International Journal of Sciences & Applied Research

www.ijsar.in

PHARMACOGNOSTIC STUDY AND DEVELOPMENT OF HPTLC FINGERPRINTS PROFILE OF *GLORIOSA SUPERBA* LINN.- STEM

Sanjay Pratap Singh[1], Rajesh Garg[2] & Manoj Tripathi[3*]

[1]Research Scholar- Awadhesh Pratap Singh, University, Rewa (M. P.)
[2]Department of Botany, Government (Auto.) P.G. Collage, Satna (M.P.)
[3*]Arogyadham, Deendayal Research Institute, Chitrakoot, District Satna (M.P.)
Email: trimanoj391@gmail.com

ABSTRACT

Gloriosa superba Linn. Family Liliaceae is a branched herbaceous climber, common in low forests almost throughout India including Andaman Islands up. It is frequently found within the hedges and road side bush about human habitations. It is also found along the forest paths of dry and moist deciduous forests in addition to the scrub jungles of the country. It is used in traditional medicinal and ayurvedic system of medicines. All parts of the plant are used for the treatment of various human as well as disorders. The present communication provides a detailed account of the pharmacognostic study carried out of Gloriosa stem. The study includes macroscopy, microscopy and powder microscopic studies, preliminary phytochemical investigation, physicochemical tests, heavy metal tests, screening of microbiological parameters and development of HPTLC (High Performance Thin Layer Chromatography) fingerprints profile. Physicochemical parameters were performed and found LOD was found 6.23% w/w, total ash value 6.88% w/w, acid insoluble ash value 0.25% w/w, alcohol soluble extractive value 18.46% w/w and water soluble extractive value 22.64%w/w. HPTLC (High Performance Thin Layer Chromatography) fingerprints profile of methanolic extract was done by using mobile phase toluene: ethyl acetate (7:3). TLC plate was derivatized by using 5% Methanolic-sulphuric acid derivatizing reagent. Major spots Rf values and colour were recorded at 366nm, after derivatization 366nm and UV light. Quantitative microbiological tests were performed and specific pathogens found absent such as *Staphylococcus aureus*/gm, *Salmonella sp.*/gm, *Pseudomonas aeruginosa*/gm, *Escherichia coli*, where total microbial count (TBC), and Yeast & Mould found under WHO limits. Heavy metals such as Pb, Cd, As, & Hg were tested and found under WHO limits Established parameters can be used as standards for quality control and identification of the plant in herbal compound formulations and also preparation of a monograph of the plant.

Keywords: *Gloriosa superba*, Pharmacognostic, Phyto-chemical analysis, Physicochemical

INTRODUCTION

Kalihari *Gloriosa superba* family Liliacea having numerous medicinal properties due to its present of several bioactive compounds (Maroyi A, van der Maesen LJG, 2011; Padmapriya S, *et. al.*, 2015; Banu HR, & Nagarajan N. 2011) The plant has been used as a medicine from ancient time. Kalihari is a herbaceous creeper plant about upto 6 meter high. It is also known as

semi-poisonous drugs in Indian Medicine system (Chaudhary S, *et. al*., 2018; Gupta R K *et. al*., 2012). Various types of phyto-constituents such as carbohydrates, glycosides, steroids, flavonoids lipids, phenolic compounds, alkaloids and terpenoids are present in the all part of the plant (Chaudhary S *et. al*., 2016 Kavina J. et al., 2011; Kavithamani D. *et., al.* 2013; Kohli G., 2017). These bio-chemicals are responsible for biological activities.

Gloriosa Plant is also known as ethno-medicinal plants in Indian medicines, it is the used for treatment of various diseases in traditionally like indigestion, leprosy, arthritis, piles, abdominal ache, infertility, intestinal worm infections, inflammations, ulcer, baldness and snakebites fever, skin infection.. The tubers are reported to exhibit anthelmintic, laxative, alexiteric and abortifacient by different communities and sources of ancient literatures such as Charak Samhita described skin diseases, itching and ailments and kapha and vata; similarly Rajanighantu- Pungent, thermogenic, eliminates deranged kapha (phlegm) and vata (wind), terminates pregnancy; Sushruta Samhita- postnatal complaints, Maudanani Nighant has mentioned as thermogenic, abortifacient and skin infections; Dhanvantari Nighantu- Leprosy, labor pain, wound infections, purgative; Bhavaprakasha has described as light abortifacient, excites pitta (bile), cures dropsy, piles, wounds, acute spasmodic pain, removes warms; Chakradatta- If smeared over the palms and feet of pregnant women, delivery of child becomes easier; Ayurveda- abortifacient, acrid, anthelmintic, antipyretic, bitter, depurative, digestive, emetic, expectorant, purgative, stomachic, tonic, thermogenic, promoting labor pain, expulsion of placenta, effective against paralysis, rheumatism, snakebite, insect bites, asthma and mentioned in Siddha- Various skin diseases (Kavina, *et. al*., 2011; Kohli *et. al*., 2017; Chaudhary *et. al*., 2018)

