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PHARMACOGNOSTIC STUDY AND PRELIMINARY PHYTO-CHEMICAL ANALYSIS OF KALIHARI (*Gloriosa superba* Linn.) TUBEROUS ROOT

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ABSTRACT

Kalihari *(Gloriosa superba* L..) Family Liliaceae is known as economical and medicinal values. It is a semi-woody herbaceous climber. It is grown as ornamental plants and found throughout India up to an altitude of 6000 fits. 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 Kalihari root. 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 5.84% w/w, total ash value 10.16% w/w, acid insoluble ash value 0.21% w/w, alcohol soluble extractive value 13.98% w/w and water soluble extractive value 25.77%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: Kalihari, Pharmacognostic, Physicochemical, Phyto-chemical analysis, HPTLC.

INTRODUCTION

Kalihari *(Gloriosa superba* L.) family Liliaceae is a semi-woody perennial or herbaceous climber. It is grown as ornamental plants and found throughout India up to an altitude of 6000fits. It is a native of tropical Africa and is now growing in many parts of tropical Asia including India, Burma, Malaysia and Srilanka.[1] It is now widely distributed throughout the tropics and worldwide as a pot plant. In Africa, its distribution is from Senegal east to Ethopia and Somalia, and to South Africa. In India it is spread from hotter southern parts to the milder mid hill zones of Himachal Pradesh, Jammu Kashmir, Uttara Kannada, Hassan, Chikmangalur, Coorg, Mysore (Karnataka); Cannanore, Palakkad, Trivandrum (Kerala) and Uttar Pradesh.[2,3] Tamil Nadu has the largest area under glory lily cultivation (upto 6000 acres) spread over seven districts viz., Karur, Tirupur, Dindigul, Salem, Ariyalur, Perambalur and Nagapattinam and holds monopoly in production of glory lily seeds with an annual production of over 600 -700 tonnes. Kalihari is the state flower of Tamil Nadu, and national flower emblem of Zambia. *Gloriosa* name is the derives from the word '*gloriosus'* which means handsome and '*superba'* from the word 'superb' means majestic or splendid. The fondness for floral beauty has also placed Gloriosa as a pot plant in gardens.[4,5]

Gloriosa superba is highly values ethno-medicinal plant due to its enormous medicinal properties.[6,7] It is used to treat the various diseases like arthritis, indigestion, fever, skin infection, cardiomyopathy, chronic ulcer, piles, leprosy, abdominal ache, inflammations, infertility, intestinal worm infections, colic, hemorrhoids, cancer baldness and snakebites. In Indian system of Medicines plant tuberous roots are used as a tonic, antiperiodic, antihelmenthic and also against snake bites. Tuber paste is externally applied for parasitic skin diseases. The tuber, pods and leaves were used to treat infections of guinea-worms, tapeworm, roundworm, liver fluke and filarial schistosomes (causing bilharzia) and tapeworm.[8,9] Tuberous root has also used to relieve neuralgia, dislocations of the shoulder, nerves, joint swellings and sprains.[10] Leaves hot extract is used for delayed puberty, delayed childbirth and sterility and menstrual problems. Leaf and unripe fruit aqueous extract mixed

with butter are applied to cure kill head lice, roots have abortifacient activities. Seeds and tubers contain valuable alkaloids viz., colchicine and colchicoside as the major constituents, which is used to treat gout and rheumatism. Due to the action of colchicoside on spindle fibre formation during cell division, the plant has been identified as a potential anti cancerous drug.[11,12,13] Despite the numerous medicinal uses attributed to this plant, there are no systematic pharmacognostical studies on the tuberous root of this 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, florescence study, microbiological screening and High-Performance Thin Layer Chromatography.

MATERIALS AND METHODS

Collection of samples

The fresh plant tuberous root of Kalihari was collected from Arogyadham campus, Chitrakoot, Satna, Madhya Pradesh in the month of March. The plant was identified and authenticated by Dr. Manoj Tripathi Senior Scientist, Arogyadham, Deendayal Research Institute Chitrakoot. The voucher specimen (AD/AS/346/2022) 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.

Macroscopic study

Macroscopic or organoleptic characters Kalihari tuberous root like appearance, colour, odour and taste were evaluated.

Microscopic study

Fresh root 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.

