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Microscopical and phytochemical exploration of traditionally utilized new-fangled stem buds from *Ficus religiosa* L and *Ficus benghalensis* L

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Abstract
Different components of the plant belonging to the family Moraceae are particularly used for the treatment of various diseases in traditional systems and folk medicine. The plants *Ficus religiosa* L. and *Ficus benghalensis* L. contain various pharmacologically effective components showing analgesic, anti-inflammatory, antioxidant, hepatoprotective, anti-diabetic, anti-platelet, and thrombolytic activities. It is the common component of various Ayurvedic and other traditional formulations for the treatment of blood disorders. Now a day the requirement for quality herbal drugs has increased day by day. The present study was planned to carry out microscopical, phytochemical, and analytical standards for the new fangled stem buds of *F. religiosa*, and *F. bengalensis*. Phytochemicals like reducing sugar, steroids, triterpenoids, saponin, anthraquinones, and phenolic compounds, were observed in the methanol extract of both plants. In TLC examination of methanol extract showed 4 spots in both plants revealed at wavelengths 254 and 365 nm. A peak (R_F = 0.60) matched to the Vanillic acid, acid established the presence of phenolic compounds. These microscopical and phytochemical parameters will support future research in pharmacological investigation, standardization, and pharmaceutical preparations.

Keywords: *Ficus religiosa*, *Ficus benghalensis*, new fangled stem buds, microscopical studies, phytochemical exploration, phenolic compounds, vanillic acid

Introduction
Ficus religiosa L. and *Ficus benghalensis* L**.** is a species of fig native to the subcontinent of India and China and belongs to the family Moraceae [1]. *F. religiosa* is also known as the bodhi tree, pippala, peepul and peepal tree in India. This tree is thought to have religious importance in three main convictions which originated on the Indian subcontinent, Buddhist Hindus and Jain. It is inherent to tropical Asia but now has been spread and cultured around many of the domains. F. religiosa is traditionally used for about 50 types of disorders comprising diabetes, asthma, diarrhea, gastric problems, epilepsy, inflammatory disorders, ulcers, sexual disorders, vaginal problems, leucorrhoea, menorrhagia, and inadequate lactation [2]. *Ficus benghalensis* is a huge evergreen, normally called a Banyan tree, and it is also thought to be sacred in certain places and used in traditional systems of medicine like Ayurveda, Siddha, Unani, and Homoeopathy. The active compounds of this plant are thought to be very efficacious in various disorders such as diabetes, dysentery, diarrhea, leucorrhoea, menorrhagia, nervous disorders, astringent, and blood disorders [3].
The flowers and new-fangled stem buds of the plants are rich sources of phenolic compounds like flavonoids and their glycosides. New-fangled stem buds of *F. religiosa* and *F. benghalensis* recently has been investigated for the treatment of thrombotic illnesses. New-fangled stem buds of both plants were also investigated for the presence of secondary metabolites, which were mostly polyphenols, flavonoids, and triterpenoids. These compounds are powerful natural antioxidants with anti-inflammatory, antiplatelet, and anticoagulant activities [4-5].
Although there has been major research work on the different parts of the plant, there is no scientific data existing on the standardization of New-fangled stem buds of *F. religiosa* and *F. benghalensis*. So in the current study microscopical, phytochemical study, evaluation of physical and chemical properties, fluorescence analysis, phenolic content, and TLC fingerprinting were carried out.

Materials and Methods

Collection and authentication of plant materials: The new-fangled stem buds of *F. religiosa* and *F. benghalensis* are collected in February 2023 located on the side of Kursi Road, Lucknow, UP, India, and authenticated from NBRI, Lucknow. Receipt no. LWG-109223 and LWG-109224 were reserved for supplementary reference.

Chemicals, drugs and instruments: Folin-Ciocalteu reagent was acquired from Sigma Aldrich Co MO, USA. Precoated HPTLC plates $60F_{254}$ and solvents for extraction were purchased from Merck, India. The standard phenolic compound vallinic acid was acquired from Sigma-Aldrich, UK.

