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EVALUATION OF PHYTOCHEMICAL AND PHARMACOLOGICAL ASPECTS OF EPIPHYTIC ORCHID *LUISIA ZEYLANICA* LINDL.

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Keywords:

GC-MS analysis, Antimicrobial, Anticancer, *Lusia zeylanica*

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ABSTRACT: The present study was carried out to evaluate phytochemical composition and pharmacological activities of leaf extract of *Lusia zeylanica* by using different solvents. The solvents like n-hexane, ethyl acetate and methanolic were used to extract dried leaf material of *L. zeylanica*. These extracts were analyzed for phytochemical constituents with GC-MS analysis and antimicrobial activity tested against four bacterial strains and three fungal strains by using the agar diffusion method. *In-vitro* anticancer activity was done against two cancer cell lines (MCF-7 and HeLa cell line) by using MTT assay. Phytochemical analysis revealed the presence of coumarins, flavonoids, glycosides, phenols, saponins, tannins, and terpenoids. GC-MS analysis determines the presence of 11 compounds in ethyl acetate, 7 compounds in methanolic extracts and a total of 7 unknown compounds respectively. A significant cancer cell growth inhibition was observed for two extracts with IC_{50} values ranging between 18.36 µg/ml to 67.914 µg/ml. Our result shows this plant is a promising source of phytocompounds with potential antimicrobial and anticancer activity.

INTRODUCTION: In developing countries, infectious diseases are a major threat to public health. In India large number of people still rely on ethnomedicine to treat serious diseases including cancer and different types of inflammations. Contrary to synthetic drugs ethnomedicinal plants are used to cure various infectious diseases. Many drugs obtained from plant origin have no side effects and have enormous therapeutic potential to treat infectious diseases [1].

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World Health Organization has also recognized the importance of ethnomedicine/ traditional medicine in the healthcare sector.

Recent ethnomedicinal, pharmacological studies on orchids indicate that these plants have immense potential on the treatment of various diseases such as neurodegenerative disorders, anticonvulsive, anti-cancer, antidiabetic *etc*. [2, 3] Orchids are one of ethnobotanical interest linking aboriginal man with plants for medicine [4]. Numerous orchid species possessing cultural values have been used in herbal medicines and also food supplements by the tribal people across the world [5]. Though there has been tremendous progress in medicinal plant research, orchids have not been exploited fully for their medicinal application. Orchid extracts and purified compounds are shown to exhibit several bio-

activities such as antimicrobial, antioxidant, anthelmintic, insecticidal, antiviral, analgesic, antipyretic, antiallergic, wound inflammatory activity [6].

Luisia zeylanica is a widely distributed epiphytic orchid found in Eastern Ghats of Visakhapatnam district. It is one such plant showing multifarious Ethnomedicinal properties such as Abscesses, emollient, chronic boils, burns, fractures and tumors [3, 7]. The knowledge of medicinal plant's active chemical constituents would be further valuable in discovering the actual value of ethnomedicine. Hence, scientific validation of ethnomedicinal plants provides evidence-based alternative medicines, which form the basis of the discovery of new drugs.

Antioxidant and phytochemical analysis of *L. zeylanica* leaf extracts showing the presence of various bioactive compounds [8]. To validate the ethnomedicinal properties of *L. zeylanica,* the present study was attempted to evaluate the phytochemical composition, antimicrobial and anticancer activities by using various solvent extracts of leaf material.

MATERIALS AND METHODS: In the present study ethnomedicinal orchid *L. zeylanica* with voucher number ANUBH01210 is collected from Paderu, Visakhapatnam District, Andhra Pradesh. Fresh healthy leaves were collected and thoroughly washed with distilled water to remove dust particles and shade dried at room temperature for ten days. The dried material was made into coarse powder by means of an electrical grinder. The dried powdered leaf material of (200 g) was Soxhlet extracted with n-hexane, ethyl acetate and methanolic solvents for about 12-15 h. The crude extracts of different solvents were evaporated by a vacuum rotary evaporator (Buchi Labortech Ag, model l, R-215) under reduced pressure. The various solvent extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The dried extracts were kept in the refrigerator at 4 °C until use.

Preliminary Phytochemical Screening: The dried extract of various solvents hexane, chloroform, ethyl acetate and methanolic were preliminarily screened by using standard procedures/tests [9, 10, 11].

Test for Alkaloids (Dragendroff's Test): 2 ml of each extract was acidified with few drops of dilute hydrochloric acid. To this acidic medium, 1 ml of Dragendroff's reagent (Potassium bismuth iodide) was added. An orange or reddish-brown precipitate produced indicates the presence of alkaloids.

Test for Flavonoids (Shinoda Test): The presence of flavonoids can be confirmed by treating the alcoholic plant extract with few fragments of magnesium ribbon and hydrochloric acid. The reaction mixture develops pink, scarlet, or crimson red color, indicates the presence of flavonoids.

Test for Saponins (Foam Test): 1 ml of each extract shaken with 10 ml of distilled water and it was agitated in a graduated cylinder for 10 min. The formation of persistent honey-comb like froth indicates the presence of saponins.

Test for Quinones: A small amount of extract was treated with concentrated HCl and observed for the formation of a yellow color precipitate.

Test for Tannins (Lead Acetate Test): To 2 ml of each extract add a few drops of 10% Lead acetate were added. The appearance of a white precipitate indicates the presence of tannins.

Test for Terpenoids and Steroids: To 1ml of the extract, 50% H_2SO_4 is added along the sides of the test tube, to which a mixture of methanolic HCl and acetic anhydride was added carefully. If there is any change in color, from green to blue-green (sometimes *via* red or blue) indicates the presence of terpenoids and steroids.

Test for Phenols: When 0.5 ml of $FeCl_3$ (w/v) solution was added to 2 ml of rest solution, the formation of an intense color indicates the presence of phenols.

Coumarins: A few drops of alcoholic sodium hydroxide were added to the 2 ml of extract. The formation of yellow color indicated the presence of coumarins.

Test for Glycosides: 2 ml of extract was mixed with a little anthrone and add few drops of conc. H_2SO_4 and warmed gently over a water bath. The presence of glycosides was identified by dark green color formation.

Resins: 2 ml of extract was treated with acetone. To this, a small amount of water was added and shaken. The appearance of turbidity indicates the presence of resins.

GC-MS Analysis: The GC-MS analysis of solvent extracts from leaves was performed using an Agilent GC-MS (model-7890 A, MS 5975) equipped with an HP- 5MS fused capillary column (30 m × 0.25 mm ID × 0.25 um Film Thickness). Inert helium gas was used as a carrier gas at a constant flow rate of 1ml/1 min. An aliquot of 2µl of two solvent extract solutions of the sample was injected into the column with an injector temperature of 280 °C. Mass transfer line and injector temperature were set at 220 °C and 300 °C, respectively. The oven temperature was programmed from 50-150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 C/min. For gas chromatography-mass spectroscopic detection, an electron ionization system with ionization energy 70eV was used and the detector was operated in scan mode from 40-500 amu. The total running time was 55.3 min.

Interpretation of Mass Spectrum (MS): Interpretation on GC-MS was conducted using the National Institute of Standard and Technology (NIST) Data Base Library 2.0 version having more than 62,000 patterns. The spectrum of the unknown component stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Microorganisms: The antibacterial activity of the crude extracts was determined by using both gram-positive and gram-negative bacteria. Three-gram positive bacteria namely *Bacillus megaterium, Lactobacillus acidophilus, Enterococcus faecalis* and three gram-negative bacteria namely *Klebsiella pneumoniae, Proteus vulgaris* and *Escherichia coli* were taken for study. For antifungal activity, *Candida albicans* and *Aspergillus flavus* were used for the study

Antimicrobial Activity: Nutrient agar (NA) and Czepak dox agar medium were used for the test of bacteria and fungi. Both mediums (100 ml) were sterilized at 15 lbs pressure (121 °C) for 15 min, cooled and inoculated with 0.1 ml of bacterial and fungal test suspension. Now the mixed medium was poured into Petri plates under aseptic conditions and allowed for solidification. Four wells of about 5 mm diameter were punched with a sterilized cork borer. Different concentrations of solvent extracts (50 µl, 100 µl, and 150 µl) were added to each well, and the addition of the solvent alone served as control. The inoculated bacterial plates were incubated at 37 °C for 24 h and fungal plates were incubated at 28 °C for 48 h. The diameter of the inhibition zone was measured in millimeters.

Anticancer Activity by MTT Assay: The two solvent extracts (Ethyl acetate and Methanolic) 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 (6×10^5) into 96 well micro-titer and incubated overnight at 37 °C for 48 h. 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 used to calculate IC_{50} dose values [12].

RESULTS AND DISCUSSION: Preliminary phytochemical screening of the different solvent extracts like hexane, ethyl acetate and methanolic extract of leaves in *L. zeylanica* revealed the presence of various chemical compounds such as alkaloids, coumarins, flavonoids, glycosides, phenols, saponins, tannins, steroids and terpenoids **Table 1**. The secondary metabolites may be responsible for the medicinal properties of medicinal plants [13]. In the present investigation, results suggest phytochemicals such as flavonoids, phenols, terpenoids, saponins and tannins may be responsible for antimicrobial and biological activities [14, 15].

Based on the results of preliminary phytochemical analysis GC-MS analysis carried out for the identification of bioactive compounds from GC fractions of the methanolic and ethyl acetate extracts of *Lusia zeylanica*. GC-MS is one of the important technological tools used to identify the profile of phytocompounds in plant species [16, 17].

