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RESEARCH ARTICLE

Phytochemical Screening, Antioxidant, Antimicrobial and Flavonoid Analysis of *Gloriosa superba* Linn. Rhizome Extracts

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Abstract

Gloriosa superba Linn. is a perennial climber belonging to family Colchicaceae, an important medicinal plant widely used in several indigenous medicinal formulations. Due to indiscriminate collection from natural habitat it has become endangered. In the present study, phytochemical, antimicrobial and antioxidant activity of the rhizome extracts of *Gloriosa superba* Linn. were evaluated. Phytochemical screening indicated that rhizomes are rich in a variety of primary and secondary metabolites such as carbohydrates, alkaloids, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins and minerals. Present investigation shows DPPH anti-scavenging activity in organic extracts such as methanol (87.69%), chloroform (43.91%), acetone (77.58%) and water (71.07%). The study highlights the biochemical and ethnopharmacological significance of *Gloriosa superba* Linn.

Keywords: *Gloriosa superba* Linn., rhizome, phytochemicals, antioxidant, antimicrobial, flavonoid.

Introduction

Gloriosa superba Linn. (Family: Colchicaceae) is a semi-woody herbaceous climber found throughout India up to an altitude of 6000 ft (Ghosh *et al*., 2002). It is a native of tropical Africa and is now growing in many parts of tropical Asia including India, Burma, Malaysia and Srilanka (Jayaweera, 1982; Sing, 2006). The altitudinal range of species is up to 2100 m above mean sea level and in India it is spread from hotter southern parts to the milder mid hill zones of Himachal Pradesh, Jammu Kashmir and Uttar Pradesh (Anon, 1956; Chandel *et al*., 1996). It is known as 'Malabar glory lily' in English, in Hindi as 'Kalihari', in Sanskrit as 'Agnisikha' and its trade name is 'Glory lily' (Ambasta, 1986; Pulliah, 2002). In world market, glory lily is considered as rich source of colchicines and gloriosine (Trease and Evans, 1983). Glory lily is an industrial medicinal crop in South India, for its high colchicine content, which is still collected from wild. Due to its over-exploitation in wild as well as problems faced during field cultivation, it was on the verge of extinction (Anon, 1997; Shivkumar *et al*., 2002). *Gloriosa superba* tuber and seeds have similar medicinal properties (Bhakuni and Jain, 1995). Different parts of *G. superba* have wide variety of uses especially in traditional system of medicine. The tuber is used for the treatment of bruises and sprains, colic, chronic ulcers, haemorrhoids, cancer, impotence, nocturnal seminal emission and leprosy and also for labour pains and abortions (Kala *et al*., 2004). *Gloriosa superba* is also used in wounds, skin related problems, fever, Inflammation, piles, blood disorders, uterine contractions, general body toner and poisoning (Haroon *et al.,* 2008).

The *G. superba* tuber is also used as abortifacient, anthelmintic, tonic, stomachic and anti-inflammatory drug. Root tuber with sesamum oil will reduce the pain in arthritis affected joints (Abhishek *et al*., 2011). Roots are acrid, anthelmintic, antipyretic, bitter, digestive, expectorant, highly poisonous and promoting expulsion of the placenta. Root paste is effective against paralysis, rheumatism, snake bite and insect bites (Chitra and Rajamani, 2009). The tuberous roots are useful in ulcers, bleeding piles, skin diseases, leprosy and snakebites (Ambasta, 1986; Chandel *et al.,* 1996). It is also considered useful in promoting labor and expulsion of placenta. Seeds are used for relieving rheumatic pain and used as a muscle relaxant (Nadkarni, 1996). Several colchicine-related alkaloids have been isolated from tubers and seeds. They are mostly dimethyl substitutes and include cornigerine, which is a potent antimitotic and muscle relaxant. A plant can contain up to 0.9% colchicine and 0.8% colchicoside. Colchicine is a powerful antimitotic agent that blocks or suppresses cell division by inhibiting mitosis, the division of a cell's nucleus. The chemical constituents of the tuber are known to be very poisonous to fish (Hemaiswarya *et al.,* 2009). The alkaloids from plants (Colchicine and Gloriosine) are used in treatment of gout and rheumatism (Nadkarni, 1996). In the present study, we report a high-throughput micro-scaled method which enables digestion of small quantities of plant samples for subsequent elemental profiling by Inductively Coupled Plasma (ICP) spectrometry, antioxidant activity, antimicrobial activity and determination of flavonoids by HPTLC.

