

# Pharmacognostic and Phytochemical Evaluation of Leaf of *Jatropha nana* var. *bengalense* C.H. Rahaman and S. Mondal: An Endemic Member of Euphorbiaceae

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## ABSTRACT

**Background:** Pharmacognostic and phytochemical studies helps to maintain the quality of herbal products and purity of crude drugs by means of standardized parameters which will lead to developing the safest and efficacious natural products. **Objectives:** This study was undertaken to carry out pharmacognostic and phytochemical investigation of the leaves of the *Jatropha nana* var. *bengalense* (Euphorbiaceae), an ethnomedicinal plant. **Materials and Methods:** Epidermal Micromorphology, physical and physicochemical constants like ash value determination and UV-fluorescence study have been employed for pharmacognostic evaluation. For qualitative phytochemistry, microchemical color reaction test and TLC studies performed. Variations in different biochemical parameters of leaves of different developmental stages have been examined and the obtained data statistically analyzed following standard methods. **Results:** Typical paracytic type of stomata is found in hypostomatic condition distributed in irregular-shaped epidermal cells. Index of these stomata was found 20.251%. Phytochemical analysis in qualitative and quantitative approaches has also been done to describe its chemical variability. Ash value was found above 8% which shows more than 50% solubility in acid whereas water and ethanol solubility is found to be just below and above the 30% respectively. In UV- fluorescence study very distinctive colour changes of the powdered leaf have been recorded. Alcoholic extracts have shown positive results for presence of most of the phytochemical groups. Quantitative phytochemical analysis confirms that a range of phytochemicals are present in good amount in the leaves of this medicinal plant which could vary in amount in different developmental states. Thin Layer Chromatography has been carried out on ethanolic extract in two different solvent systems which show 6 different Rf values further indicates the presence of important phytochemical derivatives. **Conclusion:** This study will finally be needful for standardization of identification of the genuine drug, its adulterants and of the chemical constituents present in the leaves and finally bioprospection. **Key words:** Adulteration, Bioprospection, Euphorbiaceae, *Jatropha nana* var. *bengalense*, Pharmacognosy.

## INTRODUCTION

Natural products blessed human life by providing food and medicines from time immemorial. For last couple of centuries there have been stormy interests on plants of medicinal properties in developing countries since plant-based therapies have been reported safe and having negligible side effects when compared to synthetic drugs. However, of the estimated 350,000 plant species worldwide only a small percentage has been investigated phytochemically and even smaller percentage of it has been properly studied in terms of their pharmacological properties.<sup>[1]</sup> There are reports on use of 119 chemical compounds by pharmaceutical companies that derived from or modelled after naturally occurring lead molecules,<sup>[2]</sup> and 74% of these come from ethnomedicinal plants.<sup>[3]</sup> The neutraceutical

and healing properties of plants lie in some biochemical compounds present in their different parts such as leaves, stems, roots, flowers, etc. The amount and concentration of biomolecules, their effectiveness depends on such type of plant parts, their developmental stages and ages and collection time in different seasons, etc. Some of the Indian medicinal plants have been pharmacologically and phytochemically characterized including some lesser known ethnomedicinal plants which hold various phytotherapeutic and nutritional properties. The *Jatropha* L. (Euphorbiaceae) is a very diverse subtropical and tropical genus comprised of about 186 species which are mainly distributed in Tropical America, Africa and Asia.

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<sup>[4]</sup> In various earlier works it has already been proved that, the family Euphorbiaceae including *Jatropha* species are good candidates for antimicrobial, insecticidal, antioxidant, larvicidal activities and biofuel production and are now widely being explored in terms of their bioprospection and drug discovery.<sup>[5-10]</sup> All parts of *Jatropha* plant have several uses in traditional ethnomedicines to cure various human and veterinary diseases.<sup>[9]</sup> In respect of abundance of phytochemical contents, *Jatropha* is one of the richest sources of phytochemicals like alkaloids, lignins, cyclic peptides, diterpenoids, triterpenes and other diverse bioactive compounds,<sup>[10,11]</sup> and can be employed in nutritional, agricultural and pharmaceutical industries.<sup>[12]</sup> Till date, seven species of Indian *Jatropha*, viz., *J. curcus*, *J. gossypifolia*, *J. integerima*, *J. podagrica*, *J. multifida*, *J. glandulifera*, *J. nana* var. *nana* have been pharmacologically and pharmaceutically validated earlier, however, no work has earlier been done on *Jatropha nana* var. *bengalense* Rahaman and Mondal, a rare ethnomedicinal plant.<sup>[13,14]</sup> In this paper we are first time reporting the biochemical profile of leaves of this rare and endemic member of Euphorbiaceae. The study would be helpful in crude drug authentication and bioprospection of the same in future.

