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IDENTIFICATION AND AUTHENTICATION OF DRY SAMPLES OF SOME MEDICINAL PLANTS USING LEAF EPIDERMAL FEATURES AS MARKER

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ABSTRACT
Herbal medicine is the oldest and still the most widely used system of medicine in the world today and they are made exclusively from plants. However, most of these medicines or drugs are adulterated due to lack of proper identification of the plant samples. Method of checking adulteration of drug plants is the main focus of this study. The identification and authentication of dry samples of some medicinal plants were carried out using anatomical features. Twenty-five (25) plants materials were collected in Ibadan and Ilorin, Nigeria. The plants studied include Azardiracta indica, Newboudia leavis, Polyalthia longifolia, Cymbopogon citratus, Anarcardium occidentalis, Nicotiana tobbaccum, Jatropha curcas, Chromoleana odorata, Mangifera indica, Terminalia catappa, Ocimum gratisimum, Morus messosygia, Morinda lucida, Psidium guajava, Vitellaria paradoxa, Annona senegalensis, Vernonia amygdalina, Gliricidium sepium, Ravoulvia vomitora, Telferia occindentalis Citrus aurantifolia, C. limon, C. paradisi and C. sinensis. Leaf epidermal anatomy of these selected plants showed no major variations in stomatal complex types, frequency, size and shape of stomatal cells, epidermal cell wall and trichomes between fresh and dry samples. The variations that occur were between different species but not within species. Leaf epidermal anatomy, therefore, proved to be a significant tool for resolution of taxonomic confusion of dried samples of these plants.
KEY WORDS: *leaf epidermis anatomy, medicinal plants, leaf samples, taxonomy*

INTRODUCTION

Herbal medicine is a popular means of medical management in different part of the world such as Africa, India, and China. Practitioners and individuals use both fresh and dried specimens of herbs. The source of dry specimen may be obtained in the local market where adulteration of herbal materials takes place or herbarium specimen may be used. Epidermal structures and stomata ontogeny of some medicinal plants found in Nigeria have been found to be relevant in their recognition (Gill & Karatela, 1985). Many workers such as Edeoga & Osawe (1996), and Mbagwe & Edeoga (2006) stressed that epidermal and cuticular traits of plant epidermal cells, types, and arrangement of stomata, size and shape of trichomes and number of vascular bundles could serve as vital tools in solving taxonomic problems in plants.

Over 80% of the medicinal plants used are predominantly collected from the wild or local markets where they are adulterated; collectors often rely on their experience in identifying

the species of plants being collected. Services of specialists such as taxonomist are rarely availed for authentication (Anon., 2003). Thus, it is not uncommon to find admixtures of related/allied species and infrequently also of other unrelated genera. Among the reasons attributed for species admixtures are the apparent confusion in vernacular names between indigenous system of medicine and local dialect, non-availability of authentic plants, similar in morphology features (Mitra & Kannan, 2007). The possibility of admixture is particularly high when the species in question co-occur with morphological similar species. Frequently, admixtures could also be deliberated due to adulteration (Mitra & Kannan, 2007). The consequence of species admixtures can range from reducing the efficacy of the drug to lowering the trade value (Wieniawski, 2001), besides threatening the safety of herbal medicines (Song *et al*., 2009).

Considering the adverse consequence of such species admixture may have on the eventual drug efficacy. It is imperative that the admixtures are avoided in raw herbal trade and where existing, methods developed to identify the admixtures in recent years, efforts have been made to accurately identify medicinal plants used in treating ailments to ensure the purity, quality, and safety (Jayasinghe *et al*., 2009). Adulteration can occur due to ignorance or intentional substitution with cheaper plant material and may cause damage to human body (Jordan *et al*., 2010). Therefore, authentication is necessary. The general approach to herbs identification is dependent on morphological, anatomical, chemical and molecular (Stern *et al*., 1994; Li *et al*., 2005; Li *et al*., 2010) techniques.

