Original Research Article

A STUDY ON PHYTOCHEMICAL AND ANTICANCER ACTIVITIES OF EPIPHYTIC ORCHID *AERIDES ODORATA* Lindl.

ABSTRACT

Aim: The present study was carried out to evaluate the phytochemical composition and anticancer activities of leaf extract of *Aerides odorata*, 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 analyzed 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 significant cancer cell growth inhibition with IC_{50} value, ranging between 26.211 µg/ml to 59.061 µg/ml.

Conclusion: Methanol is the best solvent and its activity. Our result showed *Aerides 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]. *Aerides 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 lead 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* was 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 (250 g) was extracted with Soxhlet apparatus with n-hexane, ethyl acetate and methanol solvents for about 12-15hr at room temperature of 35-40^{0}C. Finally, crude extracts of different solvents were concentrated in a vacuum rotary evaporator (Buchi Labortech Ag, model l, 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 2µl 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^{ev}. 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 (6X10^5) into 96 well micro-titer 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 phytocompounds in plant species [19, 20]. GC-MS analysis carried out based on the results of preliminary phytochemical analysis. Methanol 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-Trimethy cyclopentyl)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), β-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-α,α,4a,8-tetramethyl - (Fig. 4A), (2R-cis)-, 2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl) (Fig. 4B), -, m-Toluylaldehyde(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 α-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

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 µg/ml on two different cell lines *MCF-7* and *HeLa* (Plate 1 and 2; Plate 3 and 4). The results of the cytotoxicity of *A. odorata* two solvent extracts on both the cell lines are shown in Table 4, 5. The data suggest that the methanol 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 µg/ml showed the highest growth inhibition 61.128% on *MCF-7* cell lines as compared to the methanol extract having 60.69%. The recorded IC_{50} (50% of growth inhibition) value for methanol extract was 26.211µg/ml and 41.094 µg/ml in ethyl acetate extracts. It indicates that the methanol extract exhibit significant cytotoxicity effect on *MCF-7* cell lines.

In the present study, the 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_{50} (50% of growth inhibition) value for methanol extract was 52.167µg/ml and 59.061µg/ml in ethyl acetate extract. 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.

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	H2SO4 test	-	+	+
7	Resins	Acetone H2O 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

S.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-Trimethy cyclopentyl)phenol	$C_{15}H_{22}O$	218.34	0.56	Anticancer [21]
2	4.5167	1,3-Propanediol	$C_3H_8O_2$	76.095	7	-
3	5.8	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.131	1.74	Antibacterial [22]
4	6.1167	Butanamide	C_4H_9NO	87.122	6.58	-
5	9.2667	Phenyl(piperidin-3-yl)methanone	$C_{12}H_{15}NO$	189.258	4.76	Anticancer [23]
6	16.65	4-Methyl-2-pentadecyl-1,3-dioxane	$C_{20}H_{40}O_2$	312.538	0.64	Antibacterial and Antifungal [24]
7	19.99	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	296.539	2.72	Anticancer [25], antihelmintic and anti-inflammatory [26]
8	20.0333	β-Selinene	$C_{15}H_{24}$	204.357	6.93	Antioxidant and anti-inflammatory [27]
9	22.9833	Longipinocarvone	$C_{15}H_{22}O$	218.34	2.03	-
10	31.2167	(E)-5-Methylundec-4-ene	$C_{12}H_{24}$	168.324	1.69	Anticancer and Antitumor [26]
11	41.4167	Methyl heptadecanoate	$C_{18}H_{36}O_2$	284.484	2.8	Catechol-O-Methyl-Transferase Inhibitor [26]
12	41.5003	Hexadecan-1-ol 1-	$C_{16}H_{34}O$	242.447	14.72	Skin diseases [28]
13	47.9833	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$	270.457	4.63	Methyl guanidine inhibitor [26]
14	50.0607	2-O-(2-Ethylhexyl) 1-O-pentadecyl oxalate	$C_{25}H_{48}O_4$	412.655	1.55	Anticancer, Antitumour and Inhibit production of tumour necrosis factor [26]
15	58.2667	Squalene	$C_{30}H_{50}$	410.73	2.15	Antibacterial, Antioxidant, pesticide, Antitumour, anti-cancer, preventive, Immunostimulent, Chemo preventive, Lipoxygenase-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. odarata* by using GC-MS analysis

S.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-α,α,4a,8-tetramethyl-, (2R-cis)-	$C_{15}H_{26}O$	222.372	6.9167	Antimicrobial [31]
2	2.41	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{12}H_{20}O$	180.291	8.15	-
3	2.3	m-Toluylaldehyde	$C_{17}H_{34}O_2$	270.45	12.6667	Anticancer and antidote [26]
4	1.21	Methyl (2E) - 3-phenyl - 2-propeonate	$C_{10}H_9DO_2$	162.188	15.4833	Anticancer, antitumour and Cytochrome-P450-2E1-Inhibitor [26]
5	4.44	1,2,3-Propanetriol, diacetate	$C_7H_{12}O_5$	176.168	22.6667	Cellular narcotic and fragrance agent [32, 33]
6	17.11	5-Ethyl-2-methyl-2,3-dihydrofuran	$C_7H_{12}O$	112.172	29.8	Methyl guanidine inhibitor[26]
7	4.17	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310.522	31.4833	Acidifier [26], Antimicrobial[34]
8	4.77	Ethyl α-D-glucopyranoside	$C_8H_{16}O_6$	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)	$C_{10}H_{16}O$	152.237	35.3137	Antibacterial [36]
10	6.45	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (Nerolidol)	$C_{15}H_{26}O$	222.372	38.75	Antimicrobial, antioxidant, anti-nociceptive, anti-inflammatory and anti-cancer [37]
11	6.53	Erucic acid	$C_{22}H_{42}O_2$	338.576	40.4167	Antibacterial [38]

12	2.48	(9Z,12Z)-Octadeca-9,12-dienoyl chloride (Linoleoyl chloride)	$C_{18}H_{31}OCl$	298.895	43.15	Antimicrobial [26]
13	12.32	(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (farnesol)	$C_{15}H_{26}O$	222.372	43.4833	Antifungal [39]; Anticancer and antitumour [26]
14	4.47	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	292.463	55.9667	Anticancer, Antimicrobial, Antioxidant and Hyperchloesteralemic [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	-

