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Original Article

PHARMACOGNOSTIC STANDARDIZATION, PRELIMINARY PHYTOCHEMICAL SCREENING AND TLC FINGERPRINTING OF THE BARK OF *CASCABELA THEVETIA* L.

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ABSTRACT

Objectives: In this study, systematic pharmacognostic study and preliminary phytochemical screening of the bark of *Cascabela thevetia* L. were carried out.

Methods: The selected plant part was collected, processed and stored in an airtight container. From the bark different pharmacognostic studies like macroscopic and microscopic evaluation, physicochemical parameters, fluorescence analysis were done. Powdered bark was successively extracted by petroleum ether, chloroform, ethyl acetate, and methanol using a Soxhlet apparatus and finally macerated with the hydro-alcoholic solvent system (30:70). The preliminary phytochemical analysis and thin layer chromatography of the extracts were done to find the nature and number of the different phytoconstituents present.

Results: Transverse microscopy reveals the presence of crystal oxalate, cork cell, starch granules, vascular bundle, phloem fiber, parenchyma cells, and collenchyma cells. Powder microscopy also showed the presence of cork cell, fiber and calcium oxalate crystal. Results obtained in a different physicochemical analysis like total ash, acid insoluble ash, water soluble ash, alcohol-soluble extractive, water-soluble extractive, and moisture content were 8.67%, 0.83%, 5.33%, 4.53%, 12.27%, and 7.83% respectively. Phytochemical analysis showed the presence of alkaloid, flavonoid, triterpenoid, phytosterol, tannin, saponin, anthraquinone, carbohydrate and fatty acid in the different extracts. TLC (Thin Layer Chromatography) study revealed 4 spots in petroleum ether, chloroform, ethyl acetate, and methanol extracts and 3 spots in the Hydro-alcoholic extract with different solvent systems.

Conclusion: The results obtained from the study will provide a reliable basis for identification, purity, and quality of the plant.

Keywords: *Cascabela thevetia* L., Pharmacognostic study, Phytochemical screening, TLC

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INTRODUCTION

Pharmacognostic study of the crude drug is a very essential part for the quality control process of plant materials used in herbal medicine. It basically deals with standardization, authentication and systematic study of plant-based crude drugs through morphological, physicochemical and phytochemical analysis. Pharmacognostic studies ensure the identity of a plant and lay down some standardization parameters which help in identification of the plant and prevent adulterations. Similarly, phytochemical analyses give an idea about the different class of phytoconstituents present in the crude drug which are responsible for the different therapeutic activity. All These studies ensure the reproducible quality of crude drugs and their safety and efficacy as herbal medicines [1] *Cascabela thevetia* L. (Local Name: Halodhiya karabi) is a shrub or small tree grown as an ornamental plant in America, Africa, Asia, Malaysia and Pacific islands. The plant has numerous medicinal importance like antidiabetic, antimalarial, antimicrobial, antioxidant etc. as found in ethnomedicinal reports and research articles [2-9]. The plant contains a higher amount of cardiac glycosides especially in the fruit which makes the plant very toxic. But from the literature survey, it is found that the bark of the plant has several important traditional uses for treating different diseases and disorders including diabetes and malaria [2, 3]. Hence in this study, the bark of the plant was selected to determine different pharmacognostic parameters and for preliminary phytochemical screening which was not done yet. This study will provide a reliable basis for identification, standardization and quality control of this traditional medicinal plant.

MATERIALS AND METHODS

Chemicals

All the chemicals like solvents and reagents used in the study were procured from HiMedia Pvt. Ltd. and Research Lab.

Collection and identification of plant material

Barks of the *Cascabela thevetia* L. were collected from Longpotia, Sivasagar, Assam, India during the month of July 2014. The plant was identified and authenticated by Dr. A. A. Mao, Scientist-E, Botanical Survey of India, Eastern Regional Centre, Shillong via letter of reference no. BSI/ERC/Tech/Identification/2014/361 on 26 August 2014. After collection, barks were cleaned properly, cut into small pieces and dried under shade for two weeks. The dried barks were pulverized in a mechanical grinder to a coarse powder and stored in an airtight container free from moisture for future work.

