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DETERMINATION OF BIOACTIVE COMPOUNDS FROM THE PETROLEUM ETHER LEAF EXTRACT OF *MORINGA OLEIFERA* AND *PHYLLANTHUS EMBLICA* USING GC-MS ANALYSIS

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ABSTRACT

Objective: The aim of the present study was to determine the presence of number of biologically active phytoconstituents in the petroleum ether leaf extracts of *Phyllanthus emblica* and *Moringa oleifera* using Gas Chromatography- Mass Spectrometry (GC-MS). **Methods**: In the present investigation, petroleum ether extracts of *Phyllanthus emblica* and *Moringa oleifera* leaves were screened for the presence of potent bioactive phytocompounds using FT-IR and GC-MS analysis which could be a possible source of extracting the therapeutically useful products. **Results**: FT-IR analysis of both the plant extracts exhibit the presence of different functional groups ranging from O-H Alcohol, C-H Alkanes, Esters C=O stretch, C-N amine, Ketone, C=O as their key functional groups. The major bioactive components present in the petroleum ether leaf extract of *Moringa* (PELMO) screened by GC-MS analysis revealed the presence of eight bioactive compounds specifically Unsaturated alcohols, Saturated hydrocarbons, Unsaturated Fatty acid, Alkane hydrocarbon, Vitamin E, Epoxide and Triterpenoids whereas *Phyllanthus emblica* (PELPE) demonstrated the presence of seventeen bioactive compounds which includes Alcoholic compound, Saturated hydrocarbons, Unsaturated Fatty acid, Fatty Alcohol, Alkane hydrocarbons, Vitamin E, Ester compounds, Plant Sterols and Triterpenes. **Conclusion**: Based on the results obtained in this study, it could be concluded that the crude extract of *M. oleifera* and *P.emblica* leaf powder contains

various bioactive chemical compounds of medicinal properties which further justify the application of these traditional plants in the discovery of novel therapeutics.

Keywords: GC-MS, FT-IR, Petroleum ether extract, Phyllanthus emblica, Moringa oleifera.

INTRODUCTION

The world population relies mainly on plants for food, fuel and medicine. Since thousands of years, plants are used in traditional medicine due to their therapeutic potential and the exploration on medicinal plants favours the innovation of novel drug candidates used against diverse diseases (Sheela *et al*., 2013). Traditional plant based medicines are reaching popular for the treatment of several disease as it is free from side effects and cost effective when compared to the existing allopathic drugs.

Infrared spectroscopy is one of the predominant analytical techniques for providing the possible chemical identification. The FTIR spectrum is used to identify the functional groups of the bioactive compounds in the plant extract based on the peak value ratio in the Infrared radiation region. In this modern period, the gas chromatography and mass spectroscopy (GC-MS) studies have been more progressively useful for the investigation of most of the medicinal plant because this technique has established to be a precious method for the chemical analysis of non polar components and volatile essential oil, fatty acids and lipids (Khare, 2007). GC-MS is the excellent practice to determine the bioactive components of alcohols, alkaloids, acids esters, long chain hydrocarbons, steroids, amino acid and nitro compounds (Muthulakshmi *et al*., 2012). Association of chromatographic and spectroscopic methods is essential in analytical chemistry since it offers high sensitivity and selectivity, also has immense value in modern natural product analysis which may further facilitate an insight of the medicinal applications of the traditional plant.

Moringa oleifera Lam (*Moringa peregrine*) commonly known as drumstick or horse radish tree is a single genus family with fourteen known species of Moringaceae family. It is a medium sized fast growing evergreen tree that usually grows about 10- 12 m in height. *Moringa oleifera* is the most widely known and utilized species wildly cultivated all over India in hedges and home yards (Morton, 1991). Almost all the parts of the plant are reputed for traditional medicine practices (Dhakar *et al*., 2011). The pharmacological studies showed that various parts of *M. oleifera* possess hypotensive, antioxidant, antibacterial, antifungal, anti inflammatory, antitumor, hypoglycemic, hypolipidaemic, antiatherosclerosis,

antihelmintic, antiulcer, diuretic, and anti-HIV activities (Mbikay, 2012 and Garima *et al*., 2011). The leaves are highly nutritious, being a significant source of essential amino acids, protein, beta-carotene, vitamins, iron, and potassium (Anwar *et al*., 2007). *Moringa oleifera* leaf extract showed the presence of phytochemicals and its antibacterial, antioxidant activities. (Malliga Elangovan *et al*., 2014).

