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The use of microstructures in the authentication of powdered drug plants

[1§]Abdulrahaman Alanamu Abdullahi, [2]Al Sahli Abdulaziz Abdullah,[1]Tinuola Abimbola Aluko, [1]Adeniran Sunday Adebunmi, [2]Sagaya Abdulquadri

[1]Applied Plant Anatomy and Wood Technology Laboratory, Department of Plant Biology, University of Ilorin, Ilorin, Nigeria

[2]Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia

[§]Corresponding author: **Abdulrahaman Alanamu Abdullahi**. Email: abdulrahamanaa@unilorin.edu.ng

Abstract

Adulteration and substitution of herbal drugs are trending issues in the herbal industry, posing a serious threat to commercial natural product research. The anatomy of powdered and non-powdered samples of plant species were compared to ascertain their similarities. Air dried powdered leaf samples and unground or intact leaves, flowers and barks of eight medicinal plant species, namely, *Vernonia amygdalina*, *Ocimum gratisimum*, *Trichilia monadelpha*, *Bridelia ferruginea, Lophira alata*., *Alstonia boonei, Dialium guineense* and *Enantia chlorantha* were studied anatomically with the aim of identifying the original plant parts used in the preparation of the drugs. The microscopic studies of leaves of *V. amygdalina* and *O. gratisimum* revealed the presence of similar stomatal complex types and trichomes in both ground and unground samples. The anatomy and palynology of *T. monadelpha* flower revealed that bipolar, inaperturate, monopolar, monoporate, tetracolporate and triporate pollens are present in both the ground and unground samples. The microscopic study of the barks of *L. alata, B. ferruginea, A. boonei, D. guineense* and *E. chlorantha* also showed similar cells in ground and unground samples. The anatomical features are, therefore, elucidated for authentication of the originality of the medicinal plants studied.

Keywords: Adulteration, authentication, palynology, plant anatomy, microstructure, plant drug

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INTRODUCTION

Herbal medicines play an important role in all traditional medical systems. Herbal medicine is a victory of therapeutic variety among the general public. Botanical medicine, also known as phytomedicine, is defined as the use of a whole plant or a component of a plant to prevent or treat sickness. (Kumar, 2005). Individuals and communities alike benefit greatly from medicinal plants. Plants have medical value because they contain chemical compounds that have a specific physiological effect on the human body. Alkaloids, tannins, flavonoids, and phenolic chemicals are the most important bioactive elements of plants. Many of the region's medicinal plants are also used as spices and food plants. They are also occasionally added to foods meant for pregnant women and nursing mothers for medicinal purposes (Okwu, 1999). Traditional medicine uses medicinal plants, which are known to contain a variety of compounds and are used to cure a number of maladies. Most important is that they are taken by the majority of the population because they are inexpensive and available (Sofowora, 1982). According to the World Health Organization (WHO), roughly 80% of the population in underdeveloped nations relies nearly entirely on traditional medicine (Adeyemi *et al*., 2009).

The lack of standardization and quality control profiles is one of the accusations leveled at herbal medicine. The correct identification of the species in question, whether fresh, dried, or powdered, is critical in terms of quality control (Springfield *et al.* 2005). In the formulation and administration of herbal medicine, incorrect species classification and substitution pose a serious threat (Opara, 2004). Some plants appear so similar to the untrained eye that they are frequently confused. Over 80% of medicinal plants are taken from the wild or from local markets, where they are sometimes contaminated; collectors frequently rely on their skill in identifying the kind of plants being collected (Menon, 2003). Plant taxonomists and other specialists are rarely used for authentication. As a result, mixing of related/allied species, as well as other unrelated taxa, is fairly unusual. The apparent mismatch in vernacular names between the traditional system of medicine and local dialect, the lack of legitimate plants, comparable morphological traits, and other factors have all been blamed for species admixtures (Mitra and Kannan, 2007).

