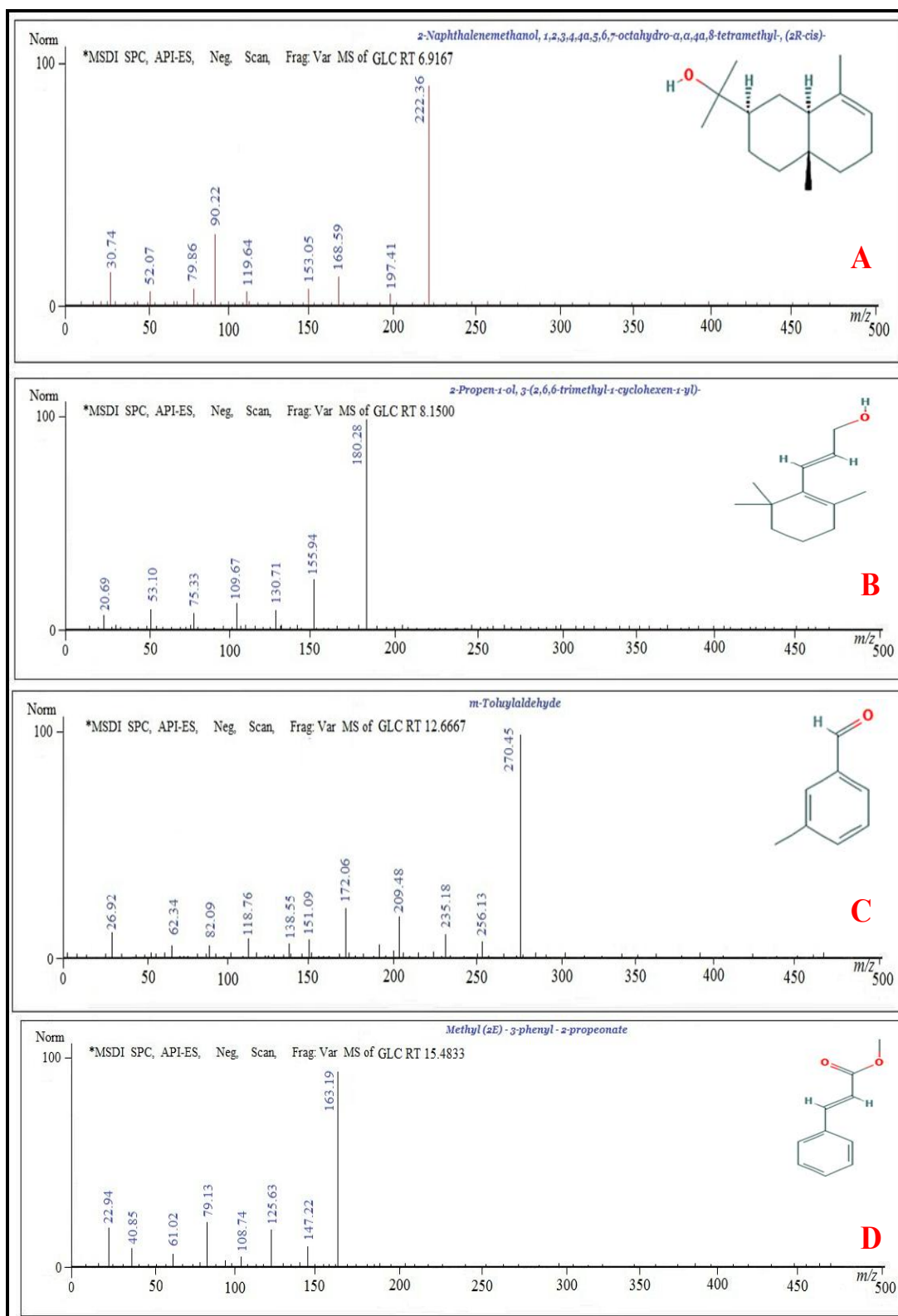


Fig. 4(A-D). Phytocompounds identified in Methanol leaf extract of *A. odorata*

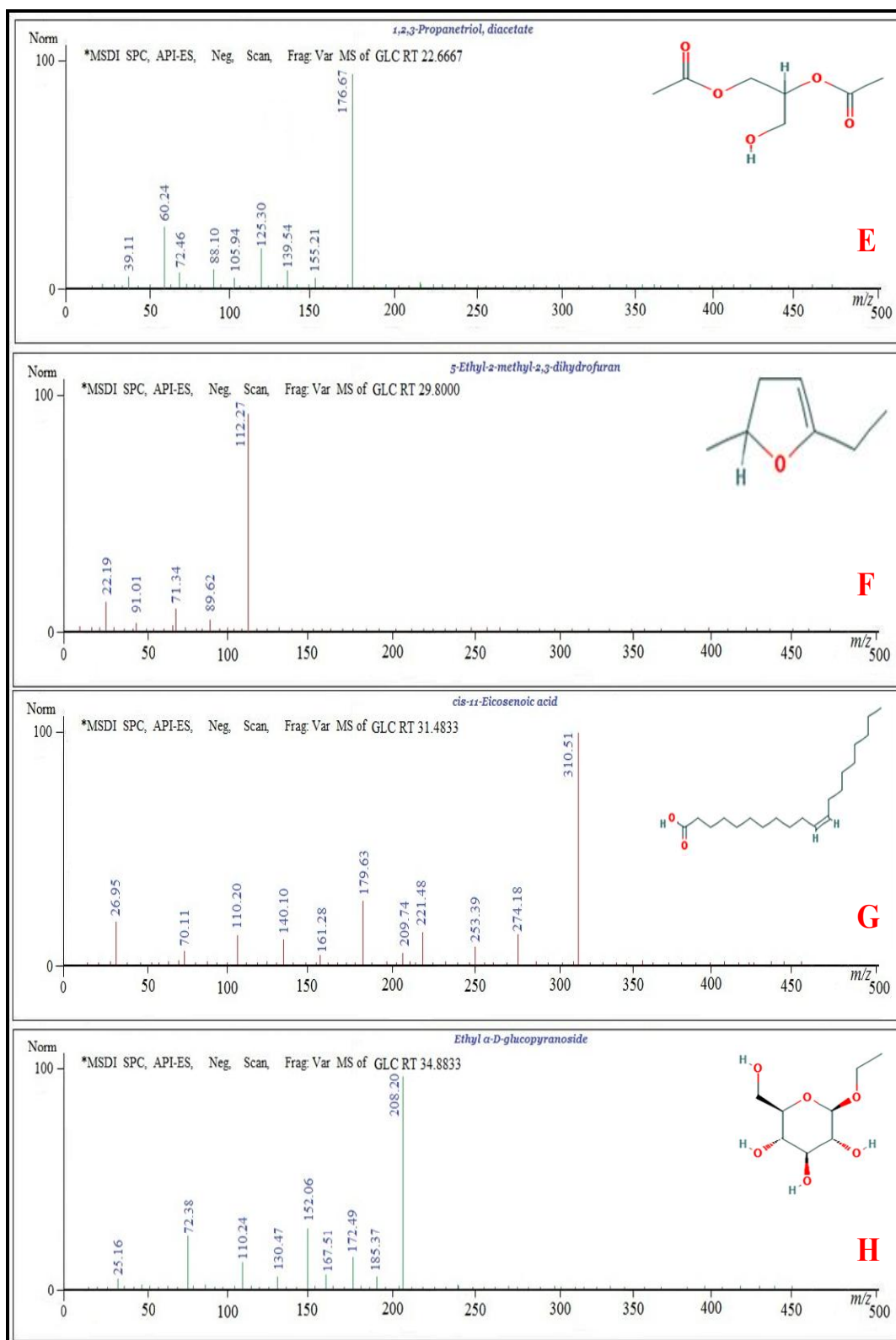


