International Journal of Advanced Research and Review

www.ijarr.in

PHARMACOGNOSTIC STUDY AND QUANTIFICATION OF MARKER COMPOUND THROUGH HPTLC TECHNIQUE IN KUKRONDHA (*BLUMEA LACERA (BURM. F.)* DC.- ROOT

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ABSTRACT

Blumea lacera (Burm. f.) DC. Belongs to family Asteraceae is an annual herb and found in tropical and sub-tropical parts of India including Madhya Pradesh. *Blumea lacera* is described as a valuable medicinal plant in many popular systems of medicine such as Ayurveda, Homoeopathy and Unani. The plant is used as folk medicine for the treatment of various diseases *viz.* bronchitis, dysentery; wound healing, cough, cold. Fresh leaf extract is used for treating pneumonia, root used to cure mouth diseases. In the Konkan region of India, the plant is used to drive away fleas and other insects. On the basis of therapeutic characters a detailed study of Kokilasha different parts (root, stem and leaf) such as anatomical studies, physicochemical tests, preliminary phytochemical analysis, identification and quantification Cineol and Ferulic acid standard markers compounds through HPTLC fingerprints profile and detection of heavy metals, were carried out. The established parameters can be used as standards for identification and quality control of the plants in compound formulations and also preparation of a monograph of the *Blumea lacera* plant root.

Keywords: *Blumea lacera*, Cineol, Ferulic acid, Pharmacognosy, HPTLC fingerprints profile

INTRODUCTION

Blumea lacera family Asteraceae is commonly called as Kakaronda. It is annual or biennial erect, 25-110 cm tall and a camphoraceous smelling herb. *Blumea lacera* stem simple, hairy, tall, corymbosely branched, branches sometimes ascending. Leaves sessile or petiolate, elliptic to oblong, 8-10 × 3.0-4.0 cm, lanate abaxially, base attenuate, margin doubly serrate, veins 4-8 pairs. Receptacle convex, glabrous. Marginal florets 2-5-lobed, limb glandular. Central florets yellowish, 6-7 mm, with 5 triangular lobes, lobes with sessile glands and multicellular hairs. It is growing wildly in wastelands, roadside areas and also found in drying ponds along drains and river margins. It is described in Ayurveda as bitter, astringent, acrid, anti-inflammatory, styptic, opthalmic, digestive, antihelminthic, liver tonic, expectorant, febrifuge, antipyretic, diuretic and stimulant and antioxidant (Sharma PV, 1984; Jain & Tarafder 1970 and Patil & Patil 2005). *Blumea lacera* cures multifarious ailments of body under different systems of healing including Ayurveda, Homoeopathy, Unani, Siddha, and Allopathy. In Ayurveda it is used for curing Jalodar, Sandhivaat, Sotha, Atisaar, Raktagranthi, and for Vranaropan. In

Unani system it's essential oil is used as Muqawwi-e-Aam. It is also well known for its Mudirr-e-Baul, Kasir-e-Riyah, and Zof-e-Meda. In Homoeopathy system it's mother tincture is widely used for curing bleeding piles and as febrifuge. In Siddha system of healing 'Narakkarandai' is used for curing thread worm infestation, and anthelmintic. In Allopathy it is more widely and frequently used for anti-inflammatory, diuretic, antidiabetic, anthelmintic, antipyretic, antimicrobial, anti-atherothrombosis, expectorant, and anxiolytic activities (Dravya Gun Vigyan 2011 & Gour, R.D., 1999).

