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EVALUATION OF MACROSCOPICAL AND MICROSCOPICAL STUDY, PHYTOCHEMICAL ANALYSIS, TLC AND HPTLC FINGERPRINTING OF *BAUHINIA PURPUREA* LINN. LEAVES

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Keywords:

Macroscopy, Microscopy, TLC, HPTLC, Phytochemical analysis

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ABSTRACT: Objective- To rationalize the Macroscopical and Microscopical study, Phytochemical analysis, TLC and HPTLC fingerprinting of *Bauhinia purpurea* Linn. Leaves. **Methods-** The crude ethanolic extract of leaves of *Bauhinia purpurea* Linn. was using the macroscopical characters (size, shape, colour, odour, taste, surface, texture, venation, margin, base, and petiole) powder microscopical study, the powder was stained with phloroglucinol and concentrated HCl to study the lignified cells, lignified parenchyma, trichomes, fibres, xylem vessels, mesophyll, palisade cells and stomata, etc, Preliminary phytochemical tests (Glycosides, Saponins, Tannins, Flavonoids etc.) and Preliminary photochemical investigation of TLC and HPTLC. **Results:** An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of fresh as well as dried crude samples of leaves phytochemically, macroscopically and microscopically. TLC and HPTLC fingerprint profiling were carried out and the salient qualitative and quantitative parameters are reported. **Conclusion:** The present study will provide referential information for correct identification and help in checking adulteration in market sample used in the preparation of various herbal medicines. The present observation will also be helpful in Macroscopical and Microscopical study, Phytochemical analysis, TLC and HPTLC fingerprinting of *Bauhinia purpurea* Linn. Leaves.

INTRODUCTION: *Bauhinia purpurea* Linn. (Caesalpinaceae) is an ornamental plant found throughout subtropical, India, North and South America, Nepal, Australia, Africa and United Kingdom. The plant is commonly known as Mandarai in Tamil and khairwal in Hindi [1].

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The flavone glycoside, 5,6-dihydroxy-7-methoxy flavone 6-O-beta-Dxylopyranoside,was isolated from the chloroform-soluble fraction of the ethanolic extract of *Bauhinia purpurea* stems, and a new flavone glycoside, 6,4' dihydroxy 3- prenyl 3,5,7,5' tetra methoxy flavone-6-O-a-Lrhamnopyranoside, has been found in the seed of *bauhinia purpurea* [2, 3].

The leaf extracts of *B. purpurea* is widely used in traditional medicines for the treatment of ailments such as sores, wounds and Diarrhoea; the root is carminative; the stem bark is credited with astringent properties and is used in Diarrhoea; a

decoction of the bark is used in Diarrhoea; the flower buds are reported to have laxative and anthelmintic properties [4].

The various type of ethnomedicinal uses of *B.purpurea* are shown in (**Table 1**). It is also reported for its anti-diarrheal, anticancer and thyroid gland stimulating properties [5-8] Hence, country traders often subject it to adulteration / substitution.

FIG.1: LEAVES AND FLOWER OF *BAUHINIA PURPUREA*

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FIG. 2: AUTHENTICATION OF SPECIMEN RECORD CARD

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TABLE 1: ETHNOMEDICAL INFORMATION FOR *BAUHINIA PURPUREA* LINN

Plant part	Uses
Flowers	Laxatives
Roots	Carminative
Root bark	Mixed with curd and used in hemorrhoids. Its paste with dried ginger applied internally in the treatment of goiter.
Stem bark	Astringent in diarrhea
Bark	Decoction is used as a wash in ulcer
Flower buds	Eaten as a vegetable, laxative, anthelmintic , useful in piles and blood dysentery
Other	Dropsy, anasarca, pain, rheumatic thigh swelling, deer-epilepsy, convulsion, delirium febris, animal bite, datura intoxication and anti thyroid

MATERIALS AND METHODS:

Plant Material:

The plant material was collected from the local park (Narayan Bagh) of Jhansi in the month of November 2014. The plant was identified by local people of that park and authenticated by Dr. Gaurav Nigam (Asst. Professor) Department of Botany, Bundelkhand University, Jhansi (U.P.) India. A herbarium specimen of the plant (BU/Bot./Spe./Pha./11-2015/01) was preserved in the Department of Pharmacognosy of our Institute for further reference. The leaves were separated and dried under shade, pulverized by mechanical grinder, passed through 40 mesh sieve and stored in a closed vessel for further use.