Gloriosa superba plant has contains various types of phytochemical. Tuberous root has present highly active compound alkaloid, colchicine and gloriosine. such as cornigerine, lumicolchicine, 3-demethyl-Nformyl- N-deacetyl-lumicolchicine, 3-demethyl-glumicolchicine, 3-demethyl colchicines, colchicocide, tannins and superbine have been also present in various parts of the plant. glycoside, 3-o-dimethylcolchicine-3-o-α-Dglucopyranoside, β-sitosterol, lumiccolchicines, 2-hydroxy-6-methoxy benzoic acid (Suri *et.al.*, 2001; Clewer *et. al*., 1915). Tuber root part of plant are found colchicines, benzoic and salicylic acid, gloriosine, tannins, 1,2-didemethyl colchicine, 2,3-didemethyl colchicine, sterols and resinous substances like as colchicines, 3-demethyl colchicine, N-formyl, N-deacetyl colchicines, colchicocide, and superbine. Colchicine is the major compound found in tuber root, seed and another important compound also found gloriosine In seeds 0.25% colchicine apart from containing sitosterol, glucoside, β-and gamma lumicolichicines, β-sitosterol, flucoside, colchicine glycoside, 3-0-demethyl colchicine 3-O-alpha-D- glucopyranoside (Veeraiah, S., and Jaganmohan Reddy, K, 2012) and 2-H-6-MeO benzoic acid and luteolin and N-formylde-Me-Colchicine 20. Found in flowers (Suri *et. al*., 2001). Despite the numerous medicinal uses attributed to this plant, there are no systematic pharmacognostical studies on the stem Karihari plant have so far been carried out. Hence the present work deals with the morphological, anatomical evaluation, physicochemical tests, preliminary phytochemical screening, heavy metals test, microbiological screening and High-Performance Thin Layer Chromatography.

MATERIALS AND METHODS

Collection of samples

The fresh plant of Gloriosa stem was collected from Arogyadham campus, Deendayal Research Institute, Chitrakoot, Satna, (M. P.) in the month of October. The plant was identified and authenticated by Dr. Manoj Tripathi Senior Scientist, Arogyadham, Deendayal Research Institute Chitrakoot. The voucher specimen (AD/AS/266/2022) prepared as per standard procedure [14] and maintained in the herbarium of department of the botany, APS University, Rewa (M.P.) for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of High Performance Thin Layer Chromatography fingerprint profile (Anonymous, 2017).

Macroscopic study

Macroscopic or organoleptic characters Gloriosa stem like appearance, colour, odour and taste were evaluated of the Gloriosa stem.

Microscopic study

Fresh stem section was cut by free hand sectioning and numerous sections examined Microscopically[15]. Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 21l with Digi-eye camera using Caliper plus version 4.2 software (Evans WC and Trease, 2003).

Powder microscopic study

The dried stem was powdered and completely passed through 355 µm IS Sieve (old sieve number 44) and not less than 50% passel on through 180 µm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, poured out the water without loss of material. Mounted a small portion in glycerin were used to all characters of the Gloriosa stem, small quantity of sample cleared by heating with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, another small quantity of sample stained with sudan red solution and mounted with glycerin, all mounted slide were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope (Kokate CK, 2006 & Mukharjee, PK 2002).

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, Hexane soluble extractive; alcohol soluble extractive value, total ash value, acid insoluble ash value was calculated (Anonymous, 2010; Mitra R and Mehrotra S, 1980).

Preliminary phyto-chemical investigation

Preliminary phyto-chemical tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, carbohydrates, proteins and saponins (Tripathi M and Sikarwar R L S , 2014 & 2015).

High Performance Thin Layer Chromatography (HPTLC) fingerprint profile

For High performance thin layer chromatography, the powdered 5 gm of sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The sample, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of toluene*: ethyl acetate* (7: 3 v/v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, Thin Layer Chromatography plate was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at UV light with Win cat software and R_f values noted (Tiwari *et., al.;*,2015; Venugopal *et. al.,* 2015).