Materials and methods

Chemicals: All solvents were distilled prior to use. TLC was performed on silica gel 60 F254 (Merck). All reagents and solvents were purchased from Merck Chemicals. The standard flavonoids were purchased from Sigma Aldrich. The UV-Vis spectra were recorded on a Shimadzu UV-1700 spectrophotometer. The HPLC were recorded on Agilent Technologies 1200 series Quaternary. Minerals detection was performed by using CEM Mars 6 microwave digester and Teledyne Leeman, ICP OES model Prodigy Dual View. The HPTLC were recorded on CAMAG LINOMAT-5.

Sampling: Fresh sample bulbs of *Gloriosa superba* Linn. Were collected during monsoon (June 2012 to September 2012) from Vajreshwari and Khandus villages of Karjat region of Western Ghats of Maharashtra (Fig. 1 and 2). The plants were identified and authenticated using herbarium collection at Botany research laboratory, DST-FIST School of Life Science, SRTM University, Nanded (MS). Fresh rhizomes were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The shade dried material was ground into coarse powder using mechanical grinder (Panasonic make). This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in airtight containers at room temperature till further phytochemical screening of secondary metabolites.

Fig. 1. Habit of *Gloriosa superba*.

Fig. 2. Rhizomes of *Gloriosa superba*.

Soxhlet extraction: Exhaustive soxhlet extraction was performed using a classical soxhlet apparatus with accurately weighed 10 g of the drug powder for 18-40 h. Extraction was performed with water, methanol, chloroform and acetone as the extracting solvent. Extraction was conducted for 6-8 h/d and finally all the extracts were evaporated under vacuum. Water, methanol, chloroform and acetone extracts of rhizome were prepared according to standard methods (Harbone, 1998). Nitrogen gas was purged through these extracts to prevent oxidation of secondary metabolites. The extracts were sealed in airtight containers and stored at -4°C.

Phytochemical screening: Phytochemical screening of active plant extracts was done by following the standard methods (Khandelwal, 1999) for the qualitative analysis of various phytochemicals such as alkaloids, carbohydrate, glycosides, saponins, flavonoids, phenols, vitamin C and vitamin E which could be responsible for antioxidant activity.

Antiscavaging activity: DPPH solution (0.1 mM) was prepared in methanol by dissolving 0.0394 g DPPH in 1000 mL methanol. The solution was kept in darkness for 30 min to complete the reaction. The free radical scavenging activity of the crude extracts was determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH). Antioxidant activity was measured according to Brand-Williams *et al.* (1995). Wherein, the bleaching rate of stable free radical, DPPH was monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbed at 570 nm, but upon reduction by an antioxidant or radical species its absorption decreases. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $[(ABS_{control} - ABS_{sample})/(ABS_{control})] \times 100$, where $ABS_{control}$ is absorbance of negative control and ABS_{sample} is the absorbance of the reaction mixture containing the sample extract.

Mineral analysis

Micro-scaled digestion: CEM-MARS 6 microwave oven was used for micro-scaled digestion. About 0.5 g of herbal samples were weighed and transferred to CEM-Xpress vessels. About 8-10 mL of conc. HNO_3 was added to the samples. The samples were pre-digested for 10-15 min prior to capping the vessels. The CEM-Xpress vessels were assembled for microwave irradiation. The microwave program was adjusted with respect to the number of vessels and reference to the guidelines of CEM at 1000 W with 100% level. About 25 min ramping period was used to reach the digestion temperature of 180°C which there upon was maintained for 15 min. The CEM-Xpress vessels were kept in fume hood for cooling and to release the pressure by uncapping. The contents were transferred to 50 mL volumetric flasks made with distilled water. The solutions were filtered prior to use (Table 1).

Table 1. Instrumental characteristics and setting for ICP-OES: Spectrometer Leeman Lab's simultaneous ICP-OES Prodigy XP dual system.

	Parameters range		Actual parameters
	Min	Max	
Power	0.1	2.0	1.1 Kw
Coolant flow	5	20	18 L/Min
Auxiliary flow	0.0	2.0	0.2 L/M
Nebulizer flow	5	60	34 psi
Plasma torch	-	-	Dual
Spray chamber	-	-	Cyclonic
Nebulizer	-	-	Concentric
Sample aspiration rate	0.5	2.0	1.4 mL/min
Replicate read time	-	-	40 sec per replicate for axial

For calibration, Leeman and Thomas Baker standard samples were used as the reference for the calibration range. The spray chamber, nebulizer and torch assembly was completely cleaned to eliminate any form of contamination. Plasma was stabilized for 15 min by flushing with distilled water. An instrument calibration was performed to check the wavelength shift and the same was successful with a minimum deviation of <10% with master scan. Diluted samples were used for further analysis by using Teledyne Leeman, ICP.