MATERIAL AND METHODS

Collection, Authentication of plant and Preparation of herbarium

Fresh and fully grown plant of *Blumea lacera* was collected from Arogyadham campus, Deendayal Research Institute, Chitrakoot, Satna (M.P.) India, in the month of October, 2022. Plants materials authenticated by Dr. Rashmi Singh, professor & head, Government Autonomous Post Graduate, Collage, Satna (M.P.). Prepared the herbarium of *Blumea lacera* plant and deposited (voucher specimen No. Govt./PGC/418), in the unit of herbarium under department of botany, Government Autonomous Post Graduate, collage, Satna (M.P.). (Evans W.C., Trease, 2002)

Preparation of Sample

Fresh roots of *Blumea lacera* was used for Pharmacognostical studies (preparation of herbarium, macroscopic and Microscopic studies. While under shade dried roots of *Blumea lacera* was powdered and stored in airtight containers and used for physicochemical studies, phytochemical, HPTLC and heavy metals studies (Khandelwal K.R., 1998).

Macroscopic and Microscopic study

Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated. Fresh root section was cut by free hand sectioning and numerous sections examined microscopically. Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 21*l* with Digi-eye camera using Caliper plus version 4.2 software (Gupta A.K.,2003 & Kokate C. K.,2006).

Powder microscopic study

About 2 g of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerine, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin. Treat a few mg with iodine solution and mount in glycerine, about 1 g of powder warmed over water bath with Chloral hydrate solution till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40X x 10X magnification of the Trinocular Research Microscope (Sholapur & Patil 2013).

Physico-chemical parameters

Physico-chemical tests were performed and set up the certain standards for *Blumea lacera* root in order to avoid the batch-to batch variation and also to check their adulteration and quality. Physicochemical tests were includes moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value and acid insoluble extractive value (Anonymous, 2009 and Anonymous, 2007).

Heavy metals tests

Heavy metals are toxic and generally occur through earth in plants. Mainly four types of heavy metals harmful for us they are Pb, Cd, As and Hg. These heavy metals detected through Atomic Absorption Spectrophotometer as per standard method (Anonymous, 2007).

Preliminary phytochemical studies

Phytochemical tests for screening and identification of bioactive chemical constituents present in the *Blumea lacera* root, various tests were performed in different solvent system like that petroleum ether, benzene, chloroform, acetone, ethanol, methanol and water were used for the preliminary photochemical screening (Freudenberg & Weinger, 1962).

High Performance Thin Layer Chromatography (HPTLC)

For High-performance thin layer chromatography, about 5gm accurately weighed *Blumea lacera* root, stem and leaf powder with 100 ml of methanol (3 X 100) in a Soxhlet apparatus separately and extracted for 6 hours. Filtered and concentrated the extracts under a vacuum oven to get the residue. Dissolved 100 mg of samples extract residue in a 10ml (10mg/ml) volumetric flask and make up the volume with methanol to get the working test solution separately (Anonymous, 2015).

Preparation of Standard Solution- (Cineol, Ferulic acid and Stearic acid Standards)

For preparation of the standard marker working solutions, 10mg of Cineol, Ferullic acid and Stearic acid were dissolved in a 10 ml volumetric flask and made up the volume with methanol separately. Then transferred 1 ml of stock solution to a 10 ml volumetric flask and made up the volume with methanol separately (0.1mg/ml). From the solution, prepared standard solutions by transferring aliquots (0.1, 0.2, 0.3 and 0.4 ml) corresponding to (1, 2, 3 and 4 ug/ml) of stock solution to 10ml volumetric flasks and made up the volume in each case to 10 ml with methanol.

High performance thin layer chromatography (HPTLC) study of the methanolic extracts of *Blumea lacera* root, stem and leaf with Cineol, Ferulic acid, and Stearic acid standard marker spots applied in precoated TLC plate. Samples (root, stem and leaf) as well as standard markers (Cineol, Ferulic acid, and Stearic acid) were applied by spotting test solution 8 µl (each test solution root, stem and leaf) on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part. And apply 1.0, 2.0, 3.0, and 4.0, µl standard markers Cineol, Ferullic acid, and Stearic acid, 0.1, 0.2, 0.3, and 0.4 ml on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe and standard markers, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin the plate and 10 mm part (Venugopal. et.al.2015).

