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Determination of phytochemicals in *Gloriosa superba* flower extract using gas gromatography and mass spectroscopic technique

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Abstract
Plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. Gas chromatographic-mass spectrometry (GC-MS) is a very powerful and ubiquitous analytical technique. It is often the analytical method of choice in toxicology, forensics, food science, and environmental research. In the present study to investigate the phytocomponents in *Gloriosa superba* flowers using GCMS. Twenty one chemical constituents have been identified from extract of *Gloriosa superba* flowers by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The prevailing compounds were 2-Octylcyclopropene-1-heptanol, Hexadecanoic acid ethyl ester, Timonacic, l-(+)-9, 12, 15-Octadecatrienoic acid, Phytol and 1,2-Benzenedicarboxylic acid. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Keywords: Gas chromatographic-mass spectrometry, *Gloriosa superba* flower, phytocomponents, Biological activity

Introduction
Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines Sathyaprabha *et al.* (2010) [12]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations Mathekaga and Meyer (1998) [8].
Phyto chemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function Harborne (1986) [2].
Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) Liu (2004) [6]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits Hamburger and Hostettmann (1991) [1]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals Roberts and Xia (1995) [9].
Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants Ronald Hites (1997) [10].

Materials and methods
Plant materials
The fully mature flowers of *Gloriosa superba* were collected in January 2015 from Thanjavur,

Tamil Nadu, Kerala and Karnataka states, India from a single herb. The flowers were identified and authenticated by Dr. S. John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamilnadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

Preparation of alcoholic extract

The collected *Gloriosa superba* flowers were washed several times with distilled water to remove the traces of impurities from the flowers. The flowers were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Gloriosa superba* flowers extract was stored in refrigerator until used.

GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0

Results and discussion

Gas chromatographic-mass spectrometry (GC-MS) is a very powerful and ubiquitous analytical technique. It is often the analytical method of choice in toxicology, forensics, food science, and environmental research. GC can well separate complex mixtures, and MS can detect these compounds. GC plays a role in separation and introduces target substances into MS system by directly injecting analytes into chromatographic column or introducing analytes into chromatographic column after injecting and heating. The chromatographic column is heated thermostatically or program-controlled. Each component is separated by the difference of thermodynamic properties (the difference of boiling points and the difference of selective absorption in the stationary phase) and the different distribution in stationary phase and mobile phase (carrier gas). MS is in fact a detector, mainly including ionization source, mass analyzer, and electron multiplier tubes. Target substances enter into MS through GC and ionized into gaseous ions in the ionization source and then enter into mass analyzer. Ions with different mass-to-charge ratio are sequentially separated and reach the electron multiplier, generating electrical signal, in order to give the 3D information of the target substances, making qualitative analysis more accurate by using ion fragment information Lisec *et al.* (2006) [5].

In the present study twenty one chemical constituents have been identified from extract of *Gloriosa superba* flowers by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The prevailing compounds were 2-Octylcyclopropene-1-heptanol, Hexadecanoic acid ethyl ester, Timonacic, l-(+)-9,12,15-Octadecatrienoic acid,, Phytol and 1,2-Benzenedicarboxylic acid. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results in Figure 1, Table 2 and 3.