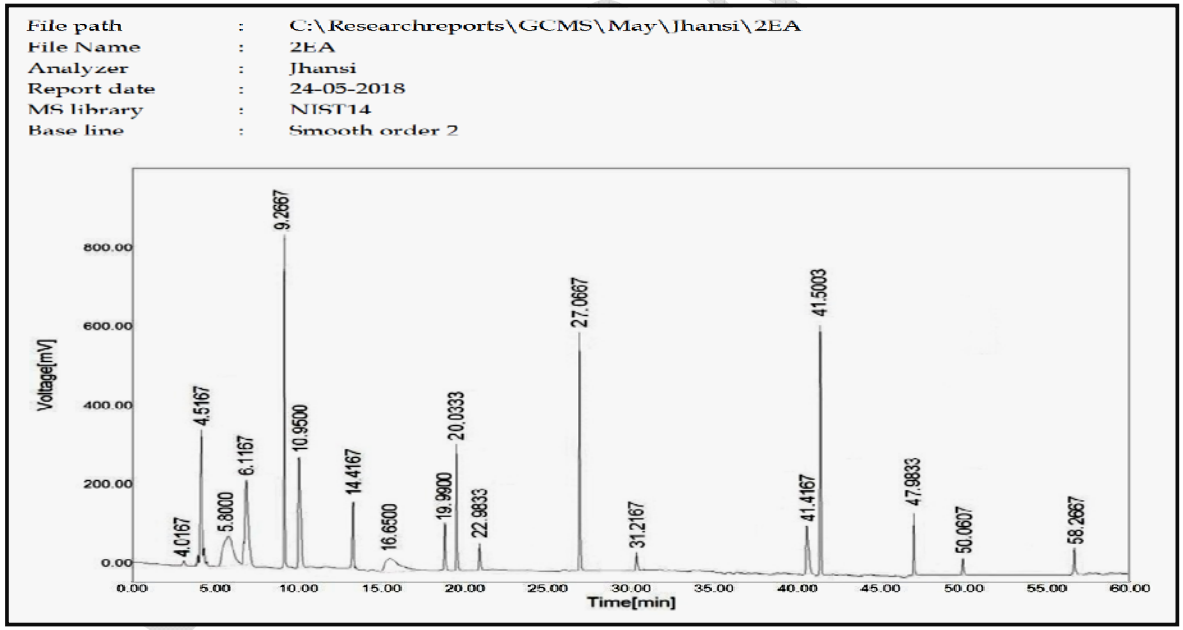

Fig. 1. GC-MS chromatogram of ethyl acetate leaf extract of *A. odorata*

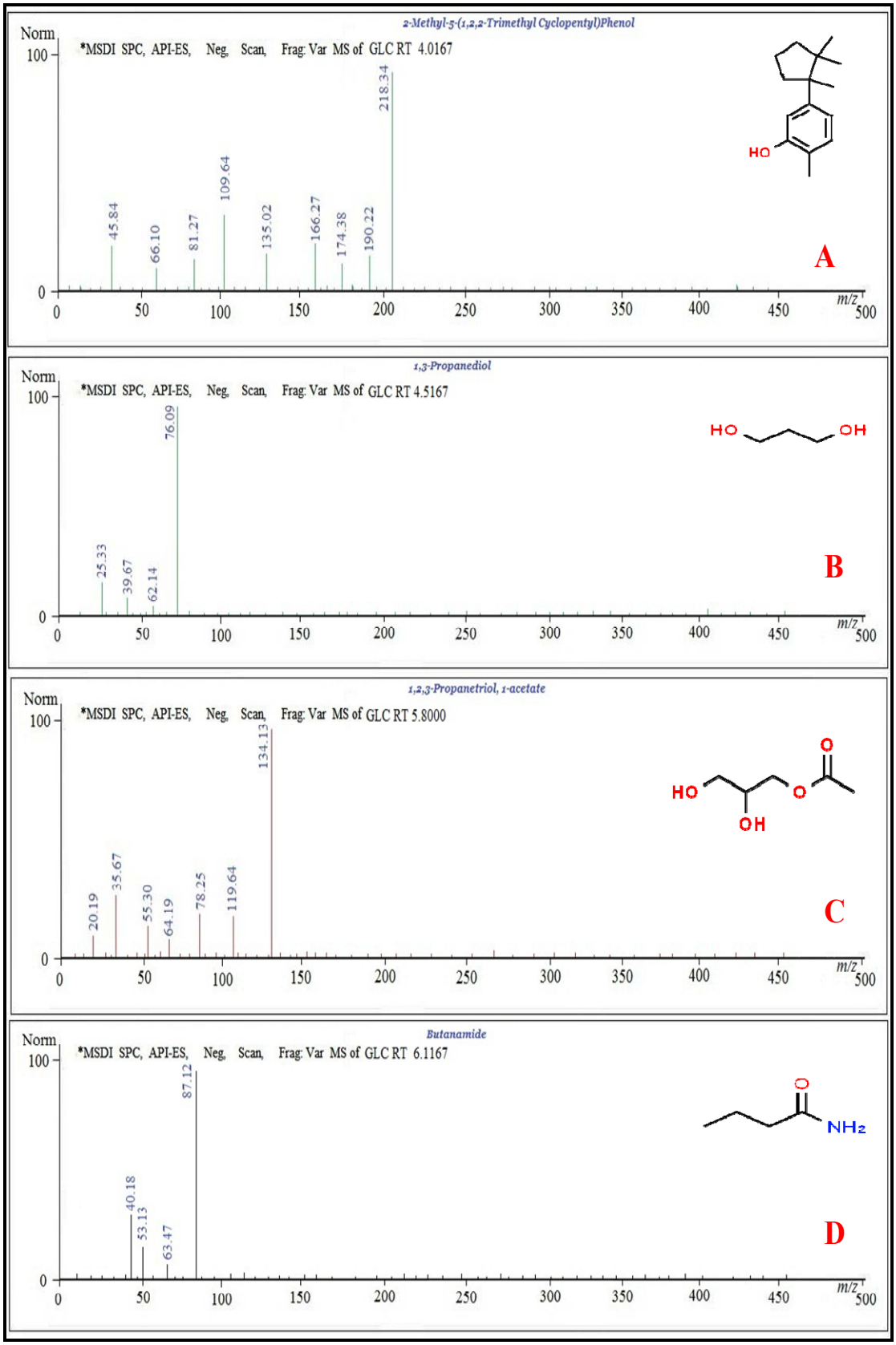

Fig. 2(A-D). Phytocompounds identified in ethyl acetate leaf extract of *A. odorata*

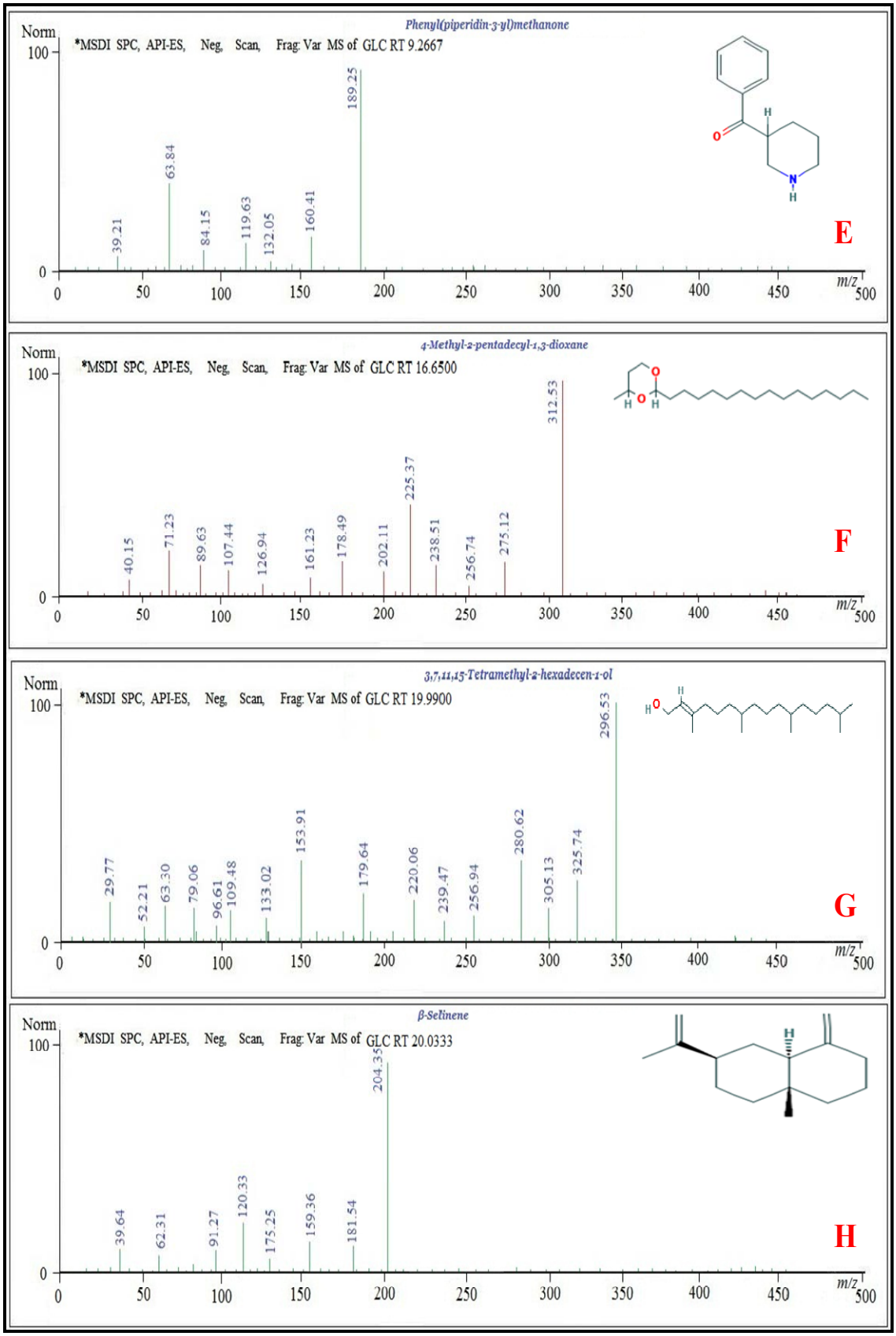

Fig. 2(E-H). Phytocompounds identified in ethyl acetate leaf extract of *A. odorata*

