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Ethnicity and Scientific validation of West Bengal Amla (*Phyllanthus emblica* L.) with special reference to GC-MS screening.

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Abstract

The present investigation was carried out to analyze the active constituents present in fruit of *Phyllanthus emblica* L. (Phyllanthaceae) consumed by the tribal people of west Bengal for the treatment of various disease alignments. *Phyllanthus emblica* L., a deciduous tree of small to medium size up to 5.5 meters representing a large group of phyto-chemical reservoir of medicinal uses in different disease like diabetes, liver disorder, snake venom neutralizer, diarrhoea, indigestion, anti-tumour, anti-carcinogenic, anti-ulcer, antioxidant, anti-inflammatory activities etc. supporting its ethnicity for the traditional healer. Seventy compounds are identified by ethyl acetate extract of this fruit using Gas Chromatography-Mass Spectrometry (GC-MS) analysis having enormous medicinal potentialities. Besides this, quantitative estimation of various bioactive constituents reveals the presence of tannins, carbohydrates, acidic compounds, poly phenols, vitamin –C, etc in different proportions.

Keywords: *Phyllanthus emblica* L., GC–MS, Phyto-constituents, Ethnicity.

Introduction:

The fruit or fruit pulp of *Phyllanthus emblica* L. is a reputed drug of Ayurvedic, Unani, Siddha and Homoeopathic systems of medicine and is believed to increase defence against diseases (Sachan *et al.,* 2013).The fruit primarily contains tannins, alkaloids, phenolic compounds, amino acids, carbohydrates, vitamin C and other compounds especially the essential nutrients (Sachan *et al.,* 2013). Fresh or dried fruit is one of the important herbal drugs used traditionally both as a medicine and as a tonic to build up lost vitality and vigour (Krishnaveni and Mirunalini, 2010). In Unani medicine, it is described as a tonic for heart and brain.

According to the two main classic texts on Ayurved, Charak Samhita and Sushrut Samhita, Amalaki is regarded as “the best among rejuvenative herbs”, “useful in relieving cough and skin disease” and “the best among the sour fruits” (Patel and Goyal, 2012).

Amla is acrid, cooling, refrigerant, diuretic and laxative. Dried fruit is useful in haemorrhage, diabetes (Mehta et al. 2009), ulcer (Sairam et al., 2002), diarrhoea (Nadkarni, 1999 and Singh *et al.,* 2011), Liver disorder (Bhattacharya *et al.,* 2000), Snake Venom Neutralizer (Alam and Gomes, 2003), Reducing Cholesterol (Anila, and Vijayalakshmi, 2002), Fevers (Nadkarni and

Nadkarni, 1999) and also for Cancer (Sancheti *et al*., 2005).

Some of the herbal formulations adheres to scientific methodology and has been generated based on reasonably sound data whereas most of them are prepared by unregistered manufacturers without license do not follow the Good Manufacturing Practice (GMP) or Indian System of Medicines (ISM) standards (Bigoniya, 2013). As *Phyllanthus emblica* L. is a natural product, our society believes that the fruit is safer than conventional pharmaceuticals (Bigoniya, 2013) irrespective of their proper doses and proper application. So it is urgent to validate phyto-constituents scientifically in terms of its efficacy and safety.

Materials and Methods:

Collection of plant material:

In West Bengal the flowering season of *Phyllanthus emblica* L. was observed to occur from the last week of March to the middle of April. The flowering reached its peak in the end of April. The fruiting season is exceptionally long. The fruit in this area become fit for harvesting in November. They can be retained on the tree up to March without any significant loss in quality or yield. The picking of fruits is generally done by the villagers in December to February.

Fruits were collected from the Jhargram Binpur Region, West Bengal, in the month of January, 2016. Fresh *Phyllanthus* flesh was washed with tap water air dried for a week in room temperature (26 ± 2 ˚C) and then grounded in an electrical grinder, stored and kept for further use.

Preparation of fruit extract:

The fruits of the plant were extracted with Ethyl acetate and analyzed using gas chromatography- mass spectrometry (GC-MS). The mass spectra of the compounds were matched with the National Institute of standards and technology (NIST) library. The phyto-chemical analysis and GC-MS profiling of the fruit extract was carried out.