The compounds were identified through mass spectrometry attached with GC and Later these compounds were confirmed based on the peak area, retention time and molecular formula were presented in **Table 5**. The mass spectra analysis of ethyl acetate extract chromatogram **Fig. 1** reveals the presence of 11 phytocompounds and 3 unknown compounds in **Table 2**. The compounds found in ethyl acetate extract are 2,2-Dimethyl-3-propyloxirane **Fig. 2A**, Hydroperoxide, 1-ethylbutyl **Fig. 2B**, Ethanone, 1-cyclohexyl-**Fig. 2C**, Cyclopentanol, 1, methyl **Fig. 2D**, 9,12,15-Octadecatrien-1-ol **Fig. 2E**,4-Methyl-1,3-dioxane **Fig. 2F**, 5-Oxotetrahydrofuran-2- carboxylic acid **Fig. 2G**, Unknown compound **Fig. 2H**, Methyl cis-10-heptadecenoate **Fig. 2I**, (E)-9-Octadecenoic acid ethyl ester **Fig. 2J**, Unknown compound **Fig. 2K**, Triacontane **Fig. 2L**, Methyl 15-methyl-hexadecanoate **Fig. 2M**, Unidentified compound 3 **Fig. 2N**.

Similarly, methanolic extract chromatogram **Fig. 3** consists of 7 phytocompounds and 4 unknown compounds in **Table 3**. The compounds in methanolic extract are (E)-1-[Bis[(E)-but-2-enoxy] methoxy] but-2-ene **Fig. 4A**, Unknown compound-1 **Fig. 4B**, Kaempferol 3-glucoside **Fig. 4C**, n-Tridecanoic acid methyl ester **Fig. 4D**, Unknown compound-2 **Fig. 4E**, 1, 4-Dimethyl-1,4,6,7-tetrahydroimidazo [4, 5-e] [1,4] diazepine-5,8-dione **Fig. 4F**, Phthalic acid, butyl hexyl ester **Fig. 4G**, Unkown compound-2 **Fig. 4H**, (Z)-Icos-13-enoic acid **Fig. 4I**, Unknown compound-3 **Fig. 4J**, Octadecanoic acid, ethyl ester **Fig. 4K**.

Most of the reported phytocompounds have various biological activities, which were represented in **Tables 2** and **3**. The present study supported by previous GC-MS studies on Orchids, they have various bioactive compounds with therapeutic potential [18, 19, 20].

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF *L. ZEYLANICA*

S. 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_2SO_4 test	-	-	-
7	Resins	Acetone H_2O test	-	-	+
8	Saponins	Foam test	-	-	+
9	Tannins	Braemer's test	-	-	+
10	Steroids	Salkowski test	-	+	-
11	Terpenoids	Salkowski test	-	+	-