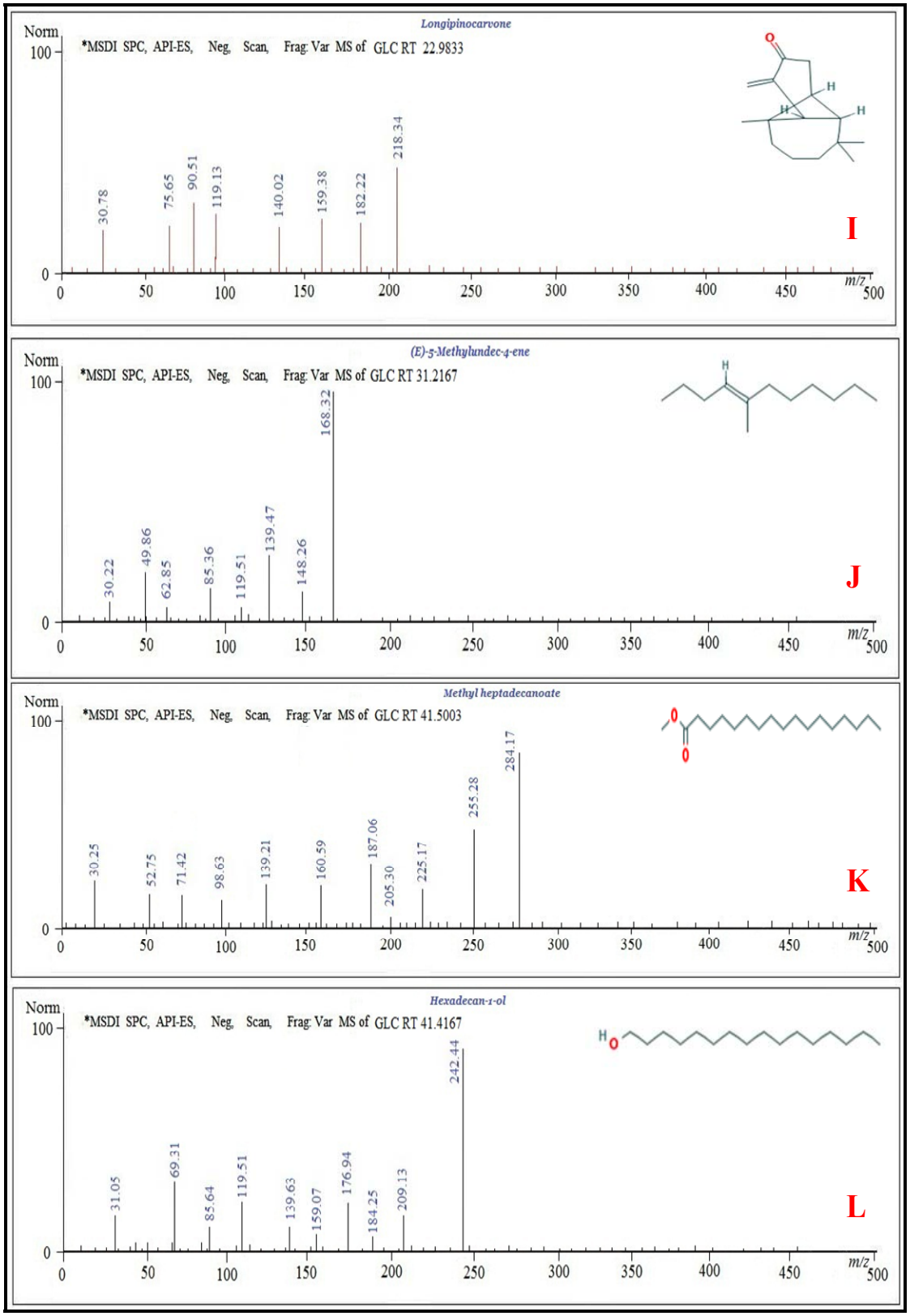

Fig. 2(I-L). Phytocompounds identified in ethyl acetate leaf extract of *A. odorata*

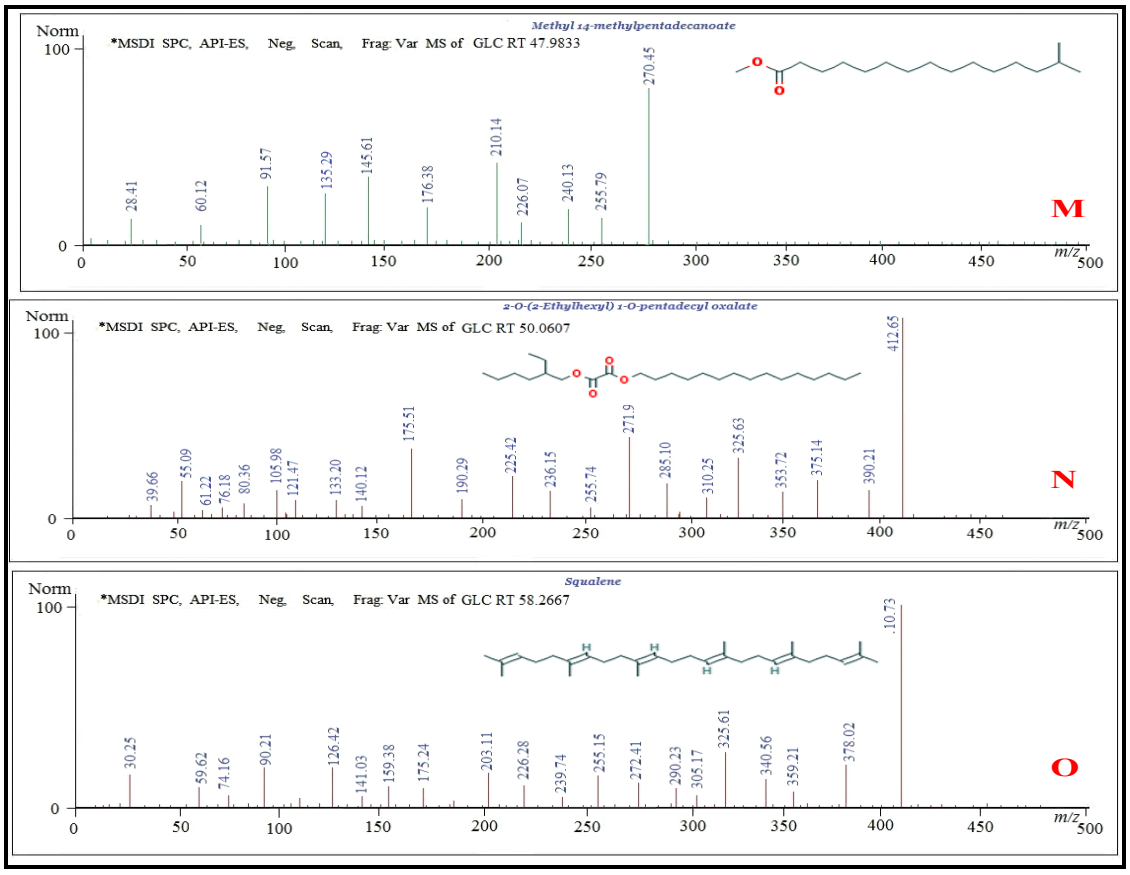

Fig. 2(M-O). Phytocompounds identified in ethyl acetate leaf extract of *A. odorata*