Macroscopy:

The observed macroscopical characters of leaves of *B.purpurea* Linn were as follows:

- Size- 8-15 cm in diameter&10-20cm long
- Shape- Shallowly cordate
- Colour- Green
- Odour- Odourless
- Taste- Slightly bitter

- Apex- marginate
- Margin- Sinuate
- Base- Stipulate
- Venation- Parallel

Microscopy:
The powder was stained with phloroglucinol and concentrated HCl to study the lignified cells, lignified parenchyma, trichomes, fibres, xylem vessels, mesophyll, palisade cells and stomata, etc. The powder was also stained with N/50 iodine solution to detect the presence of starch. A small portion of powder was mounted in water to identify calcium oxalate crystals [9]. Quantitative microscopy was determined by methods prescribed by Trease and Evans [10].

The ash values, alcohol soluble and water soluble extractives values and Loss on drying of leaves were determined as per the Indian Pharmacopoeia methods [11]. The crude fiber content was done by Dutch process. The behaviour of the powdered leaves with different chemical reagents was studied [12]. The fluorescence characters of the various extracts and powdered leaf with different chemical reagents were observed under day light and UV light (254nm & 366nm), by following procedure reported by Kokoshi et al.

Measurements of the cells/ tissues were made with the help of micrometer under a compound microscope. Other extractive values were determined successively starting from petroleum ether (60-80°), chloroform, acetone, ethylacetate, methanol and distilled water by using soxhlet extraction apparatus [13]. For this purpose the powder (100g) was successively hot extracted with 300ml of above solvents for 72 h. Before switching over to the next solvent, the powder under extraction (marc) was dried to remove the traces of earlier solvent. The dried extractives were obtained after evaporation of solvent under reduced pressure.

The angle of repose of powder was determined by the funnel method. The accurately weighed powder was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powders. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation,

$$\mathbf{Tan\ q = h/r}$$

Where h and r are the height and radius of the powder cone [14].

For the determination of bulk density, 2 g of powder, previously lightly shaken to break any agglomerates formed, was introduced into a 10 ml, measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 seconds intervals. The tapping was continued until no further change in volume was noted. Bulk density was calculated for dried powdered drug using following formula[15]:

Bulk Density = Mass of Powder/ Bulk volume.

Histological studies:
The transverse section of the leaf of *B.purpurea* Linn showed the following characters:

Lamina:
Isobilateral nature; upper epidermis showed polygonal shaped cell, arranged in single layer; mesophyll showed upper palisade cell, single layered, elongated and compactly arranged; spongy parenchyma were thin walled, loosely arranged and embedded with xylem vessels; thin walled compactly arranged lower palisade; lower epidermis as similar to upper epidermis; conical and unicellular trichomes covered with thick wall (**Fig.3**).

FIG. 3: GROSS HISTOLOGICAL SECTION OF *BAUHINIA PURPUREA*

Midrib:

Upper epidermis was multilayered; showed upper palisade cells, arranged in single layer, elongated and compact; spongy parenchyma were thin walled, loosely arranged and embedded with xylem vessels; endodermal layer showed single layered cell surrounding the vascular bundles; lower epidermis as similar to upper epidermis; conical and unicellular trichomes (**Fig.4**) covered with thick wall.

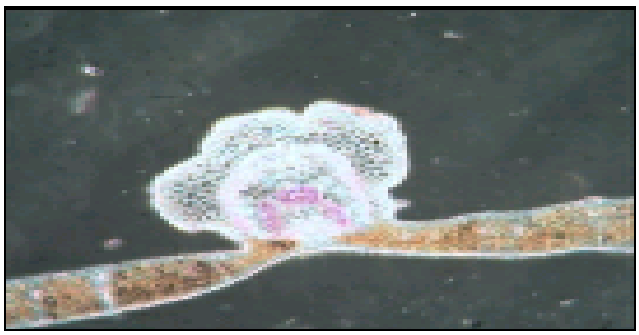

FIG. 4: UNICELLULAR TRICHOMES

Phytochemical investigation:

The various qualitative chemical tests (**Table 2**) have shown the presence of phytosterols, flavonoids, fixed oils, phenolic, tannins, glycosides and saponins in huge amount; whereas, alkaloids, aromatic acids, carbohydrate, proteins and aminoacid, triterpenoids, gums, mucilage and volatile oils were totally absent in the leaf extract of this plant.

