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Phytochemical analysis of *Gloriosa superba* L. using GC-MS from five different ecotypes of Tamil Nadu State, India

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ABSTRACT

Gloriosa superba L is an important medicinal plant and its seeds, tubers are used for medicine. To investigate the phyto-components of *Gloriosa superba* L were collected from various habitats of Tamil Nadu state, India. In the present study, the phyto-components from the tubers of *Gloriosa superba* L cultivars from Sirumalai (GA1) Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) were extracted with ethanol and the composition of chemicals and its concentration in the tubers were determined by Gas Chromatography – Mass spectrometry (GC-MS) analysis. Among the phyto-components GA1 showed 15 phyto-components, GA2 showed 13 phyto-components, GA3 showed 8 phyto-components, GA4 showed 14 phyto-components and GA5 showed 13 phyto-components. GA1, GA2, GA4 and GA5 ecotypes possessed higher phyto-components. Colchicine an important alkaloid of *Gloriosa superba* L was found in GA2, GA3, GA4 and GA5 accessions in good concentration. The results reveals that the geographical origin and climatic condition of a accession caused polymorphisms in the accumulation of phyto-components, its composition and morphological traits in *Gloriosa Superba* L originating from different ecotypes of Tamil Nadu state.

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INTRODUCTION

Gloriosa Superba L. belongs to the family Colchicaceae. This plant is popularly known by other terminologies such as “Glory Lily”, Kalihari, Ognisikha etc., due to the presence of wavy edged yellow and red flowers. *G. superba* is a perennial climbing glabrous herb with tuberous root. All the parts of the plant especially tuber is poisonous. The plant species contain high amount of Colchicine, which is a toxic alkaloid. It also contains alkaloid Gloriocine. The seeds of *Gloriosa superba* L is an important source for Colchicine extraction. The plant almost distributed throughout India and also found in forests of Tamil Nadu.

In some Districts, farmers cultivate the plant species for its seeds. Tuber is a rich source of alkaloid colchicine [1]. Most of the medicinal plants were the best source to get a variety of new herbal products [2]. In current scenario WHO estimated

80% of the people from all over the world are interested towards traditional medicine. Medicinal plants are commonly known as people’s friend, providing the food, fuel and medicine [3]. Medicinal plant resources are major sources of Indian traditional and modern medicine [4&5].

The tubers are commonly used for the treatment of inflammation, ulcers, bleeding piles, white discharge, scrofula, skin diseases, leprosy, indigestion, helminthes, snake bites, intermittent fever, baldness and debility [6&7]. Most of the research findings are plant oriented products towards identifying the chemical compounds especially secondary metabolites [8].

Mostly medicinal plants are warrantly being screened for their biological and pharmacological activities such as anti-diabetic, antioxidant, antimicrobial, laxative and anti cancer activities [9-14]. India was sourced with its rich traditional knowledge and has heritage of herbal products and medicines and enhanced its

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richness with varying biodiversity [15]. The present investigation was carried out to evident the presence of phyto-components with varying quality and quantity in the samples of *Gloriosa superba* L collected from five different accessions of Tamil Nadu State, India.

MATERIALS AND METHODS

Plant Collection

The five ecotypes of *Gloriosa superba* L cultivated in the places such as Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) belongs to the districts such as Dindigul, Tiruppur, Trichy, Salem and Nagapatinam respectively (Figure 1). They were indentified and authenticated by the Botanical Survey of India, (Southern Circle), Coimbatore, Tamil Nadu, India. The plants were deposited in the department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India.

Geographical Location of Study Area

Sirumalai (GA1) is a region of 60,000 acres (200km²) situated 25 km from Dindigul town. The latitude is 10°11'39.28"N and longitude is 77°59'48.14"E. Elevation is 1092.63 Meters (3584.75 Feet). Mulanoor (GA2) is located in Tiruppur district. Mulanur is located at 10.77°N 77.72°E. It has an average elevation of 238 metres (780 feet). Mulanoor is a part of Glory lily market.