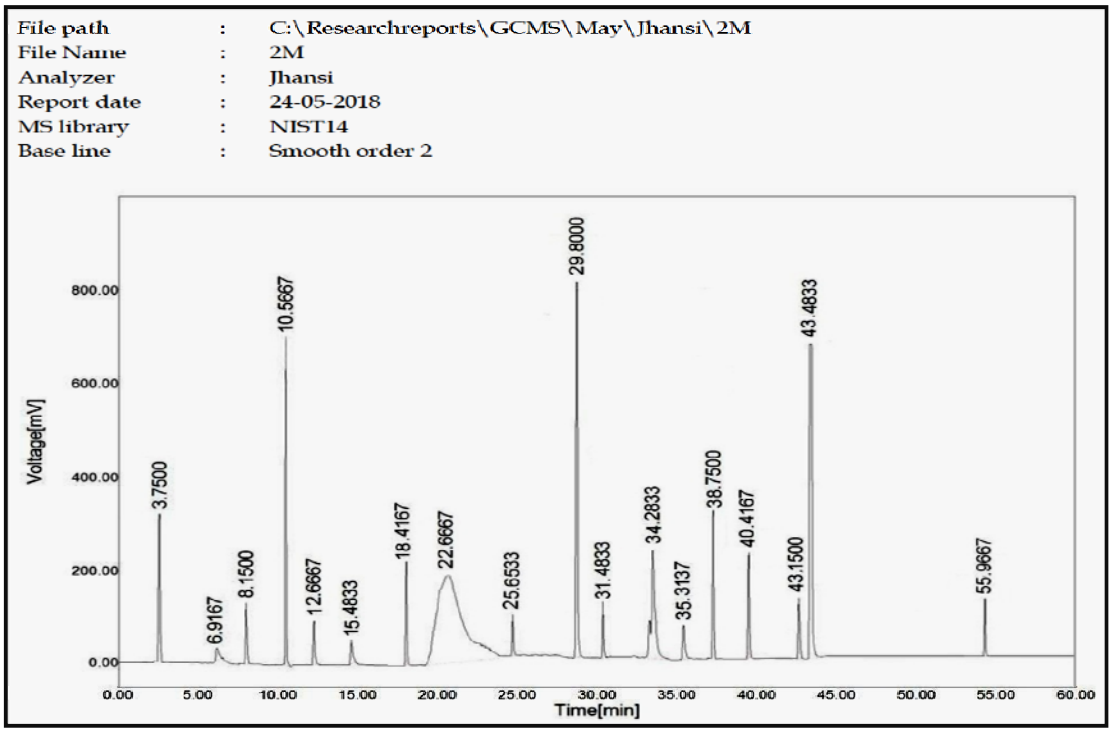

Fig. 3. GC-MS chromatogram of methanol leaf extract of *A. odorata*

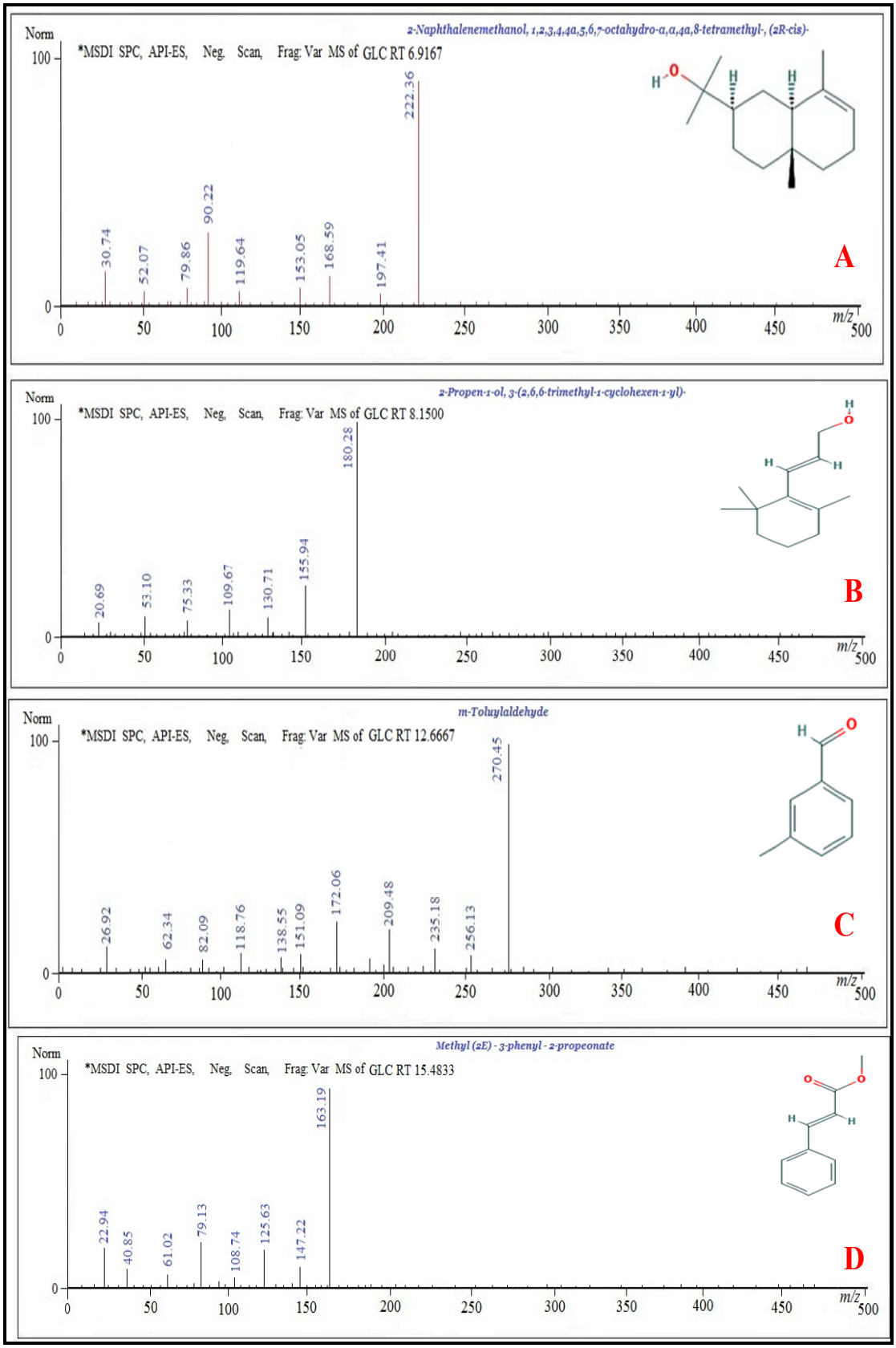

Fig. 4(A-D). Phytocompounds identified in Methanol leaf extract of *A. odorata*

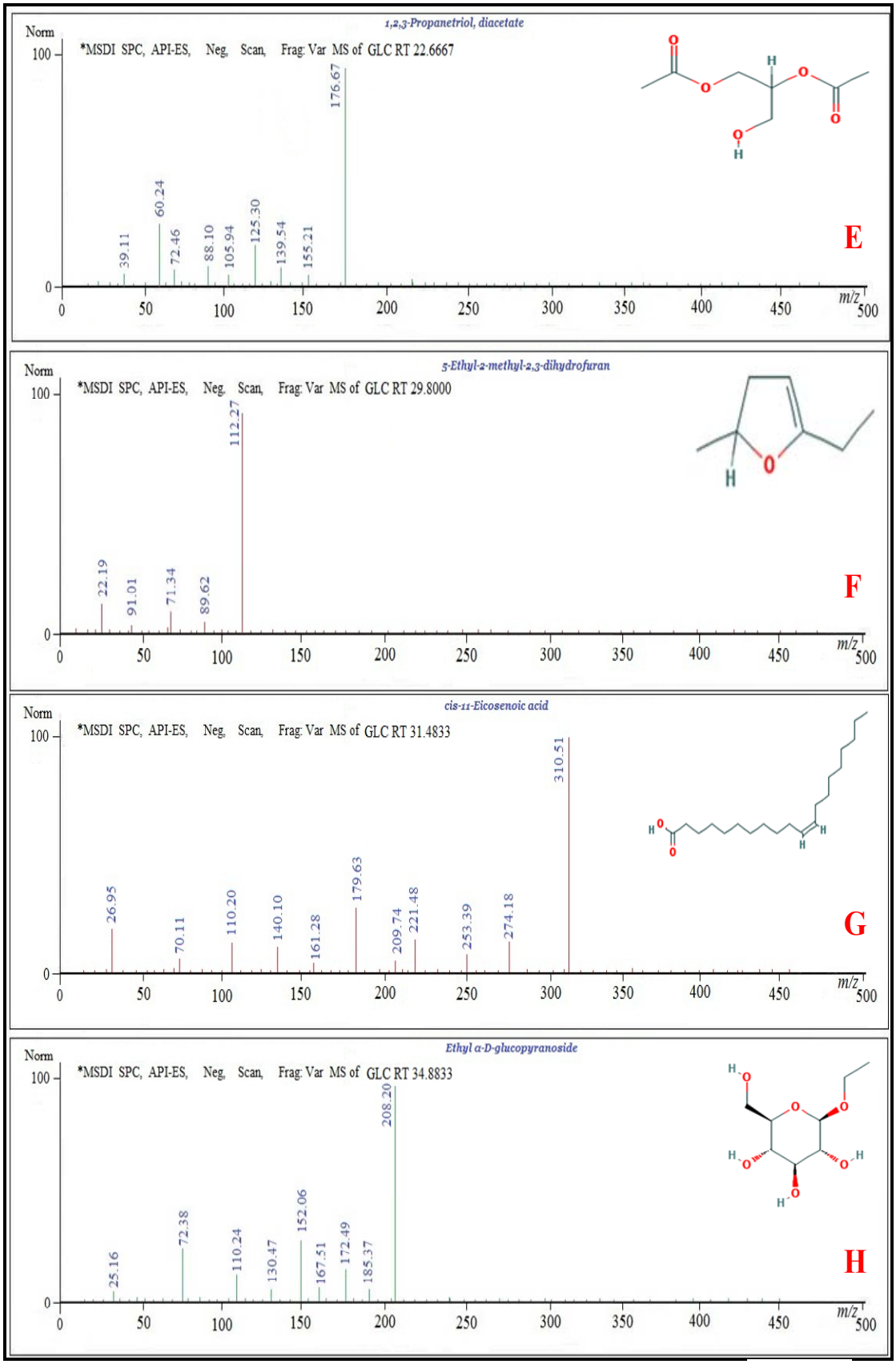

Fig. 4(E-H). Phytocompounds identified in Methanol leaf extract of *A. odorata*

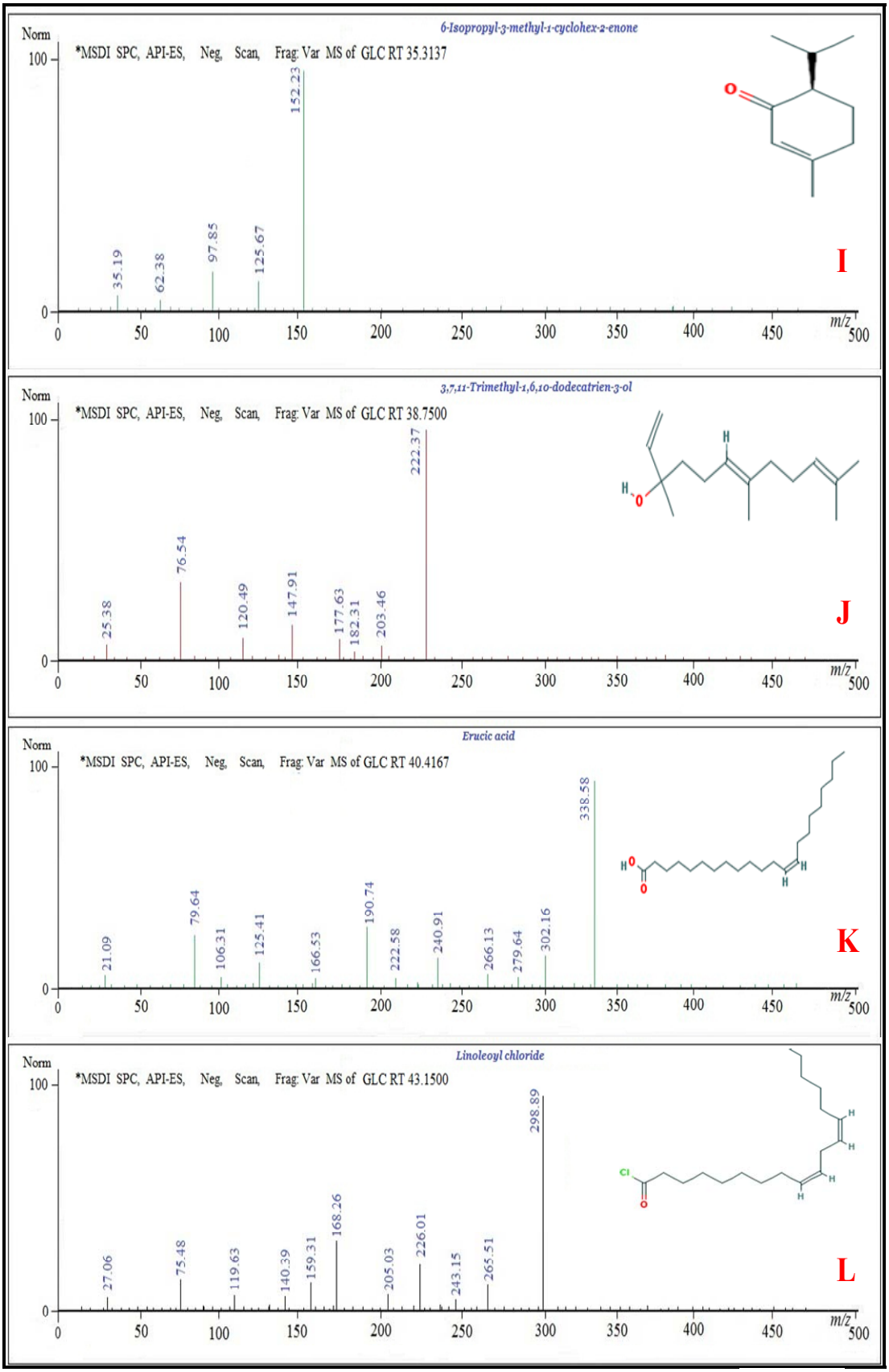

Fig. 4(E-I). Phytocompounds identified in Methanol leaf extract of *A. odorata*

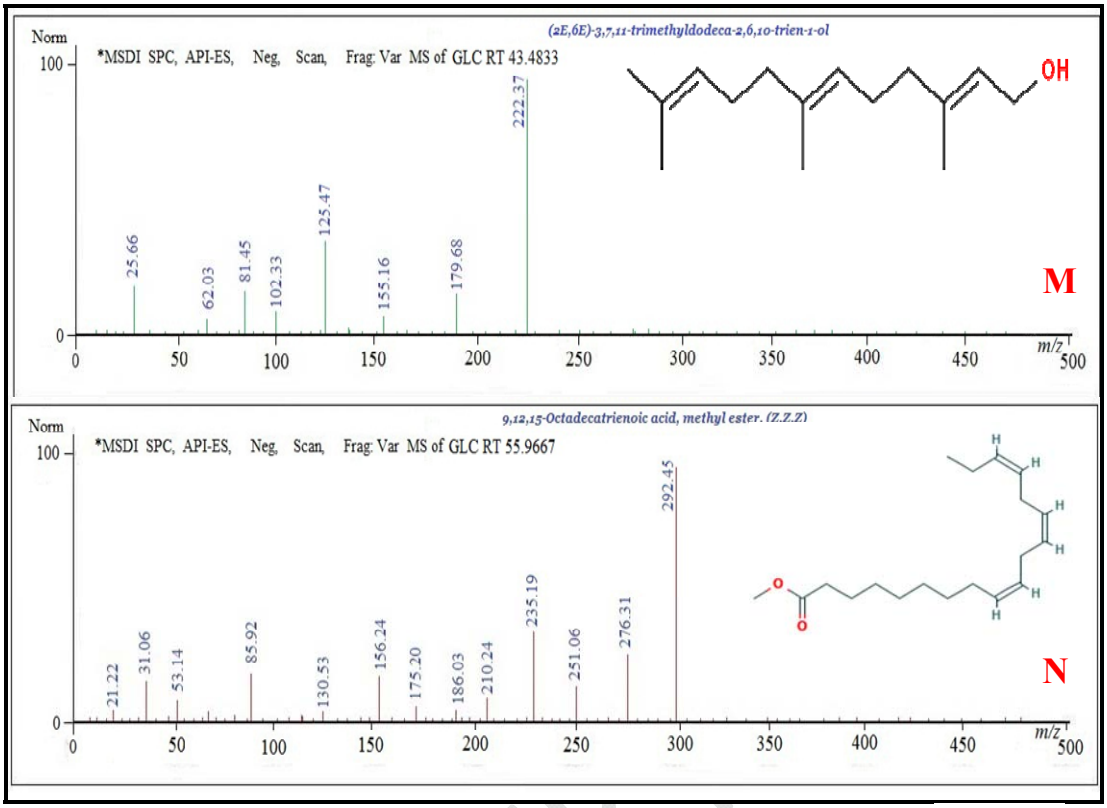

Fig. 4(M-N). Phytocompounds identified in Methanol leaf extract of *A. odorata*

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_{50} (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		

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

Cell line	Concentration (µg/ml)	Absorbance at 570nm			Average	Average-Blank	% Viability	IC_{50} (µg /ml)
