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Pharmacognostic and physicochemical characterisation of potential plants for anti-diabetic herbal formulations

Sudem Brahma, Banjai Mochahary, Mrinal Kalita & Arvind Kumar Goyal*

Department of Biotechnology, Bodoland University, Kokrajhar 783 370, Assam, India

*Email: arvindgoyal210883@gmail.com

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Abstract

In recent years, mankind has relied largely on herbal medicines to treat a variety of ailments. The aim of the current study is to investigate the pharmacognostic and physicochemical characterisation of some medicinal plants such as *Bambusa balcooa* (leaf) (BBL), *Phyllanthus emblica* (fruit) (PEF), *Hodgsonia heteroclita* (fruit pulp) (HHP), and *Punica granatum* (fruit peel) (PGP) used by the local *Bodo* tribe for the treatment of diabetes, which can be combined together to develop a novel polyherbal formulation. The preliminary phytochemical screening, microscopic evaluation, organoleptic and flow properties and qualitative heavy metal estimation was carried out using standard protocols. The preliminary phytochemical screening revealed the existence of carbohydrates, phenolics, alkaloids in all. However, amino acids were present in *P. granatum* and *P. emblica*, whereas triterpenoids were inferred in *P. officinalis*. Microscopical analysis of crude showed the presence of stone cells (BBL, HHP and PGP), xylem (PEF, HHP and PGP), trichome (PGP), fibres (all) and epidermis (PEF). The macroscopical study of crushed powder was overall acceptable to sense organs. The physical evaluation of flow properties was found to be good for *P. emblica* fruit when compared to others which was fair to passable. The heavy metal test showed the absence of bismuth, cadmium and lead in all species. Accordingly, the results obtained from the study is endowed with essential information for the authentication and quality assessment of these herbal drugs.

Keywords

Bambusa balcooa, herbal formulation, *Hodgsonia heteroclita,* Pharmacognostic, *Phyllanthus emblica, Punica granatum*

Introduction

Plants being the main source of nutrients to mankind, plays a substantial role in the health care system and prevention of many diseases (1). Since ancient times, plants and their products have been used to prevent or treat many illnesses, including diabetes (2). Herbal formulations combined become more effective than the single herb, probably due to their catalysing effect with one another. In recent years, the claims of medicinal efficiency and lack of toxicity of many plants have been scientifically ascertained. (3). Standardisation is essential for herbal formulations to measure the quality and control of herbal ingredients during the manufacturing process (4). World Health Organization (WHO) has acknowledged the indispensable relationship between the people belonging to developing nations and medicinal plants for their health care and thus took the necessary initiative to formulate the guidelines to maintain the quality and standards of the polyherbal formulations (PHFs) worldwide (5). Ayurveda, popularly known as "Mother of All Healing", is made up of two Sanskrit words, "*ayur* (life) and *veda* (science or knowledge)" (6). The exclusivity of this ancient system lies in the immeasurable diversity of healing processes used, such as animal juices, herbal formulations and natural energies (sun and water) (6). At present, there is a need for the development of a safer drug for the treatment of several ailments. Thus, there is a growing interest in the plants and traditional systems of medicine in the pharmaceutical industry for drug discovery and development (7). Hence, 4 different plant species and parts such as *Bambusa balcooa* (leaf), *Phyllanthus emblica* (fruit), *Hodgsonia heteroclita* (fruit pulp) and *Punica granatum* (fruit peel) were selected based on traditional claims by the Bodo tribe of Assam for the treatment of diabetes.

Bambusa balcooa Roxb. (Poaceae) is locally called Owa burkha by Bodos (8, 9). It possesses various phytoconstituents like flavonoids, saponins, resins, fixed oils, phytosterols, phenolics and tannins, which could be used in curing diseases and drug formulations (10). *B. balcooa* has also been reported to possess antidiabetic activity because of the presence of three compounds rutin, gallic acid and β sitosterol as reported (3).

