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LUNAR IMPACT ON BIOSYNTHESIS OF SECONDARY METABOLITS IN PLANTS *Clerodendrum inerme*(L.).Gaertn.

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Abstract:
The moon is the only natural satellite revolving around our earth and has some influence.The gravitational forces cause periodic changes to the water levels on the Earth's surface. From the ancient period, the peoples are linked the moon with the religious myth.The sacredness of the moon has been connected with the basic rhythms of life and the universe. Lot of studies find out that the influence of full moon and new moon on animals and plants. Hence, the present study has been programmed to evaluate the lunar impact on the phytocompounds of plants Clerodendrum inerme(L.).Gaertn. The results of the present study showed the phytocompounds appearance was differed in different lunar periods. Quantitatively, the amount of phytocompounds in full moon periods was comparatively high when compared to new moon period.
Key Words: New moon, Full moon, Phytocompounds, FTIR,Clerodendrum inerme(L.).Gaertn, Lunar impact.

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INTRODUCTION:

Even though the scientific evidences are meager, the lunar phenomena are universally accepted from the ancient period and it has lot of stories. People believe that abnormal human behavior peaks around the full moon period and new moon period. i.e., the increasing of intensity of psychotic disorders, violence and other deviant behavior such as homicides, emergency hospital admissions, and crisis incidents were found by researcher. Usually, the full moon and dark moon influences are not only in man and also in animals, plants and non living things.In animals, the lunar cycle may affect hormonal changes early in phylogenesis of insects, reproduction the hypothalamus-pituitary-gonadal axis of fishes, daily variations in melatonin and corticosterone disappear during full-moon days of birds. The lunar cycle also exerts its effects on taste sensitivity, Serum melatonin levels,synaptic bodies, ultra structural organellesand the ultrastructure of pineal gland cells, magnitude of the humoral immune response in laboratory rats and sheep erythrocytes (Martinez-Soriano *et al*, 2002). It is suggested that melatonin and endogenous steroids may mediate the described cyclic alterations of physiological processes.(Zimecki,2006),In the Indian palm squirrel *Funambulus pennanti*, seasonal changes in several immune parameters, such as total blood leukocytes, blastogenic response of blood, and thymus and spleen lymphocytes were studied (Haldar *et al*,2001).Slight but statistically significant lunar rhythmicities were observed in the germination of European Spruce (*Picea abies* Karst.). In this plant rate of germination was differed from sowings shortly before Full Moon, compared to those shortly before New Moon (Zürcher,1992) Hence, the present has been programmed to evaluate the lunar influence such as new moon and full moon impact on plant phytocompounds of *Clerodendrum inerme*(L.).Gaertn.

MATERIALS AND METHOD:

Collection and preparation of plant material

Leaves of plant *Clerodendrum inerme* is harvested from Thiruvalluvar University campus,Vellore,Tamilnadu,India(latitude 13°1’47.79″N, longitude 49°12′40.34°″E). The plant *Clerodendrum inerme* is growing enormously just like a fence line on both side of road inside the campus for ornamental purpose. The harvesting for the present study was performedonly at night time - 10 P.M of full moon, new moon and, in-between full moon and new moon days of April -2016(Summer month) and bring immediately to laboratory. The dirty materials, dust particles, insects eggs adhere with the plants were removed by washing with double distilled water. These cleaned leaves were shade dried at room temperature (30-30°C) for 10 days period to avoid the destruction of phytocompounds.After the complete removal of moisture content, the dried leaves were grounded well upto get fine powder. The powder of *Clerodendrum inerme*was packed inside the thimble of Soxhlet and plant extract were obtained by using solvent methanol. The plant extract obtained from leaves collected from both full moon and new moon period was taken to GC/MS analysis using Turbo Pro.