Proper control of starting materials is critical for ensuring repeatable quality of herbal products. Authentication is the initial stage in assuring the quality of starting material. Despite current technology, the global health organization (WHO, 2000) states that the macroscopic and microscopic description of a medicinal plant is the first step toward verifying the identity and degree of purity of such material and should be done before any test is performed. Adulteration is the practice of partially or completely replacing the original crude drug with other substances that are either free of or inferior in therapeutic and chemical properties, or adding low grade or spoiled drugs or entirely different drugs that are similar to the original drugs substituted with the goal of increasing profits (Kokate *et al*., 2007; Mukherjee, 2002). The trust in herbal medicines has dwindled as a result of adulteration (Dubey *et al.,* 2004). One of the most serious problems in promoting herbal goods is adulteration in market samples. Researchers have contributed to the verification of adulterations and authentications (Tewari, 1991; Gupta, 2003; Saraswathy, 2001).

Many commercially accessible medicinal plants are still unable to be authenticated or recognized using morphological or histological properties, necessitating the application of anatomical features. The epidermal features and stomata ontogeny of some Nigerian medicinal plants have been discovered to be important in their identification (Gill and Karatela, 1985). Plant epidermal and cuticular traits, type and arrangement of stomata, size and shape of trichomes, and number of vascular bundles, according to Edeoga and Osawe (1996) and Mbagwe and Edeoga (2006), could be useful tools in resolving taxonomic difficulties in plants.

In this study, eight medicinal plants, namely *Alstonia booeni* (L.) R. Br., *Bridelia ferruginea* Willd., *Dialium guineense* Willd., *Enantia chlorantha* Oliv., *Lophira alata* Banks ex Gaertn., *Ocimum gratissimum* L., *Trichilia monadelpha* (Thonn) JJ De Wild. and *Vernonia amygdalina* Delile were used. These chosen plants exhibit different, unique characters and are used for curing ailments. For instance, *T. monadelpha* of the family Meliaceae, known and called “Itana” or “Ajanrere” among the Yorubas of Western Nigeria is an important medicinal plant, and particularly a bark decoction or the pulped bark are applied externally to wounds, sores, skin affections including yaws, lumbago and oedema. A bark decoction is drunk to soothe cough, as an

analgesic and anthelmintic, and to treat gonorrhea and syphilis, whereas small amounts of pulped bark are eaten or applied as an enema to treat gastrointestinal complaints. Bark decoctions serve as an aphrodisiac, ecbolic and abortifacient. A leaf decoction is taken to treat heart complaints, and pounded leaves to treat gonorrhea and lumbago (Silvia *et al.,* 2015). *Vernonia amygdalina*, a member of the Asteraceae family, is commonly called bitter leaf in English because of its bitter taste. *V. amygdalina* is used in traditional medicine for diseases such as diarrhea, fever, and tonic and as antihelmentic (Dalziel, 1956). The plant is also used as a vegetable in food preparation. *Occimum gratissimum* belonging to Lamiaceae family is a medicinal plant, it has been used in the traditional treatment of various ailments such as diarrhea, fever, pneumonia and skin diseases (Holetz *et al.* 2003). Also, several workers have reported on the antimicrobial/antibacterial and antifungal properties of the plant (Ngossoum *et al*., 2003; Iwalokun *et al*., 2003).

The aim of this study, therefore, is to use the knowledge of plant anatomy (presence of microstructures in the powdered and non-powdered samples) to identify the constituents of plant drugs in order to authenticate their originality.

MATERIALS AND METHODS

Plant material collections and preservation

The plant materials (i.e. leaves, flowers and barks) of eight medicinal plants (Table 1) were collected from the natural habitat and some were bought from the local markets in Ilorin, Kwara State, Nigeria. The plants were identified in the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The collected plants were washed with distilled water to remove dust and adhering materials and were preserved by spreading them on the newspaper and dried for 3 – 4 days in the shade.