GC-MS analysis and Identification of components:

GC analysis was conducted on a Factor four™ capillary column (VF-5ms, 30 m, 0.25 mm id, 0.25 µm film thickness; Varian, Middelburg, The Netherlands) with the following conditions: constant flow of Helium, 0.8 mL /min; initial inlet temperature, 70^{o}C ramped to 280^{o}C at 200^{o}C/min after a 20 s delay and held for 5.0 min; injection volume, 8 µL (LVI) in the liner with an open purge valve (40:1 split ratio) for 18 s, closed until 4.0 min, and open again (30:1) until the end of the run; oven temperature program, 70^{o}C for 2 min, then 20^{o}C/min ramp to 180^{o}C followed by a 2^{o}C/min ramp to 220^{o}C and held for 30 sec, again 100^{o}C/min ramp to 285^{o}C and held for 5 min, followed by 100^{o}C/min ramp to 295oC and held for 2 min. The MS instrument transfer line temperature was 280^{o}C, with 220^{o}C ion trap and 120^{o}C manifold temperatures. Full-scan (40–650 m/z) EI (auto) mode with 20 µA filament current was used for MS analysis from 9.5–35.00 min, which gave 0.92 s/scans (3µscan). Target automatic gain control was 20,000, and the multiplier voltage was 1450 V. Baseline offset -5, peak find with S/N of the quantifier ion at least 3 and peak width 2 s was set as the parameters for processing the peaks in the chromatograms.

Minimum similarity match with regards to the NIST library spectra was kept at 500 (reversed fit). Quantification was done on the basis of diagnostic ion and the peak assignments and integration were automatically done through software.

The name, molecular weight, percentage of peak area of the components of the test materials were ascertained (Table 2).

Result:

The Phyto-chemical screening for presence of different phyto-constituents in *Phyllanthus emblica* fruit extracts are presented (Table 1).

Sl	Testing parameters	Test method	RESULT	UNIT
1	*Foreign matter*	AOAC/DGHS	NF	G/100G
2	*Insect infection*	VISUAL	NF	G/100G
3	*Total Ash content*	AOAC 941.12	0.40	G/100G
4	*Acid insoluble ash*	AOAC 941.12	0.03	G/100G
5	*Moisture content*	AOAC 931.04	78.71	G/100G
6	*Total polyphenol*	ISO 14502 (PART -1): 2005	17.68	G/100G
7	*Total Carbohydrate*	BY DIFFERENCE (REF. AOAC 986.25)	20.21	G/100G
8	*Tannin Content*	GEN /SOP/ CALLAB-02	16.24	G/100G
9	Vitamin C (Ascorbic acid)	AOAC 967.21	194.44	MG/100G

Table 1: Phyto-chemical screening for presence of different phyto-constituents in *Phyllanthus emblica* L. fruit extracts.

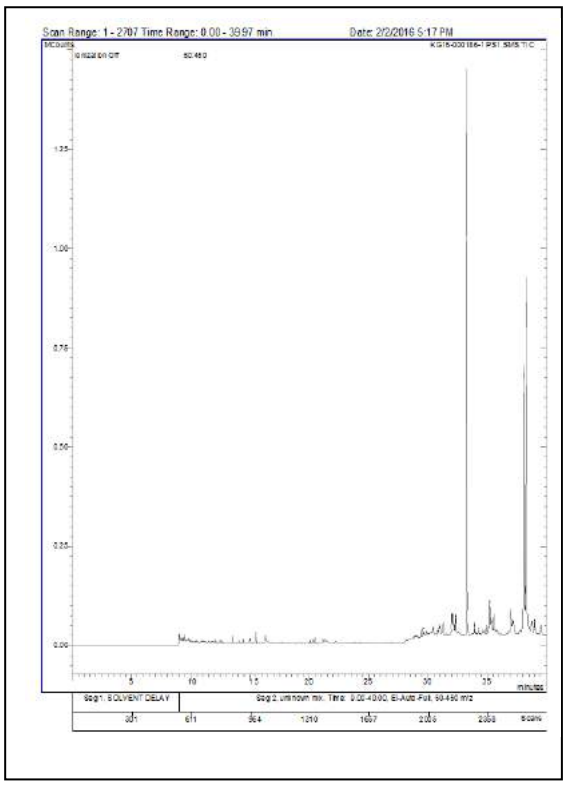


Diagram: 1. GC-MS Chromatogram obtained from the ethyl acetate fruit extract of *Phyllanthus emblica* L.