## MATERIALS AND METHODS

### Collection of plant material and identification

*Jatropha nana* var. *bengalense* was collected from Chorchor forest of Birbhum district of West Bengal during full bloomed season up to its senescence period (May to September in 2020). Detailed morphological study of the collected plant species was carried out and identification was confirmed with the help of authentic literature.<sup>[4,13]</sup> Voucher specimens are deposited in the Herbarium section of M.U.C. Women's College, Burdwan for future references. For experimental purpose, fresh young, mature and senescent leaves were collected (Plate 1, Plate 2) time to time from the field throughout the season and kept separately for further study.

### Botanical characters

*Jatropha nana* var. *bengalense* (Euphorbiaceae) is a rare medicinal plant and endemic to the dry deciduous Sal (*Shorea robusta*) forests of West Bengal<sup>[13]</sup> and Jharkhand.<sup>[14]</sup> Local Santal tribal people known it as 'Bireradam' (In West Bengal) or as 'Birpinde' (in Jharkhand).

A glabrous undershrub reaching up to 55 cm high. Roots are large, tuberous, irregularly armed-shaped and fleshy with watery juice, 5–35 × 1–11 cm. Leaves alternate, ovate or obtriangular, entire or 3-lobed up to below the middle or up to above the middle, 12–17.5 × 13–17 cm; central lobe the largest, with 3 distinct nerves; petiole or rarely sessile. Stipules paired; each with 2–8 linear-filiform branches of different lengths, 5–10 mm × c. 1 mm. Inflorescences in terminal, paniculate cymes; flowers small, green, unisexual, hypogynous, bracteate, pedicellate. Male flowers: 6–9 × 2.5–3 mm. Female flowers: c. 10 × 3 mm; calyx 4–5 × c. 3.5–4.5 mm, glabrous, lobes 5, lanceolate, subacute. Fruit



**Plate 1:** Habitat of *J. nana* var. *bengalense*

**Plate 2:** Leaves of 3 different growth stages.

capsule, subglobose, 3-lobed, slightly wrinkled. Seeds 3, triangular-ovoid; testa reticulately ribbed, with prominent brownish caruncle.

Flowering and fruiting time is May to September.

Mode of propagation is followed by seeds.

### Ethnomedicinal uses

Santal tribal people of the Chorchor forest and Garh-jungle forest areas of West Bengal use the whole plant to combat the malnutrition and poor lactation of their domestic animals like cows, goats and buffalos. Tribal medicine men of Jharkhand use this plant in the treatment of menstrual problems, malnutrition,<sup>[14]</sup> and muscular pain.

### Foliar epidermal micromorphology

Leaf sample of the plant (apex, middle and base of the lamina) were cleared following the Bokhari's method<sup>[15]</sup> to study the foliar epidermal characters like epidermal cells, stomata, and crystals. Then the cleared leaf samples were mounted in slides with 10% glycerine and 1% aqueous safranin solution. Micromorphological characters were observed under compound light microscope [Olympus microscope, Model: CH-20i fitted with camera] and suitable photographs were taken. Measurements of cells were recorded with standardized ocular micrometer.

### Physicochemical analysis

Collected leaves of the plant were washed with water, cut into small pieces and dried under shade. The dried leaf samples were mechanically grounded into by using blender, sieved and stored in an air-tight container. Physical constants like ash value determination<sup>[16,17]</sup> and UV-fluorescence nature<sup>[18]</sup> of the powder were studied following the standard methods.

Ash value: The residue left after incineration of the crude drug is designated as ash which usually represents the inorganic salts present in the crude drug.