Exact identification of medicinal plants is necessary to ensure safety, because in most cases medicinal plants are knowingly or unknowingly substituted or adulterated with similar species or varieties (Kiran *et al*., 2010). Many medicinal plants commercially available still cannot be authenticated or identified using their morphological or histological characteristics, hence, the use of anatomical characteristics. Plants of different species may possess similar morphology in dried state, and is being sold locally or used clinically as a replacement at relatively low prices under the same trade name, and this has reduced the efficacy and quality of the plants.

The world health organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine; the medicinal plants play a major role and constitute the backbone of the traditional medicine. Despite the modern techniques, identification of plants, according to the world health organization (WHO, 2000), the macroscopic and microscopic description of a medicinal plants is the first step towards establishing the identity and the degree of purity of such material and should be carried out before any test is undertaken.

Anatomical studies have been used successfully to clarify taxonomic status and help in the identification of different species (Gilani *et al*., 2002; Ianovici *et al,* 2011). When herbs are dried and folded, identification and authentication becomes rather difficult. Hence, an approach to overcome the problem of identification is the use of leaf anatomical features of the plant materials. In this work, the dry and fresh leaf samples of twenty-five medicinal plants were studied anatomically to establish relationships between them.

MATERIALS AND METHODS

Study materials. Leaves of twenty-five species of different genera of angiosperm (Table 1) were collected both at University of Ilorin main campus, Ilorin, Nigeria and Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The specimens were identified and authenticated at the Forest Herbarium, Ibadan (FHI), Nigeria and Herbarium Unit of Department of Plant Biology, University of Ilorin, Nigeria. The field collection was done for a period of seven months between September, 2011 and March, 2012. Both fresh and dried leaves samples were used. The dried samples were in form of herbarium specimens.

TABLE 1. List of some selected medicinal plants

Species	Families	Common name	Medicinal Uses
Anacardium occidentalis	Anacardiaceae	Cashew (E), Kaju (Y)*	Malaria, elephantiasis, leprosy, ringworms, scurvy, diabetes, warts, typhoid fever, caries; Antihelmintic (Olowokudejo *et al.*, 2008).
Annona senegalensis	Annonaceae	wild custard apple, wild soursop (E), Abo (Y)	Blood purification (Awoyemi *et al.,* 2012).
Azardiracta indica	Meliaceae	Neem plant (E), Dongoyaro (H, Y)	Malaria, jaundice, syphilis, eczema, ringworm, sore throat, emetic, laxative (Olowokudejo *et al.*, 2008).
Baphia nitida	Fabaceae	Irosun (Y)	Constipation, skin diseases, venereal diseases, ringworm, enema, flatulence, smallpox (Olowokudejo *et al.*, 2008).
Chromoleana odorata	Asteraceae	Akintola (Y)	Antimicrobial, dysentery, headache, fever, malaria, toothache, haemostatic, skin diseases (Olowokudejo *et al.*, 2008).
Citrus aurantifolia	Rutaceae	Lime (E), Orombo weere (Y)	High blood pressure, stroke, low sperm count and cough (Awoyemi *et al*., 2012).
Citrus limon	Rutaceae	Lemon (E), Osan lemu (Y)	Applied locally, the juice is a good astringent and is used as a gargle for sore throats.
Citrus paradise	Rutaceae	Grape (E), Osan paya(Y)	Antibiotic (Aiyeloja and Bello, 2006).
Citrus sinensis	Rutaceae	Sweet Orange (E), Osan mimu (Y)	Leaf decoction with salt is taken orally for digestive tract ailments, nerve disorders, fever, asthma, blood pressure, general fatigue and vomiting (Orwa *et al*., 2009)
Cymbopogon citrates	Poaceae	Lemon grass (E), Ewe tea (Y)	Malaria, Rheumatism and nervous-disorder (Awoyemi *et al*., 2012).
Gliricidium sepium	Papilioniaceae	Agunmaniye (Y)	Rheumatism (Lawal *et al.,* 2010)
Jatropha curcas	Euphorbiaceae	Botuje, Lapalapa (Y)	Ringworm, eczema, scabies, fever, Guinea worm, herpes, rectal eczema, black tongue, whitlow, impotence, irregular menses, convulsion, small pox (Olowokudejo *et al.*, 2008).
Magnifera indica	Anacardiaceae	Mango (E), Mangoro (Y)	Malaria, diarrhoea, diabetes, hypertension, haemorrage, insomnia, insanity, asthma, cough; Astringent, anthelmintic, emmenagogue (Olowokudejo *et al.*, 2008).
Morinda lucida	Rubiaceae	Oruwo (Y)	Fever, antimalarial (Lawal *et al*., 2010).
Morus mesozygia	Moraceae	African mulberry (E) Aye (Y)	The local population use the roots, the stem and the leaves to treat traditionally: syphilis, dermatitis, rheumatism, asthenias, fever and malaria (Burkill, 1997).
Newboudia leavis	Bignoniaceae	Akoko (Y)	Infertility (Lawal *et al*., 2010); anti-inflammatory and analgesic (Olajide, 1997).
Nicotiana tobaccum	Solanaceae	Tobacco (E), Taba (Y)	Hot water extract of the fresh leaf is taken orally as a sedative (Adesina, 1982).