Hodgsonia heteroclita Hook. f. & Thomson, commonly known as the Chinese lard plant, belongs to the family Cucurbitaceae and is locally known as Hagrani jwgwnar among the Bodo (11-13). Different parts of the plant, like the seed, fruit pulp are known for medicinal properties. *H. heteroclita* being bitter in taste, is used in the traditional system of medicine for curing diabetes by the Bodo tribe (11). *H. heteroclita* has also been reported to possess anti-diabetic properties (12, 13). The presence of caffeic acid could be responsible for anti-diabetic as reported (13).

Phyllanthus emblica L. (Phyllanthaceae), universally recognised as Indian gooseberry or amla, is the most important plant used in the traditional system of medicine in India, including folklore, Ayurveda and Unani (14). Different parts of the plant have been reported to treat various diseases like the common cold (14), fever (14), anti-inflammatory (14), hair tonic (14), anti-diabetic (14), anti-cancerous (15), hypolipidemic (16), antibacterial (16), antioxidant (16), hepatoprotective (16), gastroprotective (16), chemopreventive (16), and antimutagenic activity (16), anti-viral (17). The anti-diabetic activity of amla is probably due to the presence ellagic acid, estradiol, sesamine, kaempferol, zeatin, quercetin, and leucodelphinidin (18).

Punica granatum L., popularly designated as pomegranate, belongs to the family Punicaceae. Traditionally in the Indian sub-continent, it is known as 'Anar' or 'Dadima' (19, 20). *P. granatum* is an extensively used medicinal fruit of the indigenous system of medicine (21). *P. granatum* has a plethora of medicinal uses like anti-microbial, hepatoprotective, cardioprotective, anti-hyperglycemic, anti-inflammatory, anti-hypertensive, anti-anaemic, antioxidant, immunomodulatory properties, anti-cancer (22, 23). The presence of valoneic acid dilactone (VAD) isolated from fruit rinds of *Punica granatum* might be associated with the anti-diabetic activity of *P. granatum* (24).

Materials and Methods

Plant material selection, collection and preparation of extract

Matured fruits of *Phyllanthus emblica* and *Punica granatum* were collected from the local market, *Hodgsonia heteroclita* from the forest of Kokrajhar district and leaf of *Bambusa balcooa* was collected from the Bambusetum, Bodoland University, Kokrajhar, BTR, Assam, India (Table 1). The voucher specimens were deposited at Botanical Survey of India, Central National Herbarium, Howrah and was identified and authenticated vide letter no. CNH/Tech.II/2021/43 date 26-11-2021.

Table 1. List of plants with information on parts used, vernacular names and GPS coordinates of the collection site

Botanical Name	Part Used	Vernacular names		GPS coordinates	
		Bodo Name	Assamese Name	Latitude	Longitude
Bambusa balcooa Roxb.	Leaf	Owa burkha	Bhaluka bah	26.4694332 °N	90.292971 °E
Phyllanthus emblica L.	Fruit	Amlai	Amalaki	26.4720043 °N	90.2979632 °E
Hodgsonia heteroclita Hook.f. & Thomson	Fruit pulp	Hagrani jwgwnar	Not Known	26.4011 °N	90.2729 °E
Punica granatum L.	Peel	Dalim	Dalim	26.5288799 °N	90.2495364 °E

The individual plant parts such *Bambusa balcooa* (leaf), *Phyllanthus emblica* (fruit), *Hodgsonia heteroclita* (fruit pulp) and *Punica granatum* (fruit peel) used by the local *Bodo* tribe for the treatment of diabetes were dried at room temperature and powdered using mechanical grinder. The powder was sieved using sieve of 600 µm mesh size and stored in airtight glass bottles for analysis. The powder was subjected to Soxhlation using double distilled water (1:10 *w/v* ratio of the sample and solvent). The extraction was carried out for 6 hrs at the boiling temperature and evaporated under pressure at 50 °C and stored at 4 °C for further experimental analysis.