Figure 1(A-C): Collection of fruit sample and conversation with traditional healer.

In the GC-MS analysis the mass spectra of identified compounds from Ethyl acetate fruit extract of PE were matched with those found in the NIST spectral database are given (Table

2) and the chromatographic peak are represented (Fig.1).

Peak	Name of the compound	MF	MW	%PA
1.	Citronellyl propionate	C13H24O2	212	81.0
2.	1-Methyl -4 isopropyl-cyclohexyl 2-hydroperfluorobutanoate	C14H20F6O2	334	95.0
3.	Citronellyl acetate	C12H22O2	198	69.0
4.	3,7,11,15-Tetra methyl-2 hexadecen-1-ol	C20H40O	296	81.0
5.	Bicyclo(2.2.1) heptane,1,3,3-trimethyl-	C10H18	138	81.0
6.	7-Octadecyne,2-methyl	C19H36	268	81.0
7.	Bicyclo(2.2.1) heptane,1,7,7-trimethyl-	C10H18	138	95.0
8.	Bicyclo(3.1.1) heptane,2,6,6-trimethyl-	C10H18	138	55.0
9.	Bicyclo(2.2.1) heptane,2,2,3-trimethyl-endo-	C10H18	138	95.0
10.	Cyclohexane,1-methyl-4-(1-methylethenyl)-,cis-	C10H18	138	81.0
11.	7-tridecanol,7,ethyl-	C15H32O	228	143.0
12.	Tetradecanoic acid,10,13,di-methyl-,methyl ester	C17H34O2	270	74.0
13.	Hexadecanoic acid, methyl ester	C17H34O2	270	74.0
14.	Tridecanoic acid, methyl ester	C14H28O2	228	74.0
15.	Pentadecanoic acid, 14- methyl, methyl ester	C17H34O2	270	74.0
16.	Hexadecanoic acid, methyl ester	C17H34O2	270	74.0
17.	Decanoic acid, methyl ester	C11H22	186	74.0
18.	Hexadecanoic acid, methyl ester	C17H34O2	270	74.0
19.	Pentadecanoic acid, 14- methyl, methyl ester	C17H34O2	270	74.0
20.	Capric acid methyl ester	C11H22O2	186	74.0
21.	Phendimetrazine	C12H17NO	191	57.0
22.	Tetracontane,3,5,24-trimethyl-	C43H88	604	57.0
23.	3-methyl-2-(2-oxopropyl) furan	C8H10O2	138	57.0
24.	Hexacontanoic acid	C60H120O2	872	57.0
25.	1-pentacontanol	C50H102O	718	396.0
26.	2-HEXYL-1-Octanol	C14H30O	214	57.0
27.	1-decanol,2-hexyl-	C16H34O	242	57.0
28.	N-Hexatriacontane	C36H74	506	57.0
29.	Octatracontane,1-iodo-	C48H971	800	57.0
30.	N-Dotriacontane	C32H66	450	57.0
31.	Octadecane	C18H38	254	57.0
32.	N- Hexadecane	C16H34	226	57.0
33.	N-Pentadecane	C15H32	212	57.0
34.	N-Hexatriacontane	C36H74	506	57.0
35.	Stigmast-5-en-3-ol,oleate	C47H82O2	678	396.0
36.	N-Dotriacontane	C32H66	450	57.0
37.	Ergost -5-en-3-ol,acetate,(3,beta,24R)-	C30H50O2	442	43.0
38.	N-Hexacosane	C26H54	366	57.0
39.	Beta-sitostero acetate	C31H52O2	456	396.0
40.	N-Tetracosane	C24H50	338	57.0
41.	Oleyl alcohol	C18H36O	268	55.0
42.	Bicyclo(4.1.0) heptane,7-pentyl-	C12H22	166	67.0
43.	Pentadecanal	C15H30O	226	82.0
44.	(Z)-14-Tricosenyl formate	C24 H46O2	366	55.0
45.	1,22-docosanediol	C22H46O2	342	55.0
46.	Myristaldehyde	C14H280	212	57.0
47.	2(1H)-Benzocyclooctenone,decahydro-10a-methyl-,trans	C13H22O	194	55.0
48.	1-Eicosyne	C20H38	278	82.0
49.	Cycloheptanol, 3-(3,3-dimethyl butyl)-	C13H26O	198	57.0
50.	Spiro (3.5) nonan-1-one, 5-methyl-,trans-	C10H160	152	81.0
51.	Ergost -5-en-3-ol,acetate,(3,beta.,24R)-	C30H50O2	442	43.0
52.	Ergost -5-en-3-ol,acetate,(3,beta.)-	C28H48O	400	43.0
53.	Ergost -7-en-3-ol,acetate,(3,beta.)-	C28H48O	400	43.0
54.	1,3,3-Trimethyl-1-(2'-trimethylsilyloxyphenyl)-6-trimethylsilyloxyindane	C24H36O2Si2	412	397.0
55.	1,3,3-Trimethyl-1-(4'-trimethylsilyloxyphenyl)-6-trimethylsilyloxyindane	C24H36O2Si2	412	397.0
56.	Campesterol	C28H480	400	107.0
57.	Stigmast-5-en-3-ol,oleate	C47H82O2	678	396.0
58.	Ergostane-3,12-diol,(3. alpha.,5.beta,12. alpha)	C28H50O2	418	43.0