Ocimum gratisimum	Labiatae	Scent Leaf (E), Efinrin (Y)	Cough, diarrhoea, convulsion, fever, cold, bronchitis, diabetes, pile, antimicrobial, antibacterial, antihelmintic,insect repellant; colic (Olowokudejo *et al.*, 2008).
Polyalthia longifolia	Annonaceae	Mast Tree, Azoka Tree, Sorrow-less tree (E)	The plant has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis (Kirtikar and Basu, 1995).
Psidium guajava	Myrtaceae	Guava (E), Gurofa (Y)	The leaves of the guava tree in decoction ulcers, vaginal and uterine problems and where an astringent remedy is needed (Taylor, 2005). Also, it has been used for fevers, worm infections, kidney dysfuctions, epilepsy, and diabetes and even for cerebral infections (Taylor, 2005).
Ravoulvia vomitoria	Apocynaceae	Swizzle stick (E), Asofeyeje (Y)	Leaves for treating yellow fever, internal pains, gastroenteritis, constipation and mental disorder. It is useful in the lowering of blood pressure (Amole, 2003).
Telferia occindentalis	Cucurbitaceae	Pumpkin (E), Ugwu (I)	Convulsion, gastrointestinal disorders, blood tonic, anaemia (Olowokudejo *et al.*, 2008).
Terminalia catappa	Combretaceae	Almond (E), Furutu (Y)	The juice of the leaves is used as a folk remedy against various ailments that damages the skin, such as scabies and leprosy, the leaves themselves are used to dress rheumatic joints (Chikezie, 2011).
Vernonia amygdalina	Asteraceae	Bitter leaf (E), Ewuro (Y)	Measles, stomach ache, ringworm, toothache, gingivitis, pneumonia, malaria, diabetes (Olowokudejo *et al.*, 2008).
Vitellaria paradoxa	Sapotaceae	Shea butter tree Igi Emi, Igi Ori (Y)	Nasal decongestion, catarrh, hypertension, diuretic, anthelminthic (Olowokudejo *et al.*, 2008).

*Language E=English; Y=Yoruba; I=Igbo

Isolation of leaf epidermal layer. Leaf segments of an area of 1cm square from 35 leaves of each species were cut and immersed in 20% chromium trioxide for maceration (Alvin & Boulter, 1974). The upper (adaxial) and lower (abaxial) epidermal surfaces were separated using dissecting needle and forceps after been rinsed in clean water. A portion of the macerated sample was taken for microscopic studies. The samples are removed from the sample bottles using force pans placed in 1% aqueous solution of safranin for about 3-5 minutes. Excess stain was rinsed off the surface with clean water. The stained surfaces were placed on slides, and a drop of glycerin added to the stained surface, then covered with a cover slip. Observation was made on the microscope to determine epidermal cell type and size, stomatal complex type and size, stomatal index and stomatal density, and presence or absence of trichomes. All observations were recorded with figures and tables.

