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GC-MS analysis of phytochemical compounds present in the rhizome of *Gloriosa superba* L.

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Abstract
The present study focused on the evaluation of medicinally active compounds from tuber of *Gloriosa superba* by Gas chromatography mass spectrometry (GCMS). The rhizomes of the *Gloriosa superba* were collected, washed, shade dried and powdered. The methanol extract was prepared using soxhlet apparatus and the phytochemicals were screened from this crude methanol tuber extract of *Gloriosa superba*. The results indicated the presence of important secondary metabolites such as glycosides, flavonoids, alkaloids, saponins, coumarins, terpenoids, steroids and tannins. Further the extracts were subjected to Gas Chromatography Mass Spectroscopy for the identification of biochemical components present in the rhizome. The results showed that the rhizome containing a wide range of medicinally active phytochemicals so that it could be used as a pharmaceutically potent plant.

Keywords: *Gloriosa superba*, phytochemicals, alkaloids, medicinal plants, GCMS, rhizome

1. Introduction
Various kinds of pharmaceutical compounds from plants were used as alternative source for the development of medicines. When compared to other systems of medicine, Ayurveda uses more plant based medicines for the treatment of ailments. Because of the modern life style numerous new diseases are being identified lately. The remedies for all diseases could be present in nature but most of potentially valuable treasures in medicinal plants as bioactive compounds are unexplored and underutilized. A healthy new generation could be formed due to the systematic use of these valuable medicinal compounds and plants [1].
Gloriosa superba (Liliaceae) is one of the herbaceous medicinal climber which is a striking tuberous plant with brilliant wavy edged yellow and red flowers that appears from November to March every year [2]. It is used to cure cancer, gout, asthma, leprosy, arthritis, piles, ulcers and act as abortifacient, anthelminthic and anti-inflammatory agents etc. It is also used to treat intestinal worms, bruises, infertility, skin problem and impotence. The plant is one of the seven upavishas in the Indian medicine, which cure many ailments but may prove fatal on misuse. The tuberous root stocks of *Gloriosa superba* boiled with *Sesamum* oil reduces arthritis pain in joints [3]. The high medicinal value of this plant could be due to the presence of many secondary metabolites which act as bioactive compounds against diseases [4]. Present study intended to analyze these active compounds in roots of *Gloriosa superba* responsible for its medicinal properties.

2. Materials and Methods
2.1 Preparation of plant material
The rhizomes of the *Gloriosa superba* were collected, washed, shade dried and powdered. The powder was preserved in air sealed polythene cover for further evaluation.

2.2 Preparation of plant extract
The dried powdered tubers were defatted with petroleum ether (60 to 80 ^{0}C) by hot extraction method in a soxhlet apparatus. The defatted powder materials were further extracted with methanol and concentrated methanol extracts were used for the analysis.

2.3 Qualitative evaluation of phytochemicals
The different types of secondary metabolites such as alkaloid, steroids, glycosides, flavonoids, terpenoids, tannin, saponins and coumarins present in the crude methanol tuber extract were detected through the preliminary phytochemical studies using different standard tests [5].

2.4 Alkaloids (Dragendroff's test)
The plant extract was heated on boiling water with 10 ml of 2% sulphuric acid and were heated for 2 minute. After cooling a few drops of Dragendorff's reagent was added to it. The orange brown precipitate revealed the presence of alkaloid.

2.5 Steroids (Liebermann Burchard Reaction)
The plant extract was added with 0.5 ml of acetic anhydride and 0.5 ml of chloroform and followed by a few drops of concentrated sulphuric acid was poured slowly. The presence of steroids is indicated by the formation of green bluish colour.

2.6 Terpenoids (Salkowski Reaction)
The plant extract was dissolved in 2 ml chloroform and then 3 ml of concentrated sulphuric acid was added along the side slowly. The reddish brown inter phase revealed the presence of terpenoids.

2.7 Glycosides (Keller - Kiliani test)
The plant extract was added with glacial acetic acid, ferric chloride and concentrated sulphuric acid. The reddish brown ring at the junction of two layers indicated the presence of glycosides.

2.8 Flavonoids (Shinoda test)
The plant extract solution was heated with 1.5 ml of 50% methanol solution and followed by addition of metal magnesium. Then 5-6 drops of concentrated hydrochloric acid were poured slowly. The formation of red colour indicated the presence of flavonoids.

