Original Article

Pharmacognostical and phytochemical studies of *Atibala* (*Abutilon indicum* [Linn.] sweet) fruit

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Abstract

Background: *Abutilon indicum* (Linn.) Sweet (*Malvaceae*), generally called as "*Atibala*" is a plant of high medicinal importance. The plant possesses several beneficial effects such as cooling, laxative, digestive, analgesic, anti-inflammatory, astringent, diuretic, expectorant, antihelmintic, aphrodisiac, and demulcent which is widely used in the Ayurveda system of medicine. **Aim:** The current study is aimed to establish the macroscopy, powder microscopy and physicochemical analysis of *A. indicum* fruits. **Materials and methods:** The Pharmacognostical studies on *A. indicum* fruits, including parameters such as morphological evaluation, powder microscopy, ash values, foreign organic matter, extractive value, phytochemical, fluorescence studies, and high-performance thin-layer chromatography fingerprint profile, are established in the current study. **Results:** Fruit powder microscopy has shown diagnostic characteristics such as stellate hairs of different sizes, testa, lignified endocarp, and palisade cells. The loss on drying value of fruit powder was 7.7% w/w. The total ash values of the drug were found to be 10.5% and acid insoluble ash 2.4% w/w with respect to the air-dried crude drug. Water-soluble and alcohol-soluble extractives were found to be 9.64% w/w and 9.04% w/w, respectively. **Conclusion:** Phytochemical characterization of aqueous, alcoholic extracts of *A. indicum* fruit revealed the presence of proteins, carbohydrates, phenols, flavonoids, saponins, and steroids. The powder microscopical and phytochemical studies observed in this study can serve as a valuable resource for the authentication of *A. indicum* fruits.

Keywords: *A. indicum,* extractive values, fluorescence analysis, powder microscopy

Introduction

Abutilon indicum (Linn.) Sweet is an annual herb mainly found in the hotter areas of India with a characteristic hairy coat all over the shrub, bearing golden yellow color flowers. It grows up to an elevation of 600 m, available as a weed on roadsides and other dumping places in plains and hills.[1] In *Ayurveda*, the bark is recommended as a febrifuge, anthelmintic, and alexeteric. In *Ayurveda*, it is recommended in the conditions like *Tridoshaja* diseases (diseases caused by vitiation of *Vata*, *Pitta,* and *Kapha*),[2] allays thirst, vomiting and diminishes perspiration. The root is used in the treatment of uterine hemorrhagic disorders. The plant milk is effective against urinary disorders. In Unani treatment, the bark is prescribed in urinary complications and strangury.[3] Leaves are recommended for all kinds of inflammation, piles, lumbago, and toothache. From ancient days infusion of roots is prescribed in fever, hematuria, and leprosy. Decoction of leaves is used in bronchitis, diarrhea, gonorrhea, toothache, tender gums, and bladder inflammation.[4] The extracts from various parts of the plant have been scientifically reported for their pharmacological actions. *A. indicum* roots alcoholic extract showed significant aphrodisiac activity at 400 mg/kg in experimental animals.[5] Various fractions of roots reported significant analgesic anti-inflammatory activity.[6,7] Among various fractions of the whole plant, ethyl acetate fraction was reported for its maximum antioxidant activity.[8] The alcoholic extract of the whole plant showed significant anti-inflammatory activity.[9] Due to its mast cell stabilizing and anti-inflammatory activity of methanolic extract of aerial

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parts of *A. indicum* it has been reported in the treatment of asthma.[10] Ethanolic extract of leaves reported to possessing potent anti-bacterial,[11] antifungal,[12] antidiarrheal,[13] and immunomodulatory activity.[14] The ethanolic extract was also reported for its anti-inflammatory (IC_{50} 8.89 µg/mL) and anti-proliferative activity (IC_{50} 85.2 µg/mL).[15] Like all other parts, fruits are also reported for considerable medicinal uses. They are used in the management of piles, gonorrhea, and cough. Decoction of the fruit in combination with ammonium chloride on oral administration treats hemorrhagic septicemia. Powder of seeds with water is useful as aphrodisiac and laxative.[16] Ethyl acetate and chloroform fractions of fruits have shown strong antioxidant potential.[17] Seeds aqueous extract has been reported for its diuretic activity at 200 and 400 mg/kg doses.[18] Seed oil is reported for its anti-bacterial and antioxidant activities.[19] Despite the great significance of *A. indicum* fruits and seeds, much work has not been reported on pharmacognostic standardization.[20,21] Hence, an attempt was made to carryout detailed study of macroscopy, organoleptic characters, powder microscopy, physicochemical parameters, preliminary phytochemical and fluorescence analysis of *A. indicum* fruits.