Determination of total ash value: Fine powdered drug (5g.) was taken in a tarred silica crucible and incinerated at 650°C in the muffle furnace for 6 hr. It makes the powder free from moisture and carbon. Ash was cooled and weighed, and the percentage of total ash was calculated by the following formula:

$$\text{Total ash value(\%)} = \frac{\text{Weights of the ash}}{\text{Weight of the dried plant drug taken}} \times 100$$

Determination of acid-insoluble ash value: A fixed amount of ash was mixed with 30 ml of 2N HCL and boiled for 5 min. This ash solution was then separated using Whatman 41 filter paper. The insoluble matter was collected from filter paper, completely dried and weighed. Finally the percentage of acid insoluble ash with reference to the air-dried drug as calculated.

Determination of water-soluble ash value: A fixed amount of ash was mixed with 30 ml of water for 5 min with frequent shaking. Using a Whatman 41 filter paper the insoluble matter was separated out. The matter was ignited for 15 min until loss of all moisture of it and a final weight was taken. The percentage of water soluble ash was calculated with reference to the air-dried drug.

### Fluorescence analysis

Here in this study, different chemical reagents were mixed with the powdered drug and observed distinctive colour changes under UV-light (365nm). Very distinct colour changes were recorded and compared those with the colours of powdered drug as seen under visible light.

## Phytochemical screening

Qualitative phytochemical test (Microchemical colour reaction tests) of different solvent extracts of leaf powder:

Leaves of the collected plant were air dried and grounded to make fine powder. 5 g. of this leaf powder was successively extracted by cold maceration technique in 60 ml of each of the solvents like methanol, ethanol, and distilled water. Then the preliminary qualitative microchemical tests of the leaf extracts were performed using various reagents for detection of different phytochemical groups following standard methods.<sup>[19-22]</sup>

## Quantitative analysis for evaluation of phytochemicals

The variability of biochemical and nutritional quality of three kinds of leaves of *J. nana* var. *bengalense* (young, mature and senesced leaves collected time to time in different months over a year) were estimated by subjecting the fresh undamaged leaves to various biochemical analysis, such as total carbohydrates,<sup>[23]</sup> total proteins,<sup>[24]</sup> total lipids,<sup>[25]</sup> total nitrogen,<sup>[26]</sup> total amino acids,<sup>[27]</sup> total phenols,<sup>[28]</sup> total flavonoids,<sup>[29]</sup> and moisture,<sup>[30]</sup> Determination of each biochemical analysis was repeated for three times. To test the significance level among different phytochemical constituents of the three different kinds of leaves of different developmental stages, Tukey Test (HSD) along with *F* values of one-way ANOVA statistics were performed.<sup>[31,32]</sup>

## Thin Layer Chromatography

Fresh, mature leaves of the plant (50g.) were harvested randomly, dried under shade, and grounded to make it a fine powder. Dried leaf powder was extracted in ethanol at room temperature for 24 hr in a closed 250 ml conical flask. The ethanolic extract was then filtered and evaporated in a rotary evaporator (<37°C) to dryness. The dried extract was dissolved in 2ml ethanol and subjected to thin layer chromatography (TLC) in different solvent systems like ethanol: hexane (9:1) and ethyl acetate: ethanol(9:1) and repeated three times in each solvent system by using preparative TLC plate pre coated with Silica gel G. Finally, the *R<sub>f</sub>* values were determined by following the formula –

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

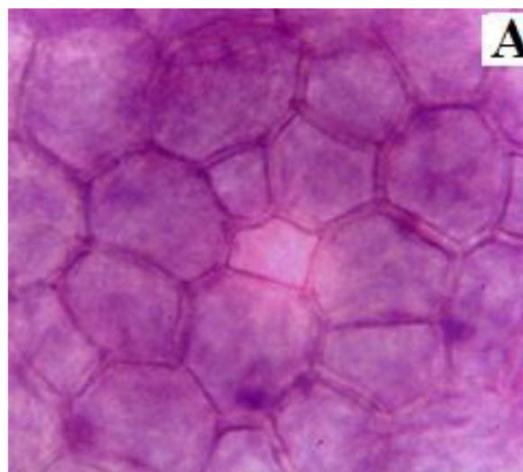
## RESULTS

### Micromorphology

Epidermis: Cells are irregular in shape. Cell walls are wavy in upper epidermis. Lower epidermis shows presence cells with straight to wavy cell wall outline. Cell size on upper epidermal surface is 50.955  $\mu\text{m} \times 36.152 \mu\text{m}$  and on lower surface, the size is 34.469  $\mu\text{m} \times 21.161\mu\text{m}$ . Frequency of the epidermal cell is 738.995/mm<sup>2</sup> on the upper surface