Fig. 4(E-H). Phytochemicals identified in Methanol leaf extract of *A. odorata*

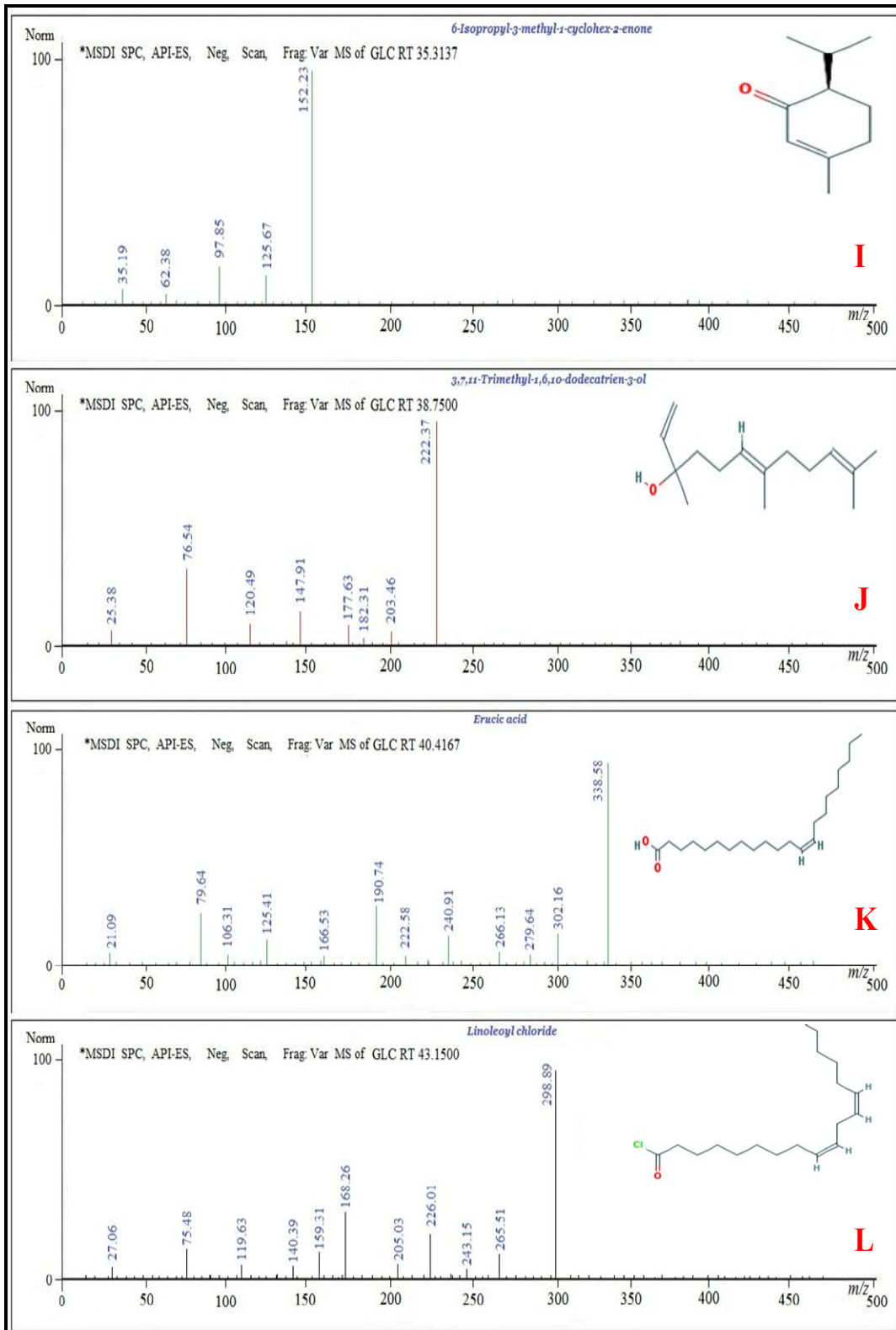


Fig. 4(I-L). Phytocompounds identified in