INTRODUCTION

Medicinal plants have been used for thousands of years for their benefits in treating many diseases. Medicinal plants have provided therapeutic properties that have been used in healing various diseases. They range from plants that are used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparations. Herbal medicine is one the oldest forms of medical treatment, it is considered one of the forerunners of the modern pharmaceutical trade (Iwo et al., 2000). Observation, characterization, and application of specific plant parts to cure certain diseases were greatly neglected in the past. Adulteration of the herbal products hinders both scientific research and the appropriate treatment application for curing ailments. In this study, microscopic and elemental analyses were attempted for the purpose of identifying morpho-anatomical characteristics of leaves from Adhatoda vasica Nees., stems from Alstonia scholaris L. R. Br., Ephedra

Microscopic characterization of some medicinal plants and elemental analysis of Triphala (three fruits) with anticarcinogenic properties

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ABSTRACT

Medicinal plants are the local heritage and assets to a country that are to be collected, preserved, characterized, scientifically analyzed, and utilized in treating particular ailments. Several important medicinal plants from different parts of the world are yet to be indexed, characterized, and tested for their medicinal values. Adulteration (mixing cheaper materials with the original herb) is a prime concern in herbal medicine for which proper scientific characterization of specific plant parts of each is essential. In this study, characterization of some medicinally important plant species (Alstonia scholaris, Ephedra sinica, Zingiber officinale, Phyllanthus emblica, Terminalia bellirica, and Terminalia chebula) was done. Anatomical studies of transverse sections of the leaves of Adhatoda vasica Nees., stems of A. scholaris L. R. Br., and E. sinica Stapf. and fine sections of rhizomes from Z. officinale Rosc. were carried out. The characteristic presence of typical vascular bundles following double staining methods and distribution of specific types of tissues helped us to identify and standardize the constant anatomical features. The standardized anatomical features helped us to detect the particular medicinal plants species. A combination of three fruits with medicinal values is called “Triphala” consisted of “Amalaki” (P. emblica), “Bibhitaki” (T. bellirica), and “Haritaki” (T. chebula), used for boosting immunity. In the Ayurvedic medicine, Triphala is recommended as an aqueous drink. In this study, microscopic and elemental analyses were attempted for the purpose of identifying morpho-anatomical characteristics of Triphala components, which could serve as markers for the authentication of this herbal product. Thinly cut sections and macerated tissue samples of three fruit samples were mounted in 70% glycerin and observed with a Leica DM750 microscope equipped with LV-4.4 software. Starch grains, epidermal tissue, and cell wall structures were observed and compared for each species. Brown and yellow storage products, tannin, and oil droplets were found in globular cells of T. bellirica and T. chebula. Elemental analysis was carried out using an Elementar CHNS analyzer (vario MICRO cube) to determine total carbon, nitrogen, and sulfur in fruit tissue. The results showed a significant variation in the amounts of carbon between samples.

KEY WORDS: Adhatoda, Alstonia, Ephedra, microscopic characterization, Triphala, Zingiber

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sinica Stapf., and rhizome from Zingiber officinale Rosc. We also analyzed and characterized the components of Triphala, which could serve as markers for the authentication of this herbal product. Characterizing and indexing of medicinal plants of the world are extremely important today because of the great demand for medicinal compounds curing different ailments. Z. officinale has been used for multiple millennia in Asia, Arabia, and India as a medicine. It cures upset stomachs, diarrhea, and nausea. The ginger oils can be used as a preservative against microbial spoilage in food items. Adhatoda is located throughout India and a well-known drug plant in Ayurvedic and Unani medicine. It has been used for the treatment of various diseases and disorders, particularly for the respiratory tract ailments. The leaf extracts of Adhatoda are useful for the treatments of arthritis and rheumatic pain. Leaf extract is an important treatment for malarial fever. The extracts possess uterine stimulating activity, and the leaves can alleviate post-partum hemorrhage (Claeson, 2000). Alstonia is located in the Indian subcontinent and Southeast Asia. It is available for the traditional use of this plant for its cardiotonic, antiabetic, and antiarthritic properties. It has been reported as antimicrobial, antiamebic, antiarrhexial, antiplasmodal, hepatoprotective, immunomodulatory, antiabetic and antiarthritic properties, anticancer, antiasthmatic, free radical scavenging, antioxidant, analgesic, anti-inflammatory, antilacular, antifertility, and wound healing activities. Many isolated constituents from A. scholaris lack the reports of pharmacological activities, which support its further pharmacological studies. The higher dose caused lethargy in the rats. It was also more toxic, causing 30% mortality, a significant reduction in the final body weight and deformity in the various organs. High doses of A. scholaris elicit marked damage to all the major organs of the body; thus, usage of high doses for prolonged periods should be undertaken with caution. Ephedra has been predominantly grown in China. It is a stimulant which constricts blood vessels and increases blood pressure and heart rate. It is popular to use as a dietary supplement to lose weight. Ephedra is one of the most dangerous: Over 800 injuries have been reported by users and doctors to the USFDA and various state medical bodies, including more than 50 deaths. Most of the cases involve the heart attacks or high blood pressure leading to bleeding in the brain or stroke (Anonymous, 2015).