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

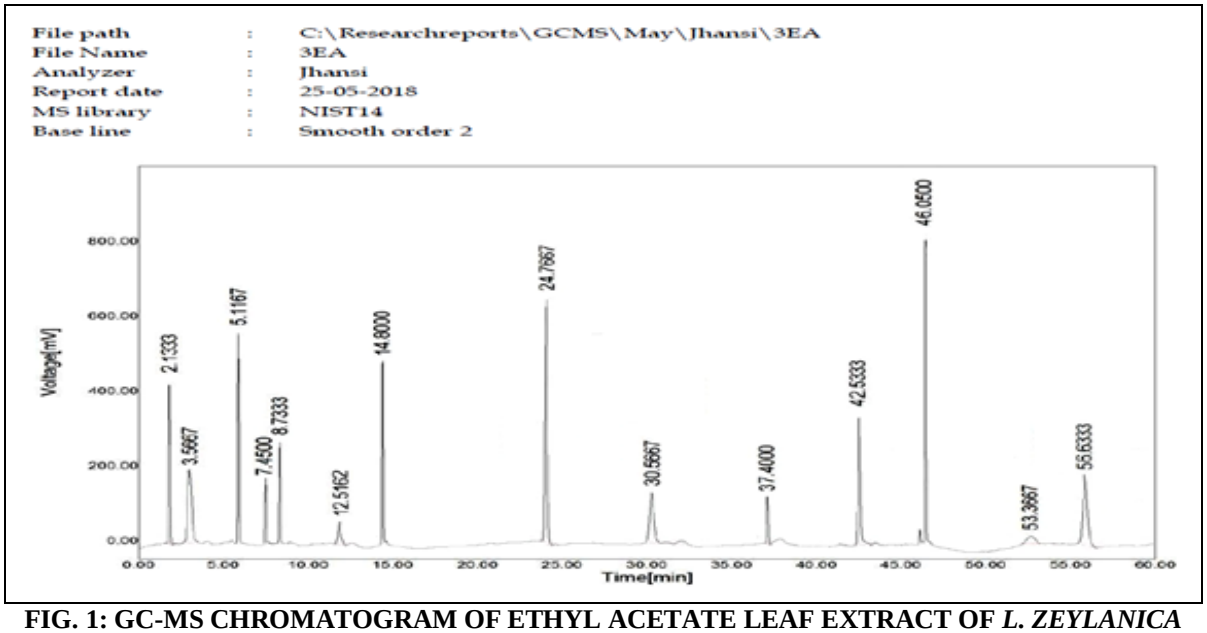


FIG. 1: GC-MS CHROMATOGRAM OF ETHYL ACETATE LEAF EXTRACT OF *L. ZEYLANICA*

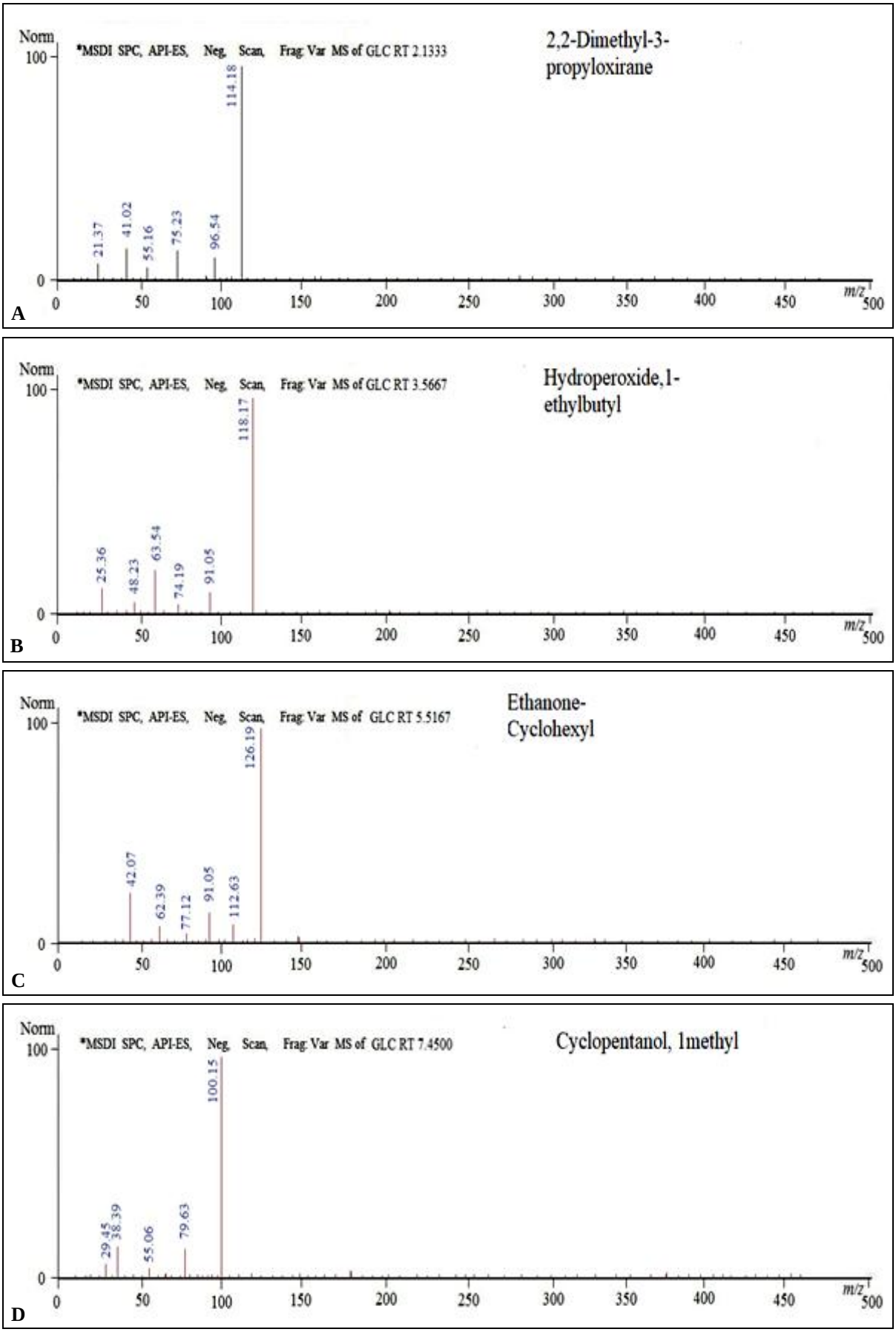


FIG. 2(A-D): GC-MS CHROMATOGRAM OF PHYTOCHEMICALS IDENTIFIED IN ETHYL ACETATE LEAF EXTRACT OF *L. ZEYLANICA*

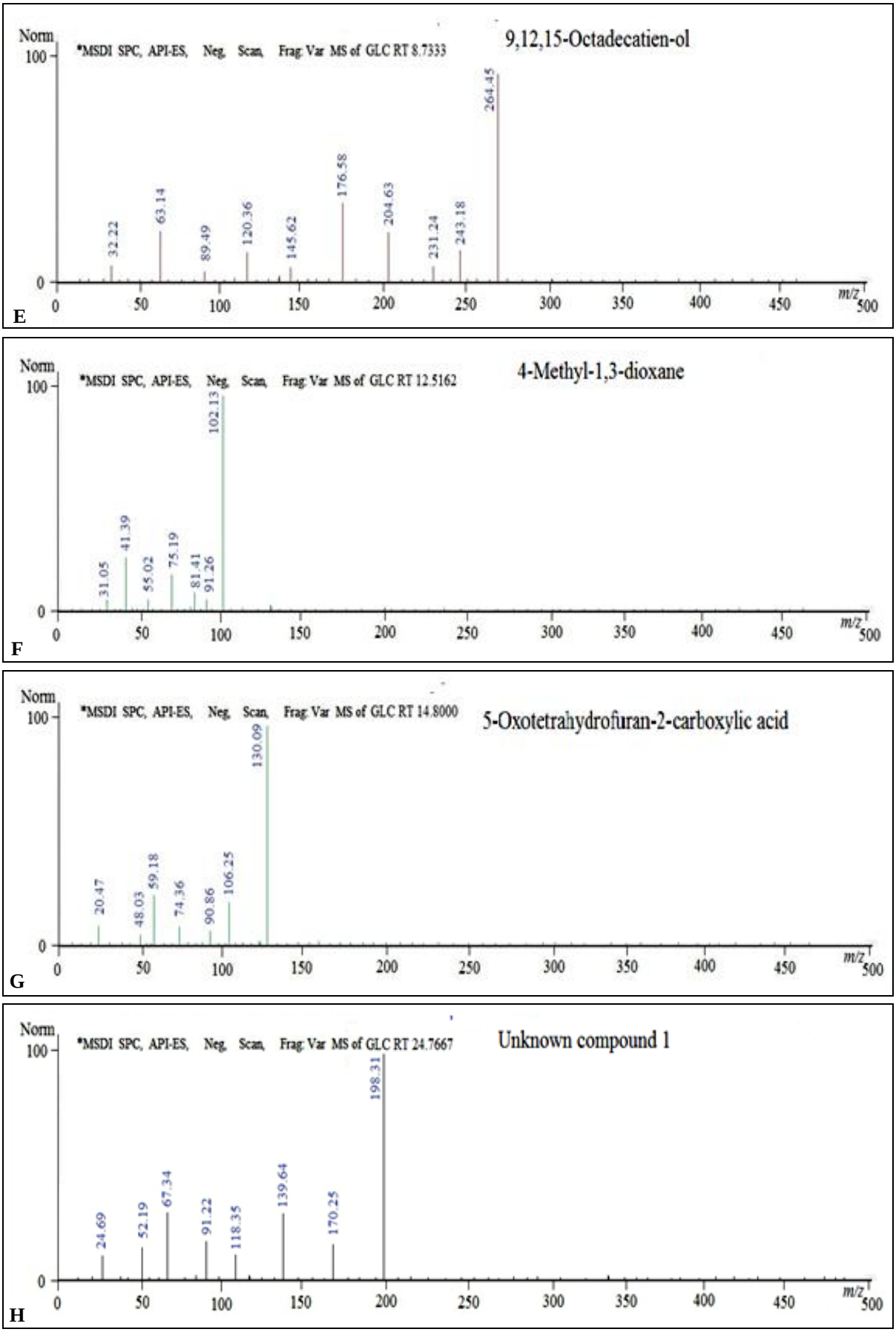


FIG. 2(E-H): GC-MS CHROMATOGRAM OF PHYTOCHEMICALS IDENTIFIED IN ETHYL ACETATE LEAF EXTRACT OF *L. ZEYLANICA*

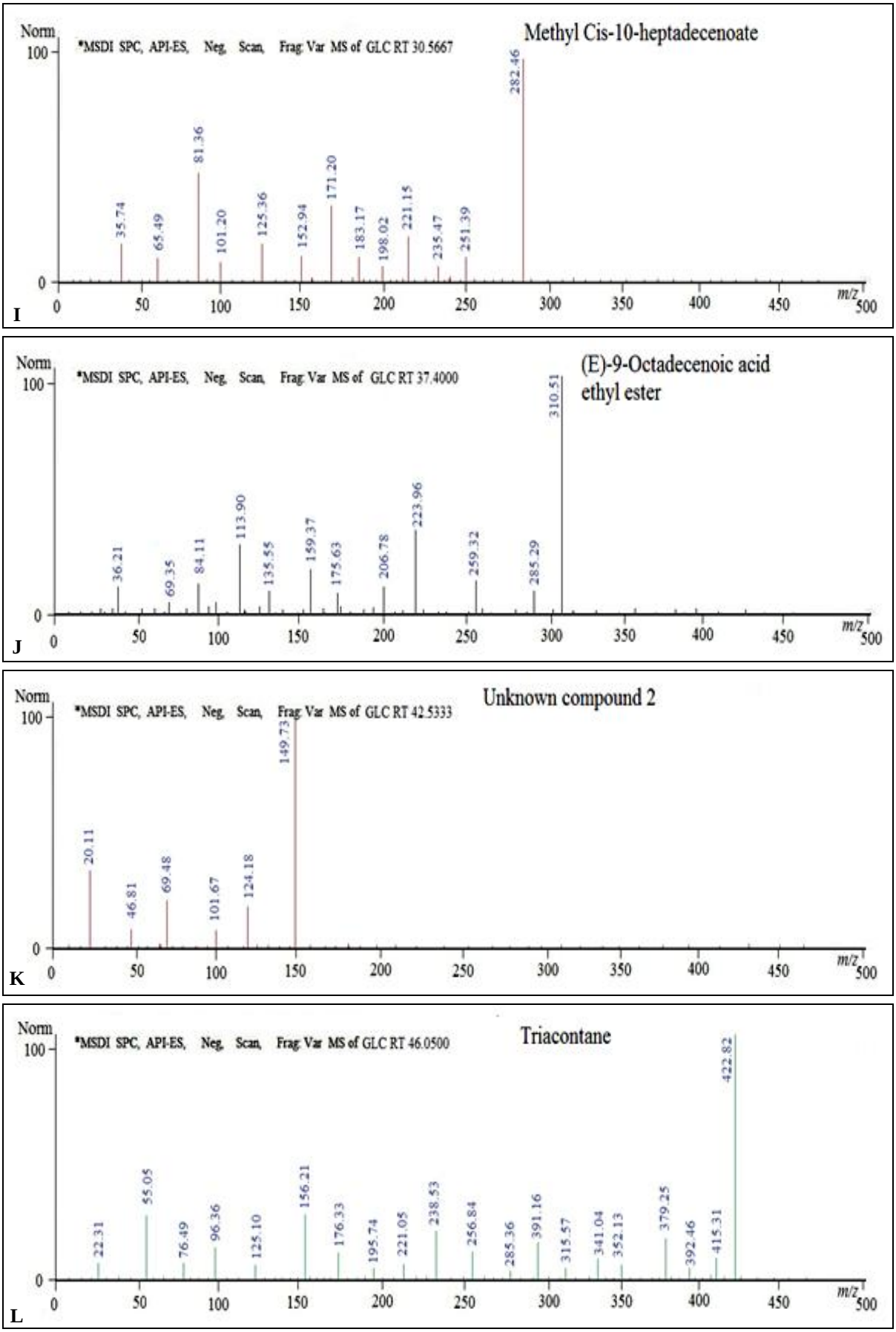
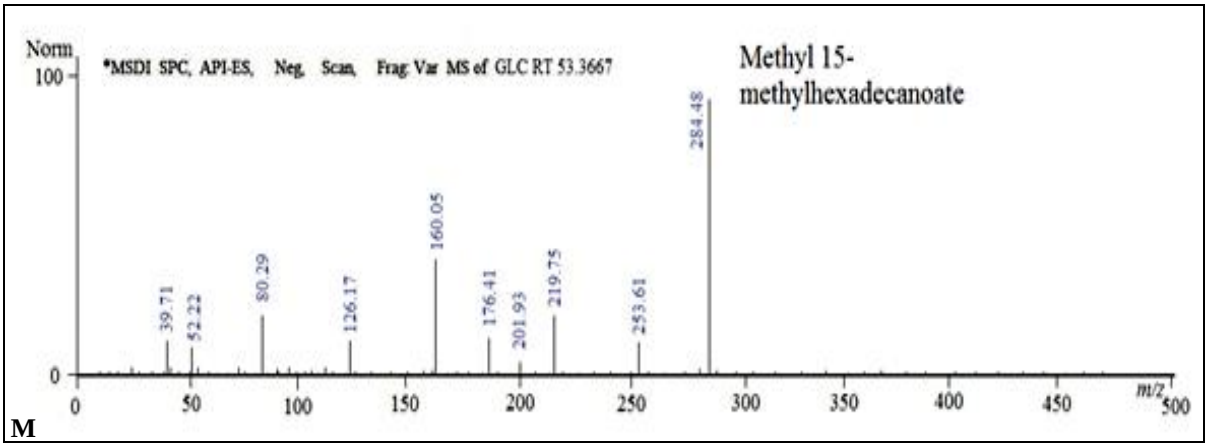