Determination of frequency of stomatal complex types. Using the field of view at X40 objective as quadrant the number of subsidiary cells per stoma was noted to determine the frequency of different complex type present in each specimen. Frequency of each complex type was expressed as percentage occurrence of such complex type based on all occurrences (Obiremi & Oladele, 2001). Terminologies for naming stomatal complex type would follow those of Dilcher (1974) and Metcalfe & Chalk (1988).

Determination of stomatal density and stomatal index. The stomatal density (SD) was determined as number of stomatal per square millimeter (Stace, 1965). SD = Number of stomatal in 0.152mm^2 field of view 10.152.

Stomatal index (SI) was determined as follow:

$$SI = S/E + S \times 100$$

Where: SI = stomatal index; S= number of stomatal per square millimeter; E= number of ordinary epidermal cell per square millimeter.

Determination of stomatal size (SS). The mean stomatal size of specie was determined by measuring length and breadth of guard cells multiplied by Franco's constant using an eye piece micrometer. A sample of 35 stomata was used. The method follows that of Wilkinson (1979).

$$SS = L \times B \times K$$

Where L=length; B=Breadth; K = Franco's constant = 0.78524

Determination of epidermal cell size. Epidermal cell size was determined as product of length and breadth of cell using eye piece micrometer, and a sample size of 35 cells was used.

Determination of trichome types. Shapes of trichome types were keenly observed and terminologies used for naming them followed those used by Dilcher (1974) and Metcalfe & Chalk (1988).

Determination of frequency of trichome types. Frequency of each trichome type was expressed as percentage occurrence of such trichome type based on all occurrences.

Determination of trichome density and index. The trichome density was determined as the number of trichomes per square millimeter. Trichome index was determined as number of trichomes per square millimeter divided by number of trichomes plus number of epidermal cell per square millimeter multiplied by 100.

Statistical analysis. All data were reported and analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Computer software was used was SPSS. A probability value of 0.05 was used as bench mark for significant difference between parameters.

RESULTS AND DISCUSSION

From the study carried out on the twenty-five (25) different species of plants having medicinal values taken from different plant families (Table 1). The species were found to have variations in their stomatal density, stomatal frequency, stomatal complex types, epidermal cell wall size and trichome distribution. However, these features are similar in both fresh and dry samples of the same species though with some quantitative insignificance differences. Table 2 and Fig. 1 showed the leaf features such as stomata, trichomes and ordinary epidermal cells in the 25 plants studied.

Anacardium occidentale. Stomatal complex type found in abaxial surface is paracytic but lacks stomata on the adaxial surface, showing that the plant is hypostomatic (stomata occurrence on lower surface only). It has a stomata index of about 7.99% on the abaxial surface. Stomatal density and stomatal size on the abaxial surface are 25.55mm² and 74.75µm respectively (Table 2). Stomatal frequency is 100%. The epidermal cells found on the adaxial surface of the plant are pentagonal and isodiametric on the abaxial surface (Figs. 1i & 1ii). Trichome is absent.

Annona senegalensis. Stomatal complex type found in abaxial surface is paracytic and brachyparacytic on the adaxial surface, showing that the plant is amphistomatic (stomata occurrence on both surfaces). The stomatal frequency on both surfaces is 100%. The stomata index is about 12.25% on the adaxial surface and 19.50% on the abaxial surface, while stomatal

size on the adaxial surface is about 23.25µm and 73.00µm on the abaxial surface. Stomata density on both abaxial and adaxial surface is 56.80mm² and 30.20mm² respectively. The stomata occurrence or distribution is greater on the abaxial surface than the adaxial surface (Table 2; Figs. 1iii & 1iv). The epidermal cells found on the adaxial surface of the plant are tetragonal and irregular in the abaxial surface.