Preliminary Phytochemical Screening

The prepared extracts were subjected to the preliminary phytochemical test to detect the presence or absence of phytochemical constituents like alkaloids, carbohydrates, phenolics, amino acids and triterpenoids as per standard protocols of Trease and Evans (25) with modification (1, 26).

Organoleptic evaluation

Organoleptic evaluation of food products is important in ascertaining the censoring acceptability or rejection of foodstuffs available in the market (27). The texture, aroma, flavour/ taste and colour of crushed powder were recorded using various sense organs.

Microscopic study

Individual powdered samples were mounted on a clear glass slide using water and covered with a coverslip. The slides were visualised under the binocular microscope (Labomed Vision 2000) and the photographs were taken using a Samsung Galaxy phone.

Determination of physical characteristics of powder

Angle of repose

The angle of repose determines the flow rate of the powder. The angle of repose was done using the funnel method. The powder (15 g) was allowed to flow through the funnel till the heap of the powder touched the tip of the funnel placed above the graph paper placed on the horizontal surface. The diameter of the powder cone was recorded, and the angle of repose was calculated using the following formula (28)

$$\text{Angle of repose} = \tan - 1h / r$$

where, h= height of pile r= radius of the pile

Bulk density

The powder was sieved through the muslin cloth, and apparent bulk density was measured by pouring 15 g of powder into a 100 ml measuring cylinder without compacting, and initial reading was noted. The bulk density was calculated by using the following formula (28)

$$Db = M / Vb$$

where, M= the mass of powder, Vb = the bulk Volume of the powder, Db = bulk density

Tapped density

After measuring the bulk density, the cylinder containing the powder was tapped manually for 500 times until further change in volume was noted. The tapped density was calculated using the following formula (28):

$$\rho tap = M / Vf$$

where, ρtap = Tapped density, M = Weight of the powder, Vf = Tapped volume.

Carr's index

It indicates the powder flow properties of the powder. It is expressed in percentage and is calculated according to the following formula (28).

$$\text{Carr's index (\% compressibility)} = 100 \times (1 - D_b / D_t)$$

where D_b = Bulk density, D_t = Tapped density

Hausner ratio

Hausner ratio is an indirect method of quantifying powder. It was calculated by the following formula (28)

$$\text{Hausner ratio} = D_t / Db$$

where D_b = Bulk density and D_t = Tapped density.

Qualitative estimation of heavy metals

Procedure outlined (29) was followed to qualitatively determine the occurrence of heavy metals like cadmium, lead and bismuth in different plant parts. It is determined to ascertain the safe use of plants.

Results and Discussion

Preliminary phytochemical screening

The current experiment was conducted to estimate the pharmacognostic and physicochemical characterisation of potential plants for anti-diabetic herbal formulations. The therapeutic potential of the plants is attributed to the occurrence of secondary metabolites like alkaloids, carbohydrates, phenolics, amino acids, etc. Among all the extracts, *E. officinalis* revealed the presence of alkaloids, carbohydrates, phenolics, amino acids and triterpenoids. Similar observations were previously reported (30-32). However, It was (30) reported that *E. officinalis* was devoid of triterpenoids during preliminary screening (Table 2). Among the different tests conducted, *P. granatum* fruit peel showed the absence of triterpenoids which was in conjunction with the earlier study (33). It was also reported the absence of alkaloids and amino acids (33). However, they documented the presence of carbohydrates. Recent studies conducted also reported the presence of carbohydrates, alkaloids, amino acids and phenolics in pomegranate peel (34, 35). Aqueous extract of *B. balcooa* leaf and *H. heteroclita* fruit pulp showed the presence of carbohydrates, phenolics and alkaloids, whereas amino acids and triterpenoids were absent in both samples. As per one report (10), Alkaloids were absent in *B. balcooa,* which is contradictory to our results. Likewise, reports are on the presence of alkaloids, carbohydrates and phenolics in *H. heteroclita* (36, 37). Thus, the presence of these secondary metabolites in the different plants may be the factor behind the anti-diabetic activity. The phytochemical analysis is tabulated in Table 2.