Vitamin E analysis by HPLC: Standard dl α-tocopherol acetate (96%) (Vitamin E) manufactured by Merck was used for calibration of standard curves. About 1 mg of dl α-tocopherol acetate was dissolved in 1 mL HPLC grade methanol. The dilutions of 100, 50, 25 and 10 µg/mL was prepared. The pretreated sample extracts and stock solutions were filtered through 0.45 µm syringe filters.

Reverse phase HPLC method: The concentration of α-tocopherol (Vitamin E) in the extracts was determined by Agilent Technologies 1200 series Quaternary system, equipped with auto sampler, quaternary pump, degasser, column oven and a DAD detector. The spectral data was collected at UV detection at 220 nm. The solvent system of acetonitrile and water (95:5) was used a gradient mobile phase on Agilent ZORBAX 300 SB column (4.6 × 150 mm × 5 µm) at a flow rate of 1.0 mL/min, 10 µL injection volume and detection was optimized at 220 nm with 15 min separation time.

Vitamin C: About 0.25% ethanolic solution of DCPI (2,6-dichlorophenol-indophenol sodium salt) was prepared for the detection of Vitamin C. To 0.5 mL of sample extracts, 2 drops of DCPI indicator was added. The blue coloration changed to red confirmed the presence of vitamin C. The test was carried out for all the extracts.

Antimicrobial activity: Antimicrobial activity of the extracts was determined by disc diffusion method. Disc of 4 mm dia were cut out from Whattman No. 1 filter paper. They were sterilized by autoclaving and stored in aseptic conditions in a test tube. During the time of treatment, the disc was taken out from the test tube with the help of forceps. The crude extract taken from the plant was pipetted out (20 mg) and poured into a clean autoclaved petri dish. Filter paper disc was placed in the petri dish for 20 min to make filter paper disc fully saturated with the extract. With the help of a forcep, one filter paper disc was placed on nutrient media. Another filter paper was saturated with methanol and after 30 min it was placed on the media as control. Likewise, saturated filter papers with chloroform, acetone and petroleum ether were used as control. Gentamicin and Bavistin were used as standards in antibacterial and antifungal study respectively (Elgayyar *et al*., 2001). After the spreading of microbes into the agar plate, the disc was prepared with plant extract. The discs are impregnated with plant extract and placed in the plate containing pure culture and kept for incubation overnight. After 1 d, zone of inhibition was noted.

Flavoinoids analysis by HPTLC: The standards Quercetin, Kaemferol, Catechin gallate, Rutin hydrate and Hesperdin were procured from Sigma Aldrich USA. All the standard solutions were prepared in ethanol where as hesperdin in water. Chromatography was performed on silica gel $60F_{254}$ (10 cm X 10 cm; 25 mm layer thickness; Merk) with aqueous, methanolic, chloroform and acetone extracts of *G. superba* rhizome. The fraction residues were collected and (10 µL) subjected for HPTLC (CAMAG, Switzerland) analysis. The fractions were impregnated on silica gel $60F_{254}$ TLC plate. The plate was air-dried and then inserted in CAMAG-twin through lass chamber containing solvent system of composition with ethyl acetate, acetic acid, formic acid and water (100:11:11:27) as a gradient mobile phase for 20 min. The well eluted TLC plate was then dried at 105°C for 15 min and scanned using Scanner 3 (CAMAG, Switzerland) at 254 and 366 nm using Win Cat 4 software.

Results and discussion

Optimization of extraction method: In order to extract the phytochemicals from plant samples efficiently, variables involved in this procedure were optimized, including extraction solvent (water, methanol, chloroform, acetone, 100%), extraction method (Soxhlet, reflux, percolation) and extraction time (18-40 h). The extraction time in water was 40 h. The biomass was refluxed for 40 h and then it was dried naturally for 2-3 d. To the dried biomass, 100% methanol was added and the reaction was percolated for phytochemicals. The methanolic fraction was collected in amber coloured bottle under nitrogen atmosphere. The material was dried for 5-6 h. The procedure was repeated for chloroform and acetone. The extraction time was optimized for all the samples. All the extracts were preserved under nitrogen atmosphere in amber coloured bottle.