Morphological and microscopical evaluation: Morphological characteristics of the new-fangled stem buds of both plants were verified by inspecting the sample with the naked eye. The transverse section was cut by free-hand sectioning, stained with safranin, and put on a glass slide, and mounted in glycerin. The glass slides were observed under (10X and 40X) compound microscope and pictures of the transverse sections were taken by a camera attached to the microscope [6].

Physicochemical parameters: According to the Indian Pharmacopoeia methods, quantitative analysis of new-fangled stem buds of both plants were carried out with help of physical and chemical parameters such as Loss On Drying (LOD), total ash, acid insoluble ash, water soluble ash, sulphated ash and crude fiber content [7].

Extractractive value: The collected new-fangled stem buds of both plants were shade-dried, powdered, and passed through a sieve (#40 mesh size). 200g of powdered material was successively extracted by Soxhlet extraction by using Pet.ether, chloroform, and methanol (Table 2). All the extracts were dried under reduced pressure and controlled temperature and yields of dried extract in Pet. ether, chloroform, and methanol of both plant materials were estimated. Preliminary phytochemical screening of all extractives of both plants was done to check the existence of chemical constituents [8].

Fluorescence analyses: The powdered crude materials of both plants were subjected to fluorescence with the help of various chemical reagents under day daylight UV light (254 nm and 365 nm) on glass slides [9].

Estimation of total phenolic contents: The methanol fraction extractives of both plant materials were estimated for the total phenolic content by using Folin-Ciocalteu reagent. An aliquot of methanol extracts was dissolved in distilled water, and the volume was made up to 1.0 ml in the different test tubes. After that, add 0.5 ml Folin Ciocalteu reagent and 2.5 m (22% w/v) sodium carbonate mixture into the distinct test tubes. The contents of the test tubes were centrifugated for 40 min. and are kept in a dark place. Finally, the absorbance of each mixture was taken at 650 nm and total phenolic contents were estimated as vanillic acid equivalents/µg from the extract [10].

Examination of TLC: Methanolic extract of both plants and standard vanillic acid dissolved in methanol and applied on pre-activated plates. The solvent system for elution is comprised of Chloroform and Methanol in a ratio of 6: 4. Pre-saturated (30 min) chromatographic twin through a vertical glass compartment was used to evolve the plate and anisaldehyde-sulphuric acid is used as sprying agent [11].

Results

Morphological study

Fresh new fangled stem buds of *F. religiosa* was apical buds present at the apex of the stem and is responsible for increase in length. It is conical, smooth, greenish brown to puff color, 3–5 cm long, 1.6 to 2.2 cm thick with a faint odor and bitter taste. After drying it appears crimped, striated surface, and fractured (Fig. 1).

Fig 1: (A) *Ficus religiosa* twig and (B) Fresh new fangled stem buds of *F. religiosa*

Fresh new fangled stem buds of *F. benghalensis* was apical buds present at the apex of the stem and are responsible for the increase in length. It is conical, hairy, pinkish brown to chocolate color, 4–5 cm long, 2.6 to 3.2 cm thick with a faint odor and bitter taste. After drying it appears crimped, striated surface, and fractured (Fig. 2).

Fig 2: (A) *Ficus benghalensis* twig and (B) Fresh new fangled stem buds of *F. benghalensis*

Microscopical study

In the transverse section (T.S.) of Fresh new fangled stem buds of *F. religiosa* showed a peculiar feature of the stem having a circular outline, smooth surface, and seen with, a reduced cortex, wide steal composed of loosely arranged xylem vessels and fibers separated by medullary rays (Fig. 3). Cork layer is absent and Cortex is composed of polygonal, collenchymatous cells; in which starch grains and latex cells were present. The phloem is composed of sieve tubes and parenchyma with extensive medullary rays under which rectangular-shaped cambium cells are present. The xylem consists of xylem vessels of various sizes; and occurs in singles and groups of 3–5 cells.