59.	Lanost-8-ene	C30H526O	412	397.0
60.	Beta. -sitosterol	C29H50O	414	43.0
61.	Clenbuterol	C12H18Cl2N2O	276	203.0
62.	Ethanone,1,1'-(6-hydroxy-2,5-benzofurandiyl)bis-	C12H10O4	218	203.0
63.	Manganese,.pi.-cyclohexadienyl(hexamethylbenzene)	C18H25Mn	296	218.0
64.	1-hydroxypyrene	C16H10O	218	218.0
65.	9,11-dimethyltetracyclo(7.3.1.0(2.7).1(7.11))tetradecane	C16H26	218	203.0
66.	Clovene	C15H24	204	41.0
67.	Aciphyllene	C15H24	204	95.0
68.	8-Amino-5-benzyloxy-6-methoxy-4-methylquinoline	C18H18N2O2	294	203.0
69.	1-Naphthalenol,decahydro-4a-methyl-8-methylene-2-(1-methylethyl)-,acetate,(1S-(1.a.,2.b.,4a.al.,8a.al.))-	C17H28O2	264	43.0
70.	2H-Cyclopropa(a)naphthalene-2-one,1,1a,4,5,6,7,7a,7b-ocah,1,7,7a-tetramethyl-,(1a.al.,7.al.,7a.al.,7b.al.)-	C15H22O	218	218.0

Table 2. Compounds Identified in Ethyl acetate extract of Phyllanthus emblica L. fruit by GC-MS (MF=Molecular formula, MW=Molecular weight, %PA=Percentage of Peak area).