Materials and methods

Collection of fruits and identification

The fruits of *A. indicum* were collected in the month of December. The plant authentication and identification were made by scientists and taxonomists of the botanical survey of India. A voucher specimen of *A. indicum* is maintained in the department of pharmacognosy. Fresh fruits were used to study the macroscopical parameters, whereas shade-dried fruit powder was used for the microscopical, physicochemical investigations. The complete research work was carried out at Central Ayurveda Research Institute, Kolkata.

Chemicals and instruments

All the solvents and chemical reagents used for the study were of analytical grade. Simple microscope, compound microscope, watch glass, microscopic slide, and other common glassware were used in this experiment. Photomicrographs were taken with Olympus CX21i LED microscope attached to the Magcam DC14 camera. Chloral hydrate, phloroglucinol, iodine, and picric acid are the major chemicals used for powder microscopy. Chemical reagents such as Dragendorff's, Millon's, and Molisch's reagents are used for phytochemical studies.

Macroscopy

Macroscopy was done by observing the fruits and seeds under a simple microscope, observed the color, size, texture, etc., Different macroscopic parameters of the fruits and seeds like shape, odor, and taste were also noted.[22]

Powder microscopy

The coarse powder (40#) of *A. indicum* fruits was studied under the compound microscope. The fruit powder was macerated in chloral hydrate reagent. A little quantity of sample was taken on a microscopic slide; 1–2 drops of phloroglucinol reagent was added, and a cover slip was placed above the sample. Then, microscopic slides were sealed with paraffin wax. The prepared slides were mounted and examined under the microscope. Tracing of characteristic structures and cell components was done.[23,24]

Photomicrographs

Photomicrographs of the different cellular tissues in the macerated sample were taken with various magnifications by a Magcam DC14 camera attached to the microscopic unit. For normal observations, ×10 was used for the study of cellular structures, and for micro-observations, ×40 was employed.[25]

Physicochemical studies

Physicochemical parameters, such as loss on drying, total ash, acid-insoluble ash, alcohol- and water-soluble extractives, as well as the foreign organic matter of fruit powder, were determined according to standard methods.[26] Air-dried sample was used for all these quantitative studies. Extractive values with ethanol and water were determined.[27]

Phytochemical screening

Phytochemical screening was performed using standard procedures. The alcohol- and water-soluble extracts of fruit powder obtained were diluted with respective solvents and subjected to chemical tests for the detection of different phytoconstituents such as alkaloids, glycosides, phenols, volatile oils, and flavonoids.[28,29]

Fluorescence analysis

The chemical nature of the active constituents can be determined by fluorescence analysis. By using standard methods, fluorescence analysis of fruit powder was carried out.[30] The shade-dried powder and after treatment with various chemical reagents such as 1N hydrochloric acid, 5% sodium hydroxide, 50% sulfuric acid, 5% acetic acid, and 50% nitric acid and their fluorescence behaviors were observed in ordinary visible light and also under ultraviolet (UV) light (245 nm and 366 nm).

High-performance thin-layer chromatography analysis

The alcoholic and hydroalcoholic extracts were dissolved in high-performance liquid chromatography-grade methanol (100 mg/1 ml). The solution was centrifuged at 3000 rpm/5 min and used for high-performance thin-layer chromatography (HPTLC) analysis. 15 µl of the alcoholic and hydroalcoholic extract and 10 µl of the hydroalcoholic extracts were loaded as 8 mm band length in the 10 × 10 precoated Silica gel $60F_{254}$ aluminum supported thin layer chromatography (TLC) plate using a Hamilton syringe and CAMAG Automatic TLC sampler 4 instrument. The plate was developed (up to 90 mm) in a twin trough chamber with ethyl acetate: Toluene: Acetic acid (3:7:0.5 v/v) mobile phase. After development, the plate was kept in the photo-documentation chamber (CAMAG TLC Scanner-180114) and captured the images with WIN CATS software [version 1.4.6 WinCATS Software (V 1.4.6); Camag (Muttenz, Switzerland)] at UV366 and 254 nm.[31,32]