Table 2. Preliminary phytochemical screening of rhizome extracts of *G. superba.*

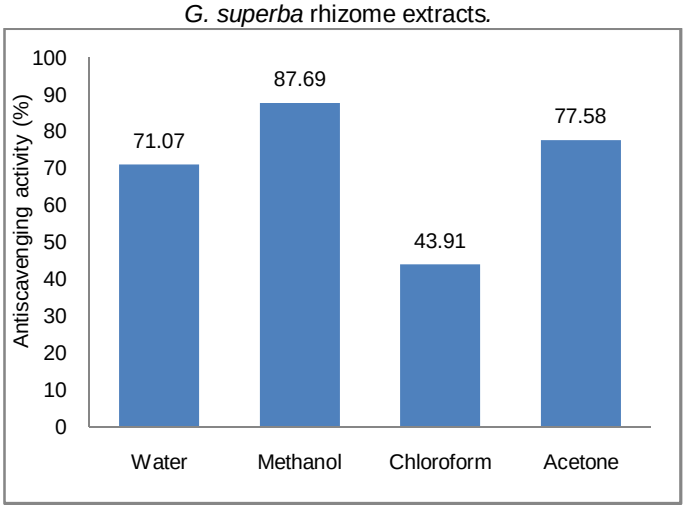
Constituents	Test	Observation	Inference			
			S1	S2	S3	S4
Carbohydrates	Benedict's reagent	Red precipitate	+	+	+	+
Alkaloids	Mayer's reagent	White precipitate	-	+	-	-
Glycosides	Borntranger's reagent	Pink coloration	+	+	+	+
Saponins	Foaming	Frothing persisted for 10-15 min	+	+	-	-
Flavonoids	Shinoda	Pink-Red coloration	+	-	-	-
Phenols	Ferric chloride	Dark brown coloration	-	+	+	+
Vitamin C	2,6-dichlorophenol-indophenol sodium salt	Red coloration	+	-	-	-
Vitamin E	HPLC method	-	-	-	+	-

S1=Water, S2=Methanol, S3=Acetone, S4=Chloroform.

Phytochemical screening: It is known that plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins and volatile oils. It is necessary to identify the phytochemical components of local medicinal plants usually employed by herbalists in the treatment of diseases (Banso and Adeyemo, 2007). The presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts. The successive soxhlet extract of seeds and tubers were extracted using hexane, chloroform and methanol in ascending order of the polarity. Preliminary phytochemical screening revealed that the extracts contain alkaloids, glycosides, steroids, terpenoids and tannins in all extracts (Senthilkumar, 2013). *Gloriosa superba* tuber showed the presence of carbohydrates, alkaloids and flavanoids (Muthukrishnan and Subramanian, 2012). The results of tubers and leaves of *G. superba* revealed the presence of various classes of compounds such as alkaloids, flavonoids, saponins, glycosides, steroids, phenols and tannins. The results of the various phytochemical tests indicated that the plant to be rich in various biologically active compounds which could serve as potential source of the crude drugs that can be used as a complementary source of traditional medicines (Banu and Nagrajan, 2012). Phytochemical screening of rhizome extracts of *G. superba* revealed presence of different phytochemicals. Indeed phytochemical investigations of this plant have resulted in occurrences of carbohydrates, alkaloids, glycosides, saponins, flavonoids, phenols, Vitamin E and Vitamin C. Table 2 illustrates the results of phytochemical screening of all the extracts of *Gloriosa superba*. The qualitative analysis of carbohydrates (Benedict's reagent test) and glycosides (Borntranger's reagent) were carried out in all extracts i.e. aqueous (S1), methanol (S2), acetone (S3) and chloroform (S4) extracts. The solutions turning red and pink confirmed the presence of carbohydrates and glycosides respectively. The hydrophilic carbohydrates and glycosides were present in water (S1) whereas hydrophobic carbohydrates and glycosides were detected in rest of the organic solvents (S2-S4). Mayer's test of extract S2 displayed appearance of white turbidity for alkaloids. The alkaloids were absent in S1, S3 and S4 extracts. The dark brown coloration test for phenols was observed in S2-S4 extracts. The water soluble phenols were absent in all the extracts. The extracts S1-S4 were shaken with distilled water. The persistence of froth in S1, S2 was observed, indicated the presence of saponins. The hydrophilic flavonoids were detected in extract S1. The water soluble vitamin C was found in S1 and vitamin E was qualitatively analyzed by HPLC method in extract S3 of *Gloriosa superba.*