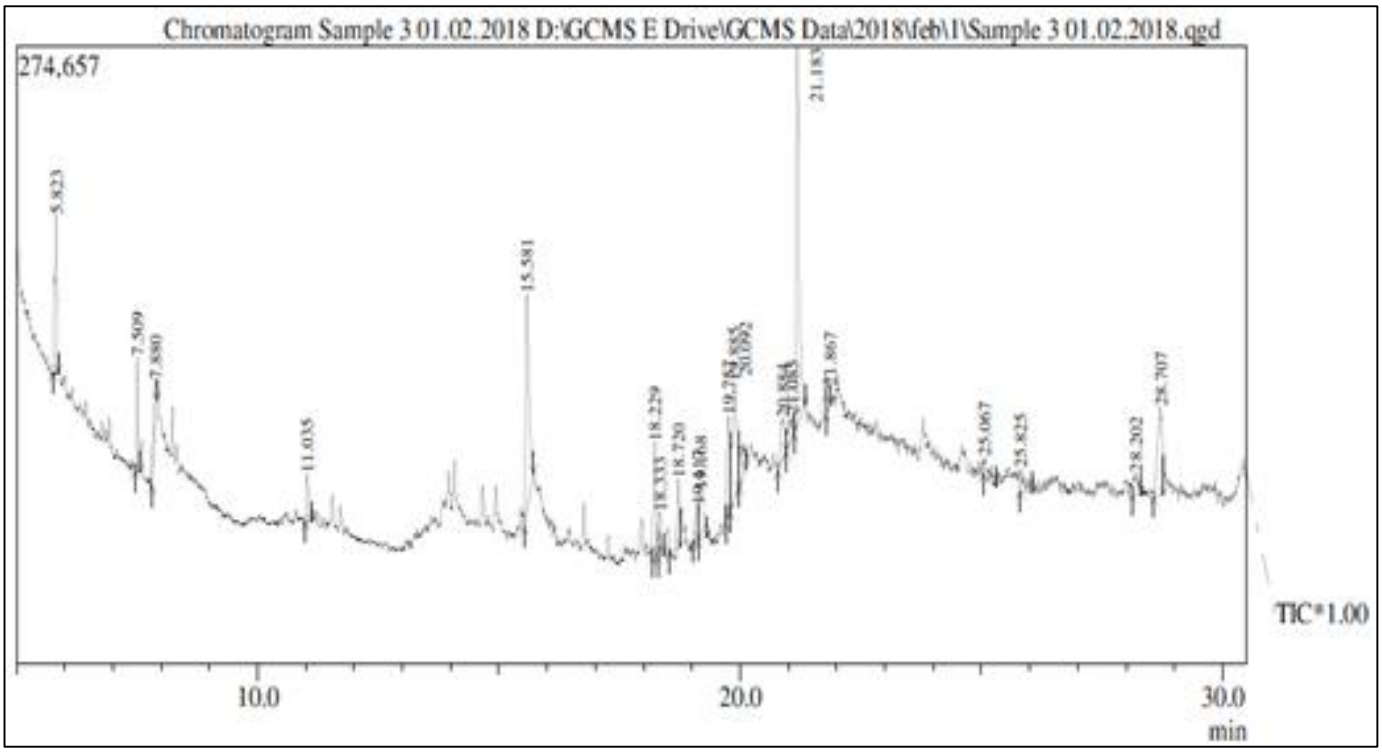

Fig 1: Chromatogram obtained from the GC-MS with the *Gloriosa superba* flowers extract

Table 1: Components identified in ethanolic extract of *Gloriosa superba* flowers (GC- MS study)