Macroscopic study

Different organoleptic and macroscopic characters of the fresh and dried bark of *Cascabela thevtia* L. like color, odor, shape, size, and texture were observed and recorded [10].

Microscopic study

Few dried barks were soaked in water for sometimes till get soften and then cut into slices with the help of a sharp blade. Thin transverse sections were taken in a watch glass and treated with chloral hydrate solution. The sections were stained with staining reagents like a mixture of phloroglucinol and concentrated hydrochloric acid, safranin separately. Glycerol was used as mounting fluids for stained sectioned and observed under microscope and photomicrographs were taken with Leica Digi 3 Photomicrographic unit at 10X magnification. Powdered materials of barks were sieved through sieve no. 60. A pinch of powder was taken on a glass slide, treated with chloral hydrate solution, washed with water and mounted with glycerol after staining with different reagents as mentioned above. Then slide was observed under a microscope using 10X and 40X magnification for different characters and photographs were taken [10].

Physicochemical parameters

Different physicochemical parameters such as ash values, moisture content, extractive values were determined according to the well established standard official procedure [10].

Fluorescence analysis

The behavior of the powdered bark with different chemical reagents and their fluorescence characteristics were observed under ultraviolet (254 and 366 nm) and visible light. The instrument used for observing fluorescence was UV-Viewer Ultraviolet Fluorescence Analysis Cabinet, MAC® Macro Scientific Work.

Extraction of the plant material

The air-dried powdered bark of *Cascabela thevetia* L. was packed in a Soxhlet extractor and extracted successively with the solvents like petroleum ether (60-80 °C), chloroform, ethyl acetate, methanol and hydro-alcoholic (30:70). After extracting with petroleum ether for about 12 h, the powdered material (marc) was first air dried and then oven dried below 40 °C. Then the marc was extracted with chloroform for about 12 h and after completion, it was dried in a similar manner. The marc was further extracted with ethyl acetate, methanol in the same way. Finally, the powdered marc was cold macerated with Hydro-alcoholic solvent (Ethanol: water = 70:30). Each extract was concentrated by distilling off the solvent and evaporate to dryness in a petri dish on a water bath. The concentrated extracts were dried and stored in a desiccator for use in subsequent experiments.

Preliminary phytochemical analysis

The different solvent extracts of the bark of *Cascabela thevetia* L. obtained were separately tested for the presence of various plant constituents such as alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, carbohydrates, saponins, triterpenoids, anthraquinones and fatty acids as per the standard procedure available [11, 12].

Thin layer chromatography

Glass plates of 3×10 cm size were coated with silica gel G (Himedia Laboratory, Mumbai, India) with the help of spreader to a layer of thickness approximately 0.25 mm. Then the plates were first air-dried and activated at 110 °C for 30 min in a hot air oven. After cooling the plates were kept in a desiccator for further use. About 5 µl of each extract solutions (10 µg/ml) was used to spot with the help of fine bore capillary tubes 2 cm above the base of the plate so that the spots were not directly immersed in the mobile phase in the developing chamber. The mobile phase for the different extracts was selected according to polarity and finalized by performing multiple time trial and error process. It gave the best solvent mixture for every extract with a good separation of the components (table 1).

After spotting the extracts on the plate chromatograms were developed in a chromatographic chamber keeping the plats at an angle of 75 °C using different solvent systems at room temperature. In all the cases solvent systems were allowed to run to a distance of 2 cm below the top of the plates. After the complete running of the solvents, the plates were removed from the chamber and allowed to air dry. Then the plates were sprayed with derivatizing reagents like the anisaldehyde-sulfuric acid solution and 10% methanolic sulfuric acid for the visualization of the different compounds and retention factor (R_f) values of the compounds were calculated using the formula given below.