RESULT AND DISCUSSION

Macroscopic characters

Blumea lacera externally root colour is light brown, faintly yellowish in internally, taste slightly astringent, and disagreeable characteristics odour. Root is cylindrical, highly tortuous,

often attached with a woolly stout crown, measuring 1.5-3.5 cm in diameter, about 2-11cm in length, surface rough, finely longitudinally striated and wrinkled running spirally and getting anastomosing at places, transversely cracked and corrugated, rarely grooved, often exhibiting lateral root scars; fracture outer short, inner fibrous (Fig.1&2).

Fig. 1 -*Blumea lacera* plant

Fig. 2 -*Blumea lacera* root

Microscopic study

Diagrammatic Transverse Section of the root is irregular circular in outline and shows a wide central wood occupying almost 2/3rd area of the section, encircled by a narrow bark (Fig.3a).

Detailed Transverse Section (TS) of the root shows outer 2 to 5 rows of cork often getting obliterated at places followed by 2 to 5 rows of parenchymatous cortex, endodermis is distinct, underneath of this lies a narrow zone of pericycle characterized with a row of oil cells, phloem, wider parenchymatous zone, traversed with small groups of fibres lying towards the inner zone, few of them being in very large conical patches with their broad base embedded in the innermost region of the phloem and reaching almost upto the region of pericycle; cambium distinct, xylem consists of isolated or groups of xylem vessels (Fig.3b).

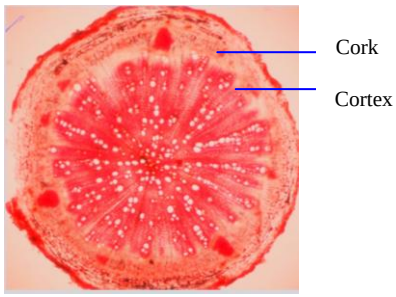
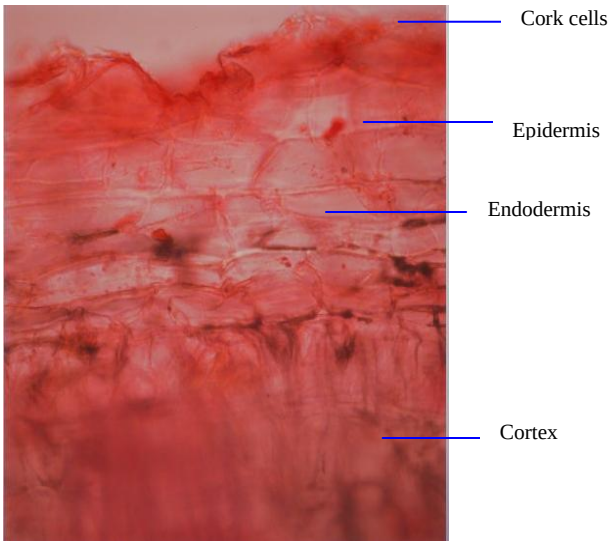


Fig. 3a- Diagrammatic TS of *Blumea lacera root*



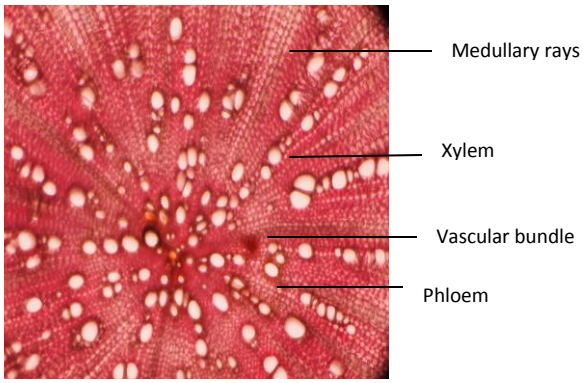


Fig. 3b- Detailed TS of *Blumea lacera root*

Powder microscopic characters

Blumea lacera root 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, thick walled cork cells in surface view, cork cells in sectional view. Fragments of ongitudinally cut reticulate vessels and prismatic crystals of calcium oxalate and fibrous projections (Fig.4a-4e.).