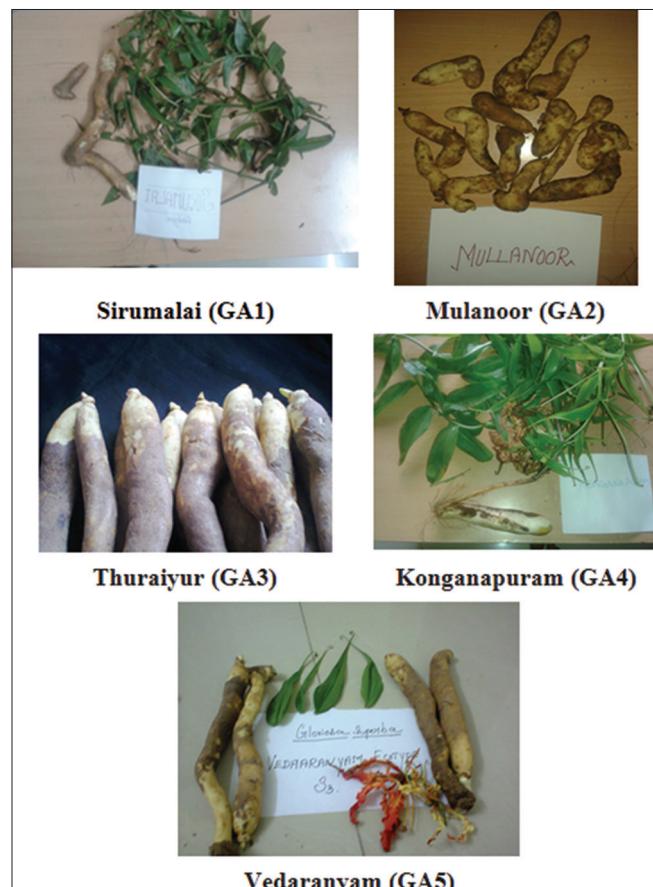


Figure 1: Morphological view of different type of ecotypes

Thuraiyur (GA3) latitude is 11° 8' 29.2380" N and longitude is 78° 35' 40.1100" E. Situated in Tiruchirappalli district. Konganapuram (GA4) is located at 11.58°N 77.92°E. It has an average elevation of 300 metres (1000 feet). Situated in Salem district. Vedaranyam (GA5) belongs to Nagapatinam district. The latitude is 10°22'27.15"N and longitude is 79°51'27.66"E. The elevation is 2.36 Meters (7.73 Feet).

Preparation of Powder and Extract

One kilogram of tubers was shade dried and powdered. The powder extracted with ethanol for 8 hours by using Soxhlet apparatus. After 8 hours, the extract was filtered through muslin cloth, evaporated under reduced pressure condition and vacuum dried to get the viscous residue. Ethanolic extract of *Gloriosa superba* L was used for GC-MS analysis. 2 μ l of the ethanolic leaf extract of *Gloriosa superba* L employed for GC-MS. Yield of extract was calculated by yield (g/100g) = (W1x100)/W2, where W1 is the weight of the extract residue obtained after solvent removal and W2 is the weight of tuber taken.

GC-MS Analysis

GC-MS analysis on the ethanolic extract of *Gloriosa superba* L was carried out in the Indian Institute for Crop Processing Technology (IICPT), Thanjavur. GC clarus 500 perkin Elmer system comprising a Aoc – 20i auto sampler and gas chromatography interfaced to a pass petrometer (GC- MS) instrument was used, applying following conditions[16].

Column

Elite-1 silica capillary column (30 mm x 0.25mm ID x M μ M df composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV was used.

Carrier Gas

Helium (99.99%) generally used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 μ l was employed (split ratio of 10:1) at injector temperature 250°C and ion source temperature 280°C. The oven temperature generally programmed from 110°C, with an increase of 10°C/min, to 200°C, then 5°C/min up to 280°C, finally ending with a 9 min isothermal at 280°C.

Spectra

Mass spectra were taken at 70eV with a scan interval of 0.5 seconds and fragments from 45 to 450 Da. GC total running time is 36 minutes. The plant extract was dissolved in methanol and filtered with polymeric solid phase extraction (SPE) column and analyzed in for different components.