FIG. 2(I-L): GC-MS CHROMATOGRAM OF PHYTOCHEMICALS IDENTIFIED IN ETHYL ACETATE LEAF EXTRACT OF *L. ZEYLANICA*



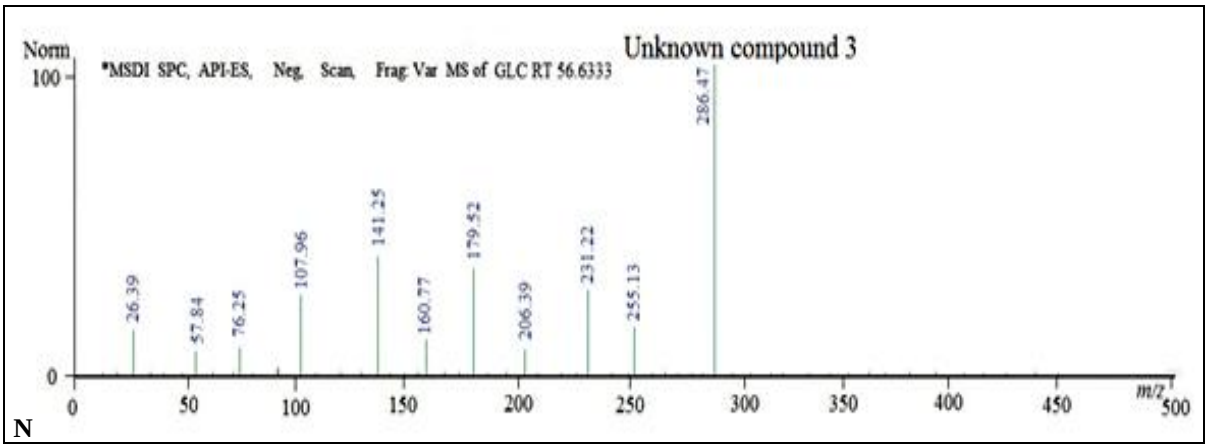


FIG. 2(M-N): GC-MS CHROMATOGRAM OF PHYTOCHEMICALS IDENTIFIED IN ETHYL ACETATE LEAF EXTRACT OF *L. ZEYLANICA*

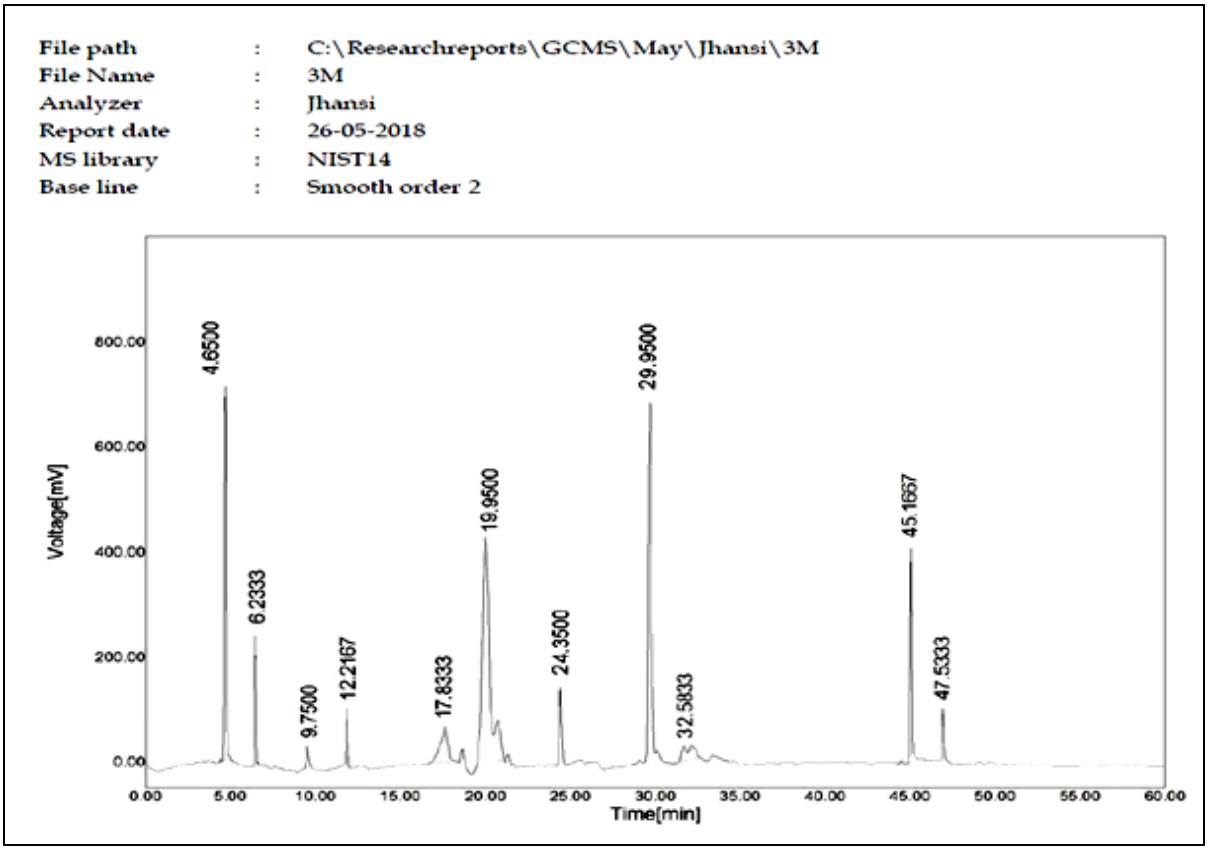


FIG. 3: GC-MS CHROMATOGRAM OF METHANOLIC LEAF EXTRACT OF *L. ZEYLANICA*

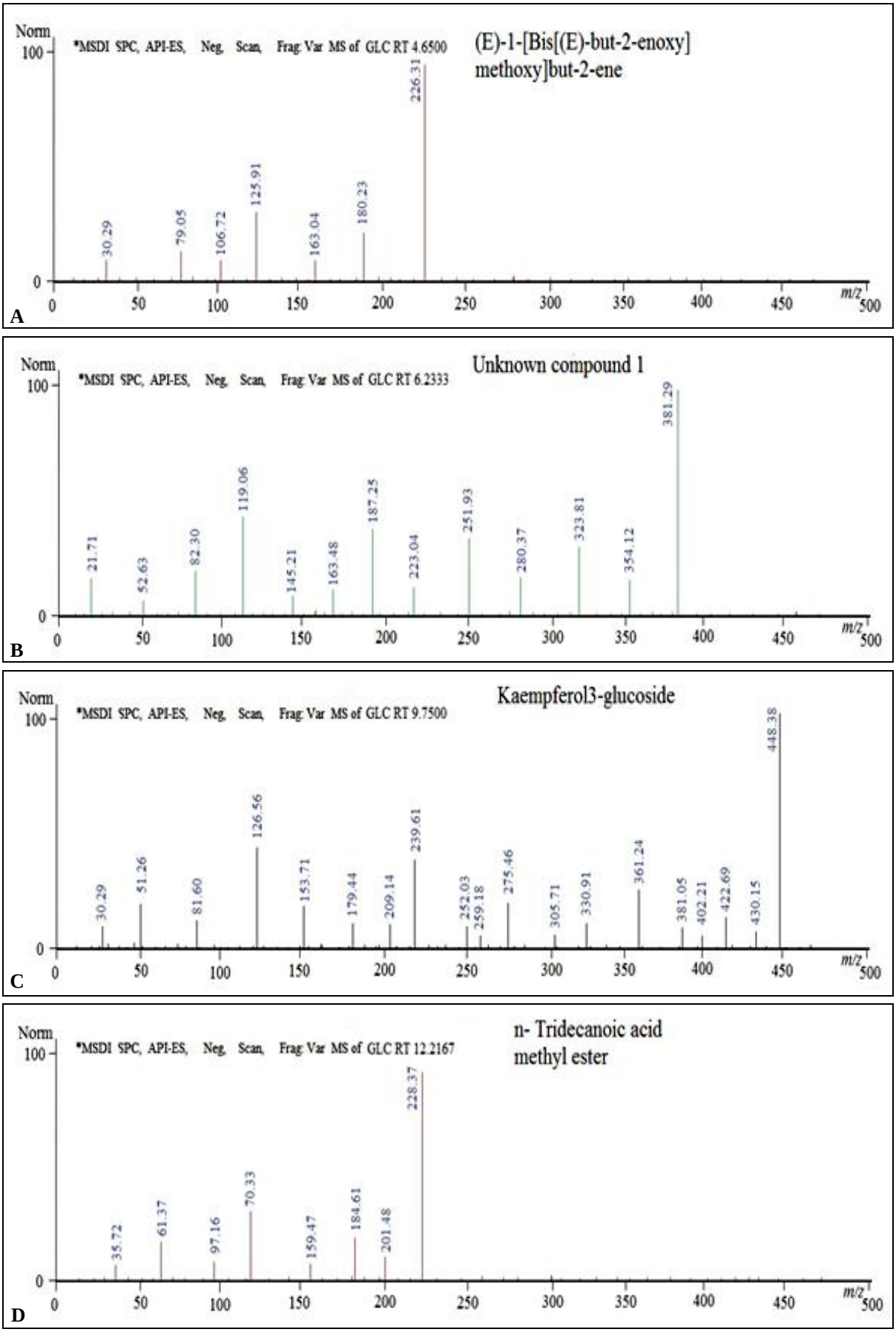


FIG. 4(A-D): GC-MS CHROMATOGRAM OF PHYTOCHEMICALS IDENTIFIED IN METHANOLIC LEAF EXTRACT OF *L. ZEYLANICA*

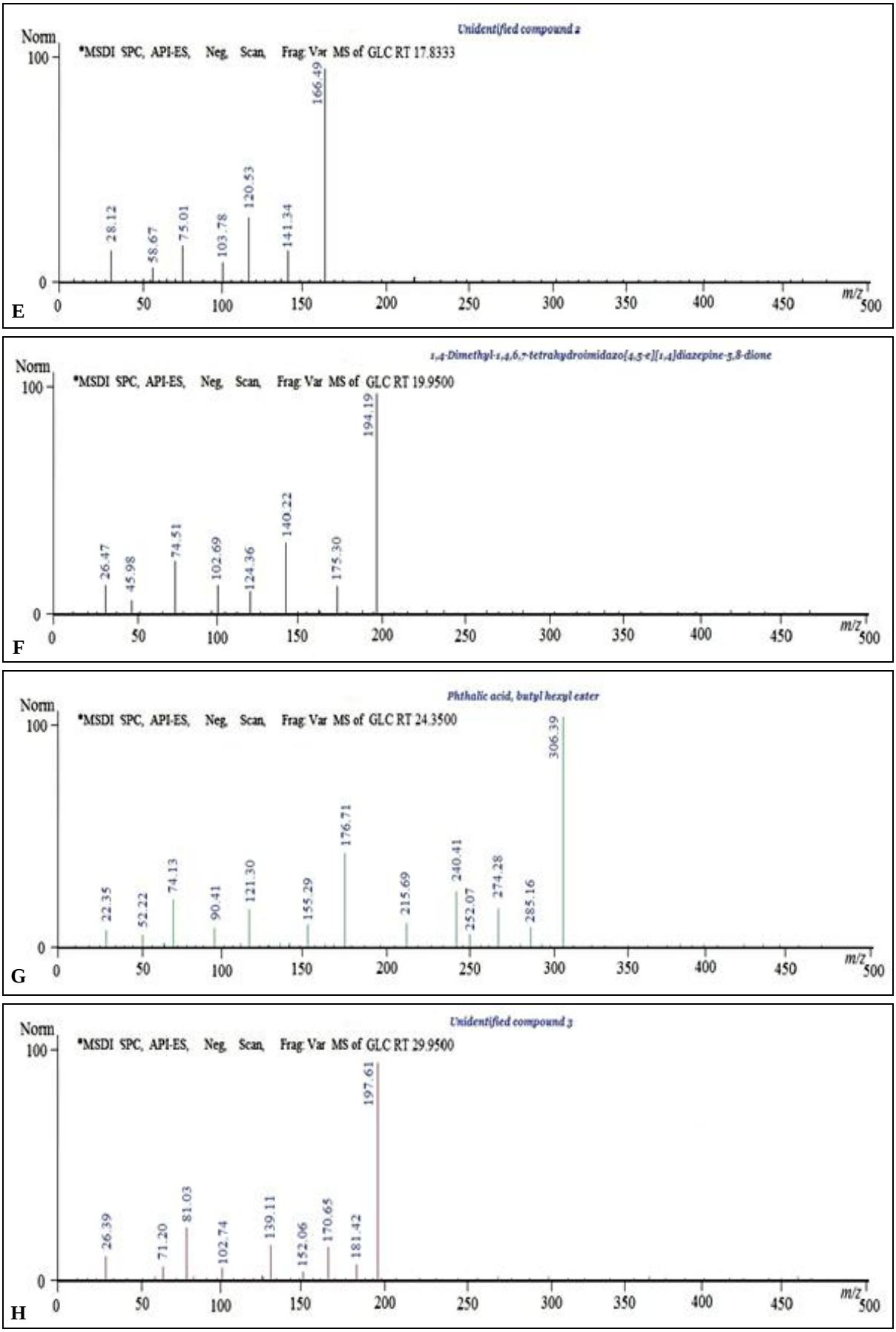


FIG. 4(E-H): GC-MS CHROMATOGRAM OF PHYTOCHEMICALS IDENTIFIED IN METHANOLIC LEAF EXTRACT OF *L. ZEYLANICA*

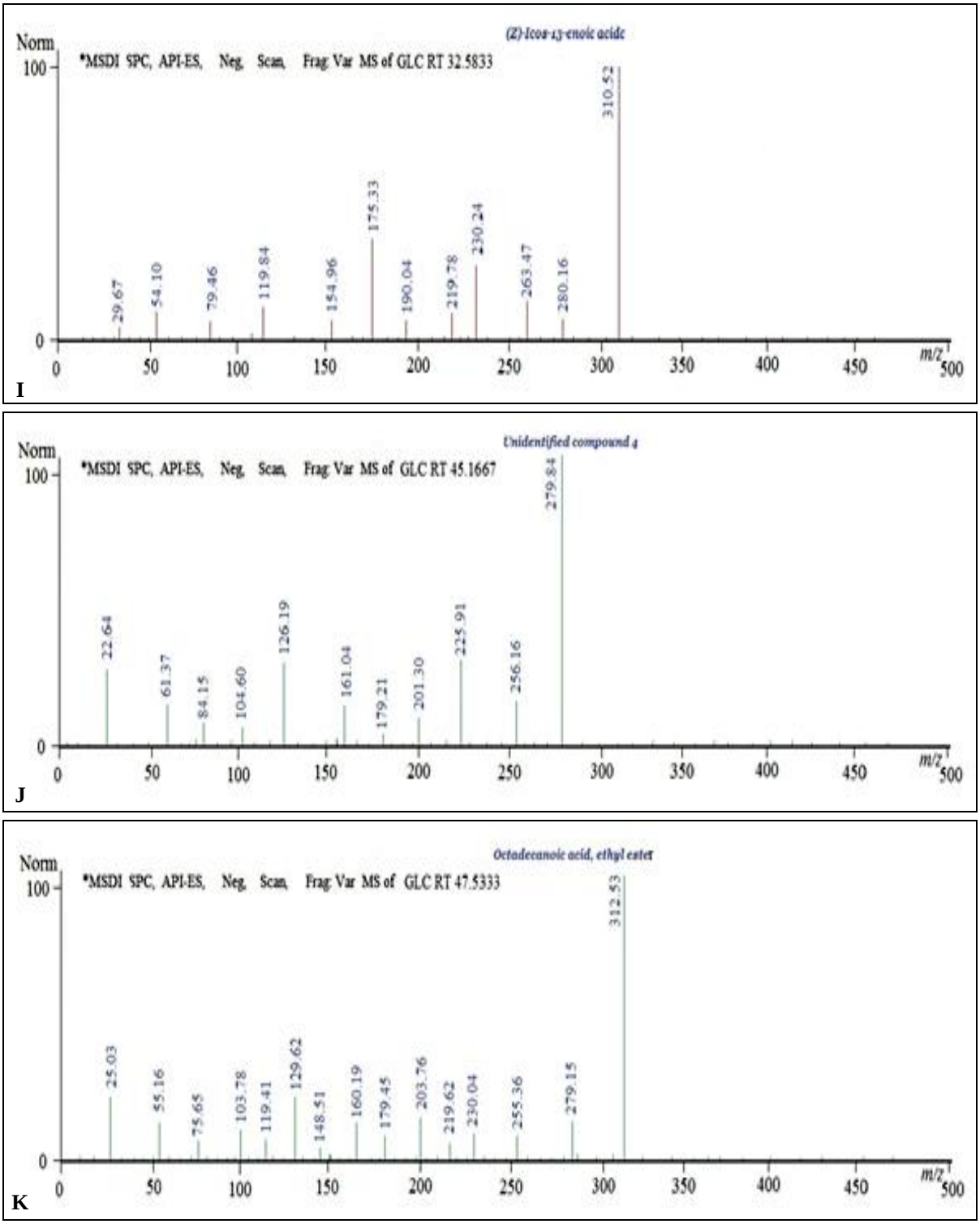


FIG. 4 (I-K): GC-MS CHROMATOGRAM OF PHYTOCHEMICALS IDENTIFIED IN METHANOLIC LEAF EXTRACT OF *L. ZEYLANICA*

Based on the results of phytochemical analysis Ethyl acetate and methanolic crude extracts were selected for antimicrobial activity. In the present study highest zone of inhibition was observed in ethyl acetate extract (200 µl) against *Lactobacillus acidophilus* (18 mm), whereas methanolic extract (200 µl) showed the highest zone of inhibition against *Candida albicans* (18 mm) **Plate 1 A-D**; **Table 4**. No zone of inhibition observed against both extracts for *Pencillium citrinum*. The degree of solubility of orchid phytoconstituents differs for various solvents [21]. The present work shows significant similarities with various antimicrobial activities of orchids [22-25].