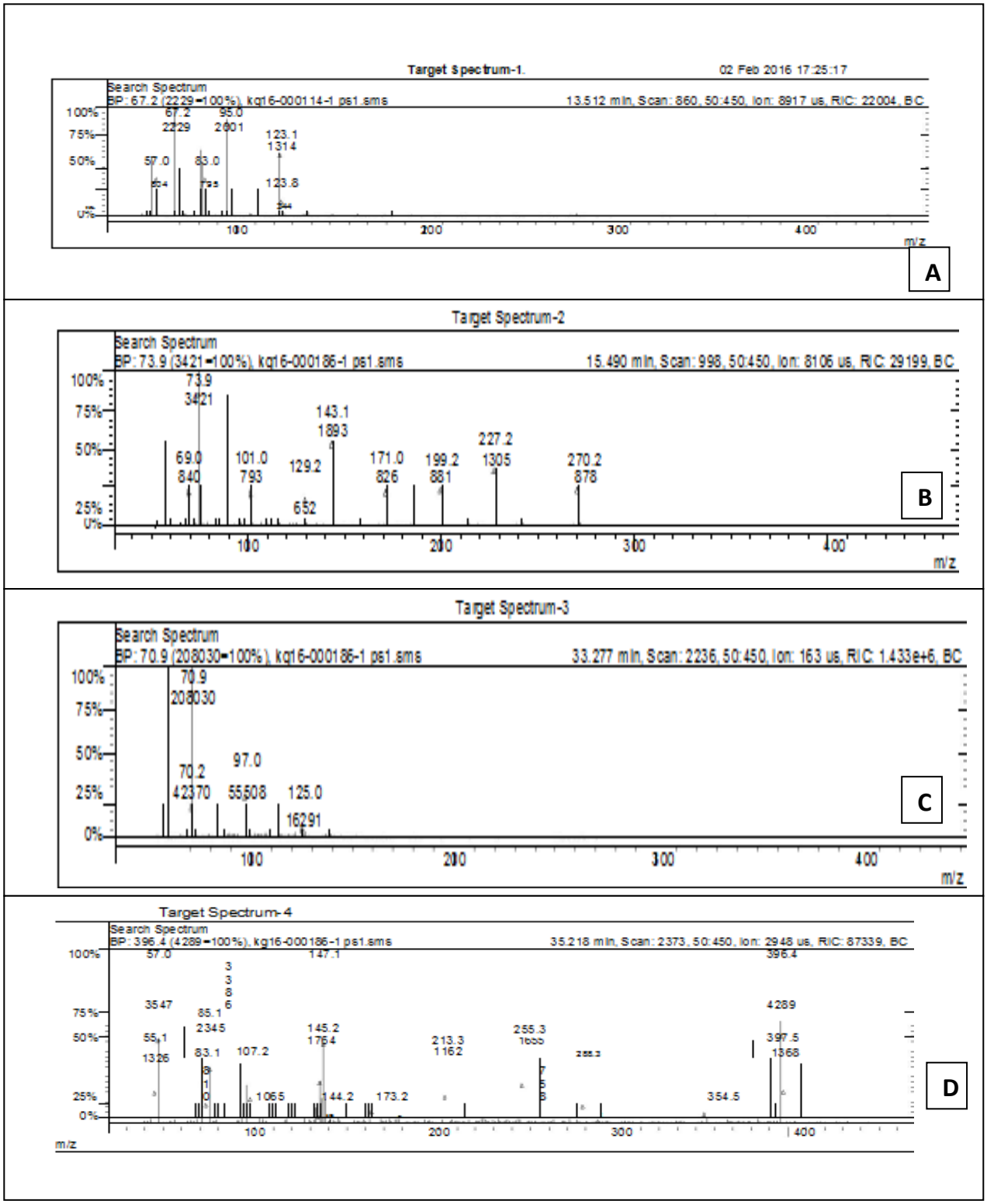
Structure and Mass Spectrum of Universal Phyto-components identified by GC-MS in ethyl acetate Extracts (Data represents 7 target spectrum with best 10 hits of match components obtained from the ethyl acetate extract of *Phyllanthus emblica* L. fruit) were examined. Biological activities of different phyto-constituents are listed (Table 3).

Sl. No.	NAME OF THE COMPOUND	BIOLOGICAL ACTIVITY
1.	Citronellyl propionate	Antimicrobial.
2.	1-Methyl -4 isopropyl-cyclohexyl 2-hydroperfluorobutanoate	Methyl-Donor
3.	Citronellyl acetate	Irritant
4.	3,7,11,15-Tetra methyl-2 hexadecen-1-ol	Oligosaccharides Provider
5.	Bicyclo(2.2.1) heptane,2,2,3-trimethyl-endo-	Endocrinprotective
6.	Tetradecanoic acid,10,13,di-methyl-,methyl ester	Inhibit Production of Uric Acid
7.	Hexadecanoic acid, methyl ester	Acidifier, Inhibit Production of Uric Acid
8.	Tridecanoic acid, methyl ester	Urine-Acidifier
9.	Pentadecanoic acid, 14- methyl, methyl ester	Increase Aromatic Amino Acid Decarboxylase activity
10.	Hexadecanoic acid, methyl ester	Acidifier, Inhibit Production of Uric Acid
11.	Decanoic acid, methyl ester	Urine-Acidifier
12.	Hexadecanoic acid, methyl ester	Inhibit Production of Uric Acid
13.	Pentadecanoic acid, 14- methyl, methyl ester	Acidulant
14.	Capric acid methyl ester	Acidifier
15.	Hexacontanoic acid	Urine-Acidifier
16.	N-Hexatriacontane	Narcotic, Natriuretic and Nauseant
17.	N-Dotriacontane	Inhibit Production of Tumor-Necrosis-Factor
18.	N- Hexadecane	Antitumor, Anaphylactic
19.	Stigmast-5-en-3-ol,oleate	Endrocrin-Tonic,energizer.
20.	N-Dotriacontane	Antitumor, Anaphylactic.
21.	Ergost -5-en-3-ol,acetate,(3,beta,24R)-	Endocrinactive
22.	N-Hexacosane	Antitoumer
23.	N-Tetracosane	Antitoumer, narcotic
24.	Oleyl alcohol	Detoxicant (Alcohol)
25.	2(1H)-Benzocyclooctenone,decahydro-10a-methyl-,trans	Hemorrhagic
26.	Ergost -5-en-3-ol,acetate,(3,beta.,24R)-	Endoanesthetic
27.	Ergost -5-en-3-ol,acetate,(3,beta.)-	Encephalopathic
28.	Ergost -7-en-3-ol,acetate,(3,beta.)-	Endrocrin-Tonic,energizer
29.	Stigmast-5-en-3-ol,oleate	Endrocrin-Tonic,energizer
30.	Lanost-8-ene	Energizer
31.	Ethanone,1,1'-(6-hydroxy-2,5-benzofurandiyl)bis-	Testosterone-Hydroxylase-Inducer
32.	Manganese,.pi.-cyclohexadienyl(hexamethylbenzene)	Pituitary-sensitizer
33.	8-Amino-5-benzyloxy-6-methoxy-4-methylquinoline	Increase Aromatic Amino Acid Decarboxylase Activity