Organoleptic parameters

Organoleptic properties constitute an important role in industrial production, carrying or augmenting the consistency of the formulation, ameliorating patient compliance and ascertaining overall product performance (38). The organoleptic evaluation of plants relating to their texture, aroma, flavour/ taste and colour were recorded and summarised in Table 3.

Powder microscopy

Microscopy plays a vital role in the identification of impure drugs, and it is considered an unavoidable step before undertaking any test (39). The structural and cellular features of crude powder help in the primary identification and authentication of the plant to be used as pharmaceutical materials (REF in link). Stone cells are observed in all the samples except in *E. officinalis,* and its primary function is to provide strength or support soft tissues. Epidermis was seen in *H. heteroclita*. The epidermis helps in the exchange of gases into the cell and protects against the loss of water in plants. Trichome was observed in *H. heteroclita* and *P. granatum*. Trichomes are the epidermal outgrowth and help in water absorption and minerals. Xylem tissue was observed in *E. officinalis*, *H. heteroclita* and *P. granatum.* Xylem helps in the conduction of water minerals nutrient upward from root to leaves. Fibre was observed in all the samples. Fibres are part of the supporting tissues and provide mechanical support and

Table 2. Preliminary phytochemical screening of various plant parts under study

Constituent	Chemical Test	Procedure	*Bambusa balcooa* (Leaf)	*Phyllanthus emblica* (Fruit)	*Hodgsonia hetroclita* (Fruit pulp)	*Punica granatum* (Fruit peel)
Alkaloids	Mayer's reagent test	Extract+ Dil. HCl + 3mL Mayer's reagent	+	+	+	+
	Dragendroff's test	Extract + Dil. HCl + 3mL Dragendroff's reagent	White ppt	Yellow ppt	White ppt	Bright Yellow ppt
Carbohydrate's	Fehling's test	1mL Fehling A+ 1mL B Fehling mixed and boiled for a minute	Brick red	Brick red	Brown red	Brick red
	Benedict's test	2 mL extract + Few drops of Benedict's reagent + Boiled for 2 min	Green	Red	Yellow	Brick red
	Molisch's test	Extract + Few drops of Molisch's reagent + Conc. H_2SO_4	Violet ring	Violet ring	Light	Violet ring
Phenolics	$FeCl_3$	Extract + $FeCl_3$	Brown	Greyish	Light brown	Deep black
	Lead acetate test	Extract + Lead acetate	White ppt	White ppt	White ppt	Deep black
Amino acids	Millon's test	Extract + Few drops of Millon's reagent	ND	Red colour	ND	Red colour
Triterpenods	Salkowski test	Extract + Few drops of chloroform + few drops of conc. H_2SO_4	ND	Red colour	ND	ND

ND= Not Detected; Brick red (Fehling's test)= presence of reducing sugars; Green (Benedict's test)= traceable, yellow= small, red=moderate; Red and Brick red (Millon's test) = presence of tyrosine; white ppt (Lead acetate) = phenolics, Yellow= flavonoids; Red (Salkowski)= steroids; ppt= precipitate