Phyllanthus emblica Linn (*Emblica officinalis*, Amla, Indian Gooseberry) belongs to the Euphorbiaceae family is widely used for medicinal purpose for over 2,000 years. Tree is normally reaching about 18 meters (60 feet) and rarely up to 30 meters (100 feet) in height (Morton, 1987). The plant is indigenous to a large area ranging from Nepal, Southern India and Sri Lanka, throughout South-East Asia to Southern China. All parts of of this plant are used medicinally with a vast range of applications including antioxidant, antibacterial, antidiabetic, hypolipidemic, antiulcerogenic, hepatoprotective, gastroprotective, and chemopreventive properties. It is useful in the treatment of haemorrhages, diarrhoea, dysentery, anaemia, jaundice, diabetes, fever, bronchitis and cough (Krishnaveni *et al*., 2010).

GC-MS is a powerful technique used for many applications which is very highly sensitive and specific in nature. Generally its application is oriented towards the specific detection and potential identification of compounds based on the molecular mass in a complex mixture. The combination of a principle separation technique (GC) with the best identification technique (MS) made GC–MS an ideal for qualitative and quantitative analysis for volatile and semi-volatile compounds (Karthishwaran *et al*., 2012). Therefore, an attempt was made to screen the bioactive compounds, evaluate the bioactive potential and characterize them by GC-MS analysis.

The objective of the study is to identify the possible functional groups and organic compounds present in the active fraction of petroleum ether extract of *Moringa oleifera* and *Phyllanthus emblica* leaves using spectroscopic (FT-IR and GC-MS) studies. This may provide an insight in its use in traditional medicine.

MATERIALS AND METHODS

Plant Collection: The fresh mature healthy leaves of *Phyllanthus emblica* and *Moringa oleifera* plant were collected during the month of November from Algar kovil hills, Madurai, Tamil nadu, India. The selected plants were authenticated by Dr. P. Jayaraman, Institute of

Herbal Botany, Plant Anatomy Research Centre, Chennai-05. Leaves were shade-dried at room temperature to avoid loss of essential oil and milled with the aid of grinding machine.

Preparation of Plant extract

The extraction process was carried out using soxhlet extraction method. About 200gm of dry powdered plant material was extracted in Soxhlet apparatus with 250ml of petroleum ether for 8 hours. After extraction, the solvent was removed using rotary vacuum evaporator to give a concentrated extract at 60˚C in a water bath. It was then dried aseptically with the help of drier and subjected to spectroscopic analysis.

FT-IR analysis

Fourier transform infrared spectroscopy (Thermo Scientific Nicolet 1S5 FTIR) was used to analyze the petroleum ether leaf extract of *Moringa oleifera* and *Phyllanthus emblica*. The spectrum was focused in the IR ranges between 600cm-4000cm by KBr pellet technique. The spectrums of leaf extract were recorded.

GC-MS Spectroscopy

GC-MS technique was used in this study to identify the phytochemicals present in the extracts; The GC-MS analysis of the crude extracts were performed using Perkin Elmer system (GC clarus 600, USA) equipped with a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-5MS fused silica capillary column (30.0 m× 0.25 mm ID, 250 µm df). Detection was operating in electron impact mode with ionization energy of 70 eV with helium (99.999%) as a carrier gas at a constant flow of 1mL/ min. The injection volume of 1 µL of sample was employed (split ratio of 10:1) injector temperature was at 240°C. The oven temperature was programmed at 60°C (isothermal for 2 min), with an increase of 10°C/min to 300°C/min for 6 min.

Identification of Compounds

Interpretation of mass spectrum of the unknown component was conducted by comparing the mass spectra with the spectrum of the known components stored in the data system National Institute Standard and Technique library (NIST-2008, Turbo mass Ver. 5.4.2). The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The name, molecular weight, structure and mass fragmentation of the components of the test materials were ascertained.

RESULT

The present study describes a detailed analysis of the phytoconstituents present in the petroleum ether leaf extract of *Moringa oleifera* and *Phyllanthus emblica.* FTIR analysis of petroleum ether leaf extracts of Moringa oleifera (illustrated in **Table.1 and Fig.1**) and *Phyllanthus emblica* leaf extract (**Table.2 and Fig.2**) gave results that exhibit the presence of different functional groups ranging from O-H alcohol (3120-4000 cm-1 Strong and Broad), C-H alkane (2850-3000 cm-1 2 Strong peaks), =C-H-O aldehyde (2800-3000 cm-1 medium peak), Acyclic ketone (1625-1750 cm-1 strong), Esters C=O stretch (1650-1755 cm-1 strong), C=O amide (1600-1670 stretching), N-H amide (1550-1640 bending), Aliphatic alkanes H-C-H bend (1440-1500) as their functional groups.