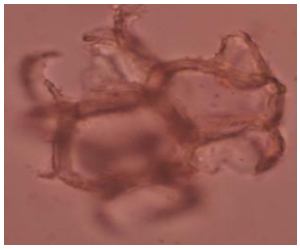


Fig.4a-Cork cells in surface view

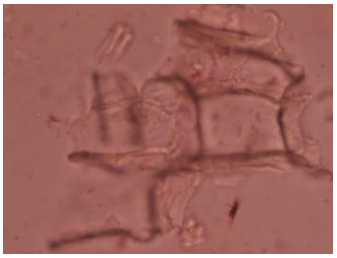


Fig.4b-Cork cells in sectional view

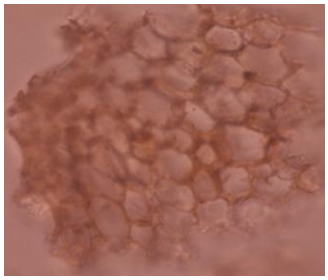


Fig.4c -Cortical parenchyma filled with starch grains and prismatic crystals of calcium oxalate

Fig.4d-Reticulate thickening

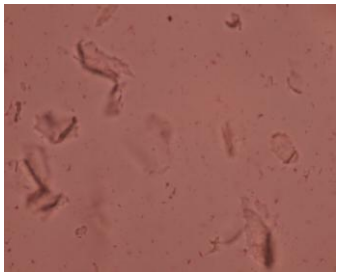


Fig.4e-Crystals and fibrous projections

Physico-chemical analysis

The physico-chemical tests such as Loss on drying on 105^0C, water such as extractive values, alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. The results are expressed as mean (n=3) ± standard deviation in w/w. *Blumea lacera* root 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. The loss on drying value obtained is an indicative of amount of moisture content could prevent bacteria, fungal or yeast growth.Water soluble extractive value is higher than the alcohol soluble extractive value. The extractive values, indicates the amount of active constituents in given amount of plant material when extracted with respective solvent and 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. total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of the ingredients. Ash value is useful in determining authenticity and purity of the drug and also these values are important quantitative standards.

Preliminary Phytochemical tests of *Blumea lacera*

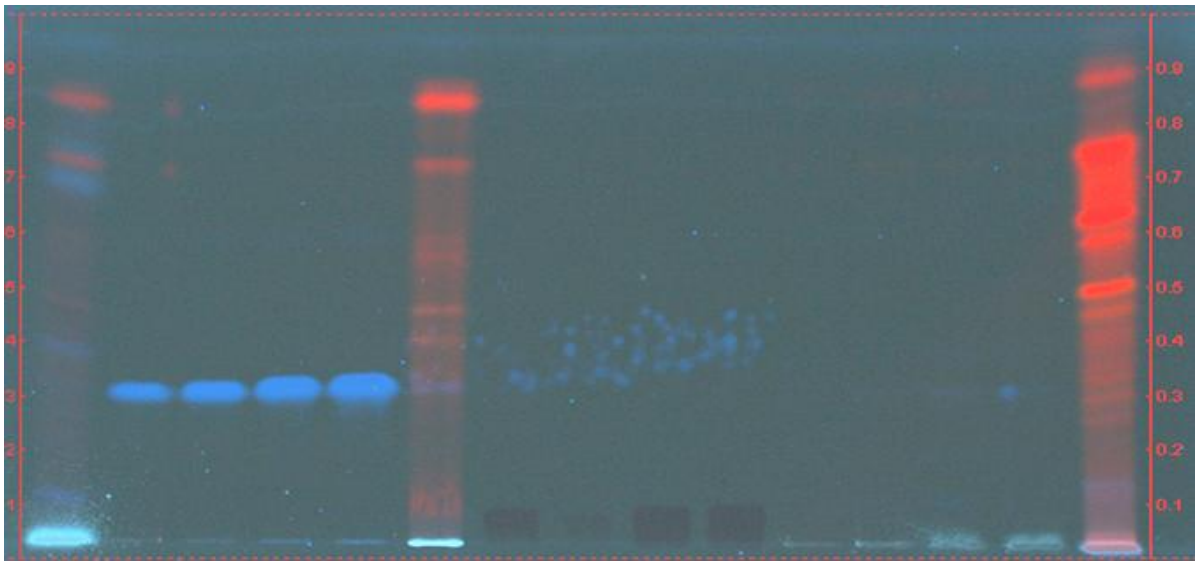
Preliminary phytochemical analysis was performed in petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water of *Blumea lacera* root, powder was carried out. It was observed that the phytochemical higher present in aqueous extract than the other extracts. The results indicated that the alkaloids and flavonoid were present in chloroform, acetone, methanol and water extracts of *Blumea lacera* root. Saponins were present only in methanol and water extracts of *Blumea lacera* root. Methanol and water extracts of *Blumea lacera* root contained carbohydrates. Acetone, methanol and water extracts of *Blumea lacera* root were found to contain tannins and phenolic compounds.