Powder microscopic study

The dried tubers 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 Kalihari root, 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.[16]

Fluorescence study

Fluorescence study was carried out of G*loriosa superba* tuberous root power separately in various mounts.[17]

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.[18, 19]

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.[20,21,22]

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.[23, 24]

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[25,26] to determine the microbial load in three samples of Kalihari tuberous root powder.

Enumeration of *Staphylococcus aureus*/gm

Enumeration of *Salmonella sp*./gm

Enumeration of *Pseudomonas aeruginosa*/gm

Enumeration of *Escherichia coli*

Determination of total microbial count (TBC)

Determination of 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. tuber is externally brown and internally buff colour, taste acrid and bitter, odour characteristics, fracture short mealy. The surface of the arms is covered with thin skin getting peeled off easily. Tuberous root stock when intact is not straight but always bent and resembling to shape of a plough. Its lower are being fairly stout, cylindrical, slightly flattened dorsi-ventrally, exhibiting small circular rootlet scars and measuring 5 to 6 cm in length and 2 to 3 cm in width. The upper arm of the rhizome is longer, cylindrical with circular marking on the surface measuring 6 to 8 cm in length (**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.

Detailed Transverse Section (TS) of the rhizome 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. 2a, & 2b).**

Powder microscopic characters

Kalihari root powder colour is whitish brown, taste not characteristics and odour astringent.

Under microscope powder showed abundant simple and compound starch grains of various shapes and sizes scattered as such or embedded in the parenchymatous cells of the ground tissue. Fragments of outer scales in surface view exhibiting thick, straight anticlinal walls embedded with rows of idioblast studded with brown colouring matter. Fragment of epidermis with straight anticlinal walls and embedded with anomocytic and anisocytic stomata. Fragments of longitudinally cut annular and reticulate vessels (**Fig. 3)**

Fluorescence study

Fluorescence study was carried out of G*loriosa superba* tuberous root power separately in various mounts and observed through UV spectrophotometer at UV light, 254nm and 366nm, colours were recorded. Results are given in **Table 1.**

Physico-chemical analysis

The physico-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. Physicochemical results were found such as LOD was found 5.84% w/w, total ash value 10.16% w/w, acid insoluble ash value 0.21% w/w, alcohol soluble extractive value 13.98% w/w and water soluble extractive value 25.77%w/w.

Heavy metals tests

Heavy metal elements (Pb, Cd, As and Hg) test were performed and results were found such as Lead (Pb) 6.7949 ppm, Cadmium (Cd) 0.0624 ppm, Arsenic (As) 7.2396 ppb and Mercury (Hg) 16.4178 ppb under limits as per guideline of WHO/ API 10 ppm, 0.3 ppm, 03 ppm and01 ppm respectively.

Microbiological limit tests

Kalihari tuberous root powder microbiological analysis 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 40cfu/g and yeast & moulds found 65 cfu/g. Microbiological profile of the *Gloriosa superba* tuberous root 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.

Preliminary phyto-chemical investigation

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

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract three spots of the Kalihari root 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.

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Kalihari tuberous root. 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 Kalihari root 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. All findings are indicating samples are genuine and free from any adulterations. These finding could be helpful in identification and authentication of Kalihari root.

Table 1: Fluorescence study of *Gloriosa superba* tuberous root.

S. No.	Drug powder+ Chemical	Observation in UV light	Observation in 254nm	Observation in 366nm
1	Powder	Cream	Creamish white	Creamish white
2	Drug powder + Distilled water	Cream	Whitish cream	Creamish white
3	Drug powder + Nitrocellulose	White	Light yellow	Yellowish green
4	Powder + Acetic acid	Dark black	Brownish white	Blackish brown
5	Powder + 50% KOH	Sky blue	Greenish white	Turmeric yellow

6	Powder + 1N HCL	Light red	Light brown	Brownish yellow
7	Powder + 1N NaOH water	Pale yellow	Greenish yellow	Pale yellow
8	Powder + H2SO4	Dark black	Dark green	Light black
9	Powder + Iodine water	Sky blue	Bluish yellow	Blue
10	Powder + 1N NaOH methyl	Blue	Yellowish green	Yellowish blue
11	Powder + 50%H2SO4	Brownish black	Greenish brown	Light yellow
12	Powder + 50%HNO3	Brownish black	Dark brown	Dark yellow