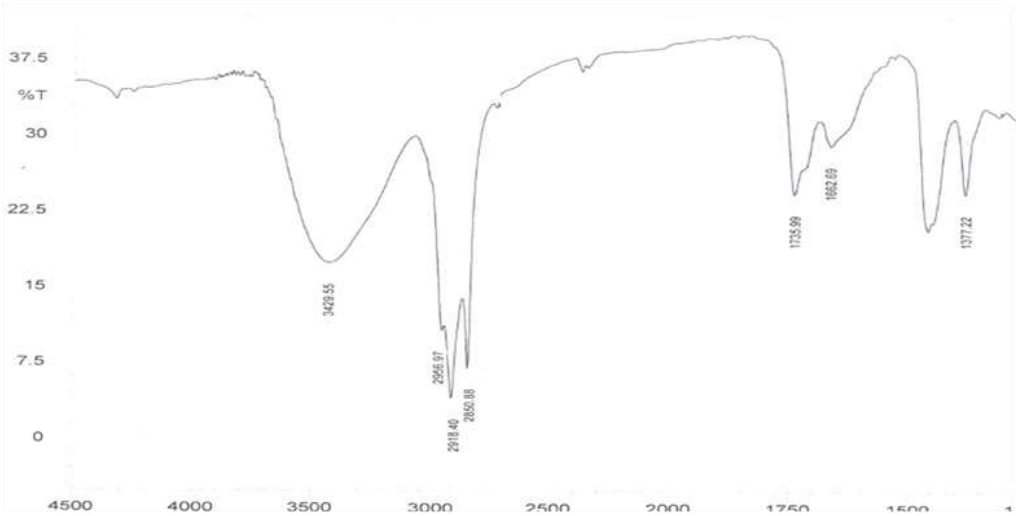


Figure 1: FT-IR Peaks of Peteroleum ether leaf extract of Moringa oliefera (PELMO)

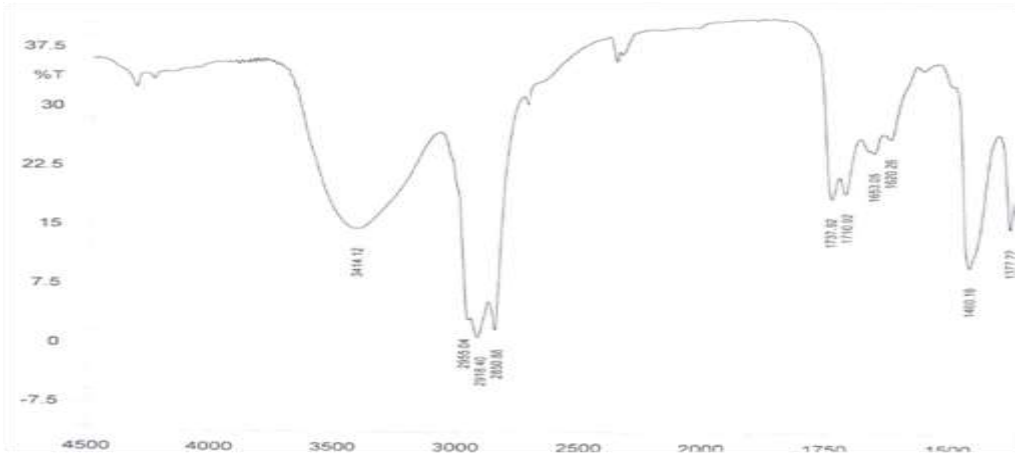


Figure 2: FT-IR Peaks of Peteroleum ether leaf extract of Phyllanthus emblica (PELPE)

Table-1: FTIR peak value of petroleum ether leaf extract of *Moringa oleifera*

S.No	Peak Value	Peak Intensity	Functional group
1	3429.55	17.327	Alcohol (O-H Stretch)
2	2956.97	10.698	Alkanes (SP^3, C-H bond)
3	2918.4	3.995	Alkanes (SP^3, C-H bond)
4	2850.88	6.984	Alkanes (SP^3, C-H bond)
5	1735.99	24.235	Esters (C=O stretch)
6	1662.69	29.034	Amides (C=O stretch)
7	1377.22	24.272	Nitro groups (N=O bend)
8	1244.13	30.24	Ethers (C-O stretch)
9	1166.97	28.78	Ethers (C-O stretch)
10	1087.89	28.119	C-N amine
11	1035.81	29.867	Unknown compound
12	893.07	36.674	Unknown compound
13	853.21	32.777	Unknown compound
14	723.33	31.237	Unknown compound
15	578.66	36.357	Unknown compound

Table-2: FTIR peak value of petroleum ether leaf extract of *Phyllanthus emblica*