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the methanolic extracts of *Blumea lacera* root, stem and leaf with Cineol, Ferulic acid, and Stearic acid standard marker spots applied in precoated TLC plate. Samples (root, stem and leaf) as well as standard markers (Cineol, Ferulic acid, and Stearic acid) were applied by spotting test solution 8 µl (each test solution root, stem and leaf) on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part. And apply 1.0, 2.0, 3.0, and 4.0, µl standard markers Cineol, Ferullic acid, and Stearic acid, 0.1, 0.2, 0.3, and 0.4 ml on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe and standard markers, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin the plate and 10 mm part. The plate was developed using a mobile phase consisting of *toluene: ethyl acetate* (7:5v/v). Linear ascending development was carried out in a 20x20cm twin through glass chamber equilibrated with the mobile phase. The optimized chamber saturation time for the mobile phase (20 ml) was 30 min at room temperature. The length of the chromatogram run was 8.5 cm. Subsequent to the development, a thin layer of chromatography plate was dried at room temperature. The peak area for samples

and standards were recorded with the camera photo documentation system Camag Reprostar 3 and the plate was scanned densitometrically with the help of Scanner 4. Record the respective areas and prepare a calibration curve by plotting peak area *vs* concentration of standard markers Cineol, Ferullic acid, and Stearic acid. Major spots R_f values with colour were recorded after derivatization at 366nm. Major spots of R_f values after derivatization at 366nm major spots R_f values are 0.30 sky blue, *Blumea lacera* root and stem with Cineol standard marker, 0.30 sky blue *Blumea lacea* root and stem with Stearic acid, standard marker. It is observed that the Cineol is higher present in *Blumea lacera* root range from 0.42 to 0.46 percent than the stem range 0.12 to 0.14 percent. While Stearic acid was present higher in stem than the root, but Ferulic acid was absent in root, stem and leaf. The percentage of Ferullic acid ranges from 0.10 to 0.12 percent in the *Blumea lacera* stem analyzed, while 0.12 to 0.14 percent 0.12 to 0.14 percent **(Fig.5a, 5b &5c).**

Fig. 5a: HPTLC Fingerprint profile of test solution of *Blumea lacera* (Root, Stem & Leaf) at 366nm after derivatization



Abbreviation- Track 1: test solution of *Blumea lacera* root*;* **Track 2-5** Cineol standard; **Track 6:** test solution of *Blumea lacera* stem; **Track 7-10:** Ferulic acid standard; **Track11-14:** Stearic acid standard; **Track 15:** test solution of *Blumea lacera* leaf.