Results and Discussion

Macroscopy

Fresh fruits are green in color, schizocarpic, circular in shape, consisting of 11–16 radiating densely pubescent mericarp per fruit, each mericarp flattened, somewhat boat shaped, black when ripe 1–2 cm in diameter, consisting of 2–3 seeds. Seeds reniform, hairy, dotted, minutely scrobiculate, brownish-black in color, and 3–5 mm in diameter. Odour characteristic, taste bitter. Figure 1 shows the morphology of fruits and seeds.

Powder microscopy

Fruit powder is grayish green color, with no specific odor and taste. It shows diagnostic microscopic characters like different sizes of lignified stellate hairs with large lumen, thick-walled epidermal cells, round to oval-shaped collenchymatous cells of hypodermis. Lignified endocarp cells having cuticular striations and few pits, palisade cells with light line, thin-walled endosperm with oil globules were observed. Group of scalariform vessels, group of fixed oil globules, brownish matter, thick-walled stone cells, elongated cells of testa and cotyledon parenchymatous cells were noticed. The observations are summarized in Figure 2.

Physicochemical studies

The results of physico-chemical parameters are depicted in Table 1. The total ash and acid insoluble ash values are 10.5% and 2.4%, respectively. The extractive values for various solvents, such as ethanol and water, were found to be 9.64% and 9.04%. The moisture content was calculated through loss on the drying method, and value was found to be 7.7%.

Phytochemical screening

The results of the phytochemical screening are shown in Table 2. Preliminary phytochemical screening revealed that aqueous and ethanolic extracts are a rich source of carbohydrates, proteins, phenols, flavonoids, and saponins. Steroids are present only in ethanolic extract. Phenols are more in aqueous extract than alcoholic extract. Saponins are rich in alcoholic extract than aqueous extract.

Fluorescence analysis

The fruit powder and various extracts were treated with UV radiation of long and short wavelengths and ordinary visible light to study the fluorescence characteristics. They emitted

Table 1: Physicochemical parameters of *Abutilon indicum* fruit

Parameters	Values obtained in percentage (w/w%)
Foreign matter	0.52
Loss on drying	7.7
Total ash	10.5
Acid-insoluble ash	2.4
Water-soluble extractives	9.64
Alcohol-soluble extractives	9.04

Figure 1: Macroscopy of *A. indicum* fruit and seed. (a) Fruits; (b) Seeds. *A. indicum: Abutilon indicum*