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

RESULTS

Pharmacognostic study of the bark

Macroscopic study

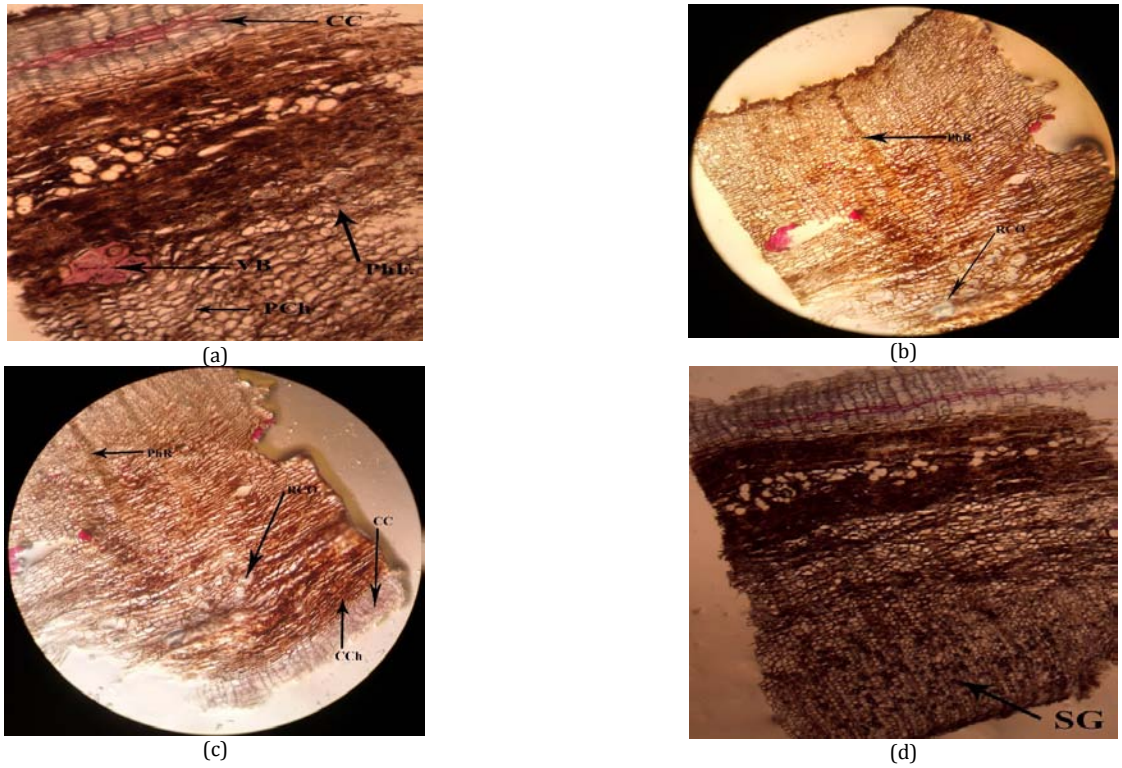
The different macroscopic and organoleptic properties of the bark of *Cascabela thevetia* L. is summarized in table 1.

Microscopic study

The different cell structures found in the transverse section microscopy and powder microscopy of the bark of *Cascabela thevetia* L. are shown in fig. 1 and fig. 2.

Physicochemical parameters

The different physicochemical parameters found in the study are given in table 2.



(a) (b) (c) (d)

Fig. 1: Transverse section of the bark of *Cascabela thevetia* L., (a) VB-vascular bundle, PhF-Phloem fibre, PCh-Parenchyma cells, (b) PhR-phloem ray, RCO-rossete crystal oxalate, (c) PhR-Phloem ray, RCO-rossete crystal oxalate, CC-Cork Cell, CCh-Collenchyma, (d) SG-starch granule

Table 1: Macroscopic evaluation of bark of *Cascabela thevetia* L

S. No.	Characteristics	Stem bark
1.	Colour of the outer surface of the bark	Greenish-brown in fresh form and dark brown in dried form
2.	Colour of the inner surface of the bark	Brownish white in fresh form and blackish brown in dried form
3.	Odor	Characteristic and woody
4.	Taste	Characteristic
5.	Shape	Hard quills
6.	Texture	Rough on the outer side and smooth in texture in the inner side