Anti-scavenging activity: Oxidation is essential to many living organisms for the production of energy to fuel biological process (Gulcin *et al.*, 2010). DPPH is a stable nitrogen centred free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidant and therefore radical scavengers (Lavanya *et al.,* 2010). Antioxidant capacity of *G. superba* rhizome extract was measured by DPPH anti-scavenging method and the results were expressed in Fig. 3. DPPH anti-scavenging activity of methanolic extract (87.69%) was higher than aqueous extract (71.07%), chloroform (43.91%) and acetone (77.58%) extracts. However, DPPH anti-scavenging values of all the extracts are higher except chloroform. These results might suggest higher medicinal suitability of alcoholic extracts in various antioxidant applications.

Fig. 3. Anti-scavenging (DPPH) activity of *G. superba* rhizome extracts.



Mineral analysis

Optimization and calibration of G. superba rhizome extracts: After optimization, a new calibration method was done by using Cu 324.754 nm, Mn 257.610, Se 196.090, Fe 259.940 and Zn 213.856 (Table 3) wavelengths for calibration. Calibration STD solutions were measured 3 times one by one with an RSD <1%. Once all the calibration standards were finished, a necessary background correction was applied for each wavelength. Samples were measured with three reproductions. Average sum of the three measurements is tabulated in the analysis report. Quantitative multi-elemental analysis by inductively coupled plasma (ICP) spectrometry depends on complete digestion of solid samples. However, fast and thorough sample digestion is a challenging analytical task which constitutes a bottleneck in modern multi-elemental analysis. Additional obstacles may be that sample quantities are limited and elemental concentrations are low. In such cases, digestion in small volumes with minimum dilution and contamination is required in order to obtain high accuracy data. We have developed a micro-scaled microwave digestion procedure and optimized it for accurate elemental profiling of plant materials. A commercially available 40-position rotor with 5 mL Poly tetra flouro ethylene (PTFE) vials, originally designed for microwave-based parallel organic synthesis was used as a platform for the digestion. The novel micro-scaled method was successfully validated by the use of various certified reference materials (CRM). The micro-scaled digestion procedure was applied on crude powder of dried plant material in small batches. The contents were transferred to 50 mL volumetric flasks and volume was made with distilled water. The solutions were filtered prior to use. Teledyne Leeman, ICP spectrometer was calibrated using Leeman standard, National Institute of Standards and Technology (NIST), USA. Diluted samples were used for further analysis.

Table 3. Accuracy of elemental concentrations in *G. superba* after micro-scaled digestion.

Elements (ppm)	*G. superba*
Zn	5.378
Cu	17.240
Mn	00
Se	593.809
Fe	12.441

Iron and copper are of great importance for life. As redox-active metal, they are involved in photosynthesis, mitochondrial respiration, nitrogen assimilation and hormone biosynthesis. Manganese is essential for plant metabolism and development and occurs in oxidation states II, III, and IV in approximately 35 enzymes of a plant cell. Zinc is important as a component of enzymes for protein synthesis and energy production and maintains the structural integrity of biomembranes. Most of the zinc enzymes are involved in regulation of DNA-transcription, RNA-processing and translation. Although the essentiality of Se to plants has not been established yet, Se is considered as a beneficial element in promoting plant growth in some plant species. We have determined five elements in coarse powder of rhizome of *Gloriosa superba* (Table 3). Thereby, the concentration of minerals in bulb extracts had different profiles and quantitative differences had been detected. The most abundant microelement was Fe in *Gloriosa superba*; whereas, Cu was found at the lowest concentration. The content of Fe was especially high followed by Mn, Zn and Cu. Selenium was not detected (Table 3).

