Pharmacognostical assessment, antioxidant ability and determination of phytomolecules using GC-MS in Gloriosa superba Linn

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Research Article

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Abstract

Gloriosa superba Linn (family Colchicaceae) is a tendril climber with cylindrical and V or L shaped rhizomes that has received much attention due to its ancient pharmacological uses and being a rich source of colchicine. The pharmacognostical profile of leaf, stem and rhizome were analysed to assure the grade of raw material in the proposed investigation. Moreover, the antioxidant capacity of ethanolic rhizome extract of *G. superba* (EREG) was assessed using DPPH radical scavenging assay. Simultaneously, we have evaluated the phytochemical profile of EREG by GC-MS and total phenolic content was performed as well. Transverse section of rhizome revealed thin layer of brownish scales that fabricate the outer skin. In addition, the cortical area made up of homogeneous fleshy and parenchymal cells filled with starch granules which is one of the superabundant diagnostic features of this plant. Pharmacognostical profile of leaf, stem and rhizome of the plant demonstrated the evidence of authenticity of this plant. Anti-scavenging capacity (IC_{50}) and total phenolic content was 77.20% and 9.54 mg, GAE/g, respectively. The GC-MS library identified two phytoconstituents as diethyl phthalate (100% peak area) and arsenous acid, tris(trimethylsilyl) ester (22.68% peak area) whose vast benefits are reported in the fields of pharmaceuticals, industry and personal care products. Frivolous research work in the field of pharmacognosy, antioxidant ability and instrumental analysis for phytomolecules provides a vast avenue for research on *G. superba*.

Introduction

Gloriosa superba Linn belongs to the family Liliaceae (Colchicaceae) is native to Africa, southeastern Asia and India. Moreover, distributed throughout the world broadly [1, 2]. This plant can be cultivated in red, loamy soils with pH range of 6.5–7.5 and good drainage. *G. superba* is a perennial, semi-woody and climbing herb upto the height of approximately 5 metres. A single fleshy, V or L-shaped cylindrical rhizome can develop one to four stems (Fig. 1). The flowers are usually bright red to orange in colour, with wavy margins and yellowish bases. In addition, the stamens reach a length of 4 cm have large anthers that shed yellow pollen in large quantities. At maturity, the fruit turns green to yellow then dark brown in colour. Moreover, each mature fruit capsule (6 to 12 cm long) contains numerous red seeds. There are number of vernacular names for this plant including Malabar glory lily in English, Kalihari in Hindi, *Agnisikha* in Sanskrit. *G. superba* one of the endangered species among the most valued medicinal plants whose traded under the name of Glory lily [3–6]. *G. superba* become noteworthy due to the abundance of colchicine. Additionally, rhizome, leave and seed of the plant impact commercially [3]. *G. superba* blamed for several ethnic pharmacological activities such as antidote for snake bites, rheumatism, haemorrhoids, colic, cancer, skin related problems, bruises, inflammation, fever, expectorant, sprains, paralysis, abortions, piles etc [7]. In addition, scientific evidences of pharmacological activities such as Anti-bacterial, Anti-inflammatory, Anti-fertility, Anti-cancer, Uterotonic and Anti-oxidant property etc were reported [8]. As known, Anti-oxidant have proficiency to quench free radicals and/or to decrease their rate of production in human bodies that cause various ailments and plants are one of the richest sources of antioxidants which include secondary metabolites as phenolic and flavonoid. Therefore, it is

essential to identify active principles in medicinal plants in order to validate traditional medicinal plants scientifically or uncover bioactive molecules for use as therapeutic drugs [9, 10]. Rhizome of *G. superba* is one of the main part where the secondary metabolites conservation is high [11]. In the current investigation pharmacognostic profile of leaf, stem and rhizome are established. Moreover, the antioxidant capacity and phytochemical profile of ethanolic rhizome extract of *G. superba* (EREG) are investigated as well.

Materials And Methods

Collection and Identification of Plant Materials

Fresh whole plant and dried rhizomes were collected from Ramna village of Varanasi, India. The collected plant specimen was identified and authenticated at Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, India (Voucher specimen no. Colchica. 2022/02).

Chemical and Reagents

All the chemicals and reagents utilized in the studies were of analytical form. Ascorbic acid, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (USA) and Sisco Research Laboratory Pvt Ltd (Mumbai). The rest of the chemicals and reagents were procured from regional vendors.

Macroscopic Evaluation

The morphological characteristics of the leaves, stem, and rhizome were assessed including their colour, shape, surface, margin, venation, apex, aroma, texture, fracture, and the presence or absence of petioles [12].