MCF-7	100	0.814	0.816	0.818	0.816	0.809	39.31	26.211
	75	0.871	0.873	0.875	0.873	0.866	42.079	
	50	0.922	0.924	0.925	0.923	0.916	44.509	
	25	0.995	0.997	0.998	0.996	0.989	48.056	
	10	1.068	1.07	1.072	1.07	1.063	51.652	
	5	1.176	1.178	1.179	1.177	1.17	56.851	
	Untreated	2.065	2.066	2.065	2.065	2.058	100	
	Blank	0.007	0.008	0.007	0.007	0		
HeLa	100	0.791	0.793	0.795	0.793	0.788	41.299	52.167
	75	0.85	0.852	0.854	0.852	0.847	44.392	
	50	0.963	0.965	0.967	0.965	0.96	50.314	
	25	1.036	1.038	1.039	1.037	1.032	54.088	
	10	1.105	1.107	1.109	1.107	1.102	57.756	
	5	1.181	1.183	1.185	1.183	1.178	61.74	
	Untreated	1.913	1.914	1.913	1.913	1.908	100	
	Blank	0.005	0.006	0.005	0.005	0		

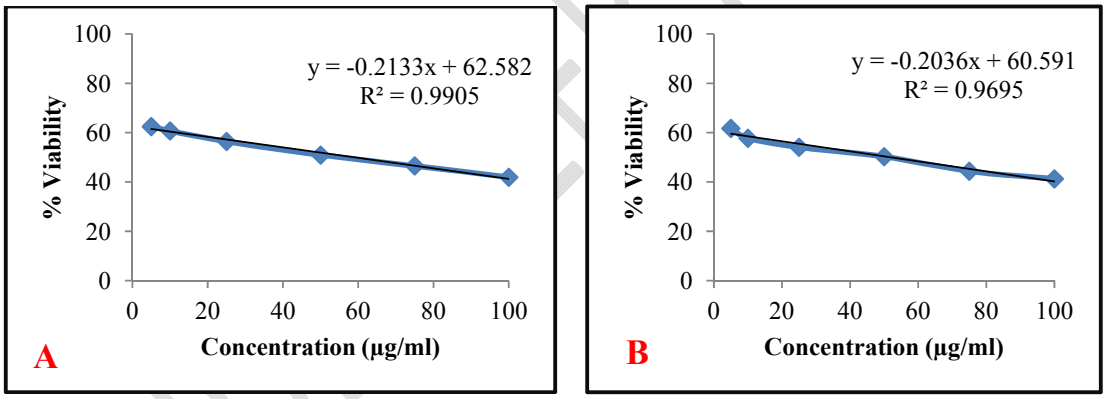

Fig. 5. A) Cytotoxic effect of ethyl acetate extract on *HeLa* Cell Line B) Cytotoxic effect of Methanol extract on *HeLa* Cell Line