S.No	Peak Value	Peak Intensity	Functional group
1	3414.12	14.494	Alcohol (O-H Stretch)
2	2955.04	2.973	Alkanes (SP^3, C-H bond)
3	2918.4	0.752	Alkanes (SP^3, C-H bond)
4	2850.88	1.696	Aldehyde (=C-H-O bond)
5	1737.92	18.54	Ketones (C=O stretch)
6	1710.92	19.151	Esters (C=O stretch)
7	1653.05	24.372	Amides (C=O stretch)
8	1620.26	26.120	Amides (N-H bend)
9	1460.16	9.819	Aliphatic alkanes (H-C-H bend)
10	1377.22	14.738	Unknown compound
11	1307.78	28.95	Amine (C-N bond)
12	1222.91	23.346	Amine (C-N bond)
13	1163.11	22.136	Amine (C-N bond)
14	1087.89	22.728	Amine (C-N bond)
15	1037.74	23.57	Alkene (=C-H stretch)
16	991.44	25.86	Unknown compound
17	893.07	32.508	Unknown compound
18	837.13	27.255	Unknown compound
19	723.33	23.634	Unknown compound
20	574.81	31.867	Unknown compound

The major chemical constituents of petroleum ether crude extract of *Moringa oleifera* and *Phyllanthus emblica* were identified by Gas Chromatogram- Mass spectrometry (GC-MS)

analysis. The active principles with their retention time (RT), Molecular formula, Molecular weight, and Concentration (Peak area %) were presented in **Table.3**.

Table-3: Major Photochemicals identified in the PELMO and PELPE by GC-MS analysis

S.No	Retention time	Name of the Compound	Molecular formula	M. Wt.	Area %
Petroleum ether leaf extract of *Moringa oleifera* (PELMO)					
1	19.475	Z6, Z9- Pentadecadien-1-ol	$C_{15}H_{28}O$	224	7.205
2	23.877	Hentriacontane	$C_{31}H_{64}$	436	6.268
3	25.273	Tetracontane 1,4 diol	$C_{54}H_{11}O$	758	29.892
4	26.223	Tetracontane-1,40-diol	$C_{40}H_{82}O_2$	594	2.224
5	26.598	Tetrapentacontane and Dotriacontane	$C_{32}H5_6$	450	4.168
6	26.898	D.L.Alpha -Tocopherol	$C_{29}H_{50}O_2$	430	26.893
7	27.799	Oxirane, Hexadecyl-	$C_{18}H_{36}O$	268	14.702
8	29.124	Beta- Amyrin	$C_{32}H_{52}O_2$	468	8.648
Petrolum ether leaf extract of *Phyllanthus emblica* (PELPE)					
S.NO	**Retention time**	**Name of the Compound**	**Molecular formula**	**M. Wt.**	**Area %**
1	22.386	Hentriacontane	$C_{31}H_{64}$	436	1.911
2	23.142	Dotriacontane	$C_{32}H_{66}$	450	3.297
3	23.867	Tetracontane	$C_{40}H_{82}$	562	6.268
4	24.572	Tritetracontane	$C_{43}H_{88}$	604	4.110
5	25.253	Pentacosane	$C_{25}H_{52}$	352	6.456
6	25.913	Unidentified compound	$C_{11}H_{15}O_{14}$	296	4.018
7	25.273	Octadecanoic acid, Methyl Ester	$C_{19}H_{38}O_2$	298	29.892
8	26.223	Unidentified compound	$C_{30}H_{50}O$	427	2.224
9	26.888	Vitamin E	$C_{29}H_{50}O_2$	430	29.633
10	27.349	Sufurous acid, Hepta decyl ester	$C_{21}H_{44}O_{38}$	376	2.895
11	27.784	Tetracontane-1,40-diol	$C_{40}H_{82}O_2$	594	6.394
12	28.244	Sufurous acid, Butyl octadecyl ester	$C_{21}H_{44}O_{38}$	376	9.57
13	28.404	Hexacosanol, Acetate	$C_{28}H_{56}O_2$	424	7.883
14	28.629	Gamma Sitosterol	$C_{29}H_{50}O$	414	9.570
15	29.114	12-Oleanen-3-yl acetate, (3 Alpha)	$C_{32}H_{52}O_2$	468	5.568
16	29.279	Tetrapententacontane	$C_{54}H_{11}O$	758	2.975

The active components present in the petroleum ether leaf extracts of *Moringa oleifera* by GC-MS analysis are (**Table.3 and Fig.3**) Z6, Z9- Pentadecadien-1-ol (7.205%), Hentriacontane (6.268%), Tetracontane-1, 40-diol (2.224 %), Tetra pentacontane (29.892 %), Dotriacontane (4.168 %), DL-α-Tocopherol (26.893 %), Oxirane (14.7%) and Beta-amyrin (8.648 %).