Table 3. Organoleptic parameters of various plant parts under study

Parameters	*Bambusa balcooa* (Leaf)	*Phyllanthus emblica* (Fruit)	*Hodgsonia hetroclita* (Fruit pulp)	*Punica granatum* (Fruit peel)
Texture	Dry, fibrous	Granular, powder	Granular, spongy	Granular
Aroma	Grassy	Fruity	Wheaties	Rancid
Flavour/ taste	Slightly sweet	Sour and sweet	Bitter	Betel nut, sweet, bitter
Colour	Fern green	Tawny brown	Beige	Sandstone orange

firm strength to the plant (40, 41). Xylem and fibre were also observed previously in *P. emblica* (30, 42, 43). Observations are on stone cells, xylem vessel, collenchyma cells of epicarp, prism type crystal of calcium oxalate and compound starch grain in *P. granatum,* whereas stone cell, xylem, trichome and fibre were observed in this study (20). However, no such study was reported for *B. balcooa* leaf and *H. heteroclita* fruit pulp. The results of powder microscopy of various plant parts are depicted in Fig. 1.

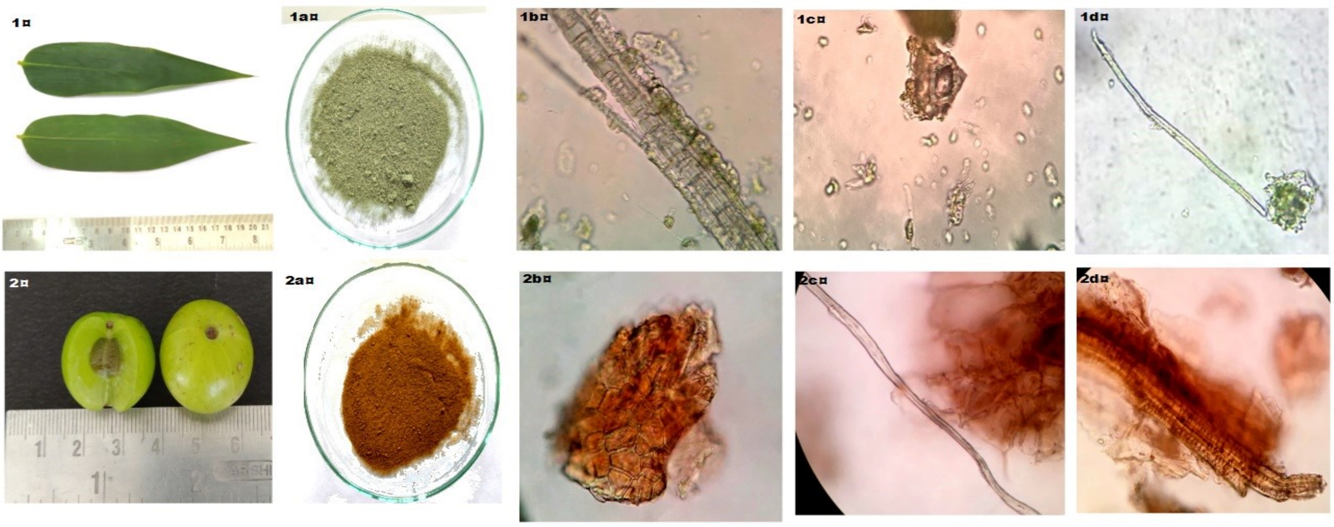

Fig. 1a. Photomicrographs of microscopic evaluation (400x) **1**. *Bambusa balcooa* leaf, **1a**. *B. balcooa* leaf powder, **1b.** Fibre bundle, **1c**. Stone cell, **1d**. Fibre, **2**. *Emblica officinalis* fruit, **2a**. *E. officinalis* fruit powder, **2b**. Epidermis, **2c.** Fibre, **2d**. Xylem.