RESULTS AND DISCUSSION:

The lunar cycle has an impact on human reproduction, in particular fertility, menstruation, and birth rate.(.Climatic (abiotic) factors often have an especially large influence on the biosynthetic levels and quality of secondary metabolites in plants (Coley, 1987).The seeds of vegetable, floral, and cereal species (lettuce [Lactuca sativa], white cabbage [Brassica oleracea], leek [Allium porrum], tomato [Lycopersicon esculentum], pea [Pisum sativum], common bean [Phaseolus vulgaris], lovage [Levisticum officinale], yarrow [Achillea millefolium], lemon balm [Melissa officinalis], monkshood [Aconitum napellus], corn [Zea mays], wheat [Triticum spp.], oats [Avena sativa], barley [Hordeum vulgare]) sown 2 days before the full moon showed better germination and stronger growth, formed more numerous inflorescences, and produced a better harvest than the seeds sown 2 days before the new moon.(Kolisko,1927,1929,1934 and 1935).The cytokinins, a group of plant growth hormones, vary with maxima at different lunar phases in the algae Ecklonia maxima and Sargassum heterophyllum. (Endres and Schad ,1997). In the present study, the GCMS analysis was done for the methanolic extract of leaf ofplant *Clerodendrum inerme* collected from full moon, new moon and normal days in between full moon and new moondays to study about the influence of new moon and full moon on biotic phytocompounds synthesis.According to the table data, *Clerodendrum inerme* leaves collected from full moon days exhibited 16 peaks at different retention time such as 5.850., 8.661.,10.341.,10.406.,10.496.,12.737.,13.693.,13.743.,14.214.,16.214.,16.384.,16.654.,18.139.,19.650.,19.810 and 27.018 indicates the presence of phytocompounds 3,7-Diacetamido-7h-S-Triazolo[5,1-C]-S-Triazole.,4h-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-.,2-Furancarboxaldehyde, 5-(Hydroxymethyl)-., 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-., 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-., 8-Quinolinol, Acetate., Sucrose., Sucrose., N-Propyl

Heptyl Ether., Endo-2,3-O-Ethylidene-.Beta.-D-Erythrofuranose., 3-Deoxy-D-Mannoic Lactone., 16-Heptadecenal., N-Hexadecanoic Acid., 2-Oxovaleric Acid, Trimethylsilyl Ester., Propanoic Acid, 2-Oxo-, Trimethylsilyl Ester and Sulfurous Acid, 2-Propyl Tridecyl Ester. Among these 16 compounds, the compounds appeared at retention time 19.810 showed the maximum area % 19.186 which indicates the presence of Propanoic Acid, 2-Oxo-, Trimethylsilyl, Ester in the methanol extract of leaf of *Clerodendrum inerme*in maximum amount. The compoundsEndo-2,3-O-Ethylidene-.Beta.-D-Erythrofuranose appeared at retention time 16.214, 8-Quinolinol, Acetate at retention time 12.737, 4H-Pyran-4-one, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl at RT 8.661 and 3-Deoxy-D-Mmannoic Lactone at RT 6.384 have the area % 13.654, 8.393 and 7.039 respectively indicated as second, third and forth large proportion of these compounds in the phytocompounds analysis. The phytocompounds traced out from the methanol extract of leaf of *Clerodendrum inerme*collected fromdark moon showed 14 compoundssuch as4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl., 2,4-Monoethylidene-l-xylitol., 3,7,11,15-Tetramethyl-2-hexadecen-1-ol., 3,7,11,15-Tetramethyl-2-hexadecen-1-ol., Pentadecanoic acid, 14-methyl-, methyl ester., Hexadecanoic acid., 11,14,17-Eicosatrienoic acid, methyl ester., 3-Heptadecen-5-yne, (z)-., Cyclohexanecarboxylic acid, 2-ethylhexyl ester., Squalene., Sulfurous acid, 2-Propyl Tridecyl Ester and Sulfurous acid, Pentadecyl 2-Propyl Ester respectively**.**Among these 14 compounds, the three phytocompounds such as 3-Heptadecen-5-yne (22.235%), (z)- at RT 19.839, Sucrose (15.669%) at RT 13.498 and Hexadecanoic acid (11.566%) at RT 13.498 appeared at first three highest percentages.i.e., The phytocompound analysis of the same plants at normal days (Days in-between full moon and dark moon) showed the existence of total of 16 compounds.Among these, the compounds such as N-Hexadecanoic acid (16.356%) at RT 18.15, 2,5-monomethylene-L-Rhamnitol (13.581%) at RT 13.568, 8-Quinolinol (12.329%) at RT 12.997, acetate proportion were present in first three position.Overall comparison of GCMS results of all three full moon (FM), new moon (NM) and normal days (ND) collected leaf extract revealedthe appearing and disappearing of some compounds. This appearance and disappearance of phytocompounds may be decided by the external climatic changes and influence of some vital forces comes from moon. Ebb and flood tides are due to lunar impact showed great diurnal variations. The same ebb and flood happened due to perigee very high when compared to diurnal variations. It is believed to same thing is happening in human and plant bodies because of both are made up of 70% of water. The water from the oceans rises to reach a maximum height, and then goes down to a minimum level, maintaining this oscillation regularly and successively. It has also been checked that this phenomenon makes itself felt in plant sap". (Translated from Restrepo,2004),It is believed to same thing is happening in human and plant bodies because of both are made up of 70% of water. The Moon is strongly connected with water; the full Moon brings more water to the plant than the new Moon" (Gabriel, 1988).Rounds (1982) found a semilunar periodicity of neurotransmitter-like substances from heart-stimulating plants, applied to the cockroach (Periplaneta africana). The weakest stimulation by the extracts of the investigated plants was, when picked at short time before new and full moon, indicating a semilunar oscillation.But in the present investigation no any significant variation in number of plant compound formation in all three full moon, new moon and normal days. But the phytocompounds appearance was differed in different lunar periods. Quantitatively, the amount of phytocompounds in full moon periods was comparatively high when compared to new moon period.