Qualitative analysis of vitamin E by HPLC: To meet the requirements for quantitative analysis, the following HPLC parameters were examined, including different columns (Agilent SB-C18 length 250 mm and 150 mm, width 4.6, particle size 5 µm), column temperature (25°C) and UV wavelength (220 nm). The best chromatographic resolution was obtained on Agilent SB-C18 length 4.6 X 150 mm, 5 µm column at 25°C. The UV detector was monitored at 200-380 nm for finger printing analysis because the peaks were observed under this wavelength. The high intense peak was observed at 220 nm. To obtain the calibration curve, working solutions of four concentrations containing vitamin E was analyzed in triplicate. The calibration curves were established by plotting peak areas versus the concentration of each analyte. In the regression equation, y = ax + b, x refers to the concentration of pure dl α-tocopherol acetate (1 g/mL), y the peak area and r the correlation coefficient (Fig. 4). Vitamins are a diverse group of organic compounds essential in trace amounts for the normal growth and maintenance of life. To ensure the adequate intake of vitamins, the human diet can be completed with a high range of multivitamin tablets and food products enriched with vitamins, in other words, these compounds are usually administered as nutraceutical or functional ingredient. They are classified as either water-soluble or fat soluble. Vitamin E is fat-soluble whereas, vitamin C is water-soluble. Vitamin E is a generic term for tocopherols and tocotrienols and it is fat-soluble antioxidant that block the production of reactive oxygen species formed when lipids undergoes oxidation. We employed reverse phase HPLC-analytical tool for qualitative estimation of vitamin E, in which HPLC has been coupled with UV detector. The lipophilic vitamin E has been detected in the methanolic extract (Fig. 5). Vitamin E is absent in chloroform and acetone extracts. The moderate antioxidant activity in organic extract suggests that the concentration of vitamin E might be less compared to that of the other antioxidants in aqueous extract. The occurrence of vitamin E suggested the antioxidant activity. The organic extracts displayed significant antioxidant activity proposed that the concentration of vitamin E might be higher along with the other natural antioxidants.

Fig. 4. Calibration curve of dl α-tocopherol acetate (96%) (Vitamin E).

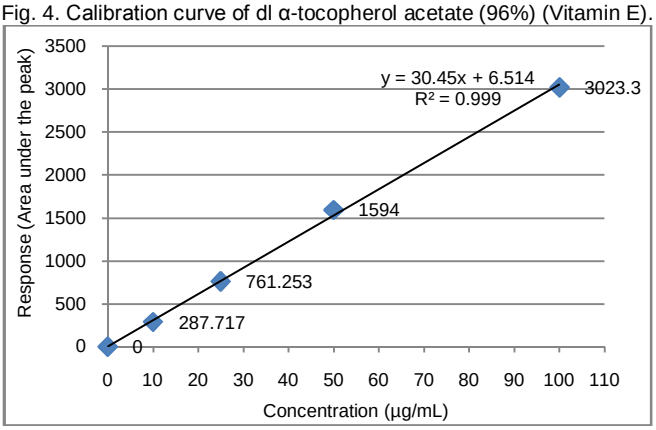
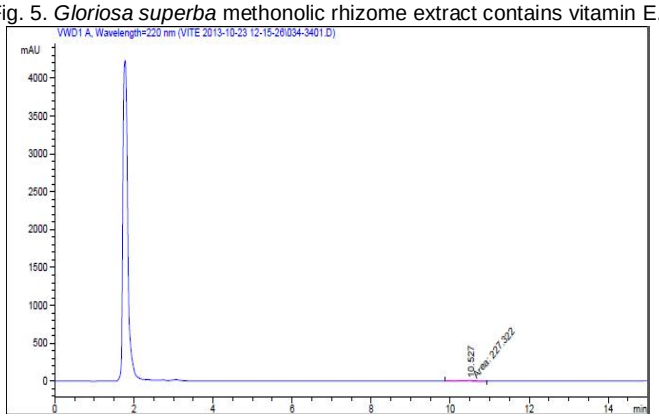


Fig. 5. *Gloriosa superba* methonolic rhizome extract contains vitamin E.



Vitamin C: Vitamin C (L-Ascorbic acid or L ascorbate) is an essential nutrient for humans and other animal species. Deficiency in this vitamin causes the disease known as scurvy in humans. This compound is also widely used as a food additive because of its antioxidant activity. The hydrophilic Vitamin C has been detected in aqueous extract (Table 2).

Antimicrobial activity: Phytochemicals from root tubers have wide spectrum action against gram-positive and gram-negative bacteria along with antifungal and mutagenic potential. The phytochemicals from tubers has passed Ames *Salmonella* mutagenicity test due to the presence of Colchicine (Shanmugam *et al*., 2009). The petroleum ether, methanol, chloroform and acetone extracts obtained from the tuber, flower and seed of *Gloriosa superba* were evaluated for antimicrobial property. All the extracts showed antibacterial activity against all the selected organisms. The maximum inhibitory activity was seen in methanol extracts. In the case of tuber, the high inhibitory activity was seen in methanol extract against *Proteus vulgaris* and *Bacillus* sp. and also in flower and seed, maximum inhibition zone obtained in methanol extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively (Nikhila *et al*., 2014). To compare with the flower and seed, tuber has the highest antibacterial activity because tuber extract showed clear inhibition zone against *Proteus vulgaris* and *Bacillus* sp. than the standard.