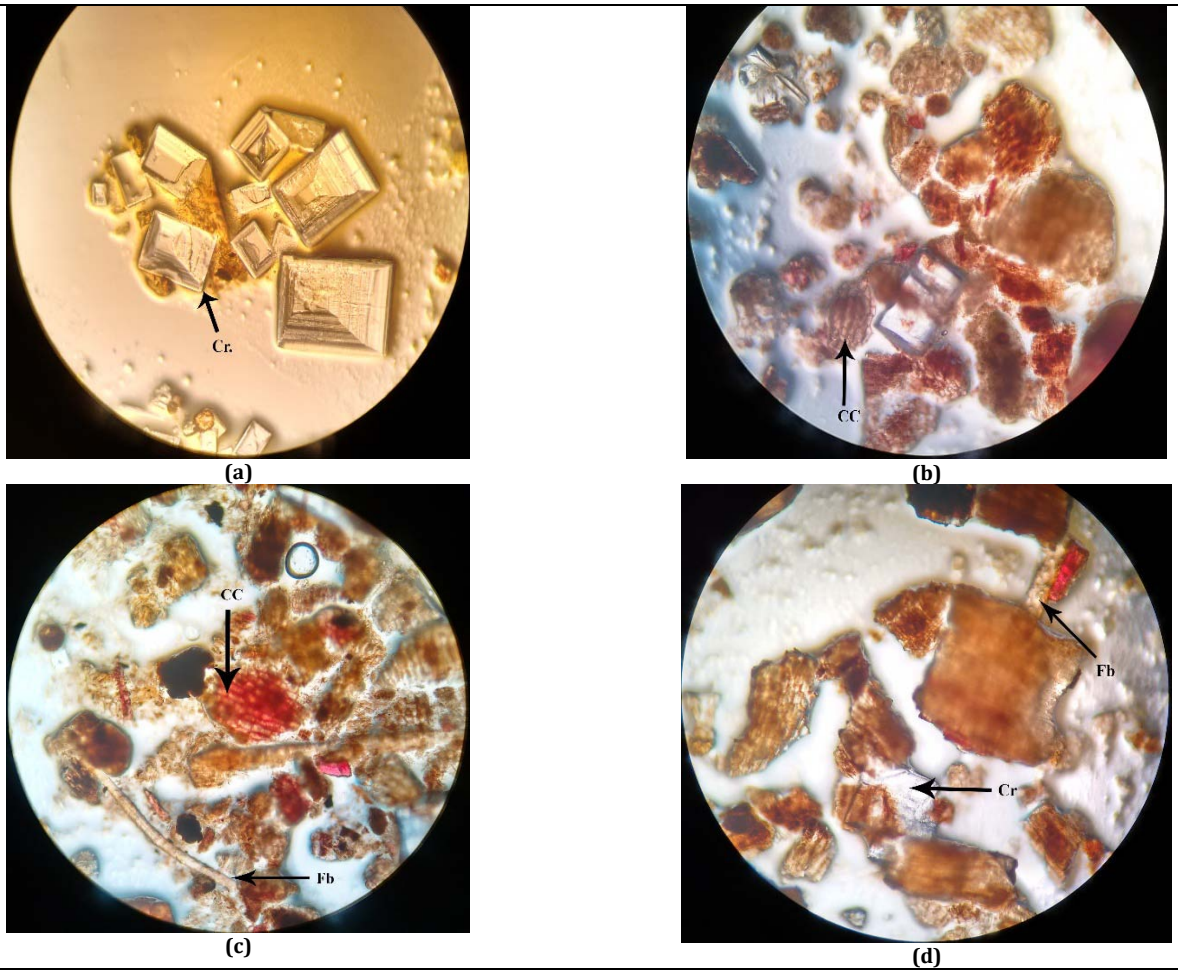


Fig. 2: Powder microscopy of the bark of *Cascabela thevetia* L., (a) Cr-Calcium oxalate crystal, (b) CC-Cork cell, (c) CC-Cork cell, Fb-Fibre, (d) Cr-Crystal, Fb-Fibre