Powdered drug preparation

Plant materials that had been dried were cleaned and cut into small bits. To obtain powder material, samples were ground in a grinder and sieved with muslin fabric. To avoid contamination, each sample was wrapped in its own piece of cotton. Fresh leaves were separated and utilized to examine microscopical characteristics.

Microscopic examination of bark

The sections and powder samples were stained with safranin, and 1 to 2 drops of glycerin was added and observed under the microscope. Fixation of bark was done by cutting and fixed in an FAA solution (Formalin-5mL + Acetic acid-5mL + 70% Ethanol-90mL). Dehydration of specimen: after 24h fixing, the bark was graded with a series of tertiary butyl alcohol (TBA) using the method of Silvia *et al.* (2015). Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12µm. The sections were stained with safranin and observed under the light microscope.

Isolation, fixation and identification of pollen

The pollen (for the ungrounded) was smeared on a glass slide with the aid of a spatula while a small portion of the powdered sample was placed on the glass slide, and two (2) drops of isopropyl alcohol (IPA) were added to it for about 5 mins in order to remove the waxy surface from the pollen. Drop of glycerine was added to it and a cover slip was placed on it. The slide was viewed with the aid of a light microscope. Observations were recorded with photomicrographs of pollens (Horrocks *et al.,* 1999) as amended by Abdulrahaman *et al.* (2013).

Determination of leaf epidermal features

The leaf segments of an area of 1cm square were cut and immersed in concentrated nitric acid (HNO_3) for maceration for 24 hours. The upper (adaxial) and lower (abaxial) epidermal surfaces were separated by using dissecting needle and forceps after being rinsed in distilled water. Small portions of the macerated leaf were stained in 1% aqueous solution of safranin for about 3 to 5 minutes. Excess stain was removed using distilled water. The specimen was then mounted in glycerin for microscopic study to determine the stomatal complex types, ordinary epidermal cells and trichomes. Terminology used in respect of the stomatal complex types and the trichome types followed those of Dilcher (1974) and Metcalfe and Chalk (1988).

Table 1: List of medicinal plants and the parts used

Plant name	Family	Local name	Common name	Part of plant used	Voucher number
***Bridelia ferruginea* Willd.**	Euphorbiaceae	Ira, iraodan, iraeju, Kirni, Ola, okukuu.	Ira	Bark	UIH 003/987
***Lophira alata* Banks ex Gaertn.**	Ochnaceae	Ekki, Pahan, uda, Kujeme, Akufo	Ironwood, meni oil tree	Bark	UIH 004/1090
***Alstonia booeni* (L.) R. Br.**	Apocynaceae	Awun, ahun, eghu, akpi	Stool wood, pattern wood	Bark	UIH 005/040
***Dialium guineense* Willd.**	Leguminosae	Awin, Icheku, Tsamiyar Kurmi	Black tamarind, tumble Tree	Bark	UIH 006/1064
***Enantia chlorantha* Oliv.**	Annonacease	Awopa or Dokitaagbo	African yellow wood	Bark	UIH 007/1091
***Vernonia amygdalina* Delile**	Compositae	Ewuro	Bitter leave	Leaf	UIH 001/1023
***Ocimumgratissimum* L.**	Lamiaceae	Efinrin	Scent leave	Leaf	UIH 002/984
***Trichilia monadelpha* (Thonn) JJ De Wild**	Meliaceae	Itana, Ajanrere		Flower	UIH 008/312