**Table 1: Foliar epidermal cell characters of the plant species.**

Leaf surface	Cell shape	Cell length ( $\mu\text{m}$ )	Cell width ( $\mu\text{m}$ )	Frequency of epidermal cell (No./mm <sup>2</sup> )	Cell wall out line	Palisade ratio
Upper	Irregular	50.955 $\pm$ 1.05	36.152 $\pm$ 1.5	738.995 $\pm$ 1.66	Wavy	21.335
Lower	Irregular	34.469 $\pm$ 0.87	21.161 $\pm$ 2	971.638 $\pm$ 1.79	Straight to wavy	

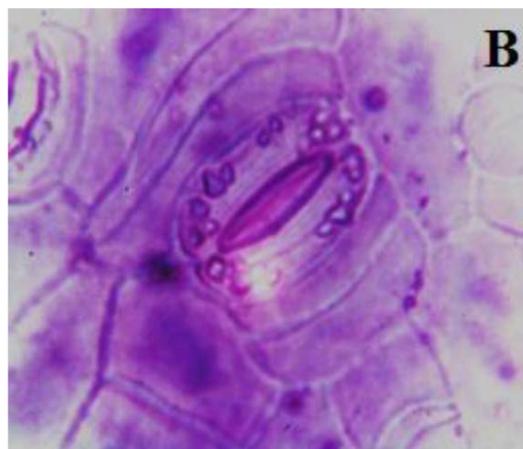


**Figure A:** Upper epidermis.

**Table 2: Stomatal features of the plant species.**

Leaf surface	Stomatal type	Stomatal length ( $\mu\text{m}$ )	Stomatal width ( $\mu\text{m}$ )	Stomatal index (%)	Stomatal frequency (No./ mm <sup>2</sup> )
Upper	-	-	-	-	-
Lower	Paracytic	36.168 $\pm$ 2.75	24.932 $\pm$ 1.59	20.251	248.263 $\pm$ 3

\*Data presented in the tables are averages of 25 observations



**Figure B:** Lower epidermis with paracytic stomata.

and it is 971.638 /mm<sup>2</sup> on the lower surface, respectively. Palisade ratio is 21.335 (Table 1; Figure A).

Stomatal complex: Leaves are hypostomatic. Only paracytic type of stomata is found. Stomatal apparatus flanked by 2–4 subsidiary cells. Stomatal size is 36.168  $\mu\text{m} \times 24.932\mu\text{m}$ . Frequency of the Stomata is 248.263 /mm<sup>2</sup>. Stomatal index is 20.251% (Table 2; Figure B).

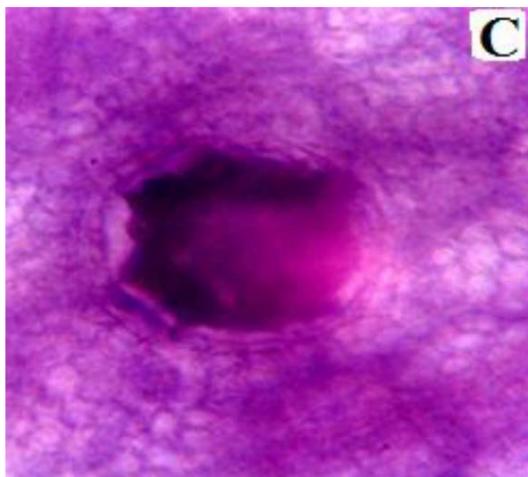
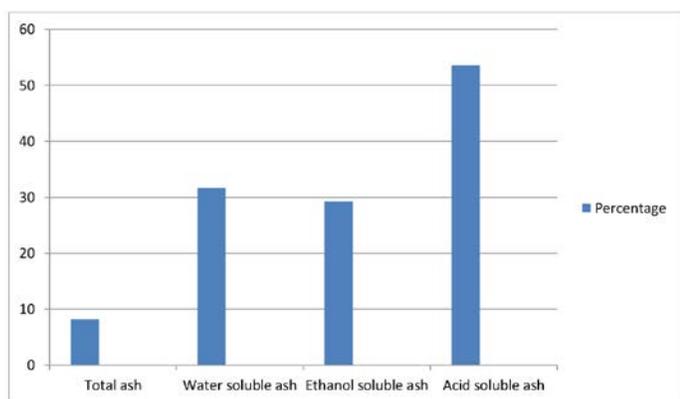
Crystals: Crystals (sphaeraphide) are present in both upper and lower epidermis of leaf. The diameter of crystals of the upper epidermis is 33 $\mu\text{m}$  and it is 28 $\mu\text{m}$  in lower epidermis (Table 3; Figure C).