Fig 1. Chromatogram of methanol leaf extract of *Clerodendrum inerme* collected infull moon days.

Qualitative Report

File: C:\TurboMass\2016.PRO\Data\FM-(16ES-0225)-.raw
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Description:
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Sample ID: FM-(16ES-0225)- Vial Number: 4

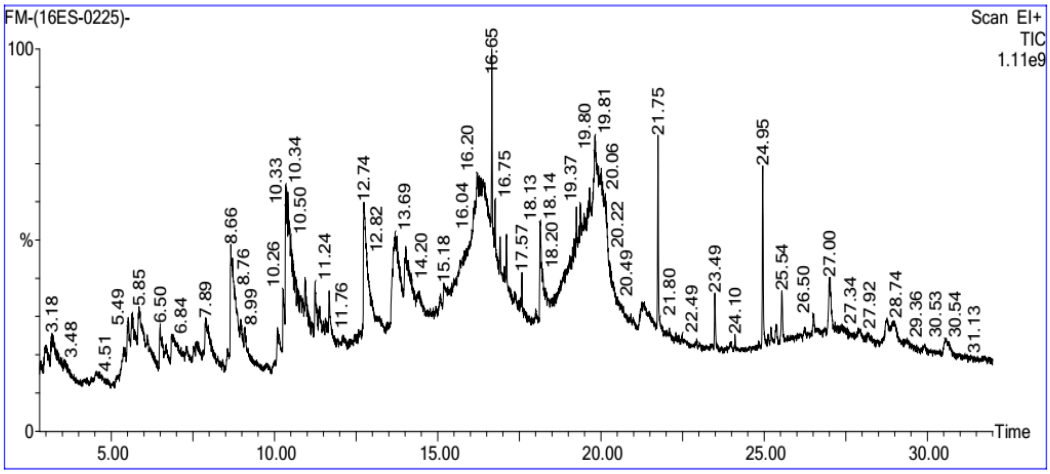


Table 2. Retention time, height,area and area percentages of spectra peaks for methanol leaf extract of plant *Clerodendrum inerme* collected infull moon days.

#	RT	Scan	Height	Area	Area %	Norm %
1	5.850	610	110,520,728	12,258,047.0	2.327	12.13
2	8.661	1172	319,174,880	44,209,404.0	8.393	43.74
3	10.341	1508	399,373,248	25,892,396.0	4.916	25.62
4	10.406	1521	369,147,488	21,632,422.0	4.107	21.40
5	10.496	1539	228,156,816	22,902,458.0	4.348	22.66
6	12.737	1987	364,795,200	51,767,324.0	9.828	51.22
7	13.693	2178	238,873,456	27,155,058.0	5.155	26.87
8	13.743	2188	224,920,352	25,031,006.0	4.752	24.77
9	14.018	2243	172,927,664	27,895,472.0	5.296	27.60
10	16.214	2682	242,864,576	68,683,904.0	13.039	67.96
11	16.384	2716	207,186,096	37,013,040.0	7.027	36.62
12	16.654	2770	557,806,912	16,293,645.0	3.093	16.12
13	18.139	3067	273,578,368	19,057,036.0	3.618	18.86
14	19.650	3369	137,393,456	10,648,973.0	2.022	10.54
15	19.810	3401	318,315,488	101,063,632.0	19.186	100.00
16	27.018	4842	163,319,152	15,242,618.0	2.894	15.08

Table 3. GCMS analysis of phytocompounds present in the methanol leaf extract of *Clerodendrum inerme* collected infull moon days.