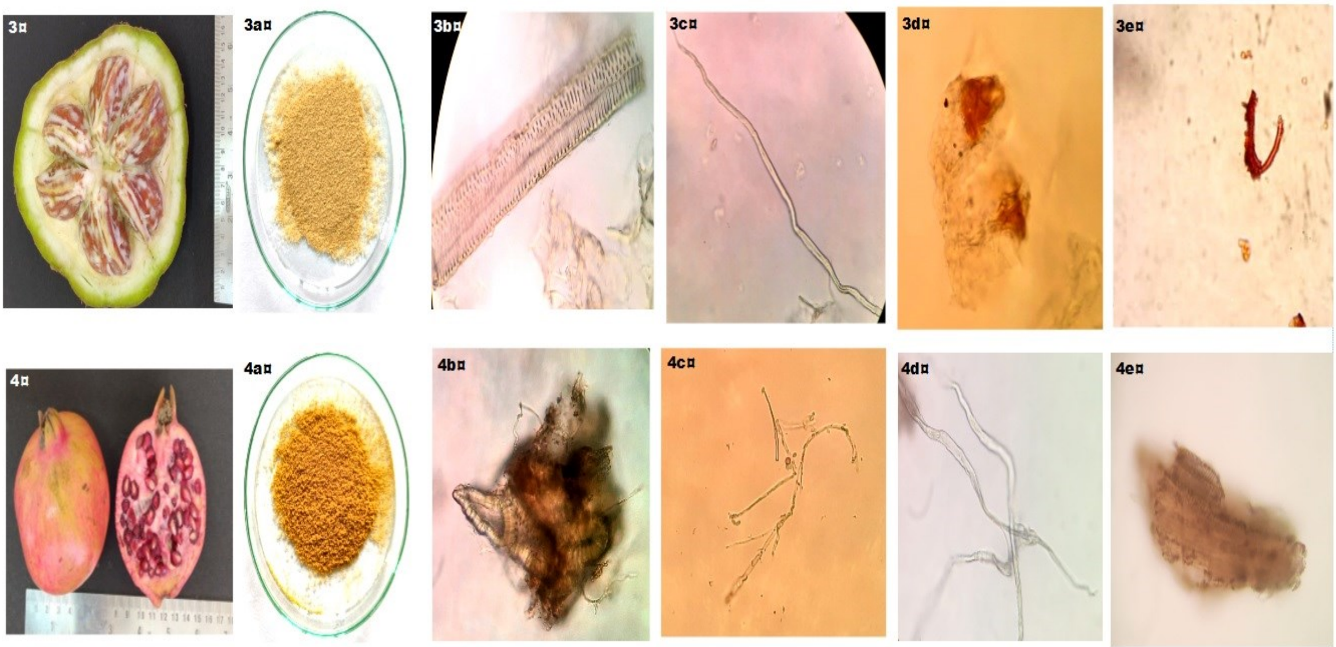

Fig. 1b. Photomicrographs of microscopic evaluation (400x) **3**. *Hodgsonia heteroclita* fruit, **3a**. *H. heteroclita* fruit pulp powder. **3b**. Xylem, **3c.** Fibre, **3d**. Stone cells, **3e**. Dwarf trichome, **4**. *Punica granatum* fruit, **4a**. *P. granatum* peel powder, **4b**. Stone cell, **4c**. Trichome, **4d**. Fibre, **4e**. Xylem.

Flow ability

Evaluation of physical parameters like the angle of repose, bulk density, tapped density, Carr's index and Hausner ratio is important in the pharmaceutical industry to determine the flow properties of drug. The angle of repose of *E. officinalis* powder was 33.99±0.49, which indicates the passable flow of powder, Carr's index was found to be 15.97±0.015 representing good compressibility and Hausner ratio was found to be 1.19±0.010, indicative of fair flow properties. These parameters were comparatively higher than reported (44). The angle of repose for *P. granatum*, *B. balcooa* and *H. heterclita* were in passbale range. The Hausner ratio was fair for *P. granatum* and *H. heterclita* but passbale for *B. balcooa*. The Carr's index for *P. granatum*, *B. balcooa* and *H. heterclita* were in the fair to passbale range. Since no work has been reported earlier, the flow ability of *P. granatum* peel, *B. balcooa* leaf and *H. heterclita* fruit pulp were evaluated for the first. The result of the physical evaluation of powder indicated that the parameters were satisfactory, as recorded in Table 4.