### Physical constant

The total ash value of the powdered leaf drug was 8.2% w/w, acid insoluble ash value was 53.65% w/w, solubility in ethanol was 29.26 % w/w and water-soluble ash value was 31.7 %w/w (Figure 1).

**Table 3: Crystal character of the plant species.**

Leaf surface	Type	Diameter ( $\mu\text{m}$ )	Dissolved in acid
Upper	Calcium Oxalate (Sphaeraphide)	33.33 $\mu\text{m}$	Dil. HCl
Lower	Calcium Oxalate (Sphaeraphide)	28 $\mu\text{m}$	

**Figure C:** Crystal of calcium oxalate (Sphaeraphides).**Figure 1:** Ash values of leaves of *J. nana* var. *bengalense*.

### Fluorescence analysis

The plant drug powder gives comparative and distinct colour changes when seen under normal visible and UV-lights when treated with chemical reagents. Addition of acetone, methanol and ethanol with the drug powder showed green, olive green and green colour respectively in visible light. Under UV-light (365 nm) the same drug sample exhibited prominent blackish-green, reddish orange and fluorescent orange colours respectively. Such colour changes are very distinctive from the colour visualized under ordinary light. Treatment with nitric acid, sodium hydroxide, hydrochloric acid, sulphuric acid and antimony trichloride showed orange-green, reddish-black, deep green, greenish-black and yellow-green colour in visible light (Plate 3a, Plate 3b) but the same powder with same chemicals results reddish-green, black-green, reddish-green, deep green colour when placed under UV-light (Table 4; Plate 4).

**Table 4: UV- fluorescence study of the leaf powder of the investigated plant.**

Sl.No.	Leaf powder treated with solvent	Under visible light	Under UV-light (365 nm)
1	Powder as such (scraped on filter paper with powder)	Pale-Green	Green
2	50 % nitric acid	Orange-green to light megenta	Reddish- green
3	5% sodium hydroxide in water	Reddish black	Blackish -green
4	35% hydrochloric acid (1N)	Deep green	Greenish black
5	80% sulphuric acid	Greenish black	Reddish green
6	Antimony trichloride	Yellowish green	Deep green
7	Methanol	Olive-Green	Reddish -orange
8	Ethanol	Green	Fluorescent orange
9	Acetone	Green	Blakish -green

### Qualitative phytochemical screening of powdered drug

Phytochemical tests of the methanolic, ethanolic and water extracts of leaf powder revealed presence of some important phytochemical groups which give clues for the possible therapeutic effects of this ethnomedicinal plant. Total nine phytochemical groups (tannins, saponins, alkaloids, flavonoids, gum, lignin, amino acids, proteins and reducing sugars) are detected in these tests. Among these groups tannins, saponins, proteins, lignin, and amino acids are present in higher amount. The signs '+', '++', '+++' indicates the degree of changes in colour found during the tests which indicates presence of specific phytochemical groups in their higher or lower concentration in a particular solvent extract (Table 5). Whereas '-' sign indicates no change in colour i.e. absence of phytochemical group or detected by respective colour reaction test.

### Quantitative phytochemical analysis of leaves of three different developmental stages

The phytochemical constituents including nutritional and some anti-nutritional components were varied in different developmental stages of leaves. Total carbohydrates, proteins, lipids, and amino acids as nutritional factors including moisture content were present in higher amount in the mature leaves relative to young and senescent leaves of the plant; while anti-nutritional factors like total polyphenols and flavonoids were lower in mature leaves than the other two kinds of leaves. All the quantitative estimations show significant difference at  $p < 0.5$  (Table 6).

