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

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. granatum and 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 anti-diabetic 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), anticancerous (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 of 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 antimicrobial, 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.

| Botanical Name          | Part Used | Vernacular names | GPS coordinates |
|-------------------------|-----------|------------------|----------------|
| **Bambusa balcooa** Roxb. | Leaf      | Owa burkha      | 26.494332°N 90.292971°E |
| **Phyllanthus emblica** L. | Fruit     | Amlai           | 26.4720043°N 90.2979632°E |
| **Hodgsonia heteroclita** Hook. f. & Thomson | Fruit pulp | Hagrani jwgwnar | 26.4011°N 90.2729°E |
| **Punica granatum** L. | Peel      | 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)

\[
\text{Angle of repose} = \tan \cdot \frac{1h}{r}
\]

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)

\[
\text{Db} = \frac{M}{V_b}
\]

where, \(M\) = the mass of powder, \(V_b\) = 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):

\[
\text{ptap} = \frac{M}{V_f}
\]

where, \(ptap\) = Tapped density, \(M\) = Weight of the powder, \(V_f\) = 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).

\[
\text{Carr’s index (compressibility)} = 100 \times (1 - \frac{D_b}{D_t})
\]

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)

\[
\text{Hausner ratio} = \frac{D_b}{D_t}
\]

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
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.

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

| Constituent | Chemical Test | Procedure | Bambusa balcooa (Leaf) | Phyllanthus emblica (Fruit) | Hodgsonia heteroclita (Fruit pulp) | Punica granatum (Fruit peel) |
|-------------|---------------|-----------|------------------------|-----------------------------|----------------------------------|------------------------------|
| Alkaloids   | Mayer’s reagent test | Extract + Dil. HCl + 3mL Mayer’s reagent | + | + | + | + |
|             | Dragendorff’s test | Extract + Dil. HCl + 3mL Dragendorff’s reagent | White ppt | Yellow ppt | White ppt | Bright Yellow ppt |
|             | Fehling’s test   | 1mL Fehling A + 1mL B Fehling mixed and boiled for a minute | Brick red | Brick red | Brown red | Brick red |
| Carbohydrate’s | Benedict’s test | 2 mL extract + Few drops of Benedict’s reagent + boiled for 2 min | Green | Red | Yellow | Brick red |
| Phenolics   | Molisch’s test   | Extract + Few drops of Molisch’s reagent + Conc. H2SO4 | Violet ring | Violet ring | Light | Violet ring |
|             | FeCl3 test       | Extract + FeCl3 | 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 |
|             | Salkowski test   | Extract + Few drops of chloroform + few drops of conc. H2SO4 | 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 heteroclita (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             |

**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 Embica officinalis fruit, 2a E. officinalis fruit powder, 2b Epidermis, 2c Fibre, 2d 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. heteroclita* were in passable range. The Hausner ratio was fair for *P. granatum* and *H. heteroclita* but passable for *B. balcooa*. The Carr's index for *P. granatum*, *B. balcooa* and *H. heteroclita* were in the fair to passable range. Since no work has been reported earlier, the flow ability of *P. granatum* peel, *B. balcooa* leaf and *H. heteroclita* fruit pulp were evaluated for the first time. 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 organoleptic and morphological features might help in authentication of the plant species, whereas the flow properties 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         | Cadmium (Cd) Procedure | Cadmium (Cd) Sample solution + NH₄OH | Cadmium (Cd) Sample solution + Potassium Ferrocyanide | Bismuth (Bi) Sample solution + NH₄OH | Bismuth (Bi) Sample solution + H₂S | 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 heteroclitia | 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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