Methanol leaf extract of *A. odorata*

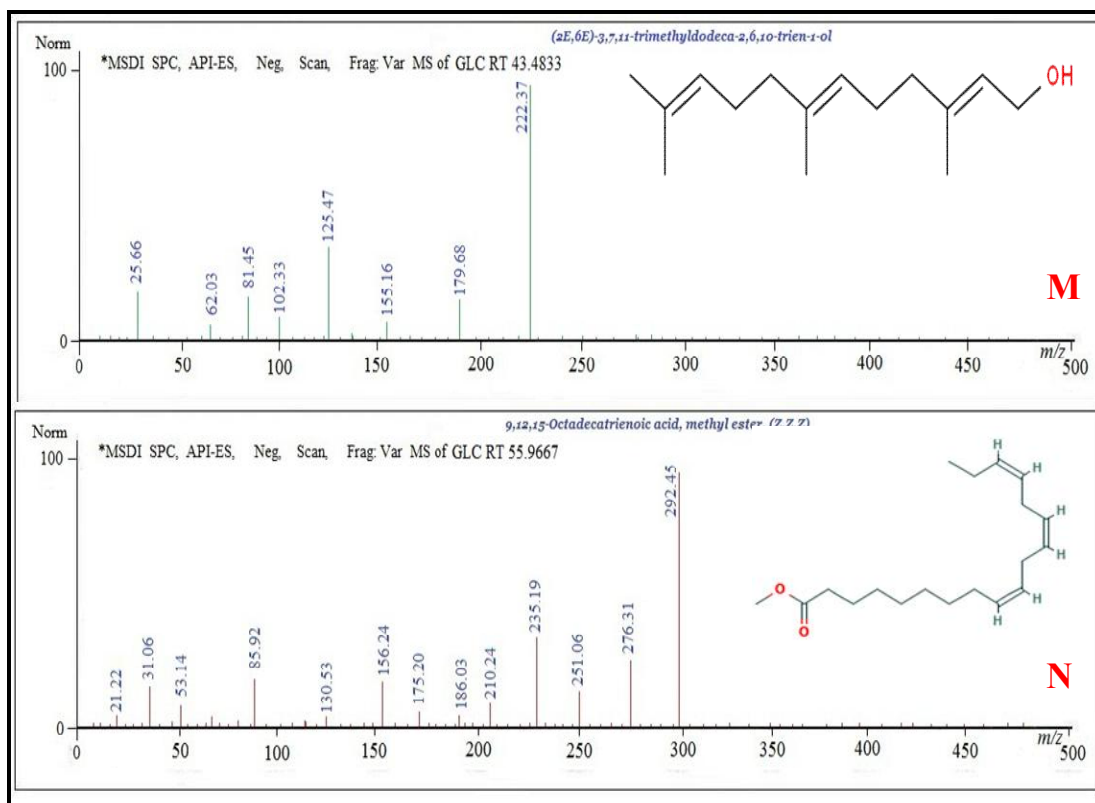


Fig. 4(M-N). Phytochemicals identified in Methanol leaf extract of *A. odorata*