2.9 Saponins
The plant extract was heated with 5 ml of distilled water for few minutes. The formation of frothing revealed the presence of saponins.

2.10 Tannins
The plant extract was treated with 2 drops of 2% ferric chloride solution and the formation of dirty green indicated the presence of tannins.

2.11 Coumarins
The extract was treated with sodium hydroxide and alcoholic sodium hydroxide. Then the concentrated hydrochloric acid was poured through the side walls of the test tube slowly. Appearance and the disappearance of yellow colour revealed the presence of coumarins.

2.12 GC-MS Analysis
Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) was used for GC-MS analysis which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. Helium was used as the carrier gas at a flow rate of 0.5ml/min and 1µl sample injection volume was utilized. The inlet temperature was maintained as 250 °C. The oven temperature was programmed initially at 110 °C for 4 min, then an increase to 240 °C. And then increased to 280 °C at a rate of 20 °C ending within 5 min. Total run time was 90 min. and the MS transfer line was maintained at a temperature of 200 °C. The source temperature was maintained at 180 °C. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass OCPTVS-Demo SPL software [1].

3. Results and Discussions
Methanol tuber extract of *Gloriosa superba* possessed various kinds of phytochemicals like alkaloid, glycosides, flavonoids, terpenoids, tannin, coumarins, steroids and phenols (Table 1). Phytochemical analysis of methanol tuber extract of *Gloriosa superba* was reported [4] in 2012 and present study almost supported the previous results except for phenolics. The previous study revealed the presence of the same phytochemicals except alkaloids that are found in the present study.
The peaks of unknown compounds from GC MS spectrum were compared with the database of known components stored in the NIST library [4, 6-11] (Gaithersburg, United States) and tabulated with the compound name, molecular formula and molecular weight (Table 2 and Figure 1). The results were correlated with the previous studies done in *Gloriosa superba*. In a previous study GC-MS analysis of the *Gloriosa superba,* presence of eight different compounds in the leaves were reported [6]. GC-MS analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana* was also reported [1]. In the present study, results showed that the rhizome possess a wide range of fatty acids, heterocyclic compound which are having anti-fungal, anti-inflammatory, antibiotic activity, skin conditioning property were identified. In comparison with the previous report, the present study confirmed that the methanolic tuber extract possess 17 different types of bioactive compounds. The first compound identified with less retention time (8.678 min) was 2-Methoxy-4-vinylphenol, while β-Amyrin trimethylsilyl ether had long retention time (31.017 min). The phytochemicals identified through the GCMS reported have various kinds of biological activities [4, 7-12] (Table 3). These phytochemicals from *Gloriosa superba* could be used for the development of powerful drugs to cure life threatening diseases.

Table 1: Phytochemical analysis of *Gloriosa superba* L.

S. No.	Phytochemicals	Results
1	Alkaloid	+
2	Terpenoids	+
3	Steroids	+
4	Glycosides	+
5	Flavonoids	+
6	Saponins	_
7	Tannins	+
8	Coumarins	+

(+) Presence, (-) Absence

Table 2: Bioactive compounds present in the methanol extract of *Gloriosa superba* tuber