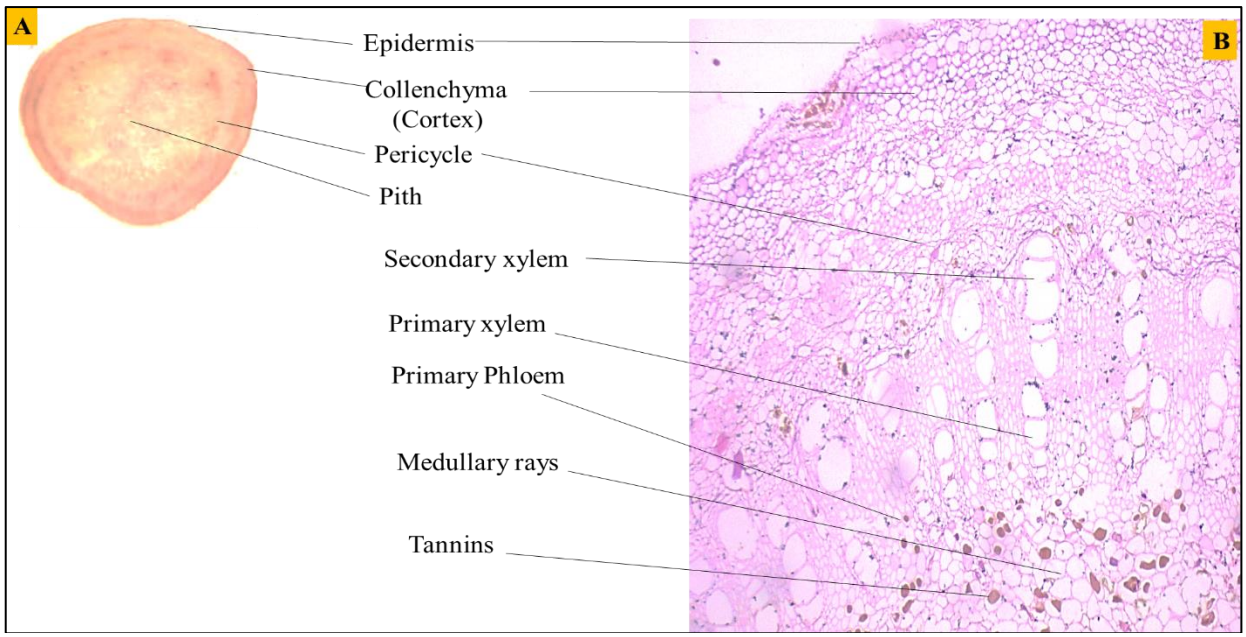

Fig 3: Microscopical features of *Ficus religiosa*, Fresh new fangled stem buds (A)10X and (B) 40X views of microscope showed xylem, phloem vessels, and medullary rays.

The microscopical features of fresh new fangled stem buds of *F. benghalensis* showed an undulated outline, glabrous surface, and seen with, a prominent cortex, wide steal composed of loosely arranged xylem vessels and fibers separated by medullary rays (Fig. 4). The cuticle is not notorious and the epidermal layer cells are thick cylindrical cells, some of them changed into epidermal hairs. It is followed by homogenous and collanchymatous cortex cells surrounding the pericycle. The secondary phloem consists of an inclusive cylinder of sieve tubes and the xylem comprises of circular, thick-walled, prolixly scattered solitary vessels (Fig. 4).