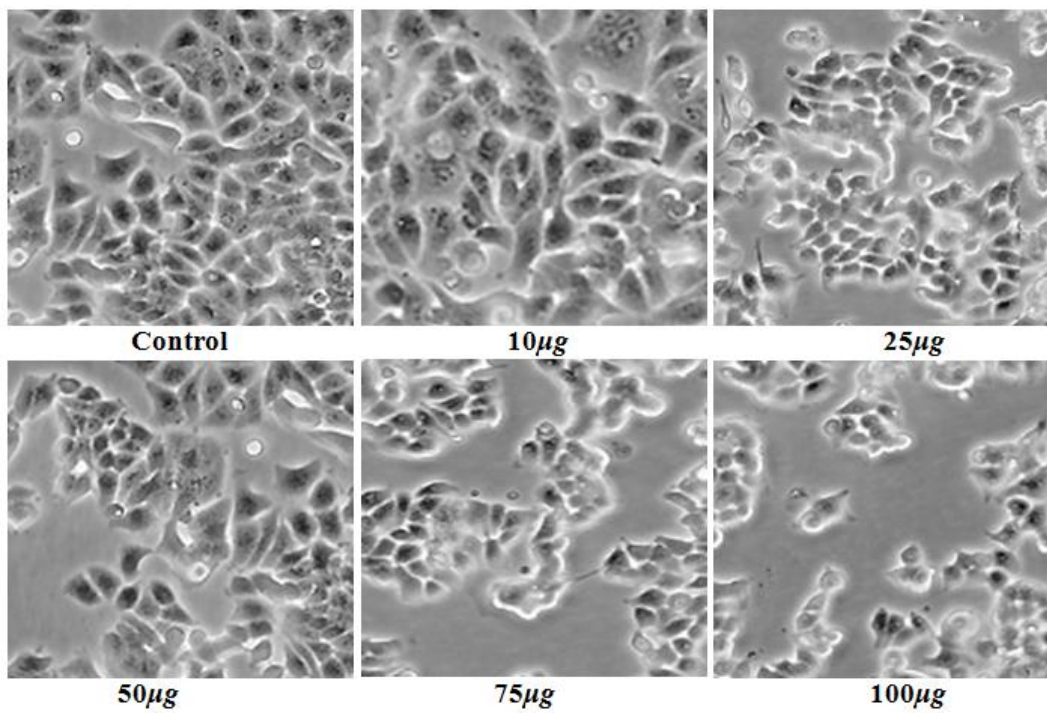
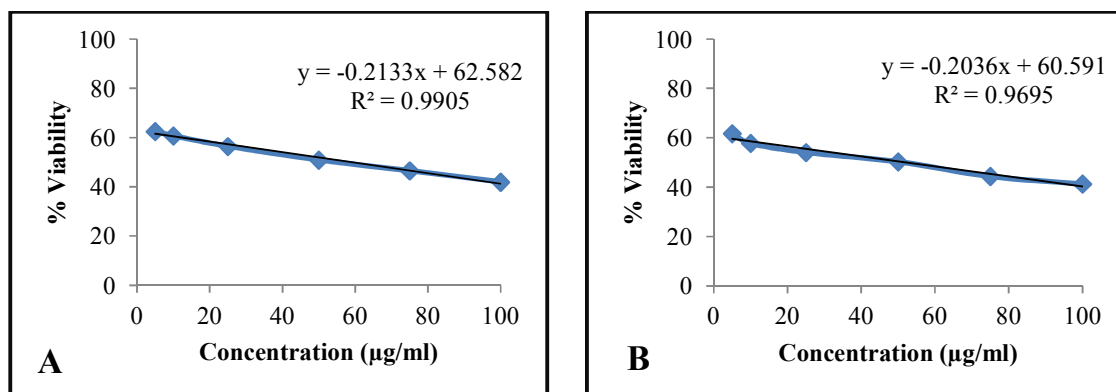
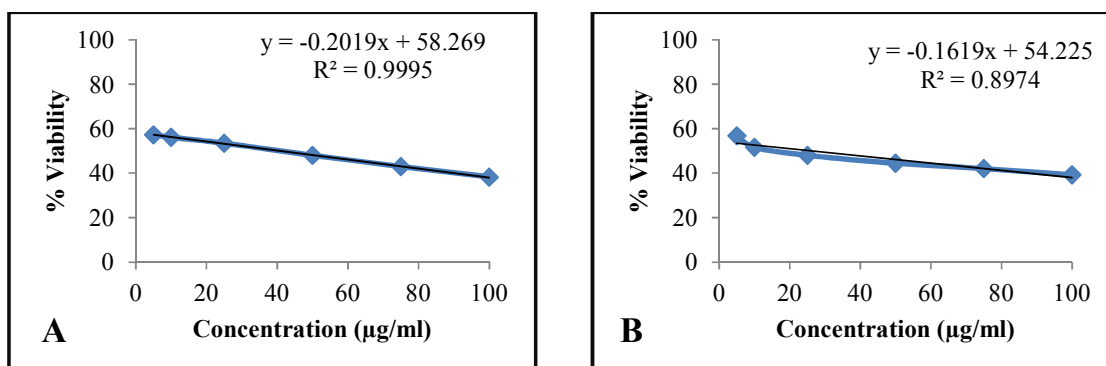


Plate 2. Cytotoxic Properties of Methanol extract on *HeLa* Cell Line



**Fig. 5. A) Cytotoxic effect of ethyl acetate extract on *HeLa* Cell Line B) Cytotoxic effect of Methanol extract on *HeLa* Cell Line**