RESULTS AND DISCUSSION

The anatomy of the powdered and non-powdered samples of the studied plant species were compared to reflect the similarities that existed between them (Figs. 1 - 3). From Fig. 1, it is clearly shown that the leaf epidermis of both fresh (ungrounded) and powdered leaves of *V. amygdalina* and *O. gratissumum*. Leaf epidermal features such as stomatal complex types, trichomes, and ordinary epidermal cells are present. Many epidermal features such as stomata, trichomes, and pollens were observed in both ground and non-ground flower samples of *T. monadelpha*. Floral anatomy of *T. monadelpha* revealed that the flower possessed unicellular trichomes in both the grounded and ungrounded samples. It was also shown through the palynological study that the flower has different types of pollens which includes; triporate pollen, bipolar pollen, tetracolporate pollen, monoporate pollen, inaperturate pollen and monopolar pollen both in the grounded and ungrounded samples (Fig. 2). Bark anatomy of *B. ferruginea*, *L. alata, A. boonei, D. guineense* and *E. chlorantha* showed some resemblances and differences in samples of ground and non-ground barks (Fig. 3).

In the herbal industries, there are many factors responsible for drug adulteration and substitution which have undermined its potency. The act of adulteration and substitution may be intentional and/or unintentional reasons depending on the aims of the herbal dwellers. Mitra and Kannan (2007) gave a list of some reasons which they assumed are the cause of unintentional adulteration such as name confusion, lack of knowledge about authentic source, similarity in morphology, lack of authentic plant, similarity in colour and careless collections of plant samples. Majorities of these reasons, if not all are prerequisites for this present study where the anatomical studies were used to proffer solution to the inherent problems of lack of trust in herbal drugs.