Fig. 1a- Kalihari Plant

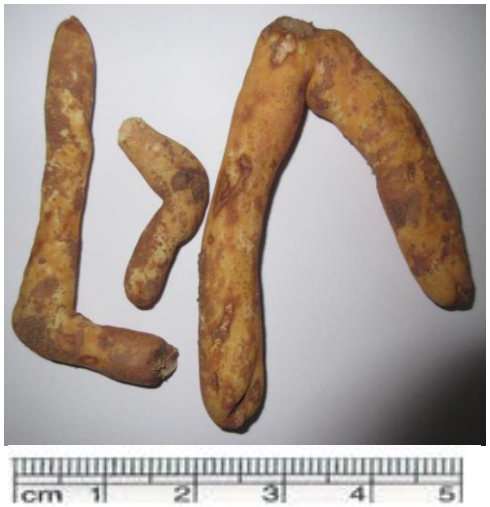

Fig. 1b- Kalihari tuberous root

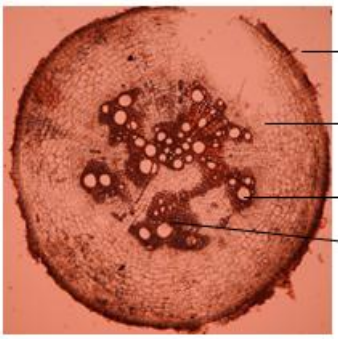

Fig. 2a- Diagrammatic TS of tuberous root

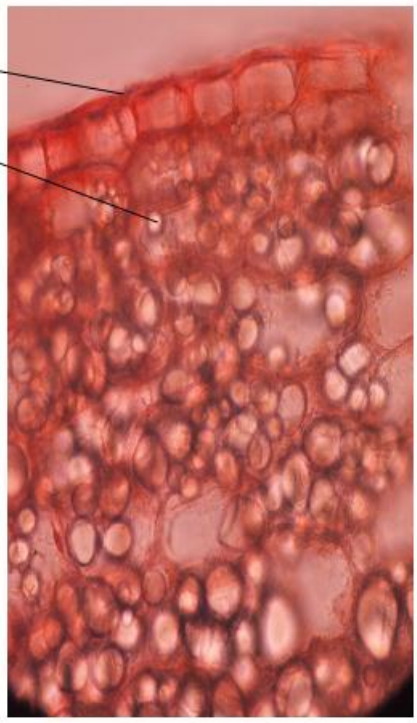

Fig. 2b- Detailed TS of tuberous root

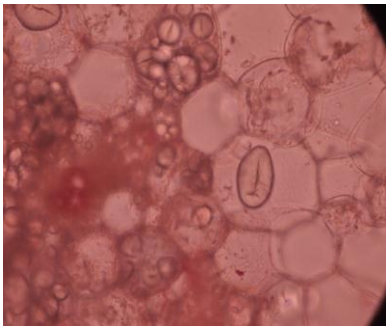

Fig. 3a

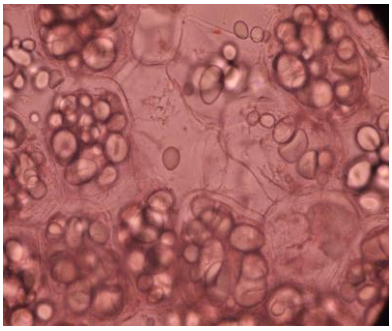

Fig. 3b

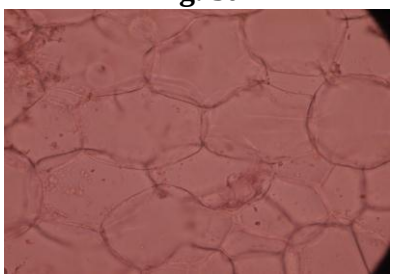

Fig. 3c

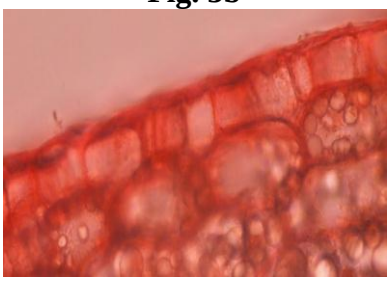

Fig. 3d

Fig. 3-3a: Parenchymatous cells embedded with Starch grains; 3b-Starch grains; 3C-Parenchymatous cells; 3D- Epidermal cell in surface view.