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF *BAUHINIA PURPUREA* LEAVES

S.no	Plant constituents	Petroleum ether	Ethanolic extract
1	Alkaloids	-	-
2	Carbohydrates	-	-
3	Glycosides	-	+
4	Saponins	-	+
5	Phenolic compound And tannins	+	+
6	Flavonoids	+	+
7	Phytosterols	+	+

Qualitative and Quantittive evaluation parameters:

The calculated Quantitative values and physical parameters of the leave of *B. purpurea* are showed in **Table 3** and **Table 4**. The fluorescence character of powdered leaf with different chemicals reagents are shown in **Table 5**.

TABLE 3: RESULT OF QUANTITATIVE MICROSCOPY OF *B. PURPUREA* LEAVES

Sr.no	Parameters	Results
1	Stomatal number (upper surface) (lower surface)	71.5 28.5
2	Stomatal index (upper surface) (lower surface)	17.26 8.72
3	Vein- islet number	83
4	Vein termination number	72
5	Palisade ratio	1:104

TABLE 4: PHYSICAL PARAMETER OF LEAVE OF *B. PURPUREA*

Sr.no	Parameters	Results(%)
1	Total Ash value	7.09
2	Acid inbsoluble value	2.67
3	Moisture content	12
4	Extractive value (water soluble) (alcohol soluble)	25.5 34.52
5	Loss on drying	12.87

TABLE 5: BEHAVIUOR OF POWDERED LEAVES ON TREATMENT WITH DIFFERENT CHEMICAL REAGENTS.

Reagents	Colour developed in day light
Powder as such	Green
1N NaOH	Greenish brown
Picric acid	Yellowish green
Glacial acetic acid	Yellowish green
1N Hcl	Pale yellowish green
1N HNO_3	Pale yellowish green
5% Iodine	Yellowish green
40% NaOH +few drops of 10% lead acetate	Yellowish green
HNO_3+ Ammonia solution	Yellow
Con H_2SO_4	Brown
5% $Fecl_3$	Reddish brown
10% sodium hydroxide +copper sulphate	Green
Acetic acid +Con H_2SO_4	Green
Acetic acid +Ferric chloride +Con H_2SO_4	Dark blackish brown
Antimony tri chloride	Green
Ammonia solution	Pale green

Thin Layer Chromatography (TLC):

"Their relative polarities which related to the type and number of functional groups present on a molecule capable of hydrogen bonding"

$$R_f = \frac{\text{(Distance travelled by solute front from origin line)}}{\text{(Distance travelled by solvent front from origin line)}}$$

Where R_f =Retention factor

The ethanolic extract of leaves of *Bauhinia purpurea* Linn was subjected to thin layer chromatography studies, to find the presence of number of compounds which support by the chemical test.

R_f value and colour of TLC spots, in solvent system of Chloroform: Xylene: Formic Acid (8.5: 1.5: few drops). These TLC spots with R_f value and colour are in **Table 6**, and TLC plate in **Fig. 5** is given below.

TABLE 6: TLC OF ETHANOLIC EXTRACT LEAVES OF *BAUHINIA PURPUREA* LINN.

Extract	Solvent System	No. of Spots	Colour of Spots	Rf value
Ethanolic Extract	Chloroform: Xylene: Formic acid (8.5:1.5:few drop)	6	Brown	0.47
			Green	0.57
			Dark	0.65
			Yellow	0.71
			Violet	0.80
			Brown	0.85
			Yellow	

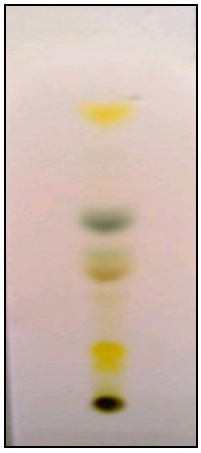

FIG.5: CHROMATOGRAM OF TLC OF ETHANOLIC EXTRACT OF LEAVES OF *BAUHINIA PURPUREA* LINN.