Microbiological limit tests

Microbial limit tests for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. Following tests were carry out as per (Choudhary *et. al.,* 2014; Tripathi *et. al.,* 2015 & Anonymous, 2007) to determine the microbial load in three samples of Gloriosa stem powder. Enumeration of *Staphylococcus aureus*/gm; *Salmonella sp*./gm; *Pseudomonas aeruginosa*/gm and *Escherichia coli;* determination of total microbial count (TBC) and Yeast & Mould. The microbiological tests were determined using specified agar media and enrichment media from Himedia, Pvt. Ltd. Mumbai.

RESULTS AND DISCUSSION

Macroscopic characters

Gloriosa superba Linn. stem is externally green and internally greenish brown colour, taste acrid and bitter, odour characteristics, stem is longer, cylindrical with circular branched climber (Fig.1a &1b).

Microscopic characters

Diagrammatic Transverse Section is oval to circular in outline and shows a layer of epidermis encircling starchy parenchymatous ground tissue with scattered vascular bundles (Fig. 2a),.

Detailed Transverse Section (TS) of the stem shows a layer of epidermis with thin cuticle followed by parenchymatous ground tissue embedded with plenty of simple and compound starch grains of various sizes and shapes, conjoint collateral vascular bundles devoid of fibres encircled by smaller sized parenchymatous sheath traversed throughout the ground tissue (Fig. 2b).

Powder microscopic characters

Gloriosa stem powder colour is whitish brown, taste not characteristics and odour astringent.

Physico-chemical analysis

Physicochemical analysis were performed and obtained the results such as LOD was found 6.23% w/w, total ash value 6.88% w/w, acid insoluble ash value 0.25% w/w, alcohol soluble extractive value 18.46% w/w and water soluble extractive value 22.64%w/w. The physic chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug.

Microbiological limit tests

Gloriosa stem powder microbiological tests of pathogenic bacteria, viz. *Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done and found that absent, while total microbial plate count (TPC) was found 55cfu/g and yeast & moulds found 80 cfu/g. Microbiological profile of the Gloriosa stem was found satisfactory under prescribed limits in WHO guidelines / Ayurvedic Pharmacopoeia of India such as for *Salmonella* sp., *Escheria coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* limits absent, where for total microbial plate count (TPC) 10^5 cfu/g and for yeast & moulds 10^3 cfu/g.

Heavy Metal tests

Heavy metal elements (Pb, Cd, As and Hg) test were performed and results were found such as Lead (Pb) 5.4521 ppm, Cadmium (Cd) 1.2390 ppm, Arsenic (As) 8.8987 ppb and Mercury (Hg) 11.4587 ppb under limits as per guideline of WHO/ API 10 ppm, 0.3 ppm, 03 ppm and 01 ppm respectively.

Preliminary phyto-chemical investigation

Qualitative phyto-chemicals were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of carbohydrates, alkaloids, tannin, flavonoids, protein and saponin.

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract three spots of the Gloriosa stem sample extract applied in precoated TLC plate. Applied 6 µl of the test solution as 8 mm bands and develop the plate in a solvent system toluene*: ethyl acetate* (7: 3 v/v) to a distance of 8 cm. Dry the developed plate in room temperature and examined. Derivatized the plate using 5% *Methanolic-sulphuric acid* reagent and heating at 105^0C till the bands are clearly visible. Major spots R_f values with colour were recorded before derivatization at 366nm, after derivatization at 366nm and at UV light. Chromatogram profile and R_f values are given (Fig. 4 & Table 4).

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Gloriosa stem. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate's important phyto-constituents. Heavy metal elements are found under limits as per guideline WHO and microbial limits test of the Gloriosa stem were found satisfactory. Total microbial plate count (TBC), Yeast & Moulds counts were reported less than the limit as per

suggested by WHO and pathogenic bacteria i.e., *Staphylococcus aureus*, *Salmonella sp*., *Pseudomonas aeruginosa* and *Escherichia coli* were found to be absent. These finding could be helpful in identification and authentication of Gloriosa stem.