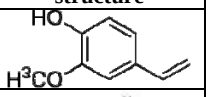
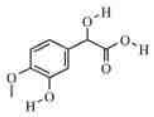
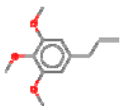
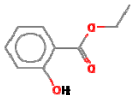
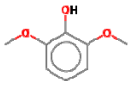
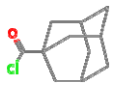
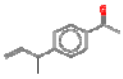
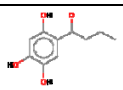
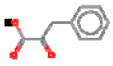
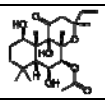
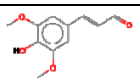
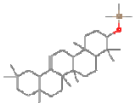
S. No.	Retention time	IUPAC Name	Molecular formula	Molecular Weight(g/mol)	Chemical structure
1	8.678	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.8	
2	11.038	3-Hydroxy-4-methoxymandelic acid	$C_9H_{10}O_5$	198.17	
3	14.312	Benzene,1,2,3-trimethoxy,5,2-propenyl	$C_{12}H_{16}O_3$	208.25	
4	14.949	Benzoic acid, 2-hydroxy Ethyl ester	$C_9H_{10}O_3$	166.17	
5	16.297	Phenol- 2,6-dimethoxy	$C_8H_{10}O_3$	154.16	
6	20.755	1-Adamantane carboxylic acid chloride	$C_{11}H_{15}ClO$	198.68	
7	21.126	1-(2-methyl-4-propoxy-phenyl, ethanone	$C_{12}H_{14}0$	174.23	
8	23.080	1-Butanone,1-(2,4,5 trihydroxy phenyl)	$C_{10}H_{12}O_4$	196.19	
9	23.443	2H-1-Benzopyran, 3,5,6,8 tetrahydro	$C_{13}H_{20}O$	192.29	
10	25.643	Benzenepropanoic acid	$C_9H_8O_3$	164.15	
11	25.956	Pentadecanoic acid	$C_{15}H_{30}O$	242.39	
12	26.935	1-Fluoroforskolin	$C_{22}H_{33}FO_7$	428.49	
13	27.223	Bicyclo[3.2.0]hept-2-ene	C_7H_{10}	94.15	
14	27.588	3,5-Dimethoxy-4-hydroxycinnmaldehyde	$C_{11}H_{12}O_4$	208.21	
15	28.427	7-Hydroxycadalene	$C_{15}H_{18}O$	214.30	
16	29.695	9,11-Octadecadienoic acid	$C_{18}H_{34}O_2$	282.46	
17	31.017	β-Amyrin trimethylsilyl ether	$C_{33}H_{58}OSi$	498.89	

Table 3: Biological activities of methanol tuber extract of *Gloriosa superba* L.

S. No.	Compound name	Activity
1	2-Methoxy-4-vinylphenol	Flavouring agent
2	3-Hydroxy-4-methoxymandelic acid	Antitumor
3	Benzene,1,2,3-trimethoxy,5,2-propenyl	Antimicrobial
4	Benzoic acid, 2-hydroxy Ethyl ester	Antimicrobial
5	Phenol- 2,6-dimethoxy	Antimicrobial
6	1-Adamantane carboxylic acid chloride	Antiviral
7	1-(2-methyl-4-propoxy-phenyl, ethanone	Antimicrobial
8	1-Butanone,1-(2,4,5 trihydroxy phenyl)	No activity reported
9	2H-1-Benzopyran, 3,5,6,8 tetrahydro	Antitumor
10	Benzenepropanoic acid	Antimicrobial
11	Pentadecanoic acid	Antimicrobial, Flavouring agent
12	1-Fluoroforskolin	Antimicrobial
13	Bicyclo[3.2.0]hept-2-ene	Antimicrobial
14	3,5-Dimethoxy-4-hydroxycinnmaldehyde	Antimicrobial
15	7-Hydroxycadalene	Antipyretic
16	9,11-Octadecadienoic acid	Antimicrobial
17	β-Amyrin trimethylsilyl ether	Anti-inflammatory, Hepatoprotective

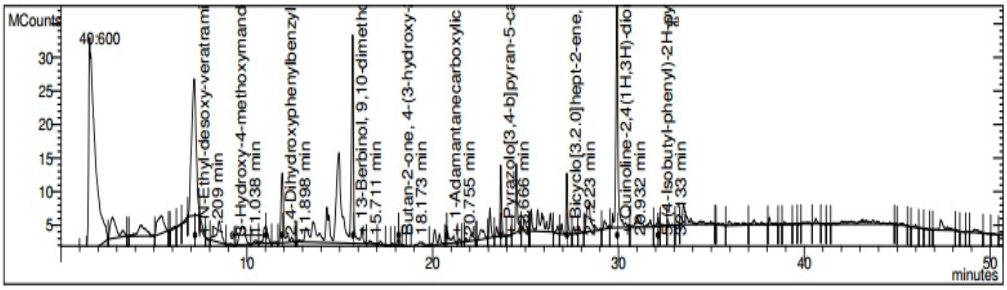


Fig 1: GC-MS result of methanol tuber extract of *Gloriosa superba*

4. Conclusion

All bioactive compounds have the ability to treat various types of diseases. GCMS analysis of the rhizomes of *Gloriosa superba* revealed the presence of 17 types of bioactive compounds with different disease curing potentialities. Isolation and utilization of these bioactive compounds could be helpful for the production of a new drug with high rejuvenating power.

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