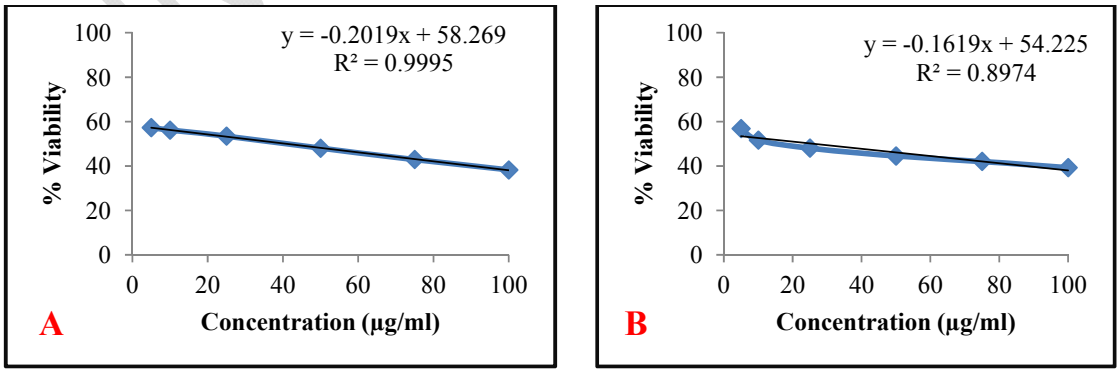

Fig. 6. A) Cytotoxic effect of ethyl acetate extract on *MCF-7* Cell Line B) Cytotoxic effect of Methanol extract on *MCF-7* Cell Line