The composition of the petroleum ether leaf extract of *Phyllanthus emblica* has been investigated through GC/GC-MS analysis. The photochemical screening showed Sixteen peaks, presented in the **Table.3 and Fig.4** which demonstrated the presence of Hentriacontane (1.911%), Dotriacontane (3.297%), Tetracontane (6.268%), Tritetracontane (4.110%), Pentacosane (6.456%), Unidentified compound (4.018%), Octadecanoic acid-Methyl ester (29.892%), Lupeol (2.224%), Vitamin E (29.63%), Sufurous acid, Hepta decyl ester (2.895%), Tetracontane-1,40-diol (6.394%), Sufurous acid, Butyl Octadecyl ester (9.57%), Hexacosanol Acetate (7.883%), Gamma Sitosterol (9.570%), 12-Oleanen-3-yl Acetate (5.568%), Tetra pententacontane (2.975%).

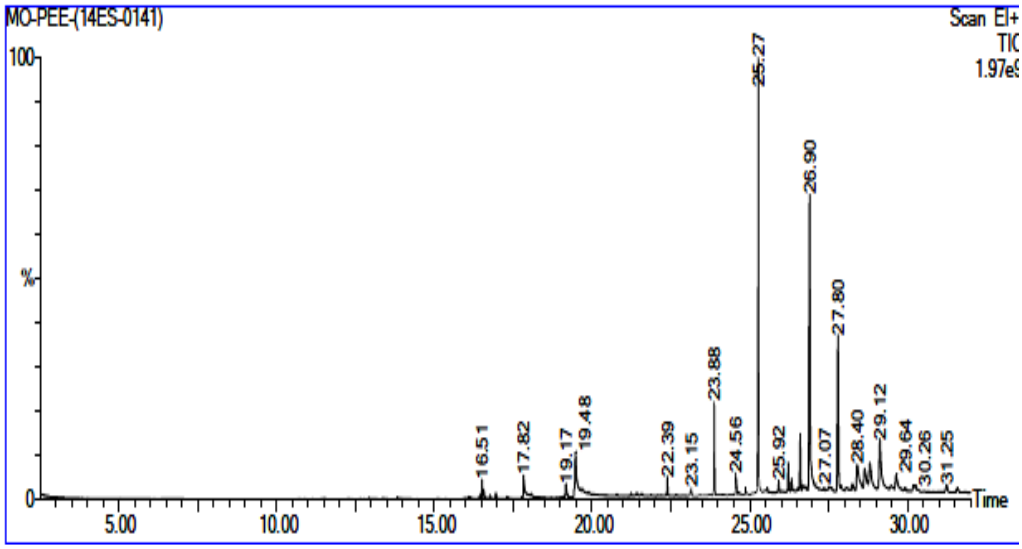


Figure-3: GC-MS chromatogram of petroleum ether leaf extract of *Moringa oleifera*