Component Identification

Here interpretation on mass spectrum GC-MS was conducted by using database of National Standard and Technology (NIST)

library, having more than 62,000 patterns. The spectrum of unknown component was compared with the spectrum

of the known components stored in the NIST library [17]. The compound name, molecular weight and structure of the components for the test materials were ascertained.

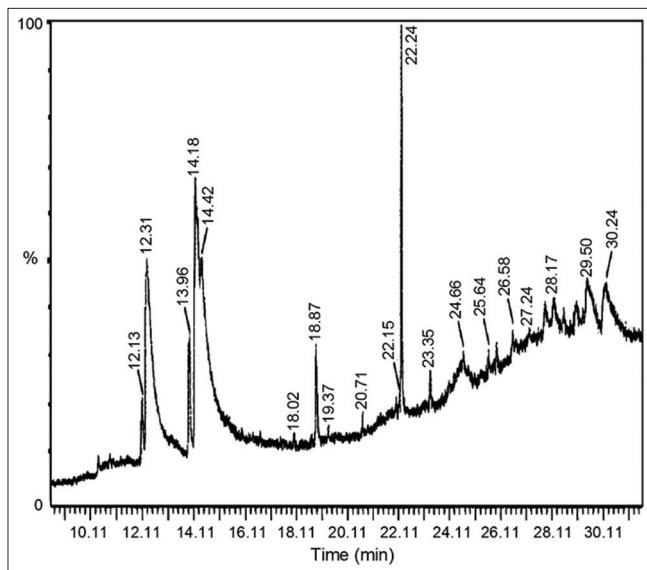


Figure 2: GC-MS chromatogram of tuber methanol extract of *Gloriosa superba* L (GA1 - Sirumalai) ecotype

RESULTS

The GC-MS analysis is the valuable method which has been increasingly applied for the analysis of medicinal plants for non-polar components, volatile essential oil, fatty acids and alkaloids. The *Gloriosa superba* L of Sirumalai accession (GA1) subjected to GC-MS analysis retrieved 15 major phyto-components.

Such as Deconoic acid, Ethyl ester, n-Hexadecanoic acid, Oleyl Alcohol, 9,12- Octadecadienoic acid (Z, Z), Dodecanoic acid, 2- Penten -1-yl ester, 1,2 – Benzenedicarboxylic acid, Diisooctyl ester, 1 – Octadecyne, Squalene, 1 – Eicosanol, Pentanoic acid, 10 – Undecenyl ester, 9 – Octadecenoic acid (Z) – Phenylmethyl ester, 6,9,12, Octadecatrienoic acid, Phenyl methyl ester (Z,Z,Z), [1, 1' Bicyclopophyl]-2-Octanoic acid, 2'-hexyl-, methyl ester, 9,12- Octadecadienoic (Z Z)- Phenylmethyl ester (Table 1 and Figure 2).

Gloriosa superba L cultivated in Mulanoor region (GA2) of Tiruppur district. This ecotypes contained thirteen different

Table 1: Phyto-components obtained through GC-MS analysis isolated from methanolic tuber extracts of *Gloriosa superba* L (GA1-Sirumalai) ecotype

S.No.	RT	Name of the compound	Molecular formulae	MW	Peak area %
1.	12.13	Decanoic acid, Ethyl ester	$C_{12}H_{24}O_2$	200	1.78
2.	12.31	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	24.34
3.	13.96	Oleyl Alcohol	$C_{18}H_{36}O$	268	1.94
4.	14.18	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	15.57
5.	18.02	Dodecanoic acid,2-penten-1-yl ester	$C_{17}H_{32}O_2$	268	0.22
6.	18.87	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.45
7.	20.71	1-Octadecyne	$C_{18}H_{34}$	250	0.36
8.	22.24	Squalene	$C_{30}H_{50}$	410	6.52
9.	23.35	1-Eicosanol	$C_{20}H_{42}O$	298	0.63
10.	24.66	Pentanoic acid, 10-undecenyl ester	$C_{16}H_{30}O_2$	254	1.68
11.	25.95	1-Octadecyne	$C_{18}H_{34}$	250	0.85
12.	27.24	9-Octadecenoic acid (Z)-, Phenylmethyl ester	$C_{25}H_{40}O_2$	372	3.69
13.	28.17	6,9,12-Octadecatrienoic acid,phenylmethyl ester,(Z,Z,Z)-	$C_{25}H_{36}O_2$	368	9.33
14.	29.50	[1,1'-Bicyclopophyl]-2-octanoic acid,2'-hexyl-,methyl ester	$C_{21}H_{38}O_2$	322	14.39
15.	30.24	9,12-Octadecadienoic(Z,Z)-,phenylmethyl ester	$C_{25}H_{38}O_2$	370	16.23