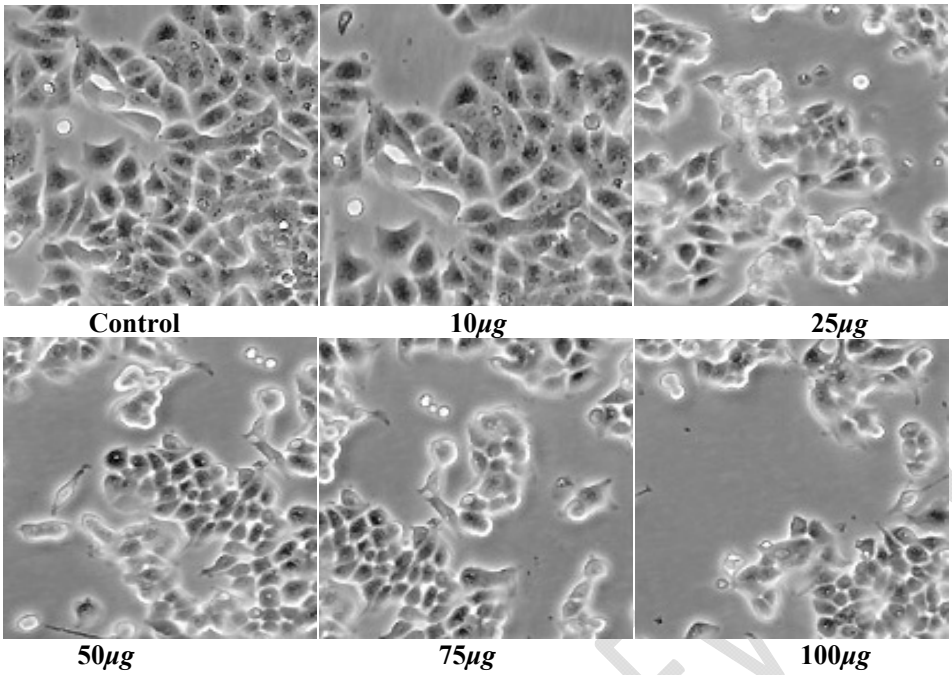

Plate 1. Cytotoxic Properties of ethyl acetate extract on *HeLa* Cell Line

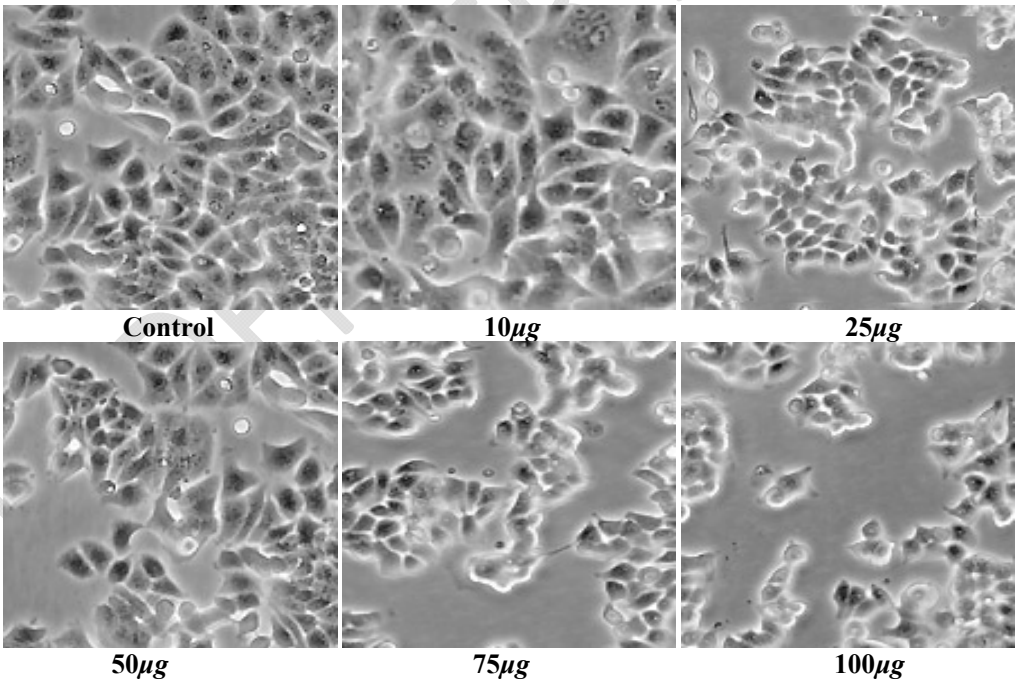

Plate 2. Cytotoxic Properties of Methanol extract on *HeLa* Cell Line

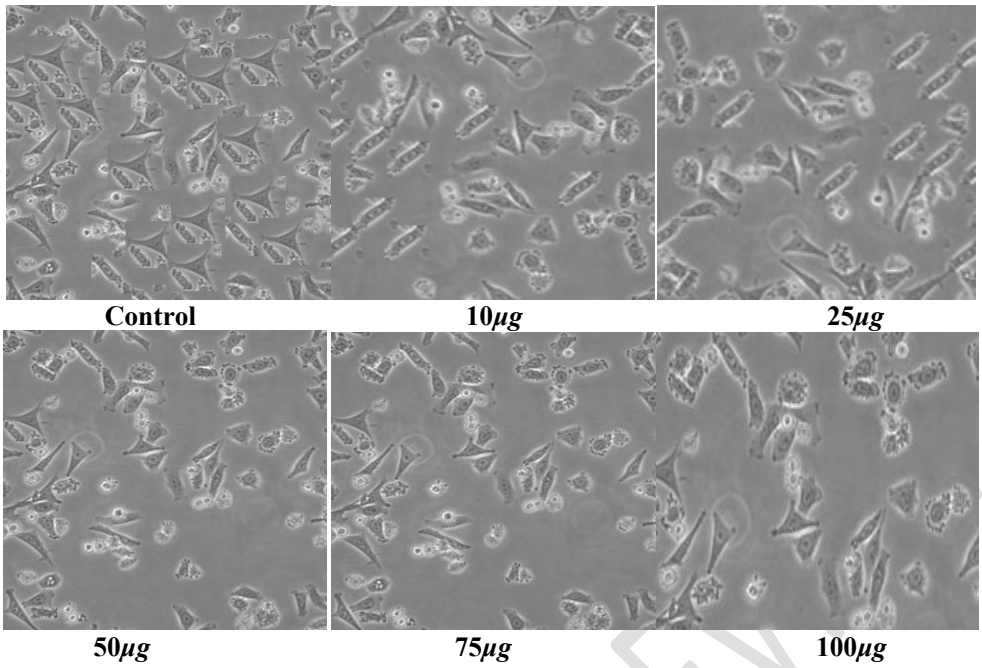

Plate 3. Cytotoxic Properties of ethyl acetate extract on *MCF -7* Cell Line

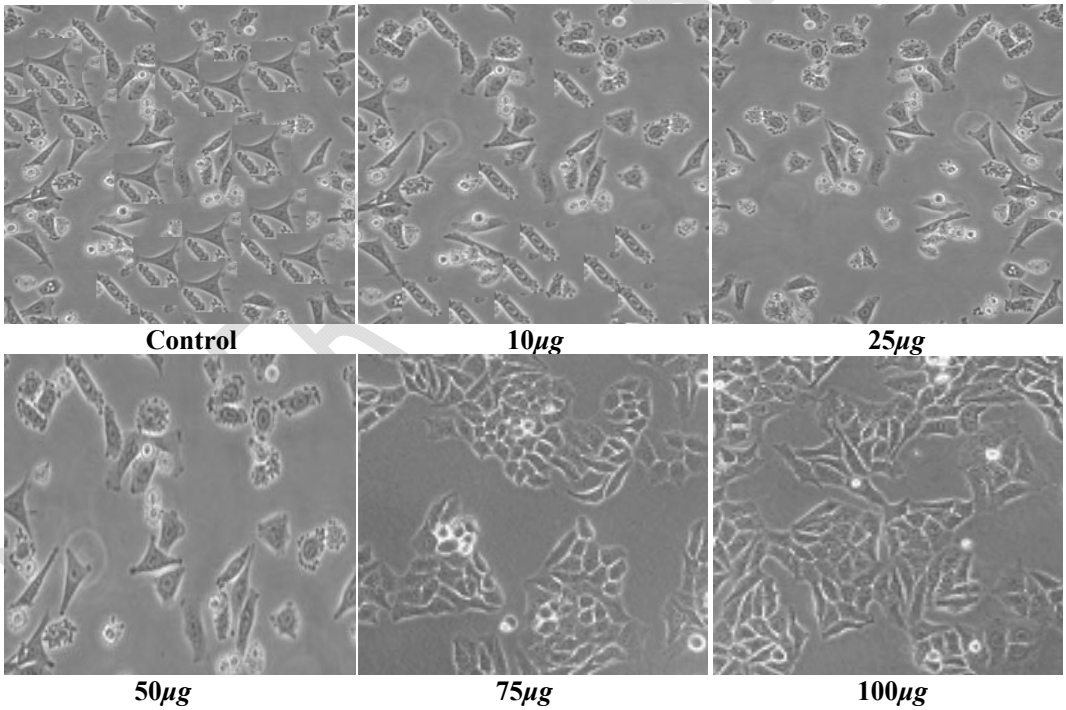

Plate 4. Cytotoxic Properties of Methanol extract on *MCF -7* Cell Line

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 (Table 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-Trimethy 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 > 5g/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 compound reported in the present study such as Phenyl(piperidin-3-yl) methanone, β-Selinene, (E)-5-Methylundec-4-ene, 2-O-(2-Ethylhexyl) 1-O-pentadecyl oxalate, Squalene, m-Toluylaldehyde, Methyl (2E) - 3-phenyl - 2-propeonate, Ethyl α-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].

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_{50} 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_{50} 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 effects 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.

ETHICAL APPROVAL AND CONSENT

It is not applicable

CONFLICT OF INTEREST

Authors do not have any conflict of interest.

REFERENCES

1. Jalal JS, Kumar Pand Pangtey YPS. Ethnomedicinal Orchids of Uttarakhand, Western Himalaya. Ethnobotanical Leaflets. 2008.
2. Pant B. Medicinal orchids and their uses: Tissue culture a potential alternative for conservation. African Journal of Plant Science. 2013; 7(10): 448-467. DOI: 10.5897/AJPS2013.1031.

3. Rosa M, Perz G. Orchids- A review of uses in traditional medicine, its phytochemistry and pharmacology. Journal of Medicinal Plants Research. 2009; 4(8):592-630
4. Shanavaskhan AE, Sivadasan M, Alfarhan AH, Thomas J. Ethnomedicinal aspects of angiospermic epiphytes and parasites of Kerala, India, Indian Journal of Traditional knowledge. 2012; 11 (2): 250-258.
5. Bulpitt CJ. The uses and misuses of orchids in medicine. Quarterly Journal of Medicine. 2005; 98:625-631.
6. Prasenjit P, Suman K. Orchids, the Marvelous Plants The NEHU Journal, 2017; 15(1): 31-40
7. Sudhakar Reddy C, Reddy KN, Raju Vatsavaya. Ethnobotanical observation on some Orchids of Andhra Pradesh, India. J. Non-Timber Forest Products. 2002; 9: 146-147.
8. Pullaiah, T. (1997). Flora of Andhra Pradesh, Scientific Publishers, Jodhpur.
9. Dash PK, Sahoo S, Bal, S. Ethnobotanical studies on orchids Niyamgiri Hill Ranges, Orissa, India. Ethnobotanical Leaflets. 2008; 12: 70-78
10. Hossain MM. Traditional therapeutic uses of some orchids of Bangladeshn. Medicinal and Aromatic Plant Science and Biotechnology, 2009; 3(1):100-106.
11. Padal SB, Sandhyasri B, Chandrasekhar P. "Tradfitional uses of Monocotyledon Plants of Aruku valley Mandalam, Visakhapatnam District, Andhra Pradesh, India." IOSR Journal of Pharmacy and Biological Sciences. 2013; 6 (2): 12-16.
12. Pankaj O. Medicinal Orchid *Nervilia macroglossa* (Hook.f.) Schltr. based herbal formulations used for male impotency in Indian traditional healing. Pankaj Oudhia's Ethnobotanical Surveys 1990- 2012. [Available at https://archive.org/ details /Pankaj OudhiaNerviliaPlicata],
13. Akhtar M, Hoque MM, Rahman M, Hossain MK. "Ethnobotanical investigation of some orchids used by five communities of Cox's bazaar and Chittagang hilly tracts districts of Bangladesh." Journal of medicinal plant studies. 2017; 5(3): 265-268.
14. Trease GE, Evans WC. Text book of *Pharmacognosy.* Balliese Tindall and Company, London. 1983; Pp: 343-383.
15. Chhabra SC, Uiso FC, Mshiu EN. Phytochemical screening of Tanzanian medicinal plants. International Journal of Ethnopharmacology 1984; 11: 157–179.
16. Harborne JB. *Phytochemical methods.* Chapman and Hall Publications, London. 1984; Pp: 288.
17. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd. Ibadan, Nigeria. 1993, 289.
18. Bhat RP. Anticancer activities of plant extracts of *Gymnacranthera farquhariana* (Hook. f. & Thomson) Warb., Myristicafatua Houtt. var. magnifica (Beddome) Sinclair and Samadera indica Gaertner. Adv. Obes Weight Manag. Control. 2017; 6: 167–171.
19. Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC-MS. Pharmacognosy Research. 2009; 1(3): 152-156.
20. Janakiraman N, Johnson M, Sathish SS: GC-MS analysis of bioactive constituents of *Peristrophe bicalyculata* (Retz) Nees. (Acanthaceae). Asian Pacific Journal of Tropical Biomedicine. 2012; 2(1): S46-S49.
21. Oon SF, Meenakshii N, Tee TT, Shamarina S, Kasim NK, Mohd Shazrul Fazry S, Yew Hoong C. Xanthorrhizol: a review of its pharmacological activities and anticancer properties, Cancer Cell Int. 2015; 15:100. DOI 10.1186/s12935-015-0255-4