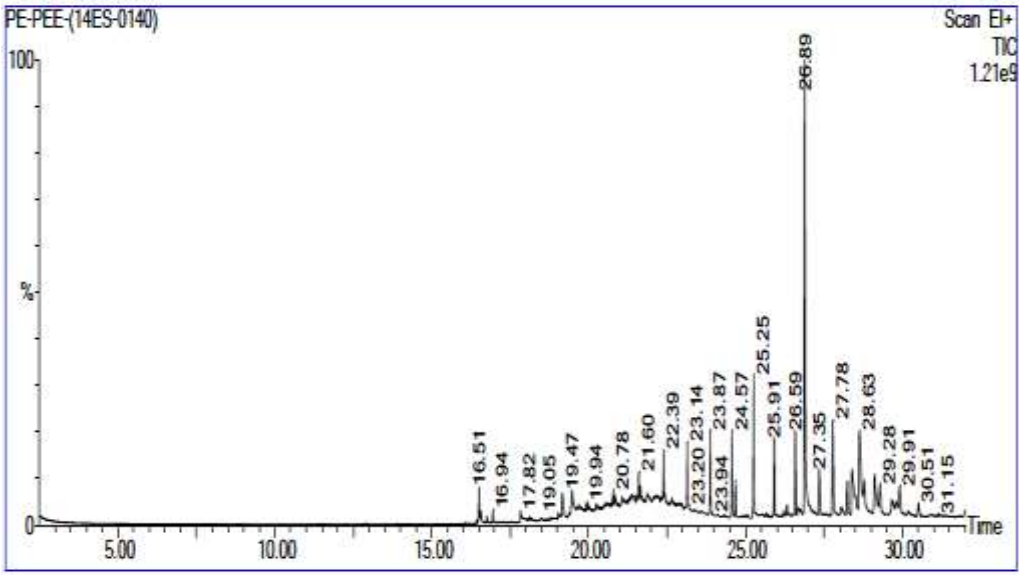


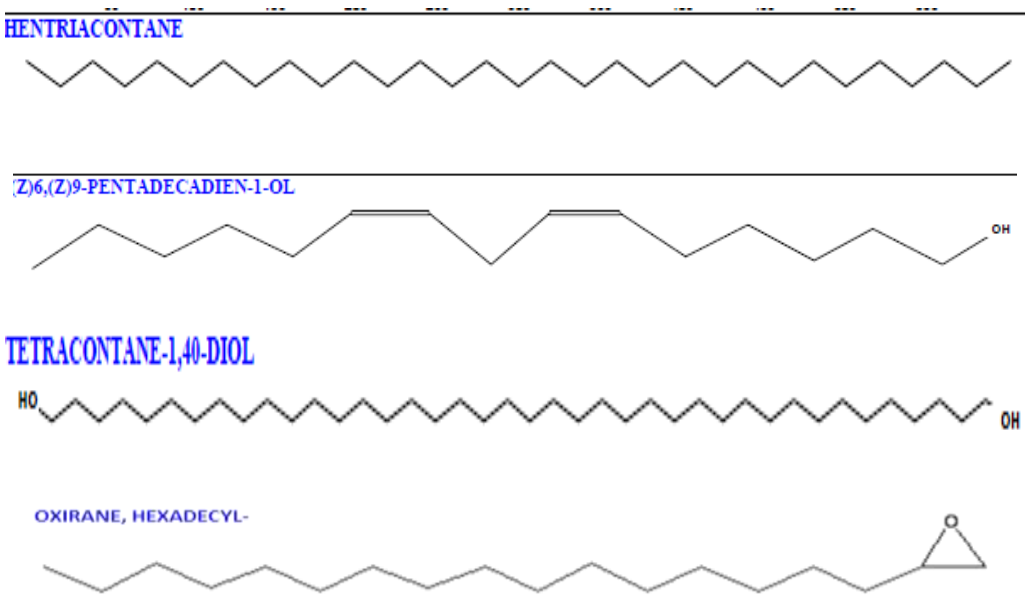
Figure-4: GC-MS chromatogram of petroleum ether leaf extract of *Phyllanthus emblica*

Some important organic metabolites which contribute the medicinal activity are listed in **Table.4**. The chemical structure of the major phytocompounds existing in the PELMO and PELPE were presented in the **Fig.5 and Fig.6** respectively.

Table-4: Activity of phyto-components identified in the Petroleum ether extract of *Moringa oleifera* (PELMO) and *Phyllanthus emblica* (PELPE) by GC-MS analysis

S.No	Name of the compound and Reference	Compound Nature	Biological Activity
1.	Z6, Z9- Pentadecadien-1-ol	Unsaturated Alcoholic compound	No activity reported
2.	Hentriacontane (Kim *et al*., 2011; Ramalakshmi *et al*., 2011)	C31-Saturated Fatty acid ester compound	Antioxidative, Protection against UV radiation, Anti-tumour activity, Anti-inflammatory and Anticancer activity
3.	Tetracontane 1,4 diol	Unsaturated Fatty acid	Anti-inflammatory activity
4.	Tetracontane-1,40-diol	Unsaturated Fatty acid	No activity reported
5.	Tetrapentacontane and Dotriacontane	Alkane hydrocarbon	No activity reported
6.	D. L. α -Tocopherol (Jim Duke, 2015)	Alcoholic compound	Antioxidant property, Anticancer, Antitumor, Anti-mutagenic, Anti-diabetic, Anti-Infertility, Anti-parkinsonian, Antialzheimeran, Antiatherosclerotic, Antistroke, Hepatoprotective, Cardioprotective, Immunomodulator, Vasodilator etc
7.	Oxirane, Hexadecyl- (Ramalakshmi *et al.*, 2011)	Epoxide	Adhesive
8.	Beta- Amyrin (Subarnas *et al*., 1993 and Jim Duke, 2015)	Pentacyclic Triterpenoid	Antibacterial, Antioxidant, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, and Hepatoprotective activities
9.	Tritetracontane	Alkane hydrocarbon	No activity reported
10.	Pentacosane	Alkane hydrocarbon	No activity reported
11.	1-Hexacosanol (Ramalakshmi *et al.*, 2011)	Long chain fatty alcohol	Antioxidant, Antibacterial, and Anti cancerous, Neuronal Growth Stimulators
12.	Octadecanoic acid, Methyl ester (Kumar et al., 2010 and Jim Duke, 2015)	Unsaturated Fatty acid ester compound	Antioxidant, Antibacterial, Anti-fungal, Anti-inflammatory, Anti-arthritic, Antihistimic, Anticoronary, Hypo-