Table 2: Values of physicochemical parameters

S. No.	Parameters		Resultant value* (%w/w)
1.	Ash Values	Total ash	8.67±0.24
		Acid-insoluble ash	0.83±0.24
		Water-soluble ash	5.33±0.24
2.	Extractive Values	Alcohol-soluble extractive	4.53±0.38
		Water-soluble extractive	12.27±0.38
3.	Moisture content		7.83±0.24

*Average of three readings±standard deviation.

Fluorescence study

The fluorescence characteristics of powdered barks with different solutions are given in table 3.

Physical characteristics of extracts

The physical characteristic of the five different solvent extracts obtained by Soxhlet extraction is given in table 5.

Table 3: Fluorescence analysis data of powdered bark of *Cascabela thevetia* L

S. No.	Particulars of the treatment	Under ordinary light	Under UV light (366 nm)
1	Powder as such	Saddle brown	Sandy brown
2	Powder+1N NaOH (Aq)	Black	Bluish-black
3	Powder+1N NaOH (Alc)	Light brown	Dark brown
4	Powder+1N HCL	Light brown	Blackish brown
5	Powder+50% H_2SO_4	Black	Brackish brown
6	Powder+50% HNO_3	Reddish brown	Black
7	Powder+Ammonia	Greenish brown	Dark brown
8	Powder+5% Iodine	Greenish brown	Black
9	Powder+5% $FeCl_3$	Greenish brown	Blackish brown
10	Powder+Acetic acid	Reddish brown	Dark brown

Table 3: Physical characteristics of the bark extracts

S. No.	Extracts	Yield (%w/w)	Consistency	Colour	Odour
1	Petroleum ether extract	3.32	Sticky solid	Greenish yellow	Characteristic
2	Chloroform extract	1.86	Solid	Dark brown	Characteristic
3	Ethyl acetate extract	2.38	Semi-solid	Dark brown	Characteristic
4	Methanol extract	13.40	Semi-solid	Dark brown	Characteristic
5	Hydro-alcoholic extract	2.52	Semi-solid	Dark brown	Characteristic

Preliminary phytochemical tests

The preliminary phytochemical screenings of the five extracts are given in table 5.

Thin layer chromatography

The TLC results of the five extracts along with photographs of the developed plates are given in table 6 and fig. 3.