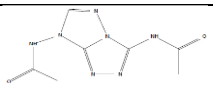
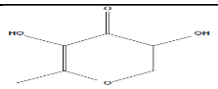
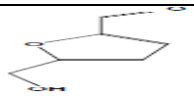
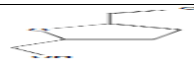
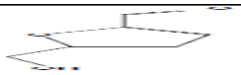
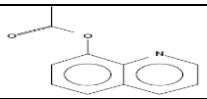
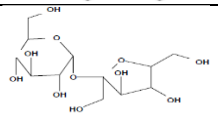
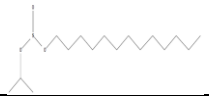
S.No	Name of the compound	Retentin time	Height	Area	Structure
1,	3,7-DIACETAMIDO-7H-S-TRIAZOLO[5,1-C]-S-RIAZOLE	5.850	110,520,728	12,258,047.0	
2.	4H-PYRAN-4-ONE, 2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL	8.661	319,174,880	44,209,404.0	
3.	2-URANCARBOXALDEHYDE, 5-(HYDROXYMETHYL)-	10.341	399,373,248	25,892,396.0	
4.	2-URANCARBOXALDEHYDE, 5-(HYDROXYMETHYL)-	10.406	369,147,488	21,632,422.0	
5.	2-URANCARBOXALDEHYDE, 5-(HYDROXYMETHYL)-	10.496	228,156,816	22,902,458.0	
6.	- 8-QUINOLINOL, ACETATE	12.737	364,795,200	51,767,324.0	
7.	SUCROSE	13.693	238,873,456	27,155,058.0	
8.	SUCROSE	13.743	224,920,352	25,031,006.0	
9.	N-PROPYL HEPTYL ETHER	14.018	172,927,664	27,895,472.0	
10.	ENDO-2,3-O-ETHYLIDENE-.BETA.-D-ERYTHROFURANOSE	16.214	242,864,576	68,683,904.0	
11.	3-DEOXY-D-MANNOIC LACTONE	16.384	207,186,096	37,013,040.0	
12.	16-HEPTADECENAL	16.654	557,806,912	16,293,645.0	
13.	N-HEXADECANOIC ACID	18.139	273,578,368	19,057,036.0	
14.	2-OXOVALERIC ACID, TRIMETHYLSILYL ESTER	19.650	137,393,456	10,648,973.0	
15.	PROPANOIC ACID, 2-OXO-, TRIMETHYLSILYL ESTER	19.810	318,315,488	101,063,632.0	
16.	SULFUROUS ACID, 2-PROPYL TRIDECYL ESTER	27.018	163,319,152	15,242,618.0	

Fig 2. Chromatogram of methanol leaf extract of *Clerodendrum inerme* collected inNew moon days.

Qualitative Report

File: C:\TurboMass\2016.PRO\Data\DM-(16ES-0224)-.raw
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Sample ID: DM-(16ES-0224)- Vial Number: 3