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. Kalihari is one of the most important plant of India and its different parts such as tuberous root, leaf, flowers and seeds are used to treat different types of human ailments and diseases. Due to its wide therapeutic importance, it is worthwhile to standardize it for use as drug.

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Conflict of interest

Authors declare no conflict of interest.

REFERENCES

1. Chopra R. N. and Chopra I. C., A review of work on Indian medicinal plants, ICMR, new Delhi, 1955.
2. Senthilkumar, M., Phytochemical screening and antibacterial activity *of Gloriosa superba* Linn. *International Journal of Pharmacognosy and Phytochemical Research*, 2013; 5(1): 31-36.
3. Maroyi A., Van Der Maesen LJG., *Gloriosa superba* L. (family colchicaceae): Remedy or poison? *Journal of Medicinal Plants Research*, 2011; 5(26): 6112-6121.
4. Kavina J., Gopi R., Panneerselvam R., *Gloriosa superba* Linn- A medicinally important plant, *Drug Invention Today*, 2011; 3(6): 69-71.
5. 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.
6. 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.
7. 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.
8. Anandhi S, Rajamani K., Studies on seed germination and growth in *Gloriosa superba* L., *Global J Res. Med. Plants & Indigen. Med.*, 2012; 1(10): 524–528
9. Chitra R, Rajamani K. Assessment of different glory lily (*Gloriosa superba* L.) accessions for biochemical tests. *Res J Med Plant*, 2010; 4: 21–5.
10. Rajagopalan, A. and Md. Abdul Khader, J.B.M. 1994. Investigation on certain aspects of growth, development, crop production and quality in glory lily (*Gloriosa superba* L.). Ph.D. (Hort) Thesis, Tamil Nadu Agricultural University, Coimbatore.
11. Farooqi, A.A., Kumaraswamy, B.K., Bojappa, K.N., Pusalkar, V.R. and Gupta, R., Plantations of the clinically important *Gloriosa superba*. Indian Horticulture, 1993; 37(4): 26–29.
12. Ghani, A. 1998. Medicinal Plants of Bangladesh (Chemical Constituents and Uses). Asiatic Society of Bangladesh, Dhaka. 25. Gooneratne, B.W.M. 1966. Massive
13. Duke, J.A., Handbook of phytochemical constituents GRAS herbs and other economic plants. CRC Press, BocaRaton, Florida, United States, 1992; 654.

14. Quality standards of Indian Medicinal Plant (published by Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi. 15th edn., 2017; 198-208.
15. Evans WC, Trease and Evans Pharmacognosy, 15th ed., (Saunders, London, 2003; 545-547.
16. Kokate CK, Practical Pharmacognosy Is ted., Vallabh Prakashan, New Delhi, 2006.
17. Mukherjee PK Quality Control of Herbal Drugs- an Approach to Evaluation ofBotanicals, 1st edn, Business Horizons, New Delhi, 2002; 695.
18. 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.
19. Mitra R and Mehrotra S, Pharmacognostical studies on Priyal: fruits and seeds of *Buchanania lanzan* Spreng. (Family: Anacardiaceae) *Bull Bot Surv India*, 1980; 22: 68-76.
20. Tripathi M and Sikarwar R L S Pharmacognostic standardization of stem bark of *Erythrina variegate* L. *Journal of the Indian Botanical Society*, 2014; 93(3&4): 248-253.
21. 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*, 2015; 7(3): 519-526.
22. Choudhary N, Siddiqui MB and Khatoon S, Pharmacognostic evaluation of Tinospora cordifolia Willd.) Miers and identification of biomarkers, *Indian J Tradit Knowle*, 2014; 13(3): 543-540.
23. Tripathi M and Sikarwar R L S Pharmacognostic Studies on Plaksa (Ficus virens Ait.) Stem Bark *Indian Journal of Natural Products and Resources*, 2015; 6(1): 27-32.
24. Tiwari A, Tripathi M and Dwivedi N, Standardization of Ayurvedic compound formulation Kutajastaka Kvatha Curna *J. Trop. Med. Plants*, 2015; *15:* 1-13.
25. Tripathi M, Sikarwar R L S, Tiwari A & Dwivedi N, Pharmacognostical identification of ingredients in Laghulai curna *Indian J Tradit Knowle*, 2015; 14(4): 531-536.
26. 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.