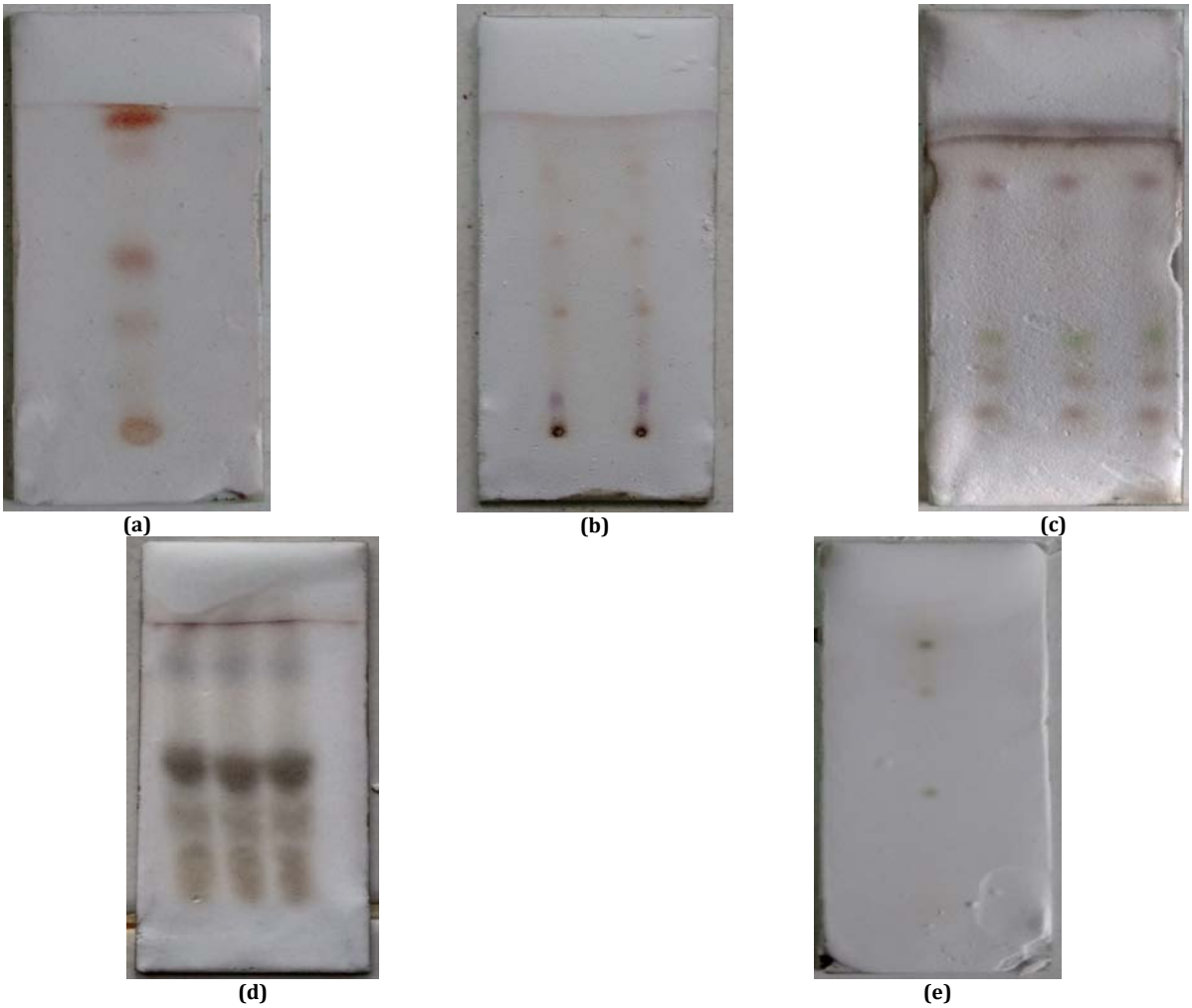


Fig. 3: TLC plates of different extracts showing the spot of compounds, (a) Petroleum ether extract (4 spots), (b) Chloroform extract (4 spots), (c) Ethyl acetate extract (4 spots), (d) Methanol extract (4 spots), (e) Hydroalcoholic extract (3 spots)

Table 4: Preliminary phytochemical screening data of bark extracts of *Cascabela thevetia* L

Plant constituents	Petroleum Ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Hydro-alcoholic extract
Alkaloid	-	-	+	+	-
Flavonoid	-	-	-	+	+
Triterpenoid	-	-	+	+	+
Phytosterol	+	+	-	-	-
Tannin	-	-	-	+	+
Saponin	-	-	-	+	+
Free anthraquinone	-	-	+	+	+
Coumarin	-	-	-	-	-
Carbohydrate	-	-	+	+	+
Protein/Amino acid	-	-	-	-	-
Fatty acid	+	+	-	-	-

(+) means present and (-) means absent.

Table 5: TLC profile of *Cascabela thevetia* L. bark extracts

Extract	Solvent system	Number of spots	R_f values	Visualizing agent
Petroleum ether	Tolune: Acetone = 7:3	4	0.28, 0.4, 0.56, 0.94	Anisaldehyde-Sulfuric acid
Chloroform	Tolune: Acetone = 6.6:4.4	4	0.20, 0.55, 0.69, 0.89	Anisaldehyde-Sulfuric acid
Ethyl acetate	Chloroform: Ethyl acetate: Methanol = 4:3:3	4	0.10, 0.29, 0.58, 0.85	10% H_2SO_4
Methanol	Chloroform: Ethyl acetate: Methanol = 9:4:0.5	4	0.12, 0.29, 0.47, 0.83	10% H_2SO_4
Hydro alcoholic	Ethyl acetate: Methanol: Glacial acetic acid = 4:8:3	3	0.36, 0.72, 0.85	10% H_2SO_4