### Thin Layer Chromatography (TLC)

The ethanolic extracts of the mature leaves were subjected to TLC. The TLC with mobile phase ethanol: hexane (9:1) showed two spots with  $R_f$  values of 0.666 and 0.5 (Plate 5) whereas with ethyl acetate: ethanol (9:1) showed four spots in visible light with  $R_f$  values of 0.968, 0.593, 0.468 and 0.406 respectively (Plate 6). These  $R_f$  values represent relative migration only whereas absolute values depend on various environmental parameters like temperature, humidity, etc. which may vary depending on locations. The TLC of plants extracts thus reports 6 different spots for various photochemicals (Table 7).

**Table 5: Microchemical test of the powdered leaf extracts.**

Sl. No.	Test for	Test performed / Reagents used	Nature of change	Extraction and degree of change		
				Methanolic	Ethanollic	Water
1	Alkaloids	Wagner's reagent	Orange-brown ppt.	++	+	-
2	Flavonoids	10 % NaOH	Magenta colour	+	-	-
3	Reducing sugars	Benedict's reagents	Brick -red ppt.	-	+	-
	Gum	Molish's test	Pink colour	-	+	+
4	Tannins	10 % aqueous Pb-acetate	Yellow ppt.	+	+++	+++
5	Saponins	1% Pb-acetate	White ppt.	+++	++	+++
6	Proteins	Millon's test	Cream colour ppt.	+++	++	+
7	Lignin	Phloroglucinol + 50 % HCL	Pink or fuchsia colour	+++	+++	++
8	Amino acids	0.2 % Ninhydrin + heat	Purple colour	+++	++	+

**Table 6: Biochemical analysis of three different developmental stages of leaves (Mean  $\pm$  SE of 3 observations).**

Phytochemicals	Young	Mature	Senesced	$F_{2,6}$	P
Carbohydrate (mg/g)	95.95 $\pm$ 1.48 <sup>a</sup>	96.87 $\pm$ 1.46 <sup>ab</sup>	58.45 $\pm$ 0.68 <sup>c</sup>	297.54	0.0001
Protein (mg/g)	9.18 $\pm$ 0.27 <sup>a</sup>	11.06 $\pm$ 0.35 <sup>b</sup>	8.65 $\pm$ 0.16 <sup>ac</sup>	21.44	0.002
Lipid (mg/g)	11.16 $\pm$ 0.44 <sup>a</sup>	11.70 $\pm$ 0.48 <sup>ab</sup>	9.13 $\pm$ 0.14 <sup>c</sup>	12.27	0.008
Nitrogen (%)	3.30 $\pm$ 0.15 <sup>a</sup>	3.84 $\pm$ 0.41 <sup>ab</sup>	2.40 $\pm$ 0.15 <sup>ac</sup>	7.38	0.024
Amino acid (mg/g)	3.16 $\pm$ 0.17 <sup>a</sup>	4.06 $\pm$ 0.08 <sup>b</sup>	2.53 $\pm$ 0.17 <sup>ac</sup>	25.44	0.001
Phenol (mg/g)	9.85 $\pm$ 0.18 <sup>a</sup>	9.16 $\pm$ 0.17 <sup>ab</sup>	7.93 $\pm$ .024 <sup>c</sup>	23.32	0.001
Flavonoids (mg/g)	8.23 $\pm$ 0.20 <sup>a</sup>	8.13 $\pm$ .017 <sup>ab</sup>	6.58 $\pm$ 0.19 <sup>c</sup>	23.74	0.001
Moisture content (%)	69.53 $\pm$ 0.37 <sup>a</sup>	66.46 $\pm$ 1.57 <sup>ab</sup>	56.13 $\pm$ 0.48 <sup>c</sup>	52.01	0.0001

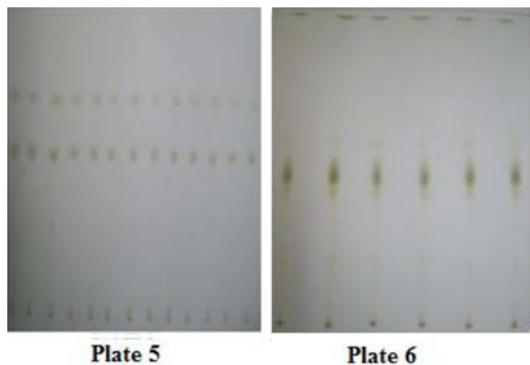
\*Within the row means followed by same letter(s) are not significantly different at 5% level by Tukey Test (HSD) along with F values (ANOVA).