			cholesterolemic, Anticancer, Hepato-protective action; Soap, Lumbricant and Cosmetics
13.	Pentatriacontane	Alkane hydrocarbon	No activity reported
14.	Sufurous acid, Hepta decyl ester	Ester compound	No activity reported
15.	Sufurous acid, Butyl octadecyl ester	Ester compound	No activity reported
16.	Gamma Sitosterol (Jim Duke, 1998 & 2015)	Plant Sterols serves a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D	Antiatherosclerotic, Anti-diabetic, Angiogenic, Anticancer, Antibacterial, Antiviral, Anti-inflammatory, Antitumour, Antidiarrhoeal, Antiinfertility, Antileukemic, Antimutagenic, Apoptotic, Hepatoprotective, Cardio-rpotective, Hypocholesterolemic, Hypoglycemic, Pesticide, Spermicide
17.	12-Oleanen-3-yl acetate (Jim Duke, 1998 and Oluwatoyin *et al*., 2012)	Triterpene	Antioxidant, Antibacterial, Anti inflammatory, Antitumor activities, Antioxidant, Anti-inflammatory, Anti-diabetic, and Anti-amylase inhibitor activities
18.	Tetrapententacontane	Alkane hydrocarbon	No activity reported



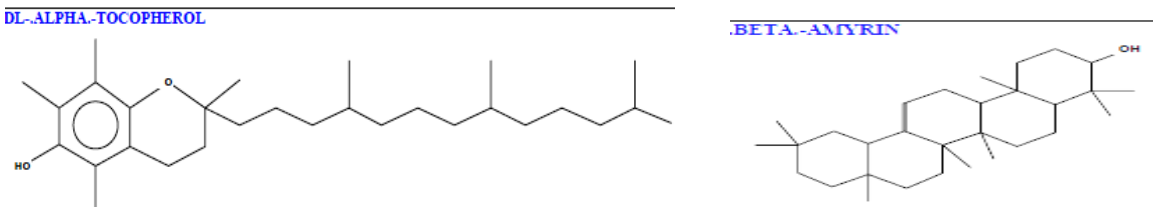


Figure-5: Chemical structure of the Major Phytocompounds in PELMO

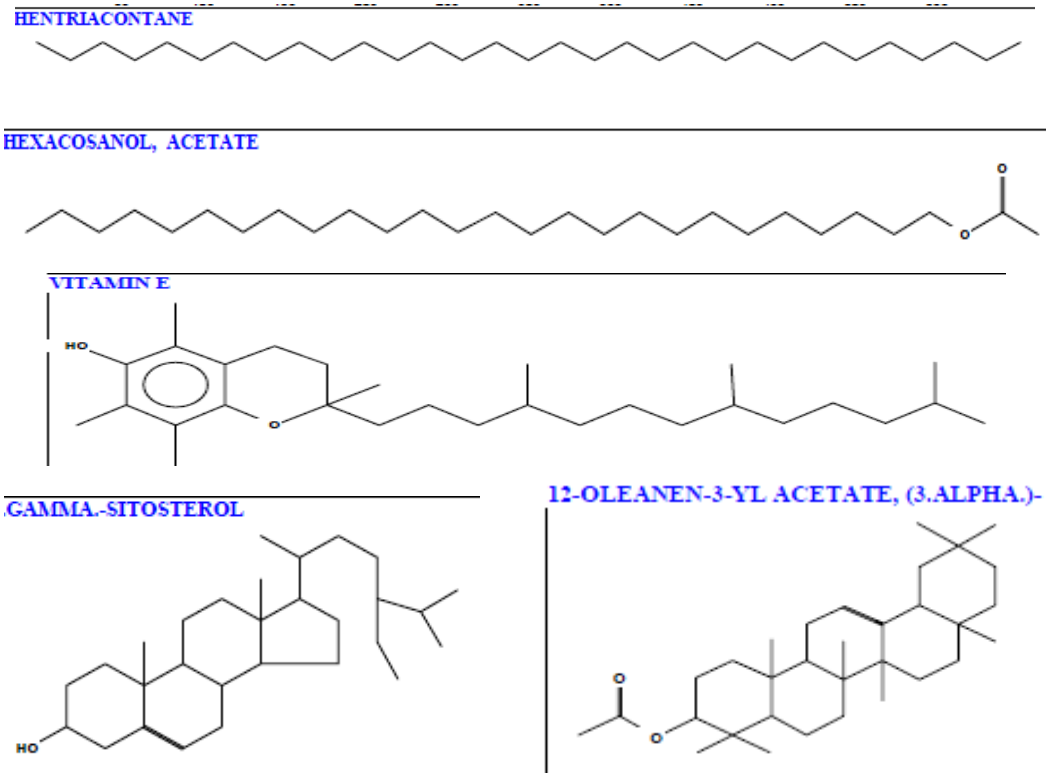


Figure-6: Structural depiction of the Major Phytocompounds in PELPE

DISCUSSION

Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs. Spectroscopic technique is a potent diagnostic tool for the qualitative and quantitative analysis of biological and pharmaceutical materials.