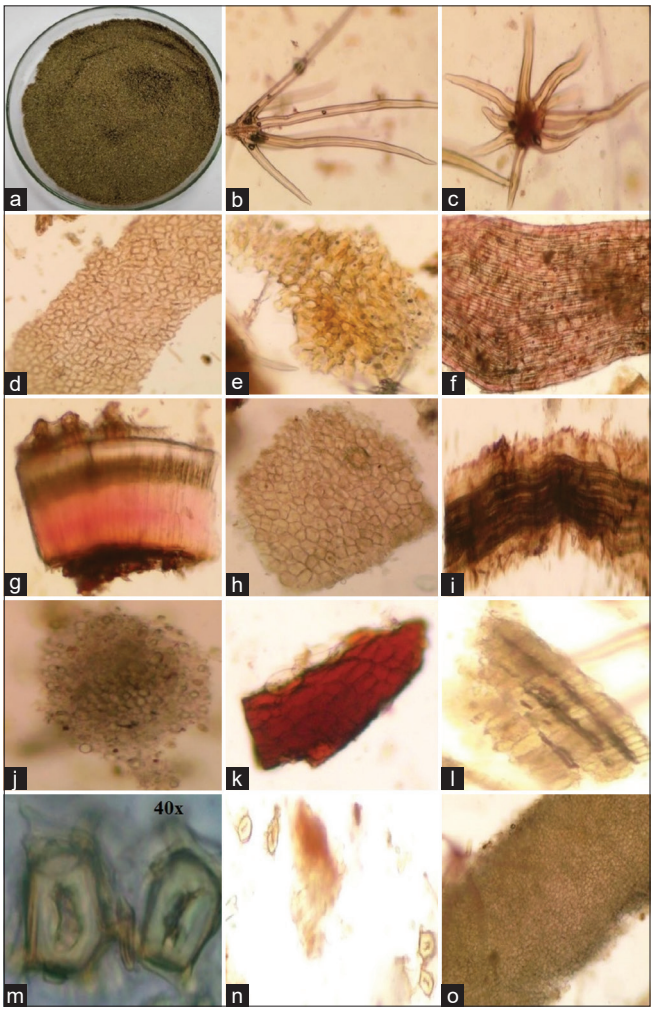

Figure 2: Powder microscopy of *A. indicum* fruit. (×10 magnification) (a) Fruit powder; (b and c) Stellate hairs; (d) Epidermis; (e) Hypodermis; (f) Endocarp; (g) Palisade cells; (h) Endosperm; (i) Scalariform vessels; (j) Group of oil globules; (k) Brownish matter; (l) Testa; (m and n) Stone cells; (o) Cotyledon parenchymatous cells. *A. indicum: Abutilon indicum*

various color radiations. The color change for the fruit powder and individual extract were distinctive and reproducible, revealing the solvent properties to the phytoconstituents, and data are represented in Table 3.

High-performance thin layer chromatography analysis

HPTLC analysis of ethanolic extract showed the presence of 9 peaks at different R_f values at 254 nm. Similarly, hydroalcoholic extract showed 10 peaks (for 10 µl sample) and 12 peaks (for 15 µl sample). When the plate was observed at UV-254 nm, fluorescence quenching was observed, which was seen as black zones on the green background of the TLC plates. At 366 nm, spots were observed in deep blue and red color. The difference in peaks number in alcoholic and hydroalcoholic extracts clearly indicate that there is a marked chromatographic difference in the chemical nature of both extracts. The results are summarized in Table 4 and Figures 3, 4.

Conclusion

The present study established the qualitative and quantitative diagnostic features of *A. indicum* fruits through morphological,

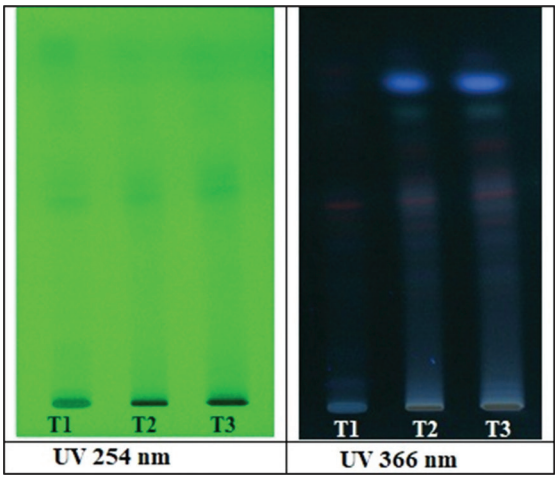

Figure 3: Developed TLC plates of *A. indicum* fruit extracts. T1 - Alcoholic extract (15 µl) T2 - Hydroalcoholic extract (10 µl) T3 - Hydroalcoholic extract (15 µl). *A. indicum: Abutilon indicum*, TLC: Thin-layer chromatography

Table 2: Preliminary phytochemical analysis of *Abutilon indicum* fruit

Test applied	Test for	AIFAE	AIFEE
Molisch test	Carbohydrates	+++	+++
Fehling's test		++	–
Benedict's test		–	–
Biuret test	Proteins	+++	+
Millon's test		+++	+++
Dragendorff's test	Alkaloids	–	–
Wagner's test		–	–
Hager's test		–	–
Mayer's test		–	–
Borntrager's test	Glycosides	–	–
Ferric chloride test	Phenols and Tannins	+	+
Lead acetate test		+++	+
Dilute Kmno^4 test		+++	+++
Shinoda test	Flavonoids	+	+
Alkaline reagent test		++	++
Foam test	Saponins	+	+++
Salkowski test	Steroids	–	++
Libermann's test		–	++