DISCUSSION

Standardization of crude drug or plant part is very much important to establish its identity and to maintain quality and clinical efficacy. For the very first time, the systematic pharmacognostic study of the bark part of *Cascabela thevetia* L. was done to establish its identity and quality as a crude drug. It is a prerequisite to establishing different pharmacognostic parameters and standards based on the macroscopic and microscopic evaluation to include a plant part or crude drug in herbal pharmacopeia or any other official monograph [13]. The bark of *Cascabela thevetia* L. is used by peoples to treat different diseases without standardization; hence this study will provide some standard data for the genuine crude drug or plant part [14]. After collection of the plant part, it was properly processed so that there was no chance for any changes or alteration in the chemical constituents present in it. The objective of drying of fresh crude materials is to prevent the enzymatic or hydrolytic reactions that may alter the chemical composition of the crude drugs. It also reduces the weight and bulk of crude drugs [15]. Macroscopic and microscopic studies are the starting point for standardization of a particular plant part which helps researchers for identification and authentication of the plant material [16, 17]. The macroscopic study has given the outer appearance in both fresh and dry form along with different organoleptic properties which are specific for the bark of *Cascabela thevetia* L. Transverse section microscopy showed the presence of different cellular structure like a vascular bundle, phloem fibre, parenchyma cells, phloem ray, cork cell, and collenchyma. Besides rossete crystal oxalate and starch granules were also found in the plant part. In powder microscopy, the same cellular structures were found in broken forms which are specific for this plant part. Burning or making ash involves oxidation of the components of the product. A high ash value is indicative of contamination, substitution or adulteration. The total ash usually contains carbonates, phosphates, silicates which include both physiological and non-physiological ash. Acid-insoluble ash indicates the contaminants with silicon materials like earth and sand and water soluble ash indication of the amount of inorganic elements present. The extractive values with a particular solvent indicate the nature of phytoconstituents present in the crude drug. Moisture content or loss on drying is a very essential parameter which helps in the preservation condition for a crude drug. All these physicochemical parameters determined for the plant were specific for the plant part and can be utilized for quality control process for the crude drug [16, 17]. Fluorescence analysis of the powdered plant material by treating with different chemicals provided the idea regarding the possible nature of the phytoconstituents present in the plant part [18]. Preliminary phytochemical tests showed the presence of a different class of plant metabolites (primary and secondary) in the different solvent extracts of the plant part. The methanol extract of *Cascabela thevetia* L. had the highest amount as well as a number of phytoconstituents compared to other solvent extracts. Overall it was found that that the bark of the plant contains many secondary metabolites like alkaloid, flavonoid, triterpenoid, phytosterol, tannin, saponin, free anthraquinone and primary metabolites like carbohydrate, and fatty acid. So it was selected for further studies. TLC was performed with aim of separating the individual components present in the extracts and to find out the suitable solvent systems which will be helpful in isolation of the components by column chromatography. The R_f values of the different spots present in the chromatograms also give an idea about the nature of the components and it can also use as a qualitative measurement for quality control process [18, 19].

All the studies carried out according to the available standard procedure which provided the different parameters for identification, authentication, and quality control process and will make easier for other researchers to work on the plant material for any other study or to incorporate in the standard monograph.

CONCLUSION

There were no previous reports on pharmacognostic parameters of the bark of *Cascabela thevetia* L., so this study will help in the quality control and standardization of the crude drug material. The reported pharmacognostic parameters from this study can be considered as distinctive enough for authentication of this crude drug or plant part and can be included as standards in the official monograph. Preliminary phytochemical screening and TLC studies of the extracts have given an idea regarding the presence of different plant secondary metabolites in the bark which will help to isolate the different bioactive compounds and to evaluate them for the pharmacological efficacy of the crude drug.