Triphala is a Sanskrit term meaning “three fruits” that include Phyllanthus emblica (Amalaki), Terminalia bellirica (Bibhitaki), and Terminalia chebula (Haritaki), used for boosting immunity. Recently, it has been found that Triphala inhibits the growth of pancreatic tumor cells in mice (Shi et al., 2008). In the Ayurvedic medicine, Triphala is recommended as an aqueous drink. Adulteration of the herbal products hinders both scientific research and the appropriate treatment application for curing ailments. In this study, microscopic and elemental analyses were attempted for the purpose of identifying morpho-anatomical characteristics of Triphala components, which could serve as markers for the authentication of this herbal product.

MATERIALS AND METHODS

Collection of Samples

We collected the plant specimens from different regions of India. A. vasica Nees., Z. officinale were collected from Mitra Garden, Bakhrabad, North 24 Pargans, West Bengal, India. North 24 Parganas is a district in southern Western Bengal, of eastern India. North 24 Parganas extends in the tropical zone from latitude 22°11’6” north to 23°15’2” north and from longitude 88°20’ east to 89°5’ east. Fresh leaves of Adhatoda (Local name Vasaka) were collected from a healthy plant from the garden, and rhizomes were dug out from the garden soil. We also collected the fresh fruits of Emblica officinale (Amalaki or Indian Gooseberry, Family: Euphorbiaceae), T. bellirica (Bibhitaki, Family: Combretaceae), and T. chebula Retz. (Haritaki, Family: Combretaceae).

Mature stems with barks from A. scholaris L., (Local name Chatim) were collected from the eastern side of the Ballygunge Science College campus, Kolkata, India.

We cut sections of the leaves from A. vasica Nees, A. scholaris L. R. Br., and stems from E. sinica Stapf. We studied the fine sections of rhizome from Z. officinale Rosc. We prepared slides from the hand cut transverse sections (T.S.) of the stems and leaves and followed by double staining with safranin (50%) and light green (90%) using differential staining and alcoholic dehydration. We collected the dried Triphala, the three fruit components from India. We examined two types of tissue samples of the Triphala after soaking them in deionized water to soften for a few days: (1) Thinly cut fine sections of to reveal the surface features, and (2) macerated fruit tissue. The tissue samples from the fruits were observed under a Leica DM750 digital microscope attached to a camera and software LAS V4.4. We captured the micrographs to compare the microscopic features of the fruits. The macerated samples revealed starch granules, plant epithelial cell, and storage products.
The elemental analysis in our investigation was carried out to determine the components of the fruits using an Elementar CHNS analyzer (vario MICRO cube); it was done to determine the percentage total carbon (TC), total nitrogen (TN), and total sulfur (TS) in this forage tissue. This method of thermal conductivity detector (TCD) measures the amount of CO₂, H₂O, SO₂, and N₂ (gas). A significant amount of carbon was recorded compared to other tissue samples and the presence of the building protein of antioxidants.

RESULT AND DISCUSSION

The characteristic vascular bundles and distribution of specific tissues helped us in the identification of the plant species under investigation. Ephedra is an herb, and the branches and tops are used to make medicine, but the root or whole plant can also be used. Ephedra is banned in the U.S. due to safety concerns. Mormon tea and ephedra are often confused. Mormon tea or American ephedra comes from Ephedra nevadensis, and ephedra or ma huang comes primarily from E. sinica. Mormon tea lacks the chemicals (notably ephedrine) that give Ephedra its effects and potentially serious side effects. Ephedra is used for weight loss and obesity and to enhance athletic performance. It is also used for allergies and hay fever; nasal congestion; and respiratory tract conditions such as bronchospasm, asthma, and bronchitis. It is also used for colds, flu, swine flu, fever, chills, headache, inability to sweat, joint and bone pain, and as a ‘water pill’ to increase urine flow in people who retain fluids (Anonymous, 2015a).

