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HPLC analysis of *Gloriosa superba* L., from five different accessions of Tamil Nadu state, India

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ABSTRACT

Gloriosa superba L is a most significant medicinal plant and especially for its seeds, tubers are used in folklore medicinal aspects as well as medicine. In the present investigations, the phyto-compounds from the different accessions of tubers of *Gloriosa superba* L cultivars from Sirumalai(GA1) region, Mulanoor(GA2)region, Thuraiyur(GA3)region, Konganapuram (GA4) region and Vedaranyan(GA5)region were extracted by ethanolic extract and the composition of chemicals and its concentration in the tubers were determined by HPLC analysis. GA1, GA2, GA4 and GA5 ecotypes possessed higher phyto-components. Colchichine is an important alkaloid of*Gloriosa superba* L was found in GA2, GA3, GA4 and GA5 accessions in good concentration. The results showed that the different geographical origin and climatic conditions of a accession causes polymorphisms in the accumulation of phyto-compounds and its composition and morphological traits in *Gloriosa Superba* L originating from different accession of Tamil Nadu state.

Key words: *Gloriosa superba* L, ethanolic extract, chemicals, phytocompounds, polymorphisms.

INTRODUCTION

Medicinal plants serve as an important source of various phytochemicals and various pharmacological characteristics. Identification of valuable plants is significant contribution in phytomedicine aspects. Phytochemicals are abundantly found in different levels in medicinal plants in various parts and are commonly used in herbal medicine and preparations for the treatment of various ailments such as cough, malaria, wounds, tooth ache and rheumatic diseases (Supritha and Radha 2018). The nature has provided a complete medicinal source of remedies to cure various ailments of human beings. Generally higher plants are manufacture of primary and secondary metabolites. Public house vast knowledge on the uses of medicinal plants.

For medicinal plant utilization is safety for the purpose of medicinal value and need to standardize to authenticity of medicinal plant species and used as quality criteria. General chromatographic methods are generally used and applied for the quality control of

medicinal plants due to its advantages and high efficiency (Celeghini et al. 2001).

As per World Health Organization (WHO) report uses than 80% of the population from the world wide commonly depend on traditional medicinal system. The medicinal value of plants lies in various chemical substances that are produce a definite physiological action on human body system. A most significant bioactive compounds of plans are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga et al. 2005).

HPLC method is very high sensitive technique for detection, identification and quantification of any chemicals in particular samples by using UV and Visible absorbance spectrophotometer (Hanchi and Golkho 2009). By comparing with the retention time of standards, phenolic compounds can be identified (Sarura et al. 2002).The presence of ascorbic acid, flavonoids and phenolic acid in medicinal plants are an appreciable amount. The studies on antioxidant activities are represent as significant to find out the biological properties of medicinal plants (Seal 2016; Paul Jasmine and Balakrishnan 2018; Paul Jasmine et al. 2020a&b).

HPLC technique is generally an analytical technique commonly used for the isolation of various natural products from the various natural resources (Thirumal and Laavce 2017). Sukarya et al. (2009) stated that medicinal plants are an important source in traditional medicinal system especially in developing countries and control many infectious diseases. There are many types of alkaloids found in medicinal plants. Colchicine is an important alkaloid of *Gloriosa superba* L. The present investigation deals with the presence of colchicine content in five different accessions of *Gloriosa Superba* tuber cultivated in various parts of Tamil Nadu State, India.

MATERIALS AND METHODS

Plant tuber collection

The difference accession of *Gloriosa superba* L. cultivated in various places such as Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) belongs to five different districts such as Dindigul, Tiruppur, Thiruchirapalli, Salem and Nagapattinam. The plant specimen authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2018/TECH/2042).The voucher specimen was deposited in the Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India.