Microscopic Evaluation

The microscopical study of *G. superba* was performed as per the procedure mentioned by Laloo et al (2013) *via* splitting thin sections of fresh leave, stem and rhizome using sharp razor. The thinnest sections were selected from the cut sections and stained with Phloroglucinol-HCl (2:1)/Wiesner and mounted with glycerin, examined under the microscope (Olympus cellSens imaging software integrates with fully motorized Bx-63 Trinocular microscope) and photographed. Furthermore, a portion of peel was removed from the lower surface of the leaf by turning the leaf over and gently separating the peel with the help of tweezers. Simultaneously, the peel was placed on the centre of the slide with the help of a brush and mounted with glycerine and then photographed to evaluate type of stomata present [13].

Preparation of the Plant Extract

The collected rhizomes of *G. superba* were cleaned, sun-dried and powdered coarsely using mechanical grinder (Mortar pestle). The coarsely powdered drug (300 g) was subjected to soxhlet extraction using ethanol (2 L) as a solvent for 72 hours. The methanol extract obtained was concentrated under reduced pressure in a rotary evaporator (JSGW rotatory vacuum flash evaporator) below 60°C temperature to obtain the concentrated extract of rhizomes. The remaining traces of solvent were removed from the extract by storing it in a vacuum desiccator for several days to get in the form of dry residue (32.55 g).

Determination of Antioxidant Activity

DPPH radical scavenging assay

The free radical scavenging ability of EREG was assessed according to the earlier reported method with minor modification. Fresh radical or DPPH (2,2-Diphenyl-1-picrylhydrazyl) solution was prepared by dissolving 4 mg of DPPH powder in 100 mL of methanol. Dilution series (0-1000 μg/mL) of ascorbic acid (stock solution) was employed to generate the standard curve. The test solution of EREG (0.5 mL) was added to 2.5 mL of methanolic DPPH. The developed mixture was thoroughly mixed and kept for 30 min at room temperature in dark. Absorbance of resulted mixture of EREG, stock solution and blank solution (mixture of 0.5 mL of EtOH and 2.5 mL of DPPH solution) was measured spectroscopically at 517 nm. In addition, percentage inhibitions of DPPH were calculated using below equation [14, 15]. % DPPH inhibition = $((A_B - A_A)/A_B) \times 100$; Where A_B: absorbance of blank solution; A_A: absorbance of the EREG solution.

Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to spectrophotometrically (765 nm) determine the TPC in EREG [16]. A standard curve was constructed employing gallic acid. The test solution of EREG was added to 2.5 mL of each aqueous reagent of Folin-Ciocalteu and Na_2CO_3 (75%). Total content of phenolic compounds in EREG was measured as milligram of gallic acid equivalents (GAE) per gram of weight. Triplicate assessments of each sample were executed.

Gas Chromatography-mass Spectroscopy

GC-MS analysis of EREG was accomplished using an Agilent 5977B EI/CI MSD: 8890/5977B series (USA) instrument model fitted with an auto sampler (G4513A) and an Agilent DB-5MS column of 30 m length, 0.25 mm internal went. diameter and 0.25 μm film thickness. Helium was used as the carrier gas with a constant flow rate of 1.322 mL/min, 13.545 psi pressure and 36.262 cm/s average velocity. Series II triple-axis detectors with high energy dynodes and long-life electron multipliers were employed for the detection. It was operated in electron impact mode while the oven and injector temperatures were programmed at 40°C and 60°C, respectively, which were gradually increased to 325°C. Phytocomponents were interpreted based on retention time values and known compounds in the NIST20 library [17].

Result

Macroscopic Characterization

The organoleptic characterization of leaf, stem, and rhizome of the plant is delineated in Table 1 and Fig. 1.

Table 1
Macroscopical characterization of leaf, stem and rhizome of *G. superba*

Characters	Plant Parts		
	Leaf	Stem	Rhizome
Colour	Shiny bright Green and	green	yellowish brown (outer) yellowish off-white (inner)
Shape	Ovate to lanceolate	erect then climber	“V” or “L”
Size	4–25 cm (length); 1.5–4.5 (width)	approximate 1–11 feet	10 to 15 cm; 2.5–3.8 cm (diameter)
Margin/ Venation	Entire/Parallel	-	-
Apex/Base	Acuminate with 1–2 cm long spiral tendril/Obtuse	-	-
Arrangement	In whorls of 3–4/ opposite/ alternate	-	-
Pendiculate/sessile	Subsessile	-	-
Taste	Bitter	bitter	acrid and bitte
Texture	Smooth	smooth	slightly rough
Fracture	-	short	short

Anatomical Description of G. superba

Leaf

A single layer of epidermis covered with medium outer wall/cuticle was present on both the adaxial and abaxial surface in the transverse section of leaf (Fig. 2a). Clearly visible single layer of collenchymatos cells was shown beneath the epidermis layer of adaxial surface. The transverse section of plant leaf showed the lower epidermal cells were larger in shape as compared to upper epidermal cells. The diagram was seen to be formed from barrel to oval-shaped of lacunae collenchyma cells. Centrally

positioned bicollateral vascular bundles, bounded on both side of xylem by sclerenchyma cells without intercellular spaces were seen. Moreover, *G. superba* is isobialeteral leaf showed anisocyctic type of stomata on the abaxial surface (Fig. 2).