34.	1-Naphthalenol,decahydro-4a-methyl-8-methylene-2-(1-methylethyl)-,acetate ,(1S-(1.alpha.,2.beta.,4a.alpha.,8a.alpha.))-	Male genital disorder.
35.	2H-Cyclopropa(a)naphthalene-2-one,1,1a,4,5,6,7,7a,7b-ocah,1,7,7a-tetramethyl-,(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)-	Male genital disorder.

Table 3: Biological activity of compounds identified in the fruits of *Phyllanthus emblica* L.

Source: Dr. Duke's : Phytochemical and Ethnobotanical databases (Dr. Duke's 1992-2016).



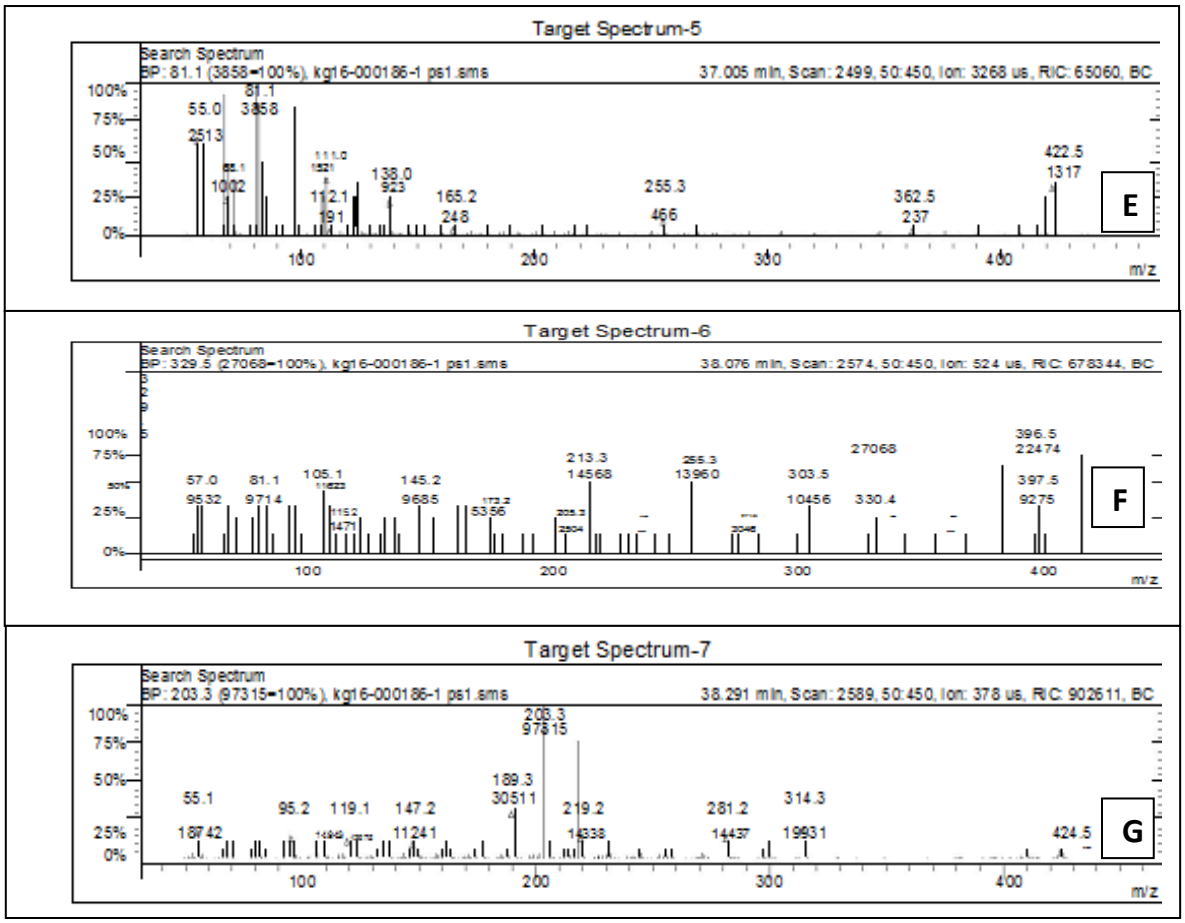


Fig.4 (A-G). Mass spectra of chemical compounds obtained by seven different target spectrums from Ethyl acetate fruit extract of *PE*.

Discussion:

The study on the active principles of ethyl acetate extract of the fruit of *Phyllanthus emblica* using GC- MS showed the presence of 70 major and minor peaks obtained from entire solution. Most of these compounds have shown proven medicinal values in the pharmaceutical industries like Bicyclo (2.2.1) heptane,2,2,3-trimethyl-endo-as Endocrin-protective, Tetradecanoic acid,10,13,di-methyl-,methyl ester as to Inhibit Production of Uric Acid, Hexadecanoic acid, methyl ester as Acidifier (Dr. Duke's 1992-2016). Pentadecanoic acid, 14- methyl, methyl ester Increases Aromatic Amino Acid Decarboxylase Activity. Pentadecanoic acid, 14- methyl, methyl ester used as Acidulant. N-Hexatriacontane used as Narcotic, Natriuretic and Nauseant. N-Dotriacontane and N-Hexadecane on the other hand Inhibit Production of Tumor-Necrosis-Factor thus helps in tumour healing. Stigmast-5-en-3-ol,oleate, Ergost -5-en-3-ol,acetate, (3,beta,24R)-,Ergost-5-en-3-ol,acetate, (3,beta.,24R)-, Ergost -5-en-3-ol,acetate, (3,beta.) and Ergost -7-en-3-ol,acetate,(3,beta.)