TABLE 2: BIOACTIVE COMPOUNDS PRESENT IN ETHYL ACETATE EXTRACT OF *L. ZEYLANICA* BY USING GC-MS ANALYSIS

S. no.	Retention time (min)	Peak area %	Name of the compound	Molecular formula	Molecular weight	Biological activity
1	2.1333	8.37	2,2-Dimethyl-3-propyloxirane	$C_7H_{14}O$	114.188g/mol	No activity
2	3.5667	4.33	Hydroperoxide, 1-ethylbutyl	$C_6H_{14}O_2$	118.176g/mol	No activity
3	5.5167	13.8	Ethanone, 1-cyclohexyl-	$C_8H_{14}O$	126.199g/mol	No activity
4	7.4500	4.44	Cyclopentanol, 1 methyl	$C_6H_{12}O$	100.161g/mol	No activity
5	8.7333	6.26	Cyclopentanol, 1 methyl	$C_{18}H_{32}O$	264.453g/mol	Antioxidant & Antibacterial[26]
6	12.5162	.94	4-Methyl-1,3-dioxane	$C_5H_{10}O_2$	102.133g/mol	Methyl guanidine inhibitor[27]
7	14.800	10.02	5-Oxotetrahydrofuran-2-carboxylic acid	$C_5H_6O_4$	130.099g/mol	Acidifier and arachidonic acid inhibitor [27]
8	24.7667	13.93	Unidentified compound 1	-	-	-
9	30.5667	3.92	Methyl cis-10-heptadecenoate	$C_{18}H_{34}O_2$	282.468g/mol	Catechol-O-Methyl Transferase inhibitor[27]
10	37.4000	3.72	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.522g/mol	Anticancer and antitumour [27]
11	46.0500	6.85	Triacontane	$C_{30}H_{62}$	422.826g/mol	Antibacterial, Antidiabetic and antitumour[28]
12	42.5333	18.04	Unidentified compound 2	-	-	-
13	53.3667	0.61	Methyl 15-methylhexadecanoate	$C_{18}H_{36}O_2$	284.484g/mol	Catechol-O-Methyl Transferase inhibitor[27]
14	56.6333	4.77	Unidentified compound 3	-	-	-

TABLE 3: BIOACTIVE COMPOUNDS PRESENT IN METHANOLIC EXTRACT OF *L. ZEYLANICA* BY USING GC-MS ANALYSIS

S. no.	Retention time (min)	Peak area %	Name of the compound	Molecular formula	Molecular weight	Biological activity
1	4.6500	23.37	(E)-1-[Bis[(E)-but-2-enoxy]methoxy]but-2-ene	$C_{13}H_{22}O_3$	226.316 g/mol	No activity reported
2	6.2333	9.00	Unidentified compound 1	-	-	-
3	9.7500	1.92	Kaempferol 3-glucoside	$C_{21}H_{20}O_{11}$	448.38 g/mol	Anti-inflammatory, antioxidant, anti-cancer, anti-diabetic [29]
4	12.2167	3.9	n-Tridecanoic acid methyl ester	$C_{14}H_{28}O_2$	228.376 g/mol	Antitumour [27]
5	17.8333	2.01	Unidentified compound 2	-	-	-
6	19.9500	14.01	1,4-Dimethyl-1,4,6,7-tetrahydroimidazo[4,5-e][1,4]diazepine-5,8-dione	$C_8H_{10}N_4O_2$	194.191 g/mol	No activity
7	24.3500	5.02	Phthalic acid, butyl hexyl ester	$C_{18}H_{26}O_4$	306.402 g/mol	Antimicrobial
8	29.9500	21.65	Unidentified compound 3	-	-	-
9	32.5833	0.83	(Z)-Icos-13-enoic acid	$C_{20}H_{38}O_2$	310.522 g/mol	Increase Zinc bioavailability[27]
10	45.1667	11.74	Unidentified compound 4	-	-	-
11	47.5333	6.55	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.538 g/mol	Antiviral, antibacterial and anticancer[30]

TABLE 4: ANTIMICROBIAL STUDY SHOWING ZONE OF INHIBITION OF SOLVENT EXTRACTS OF *L. ZEYLANICA*

Microorganism	Ethyl acetate extraxt				Methanolic extract			
	Zone of inhibition (mm)							
	50µl	100µl	150µl	200µl	50µl	100µl	150µl	200µl
Bacillus megaterium	7±0.12	10±0.15	12±0.05	14±0.11	-	4±0.09	5±0.11	7±0.07
Lactobacillus acidophilus	7±0.14	9±0.09	13±0.13	18±0.12	-	3±0.07	7±0.14	9±0.11
Klebisiella pneumoniae	5±0.09	7±0.11	8±0.14	1±0.07	3±0.09	4±0.14	6±0.12	7±0.07
Escherichia coli	6±0.11	8±0.07	8±0.09	11±0.13	-	4±0.12	5±0.19	6±0.09
Enterococcus faecalis	5±0.13	8±0.13	11±0.10	13±0.07	3±0.11	4±0.09	6±0.12	7±0.11
Proteus vulgaris	6±0.15	9±0.12	10±0.09	17±0.15	-	2±0.11	4±0.09	5±0.07
Candida albicans	7±0.10	9±0.12	11±0.15	14±0.05	3±0.12	6±0.05	11±0.07	18±0.15
Aspergillus flavus	3±0.09	5±0.11	8±0.13	13±0.14	-	-	-	-
Penicillum citrinum	-	-	-	8±0.13	-	-	-	-

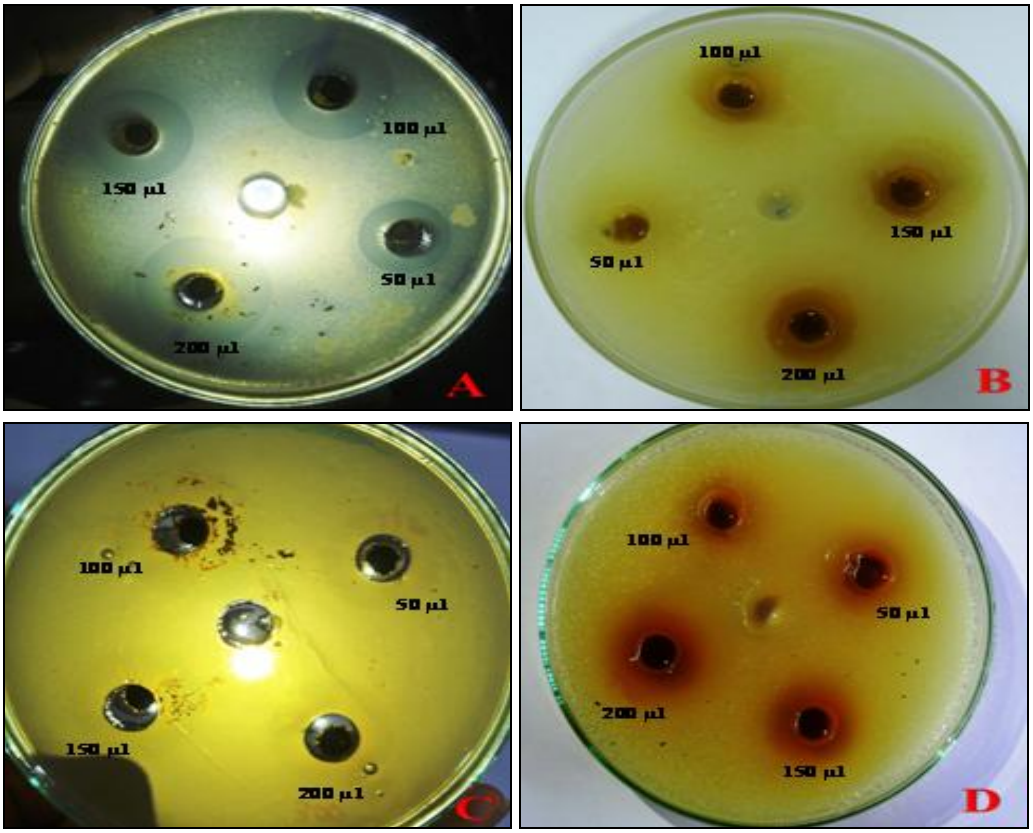


PLATE 1A-D: ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE AND METHANOLIC EXTRACTS OF *L. ZEYLANICA* ON *LACTOBACILLUS ACIDOPHILUS* (A & C), ON *CANDIDA ALBICANS* ON (B & D)

Anticancer Activity: Medicinal orchids have a significant role in the prevention of cancer and its treatment [31, 32, 33] IC_{50} value is less than 1000µg/ml for crude plant extract is toxic, while non-toxic (inactive) if it is greater than 1000 µg/ml [34]. In our study, the death rate of MCF and HeLa cell lines increase with a rise in the concentration of *L. zeylanica* leaf extract. Anticancer activity of ethyl acetate and methanolic leaf extract on MCF-7 and HeLa cell lines were shown in **Plate 2A** and **2B; Plate 3A** and **3B.** The viability percentage of MCF-7 cell line of ethyl acetate and methanolic leaf extracts at concentration 100 µg/ml reduced from 100% to 39.65% and 36.39% respectively **Table 5**. Similarly, for HeLa cell lines it was reduced to 44.44% and 40.93% in **Table 6**.