Stem

In this transverse section of dicot stem, a single layer of epidermis sheeted with a prominent cuticle was seen. Underneath the epidermis, two layers of collenchymatous hypodermis and two to three layers of spherical thick sclerenchymatous pericycles were present. The cortex and medulla region of stem was made up with uneven sized spherical and brick like shaped parenchymatous cells. The vascular bundles in the parenchyma cells arranged themselves like a ring. A total of twenty-two vascular bundles were present as inner and outer cores. Presentation of both the xylem and phloem in a same radius confirmed its collateral nature of vascular bundle. There was a periphery-to-pith arrangement in this organization with phloem towards the periphery and endarch xylem to the pith zone. In addition, both the protoxylem and metaxylem were combined to form the endarch xylem (Fig. 3).

Rhizome

The rhizome of the plant was covered with a thin layer of brownish scales, which form a skin over the tuber. Moreover, the transverse section of this brownish scale revealed two or three rows of varied-size, thin-walled, rectangular, tangentially elongated cells that fabricate the outer brown skin. The epidermis was made up of rectangular cells which surrounded by dense outer walls. The cortical area appeared to be made up of rows of homogeneous fleshy and parenchymal cells with thin, colourless walls containing starch granules. Moreover, the cortex region contained a number of small-sized, scattered vascular bundles. Large, polygonal parenchymatous cells were associated with prominent intercellular spaces that were filled with large amounts of starch grains. Most of the starch grains were oblong, rounded, or polyhedral in shape. Oftentimes there were 5–13 grains of starch in each parenchymatous cell. Furthermore, high content of starch grains in its tubers is one of the superabundant diagnostic features of this plant. Innumerable simple and collateral vascular bundles were dispersed throughout the cortex. The majority of xylem vessels seemed to be annular or spiral while the parenchymatous cells of phloem were small and thin-walled containing less prominent companion cells (Fig. 4).

Antioxidant Activity Of Ereg

DPPH anti-scavenging Activity

DPPH composed of a stable free radical, which becomes colourless in the presence of antioxidants and this capability of DPPH is used in evaluation of antioxidant assays. Moreover, the existence of an odd

electron in DPPH is responsible for the absorption of electromagnetic radiation at 517 nm and the formation of dark purple colour. The DPPH undergoes decolorization and alters the range of absorption when it accepts an electron donated by an antioxidant [18]. The antioxidant capacity (50% inhibition (IC_{50}) of EREG measured by DPPH anti-scavenging method was 77.20%.

Total Phenolic Content

TPC of EREG was found 9.54 mg, GAE/g extract which was derived from a calibration curve (y = 0.0119x − 0.0014, R^2 = 0.9969) of gallic acid (0-100 µg/mL). The values of phenolic content in this current study varied slightly compared to those in the literature. The amount of total phenolic may be slightly different from that reported in literature due to the amount of secondary metabolite, geographical variation, duration of the study, or extraction method [19].

GC-MS

In the present study, the GC-MS method was used to identify phytoconstituents in ethanolic extract of rhizome. GC-MS library data describe different compounds based on retention times and GC-MS responses. In current investigation two phytoconstituents were identified by NIST20 library as diethyl phthalate and arsenous acid, tris(trimethylsilyl) ester. The earlier phytomolecule was identified with less retention time (16.773 minute) and 100% peak area. Resulted chromatogram shown mass spectrum (Fig. 5) of diethyl phthalate in which total four peaks labelled as 149, 177, 76 and 65 *m/z* value, in which 149 was *m/z* top peak, 177 was the second highest peak. Simultaneously, Fig. 5 also goes to show the molecular structure and molecular weight of the compound, which is 222. It is known by the IUPAC name diethyl benzene-1,2-dicarboxylate and molecular formula $C_{12}H_{14}O_4$. Moreover, it is also cognized by the variety of names, including phthalic acid diethyl ester, anozol, phthalol, neantine, diethyl phthalic acid, and so on.