- are used as Endrocrin-Tonic, energizer and as Endoanesthetic (Dr. Duke's1992-2016).2(1H)-Benzocyclooctenone, decahydro-10a-methyl-, trans medicinally used to treat Hemorrhage. Manganese,. pi.-cyclohexadienyl (hexamethylbenzene) used as Pituitary-sensitizer. Ethanone, 1, 1'-(6-hydroxy-2,5-benzofurandiyl) bis used as Testosterone-Hydroxylase-Inducer.

1-Naphthalenol,decahydro-4a-methyl-8-methylene-2-(1-methylethyl)-,acetate,(1S-(1.alpha., 2.beta., 4a.alpha.,8a.alpha.))- and

2H-Cyclopropa(a)naphthalene-2-one,1,1a, 4,5, 6,7,7a,7b-ocah,1,7,7a-tetramethyl-, (1a.alpha., 7. alpha., 7a.alpha.,7b.alpha.)- Medicinally used as Male genital disorder (Dr. Duke's 1992-2016).

Conclusion:

GC-MS study indicates 70 major and minor phyto-constituents present in the ethyl acetate fruit extract of the *Phyllanthus emblica*, out of which almost 35 phyto-constituents have proved biological activities (according to Dr. Duke's : Phytochemical and Ethnobotanical databases) which justified its use for various ailments by traditional practitioners.

Present investigation provides the scientific basis to the ethno-medical usage of the fruit. However, isolation of the individual phytochemical constituents, subjecting it to biological activity, toxicity profiles are needed to be exploring in scientific way. Hence further studies are needed to be worked out on the application of individual phyto-chemical compound to the actual sufferer to treat for various ailments by medical practitioners.

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