Table 4.Antimicrobial activity of *G. superba* rhizome extracts.

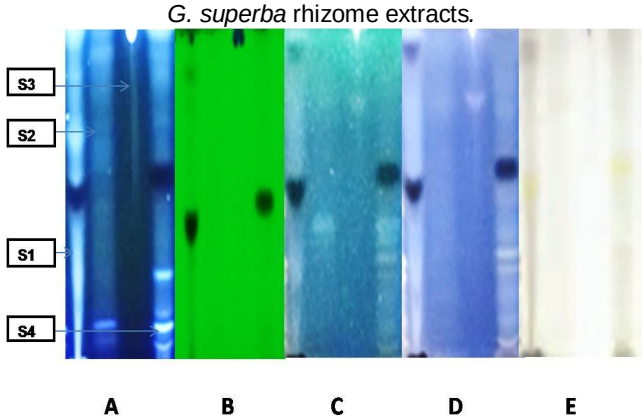
Extract	*S. typhi*	*S. aureus*	*E. coli*	*A. niger*
Acetone*	0.6	-	-	-
Chloroform*	-	-	-	-
Methanol*	-	0.2	-	-
Water*	0.6	0.8	-	-

*Diameter of zone of inhibition in cm.

The results were found to be in correlation with the previous studies (Shanmugham *et al*., 2009; Haroon *et al*., 2008; Senthilkumar, 2013). The studies revealed that the tubers and seeds of *Gloriosa superba* possessed good antibacterial activity in different solvents. Phytochemical screening and antimicrobial activity of *Gloriosa superba* were also reported against *Bacillus cereusm* (Senthilkumar, 2013). In the present investigation, maximum zone of inhibition was obtained in aqueous extract against *Staphylococcus aureus* and *Salmonella typhi*. The methanolic extracts showed 0.2 cm zone of inhibition against *Staphylococcus aureus* and acetone extract showed 0.6 cm against *Salmonella typhi.* The bacterium *E. coli* and fungus *Aspergillus niger* also didn't show any zone of inhibition. Similarly chloroform extract didn't show any zone of inhibition against all the microbes (Table 4).

Flavonoids analysis by HPTLC: Flavonoids, the most important and most diverse natural phenolics (Agarwal, 1989) have diverse chemical and biological activities including radical scavenging properties. Figure 6 showed HPTLC profiles of aqueous and methanolic extracts of rhizome of *Gloriosa superba.* In HPTLC technique, the flavonoids from methanolic extracts were determined using solvent system ethyl acetate, acetic acid, formic acid and water (100:11:11:27) as a gradient mobile phase. Figure 6 showed bands of aqueous, acetone, chloroform and methanolic extracts under 254 and 366 nm.

Fig. 6. HPTLC studies on flavonoids of *G. superba* rhizome extracts.

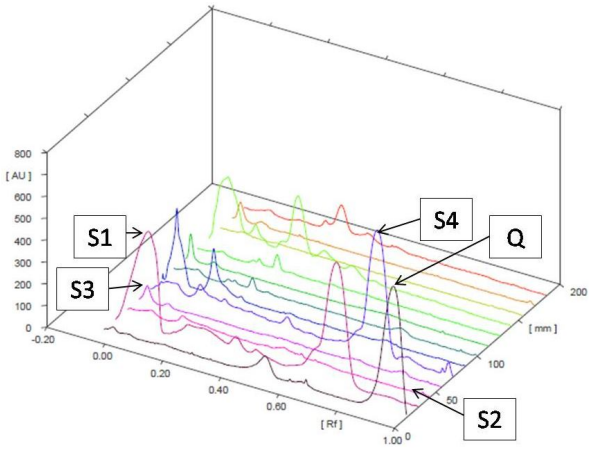


A. Water, Acetone, Chloroform and Methanol extracts under UV 254 BD
B. Water, Acetone, Chloroform and Methanol extracts under UV 366 BD
C. Water, Acetone, Chloroform and Methanol extracts under UV 254 AD
D. Water, Acetone, Chloroform and Methanol extracts under UV 366 AD
E. Water, Acetone, Chloroform and Methanol extracts under light.
S1=Water, S2=Acetone, S3=Chloroform, S4=Methanol.

Table 5. HPTLC–Flavonoids profile of water, acetone, chloroform and methanolic rhizome extracts of *Gloriosa superba* under UV 254 and 366 AD.

