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GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF *KYLLINGA TRICEPS*

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ABSTRACT:

Objective: To isolate and analyze the chemical components in crude methanolic extract from the South Indian medicinal plant of *Kyllinga* ***triceps*** by gas chromatography-mass spectrometry (GC-MS).

Methods: Preliminary phytochemical screening of the extract was carried out according to the standard method described by Brindha *et al*. The shade dried powder was extracted with methanol by using soxhlet extractor. The GC-MS analysis of methanolic extract of *Kyllinga triceps* was performed using a GC–MS equipment Thermo GC-TRACE ultra ver., 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30 Mts, ID: 0.25 mm, Film: 0.25 µm was used and flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. Crude samples which dissolved in methanol were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral library search programme.

Results: The phytochemical analysis of the extract revealed the presence of steroids, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins and amino acids. The GC-MS analysis provided different peaks determining the presence of twenty two different phytocompounds namely Methanone, (1-hydroxycyclohexyl) phenyl - (14.88%), Tricyclo [4.2.1.1 (2,5)] dec-7-en-9-ol-(6.62%), n-deutero-3-ethyl-3-phenyl-2,6-dioxopiperidine-(5.19%), 2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-(4.08 %), (E)-2-ethylidene-3-oxo-GA9 16á, 17-epoxide methyl ester (2.94%), Z)-4-chloro-2-methyl-1-phenylbut-3-en-1-one (2.05 %), etc.

Conclusion: The presence of various bioactive compounds confirms the application of *Kyllinga triceps* for various ailments. However, isolation of individual phytochemical constituents may be proceeded to find out a novel drug.

INTRODUCTION: In developing countries like India, the majority of people living in rural areas were almost exclusively using traditional medicines in treating all sorts of disease [1].

Plants make a significant contribution to health care due to the recognition of the value of traditional medicinal systems [2-3].

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Now a day's more light is being shed on the importance of medicinal plants, many of which have been used as more traditional or folk remedies, on the other hand, they are being studied and analyzed for potential biological activities that will thus explain why the local have been used them for treating various disease and illness less [4]. Herbal medicine is safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway [5]. Plant – based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits and seeds etc [6]. The medicinal actions of plants unique

to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct [7]. Screening of active compounds from plants has led to invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer [8], heart disease [9], febrifuge and antidermatosis, also used to treat diabetes. The determination of phytoconstituents is largely performed by the relatively expensive and often laborious techniques such as gas (GC) and liquid chromatography (LC) combined with specific detection schemes [10].

In the last few years, GC-MS has become firmly established as a key technological metabolic profiling in both plant and non-plant species [11-13]. *Kyllinga triceps* is an herb belonging to the family Cyperaceae. It grows in moist places. Fresh juice of the plant is used externally to wash the wounds. It is used in the treatment of indigestion [14]. Decoction of roots is used in diabetes and to relieve thirst in fevers [15]. The roots yield oil which is used to promote the action of the liver and relieve pruritus [16]. The literature search reveals that still no work have been done on this plant. Nobody has isolated this crude extracts from methanolic solvent and analyse the crude extract by GC-MS. With this knowledge the present study was intended to determine the phytochemical profile of the extract of *Kyllinga triceps* using GC-MS.

MATERIALS AND METHODS:

Collection of the plant material: The plant *Kyllinga triceps* was collected from the Tirunelveli Dist, Tamil Nadu, India in the month December 2012. The plant materials was identified and authenticated by Dr. V. Chelladurai, Retired Research officer-botany, Central Council For Research In Ayurveda and Sidha (C.C.R.A.S). Govt. of India, Tiruneveli. The collected plant material was free form disease and also free from contamination of other plants.

Preparation of plant extract: 100 gms of *Kyllinga triceps* air dried and coarsely powdered entire plant material was extracted with 500 ml methanol by using Soxhlet extractor. The sample was kept in dark for 72 hrs with intermittent shaking. Then the solvent was evaporated under reduced pressure using rotary evaporator and to obtained viscous semi solid masses (g).

Phytochemical Screening: The methanolic extract was tested for steroids, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and amino acids. Phytochemical screening of the extract was carried out according to the standard method [17].

GC-MS analysis: The GC-MS analysis of methanolic crude extract of *Kyllinga triceps* was performed using a GC–MS equipment Thermo GC-TRACE ultra ver: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film: 0.25µm was used and flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40^{o}C raised to 250^{o}C at 5^{o}C / min and injection volume was 1 micro litre. Samples which dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using Wiley spectral library search programme.