The second identified compound (arsenic acid, tris(trimethylsilyl) ester) had a longer retention time (26.020 minute) than the first one while the peak area was 22.68%. The chromatogram of arsenous acid, tris(trimethylsilyl) ester shown three peaks with 207.04, 281.01 and 73.05 *m/z* value, in which 207.04 was *m/z* top peak, while 281.01 was the second highest peak. The IUPAC name and molecular formula of arsenic acid, tris(trimethylsilyl)ester is tris(trimethylsilyl) arsorite and $C_9H_{27}AsO_3Si_3$, respectively.

Discussion

The demand for herbal medicines is increasing steadily due to their prolonged classical usage and low risk of adverse effect as in the case of modern medicine [20, 21]. Natural medicines have the advantages of being readily available, affordable, and having few or no adverse effects, but they have the drawback of being adulterated. Demand for natural medicines increases with their efficacy that escalate the chances of non-availability. The natural medication is easily adulterated with poor grade of ingredients to

satisfy the rising demand [22, 23]. The quality and quantity of bioactive molecules of medicinal plants govern their pharmacological efficacy whereas the abuse of natural substances or herbal medications begins with faulty identification [24]. However, as ecological conditions changed and species varied, some characteristics of medicinal plants from various origins started to resemble each other more and more. This led to variety-confusion and significant quality differences in herbal medicines on the market. Identification of the varieties and assurance of the quality of herbal medicines are therefore of the utmost importance [25]. and the authentication of raw material is the first step to assure purity in this route. As a result, there has been a significant rise in the standardisation of certain medicinal plants with potential pharmacological benefit in recent years. In spite of the advanced techniques, identification of medicinal plants using pharmacognostic studies is more credible. As the World Health Organization also reported that macroscopic and microscopic elucidation of a plant drug should be used as a first step to evaluate the identity and purity level of such materials [26–28]. In order to negate its substitution or adulteration, *G. superba* lacks sufficient evidence to demonstrate its authenticity. As a consequence, authors have tried to identify pharmacognostical characters in breadth that can be useful for standardizing and identifying this plant. Macroscopic competence as colour, size, shape, margin/vein, apex/base, arrangement, pendiculate/sessile, taste, texture, and fracture, were evaluated to endow elementary signals about quality variation. The notable diagnostic features of *G. superba* microscopy were centrally positioned bicollateral vascular bundles and anisocyctic stomata in TS of leaf, periphery-to-pith (phloem towards the periphery and endarch xylem towards the pith) and ring frame arrangement of vascular bundle in TS of stem, abundance of starch granules in cortical area and outer brown skin of tuber (constructed with two or three rows of varied-size, rectangular, tangentially elongated cells) in the TS of rhizome may be considered as discriminative features which may helpful in the determination of anatomical structure and establishment of relevant identity of the plant.

There is mounting evidence that free radicals play a major role in many diseases. The substances that have the ability to counteract free radicals termed as antioxidant. A growing number of people are becoming aware of the potential benefits of antioxidants in the prevention and treatment of diseases caused by oxidative stress in recent years. In addition to natural antioxidants, synthetic antioxidants are also included in the group. In spite of their widespread use, some synthetic antioxidants have been found to be harmful due to their potential toxicity and carcinogenicity. As a result of potential health benefits, natural antioxidants are expected to be an alternative to synthetic ones. So far, the antioxidant activities of many medicinal plants have been assessed, and the findings revealed that many of them are rich sources of natural antioxidants. Therefore, researchers are highly keen on the discovery for raw materials that possess strong natural antioxidants [29]. Phenolics are one of the important secondary metabolites with redox power that acts as free radical scavenger or a primary antioxidant [30]. Selection of extraction technique and solvent are blameworthy to exude bioactive molecules from the plants [31]. Hydroxyl group in a phenolic compound facilitates scavenging of free radicals and also enhance its solubility in polar solvents, therefore ethanol was chosen as the extraction solvent. In addition, the studies in recent years have showed that TPC and antioxidant activity are significantly correlated [32]. The amount of obtained total phenolic may be slightly different from that reported in literature due to the amount of secondary

metabolite, geographical variation, duration of the study, or extraction method [19]. DPPH and TPC findings of EREG has proved its antioxidant activity beyond the doubt.

In GC-MS investigation two compound were identified. In addition, a vast use of identified compound diethyl phthalate includes pharmaceutical activities, personal care products, industrial uses with antimicrobial activity, perfumes, lotions, cosmetic products, paints, flexible plastics such as toothbrushes, medical devices, automobile parts, instruments, and drug packaging [33–35]. Several phthalate derivatives have been studied that exert inhibitory activities on glycoprotein cathepsin B which upregulated in certain type of cancer [36].