Geographical Location

Sirumalai (GA1) is a region of 60,000 Acres (200 Km^2) Situated 25Km away from Dindigul. The latitude is 10º 11' 39.28" N and longitude is 77º 59' 48" E. Elevation is 1092.63 meters (3584.75 Feet). Mulanoor (GA2) is located in Tiruppur district. Mulanoor is located at 10.77º N and 77.72ºE. It has an average elevation of 238 meters (780 feet). Mulanoor is a part of Glory Lily market. Thuraiyur (GA3) latitude is11º 8'29.2380" N and longitude is 78º35'40.100" E situated in Tiruchirappalli district. Konganapuram (GA4) is located at 11.58ºN, 77.92ºE. It has an average elevation of 300 meters (1000 feet) and situated in Salem district. Vedaranyam (GA5) belongs to Nagapattinam district. The latitude is 10º22'77 27.15" N and longitude is 75º 51' 27.66" E. The elevation is 2.36 meters (7.73 feet)

Preparation of powder form the tuber

The tubers were collected from five different accessions of Tamil Nadu state, India from five different districts. The collected raw materials thoroughly washed with distilled water and dried for 3 to 4 weeks under shade dry then finally powdered by using electric blender. The dried powder material from the tubers of five difference accession were properly packed in polyethene bags and used for further extraction and laboratory purpose.

Method of Soxhlation:

Twenty grams of five different individual accession of *Gloriosa superba* L dried powder were weighed and sent for Soxhlation in 200 ml of petroleum ether at 70º C. The extracts are collected in round bottom flask and evaporated by using a rotary evaporator at 60º constant temperature with reduced pressure for two hours.

Method of Maceration

Twenty grams of powdered *Gloriosa superba* L from five different accession were placed in 200 ml of Hexane for 48 hours duration at room temperature and shaken periodically with the help of orbital rotary shaker at 100 rpm. The extracts further filtered thrice with the help of Whatmann No. 1 filter paper. Further the filtrate was evaporated under reduced pressure at 68º C temperature in a rotary evaporator for 2 hours.

HPLC analysis

The five different accessions of *Gloriosa superba* L tuber samples quantitative analysis was performed by following the method of Singh et al. (2010). The Winchrom integrator was generally used for calculation of peak area. Reverse-phase chromatography analysis was carried out in isocractic conditions using a C-18 reverse phase column (250 X 4.6 mm, particle size 2 µm, Luna µ C-18 at 25ºC. Running conditions included; injection column is 20 µl .mobile phase: Methanol : 0.4% acetic acid C800: 200 v/v); flow rate; 1ml/min; and detection at 290 nm. 2.5mg of crude extracts of *Gloriosa superba* L tuber power collected form fiver different accessions, the samples were dissolved with the help of 5 ml methanol solution. Then each sample were injected to UHPLC system with the same condition. Samples were filtered through an ultra membrane prior to injection in the sample loop. Standard solution was phenol. Colchicine solution was present in every samples and were identified by with the help of Chromatographic peaks and the retention time (Rt) of

individual standards and then confirmed through coinjection with isolated standards.

RESULTS AND DISCUSSION

The results are showed that all the five accessions studied exhibited significant variation in Colchicine and determined in all accession of *Gloriosa Superba* are represented. The Chromatogram of standards and colchicine compounds are depicted in figure 1 to 6 respectively.

Figure 1 indicated that the pure standard molecule of Colchicine has the retention time of 3.066 with highest production based on the various concentration. The different sample of *Gloriosa superba* was compared with standard Colchicine through HPLC analysis. GA1 sample of Sirumali poses the two compounds and exhibited the Rt value of 3.133 & 3.232 with equal proportion of injected volume. The retention time of 3.133 corresponds to the Colichicine with the concentration of peak value. The second peak corresponds to 3.232 indicated that major impurities or derivatives of Colichicine (Fig.2), Further it need to be tested for the biological potential. Similarly GA2, GA3, GA4 and GA5 (Fig 3 to 6) were analyzed and showed the presence of Colichicine at different level. GA2 Mulanoor posess second most major compound of Colichicine followed by GA3. The sample GA4 and GA5 possess the single peak and having 100% purity. The both sample of GA4 and GA5 had not detected any Colichicine at detectable range. The overall result concluded that GA2 having highest production of Colichicine with high purity whereas GA3 and GA1 has less quantity of production of alcoholic compounds. Multiple peak represent that the number of compounds present in it. The peak area details provided in Table 1 to 6 for all five different accessions with standard.