(+): Present in minor quantity; (++): Present in moderate quantity; (+++): Present in high quantity; (–): Absent , AIFAE: *Abutilon indicum* fruit aqueous extract, AIFEE: *Abutilon indicum* fruit ethanolic extract

Table 3: Fluorescence analysis of *Abutilon indicum* fruit

Chemical reagent	Visible light	Short UV light	Long UV light
As powder	Light green	Green	Dark green
1N hydrochloric acid	Greenish white	Greenish white	Greenish white
5% sodium hydroxide	Cream	Green	Dark green
50% sulfuric acid	Brown	Green	Dark green
5% acetic acid	Cream	Cream	Cream
50% nitric acid	Cream	Green	Dark green
Picric acid	Creamish yellow	Yellow	Bright yellow
Dilute iodine solution	Light brown	Green	Dark green
5% ferric chloride	Light brown	Light brown	Light brown
Water	Cream	Cream	Cream
Alcohol	Ash	Ash	Ash

UV: Ultraviolet

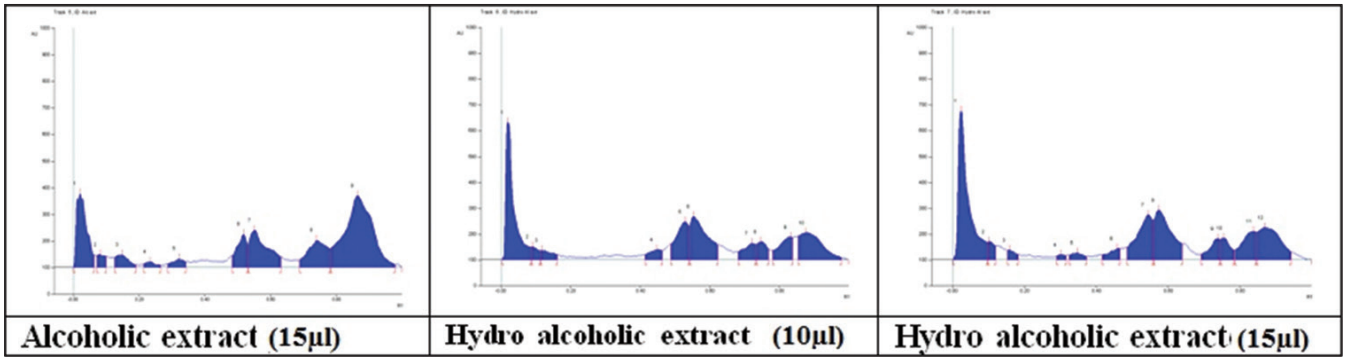

Figure 4: HPTLC chromatograms of *A. indicum* fruit extracts. HPTLC: high-performance thin layer chromatography, *A. indicum: Abutilon indicum*

Table 4: R_f values of different extracts at 254 nm

Sample	Volume applied on TLC plate (v/v) (μL)	Detection wave length (nm)	Number of peaks and R^f value
Alcoholic extract	15	254	9 and 0.02, 0.08, 0.15, 0.23, 0.32, 0.52, 0.55, 0.74, 0.86
Hydroalcoholic extract	10	254	10 and 0.02, 0.09, 0.12, 0.45, 0.52, 0.55, 0.72, 0.75, 0.83, 0.88
Hydroalcoholic extract	15	254	12 and 0.02, 0.10, 0.16, 0.30, 0.35, 0.46, 0.54, 0.57, 0.74, 0.76, 0.84, 0.87

TLC: Thin-layer chromatography

powder microscopical, physicochemical, and HPTLC analysis. Phytochemical analysis revealed that alcoholic and aqueous extracts are rich sources of saponins, phenols, flavonoids, carbohydrates, and proteins. HPTLC chromatograms of the alcoholic extract have shown nine peaks and hydroalcoholic extract has shown twelve peaks, which helps as a fingerprint for identification and standardization. This powder microscopical and phytochemical study results will help in minimizing adulteration, authentication of the original crude drug and in carrying out further research on *A. indicum* fruits.

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Conflicts of interest

There are no conflicts of interest.

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