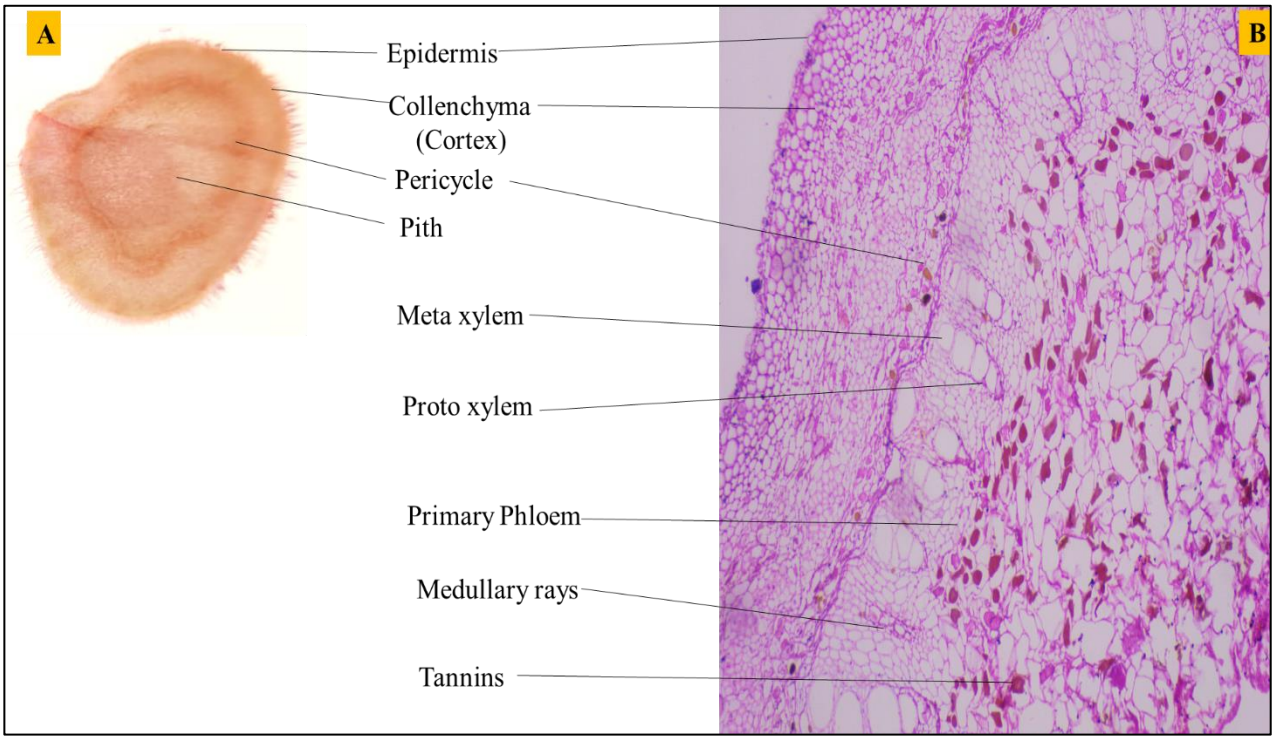

Fig 4: Microscopical features of *Ficus benghalensis*, Fresh new fangled stem buds (A)10X and (B) 40X views of microscope showed xylem, phloem vessels, and medullary rays.

Qualitative chemical test

The phytochemical test for the extractive of *Ficus religiosa* and *Ficus benghalensis* in Pet-ether, chloroform, and methanol was carried out which shows the presence of different phytoconstituents reducing sugar, steroid, triterpenoids, saponin, flavonoid, and phenolic compounds in both plants (Table 1 & 2).

Table 1: Qualitative chemical test of the methanol extracts of new fangled stem buds of *Ficus religiosa*

Plant constituents + Test reagent	Pet. ether	Chloroform	Methanol
1. Alkaloids	–	–	–
2. Reducing sugar	–	–	+
3. Glycoside			
a) Cardiac glycoside	–	–	–
b) Saponin glycoside	–	+	+
c) Anthraquinone	–	–	–
d) Flavonoids glycosides	–	–	+
4. Tannins	–	–	+
5.Phenolic compounds	–	–	+
6. Phytosterol	+	+	+
7.Terpenoids	+	+	+