Fig.5b: Standard Peak of HPTLC Fingerprint profile of test solution of *Blumea lacera*

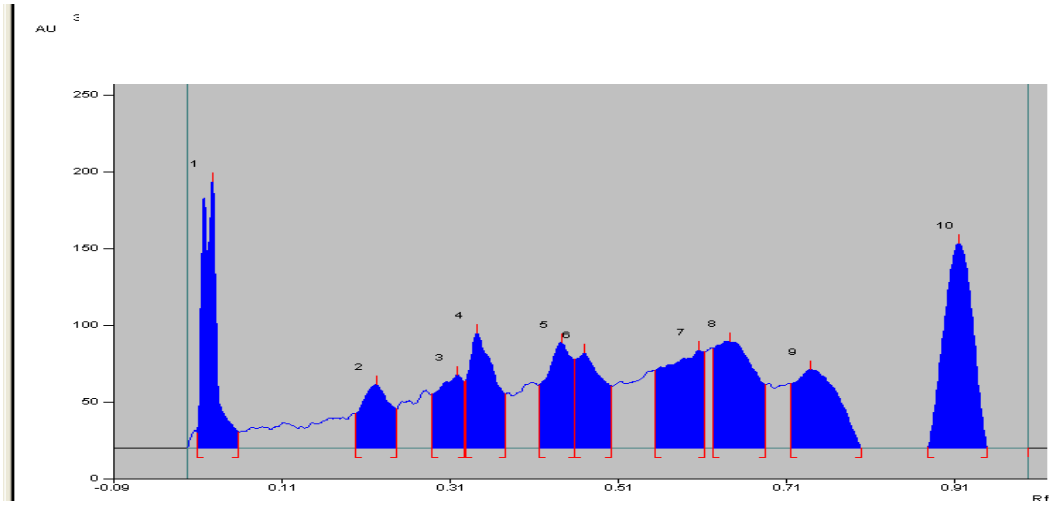
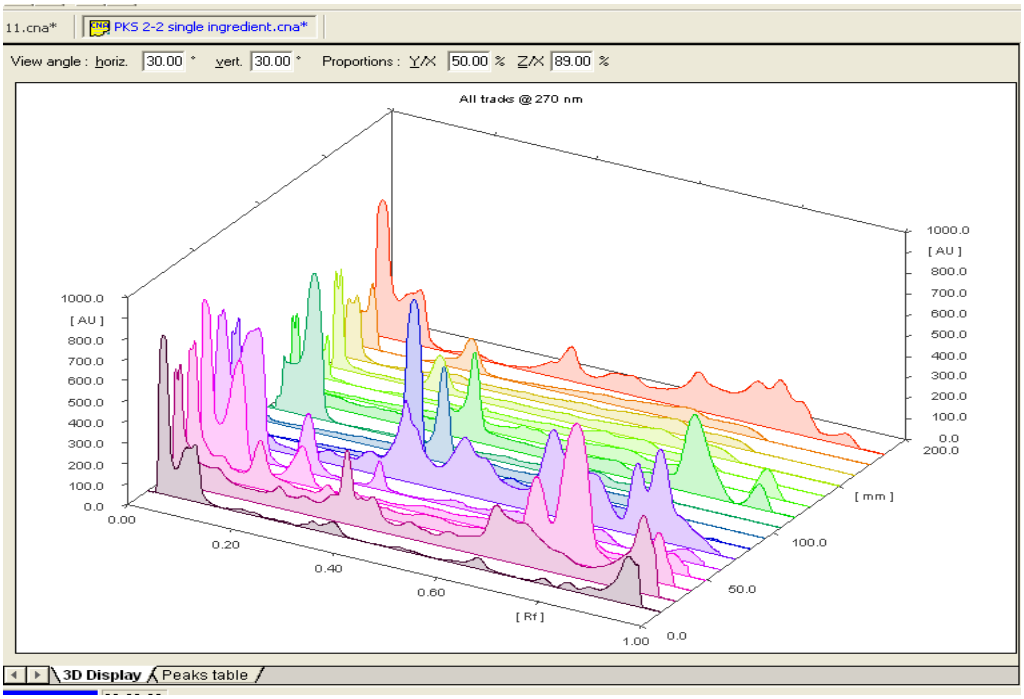


Fig.5c: Spectra of HPTLC Fingerprint profile of test solution of *Blumea lacera*



Heavy metals tests

Heavy metal elements (Pb, Cd, As and Hg) test were performed and found under limits as per guideline WHO and results are given in Table 1.