The FT-IR technique is based on the selective radiation absorption of the chemical substance in infrared region by which the molecules vibrate giving rise to maximum absorption spectrum. It is an outstanding method for the qualitative analysis because the spectrum of compound is unique with the exception of optical isomers. This method is most useful for the

identification of purity, details of gross structure; also in the field of natural products, forensic chemistry and of competitive products analysis in industries.

Various medicinal plant are flourishing foundation of secondary metabolites such as alkaloids, glycosides, flavonoids, phenol, tannins and terpenoids determined by GC-MS spectrum analysis (Adekunle *et al*., 2009). The present study has been found useful in the identification of several constituents present in the petroleum ether extract of both the medicinal plant leaves (PELMO and PELPE). Most of the compound possess medicinal properties whereas certain components reported in this work have not been reported in literature before.

Best of our knowledge and literature survey there is no report of gas chromatography and mass spectrum analysis to identify the chemical compounds from the petroleum ether extract of the *Phyllanthus emblica* leaves. In a study conducted by Abhijeet *et al*., 2013, the GC-MS outcome of the petroleum ether leaf extracts of *Moringa oleifera* (PELMO) in Maharashtra, India were Pentacosane, Nonacosanol, Docosane, Heptadecane, 2 Methyl, 9, 5, 11-Octadecatrienoic acid, methyl ester, Phytol and Octadecanoic acid, 2-(2-hydroxy ethoxy) ethyl ester. But our present study in PELMO showed the presence of Z6, Z9- Pentadecadien-1-ol, Hentriacontane, Tetracontane-1, 40-diol, Tetra pentacontane, Dotriacontane, DL-α-Tocopherol, Oxirane, and Beta-amyrin. Even though the plant leaves and the solvent are the same, not even a single phytochemical compound is found to be similar. The high variation in the chemical nature of the plant may be due to the geographical location, leaves age (young/ mature/ old), season during which the leaves are collected for analysis.

Generally in plants, high quantity of unsaturated fatty acids in the oil was advantageous from the nutritional and health aspects. Since consumption of unsaturated fatty acids will not lead to heart related diseases whereas using up of saturated fatty acids rich foods associated with assured cardiovascular disorders such as atherosclerosis, aging and cancer (Law, 2000). Sterols play a vital role in plant cell membranes, an important constituent of all eukaryotes. Plant sterols hold vital physiological activities. They are biogenetic precursors of many hormones and oviposition stimulants of some insects (Harborne, 2001).

The present study established the presence of active bioactive compounds in the crude extracts of *M. oleifera* and *P.emblica* leaves with the prediction of their respective molecular weight, formula and structure of active molecules. In our previous research work, *M. oleifera*

leaf extract showed significant enzymatic (SOD, CAT and POD) and non-enzymatic (Ascorbic acid, Reduced glutathione and Carotenoids) anti-oxidant activities (Malliga Elangovan *et al*, 2014) which may possibly due to the predominant role of Hentriacontane, Beta- Amyrin and Alpha- Tocopherol compounds in it.

CONCLUSIONS

It could be concluded that petroleum ether leaf extract of *P.emblica* and *M. oleifera* possesses a number of bioactive compounds, which may be used in the manufacture of body products, drugs, phyto-pharmaceutical and therapeutic value. The presence of various bioactive compounds such as Alkanes, Fatty acids, Steroids, Vitamin E and Triterpenes justifies the use of these medicinal plants for various ailments by traditional practitioners such as an antioxidant, antimicrobial, anti-inflammatory, antiulcer, diuretic etc.

Based on the results obtained in this study, it could be believed that the crude extract of *P.emblica* and *M. oleifera* leaf powder contains chemical constituents of pharmacological and nutritional significance. So these plants are recommended as phytopharmaceutical importance. However, it is recommended that additional work is to be carried out to isolate, characterize and elucidate the individual phytochemical constituents in these medicinal plants responsible for the bio-efficacy and bioactivity in the pharmaceutical activity which definitely give fruitful results in the discovery of novel herbal drugs.

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