Conclusion

This study offers in-depth acquaintance about the macroscopical and microscopical features of leaf, stem, and rhizome. The pharmacognostical findings dispersed in this paper can be used further as a standard for the identification and authentication of *G. superba*. We are detailing the pharmacognostic profile of leaves and stems of *G. superba*, making this report the first to include a comprehensive pharmacognostic profile of this plant which will assist researchers in properly identifying the species. Combined with the photographic atlas, this study will be a guide to correctly identify the drug and to distinguish it from substitutes and adulterants. Moreover, the findings of DPPH and TPC corroborated the antioxidant properties of EREG.

Declarations

Ethical Approval and Consent to Participate

Not applicable

Consent for publication

All authors agree to publish

Conflict of Interest

There are no any conflicts of interest

Authors contributions

Dev Nath Singh Gautam and Geeta Rai conceived and planned this research work after literature on this plant. Anupriya Singh carried out the experiment. Moreover, Ashwani Kumar contributed to the interpretation of the results. All authors discussed the results and commented on the manuscript.

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Availability of data and materials

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Figures

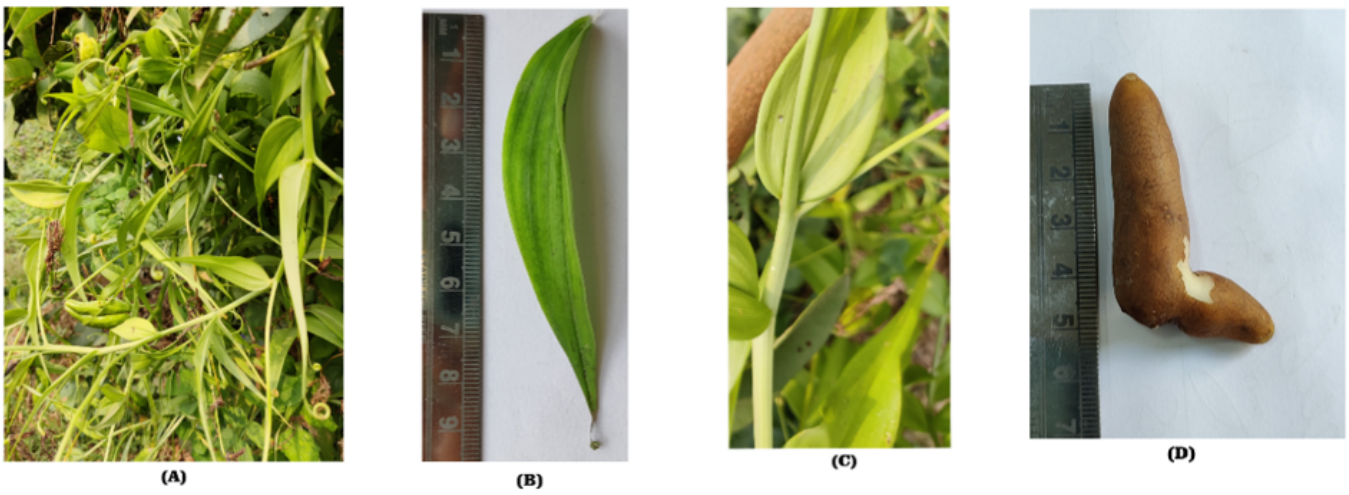

Figure 1

Portraits of *Gloriosa superba*: (A) Whole plant; (B) Leaf, (C) Stem and (D) Fresh rhizome