22. Kasture S, Pontis S, Pinna A, Schintu N, Spina L, Longoni R, Simola N, Ballero M, Morelli M. Assessment of symptomatic and neuroprotective efficacy of Mucuna pruriens seed extract in rodent model of Parkinson's disease. Neurotox Res. 2009; 15(2):111-22. doi: 10.1007/s12640-009-9011-7.
23. *https://pubchem.ncbi.nlm.nih.gov*
24. Hatice BK, Ayse Y, Emel M, Sibel D. Synthesis and Biological Activity of New 1,3-Dioxolanes as Potential Antibacterial and Antifungal Compounds. Molecules, 2011; 16: 6806-15
25. Pejin, Boris, Kojić, Vesna, Bogdanovic, Gordana. An insight into the cytotoxic activity of phytol at in vitro conditions. Natural product research. 2014; 28:1-4. 10.1080/14786419.2014.921686.
26. Dr. Duke's Phytochemical and Ethno botanical Databases. http://www.ars-grin.gov/duke/chem-activities.html.
27. Mahesh Chandra, Om Prakash, Ravendra Kumar, Rakesh Kumar B, Brij Bhushan, Mahesh Kumar, Anil Kumar P. β-Selinene-Rich Essential Oils from the Parts of Callicarpa macrophylla and Their Antioxidant and Pharmacological Activities Medicines. 2017; (4):52. doi:10.3390/medicines4030052
28. Smolinske and Susan C. "Handbook of Food, Drug and Cosmetic Excipients", CRC Press. 1992: pp. 75–76.
29. Zih-Rou H, Yin-Ku L, Jia-You F. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules. 2009; 14: 540-554. DOI: 10.3390/molecules 14010540
30. Senthil kumar, Devaki, Manohar, Babu. Effect of squalene on cyclophosphamide-induced toxicity. Clin Chem. Ata. 2006; 36(4): 335-342.
31. Nawaz S, Shareef M, Shahid H, Mushtaq M, Sarfraz M. A review of antihyperlipidemic effect of synthetic phenolic compounds Matrix Science Medica, 2017; 1 (1): 22-26
32. Greenfield S and Miller G. Fungicidal salicylaldehyde hydrazones and azines US3829492A, 1974.
33. Rolla S, Guniz Kucukguzel S. Biological activities of hydrazone, derivatives, Molecules, 2007; 12:1910-1939.
34. Arumugham S, Praveen kumar R, Ramasamy Thangaraj, Oscar FL, Edachery B, Dhanasekaran D, Nooruddin T. Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. Asian Pac J Trop Dis. 2014; 4(Suppl 2): S979-S984
35. Bogaki T, Mitani K, Oura Y, Ozeki K. Effects of ethyl-α-d-glucoside on human dermal fibroblasts Biosci Biotechnol Biochem. 2017; 81(9):1706-1711. Doi: 10.1080/09168451.2017.1353400.
36. Farid A, Shahverdi AR, Fatemeh Rafii, Fazeli MR, Amini M. Effects of Piperitone on the Antimicrobial Activity of Nitrofurantoin and on Nitrofurantoin Metabolism by Enterobactercloacae., Pharmaceutical Biology. 2007; 45(3): 230-234. DOI: 10.1080/13880200701213161
37. Chan WK, Tan LTH, Chan KG, Lee LH, Goh BH. Nerolidol: A Sesquiterpene Alcohol with Multi-Faceted Pharmacological and Biological Activities. Molecules. 2016; 21: 529. doi.org/10.3390/molecules21050529
38. Khoobchandani M, Ojeswi BK, Ganesh N, Srivastava MM, Gabbanini S, Matera R, et al. Antimicrobial properties and analytical profile of traditional Eruca sativa seed oil: Comparison with various aerial and root plant extracts. Food Chem. 2010; 120: 217-24.

39. Marcos-Arias et al.: In vitro activities of natural products against oral Candida isolates from denture wearers. BMC Complementary and Alternative Medicine. 2011; 11:119 doi:10.1186/1472-6882-11-119
40. Praveen PK, Kumaravel S, Lalitha C. "Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo", Afr. J. Biochem. Res.2010; 4(7): 191-195.
41. Akpuaka A, Ekwenchi M, Dashak DA, Dildar A. "Biological Activities of Characterized Isolates of n-Hexane Extract of *Azadirachta indica* A. Juss (Neem) Leaves", N. Y. Sci J. 2013; 6(6): 119-124.
42. Li YM, Wang HY, Liu GQ. Erianin induces apoptosis in human leukemia HL-60 cells. Acta Pharmacologica Sinica. 2001; 22:1018.
43. Zhao C, Liu Q, Halaweish F, Shao B, Ye Y, Zhao W. Copacamphane, Picrotoxane, and Alloaromadendrane Sesquiterpene Glycosides and Phenolic Glycosides from Dendrobium moniliforme. J. Nat. Prod. 2003; 66:1140-1143.
44. Li H, Yan Z, Zhou Z, Xu L, Daikonya A, Wang J. Anti-allergic agents from natural sources. Heterocycles. 2006; 68:1259-1265.
45. Won JH, Kim JY, Yun KJ, Lee JH, Back NI, Chung HG, Chung SA, Jeong TS, Choi MS, Lee KT. Gigantol isolated from the whole plants of Cymbidium goeringii inhibits the LPS-induced iNOS and COX- 2 expression via NF-kappaB inactivation in RAW 264.7macrophages cells. Planta Med.2006; 72:1181-1187.
46. Shimura H, Matsuura M, Takada N, Koda Y. An antifungal compound involved in symbiotic germination of *Cypripedium macranthos var. rebunense* (Orchidaceae). Phytochem. 2007; 68:1442- 1447.
47. Kalaiarasan A, Ahmed john S. Some Bioactive constituents of GC-MS analysis of *Bulbophyllum kaitense* rechib. Stem, Eastern Ghats of India. Int. J. Pharma and Bio Sciences. 2011; 2(4): 156-160.
48. Dandekar R, Fegade B, Bhaskar VH. GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxypetalum* leaves. J. Pharm Phytochem. 2015; 4(1): 149-154.
49. Keerthiga M, Anand SP. Bioactive Compound Evaluation of Ethanol Extract from *Geodorum densiflorum* (Lam.) Schltr. By GC-MS analysis. Int. J. Pharmal Res. 2015; 5(6): 139-144.
50. Prasad R, Koch B. Antitumor Activity of Ethanolic Extract of Dendrobium formosum in T-Cell Lymphoma: An In Vitro and In Vivo Study. BioMed Research International 2014; Article ID 753451.
51. Prasad R, Koch B. In vitro Anticancer Activities of Ethanolic Extracts of Dendrobium crepidatum and Dendrobium chrysanthum against T-cell lymphoma , J Cytol Histol. 2016; 7:4
52. Bhatt DR, Jethva KD, Zaveri MN. In-vitro cytotoxicity studies of the therapeutic orchid: Eulophia nuda, Journal of Pharmacognosy and Phytochemistry. 2018; 7(4): 680-683.
53. Divya K, Pradeep HR, Kumar KK, Venkatesh KRH and Jyothi T. Herbal drug *Swietenia mahogany* jacq- a review. Global Journal of Research on Medicinal Plants & Indigenous Medicine. 2012; 1: 557.
54. Sheel R, Nisha K, Kumar J. Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. ISRO Journal of Applied Chemistry. 2014; 7: 10-13.
55. Opdyke DLJ, Letizia C. Fragrance raw materials monographs, Food and Chemical Toxicology, Volume 20, Issue 6, 1982, Pages 637-852, ISSN 0278-6915. doi.org/10.1016/S0015-6264(82)80217-4.

56. Scortichini M, Rossi PM. Preliminary *in vitro* evaluation of antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill) J Appl Bact. 1991; 71:109–112. doi: 10.1111/j.1365-2672.1991.tb02963.x.
57. Homann T, Tag C, Biebl H, Deckwer WD, Schink B. Fermentation of glycerol to 1, 3-propanediol by *Klebsiella* and *Citrobacter* strains. *Appl Microbiol Biotechnol.* 1990; 33: 121–126.
58. Colin T, Bories A, Moulin G. Inhibition of *Clostridium butyricum* by 1, 3-propanediol and diols during glycerol fermentation. *Appl Microbiol Biotechnol.* 2000; 54: 201–205.
59. Cheng KK, Zhang JA, Liu DH, Sun Y, Liu HJ, Yang MD. Pilot-scale production of 1, 3-propanediol using *Klebsiella pneumoniae*. *Process Biochem.* 2007; 42: 740–744.
60. Zhu JG, Li S, Ji XJ, Huang H, Hu N. Enhanced 1, 3-propanediol production in recombinant *Klebsiella pneumoniae* carrying the gene yqhD encoding 1, 3 propanediol oxidoreductase isoenzyme. World Journal of Microbiology Biotechnology. 2009; 25:1217–1223.
61. Midéko JK, Fernand G, Pierre A, Marc-Abel A, Sylvie C and Jalloul B. Chemical composition and biological activities of extracts and essential oil of Boswellia dalzielii leaves, Pharmaceutical Biology. 2017; 55:1, 33-42.
62. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols DJ and McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta medica. 1982; 45(05): 31-4
63. Sohag SI, Hoque MM, Huda MK. Phytochemical screening and antioxidant activity of rare medicinal orchid *Luisia zeylanica* Lindl. Journal of Pharmacognosy and Phytochemistry, 2017; 6(4): 688-692.
64. Haridas R, Manorama S, Thekkan S. In-vitro cytotoxicity activity of malaxis rheedii sw methanol extract against hela cell line and mcf-7 cell line Asian J Pharm Clin Res. 2016; 9 (6): 244-246.