Table 2 : Phyto-components obtained through GC-MS analyzing isolated from methanolic tuber extracts of *Gloriosa superba* L (GA2–Mulanoor) ecotype

S.No.	RT	Name of the compound	Molecular formulae	MW	Peak area %
1.	4.03	2,3-Dimethyl fumaric acid	$C_6H_8O_4$	144	3.82
2.	5.78	Salicyl Alcohol	$C_7H_8O_2$	124	10.29
3.	6.73	2-[3-Hydroxy-4-methoxyphenyl]-semicarbazide	$C_8H_{11}N_3O_3$	197	2.70
4.	8.41	Benzoic acid,2-hydroxy-6-methoxy	$C_8H_8O_4$	168	9.08
5.	11.06	Undecanoic acid	$C_{11}H_{22}O_2$	186	1.26
6.	12.14	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	24.36
7.	13.81	9,12-Octadecadienoic acid,methyl ester,(E,E)-	$C_{19}H_{34}O_2$	294	2.00
8.	14.17	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	32.09
9.	17.95	2-Hydroxy-(Z)-9-pentadecenyl propanoate	$C_{18}H_{34}O_3$	298	0.23
10.	18.72	10-Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296	0.35
11.	27.91	Cholestan-3-ol,2-Methylene-,(3a,5a)-	$C_{28}H_{48}O$	400	0.96
12.	29.40	Lumicolchicine	$C_{22}H_{25}O_6$	399	10.04
13.	30.00	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	2.82

compounds. The compounds such as 2,3-Dimethyl fumaric acid, Salicyl Alcohol, 2-[3-Hydroxy-4-methoxyphenyl]-semicarbazide, Benzoic acid, 2-hydroxy-6-methoxy, Undecanoic acid, n-Hexadecanic acid, 9,12-Octadecadienoic acid, methyl ester, (E,E)-, 9,12-Octadecadienoic acid (Z,Z)-, 2-Hydroxy-(Z)9-pentadecenyl propanoate, 10- Undecenoic acid, Octyl ester, Cholestan-3-ol, 2-Methylene-, (3a,5a)-, Lumicolchicine, Ethyl iso-allocholate were identified (Table 2 and Figure 3).

Gloriosa superba L the ecotypes from Thuraiyur (GA3), Trichy district recorded eight compounds such as Lactose, Undecanoic acid, n-Hexadecanoic acid, 9,12- Octadecadienoic acid (Z, Z), 2-Hydroxy – (Z) 9-Pentadecenyl propanoate, 10- Undecenoic acid, Octyl ester, Squalene, Cis-Z-a-Bisabolene epoxide, Cholestan-3-ol, 2-Methylene,(3a, 5a)- Lumicolchicine, Vitamin A aldehyde, Lumicolchicine and Ethyl iso-allocholate (Table 4 and Figure 5).