E. sinica Stapf. (Ephedraceae)

Wood anatomy

Stems and roots of E. sinica feature a single cambium with secondary phloem that formed externally and secondary xylem, internally. Rays are chiefly multiseriate, separated in broad fascicular areas. Particular fascicular areas may experience addition of secondary xylem more rapidly than others; this lack of synchronization becomes evident in diagonal orientation of ray cells that interconnect equivalent portions of adjacent fascicular areas. In rather old, E. sinica stems (diameter depends on species) distortion in the orientation of fascicular and ray portions can occur. Vessels of E. sinica are essentially solitary; if contacts between vessels occur, they appear attributable to random vessels placement in wood in which vessel density is great. There is no tendency toward vessel grouping as there is in dicotyledons that have fiber – tracheids or libriform fibers as the imperforate tracheary element type, grouping of vessels does not occur to any appreciable extent.

Constituents and medicinal uses

E. sinica, a species of ephedra (ma huang), contains ephedrine and pseudoephedrine. It has been found to stimulate the nervous system, increase airflow into the lungs, and constrict blood vessels. In combination with caffeine, ephedra appears to cause weight loss. However, effects of ephedra or ephedrine monotherapy have been mixed. Ephedrine has been widely studied for asthma and low blood pressure. However, quality research of commercial supplements with Ephedra is lacking. Major safety concerns have been associated with ephedra or ephedrine use including high blood pressure, increased heart rate, nervous system excitation, irregular heartbeat, heart attack, and stroke (Anonymous, 2015).

Ephedra is one of the most dangerous of the dietary supplements. Ephedra has been linked to stroke and heart attack. Over 800 injuries have been reported by users and doctors to the FDA and various state medical bodies, including more than 50 deaths. Most of these cases involve the heart attacks or high blood pressure leading to bleeding in the brain or stroke (Anonymous, 2015b).
**Adhatoda vasica Nees (Acanthaceae)**

**Morphology and leaf anatomy**
It is also known under the common name Malabar nut tree, and the Sanskrit name is *Vasaka*. The plant has been used in the indigenous system of medicine in India for over 2000 years (Atal, 1980). *A. vasica* is a small bushy tree. The leaves are simple, extipulate, and petiolate; about 10-20 cm long and 3.5-6 cm broad, entire, glabrous, lanceolate, acute at base and slightly acuminate toward the apex. The petiole is short, 2-8 cm long, venation pinnate, odor slight, and tastes bitter.

Upper surface is pale and lower surface is green.

A T.S. of the leaf shows a wavy epidermis with a thin layer of cuticle. The epidermis is covered with scattered 1-3 celled warty, conical covering trichomes, and small sessile quadricellular glandular trichomes. Numerous stomata are recorded on the lower epidermis. Below the epidermis, two-layered palisade cells were noted. Sometimes cells contain cystoliths, oil globules, and calcium oxalate crystals (Mitra et al., 2008). The leaf is associated with characteristic wings on both sides (Figure 2a and b). The typical centrally placed half-moon shaped vascular bundle with other anatomical features.

**Constituents and medicinal uses**

The *A. vasica* leaves contain 0.25% crystalline alkaloid vasicine and an odorous volatile oil classified as an essential oil. The leaves are rich in quinazoline alkaloids, namely vasicine, vasicinone, deoxyvasicine, vasicol, adhathodinine, and vascinol. The leaves are being used for thousands of years in Ayurvedic medicine as an expectorant for cough, chronic bronchitis, and asthma. The leaf and root extracts are used as mild bronchial antispasmodic. The volatile principle has an antitubercular activity. The marked expectorant property is clinically proven and is being used to relieve cough, liquefies sputum, and reduces cold-related ailments. Antidiabetic, antioxidant, and antibacterial activities of leaf extracts of *Adhatoda* sp. are widely reported (Soni et al., 2008; Ilango et al., 2009).

Hepatoprotective activity of *A. vasica* aqueous leaf extract was recorded by a group of researchers. In this research, they observed this hepatoprotective activity on d-galactosamine-induced damaged liver in rats (Bhattacharyya et al., 2005). A potential use of adjunctive function against tuberculosis was recommended as well (Grange et al., 1996). Although many naturally occurring antioxidants are found in *A. vasica*, and Bajpai et al. (2015) investigated the phytochemistry, antioxidant, and free radical scavenging potential of ethanolic leaf extract of *A. vasica* (ELEAV) using different antioxidant models. The phytochemical analysis of ELEAV revealed the presence of alkaloids, flavonoids, terpenoids, saponins, phenols, and steroids. During the last 30 years, several scientific reports on oxytocic and abortifacient effects of vasicine and alkaloid derived from the plant have appeared. This leads to questions concerning the safety of *A. vasica* as an herbal medicine (Claeson, 2000).