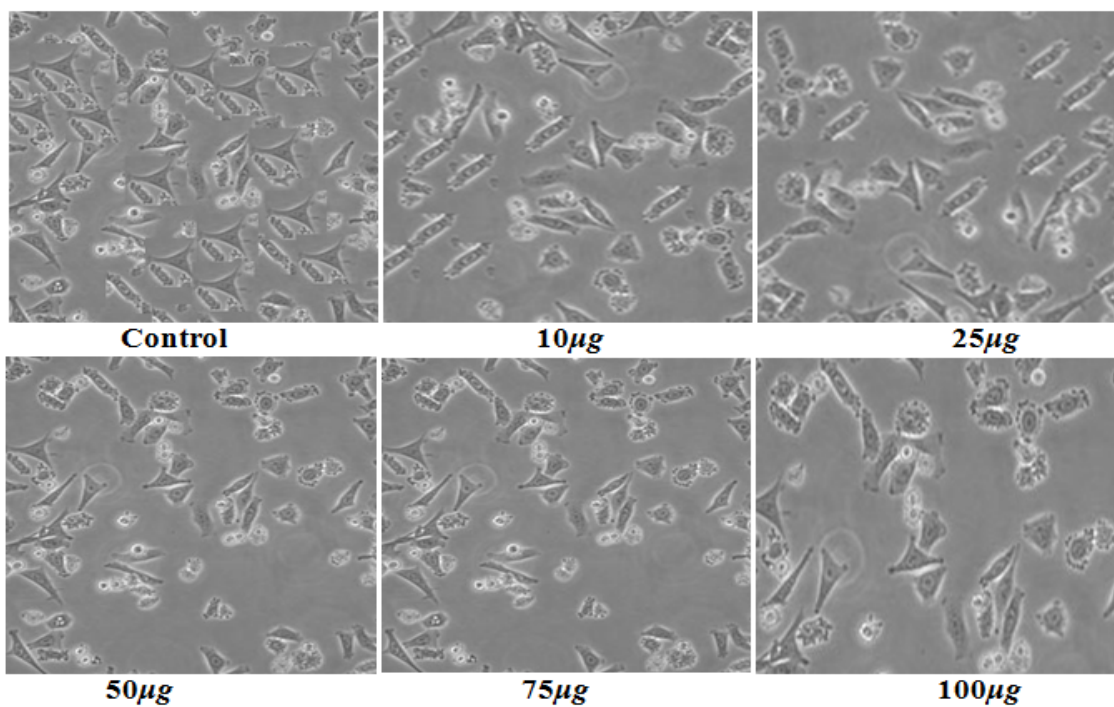
**Fig. 6. A) Cytotoxic effect of ethyl acetate extract on *MCF-7* Cell Line B) Cytotoxic effect of Methanol extract on *MCF-7* Cell Line**

#### 4. DISCUSSION

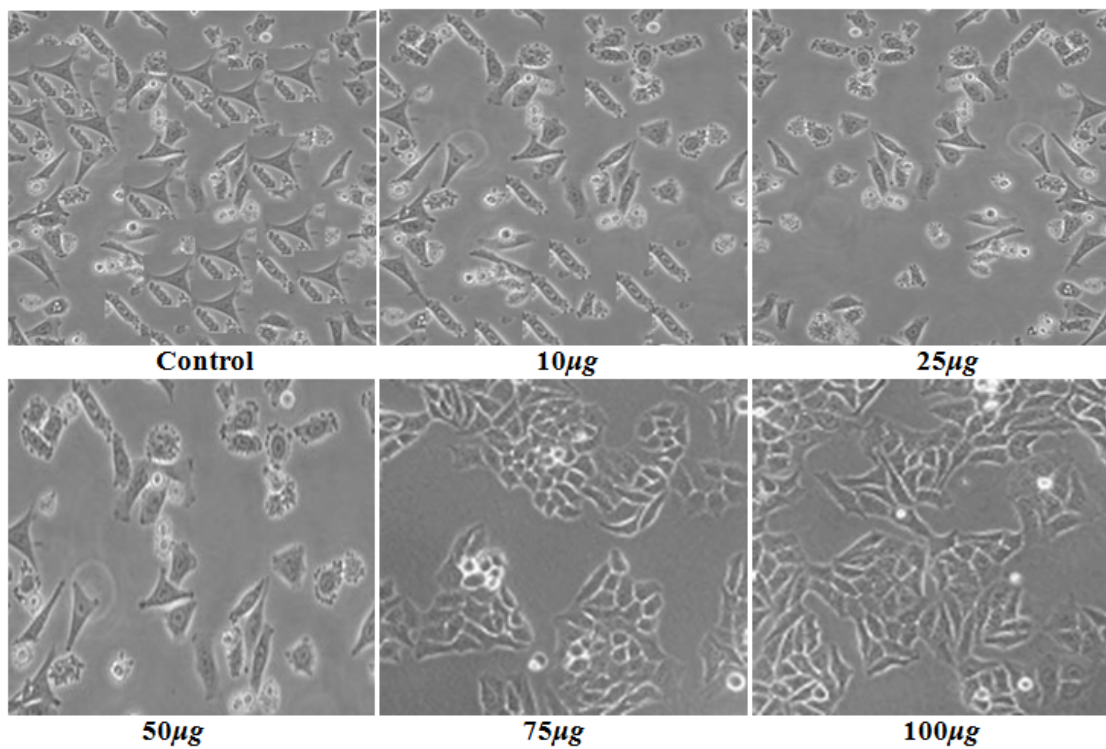
The documentary evidences on orchid metabolites and extracts proved their efficiency over number of human ailments [42,43,44,45,46, 47,48,49]. They also have a significant role in prevention of cancer and its treatment [50,51, 52]. Phytochemical analysis of different organic extracts of *A. odorata* contains fatty acids, secondary alcohols, diketones, esters and phenols. These secondary metabolites may be for various biological activities of medicinal plants [53,54]. Most of the compounds identified in ethyl acetate and methanol extracts of the plant are biologically active (Tables 2 and 3). In the present study a total of seven phytocompounds in ethyl acetate and six compounds in methanol extracts have anticancer activity. 2-Methyl-5-(1,2,2-Trimethyl cyclopentyl) phenol is also known as Xanthorrhizol. It has biological activities such as anticancer, antimicrobial, anti-inflammatory, antioxidant and