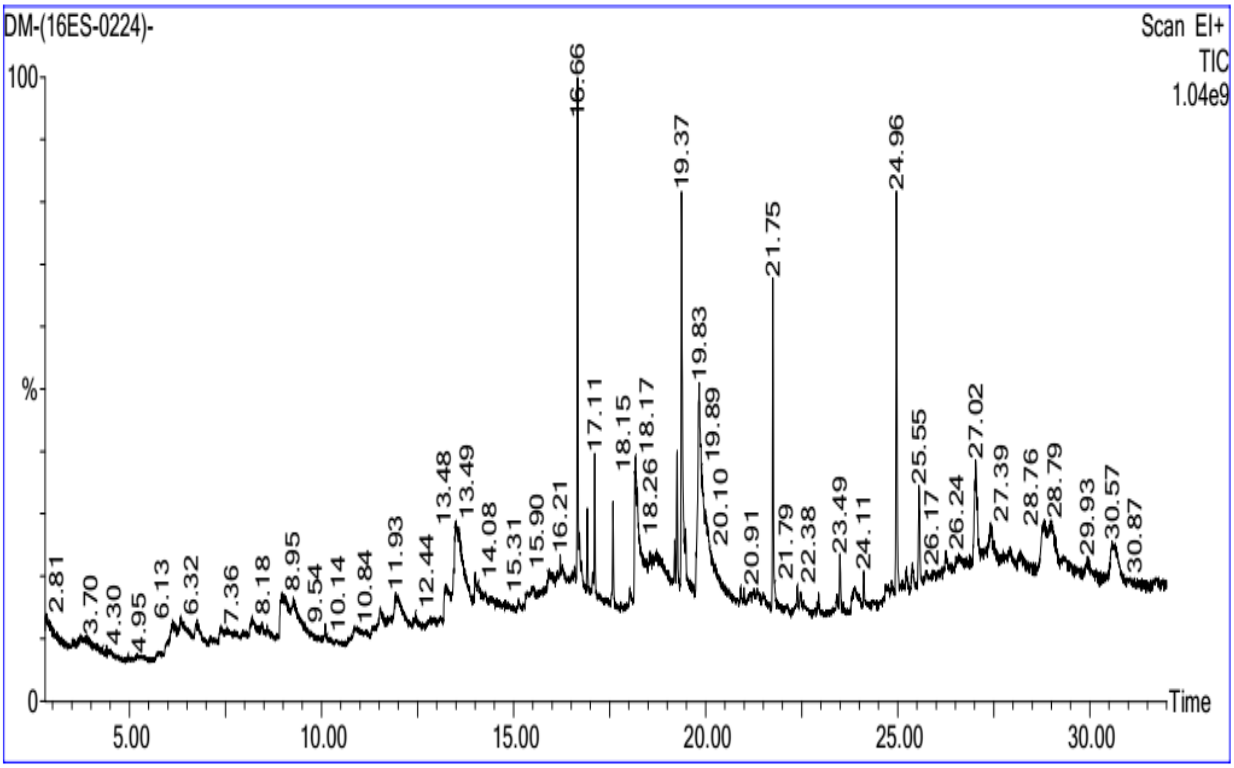


Table 4. Retention time, height,area and area percentages of spectra peaks for methanol leaf extract of plant *Clerodendrum inerme* collected inNew moon days

#	RT	Scan	Height	Area	Area %	Norm %
1	8.971	1234	60,823,800	8,679,013.0	3.335	15.00
2	13.238	2087	64,220,168	9,139,906.0	3.512	15.79
3	13.498	2139	156,637,840	40,783,648.0	15.669	70.47
4	16.664	2772	809,399,936	17,919,602.0	6.885	30.96
5	17.109	2861	225,291,344	5,264,193.5	2.023	9.10
6	17.584	2956	176,425,504	5,353,934.0	2.057	9.25
7	18.170	3073	235,648,688	30,104,218.0	11.566	52.02
8	19.255	3290	220,151,936	6,321,535.0	2.429	10.92
9	19.370	3313	635,014,464	23,238,802.0	8.928	40.16
10	19.830	3405	346,217,312	57,871,948.0	22.235	100.00
11	21.746	3788	542,666,560	13,967,585.0	5.366	24.14
12	24.962	4431	663,724,544	19,765,716.0	7.594	34.15
13	25.547	4548	168,450,448	7,231,300.0	2.778	12.50
14	27.023	4843	172,247,744	14,635,936.0	5.623	25.29

Table 5. GCMS analysis of phytocompounds present in the methanol leaf extract of *Clerodendrum inerme* collected inNew moon days.