**A. scholaris L. R. Br. (Apocynaceae)**

**Anatomy of stem**

It is a large evergreen tree up to 30 m in height. The T.S. of stem shows a wavy epidermis and a hypodermis below composed of collenchyma cells. The vascular bundle is composed of wavy xylem vessels with characteristic “grooves” in between. The centrally placed parenchyma cells formed the pith (Figure 3a-c).

**Anatomy of the bark**

The bark is grayish brown, rough, lenticellate abounding in bitter, milky latex.

**Constituents and Medicinal Uses**

The bark contains alkaloids: Achitenine, echitamine (ditmine), and echitamidine together with triterpenes: α-amyrin and lupeol. The bark is bitter, astringent, acrid, thermogenic, digestive, laxative, anthelmintic, depurative, galactagogue, stomachic, cardiotonic, and tonic. It is used to cure fevers, malarial fevers, abdominal disorders, diarrhea, dysentery, dyspepsia, skin diseases, asthma, and bronchitis (Prajapati et al., 2003; Meena et al., 2011). The wide medicinal use of this tree marked it for use as a
valuable resource of herbal medicine and henceforth being cultivated all over India.

**Zingiber officinale** Rosc. (Zingiberaceae)

**Morphology and anatomy of the rhizome**

The rhizomes are smooth, white to yellowish brown in color, irregularly branched, somewhat annulated and laterally flattened. The growing tips are covered by a few scales. The T.S. of the rhizome shows the characteristic non-lignified, spiral or reticulate vessels, long brown pigment cells, fibers, starchy parenchyma, and yellow oleoresins all over the section. The brown pigment cells distributed all over the parenchymatous ground tissue (Figure 4a and b).

**Constituents and medicinal uses**

The raw ginger is acrid, thermogenic, carminative, laxative, and digestive. It is useful in anorexia, dyspepsia, pharyngopathy, and inflammations. The dry ginger is appetizer, aphrodisiac, expectorant, anethmic, and carminative. The peelings from the rhizome cure upset stomachs, diarrhea, and nausea. Ginger contains gingerol which is an anti-inflammatory, shogaol increases bile secretion, enhances GI tract activity. Ginger prevents nausea, used in arthritis treatment, heart disease by lowering cholesterol (Ghayur et al., 2005). Adulterants are common impurities associated with *Zingiber*. *Zingiber mioga* or Japanese ginger is an adulterant with genuine ginger. Another adulterant is the powdered form of ginger called exhausted ginger.

**Investigation on Triphala**

*Triphala* is composed of three fruits that are being used as growth supplement for boosting immunity for 3000 years by the Ayurvedic Medicine in India and has been known to the Indian culture to possess powerful medicinal benefits. Cancer is a global issue and looking outside the boundaries to other countries can reveal some additional insight to cure. Cancer is second in the leading cause of death in the United States at 22% (Brown, 2011) and steadfast research although has made great comprehensive conclusions dismally, bears moderate and general treatments of such an incredible adversary of mankind’s life expectancy. *Triphala* has been used in the laboratory animals to prevent pancreatic tumor (Srivastava, 2007; Shi et al., 2008). The three fruits of *Triphala* consist of *E. officinale* (Amalaki or Indian Gooseberry, Family: Euphorbiaceae), *T. bellirica* (Bibhitaki, Family: Combretaceae), and *T. chebula* Retz. (Haritaki, Family: Combretaceae). The combination of these fruits into a powder form to mix and drink has been validated for gastrointestinal issues such as constipation, gastric hyperacidity, improved appetite, and reduced dental caries. Based on Ayurvedic evidence, each fruit exhibits unique features:

*T. chebula* “Haritaki”: Liver wellness and digestion for improved appetite and anti-inflammatory.

*P. emblica* “Amla” or “Gooseberry”: Free radical scavenging and super antioxidant.
T. bellirica “Vibhitaki” or “Bibhitaki”: Antibacterial, anti-inflammatory, and laxative.