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AUTHORS CONTRIBUTIONS

Conception and design of the work were done by Mr. Biman Bhuyan and Mr. Neelutpal Gogoi. Laboratory experiments were done by Mr. Neelutpal Gogoi with the help of Mr. Trinayan Deka. Data

compilation and drafting of the manuscript were done by Mr. Neelutpal Gogoi. Finally, a critical review of the article was done by Mr. Biman Bhuyan and Mr. Trinayan Deka.

CONFLICT OF INTERESTS

All authors have none to declare

REFERENCES

1. Chanda S. Importance of pharmacognostic study of medicinal plants: an overview. J Pharmacogn Phytochem 2014;2:69-73.
2. Buragohian J. Ethnomedicinal plants used by the ethnic communities of tinsukia district of Assam, India. Recent Res Sci Technol 2011;3:31-42.
3. Khan MH, Yadava PS. Antidiabetic plants used in Thoubal district of Manipur, Northeast India. Indian J Tradit Know 2010;9:510-4.
4. Sowjanya KM, Narendra K, Swathi J, Satya AK. Phytochemical extraction and antimicrobial efficiency of crude leaf extract of medicinal plant *Cascabela thevetia*. Int J Res Pharm Biomed Sci 2013;4:465-70.
5. Ravikumar HS, Patil TR, Gurumurthy KPH. *In vitro* antimicrobial activity of ethanol extract of *Thevetia peruviana*. BioTechnol: Indian J 2008;2:5-7.
6. Gata Gonçalves L, Nogueira JMF, Matos O, de Sousa RB. Photoactive extracts from *Thevetia peruviana* with antifungal properties against *Cladosporium cucumerinum*. J Photochem Photobiol B 2003;70:51-4.
7. Thilagavathi R, Kavitha HP, Venkatraman BR. Isolation, characterization and anti-inflammatory property of *Thevetia peruviana.* E J Chem 2010;7:1584-90.
8. Hassan MM, Saha AK, Khan SA, Islam A, Mahabub-Uz-Zaman M, Ahmed SSU. Studies on the antidiarrhoeal, antimicrobial and cytotoxic activities of ethanol-extracted leaves of yellow oleander (*Thevetia peruviana*). Open Vet J 2011;1:28-31.
9. Gogoi N, Bhuyan B. *In vivo* anti-diabetic activity evaluation of the bark of *Cascabela thevetia* L. in streptozotocin-induced diabetic rats. Int J Pharm Pharm Sci 2017;9:48-53.
10. Quality control methods for herbal materials, World health Organization, Geneva Switzerland; 2011.
11. Khare P, Kishore K, Sharma DK. A study on the standardization parameters of *Bauhinia variegate*. Asian J Pharm Clin Res 2017;10:133-6.
12. Rawat P, Bachheti RK, Kumar N, Rai N. Phytochemical analysis and evaluation of *in vitro* immunomodulatory activity of *Rhododendron Arboreum* leaves. Asian J Pharm Clin Res 2018;11:123-8.
13. Chanda S. Importance of pharmacognostic study of medicinal plants: an overview. J Pharmacogn Phytochem 2014;2:69-73.
14. Kumar CD. Pharmacognosy can help minimise accidental misuse of herbal medicine. Curr Sci 2007;93:1356-8.
15. Sonia NS, Jessykutty PC. Drying technologies for good quality crude drug production of medicinal plants-a review. Int J Med Pharm Sci 2016;6:59-72.
16. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy,* 42nd ed. Nirali Prakashan, Pune; 2008.
17. William CV. Trease and evans pharmacognosy. 16th ed. Saunders Elsevier, USA; 2009.
18. Kala C, Ali SS, Chaudhary S. Comparative pharmacognostical evaluation of *Costus speciosus* (Wild Ginger) and *Zingiber officinale* (Ginger) rhizome. Int J Curr Pharm Res 2016;8:19-23.
19. Wall PE. *Thin-layer chromatography: a modern practical approach*, cambridge, The royal Society of Chemistry; 2005.