Rhizome extract	254 nm AD				366 nm AD			
	Rf	Height	Area	Assigned substances	Rf	Height	Area	Assigned substances
Water	0.23	0.8	44028.8	Unknown	0.24	2.4	47197.6	Unknown
	0.35	26.5	938.2	Unknown	0.43	13.7	401.2	Rutin
	0.45	27.5	2127.9	Rutin	0.83	0.1	19791.6	Unknown
	0.50	8.6	584.2	Unknown				
	0.60	33.7	1080.8	Unknown				
	0.82	3.7	23743.7	Unknown				
Acetone	0.02	6.5	98.1	Unknown	0.09	15.8	1681.2	Unknown
	0.09	0.4	1028.4	Unknown	0.21	24.4	1698.5	Unknown
	0.20	13.1	601.0	Unknown	0.46	20.1	448.4	Rutin
	0.46	19.8	495.9	Rutin	0.53	23.7	1400.5	Hesperidin
	0.52	26.4	1411.7	Hesperidin	0.59	13.0	378.4	Unknown
	0.59	10.9	432.7	Unknown				
Chloroform	0.04	15.2	4886.1	Unknown	0.05	4.9	2756.1	Unknown
	0.08	1.3	426.7	Unknown	0.52	8.7	226.6	Hesperidin
	0.23	0.3	52.2	Unknown	0.71	15.9	330.2	Orientin
	0.47	11.9	379.5	Rutin	0.80	3.3	205.8	
	0.52	15.5	338.3	Hesperidin	0.91	4.1	698.2	Coumaric acid
	0.56	6.7	382.9	Unknown				
	0.71	21.1	446.0	Orientin				
	0.78	8.5	239.6	Unknown				
	0.91	4.1	1168.7	Coumaric acid				
Methanol	0.11	84.3	12197.8	Unknown	0.23	35.9	25717.8	Unknown
	0.20	28.0	3305.8	Unknown	0.27	20.5	524.2	Unknown
	0.27	6.9	414.2	Unknown	0.48	29.9	1354.9	Rutin
	0.29	19.5	291.5	Unknown	0.52	3.7	725.8	Hesperidin
	0.38	16.5	498.2	Unknown	0.60	14.3	370.8	Unknown
	0.48	33.4	1970.6	Rutin	0.84	13.3	21016.4	Unknown
	0.52	4.4	721.4	Hesperidin	0.91	4.5	792.7	Coumaric acid
	0.64	21.9	1042.8	Unknown	0.99	1.1	66.9	Vanilic acid
	0.82	32.5	24100.9	Unknown				
	0.92	0.6	1987.8	Coumaric acid				
	0.99	3.4	141.6	Vanilic acid				

Fig. 7. HPTLC peaks of standard and rhizome extracts of *Gloriosa superba.*



All peaks at 366 AD (Q-STD Quercetin, S1-Water, S2-Acetone, S3-Chloroform and S4-Methanol).

Similarly Fig. 6C, D, E showed under UV 366 and 254 AD. The methanolic extracts showed maximum bands of flavonoids including rutin. The aqueous extract showed different flavonoids, thus, HPTLC technique could be considered as an accurate precise method for the determination of flavonoids in rhizome extracts of *Gloriosa superba*. There were six different assigned substances in aqueous extract with range of Rf between 0.23 to 0.82. Same as in methanolic extract, there were eleven assigned substances including rutin. The Rf were ranging in between 0.11 to 0.99. The methanolic extract showed maximum flavonoids such as rutin, coumaric acid, hesperidin and vanilic acid as compared to aqueous rhizome extract of *Gloriosa superba* (Table 5). HPTLC peaks of standard and rhizome extracts of *G. superba* is shown in Fig. 11.

Conclusion

An improved RP-HPLC-UV-method has successfully applied for determination of dl α-tocopherol acetate in organic extracts. Similarly the results obtained from phytochemical analysis illustrates the occurrences of

various micronutrients i.e. carbohydrates, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins, alkaloids and minerals i.e. Zn, Cu, Mn, Se, Fe. The present findings for microelements and minerals suggested that their contents are responsible for significant antioxidant activity in all extracts. The detection of flavonoids by HPTLC also revels strong antioxidant activity in methanolic as well as in aqueous extracts. It showed the importance of rhizome in crude drug preparations as well as in traditional formulations. The structural characterisations of isolated flavonoids from various extracts of *Gloriosa superba* are in progress.

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