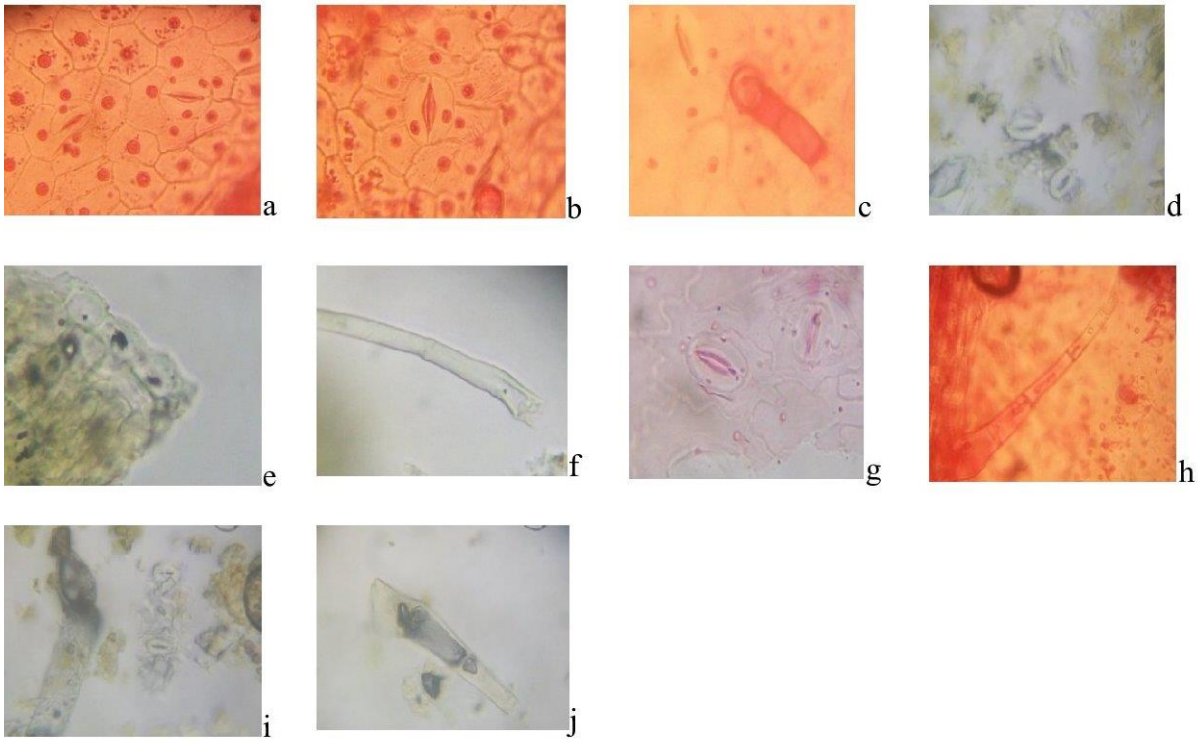

Figure 1: Structures of *Vernonia amygdalina* showing (a) tetracytic, (b) anomocytic stomata and (c) trichome in ungrounded leaf, (d) stomata, (e) epidermal cells and (f) trichome in grounded leaf. Structure of *Ocimum gratissimum* (g) stomata, (h) trichomes in ungrouded leaf, (i) and (j) trchomes in grounded leaf x600

The anatomical characters available in the powdered samples are much fewer than in non-powdered specimens. The difference is attributable to the damage of the plant cell wall during grinding preparation, causing distortion in tissue arrangements and patterns normally found in the ungrounded plant samples. This aspect of micromorphology of medicinal plants is yet to be studied keenly; hence literature is very scanty on it.

Few studies have been carried out in the aspect of proper identification of ground plant leaf samples (Adeniyi, 2009). The evaluation of a crude drug is a vital and a very essential part in establishing its exact identity and quality. Before the inclusion of a crude drug in an herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Therefore, in the present study, some diagnostic features have been evolved to identify some of the commonly found drug plants through the anatomical studies. From the work done in this study, it has been shown that tichomes are very essential in idenifying a plant. This is so because, the trichome types can still be traced even in powdered form; unlike stomatal complex type which is a bit difficulty be identified properly once in powdered form.

Presence of multicellular epidermal hairs in both ground and non-ground leaves of *V. amygdalina* is in corroboration with the findings of Ahlam and Bouran (2011). Similarly the stomatal types present are anomocytic and anisocytic. These were also reported by Metcalfe and Chalk (1988) and Ibrahim *et al.* (2004). In *O. gratissimum*, the abaxial surface showed diacytic and anisocytic types of stomata while the adaxial surface showed only diacytic stomata. The work of Hemlata *et al.* (2010) reported the presence of diacytic stomata on both the abaxial or adaxial surfaces of *O. gratissimum.* The trichomes and ordinary epidermal cells are also prominently shown in the two species (i.e. *V. amygdalina* and *O. gratissimum*).

