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GC-MS ANALYSIS OF *PHYLLANTHUS EMBLICA*L. FRUIT EXTRACT USED FOR ANTIUROLITHIATIC ACTIVITY

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ABSTRACT:
Urolithiasis is a global problem afflicting human beings for several centuries. It is also called Nephrolithiasis of kidney stones. Kidney stone formation is a complex that results from a succession of several physicochemical events including supersaturation, nucleation growth, aggregation and retention within kidneys. Medicinal plants as an alternative source for its management as they have potential phytoconstituents. These Phytoconstituents exert their beneficial effect on urolithiasis. For this study *Phyllanthus emblica* Fruit is selected which is commonly used for the treatment of kidney stone related problems. The aim of the present study is to detect the volatile organic compound present in *Phyllanthus emblica* fruit extract through Gas Chromatography and Mass Spectroscopy Analysis.

Keywords:- GC-MS Analysis, Antiurolithiatic activity, Phyllanthus emblicaL., Medicinal Plants.

INTRODUCTION :

Urolithiasis is the process of formation of deposition of calculi in the urinary tract which is considered as the third most common disorder estimated to occur in around 12% of the global population worldwide. It is a process of stone formation which occur either in the kidney(commonly known as nephrolithiasis) and or any part of the urinary tract, including the ureters (Known as ureteral stone) and Bladder (Bladder stone). This urological disorder affects about 12% of the world population. About 80% all kidney stones are composed of oxalate, Calcium and around 20% of kidney stones made of calcium phosphate which, like oxalate calcium stones, are formed as a result of increased calcium. The People with kidney stones in the US as 12 % of Males and 6% of Females and in India also has 12 % get affected but it is approximately 12 % of the world population get renal stone disease with a reappearance rate of 70-80% of Males and 40-60% females.Medicinal plant materials could be beneficial to find out efficient cure for urinary stones. Indeed, the world health organization has also paid importance to the use of herbal drugs as well as traditional medicines due to low cost and low side effects. The frequently occurring side effects by using diuretics drugs are headache, nausea, dizziness, loss of appetite gout, rheumatoid arthritis and joint pain may occur in severe cases.Herbal or natural diuretics play a major role in treating kidney related diseases. Diuretics or water pills are much stronger and more effective than the natural herbs, but the risk of side effects is much greater because of using these diuretic drugs. In the present study author chose *Phyllanthus emblica* based on its traditional prescription.

MATERIAL AND METHODS:

Material: -*Phyllanthus emblica* L. **Part used:** - Fruit

Preparation of plant extract: -

In order to isolate the active ingredients from this plant, Methanolic extract was prepared by filling the Soxhlet thimble for each extraction medium with 10 g powder material separately. In process, plant powder has been placed in

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cellulose thimble in an extractor chamber, which has been placed on the top of the collecting flask beneath a reflux condenser. With the addition of 250 ml methanol, separately to the flasks, set up was heated under reflux and when certain level of condensed solvent has been accumulated in the thimble it was siphoned off into the flask beneath. Nearly 15 cycles were repeated for 72 hours for methanol extraction medium. The extract was filtered with Whatman filter paper no.1 and evaporated in water bath at 40°C. The prepared extracts have been stored in the dark bottles in refrigerator till further use.

Gas Chromatography and Mass Spectroscopy(GCMS): -

Gas chromatography is a powerful separation technique for detection of volatile organic compounds. Combining separation and on-line detection allows accurate quantitative determination of complex mixture, including traces of compounds down to parts per trillion in some specific cases. Gas liquid chromatography commands a substantial role in the analysis of pharmaceutical product (Watson, 1999). Mass spectrometry consists basically of weighing ions in the gas phase. Mass spectrometry is an analytical technique that involved the study in the gas phase of ionized molecules with the aim of Molecular weight determination, Structural characterization, Qualitative and quantitative analysis of components in a mixture etc. (Horborn 1998).

The extract prepared for this analysis is also methanolic of 100mg/ml concentration to obtain the retention time of each compound, area%, height% and thereafter it has been search against NIST and Wiley Library to obtain the chemical name and CAS number if available with respect to peak information obtained for each compound. Private agency Sai Biosystems Private Limited, Nagpur has been used for the service and compound details has been understood with the technical person.

GC-MS analysis of the methanol extract of plants was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µmdf). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

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RESULT AND DISCUSSION:

1 GC-MS Spectra of extract obtained from *Phyllanthus emblica* L.

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	10.005	6480142	52.25	681526	36.09	9.51	1,2-Benzenedicarboxylic acid, diethyl ester (C
2	12.125	263871	2.13	14994	0.79	17.60	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-o
3	13.134	639689	5.16	166304	8.81	3.85	1,2-Benzenedicarboxylic acid, butyl 8-methyln
4	13.765	74957	0.60	29796	1.58	2.52	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-dien
5	13.892	52389	0.42	10115	0.54	5.18	OLEIC ACID, PROPYL ESTER
6	14.050	307088	2.48	55983	2.96	5.49	Hexadecanoic acid (CAS) Palmitic acid
7	14.145	850544	6.86	172792	9.15	4.92	1,2-Benzenedicarboxylic acid, butyl 2-methylp
8	14.292	79477	0.64	23486	1.24	3.38	PHOSPHINE, TRI-2-PROPENYL-
9	14.600	39900	0.32	20137	1.07	1.98	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-
10	15.233	1103430	8.90	220978	11.70	4.99	1-Octadecanol (CAS) Stenol
11	15.479	237150	1.91	43577	2.31	5.44	TETRACOSAMETHYLCYCLODODECASI
12	16.591	241698	1.95	24137	1.28	10.01	N-TETRADECANOIC ACID AMIDE
13	17.189	146819	1.18	51445	2.72	2.85	OCTADECAMETHYLCYCLONONASILOX
14	17.436	399539	3.22	90050	4.77	4.44	4-p-chorophenyl-2-dimethylamino-5-nitrosoth
15	18.120	76486	0.62	20087	1.06	3.81	1-Decanol, 5,9-dimethyl-
16	19.382	182672	1.47	52115	2.76	3.51	EICOSAMETHYLCYCLODECASILOXANI
17	20.524	75281	0.61	16101	0.85	4.68	1-(2-Methoxypropyl)-6-bromo-6-deoxy-3,4-O
18	21.450	73480	0.59	10367	0.55	7.09	1-(DECYLSULFONYL)-1-DEOXY-D-MANI
19	22.425	224432	1.81	51460	2.72	4.36	TETRACOSAMETHYLCYCLODODECASI
20	22.908	853188	6.88	133135	7.05	6.41	BIS(2-ETHYLHEXYL) PHTHALATE
		12402232	100.00	1888585	100.00		

The chromatogram obtained with the extract of *Phyllanthus emblica* L. indicated presence of total 20 different compounds. Notable amongst them are 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate, 1-Octadecanol (CAS) Stenol, BIS(2-ETHYLHEXYL) PHTHALATE, 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (CAS) N-BUTYL ISOBUTYL PHTHALATE, 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester, 4-p-chorophenyl-2-dimethylamino-5-nitrosothiaz. Choubey et al., (2010) have also reported that ethyl phthalate and Stenol have promising antiurolithiatic action.

DISCUSSION:

Lans (2006) has reported that the genus *Phyllanthus* has a long history of use in the treatment of kidney stones and some related species with medicinal significance are *P. epiphyllanthus, P. niruri P. urinaria, P acuminatus and P. emblica. P. amarus, P. nururi* and *P. urinaria* that are used in the treatment for kidney and gallstones. Barros et al., (2003) have also reported about the positive effects of aqueous extract from *Phyllanthus niruri* on calcium oxalate in vitro. More recently, Bindhu et al., (2015) have reported that *Phyllanthus emblica* extract have an inhibiting effect on the growth of struvite type crystals. Therefore, in the present study GC-MS analysis was done to found out volatile organic compound having antiurolithiatic property.

CONCLUSION:

GC-MS data concluded that the compounds which have more area of % which are more effective for antiurolithiatic activity.

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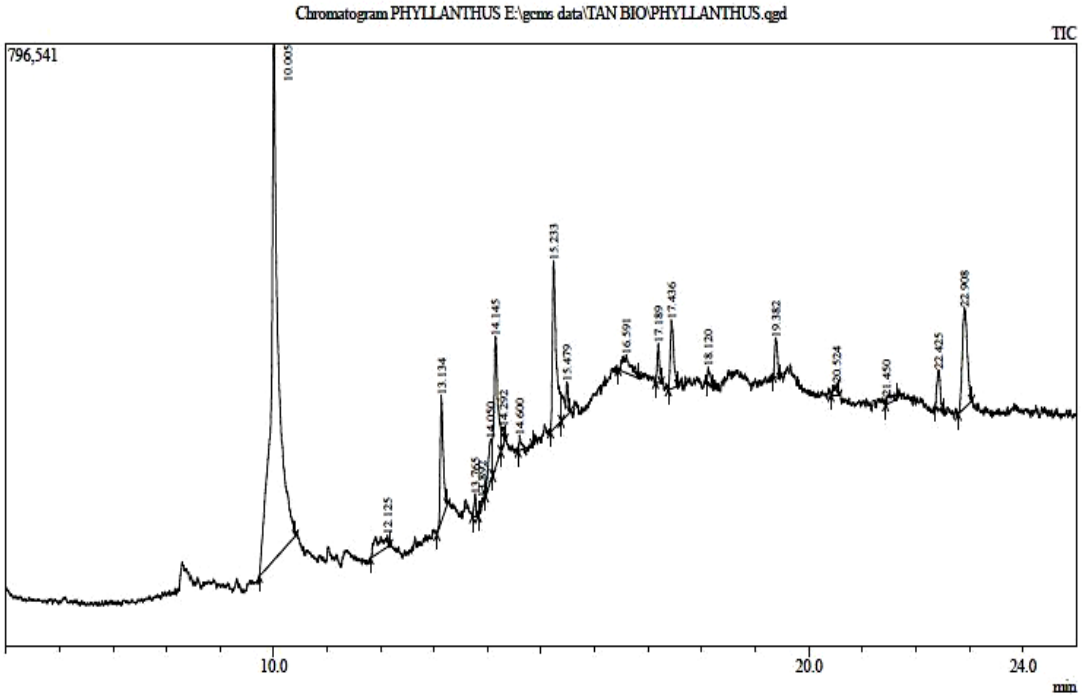

Fig 1. Chromatograph of extract obtained from *Phyllanthus emblica* L.

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