HPTLC Profile (High Performance Thin Layer Chromatography):

Ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system Chloroform: Xylene: Formic Acid (8.5:1.5: few drops) was ideal and R_f values of HPTLC Fingerprint (**Table 7**) and **Fig. 7** HPTLC finger printing of ethanolic extract on leaves of *B.purpurea* given the spots of the chromatogram were visualized at 254nm and 366nm.

TABLE 7: R_F VALUES OF HPTLC FINGERPRINT PROFILE OF *BAUHINIA PURPUREA*

R_f Value	Before Derivatization			After Derivatization	
	At 254 nm	At 366 nm	At white R	At 366 nm	At white R
R_f 1	0.12 (Black)	0.07 (Pink)	0.08 (brown)	0.10 (White)	0.11 (Brown)
R_f 2	-	0.10 (Pink)	0.13 (brown)	0.13 (Pink)	0.17 (Brown)
R_f 3	-	0.13 (Pink)	0.15 (Green)	0.16 (Pink)	0.22 (Brown)
R_f 4	-	0.15 (Pink)	0.19 (Brown)	0.23 (Pink)	-
R_f 5	-	0.17 (Orange)	0.25 (Brown)	0.29 (Pink)	-
R_f 6	-	0.21 (Orange)	-	0.30 (Faint green)	-
R_f 7	-	0.23 (Orange)	-	0.35 (Sky blue)	-
R_f 8	-	0.29 (Orange)	-	0.41 (Light blue)	-
R_f 9	-	0.32 (Faint green)	-	-	-

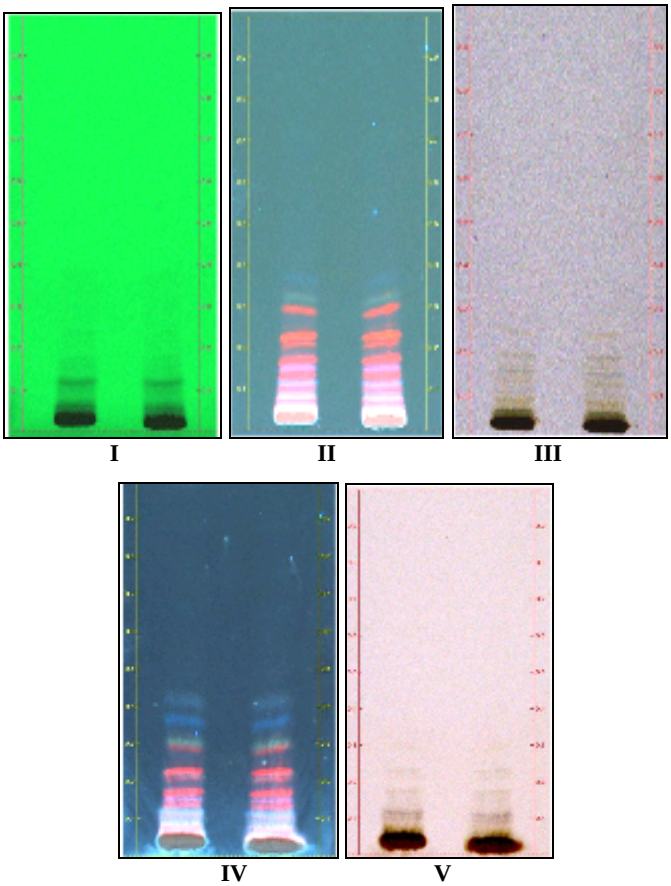

FIG. 7: HPTLC REPORT OF ETHANOLIC EXTRACT OF LEAVES OF *BAUHINIA PURPUREA* LINN.

Where **I** = 254nm (before derivatization); **II** = 366nm (before derivatization); **III** =Visible light (before derivatization); **IV** =366nm (after derivatization); **V**=Visible light (after derivatization)

RESULT: As a part of standardization study, the macroscopically examination of drug was studied. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicated the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent.

DISCUSSION: Macroscopic as well as microscopical studies of any phytodrug are the primary steps to establish its botanical quality control before going to other studies. The above mentioned parameters are helpful for the future identification and authentification of the plant in the herbal industry and in factories. The physico-chemical standards, such as ash values, extractive values, crude fiber content and fluorescence analysis, will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in future.

The leaf constants can be included as microscopical standards in Indian herbal pharmacopoeia. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may serve as a characteristic fingerprint for qualitative evaluation of fruits.

CONFLICT OF INTEREST STATEMENT: We declare that we have no conflict of interest.

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