(+) Present, (-) Absent

Table 2: Qualitative chemical test of the methanol extracts of new fangled stem buds of *Ficus benghalensis*

Plant constituents + Test reagent	Pet. ether	Chloroform	Methanol
1. Alkaloids	–	–	–
2. Reducing sugar	–	–	+
3. Glycoside			
a) Cardiac glycoside	–	–	–
b) Saponin glycoside	–	+	+
c) Anthraquinone	–	–	–
d) Flavonoids glycosides	–	–	+
4. Tannins	–	–	+
5.Phenolic compounds	–	–	+
6. Phytosterol	–	+	+
7.Terpenoids	+	+	+

Physicochemical evaluation

The physicochemical parameters such as loss on drying (LOD), ash value (Total ash; Acid-insoluble ash; Water-soluble ash, and Sulphated ash) crude fiber, extractive value for new-fangled stem buds of *Ficus religiosa* and *Ficus benghalensis* powder was performed and ressults are mentioned in (Table 3).

The percentage yields of extractive of both plants in Pet. ether, chloroform, and methanol fraction were 1.60 & 1.45%, 1.80 & 2.21%, and 6.50 & 760% w/w respectively.

The Pet. ether extract showed yellowish green-colored oily substance and chloroform extracts showed a whitish-yellow-colored extract which was less in quantity and insoluble in water. The extract in methanol of both plant materials was yellowish brown colored extract soluble in water.

Fluorescence Analysis

The fluorescence characteristics of new-fangled stem buds of *Ficus religiosa* and *Ficus benghalensis* powder were mentioned in (Tables 4 & 5). The fluorescence characters are checked in both daylight and ultraviolet light. Fluorescent lights of both higher (365 nm) and lower wavelengths (254 nm) were used in fluorescence analysis.

Total phenolic content

The total phenolic content in Pet. ether, Chloroform, and methanol extract were estimated. The methanol extract showed the highest concentration of phenolic compounds in both plants whereas Pet. ether and chloroform extracts showed non-significant amounts of phenolic compounds (Fig. 6). Polar nature phenolic compounds present in different plant sources are soluble in polar solvents like methanol, ethanol and water whereas Pet. ether and chloroform are the non-polar solvents in which phenolic compounds are not solubilized.

Table 3: Quantitative standards for powder of new-fangled *Ficus religiosa* and *Ficus benghalensis* (Values in %w/w with reference to the air-dried drug)

Parameters	*Ficus religiosa*	*Ficus benghalensis*
1) Loss on Drying	11.20%	10.23%
2) Ash Value		
a) Total ash	13.50%	11.5%
b) Acid-insoluble ash	2.45%	1.92%
c) Water-soluble ash	7.65%	6.40%
d) Sulphated ash	2.56%	2.11%
3) Crude fiber		18.27%
4) Extractive value		
a) Pet. ether	1.60%	1.45%
b) Chloroform	1.80%	2.21%
c) Methanol	6.50%	7.60%

Values in %w/w with reference to the air-dried drug

Table 4: Fluorescence characteristics for the powder of new-fangled *Ficus religiosa* under day (254 nm) and UV light (365 nm)

Treatment	Colour in Day light	Colour in Short UV (254 nm)	Colour in Long UV (365 nm)
Drug powder	Dark brown	Brown	Brown-Black
Powder + 1M NaOH	Italic brown	Light blue	Dark green
Powder + 1M HC1	Greenis brown	Bluish green	Bright blue
Powder + 50% H_2 SO_4	Bluish Green	Bluish yellow	Dark blue
Powder + HNO_3	Yellowish orange	Light orange	Dark Brown

Table 5: Fluorescence characteristics for the powder of new-fangled *Ficus benghalensis* under day (254 nm) and UV light (365 nm)