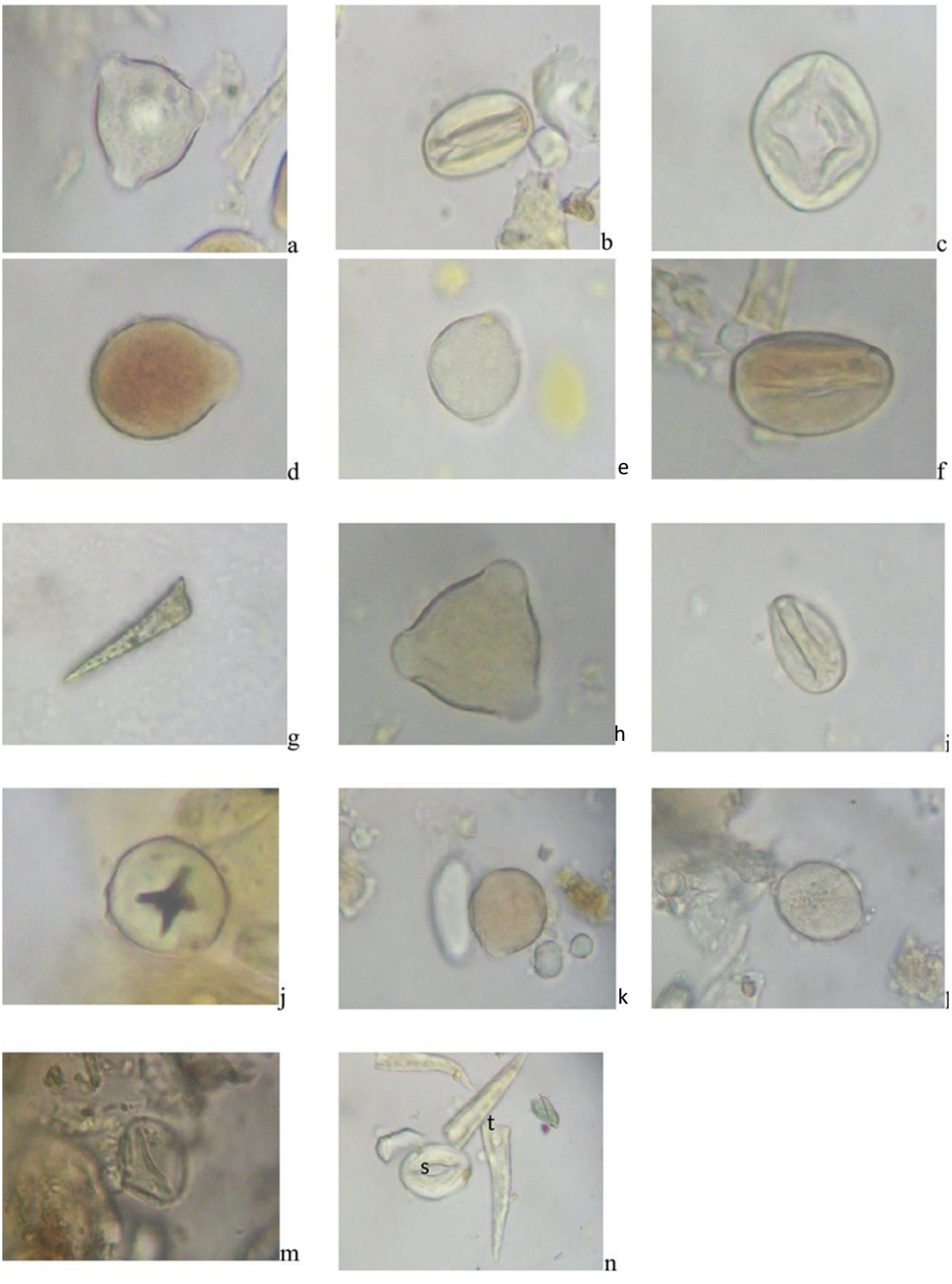

Figure 2: Leaf specimens of *Trichlia monadelpha* showing unground leaf surfaces with triporate (a), bipolar pollen (b), tetracolporate pollen (c), monoporate pollen (d), inaperturate pollen (e), monopolar pollen (f), and powdered leaf samples with unicellular trichome (g), triporate pollen (h), bipolar pollen (i), tetracolporate pollen (j), monoporate pollen (k), inaperturate pollen (l), monopolar pollen (m) and stoma and unicellular trichomes (n) x600