RESULT & DISCUSSION: Preliminary phytochemical screening of nine different metabolites (steroids, alkaloids, sugars, phenolics, flavonoids, saponins, tannins, anthraquinone and amino acids) were tested with methanolic extract of *Kyllinga triceps*. Hence the results showed the presence of steroids, alkaloids, sugar, phenolics, flavonoids, saponins, tannins and amino acids but not anthraquinone. The result of phytochemical screening of different secondary metabolites of *Kyllinga triceps* were showed in **Table 1.**

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *KYLLINGA TRICEPS*

Compounds	Methanol Extract
Steroids	+
Alkaloids	+
Sugar	+
Phenolics	+
Flavonoids	+
Saponins	+
Tannins	+

Amino acids	+

The results are relevant to GC-MS analysis leads to the identification of number of compounds from GC fractions of the methanolic extracts of *Kyllinga triceps.* They were identified through mass spectrometry attached with GC. GC-MS analysis of methanolic extract of *Kyllinga triceps* was putted into a **Table 2**. The various components present in the methanolic extract of *Kyllinga triceps* that were detected by the GC-MS are shown in Table 2.

TABLE 2: COMPOUNDS PRESENT IN THE METHANOLIC EXTRACT OF *KYLLINGA TRICEPS* USING GC-MS ANALYSIS

S. No.	RT	Name of the Compound	MF	MW	Peak area %	Compound nature
1	0.39	(E)-2-ethylidene-3-oxo-GA9 16á, 17-epoxide methyl Ester	$C_{22}H_{26}O_6$	386	2.94	Ester
2	7.46	3-(4'-Bromophenyl)-5,6-diphenylimidazo[2,1-b]thiazole	$C_{23}H_{15}BrN_2S$	430	0.68	Alkaloid
3	9.35	[22,23,24-trideuterio]-3á,14à, 25-trihydroxy-5á-cholest-7-en-6 –one	$C_{27}H_{41}D_3O_4$	432	0.80	Sterol
4	12.62	(Z)-4-chloro-2-methyl1phenylbut -3-en-1-one	$C_{11}H_{11}ClO$	194	2.05	Ketone
5	13.25	cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	0.72	Alkane
6	14.33	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-	$C_{13}H_{20}O_2$	208	4.08	Ester
7	17.13	Phenol, 2,4-bis(1,1-dimethylethyl)- (CAS)	$C_{14}H_{22}O$	206	0.43	Phenol
8	17.70	Tricyclo[4.2.1.1(2,5)]dec-7-en-9-ol	$C_{10}H_{14}O$	150	6.62	Alcohol
9	20.58	Methanone, (1-hydroxycyclohexyl)phenyl-	$C_{13}H_{16}O_2$	204	14.88	Ketone
10	22.43	4-{2,6-Dibromo-4-[(tert-butyl) azo] phenyl}morpholine	$C_{14}H_{19}Br_2N_3O$	403	0.47	Alkaloid
11	25.71	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270	0.44	Fatty acid ester
12	27.46	3,4-dihydro-4-oxochinazolin-2-carbonsaure-ethylester	$C_{11}H_{10}N_2O_3$	218	0.41	Alkaloid
13	29.01	n-deutero-3-ethyl-3-phenyl-2,6-dioxopipe ridine	$C_{13}H_{14}DNO_2$	217	5.19	Alkaloid
14	29.32	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	0.35	Ester of fatty acid
15	32.72	1-(2-Allyl-phenoxy)-3-[4-(2-hydroxy-ethyl)-piperazin-1 -yl]-propan-2-ol	$C_{18}H_{28}N_2O_3$	320	1.29	Alkaloid
16	33.42	6-(2-Trimethylsilylethynyl)-2-(4-carbomethoxypyrazin-1-yl) pyridine	$C_{16}H_{23}N_3O_2Si$	317	0.32	Alkaloid
17	36.01	3-Hydroxy-2-(p-tolyl)-3-phenylylisoindolin-1-one	C21H17NO2	315	0.44	Alkaloid
18	36.55	3methyl4(selenophenylmethyl)cinnoline	$C_{16}H_{14}N_2Se$	314	0.34	Alkaloids
19	36.86	Cholesta-5,7,9(11)-trien-3-ol, 4,4-dimethyl-, (3á)-	$C_{29}H_46O$	410	0.50	Sterol
20	37.15	3-(3",4",5"-Trimethoxyphenyl)diosmetin	$C_{25}H_{22}O_9$	466	0.71	Flavonoids
21	37.62	6-(t-Butylimino)-8-(3'-trifluoromethylphenyl)-3,4-dihyd ro-2H, 6H-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile	$C_{19}H_{19}F_3N_4S$	392	0.61	Alkaloids
22	37.97	Diisooctylphthalate	$C_{24}H_38O_4$	390	1.89	Ester

The results revealed that the presence of 22 different phytocompounds viz., (E)-2-ethylidene-3-oxo-GA9 16á, 17-epoxide methyl ester (2.94 %), 3-(4'-Bromophenyl)-5,6-diphenylimidazo[2,1-b]thiazole (0.68 %), [22,23,24-trideuterio]-3á,14à,25-trihydroxy-5á-cholest-7-en-6-one (0.80 %), (Z)-4-chloro-2-methyl-1-phenylbut-3-en-1-one (2.05 %), cyclohexasiloxane, dodecamethyl-(0.72 %), 2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-(4.08 %), Phenol, 2,4-bis(1,1-dimethylethyl)- (CAS)-(0.43 %), Tricyclo[4.2.1.1(2,5)]dec-7-en-9-ol (6.62 %), Methanone, (1-hydroxycyclohexyl)phenyl-(14.88 %), 4-{2,6-Dibromo-4-[(tert-butyl)azo]phenyl}morpholine (0.47 %), Hexadecanoic acid, methyl ester (CAS) –(0.44%), n-deutero-3-ethyl-3-phenyl-2,6-dioxopiperidine-(5.19%) , 9,12-Octadecadienoyl chloride, (Z,Z)-