CONCLUSION

Due to the side effects of modern medicines on human health, the importance and uses of herbal medicines are increasing day by day all over the world. Because the plants have natural chemicals which do not have any adverse side effects on human health. But the herbal medicines however, suffering from lack of standardization parameters and quality control. Hence the standardization and quality control of herbal drug is very important. Gloriosa is one of the most important plant of India and its different parts such as stem root, leaf, flowers and seeds are used to treat different types of human ailments and diseases like leprosy, lower pain, wound infections, purgative, cures dropsy, piles, wounds, acute spasmodic pain, removes warms, effective against paralysis, rheumatism, snakebite, insect bites, asthma and mentioned etc. Due to its wide therapeutic importance, it is worthwhile to standardize it for use as drug.

Acknowledgement

The authors are grateful to Shri Abhay Mahajan, Hon'ble Organizing Secretary, Deendayal Research Institute, Chitrakoot, Satna (M.P.) for providing infrastructure and necessary facilities.

REFERENCES

1. Maroyi A., Van Der Maesen LJG. , *Gloriosa superba* L. (family colchicaceae): Remedy or poison? *Journal of Medicinal Plants Research,* 2011; 5(26): 6112-6121.
2. Padmapriya S., Rajamani K., Sathiyamurthy V.A., Glory lily (*Gloriosa superba* L.)- A review, *International Journal of Current Pharmaceutical Review and Research,* 2015; 7(1): 43-49.
3. Banu H.R., Nagarajan N., Antibacterial potential of Glory lily, *Gloriosa superba* Linn., *International Research Journal of Pharmacy,* 2011; 2(3): 139-142.
4. Chaudhary S., Gupta R.K., Kumar A., Tarazi H., Hepatoprotective and antioxidant potential of *Nyctanthes arbor-tristis* L. leaves against anti-tubercular drugs induced hepatotoxicity, *Journal of Pharmacy and Pharmacognosy Research*, 2018; 6(3): 205-215.
5. Gupta R.K., Chaudhary S., Vaishali, Singh R.K., Ant hepatotoxic influence of aqueous extract of *Ipomoea carnea* against carbon tetrachloride induced acute liver toxicity in experimental rodents, *Asian Journal of Pharmaceutical and Clinical Research*, 2012; 5(4): 262-265.
6. Chaudhary S., Semwal A., Kumar H., Verma H.C., Kumar A., In-vivo study for anti-hyperglycemic potential of aqueous extract of Basil seeds (*Ocimum basilicum* Linn) and its influence on biochemical parameters, serum electrolytes and hematological indices, *Biomedicine and Pharmacotherapy*, 2016 (84): 2008-2013.
7. Kohli G., Kaushik D., Jangra S., Bhyan B., Chawla I., Thakur V., Ethnopharmacological profile of *Gloriosa superba*: An endangered medicinal plant, *World Journal of Pharmacy and Pharmaceutical Sciences,* 2017; 6(3): 267-285.
8. Kavina J., Gopi R., Panneerselvam R., *Gloriosa superba* Linn- A medicinally important plant, *Drug Invention Today* 2011; 3(6): 69-71.