TABLE 5: CYTOTOXIC PROPERTIES OF ETHYL ACETATE EXTRACT OF *L. ZEYLANICA* ON MCF -7 AND HELA CELL LINES

Cell line	Concentration (µg/ml)	Absorbance at 570 nm			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
MCF-7	100	0.821	0.823	0.825	0.823	0.816	39.65	
	75	0.915	0.917	0.918	0.916	0.909	44.169	
	50	1.043	1.045	1.047	1.045	1.038	50.437	
	25	1.098	1.101	1.102	1.1	1.093	53.109	
	10	1.189	1.191	1.193	1.191	1.184	57.531	48.439
	5	1.245	1.247	1.249	1.247	1.24	60.252	
	Untreated	2.065	2.066	2.065	2.065	2.058	100	
	Blank	0.007	0.008	0.007	0.007	0		
HeLa	100	0.851	0.853	0.855	0.853	0.848	44.444	
	75	0.935	0.936	0.938	0.936	0.931	48.794	
	50	0.995	0.997	0.999	0.997	0.992	51.991	
	25	1.079	1.081	1.083	1.081	1.076	56.394	67.914
	10	1.186	1.188	1.189	1.187	1.182	61.949	
	5	1.272	1.274	1.276	1.274	1.269	66.509	
	Untreated	1.913	1.914	1.913	1.913	1.908	100	
	Blank	0.005	0.006	0.005	0.005	0		

TABLE 6: CYTOTOXIC PROPERTIES OF METHANOLIC LEAF EXTRACT OF *L. ZEYLANICA* 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	0.754	0.756	0.758	0.756	0.749	36.394	
	75	0.812	0.814	0.816	0.814	0.807	39.212	
	50	0.885	0.887	0.889	0.887	0.88	42.76	
	25	0.975	0.977	0.978	0.976	0.969	47.084	
	10	1.062	1.064	1.066	1.064	1.057	51.36	18.360
	5	1.133	1.135	1.137	1.135	1.128	54.81	
	Untreated	2.065	2.066	2.065	2.065	2.058	100	
	Blank	0.007	0.008	0.007	0.007	0		
HeLa	75	0.842	0.844	0.845	0.843	0.838	43.92	
	50	0.953	0.955	0.957	0.955	0.95	49.79	
	25	1.025	1.027	1.028	1.026	1.021	53.511	
	10	1.096	1.098	1.099	1.097	1.092	57.232	49.497
	5	1.295	1.297	1.299	1.297	1.292	67.714	
	Untreated	1.913	1.914	1.913	1.913	1.908	100	
	Blank	0.005	0.006	0.005	0.005	0		
	100	0.754	0.756	0.758	0.756	0.749	36.394	

Results indicate methanolic leaf extract against the MCF-7 cell line was found to suppress cell proliferation and it showed good cytotoxicity when compared to HeLa cell lines. The lowest IC_{50} value 18.36 µg/ml observed for methanolic leaf extract on MCF-7 cell lines. It indicates that the inhibitory effect of methanolic leaf extract of *L. zeylanica* on breast cancer lines at different concentrations was found as potential chemotherapeutic agents to induce apoptosis in cancer cells.

The present results were supported by previous anticancer studies on orchids [8, 35]. Hence, the findings of this study proved that leaf extract of *L. zeylanica* has an anti-cancer effect and this species could be used to develop anticancer drugs.