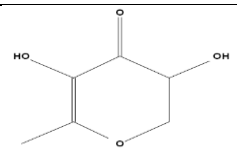
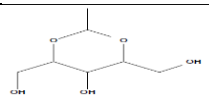
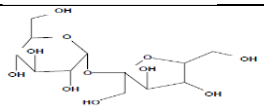
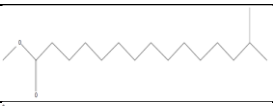
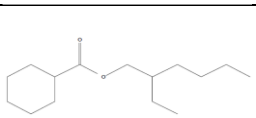
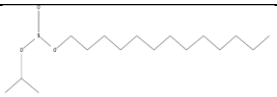
.No	Name of the compound	Retentin time	Height	Area	Structure
1,	4H-PYRAN-4-ONE, 2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL	8.971	60,823,800	8,679,013.0	
2.	2,4-MONOETHYLIDENE-L-XYLITOL	13.238	64,220,168	9,139,906.0	
3.	SUCROSE	13.498	156,637,840	40,783,648.0	
4.	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	16.664	16.664	17,919,602.0	
5.	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	17.109	225,291,344	5,264,193.5	
6.	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	17.584	176,425,504	5,353,934.0	
7.	HEXADECANOIC ACID	18.170	235,648,688	30,104,218.0	
8.	11,14,17-EICOSATRIENOIC ACID, METHYL ESTER	19.255	220,151,936	6,321,535.0	
9.	PHYTOL	19.370	635,014,464	23,238,802.0	
10.	3-HEPTADECEN-5-YNE, (Z)-	19.830	346,217,312	57,871,948.0	
11.	CYCLOHEXANECARBOXYLIC ACID, 2-ETHYLHEXYL ESTER	21.746	542,666,560	13,967,585.0	
12.	SQUALENE	24.962	663,724,544	19,765,716.0	
13.	SULFUROUS ACID, 2-PROPYL TRIDECYL ESTER	25.547	168,450,448	7,231,300.0	
14.	SULFUROUS ACID, PENTADECYL 2-PROPYL ESTER	27.023	172,247,744	14,635,936.0	

Fig 3. Chromatogram of methanol leaf extract of *Clerodendrum inerme* collected indays in between full moon and new moon (normal).

Qualitative Report

File: C:\TurboMass\2016.PRO\Data\ND-(16ES-0226)-.raw
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Description:
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Sample ID: ND-(16ES-0226)- Vial Number: 5

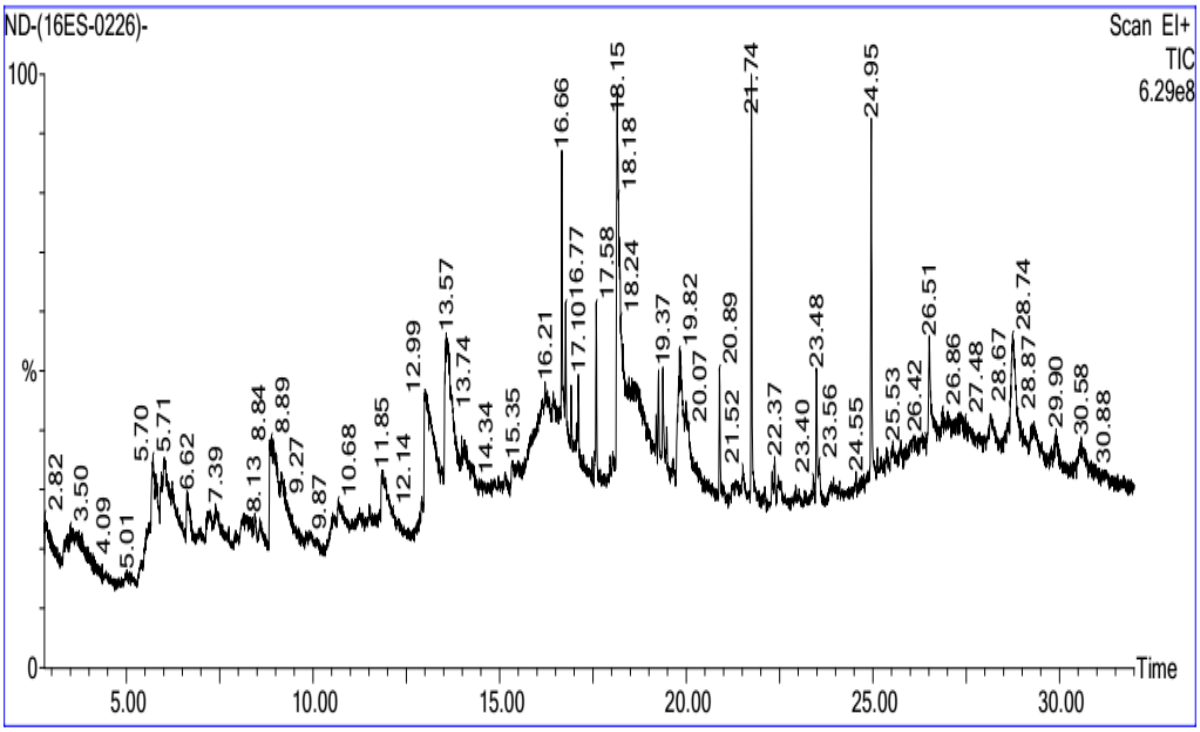
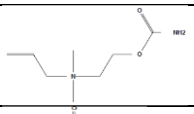
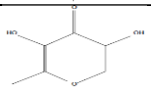
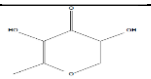
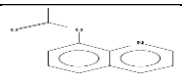
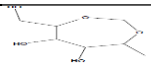
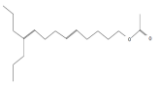
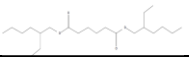
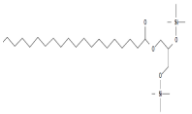
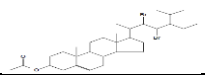