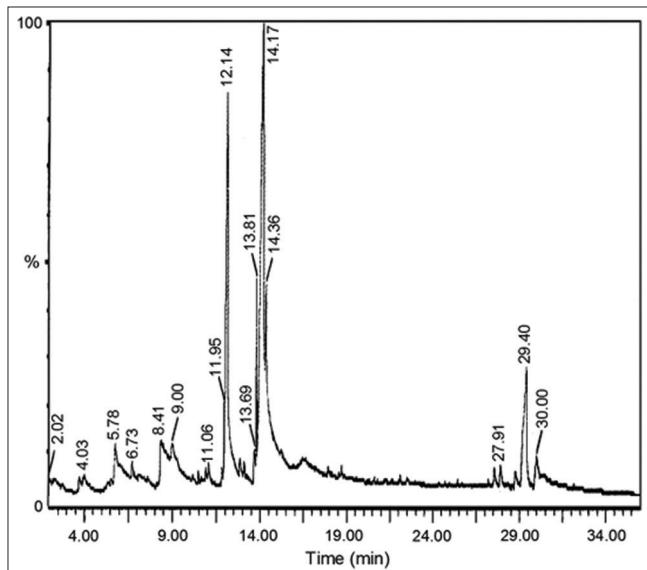


Figure 3: GC-MS chromatogram of tuber methanol extract of *Gloriosa superba* L (GA2 – Mulanoor) ecotype

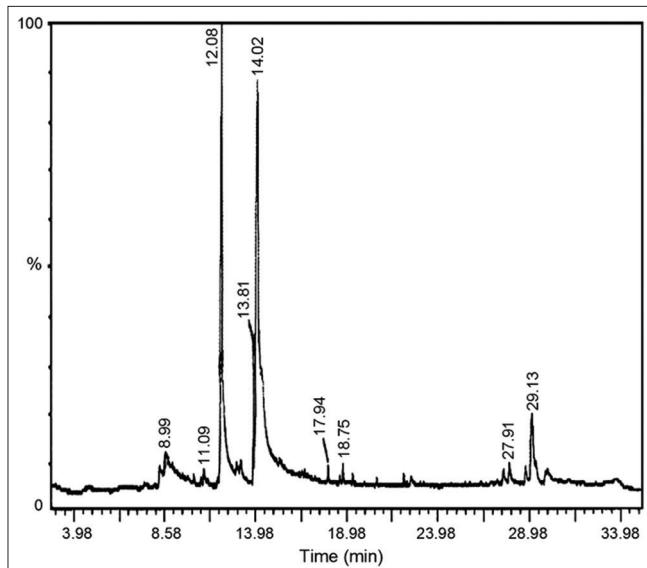


Figure 4: GC-MS chromatogram of tuber methanol extract of *Gloriosa superba* L (GA3 – Thuraiyur) ecotype

ester, Cholestan-3-ol, 2-Methylene,(3a, 5a)-, Lumicolchicine (Table 3 and Figure 4).

There were thirteen chemical compounds recorded in GA4. They are Lactose, Benzene 1,4-bis(1,1-dimethyl ether), n-Hexadecanoic acid, 9,12- Octadecadienoic acid (Z, Z), 10-Methyl-E-11-Tridecen-1-ol propionate, 2-Hydroxy – (Z) 9-Pentadecenyl propanoate, 10- Undecenoic acid, Octyl ester, Squalene, Cis-Z-a-Bisabolene epoxide, Cholestan-3-ol, 2-Methylene,(3a, 5a)- Lumicolchicine, Vitamin A aldehyde, Lumicolchicine and Ethyl iso-allocholate (Table 4 and Figure 5).

There are thirteen chemical compounds are identified in the ecotype of Vedaranyam (GA5) *Gloriosa superba* L. The chemical compounds such as lactose, Undecanoic acid, n-Hexadecanoic