(0.35 %), 1-(2-Allyl-phenoxy)-3-[4-(2-hydroxy-ethyl)-piperazin-1-yl]-propan-2-ol (1.29 %), 6-(2-Trimethylsilylethynyl)-2-(4-carbomethoxypyrazin-1-yl)pyridine (0.32 %), 3-Hydroxy-2-(p-tolyl)-3-phenylylisoindolin-1-one (0.44 %), 3-methyl-4-(selenophenylmethyl)cinnoline (0.34 %), Cholesta-5,7,9(11)-trien-3-ol, 4,4-dimethyl-, (3á)-(0.50 %), 3-(3",4",5"-Trimethoxyphenyl)diosmetin(0.71%), 6-(t-Butylimino)-8-(3'-trifluoromethylphenyl)-3,4-dihyd ro-2H, 6H-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile (0.61 %), diisooctylphthalate (1.89 %). The GC-MS spectrum confirmed the presence of 22 major components with the retention time 0.39, 7.46, 9.35, 12.62, 13.25, 14.33, 17.13, 17.70, 20.58, 22.43, 25.71, 27.46, 29.01, 29.32, 32.72, 33.42, 36.01, 36.55, 36.86, 37.15, 37.62, and 37.97 respectively **(Fig. 1).**

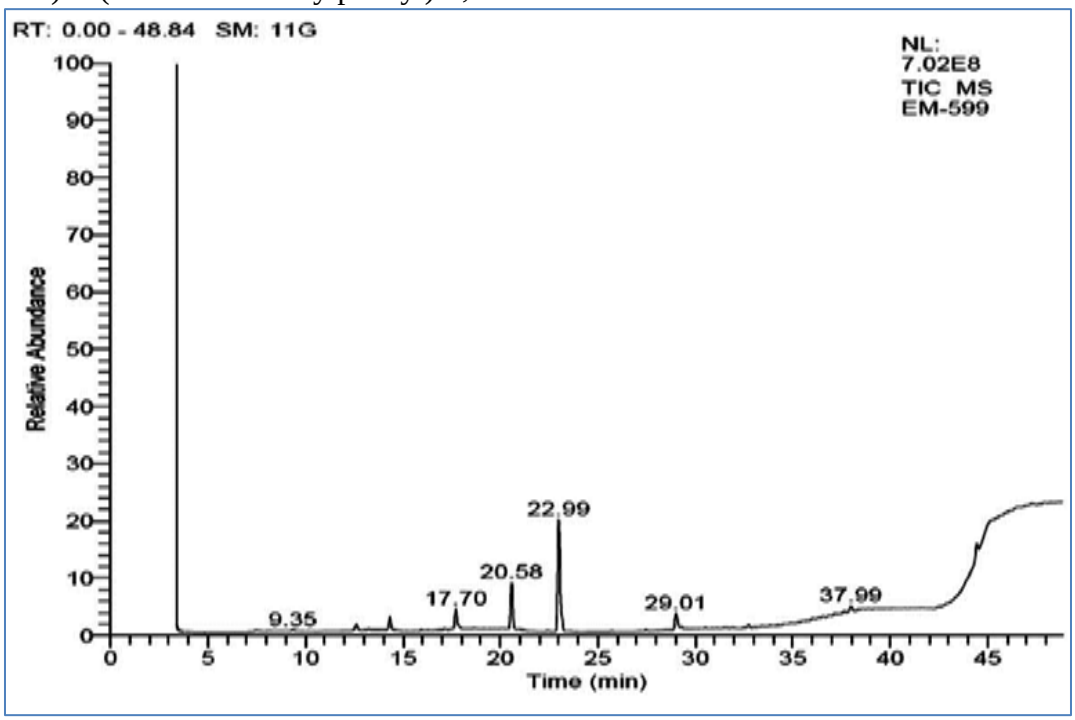

FIGURE 1: GC-MS CHROMATOGRAM OF METHANOLIC EXTRACT OF *KYLLINGA TRICEPS*

The name, molecular weight, molecular formula and structure of the component of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

CONCLUSION: The present study results confirmed the presence of steroids, alkaloids, sugar, phenolics, flavonoids, saponins, tannins and amino acids. It helps to predict the formula and structure of biomolecules which can be used as drugs. It enhances the traditional usage of *Kyllinga triceps* which possesses several known and unknown bioactive compounds. Further investigation may lead to the development of drug formulation. Evaluation of pharmacological activity in the methanolic extract is in progress.

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