Azadirachta indica. Stomatal complex type found in abaxial surface is paracytic but lacks stomata at the adaxial surface. The abaxial surface has stomatal index is 29.65% and stomatal density of about 30.36mm². Stomatal size and stomatal frequency are 52.24µm and 100% respectively (Table 2). The epidermal cells found on both surface of this plant are hexagonal. Trichome is absent on both surfaces (Figs. 1v & 1vi).

Baphia nitida. Stomatal complex type found in abaxial surface is paracytic and diacytic and paracytic on the adaxial surface. Stomatal occurrence is greater in the abaxial surface than adaxial surface. It has a stomatal index of about 9.45% on the abaxial surface and 6.23% on the adaxial surface. Stomatal density and stomatal size on the abaxial surface are 66.54mm² and 43.30µm respectively and 36.04 mm² and 53.30 µm on the adaxial surface (Table 2). Stomatal frequency on the adaxial surface is 100% and 70.50% and 29.50%on the abaxial surface. The epidermal cells found on the adaxial surface of the plant are tetragonal and polygonal on the abaxial surface (Figs. 1vii & 1viii).

Chromoleana odorata. Stomatal complex type found in abaxial surface is anomocytic and anisocytic but lacks stomata at the adaxial surface. It has a stomata index of about 6.32% on the abaxial surface. Stomata density and stomatal size on the abaxial surface are 48.58mm² and 68.20µm respectively (Table 2). Stomatal frequency is 100%. The epidermal cells found on both surfaces of the plant are isodiametric (Figs. 1ix & 1x). Trichome is absent.

Citrus aurantifolia. Stomatal complex type found in abaxial surface is paracytic but lacks stomata at the adaxial surface. The abaxial surface has stomatal index is 94.60% and stomatal density of about 39.20mm². Stomatal size and stomatal frequency are 19.69µm and 100% respectively (Table 2). The epidermal cells found on both surface of the plant are pentagonal. Trichome is absent (Figs. 1xi & 1xvii).

Citrus limon. Stomatal complex type found in abaxial surface is paracytic but lacks stomata on the adaxial surface. Abaxial surface has stomatal density of 26.55mm² and stomatal index of about 90.10%. Stomatal size and stomatal frequency are 30.30µm and 100% respectively (Table 2). Trichome is absent on both surfaces of the plant. The epidermal cells found on the adaxial surface of the plant are hexagonal and pentagonal on the abaxial surface (Figs 1xiii & 1xiv).

Citrus paradise. Stomatal complex type found in abaxial surface is paracytic but lacks stomata on the adaxial surface. The epidermal cells found on both surface of this plant are pentagonal. Trichomes are absent on both surfaces. It has a stomatal index of 25.15% and density of 32.33mm². Stomatal size and frequency of 39.20µm and 100% respectively (Table 2; Figs. 1xv & 1xvi).

Citrus sinensis. Stomatal complex type found in abaxial surface is paracytic but lacks stomata on the adaxial surface. Abaxial surface has a stomatal density of 36.00mm² and stomatal index of about 92.60%. Stomatal size and stomatal frequency are 26.40µm and 100% respectively (Table 2). Trichome is absent on both surfaces of the plant. The epidermal cells

found on both adaxial surface of the plant and on the abaxial surface are pentagonal (Figs 1xvii & 1xviii).

Cymbopogon citrates. Stomatal complex type found on both surfaces are tetracytic, showing that the plant is amphistomatic (stomata occurrence on both surfaces). The stomatal index is about 10.50% on the adaxial surface and 16.30% on the abaxial surface, while stomatal size on the adaxial surface is about 80.60µm and 73.30µm on the abaxial surface. Stomatal density on both abaxial and adaxial surface is 72.50mm² and 36.00mm² respectively (Table 2). Stomata occurrence or distribution is found to be greater in abaxial surface than adaxial surface (Figs. 1xix & 1xx). The epidermal cells found on the adaxial surface of the plant are hexagonal and pentagonal in the abaxial surface. It possesses trichomes which are evenly distributed on the surfaces of the plant.