Treatment	Colour in Day light	Colour in Short UV (254 nm)	Colour in Long UV (365 nm)
Drug powder	Light Brown	Orange Brown	Brown - Black
Powder + 1M NaOH	Brown	Green	Blue – Black
Powder + 1M HC1	Dark green	Yellow-Blue	Blue
Powder + 50% H_2 SO_4	Dull green	Orange-red	Dark Brown
Powder + HNO_3	Yellow-orange	Orange-Yellow	Chocolate color

Examination of TLC fingerprint

The TLC plates point out that the phytoconstituents present in the methanol extract of both plants were clearly 4-5 separated chemical moieties (Fig. 7). There are similar phytocomponents visualized in both plants with R_F values 0.26, 0.42, 0.65, and 0.85. The methanol extract of both plants contains chemical moieties with an equal R_F value (0.65) to vallinic acid indicating the presence of a phenolic compound.

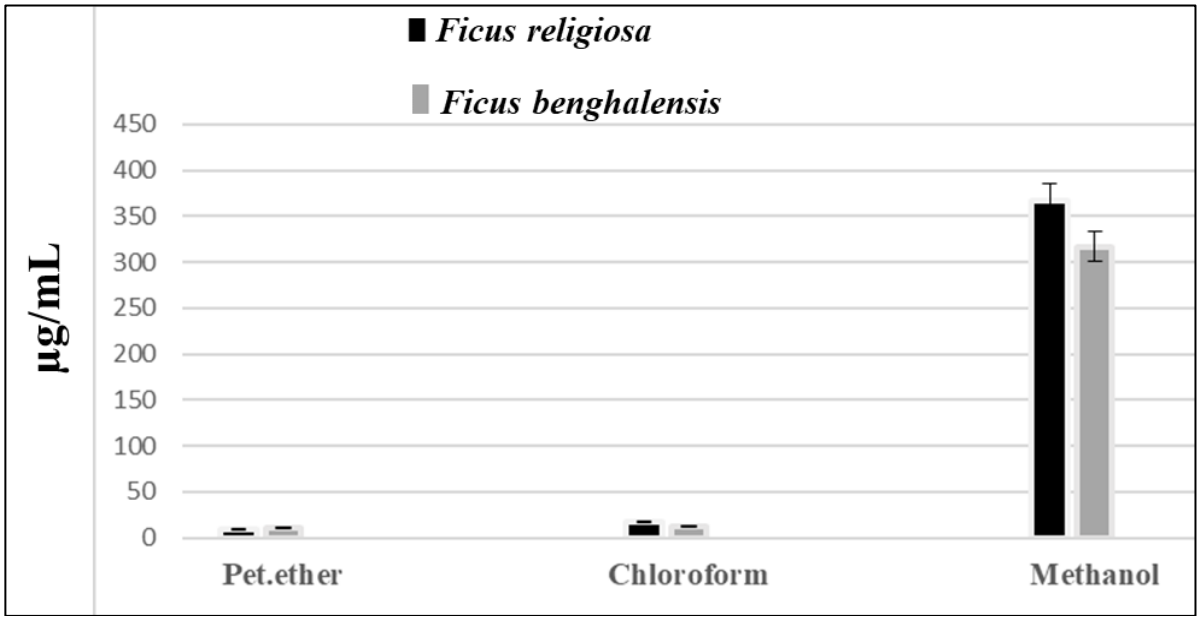

Fig 6: Total phenolic content in the extractive of *Ficus religiosa* and *Ficus benghalensis* in Pet-ether, chloroform, and methanol**.** Each value represents mean±S.D. (n = 4)