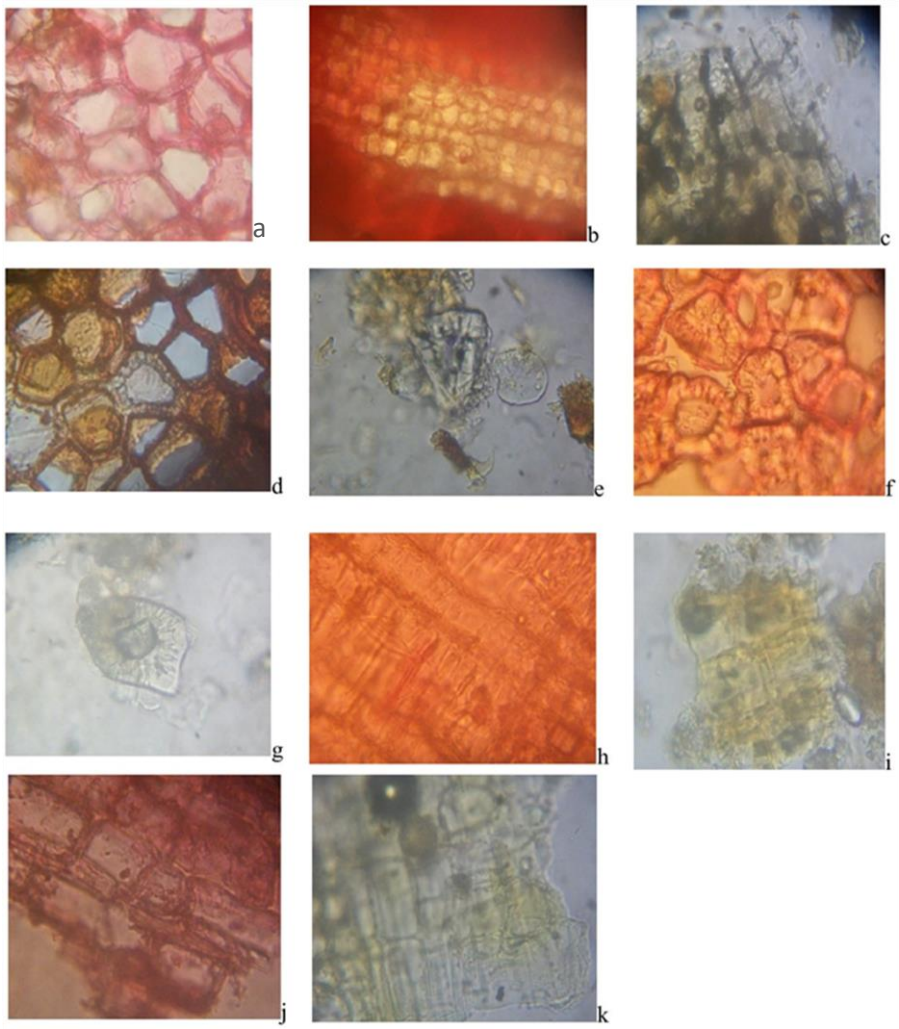

Figure 3: Anatomy of bark of *Bridelia ferruginea* [(a & b) unground, (c) ground samples]; *Lophira alata* [(d) unground, (e) ground samples]; *Alstonia boonei* [(f) unground, (g) ground samples]; *Dialium guineense* [(h) unground, (i) ground samples] and *Ecantia chlorantha* [(j) unground, (k) ground samples] x600

The presence of similar epidermal cells and cell wall patterns in the bark of *A. booeni*, *B. ferruginea*, *D. guineense*, *E. chlorantha* and *L. alata* and, same pollen types and occasional presence of trichomes and stomata in the flowers of *T. monadelpha* in both ground and non-ground samples are indications of the usefulness of anatomical evidence in crude drug plant identification. The anatomical features observed are diagnostic for a species, and hence are good characters that can be employed in delimiting taxa of plants.

It can, therefore, be concluded that the presence of microstructures in the powdered samples of the drug plants can be used to authenticate the originality of the plant materials used. The study of the leaf anatomy of *O. gratissimum* and *V. amygdalina*, flowers anatomy and palynology of *T. monadelpha* and bark anatomy of *A. booeni*, *B. ferruginea*, *D. guineense*, *E. chlorantha* and *L. alata* can serve as an evidence and an important source of information to ascertain the identity of these plants. The anatomical features of each species are diagnostic features of identifying each of these species either intact or ground form. Similar works were carried out by Pacheco-Silva and Donato (2016) and Kotina *et al*. (2014) in *Myrciaria glomerata* and *Warburgia salutaris* respectively, where the anatomy of leaf and bark were used as diagnostic features of these plants.

Therefore, it is suggested that drug plants should be subjected to various tests like the anatomical analysis, along the proximate and

phytochemical analyses, among others, to ascertain the true components of the drug plants before their release to the market in order to provide a broad basis for comparison.

Conflicts of Interest

The authors have no conflict of interest to declare.

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