Gliciridium sepium. Stomatal complex type found in abaxial surface is paracytic but lacks stomata at the adaxial surface. The stomatal index on the abaxial surface is 11.30% and stomatal density is 32.35mm². Stomatal frequency is 100% and stomatal size is 15.00µm. The epidermal cells found on the adaxial surface of the plant are tetragonal and hexagonal in the abaxial surface (Table 2; Figs. 1xxi & 1xxii). Trichome is absent.

Jatropha curcas. Stomatal complex type found in abaxial surface is paracytic and brachyparacytic at the adaxial surface. The stomatal frequency on both surfaces is 100%. The stomata index is about 39.21% on the adaxial surface and 57.56 % on the abaxial surface, while stomatal size on the adaxial surface is about 122.30µm and 142.30µm on the abaxial surface. Stomata density on both abaxial and adaxial surface is 35.00mm² and 25.00mm² respectively (Table 2). Stomatal occurrence is greater on the abaxial than on the adaxial surface (Figs. 1xxiii & 1xxiv). The epidermal cells found on the adaxial and abaxial surface of the plant is polygonal. Uniseriate trichomes are present.

Mangifera indica. Stomatal complex type found in abaxial surface is paracytic but lacks stomata on the adaxial surface, showing that the plant is hyostomatic (stomata occurrence on lower surface). The stomatal index of abaxial surface is about 17.85% and stomatal density of 28.52mm². Stomatal size and stomatal frequency are 66.52µm and 100% respectively (Table 2). The epidermal cells found on the adaxial surface are wavy and hexagonal on the abaxial surface. Trichomes are absent (Figs. 1xxxv & 1xxxvi).

Morinda lucida. Stomatal complex type is paracytic which occurrence is 100% on the abaxial leaf surface only no on the adaxial (i.e. the leaf is hypostomatic). Stomatal density is 20.50mm^2; stomatal index is 5.54% and stomatal size is 16.50µm. the epidermal cell is tetragonal in shape. There is no trichome (Table 2; Figs 1xxv & 1xxvi).

Morus messosygia. Stomatal complex type found in abaxial surface is paracytic but lacks stomata at the adaxial surface. It has a stomata index of about 15.60% on the abaxial surface. Stomatal density and stomatal size on the abaxial surface are 35.50mm² and 14.00µm respectively (Table 2). The stomatal frequency is 100%. The epidermal cells found on the adaxial surface and abaxial surface of the plant are tetragonal (Figs. 1xxvii & 1xxviii). Trichome is absent.

Newboudia leavis. Stomatal complex type found in abaxial surface is anomocytic but lacks stomata on the adaxial surface. The stomatal index on the abaxial surface is 15.90% and stomata density is 39.50mm². Stomatal frequency is 100% and stomatal size is 25.00µm. The epidermal cells found on the adaxial surface of the plant are tetragonal and irregular while it is

also irregular and pentagonal on the abaxial surface (Table 2; Figs 1xxix & 1xxx). Trichome is present on both surfaces.

Nicotiana tabaccum. Stomatal complex type found in abaxial surface is paracytic while on the adaxial surface, brachyparacytic and paracytic showing that the plant is amphistomatic (stomata occurrence on both surfaces). The stomatal frequency on adaxial surface is 40% and 100% and 100% on abaxial surface. The stomatal index is about 23.21% on the adaxial surface and 26.00 % on the abaxial surface, while stomatal size on the adaxial surface is about 70.00µm and 106.60µm on the abaxial surface. Stomatal density on both abaxial and adaxial surface is 9.80mm² and 12.60mm² respectively. Stomatal occurrence is greater on the abaxial than on the adaxial surface. The epidermal cells found on the adaxial surface of the plant are rectangular and hexagonal on the abaxial surface. There is presence of simple trichomes which are sparsely distributed on the leaf surfaces (Table 2; Figs. 1xxxi and 1xxxii).