Heavy metal test

The incidence of heavy metals in plants reveals their purity and adulteration (29). In the current study, the heavy metals, namely cadmium, bismuth and lead, were found to be absent in all samples as summarised in Table 5, which indicates no contamination of heavy metals and thus can be safely incorporated as an ingredient of various herbal formulations. Of the 4 plant parts, the detection of heavy metals was reported only for *P. emblica* fruit (45, 46), whereas the other plants were accessed for the first time.

Conclusion

Standardisation is essential for herbal drugs to measure the quality based on the number of their active constituents. Today a newer and advanced method is available to standardise herbal drugs. According to WHO, the pharmacognostic and physicochemical characterisation may be the initial step in establishing the identity and purity of herbal plants and should be conducted before performing any tests (39). The presence of various pharmacological and phytochemical constituents in *Bambusa balcooa*, *Phyllanthus emblica*, *Hodgsonia heteroclita*, *Punica granatum* reveals that they have therapeutic potentials. The oragnoleptic and micromorphological features might help in authentication of the plant species, whereas the flow poperties is essential for various purposes such as blending, filling of capsules and tablet manufacturing. Therefore, the current study might provide helpful information with respect to its identification, validation, standardisation and the nature of adulteration. However, further research study is required for the isolation, structural elucidation and screening of active principal compounds to point out the real activity of herbal.

Table 4. Flow characteristics of the powder of various plant parts under study

Batch	Angle of repose	Bulk density (g/mL)	Tapped density (g/mL)	Hausner ratio	Carr's index
Bambusa balcooa	38.08±0.25	0.31±0.010	0.39±0.005	1.26+0.005	20.83±0.100
Phyllanthus emblica	33.99±0.49	0.60±0.015	0.72±0.010	1.19±0.010	15.97±0.015
Hodgsonia heteroclita	36.62±0.25	0.26±0.005	0.32±0.005	1.25±0.005	19.88±0.010
Punica granatum	37.72±0.36	0.35±0.010	0.42±0.005	1.22±0.005	18.23±0.050

Angle of repose = 30-40 (Passable) (28), Carr's index= 12-16 (Good), 18-31 (Fair to passable) (28), Hausner ratio = 1.19-1.25 (Fair), 1.26-1.34 (Passable) (47).

Table 5. Determination of heavy metals in various plant parts

Sample solution	Procedure	Heavy metals: Cadmium (Cd) Sample solution + NH_4OH	Cadmium (Cd) Sample solution + Potassium Ferro-cyanide	Bismuth (Bi) Sample solution + NH_4OH	Bismuth (Bi) Sample solution + H_2S	Lead (Pb) Sample solution + Dil HCl (37%)	Lead (Pb) Sample solution + KI
Bambusa balcooa	Observation	No white ppt.	No white ppt.	No white ppt.	No dark brown ppt.	No white ppt.	No yellow ppt.
	Inference	Absent	Absent	Absent	Absent	Absent	Absent
Phyllanthus emblica	Observation	No white ppt.	No white ppt.	No white ppt.	No dark brown ppt.	No white ppt.	No yellow ppt.
	Inference	Absent	Absent	Absent	Absent	Absent	Absent
Hodgsonia heteroclita	Observation	No white ppt.	No white ppt.	No white ppt.	No dark brown ppt.	No white ppt.	No yellow ppt.
	Inference	Absent	Absent	Absent	Absent	Absent	Absent
Punica granatum	Observation	No white ppt.	No white ppt.	No white ppt.	No dark brown ppt.	No white ppt.	No yellow ppt.
	Inference	Absent	Absent	Absent	Absent	Absent	Absent

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Authors contributions

AKG conceptualized and designed the study. SB, BM, MK carried out the research work and acquired the data. All the authors analyzed the data and wrote the first draft of the manuscript. Finally all the authors edited the manuscript and approved the final version for submission.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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