Peak#	R Time	Area%	Molecular formula	Molecular weight	Molecular Name
1	5.823	5.87	$C_9H_{20}O_2$	160	Butane, 1,1-diethoxy-2-methyl-
2	7.509	3.13	$C_{12}H_{26}$	170	Decane, 3,7-dimethyl-
3	7.880	3.07	$C_9H_{20}O_3$	176	Propane, 1,1,3-triethoxy-
4	11.035	1.97	$C_{12}H_{26}$	170	Decane, 3,7-dimethyl-
5	15.581	13.85	$C_{20}H_{26}O_4$	330	Phthalic acid, di-(1-hexen-5-yl) ester
6	18.229	4.60	$C_{15}H_{30}O$	226	Pentadecanal-
7	18.333	2.16	$C_7H_{14}O$	114	1,3-Dimethylcyclopentanol
8	18.720	2.60	$C_{18}H_{34}O$	266	2-Octylcyclopropene-1-heptanol
9	19.117	1.77	$C_{10}H_{20}FO_2P$	222	Methylphosphonic acid, fluoroanhydride, 3-cyclohexylpropyl ester
10	19.168	3.74	$C_8H_{16}O$	128	Cyclohexanol, 1-ethyl-
11	19.757	4.75	$C_{14}H_{16}O_4$	248	(Z)-2-((Hex-3-enyloxy)carbonyl)benzoic acid
12	19.885	10.33	$C_{18}H_{36}O_2$	284	HEXADECANOIC ACID, ETHYL ESTER
13	20.092	2.71	$C_4H_7NO_2S$	133	Timonacic
14	20.884	3.45	$C_{15}H_{30}O$	226	Pentadecanal-
15	21.085	2.04	$C_{27}H_{52}O_4Si_2$	496	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-
16	21.183	19.85	$C_{20}H_{40}O$	296	Phytol
17	21.867	1.77	$C_{19}H_{28}N_4O_3$	360	1H-IMIDAZOLE-1-ACETAMIDE, 2-METHYL-5-NITRO-N-(1-TRICYCLO[3.3.1.1(3,7)]DEC-1-YLPROPYL)-
18	25.067	1.66	$C_8H_{22}OSSi_2$	222	3-Oxa-6-thia-2,7-disilaoctane, 2,2,7,7-tetramethyl-
19	25.825	1.61	$C_{12}H_{22}Si_2$	222	1,2-Bis(trimethylsilyl)benzene
20	28.202	1.71	$C_{10}H_{30}O_3Si_4$	310	TETRASILOXANE, DECAMETHYL-
21	28.707	7.37	$C_{26}H_{42}O_4$	418	1,2-benzenedicarboxylic Acid, Dinonyl Ester

Table 3: Biological Activity of phyto-components identified in the ethanolic extracts of the *Gloriosa superba* rhizome by GC-MS.

S.NO.	R. Time	Name of the compound	Biological activity **
1.	18.720	2-Octylcyclopropene-1-heptanol,	Antibacterial activity
2.	19.885	Hexadecanoic acid ethyl ester,	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alphareductase inhibitor
3.	20.092	Timonacic	Antineoplastic and antioxidant activities
4.	21.183	Phytol	Antibacterial, Anticancer, anti-inflammatory, anti-diuretic, immunostimulatory and anti-diabetic
5.	21.085	9,12-Octadecadienoic Acid	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne
6.	28.707	1,2-Benzenedicarboxylic acid,	Antibacterial, Antifouling

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database.

Karpagasundari and Kulothungan (2014) [4] screened the bioactive components of *Physalisminima* leaves have been evaluated using GC-MS. GC-MS analysis of extract of *Physalisminima* leaves revealed the existence of heneicosanoic acid (25.22), bicycle [4.1.0] hepta-2, 4-dien (27.41) octadecanoic acid (CAS), stearic acid (31.19) and octadeca-9, 12-dienoic acid (32.02).

Similarly the work was done by GC-MS analysis of bioactive components of *Hugonia mystax* L. (Linaceae). Thirteen compounds were identified. 1,2-benzene dicarboxylic acid, diisooctyl ester (48.75 %) was found to be major component followed by n- hexadecanoic acid (13.52 %), phytol (9.25 %), squalene (6.41 %), vitamin E (4.09 %), dianhydromannitol (3.56 %), 9,12–octadecadienoic acid (Z,Z)–(3.20%) and 3,7,11,15 – tetramethyl -2- hexadecen -1-ol (2.85 %).

Phytol isomer is one among the thirty compounds of the present study. Similarly Maria Jancy Rani *et al.* (2011) [7] observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* (2011) [13] in *Mimosa pudica* leaves. Similarly result was also observed in the leaves of *Lantana camara* Sathish kumar and Manimegalai (2008) [11]. Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells Inoue *et al.* (2005) [3]. Phytol, phenol, 2, 4-bis (1-phenylethyl) which are all have medicinal properties. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K. It is used along with simple sugar or corn syrup as a hardener in candies. Similar types of compounds were identified among the thirty compounds of this present study.

Conclusion

In the present study twenty chemical constituents have been identified from Methanolic extract of the whole plant of *Gloriosa superba* flower by Gas Chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of flower various ailments by traditional practitioners.

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