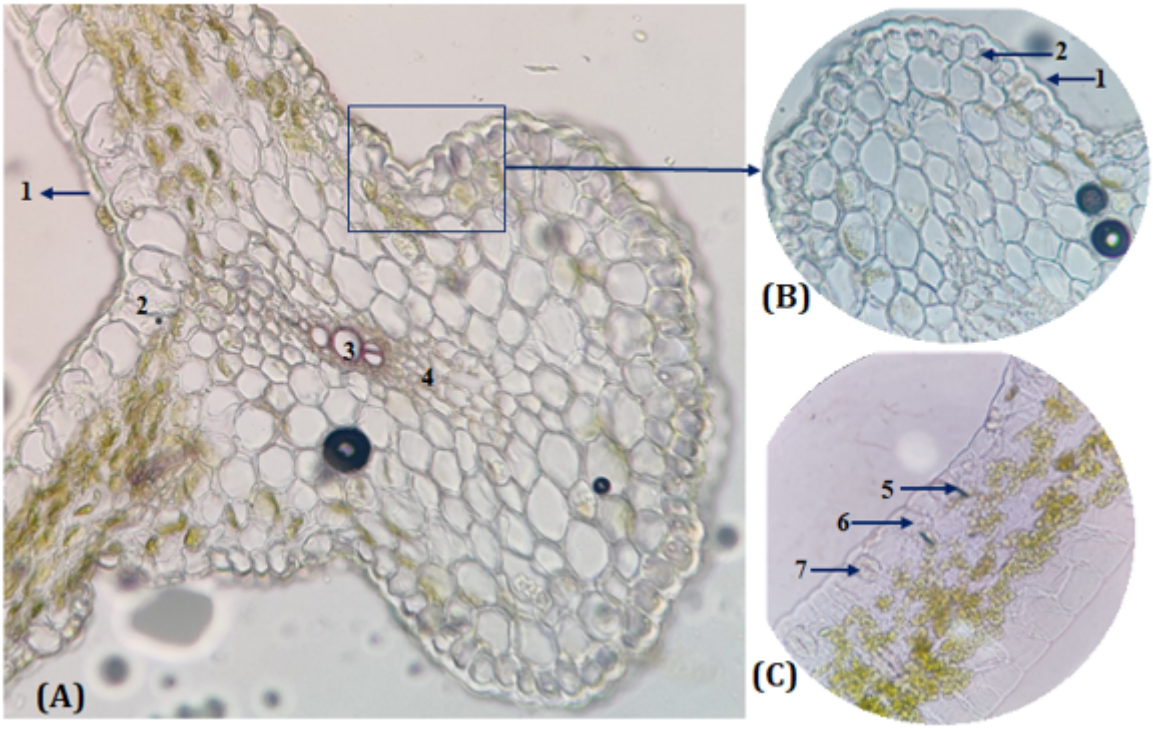

Figure 2

(A) Transverse section of *G. superba* leaf; (B) Enlarged portion of section; 1- cuticle, 2- epidermis, 3- xylem, 4- phloem; (C) Stomata; 5- stomatal pore, 6- subsidiary cell, 7- guard cell