**Table 7: R<sub>f</sub> values for different solvent system of alcohol extract.**

Mobile phase used	Observation	R <sub>f</sub> value
ethanol: hexane, (9:1)	2 spots	0.666; 0.5
ethyl acetate: ethanol (9:1)	4 spots	0.968, 0.593, 0.468, 0.406



**UV-fluorescence study of powdered leaf:** Plate 3- Under normal light (3a and 3b), Plate 4- Under UV- Light.



**Plate 5:** TLC plate for solvent system:ethanol:hexane (9:1).

**Plate 6:** TLC plate for solvent systems: ethyl acetate: ethanol (9:1)

## DISCUSSION

Here, various pharmacognostic characters of this ethnomedicinal plant have been standardized for the first time following standard methods like micromorphology, physicochemical and phytochemical parameters, etc. These characters may be considered as signature characters for identification of crude drug in its fresh as well as dried forms. According to WHO (1998), the macroscopic and microscopic evaluations of plants are the basic and reliable criterion for identification and purity confirmation.<sup>[33]</sup>

In this study, epidermal cells are found irregular in shape. Very distinct type of cell walls out line is observed which is mainly wavy in both lower and upper epidermal cells. The palisade ratio for this species is 21.335 which distinguish it among other species of Euphorbiaceae studied for their palisade ratio. Taxonomically and pharmacognostically the study of stomata is very important to identify the medicinal plants.<sup>[34,35]</sup> In foliar micromorphology of the present investigated plant, hypostomatic condition with paracytic type of stomata conforms the stomatal type reported in earlier studies in the genus *Jatropha*,<sup>[36,37]</sup> Similarly, Stomatal Index (SI) of *J. nana* var. *bengalense* was determined as 20.251 which is prominent and fixed to this species of genus *Jatropha* and makes it unique among other members of the same family.

In pharmacognosy, to make some fingerprint characters of a crude drug, physicochemical features play a crucial role and it is needed for detection of adulterants also.<sup>[38]</sup> Here physicochemical values of the powdered drug were found very distinct in the investigated species. It provides marker characters typical for the specific drug for its quality assessment as they vary from one species to another. It is applied as one of the important diagnostic tools in crude drug study. The ash value is considered as an indicator of presence of inorganic matters in the crude drugs.<sup>[39]</sup> It has

been found that the ash value of leaf is 8.2% and it is very distinct as well. This ash value of the leaf drug is an indicative for the presence of good amount of inorganic minerals like carbonate, oxalate, phosphate, silica, etc. Moreover, the differences between the acid insoluble ash value and water soluble ash value in the leaf of this plant further highlights its importance in authentication and quality control of the crude drug.

The fluorescence analysis of the drug powder is very useful to distinguish genuine drug from the adulterated one.<sup>[38]</sup> Some chemicals present in the plant drug powder fluoresce differently under different wavelength of UV-light and when the colour change of the same crude drug is compared in normal light, the distinctions in colour changes will be very helpful to distinguish the original drug with its adulterated forms. One of the convincing changes in colour is found in present investigation, when the green coloured leaf powder treated with methanol and ethanol. Such alcoholic treatment showed the reddish orange and fluorescent orange colours of the drug under UV light, respectively. Based on the above mentioned findings crude drug adulteration could be possible to investigate through easy identification of the drug in its dried and powdered forms.

Preliminary phytochemical analysis highlights the chemical nature of crude drug and its valuable phytoconstituents. A good number of metabolites such as sugars, proteins flavonoids, saponins, alkaloids are found to present in the crude methanolic, ethanolic, and water extracts of *J. nana* var. *bengalense* leaves. Degree of colour change in microchemical colour reaction tests indicates high amount of lignin and amino acids and a less amount of gum in the leaf extracts. The microchemical colour reaction tests of methanolic leaf extract of this plant detected some important phytochemical group like alkaloids, flavonoids, tannins, saponins, etc. which give clues on medicinal properties of this species. Disease curing properties of different phytochemicals have already been studied and well documented from some medicinal plants for treatment of tumour, inflammation, diarrhoea, malaria, diabetes, rheumatic pain, sexual diseases, etc.<sup>[39-41]</sup> In our study, microchemical colour reaction tests confirm the presence of different phytochemical groups like alkaloids, flavonoids, saponins, tannins, etc. which clearly indicates its therapeutic properties and its possibility towards scientific validation of its different ethnomedicinal uses.