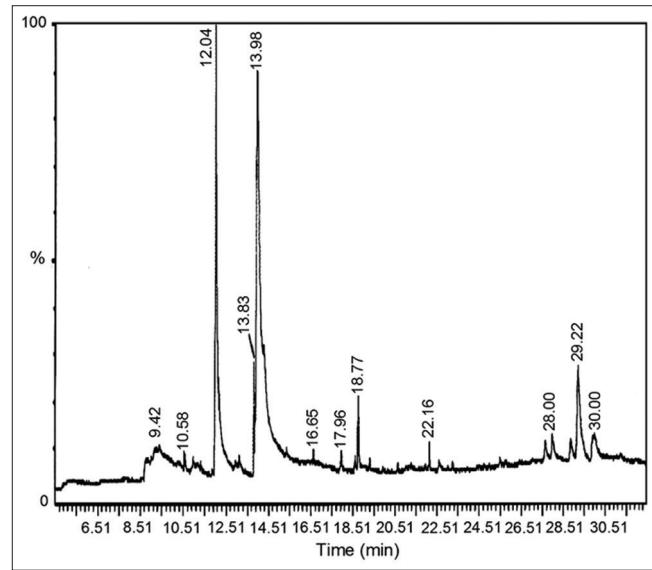


Figure 5: GC-MS chromatogram of tuber methanol extract of *Gloriosa superba* L(GA4 – Konganapuram) ecotype

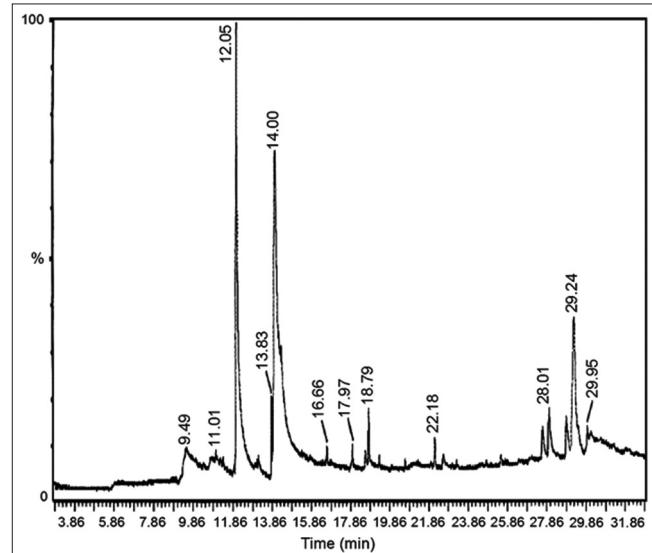


Figure 6: GC-MS chromatogram of tuber methanol extract of *Gloriosa superba* L (GA5 – Vedaranyam) ecotype

Table 3: Phyto-components obtained through GC-MS analysis isolated from methanolic tuber extracts of *Gloriosa superba* L (GA3-Thuraiyur) ecotype

S.No.	RT	Name of the compound	Molecular formulae	MW	Peak area %
1.	8.99	Lactose	$C_{12}H_{22}O_{11}$	342	5.29
2.	11.09	Undecanoic acid	$C_{11}H_{22}O_2$	186	0.83
3.	12.08	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	32.77
4.	14.02	9,12-Octadecanoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	53.30
5.	17.94	2-Hydroxy-(Z) 9-pentadecenyl propanoate	$C_{18}H_{34}O_3$	298	0.41
6.	18.75	10- Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296	0.47
7.	27.91	Cholestan-3-ol,2-methylene-,(3 α ,5 α)-	$C_{28}H_{48}O$	400	1.16
8.	29.13	Lumicolchicine	$C_{22}H_{25}NO_6$	399	5.78

Table 4: Phyto-components obtained through GC-MS analyzing isolated from methanolic tuber extracts of *Gloriosa superba* L (GA4-Konganapuram) ecotype

S.No.	RT	Name of the compound	Molecular formulae	MW	Peak area %
1.	9.42	Lactose	$C_{12}H_{22}O_{11}$	342	6.06
2.	10.58	Benzene,1,4-bis (1,1-dimethyl ether)-	$C_{14}H_{22}$	190	1.11
3.	12.04	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	22.80
4.	13.98	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	39.64
5.	16.65	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268	0.29
6.	17.96	2-Hydroxy-(Z)9-pentadecenyl propanoate	$C_{18}H_{34}O_3$	298	0.48
7.	18.77	10-Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296	1.62
8.	22.16	Squalene	$C_{30}H_{50}$	410	0.52
9.	27.66	Cis-Z- α -Bisabolene epoxide	$C_{15}H_{24}O$	220	2.64
10.	28.00	Cholestan-3-ol,2-methylene-,(3 α ,5 α)-	$C_{28}H_{48}O$	400	4.44
11.	28.87	Vitamin A aldehyde	$C_{20}H_{28}O$	284	2.28
12.	29.22	Lumicolchicine	$C_{22}H_{25}NO_6$	399	10.22
13.	30.00	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	7.89