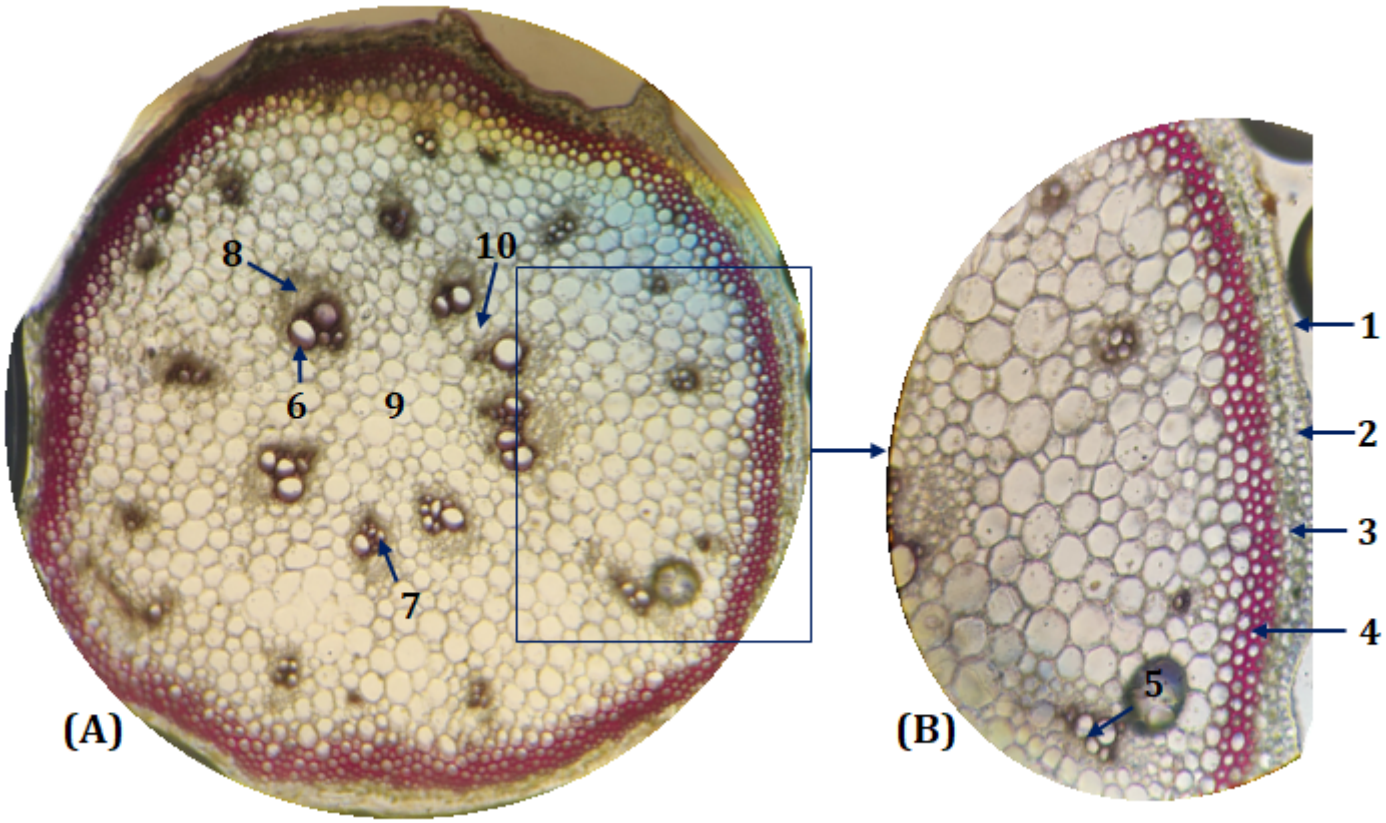

Figure 3

(A) Transverse section of *G. superba* stem; (B) Enlarged portion of section; 1- cuticle, 2- epidermis, 3- hypodermis, 4- pericycles, 5- vascular bundle, 6- meta xylem, 7- proto xylem, 8- phloem, 9- pith, 10- medullary ray