We collected the dried fruits of Triphala (Figure 5a-c) from the trees (Figure 6a-c) analyzed the component of Triphala digital microscopy and the carbon-nitrogen-sulfur (CNS) analyzer to assess the tissue and CNS content of the three fruits. We examined two types of tissue samples of the Triphala after soaking them in deionized water to soften for a few days: (1) Thinly cut fine sections of to reveal the surface features, and (2) macerated fruit tissue. The tissue samples from the fruits were observed under a Leica DM750 digital microscope attached to a camera and software LAS V4.4. We captured the micrographs to compare the microscopic features of the fruits. The macerated samples revealed starch granules, plant epithelial cell, and storage products. The elemental analysis in our investigation was carried out to determine the components of the fruits using an Elementar CHNS analyzer (vario MICRO cube); it was done to determine the percentage TC, TN, and TS in the tissue extracted from the fruits. This method of TCD measures the amount of CO₂, H₂O, SO₂, and N₂ (gas) and it has been used to determine the nutrients and active principle of fruits and vegetables in recent years (Missanjo et al., 2015). A significant amount of carbon was recorded compared to other tissue samples and the presence of the building protein of antioxidants (Figure 7).

From the microscopic examinations, we recorded starch grains and fibers (xylem vessels and tracheids). The abundance of starch granules and intense activity demonstrates the complex carbohydrates by size can play an important role in the efficacy of absorbed nutrients through Triphala’s acclaimed properties (Mukherjee et al., 2006). We also analyzed the components with a CNS analyzer. A CNS analyzer was used to detect the percentage of carbon, nitrogen, and sulfur content in the Triphala dried fruit samples. First, measures were taken to calibrate the CNS Analyzer (Figure 8a). The samples were loaded on the Aluminum boat (8c) the Aluminum boat (8c) and each sample was run within the carousel (Figure 8b) for 10 min. The temperature and time were synchronized to obtain accurate result. A few blanks and three samples of sulfanilamide were run to calibrate the equipment and standardize the run. A computer program allowed us to detect the CNS ratio presented on the computer screen that can be saved as an Excel spreadsheet (Figure 8d).

The most integral portion of the results is the percentage of carbon, nitrogen, and sulfur. The percentage shows high content of carbon:

Figure 5: (a) Phyllanthus emblica (Amla). (b) Terminalia chebula (Haritaki). (c) Terminalia belarica (Bibhitaki)

Figure 6: (a-c) The fruits of Triphala on trees: (a) Phyllanthus emblica (Amla). (b) Terminalia belarica (Bibhitaki). (c) Terminalia chebula (Haritaki)

Figure 7: (a-f) Microscopic examination on: (a) Phyllanthus emblica surface; (b) Terminalia chebula surface; (c) P. emblica internal tissue; (d) Terminalia bellirica surface; (e) T. chebula tissue; (f) T. chebula surface crystals
Determinately, Triphala has proven health benefits having unique phytochemical properties as each fruit was analyzed with a CNS analyzer (Table 1). The starch granules and viscous and nonviscous fiber present in the fruits also demonstrates the potential efficacy of absorption into the gastrointestinal tract and the ability to bind in antioxidant activity is very promising. Simply gaining knowledge from all aspects of what we consume and the amount will further enhance cancer prevention (Srivastava, 2007). “Adulteration” means mixing cheaper or unidentified materials to a comparatively expensive herbal product. Adulteration interferes with the proper treatment of ailments with herbal products. In most of the cases, the adulterated components lack the phytochemical properties and fail to cure a specific ailment. Sometimes, the adulterated products can cause significant harm to the patients by increasing the ailments without any cure. Present investigation was aimed at characterizing the Triphala by microscopic (Figure 7a-f) and elemental analysis (Table 1, Appendix). This will aid in proper identification of the three fruit components, helping to cure ailments effectively.

The uses of the natural plant botanicals are used to treat and prevent illness. They can be applied directly on the source of illness, or otherwise used in teas and extracts. This analysis obtained different indications of healing properties in a particular diminutive amount of medicinal plants. Recently, an increased interest for the herbal medicines has noted since the application of herbal medicines and plant parts for curing ailments have little or no side effect on health conditions. Adulteration of the herbal products hinders both scientific research and the appropriate treatment application for curing ailments. In this investigation, we collected leaves, roots, and fruits from authentic sources and cut T.S. to observe and standardize constant anatomical features. We used digital microscopy to capture images using light and fluorescence. For Triphala that is composed of three fruits, we observed the surface features and captured digital images after macerating the tissue at different magnifications. We also recorded the unique phytochemical properties as each fruit of Triphala by analyzing with a CNS analyzer. Identification of plants is important because all medicinal plants have specific features in each genus. Each herbal source that we investigated are proved to possess important medicinal value; however, they all possess toxic effects depending on the dose applied.

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