Table1-Heavy metals testing of *Blumea lacera root*

Metals name	Results	Actual conc. unit	API limits
Lead (Pb)	5.6732	ppm	10ppm
Cadmium (Cd)	0.0934	ppm	0.3ppm
Arsenic (As)	4.5634	ppb	03ppm
Mercury (Hg)	6.8912	ppb	01ppm

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

CONCLUSION

According to our findings, this plant may be beneficial for treating a variety of illnesses, including bronchitis, dysentery; wound healing, cough and cold. It is reasonable to assume that plants with ethnomedical significance have a substantial amount of therapeutic resources. Therefore, it is essential to characterize plant parts pharmacologically and phytochemically in order to develop, modernize, and ensure the quality of herbal compositions. Therefore the present investigation has been undertaken.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Shivesh Pratap Singh, Principal, Government Autonomous P G College, Satna (M.P.) India and Shri Abhay Mahajan, Organizing Secretary, Deendayal Research Institute, Chitrakoot, Satna (M.P.), India for providing infrastructure and necessary facilities during research work.

REFERENCES

- Sharma PV, Dravyaguna Vijayana, vol. II (vegetable drugs). Varanasi: Chaukhambha Bharati Academy, 1984; pp 794-796.
- Jain S.K. and Tarafder C.R., "Medicinal Plant lore of Santals", Econ Bot, 1970, 24, 241-278.
- Patil M.V., Patil D.A., "Ethnomedicinal practice of Nashik district, Maharashtra", Ind. J. Trad. Knowl., 2005, 4(3), 287-29
- Dravya Gun Vigyan Ki Pramukh Vanaushadhiyan. Haridwar, Uttar Pradesh: Shree Vedmata Gayatri Trust (TMD), 2011.
- Gour, R.D., 1999. Flora of the district Garhwal Srinagar, Uttarakhand, Transmedia publication.
- Evans W.C., Trease., "Text Book of Pharmacognosy", 15th ed.; ELBS London: 2002.
- Khandelwal K.R., "Practical Pharmacognosy", Nirali Prakashan, Pune, 1998, 146- 160.
- Gupta A.K., "Physical constant: Evaluation of crude drug" Quality Standard of Indian medicinal plant, Indian council of Medical Research, New Delhi, 2003, 1, 236-237.

- Kokate C. K., Practical Pharmacognosy Is ted., Vallabh Prakashan, New Delhi, 2006.
- Sholapur Hasan Pasha N and Patil Basanagouda M, Pharmacognostical and phytochemical investigations on the bark of *Moringa oleifera* Lam. *Indian J Nat Prod Resour* 4(1) (2013) 96-101.
- Anonymous, (2007). Quality standards of Indian Medicinal Plant, Published by Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi, 15^{th} ed p. 198-208.
- Anonymous, (2009). Laboratory guideline for the analysis of Ayurveda and Siddha formulations, published by Central Council for Research in Ayurveda and Siddha Deptt. Of AYUSH, Ministry of Health & Family Welfare Government of India, Janakpuri, New Delhi.
- Freudenberg K., Weinger K., "The chemistry of flavanoid compounds", Geissman A. editors, Pregamon Press, Oxford, 1962, 211
- Anonymous, (2015). Quality Standards of Indian Medicinal Plants, vol. 13, CSIR, New Delhi
- Venugopal G,; Srinivasa R C,; Rao N B,; Rao V B,; Rao V T, and Rao A P, (2015). 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), p. 519-526