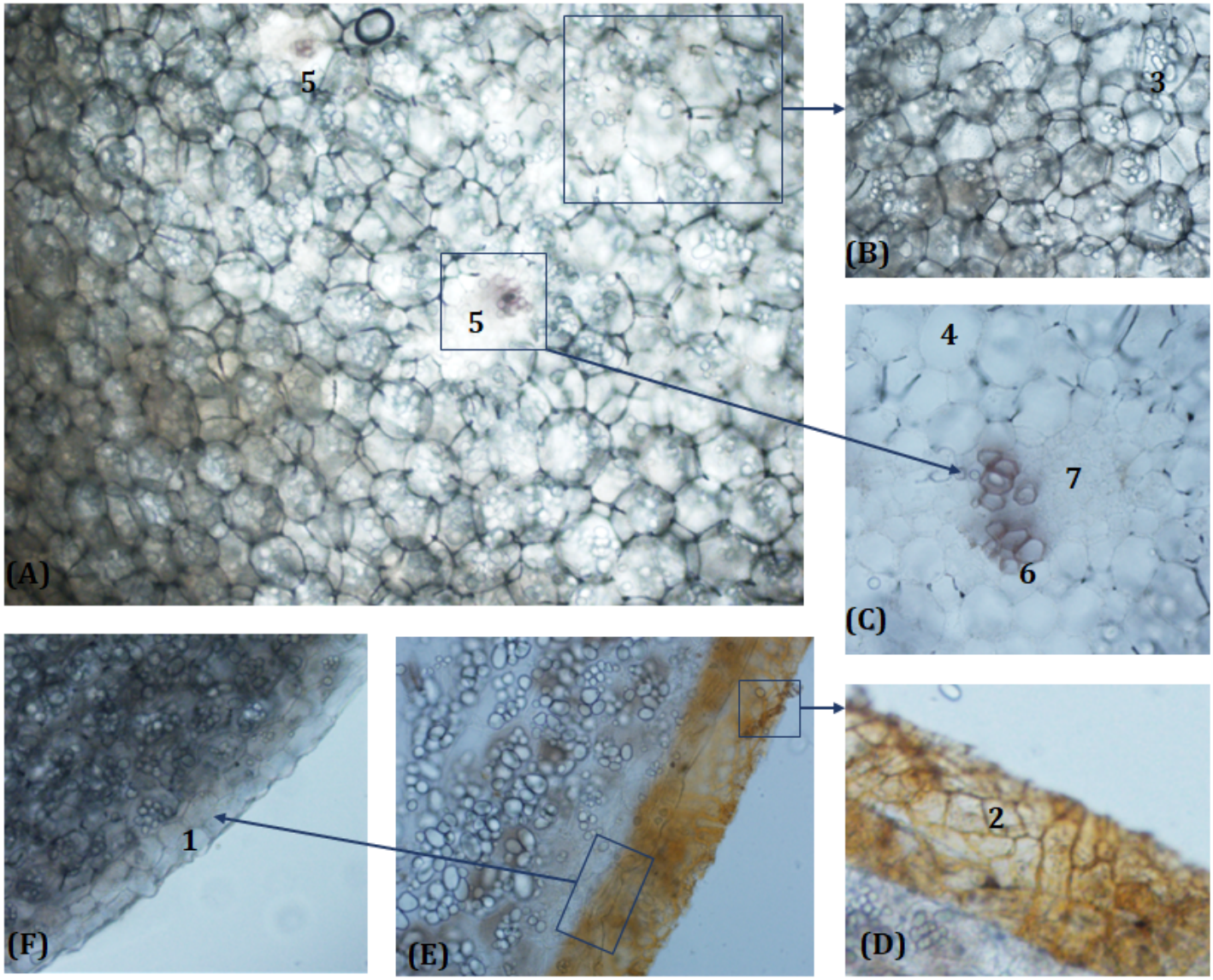

Figure 4

(A) Transverse section of *G. superba* rhizome; Enlarged portion of: (B) starch grains, (C) vascular bundle, (D) brown scale, (F) epidermis; 1- epidermis, 2- brown scales, 3- starch grains, 4- parenchymal cells, 5- vascular bundle, 6- xylem, 7- phloem

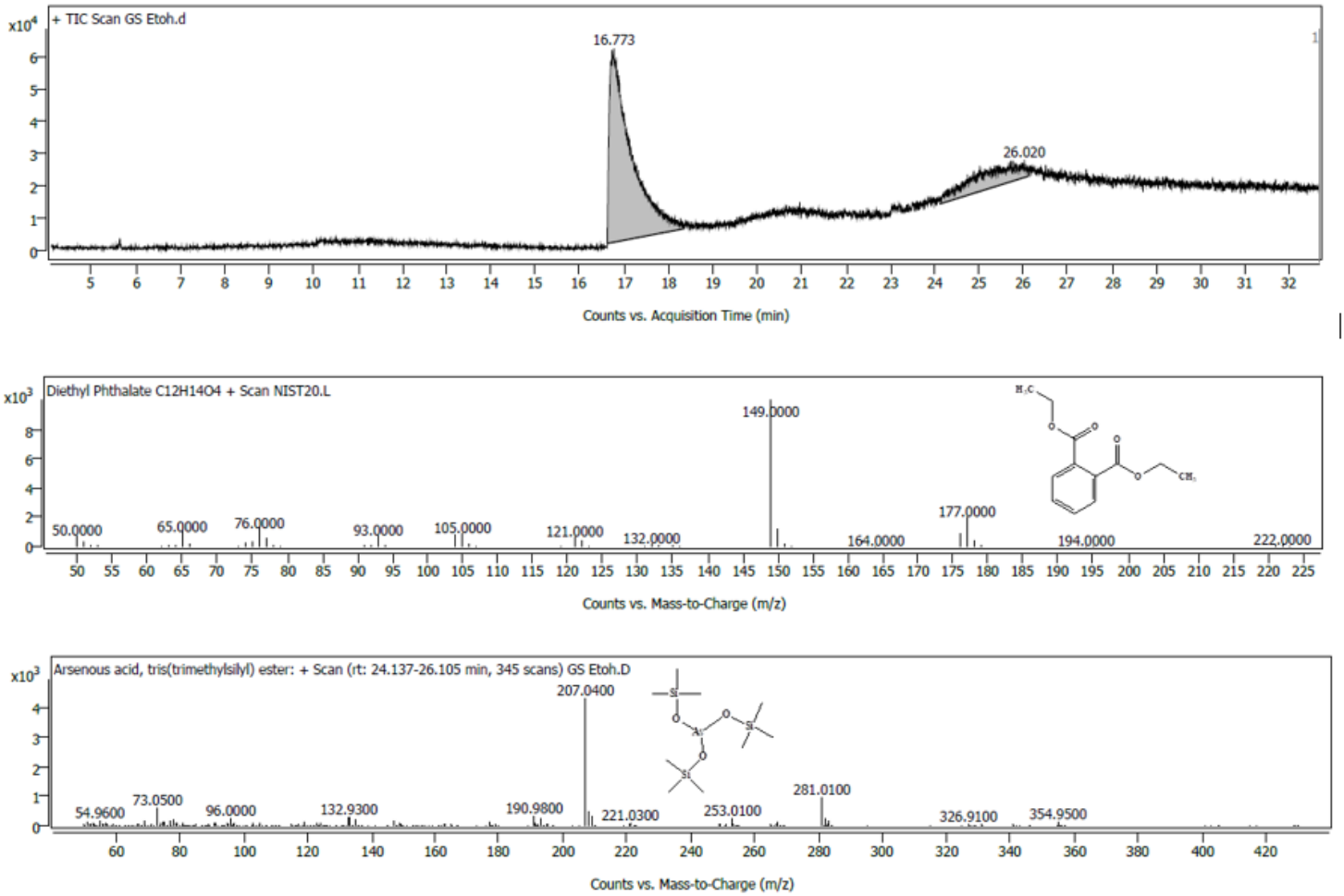

Figure 5

GC-MS chromatograms of ethanolic rhizome extract of *G. superba*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GA.png