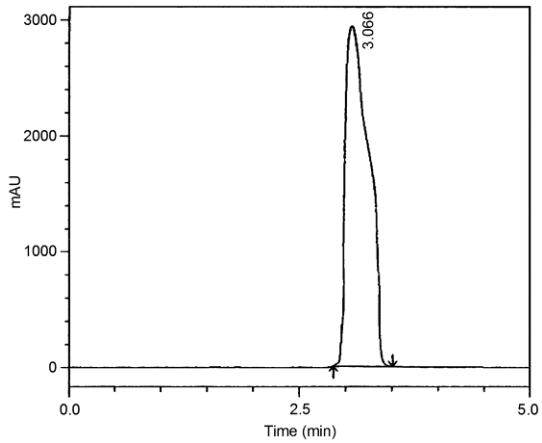

Fig. 1. HPLC chromatogram of standard Colchicine.

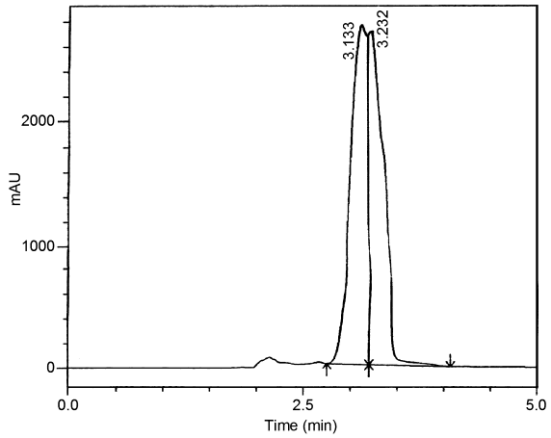

Fig. 2. HPLC chromatogram of the *Gloriosa superba* accession Sirumalai (GA1).

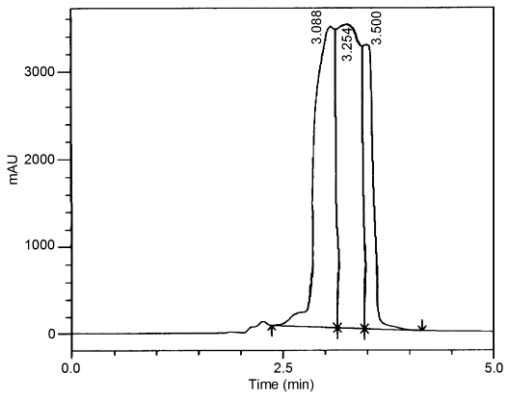

Fig. 3. HPLC chromatogram of the *Gloriosa superba* accession Mulanoor (GA2).

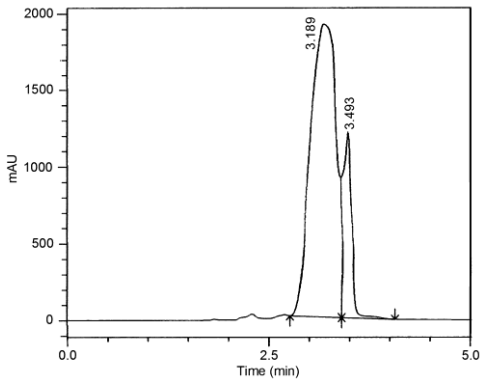

Fig. 4. HPLC chromatogram of the *Gloriosa superba* accession Thuraiyur (GA3).

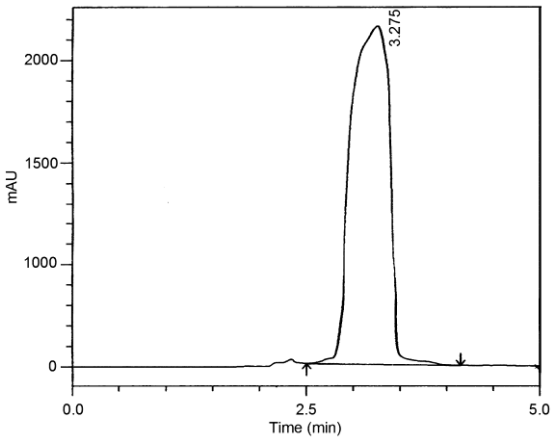

Fig. 5. HPLC chromatogram of the *Gloriosa superba* accession Konganapuram (GA5).