9. Kavithamani D., Umadevi M., Geetha S., A review on *Gloriosa superba* L., as a medicinal plant, *Indian Journal of Research in Pharmacy and Biotechnology*, 2013; 1(4): 554- 557.
10. Kavina J., Gopi R., Panneerselvam R., *Gloriosa superba* Linn- A medicinally important plant, *Drug Invention Today* 2011; 3(6): 69-71.
11. Kohli G., Kaushik D., Jangra S., Bhyan B., Chawla I., Thakur V., Ethnopharmacological profile of *Gloriosa superba*: An endangered medicinal plant, *World Journal of Pharmacy and Pharmaceutical Sciences*, 2017; 6(3): 267-285.
12. Suri O.P., Gupta B.D., Suri K.A., A new glycoside, 3-odemethylcolchicine- 3-o-alpha-d-glucopyranoside from Gloriosa seeds, *Natural Product Letters* 2001; 15(4):217- 219.
13. Clewer HWW, Green S.S., Tutin F., The constituents of *Gloriosa superba*, *Journal of Chemical Society,* 1915; (107): 835-846.
14. Veeraiah, S., and Jaganmohan Reddy, K., Current strategic approaches in ethanomedicinal plants of *Tinospora cordifolia* and *Gloriosa superba* - a review, *International Journal of Pharma and Bio Sciences*, 2012; 3(2): 320-326.
15. Anonymous, Quality standards of Indian Medicinal Plant (published by Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi. 15th edn. (2017) p. 198-208.
16. Evans WC, and Trease Pharmacognosy, 15th ed., (Saunders, London, (2003) 545-547.
17. Kokate CK, Practical Pharmacognosy Is ted., Vallabh Prakashan, New Delhi, 2006.
18. Mukherjee PK Quality Control of Herbal Drugs- an Approach to Evaluation of Botanicals, 1st edn, Business Horizons, New Delhi, (2002) 695.
19. Anonymous Laboratory guide for the analysis of Ayurveda and Siddha formulations, published by Central Council for Research in Ayurveda and Siddha, Department of AYUSH, Ministry of Health and family Welfare, Government of India, New Delhi (2010) 83-87.
20. Mitra R and Mehrotra S, Pharmacognostical studies on Priyal: fruits and seeds of *Buchanania lanzan* Spreng. (Family: Anacardiaceae) *Bull Bot Surv India* 22 (1980) 68-76.
21. Tripathi M and Sikarwar R L S Pharmacognostic standardization of stem bark of *Erythrina variegate* L. *Journal of the Indian Botanical Society* 93 (3&4) (2014) 248-253.
22. Tripathi M and Sikarwar R L S Pharmacognostic Studies on Plaksa (Ficus virens Ait.) Stem Bark *Indian Journal of Natural Products and Resources* 6(1) (2015) 27-32.
23. Tiwari A, Tripathi M and Dwivedi N, Standardization of Ayurvedic compound formulation Kutajastaka Kvatha Curna *J. Trop. Med. Plants (*2015) *15* 1-13.
24. Venugopal G, Srinivasa R C, Rao N B, Rao V B, Rao V T, Rao A P Pharmacognostic and Preliminary Phytochemical Evaluation of *Ocimum basilicum* L. var. *pilosum* (Willd.) Benth. and *O. tenuiflorum* var. CIM-AYU, *International Journal* of *Pharmacognosy* and *Phytochemical Research* 7(3) (2015) 519-526
25. Choudhary N, Siddiqui MB and Khatoon S, Pharmacognostic evaluation of Tinospora cordifolia Willd.) Miers and identification of biomarkers, *Indian J Tradit Knowle,* 13(3) (2014) 543-540.
26. Tripathi M, Sikarwar R L S, Tiwari A & Dwivedi N, Pharmacognostical identification of ingredients in Laghulai curna *Indian J Tradit Knowle* 14(4) (2015) 531-536.

27. Anonymous Protocol for Testing of Ayurvedic, Siddha & Unani Medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines Ghaziabad, 2007.

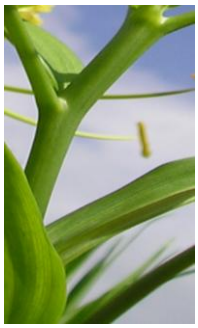
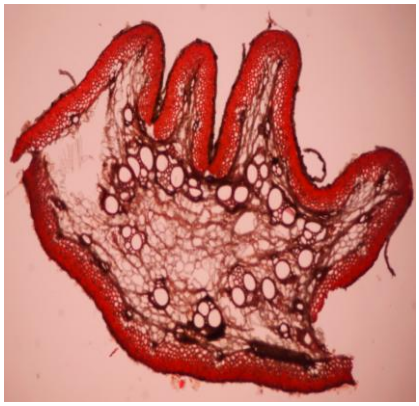


Fig.1a: fresh stem

Fig.1b: dried stem

Fig.3: stem powder



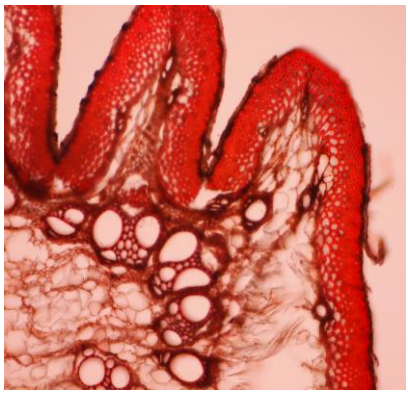


Fig.2a. Diagrammatic TS of stem

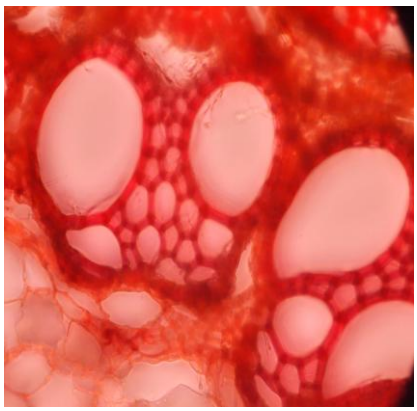


Fig.2b: Detailed TS of stem