The concentration and proportion of nutrients vary considerably within a particular species throughout its different developmental stages.<sup>[42-44]</sup> Carbohydrates provide general vitality, activity and growth of organisms whereas proteins and lipids serve as an alternative source of energy.<sup>[42,45]</sup> Furthermore, lipid is an essential component of diet and provide structural role in cellular membranes and transport of lipoproteins.<sup>[46]</sup> The protein content is generally a limiting factor for the optimal growth.<sup>[43-44,47]</sup> The biochemical components, carbohydrates, proteins, lipids, nitrogen, amino acids, flavonoids and phenols can play important role in plant defence against herbivory,<sup>[42-44,47-50]</sup> and they are free radical scavenger and have strong anticancer activity.<sup>[43]</sup> Flavonoids are most common and widely distributed form of plant phenolics. In this study, higher level of carbohydrates, proteins and lipids along with the Nitrogen, amino acids, moisture, phenols and flavonoids are recorded in young and mature leaves than senesced one.<sup>[6,47]</sup> The 6 spots which are given after the evaluation of the TLC plates possibly indicate the presence of alkaloid, tannin, saponin, phenol and flavonoid derivatives. This analysis of the investigated plant species shows its versatile chemical profile that would be a good claim for synthesis of natural products. The major nutritional compositions of *J. nana* var. *bengalense* leaves were found to include carbohydrates, proteins and lipids and the good distribution of other components in the leaves may explain its use as one of the forage feeds given to domestic animals and therefore confirms its traditional use as food for veterinary animals. Further intensive studies on nutrient contents and their different biological activity studies are to be needed to validate its ethnoveterinary uses as animal forage feed and galactagogue.

## CONCLUSION

The diagnostic characters on foliar epidermal micromorphology and physicochemical constants investigated here in the pharmacognostic study of leaf of *J. nana* var. *bengalense* will be very useful in authentication of its crude drugs. The data on the availability of different phytochemical groups will not only be supportive in quality assurance of the drug obtained from this ethnomedicinal plant, it will further highlights that the leaf of this medicinal undershrub possess various therapeutic properties. Quantitative phytochemical analysis of the leaves of different developmental stages itself qualifies for its further scientific studies in a wide panorama of pharmacological functions including antimicrobial, anti-inflammatory, antispasmodic, anticancer, etc. This information clearly focuses the promising domains of phytochemical and pharmacological studies of present investigated plant. Present investigation also highlights the scientific basis regarding traditional uses of this medicinal plant for various healing purposes. Further investigation with this plant in the line of phytochemistry and pharmacology will expose the scope for identification of the lead molecules and for development of potent bioactive natural products.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

## ABBREVIATIONS

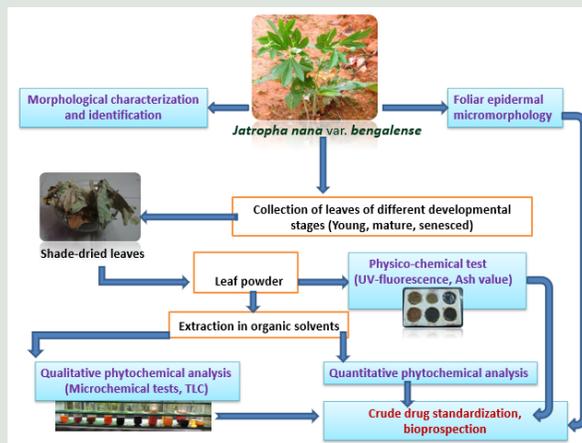
**TLC:** Thin Layer Chromatography; **UV:** Ultraviolet; **WHO:** World Health Organization; **R<sub>f</sub>:** Retention Factor.

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### GRAPHICAL ABSTRACT



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