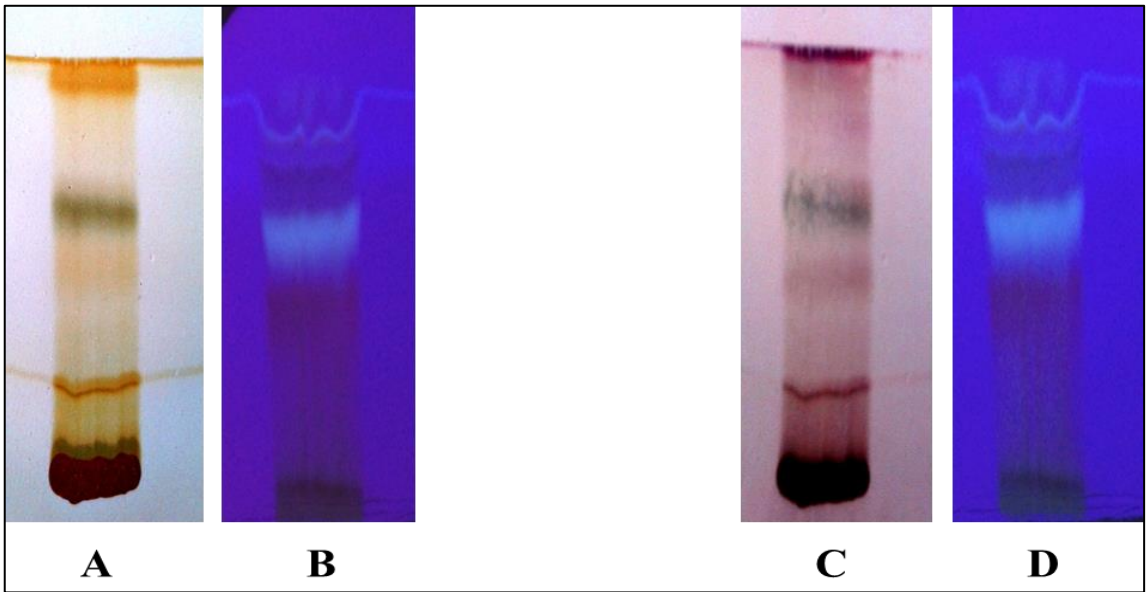

Fig 7: TLC plate A at λ_{254} nm and plate B at λ_{365} nm, methanolic extract of *Ficus religiosa* and plate C at λ_{254} nm and plate D at λ_{365} nm, methanolic extract of *Ficus benghalensis*.

Discussion

The curious organoleptic and microscopic features of *Ficus religiosa* and *Ficus benghalensis* are essential in proving the authenticity, and accurate recognition and also serve as analytical tools for the selection of these plants. The new-fangled stem buds of *Ficus religiosa* and *Ficus benghalensis* have peculiar morphological characters.

The T.S. micrograph performed on the new-fangled stem buds emphasized a lot of specific feathers like the epidermal layer, cortex, medullary rays, phloem, primary and secondary xylem, and pith are the diagnostic feathers for these plants. The T.S. of the plant material was performed by free-hand sectioning and stained with safranin.

The result for loss on drying proves that crude powder is dried in a proper manner and its storage was done in the correct manner. The value of total ash, acid-insoluble ash, and water-soluble ash, total ash is mainly peculiar in the assessment of drug purity. All the extractives of both crude drugs were subjected to qualitative chemical analysis to detect the presence of various phytoconstituents. Phytochemical analysis of both plants indicated the presence of reducing sugars, flavonoids, saponins, steroids, triterpenoids, and phenolic compounds. The Pet. ether and chloroform extracts show the existence of phytosterol and triterpenoids. Extract with methanol shows the existence of polar constituents like reducing sugar, phenols, flavonoids, glycosides, etc. These compounds were identified by TLC in both plants compared to the standard phenolic compound vallinic acid.

Conclusion

The data collected while performing the morphological, microscopical, physicochemical, and TLC fingerprint analysis mentioned above is useful in the standardization of the selected plant materials. The phytochemical study, evaluation of physical and chemical properties, fluorescence analysis, and phenolic content are very peculiar objectives for the identification of a particular crude drug. Biologically active phenolic compounds were qualitatively and quantitively estimated and can be used to treat a variety of illnesses.

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Conflict of Interest

The Authors declare that there is no conflict of interest.

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