Table 5 : Phyto-components obtained through GC-MS analyzing isolated from methanolic tuber extracts of *Gloriosa superba* L (GA5-Vedaranyam) ecotype

S.No.	RT	Name of the compound	Molecular formulae	MW	Peak area %
1.	9.49	Lactose	$C_{12}H_{22}O_{11}$	342	10.11
2.	11.01	Undecanoic acid	$C_{11}H_{22}O_2$	186	1.76
3.	12.05	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	29.81
4.	14.00	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	36.73
5.	16.66	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268	0.35
6.	17.97	2-Hydroxy-(Z) 9-pentadecenyl propanoate	$C_{18}H_{34}O_3$	298	0.83
7.	18.79	10-Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296	1.73
8.	22.18	Squalene	$C_{30}H_{50}$	410	0.59
9.	27.66	Cis-Z- α -Bisabolene epoxide	$C_{15}H_{24}O$	220	1.86
10.	28.01	Cholestan-3-ol, 2-methylene-,(3 α ,5 α)-	$C_{28}H_{48}O$	400	3.21
11.	28.87	Vitamin A aldehyde	$C_{20}H_{28}O$	284	1.96
12.	29.24	Lumicolchicine	$C_{22}H_{25}NO_6$	399	9.83
13.	29.95	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	1.21

acid, 9,12- Octadecadienoic acid (Z,Z), 10-Methyl-E-11-Tridecen-1-ol propionate, 2-Hydroxy – (Z) 9-Pentadecenyl propanoate, 10- Undecenoic acid, Octyl ester, Squalene, Cis-Z- α -Bisabolene epoxide, Cholestan-3-ol, Vitamin A aldehyde, 2-Methylene-,(3 α ,5 α)-Lumicolchicine and Ethyl iso-allocholate were reported (Table 5 and Figure 6).

The chemical compound Lactose was reported in *Gloriosa superba* L ecotypes of GA3, GA4 and GA5. 9,12-Octadecadienoic acid (Z,Z)-, n-hexadecanoic acid were reported in GA1, GA2, GA3, GA4 and GA5. Squalene was reported in GA1, GA4 and GA5 ecotypes. Vitamin A aldehyde found in GA4 and GA5 ecotypes. Lumicolchicine was present in ecotypes of GA2, GA3, GA4 and GA5 respectively. Ethyl allocholate is found in ecotypes of GA2, GA4 and GA5.

DISCUSSION

Our study reveals that *Gloriosa superba* L tuber contains various phyto-chemicals including alkaloids, steroids, triterpenoids, saponins and tannins. The results supported similar findings observed by *Listea glutinosa* [18]. GC-MS analysis of drugs revealed that the presence of various phyto-chemicals, including alkaloids and other observed structured compounds in GC- MS analysis in our findings[19].

Medicinal plant derived phyto-chemicals are an important source of medicinal plants and for eco friendly applications [20]. Plant extracts and phyto-components are active against various diseases [21]. In the present investigation, methanol extracts of tuber *Gloriosa superba* L from five different ecotypes such

as Sirumalai (GA1), Mulanoor(GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) contain various phyto-components. The results are obtained in accordance with the previous reports.

The analysis indicates that domesticated plant of *Gloriosa superba* L morphologically as well as physiologically divergent from their ancestor wild ecotypes. The present investigation reveals a comparative analysis of the phyto-components of five different accessions of *Gloriosa superba* L from Tamil Nadu state, India by using GC-MS method. Most of the accessions possess higher phyto-components in the tuber of *Gloriosa superba* L.

CONCLUSION

The present investigation on the phyto-components of *Gloriosa superba* L accessions may be utilized as an application oriented tool to find out a new source of natural antioxidants, pharmaceutical applications in a quantitative and qualitative approach.

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