Table 6. Retention time, height,area and area percentages of spectra peaks for methanol leaf extract of plant *Clerodendrum inerme* collected days in between full moon and new moon (normal).

#	RT	Scan	Height	Area	Area %	Norm %
1	6.625	765	54,241,200	5,227,275.5	2.433	14.88
2	8.886	1217	104,532,496	8,453,328.0	3.934	24.07
3	8.926	1225	94,311,224	9,442,885.0	4.395	26.89
4	12.997	2039	125,813,552	26,489,114.0	12.329	75.42
5	13.568	2153	156,074,960	29,179,514.0	13.581	83.08
6	16.664	2772	281,303,072	7,362,653.0	3.427	20.96
7	17.584	2956	175,040,576	4,330,329.0	2.015	12.33
8	18.150	3069	390,173,760	35,120,884.0	16.346	100.00
9	18.305	3100	87,772,056	5,720,568.0	2.662	16.29
10	19.815	3402	141,982,992	19,620,714.0	9.132	55.87
11	19.990	3437	79,932,048	9,034,492.0	4.205	25.72
12	21.741	3787	442,725,472	12,155,333.0	5.657	34.61
13	23.482	4135	131,306,280	4,375,959.0	2.037	12.46
14	24.952	4429	379,093,824	12,886,386.0	5.998	36.69
15	26.508	4740	121,893,424	10,734,981.0	4.996	30.57
16	28.744	5187	108,611,632	14,723,902.0	6.853	41.92

Table 7. GCMS analysis of phytocompounds present in the methanol leaf extract of *Clerodendrum inerme* collected days in between full moon and new moon (normal).

S.No	Name of the compound	Retentin time	Height	Area	Structure
1.	3-AZONIA-5-HEXENE-1-OL, N,N-DIMETHYL-, CARBAMATE ESTER, BROMIDE	6.625	54,241,200	5,227,275.5	
2.	4H-PYRAN-4-ONE, 2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL	8.886	104,532,496	8,453,328.0	
3.	4H-PYRAN-4-ONE, 2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL	8.926	94,311,224	9,442,885.0	
4.	8-QUINOLINOL, ACETATE	12.997	125,813,552	26,489,114.0	
5.	2,5-MONOMETHYLENE-L-RHAMNITOL	13.568	156,074,960	29,179,514.0	
6.	E-2-TETRADECEN-1-OL	16.664	281,303,072	7,362,653.0	
7.	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	17.584	175,040,576	4,330,329.0	
8.	N-HEXADECANOIC ACID	18.150	390,173,760	35,120,884.0	
9.	N-HEXADECANOIC ACID	18.305	87,772,056	5,720,568.0	
10.	PROPYLURE	19.815	141,982,992	19,620,714.0	
11.	SPIRO[ANDROST-5-ENE-17,1'-CYCLOBUTAN]-2'-ONE, 3-HYDROXY-, (3.BETA.,17.BE	19.990	79,932,048	9,034,492.0	
12.	HEXANEDIOIC ACID, BIS(2-ETHYLHEXYL) ESTER	21.741	442,725,472	12,155,333.0	
13.	EICOSANOIC ACID, 2,3-BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER	23.482	131,306,280	4,375,959.0	
14.	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-HEXAMETHYL-, (ALL-E)-	24.952	379,093,824	12,886,386.0	
15.	.BETA. CAROTENE	26.508	121,893,424	10,734,981.0	
16.	22,23-DIBROMOSTIGMASTEROL ACETATE	28.744	108,611,632	14,723,902.0	

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