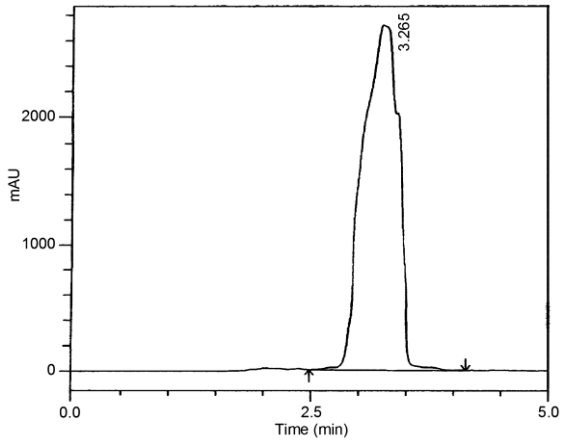

Fig. 6. HPLC chromatogram of the *Gloriosa superba* accession Vedaranyam (GA5).

Table :1 Peak area obtained for standard Colchicine

Peak No	Ref time	Area	Height	Area %	Height %
1	3.066	51186640	2935863	100.00	100.00
Total		51186640	2935863	100.00	100.00

Table :2 Peak area obtained in *Gloriosa superba* L for Sirumalai (GA1) Accession

Peak No	Ref time	Area	Height	Area %	Height %
1	3.133	34471350	2745226	53.970	50.434
2	3.232	29400390	2697995	46.030	49.566
Total		63871740	5443221	100.00	100.00

Table :3 Peak area obtained in *Gloriosa superba* L for Mulanoor (GA2) Accession

Peak No	Ref time	Area	Height	Area %	Height %
1	3.088	56189934	3448837	44.674	33.943
2	3.254	65243823	3467868	44.674	34.130
3	3.500	24610266	3244034	16.851	31.927
Total		146044024	10160739	100.00	100.00

Table :4 Peak area obtained in *Gloriosa Superba* L for Thuraiyur (GA3) Accession

Peak No	Ref time	Area	Height	Area %	Height %
1	3.189	42448081	1907711	82.979	61.130
2	3.493	8707166	1213025	17.021	38.870
Total		51155247	3120736	100.00	100.00

Table :5 Peak area obtained in *Gloriosa superba* L for Konganapuram (GA4) Accession

Peak No	Ref time	Area	Height	Area %	Height %
1	3.275	93919665	330742	100.00	100.00
Total		93919665	330742	100.00	100.00

Table :6 Peak area obtained in *Gloriosa Superba* L for Vedaranyam (GA5) Accession

Peak No	Ref time	Area	Height	Area %	Height %
1	3.265	71291856	2709219	100.00	100.00
Total		71291856	2709219	100.00	100.00

During cultivation of *Gloriosa superba* L, farmer applying various organic and inorganic fertilizers (NPK) levels in all five different accessions such as Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5). Al-Fayyad et al .(2002) reported that the presence of Colchicine alkaloid content in corms (0.052%), leaves (0.013%) and flowers (0.025%) of *Colchicum* by using different analytical techniques.

The Colchichine and Colchicoside is secondary metabolites are often influenced by the environmental and seasonal factors. On the basis of dry mass, Colchicum and *Gloriosa* contains 0.62% to 0.9% of colchicines (Chopra et al. 1956; Sarin et al. 1977). The higher content of colchicine in *Gloriosa* than in Colchicum makes it a commercially viable source of this medicine(Srivastava et al. 1977; Bellet and Gaignault 1985).

From this present investigation, we observed wide variation within the biochemical aspects of *Gloriosa Superba* from the five different accessions, which is further exploited to popularize the useful accessions for the extraction an invention of latest new drugs in future.

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