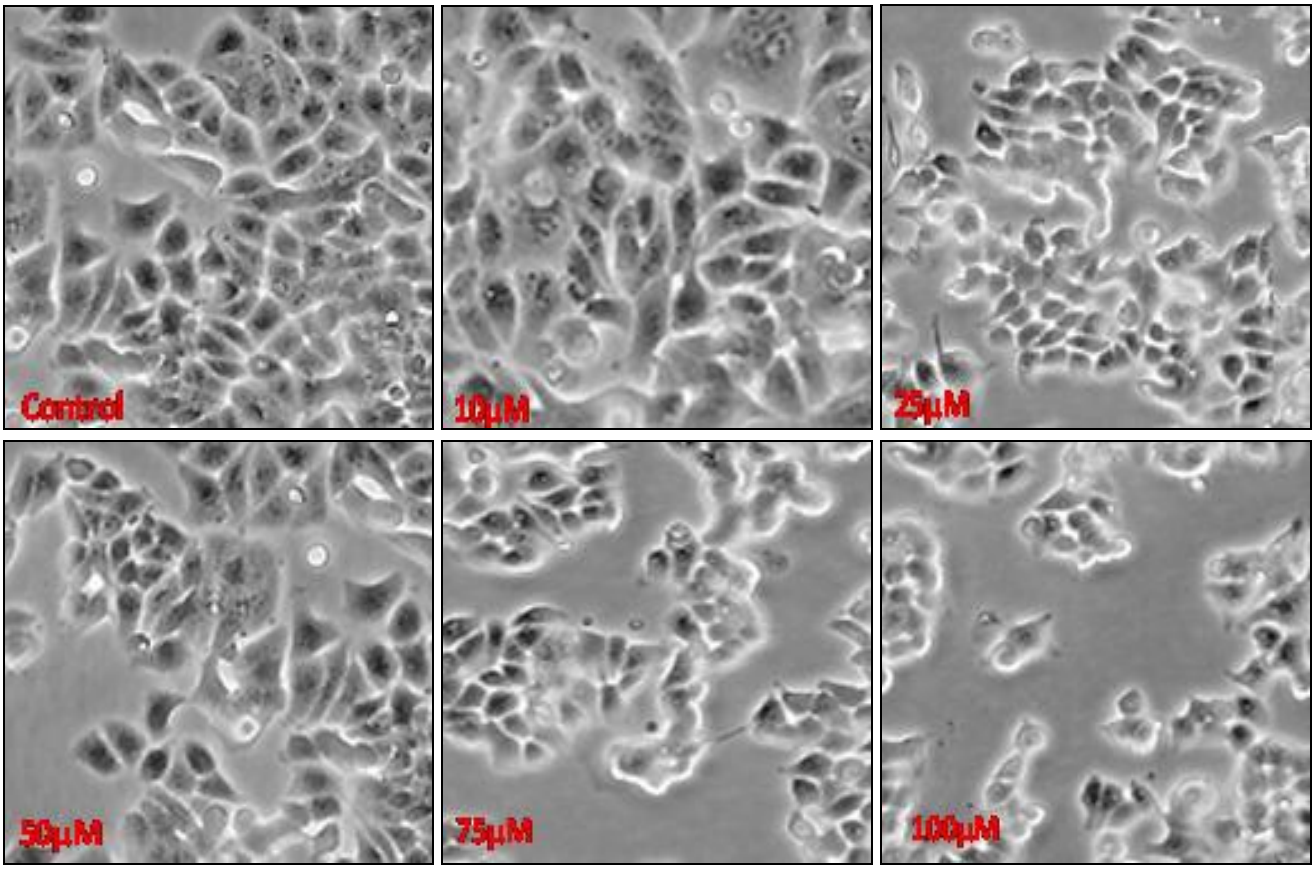


PLATE 2A: ANTICANCER ACTIVITY OF ETHYL ACETATE EXTRACT OF *L. ZEYLANICA* ON HeLa CELL LINE

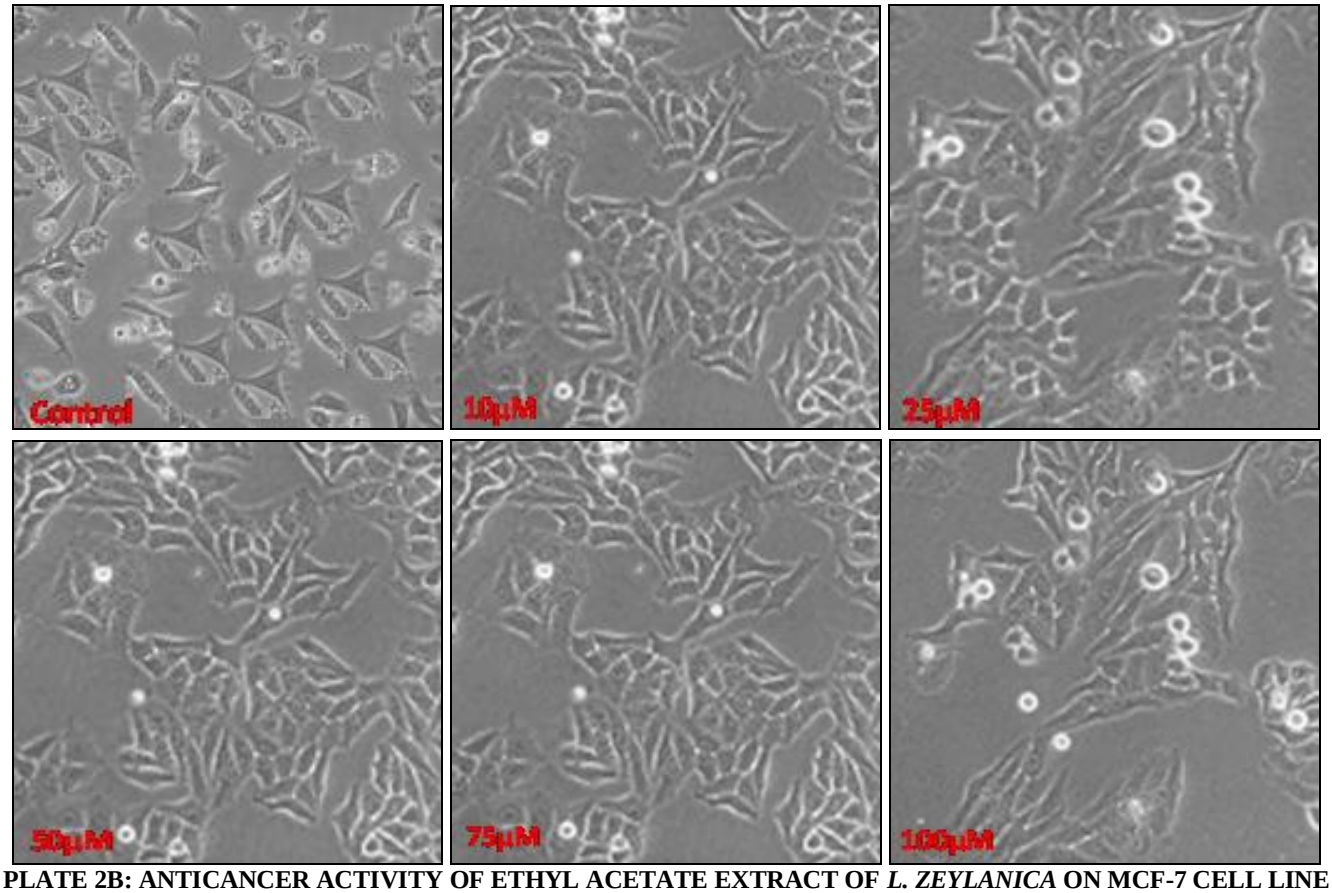


PLATE 2B: ANTICANCER ACTIVITY OF ETHYL ACETATE EXTRACT OF *L. ZEYLANICA* ON MCF-7 CELL LINE

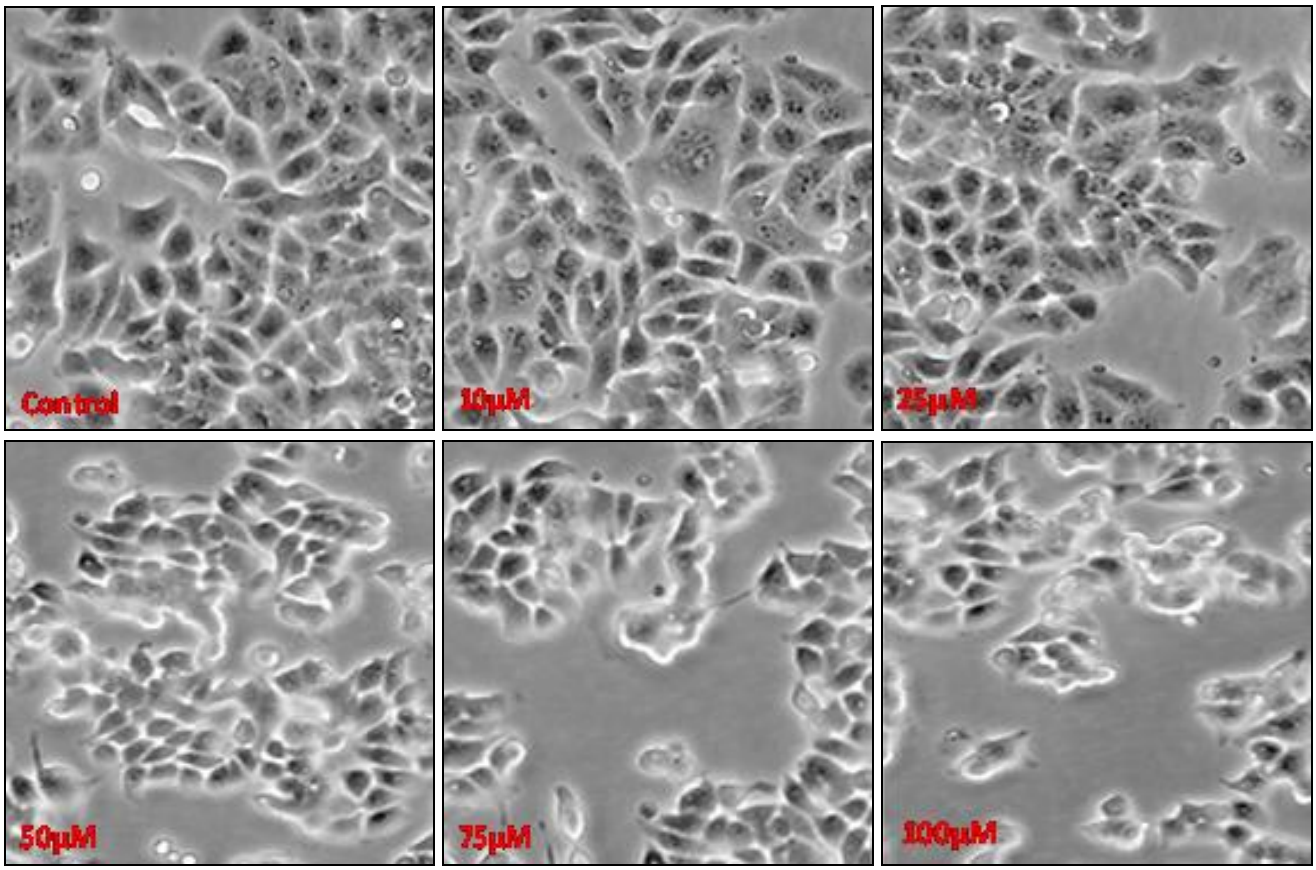


PLATE 3A: ANTICANCER ACTIVITY OF METHANOL EXTRACT OF *L. ZEYLANICA* ON HeLa CELL LINE

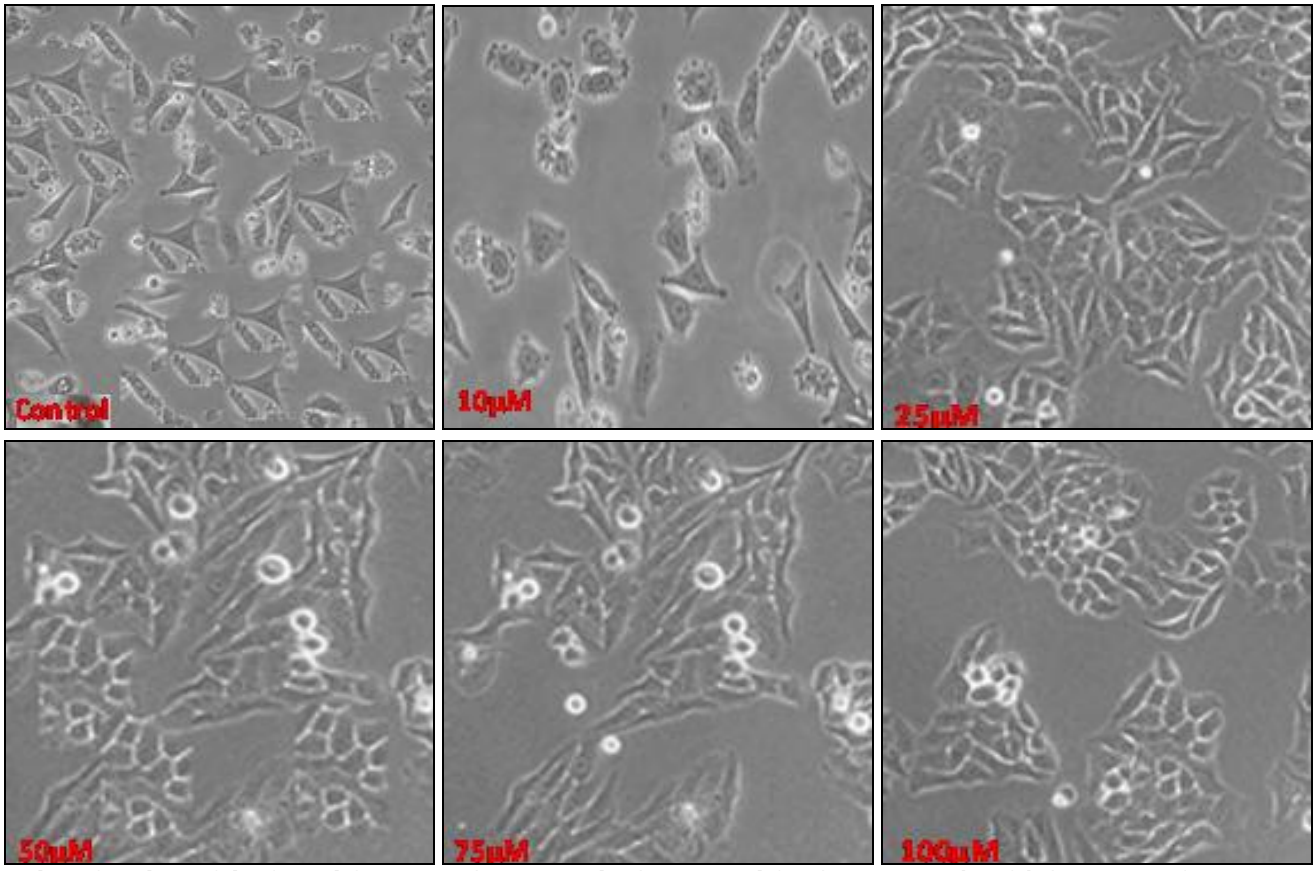


PLATE 3B: ANTICANCER ACTIVITY OF METHANOL EXTRACT OF *L. ZEYLANICA* ON MCF-7 CELL LINE

CONCLUSION: Phytochemical analysis of epiphytic orchid *L. zeylanica* confirmed the presence of bioactive compounds. The various solvent extracts of proved conclusively anti-microbial and *in-vitro* anticancer activity on MCF-7 and HeLa cell lines. Further studies should be done to isolate the unknown compounds present in the extract and develop novel drugs to treat cancer.

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