antihypertensive [21]. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol) is an unsaturated acyclic diterpenoid alkene alcohol and act as precursor of vitamin E. This compound has acute oral cytotoxicity LD50 in rats > 5 g/kg [55]. 9,12,15-octadecatrienoic acid methyl ester is an unsaturated fatty acid ester which has been shown to possess anticancer, hypocholesterolemic, antimicrobial and antioxidant activities [40,41]. Apart from this other compounds reported in the present study such as Phenyl(piperidin-3-yl) methanone,  $\beta$ -Selinene, (E)-5-Methylundec-4-ene, 2-O-(2-Ethylhexyl) 1-O-pentadecyl oxalate, Squalene, m-Toluyaldehyde, Methyl (2E) - 3-phenyl - 2-propeonate, Ethyl  $\alpha$ -D-glucopyranoside, 3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol, (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol also posses anticancer properties. Squalene acts as a defence agent against certain pathogens causing human and animal diseases along with its anticancer activity [56].





**Plate 3. Cytotoxic Properties of ethyl acetate extract on *MCF-7* Cell Line**



**Plate 4. Cytotoxic Properties of Methanol extract on *MCF-7* Cell Line**

Some compounds like 1,3 propanediol has a wide range of applications. It is used as adhesive, lubricant, antifreeze and medicine [57, 58,59,60]. Hexadecan-1-ol is a fatty alcohol more commonly used as a emulsifier agent in skin creams and lotions [28]. Longipinocarvone is sesquiterpenes compound, and also reported in essential oil of *Boswellia dalzielii* leaves [61]. The results of anticancer study reveal a death rate of *MCF-7* and *HeLa* cell lines increase with a rise in concentration of *A. odorata* leaf extract. IC<sub>50</sub> value is greater than 1000µg/mL in crude plant extract is non toxic, while toxic if it is less than 1000 µg/mL [62]. The lowest IC<sub>50</sub> value 26.211µg/mL observed for methanolic leaf extract on *MCF-7* cell lines. It indicates that the methanol extract shows significant inhibitory effect. The present results in agreement with previous reports of anticancer studies on orchids [63,64]. Hence, the findings of this study proved that leaf extract of *A. odorata* have anticancer effect and this species could be acts good source to develop anticancer drugs.

## 5. CONCLUSION

Phytochemical analysis of epiphytic orchid *A. odorata* confirmed the presence of bioactive compounds. The ethyl acetate and methanol solvent extracts has proved in vitro anticancer activity on *MCF-7* and *HeLa* cell lines. Many of the compounds reported have anticancer properties. Hence, solvent extracts of this plant act as good source of anticancer drugs.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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