Ocimum gratissimum. Stomatal complex type found on abaxial surface are paracytic and diacytic while on the adaxial surface it is anisocytic, diacytic and paracytic showing that the plant is amphistomatic (stomata occurrence on both surfaces). The stomatal index of the abaxial surface is about 36.40% and stomatal density of 17.72mm² the adaxial surface has stomatal index of about 25.11% and stomatal density of 13.26mm². Stomata size on the abaxial surface is 62.96µm and 50.58µm on the adaxial surface. Stomatal occurrence is greater on the abaxial than on the adaxial surface. Stomatal frequency ranges from 11.10-60.50. The epidermal cells found on the adaxial surface of the plant are irregular and tetragonal on the abaxial surface. Trichome is sparsely distributed (Table 1; Figs 1xxxiii & 1xxxiv).

Polyalthia longifolia. Stomatal complex type found in abaxial surface are anomocytic and anisocytic but anomotetracytic on the adaxial surface, showing that the plant is amphistomatic (stomata occurrence on both surfaces). Stomatal index on adaxial surface is 6.23% and 3.26% on abaxial surface. Stomatal density on abaxial surface is 36.20mm² and 40.90mm² on adaxial surface. Stomatal size on the abaxial surface is 10.50µm and 8.50µm on adaxial surface. Stomatal frequency on abaxial surface is 60% and 70.00 and 30.00% on adaxial surface Stomatal occurrence is greater on the abaxial surface than adaxial surface (Table 2; Figs. 1xxxvii & 1xxxviii). The epidermal cells found on both surfaces of the plant are polygonal. Trichome is absent.

Psidium guajava. Stomatal complex type found in abaxial surface is paracytic but lacks stomata on the adaxial surface. The stomatal index on the abaxial surface is 10.90% and stomatal density is 35.00mm². Stomatal frequency is 100% and stomatal size is 17.50% on the abaxial surface. The epidermal cells found on the adaxial surface of the plant are polygonal and irregular on the abaxial surface (Table 2; Figs. 1xxxix and 1xl). It possesses little trichome on the leaf surfaces.

Ravoulvia vomitora. Stomatal complex type found on abaxial surface is tetracytic but lacks stomata on the adaxial surface. Stomatal size on the abaxial surface is 74.75µm respectively. Stomatal frequency is 100%. The stomatal index on the abaxial surface is 6.98% and stomatal density is 73.71mm². The epidermal cells found on the adaxial surface of the plant are tetragonal and pentagonal in the abaxial surface (Table 2; Figs. 1xliii & 1xliv). Trichome is absent.

Telferia occidentalis. Possess polygonal epidermal cell wall on both abaxial and adaxial surfaces. It possesses hypostomatic leaves i.e. leaves having stomata occurring on only lower side of the leaves. Abaxial surfaces exhibits paracytic stomata type (stomata accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells). It has a stomatal index of about 21.00% on the abaxial surface. Stomatal density is 136.10mm². Stomatal size and stomatal frequency are 108.80µm and 100% respectively (Table 2; Figs. 1xlv & 1xlvi). Trichome occurrence is only found on the abaxial surface.

Terminalia catappa. Stomatal complex type found in adaxial surface is tetracytic and may be anisocytic, tetracytic or laterocytic on the abaxial surface. Stomata index on adaxial surface is 6.23% and 10.59% on abaxial surface. Stomatal density on abaxial surface is 12.03mm² and 9.00mm² on adaxial surface. Stomatal size on the abaxial surface is 47.50µm and 66.17µm on adaxial surface. Stomatal frequency on abaxial surface is 15.40%, 50.05% and 34.55% and 100.00% on adaxial surface. Stomatal occurrence is greater on the abaxial surface than adaxial surface (Table 2; Figs 1xli & 1xlii). This plant possesses wavy epidermal cell on both surfaces. Trichome is absent.

Vernonia amygdalina. Possess tetragonal cell epidermal cell wall on both abaxial and adaxial surfaces. It possesses amphistomatic leaves i.e. leaves having stomata occurring on both sides of the leaves. Its abaxial and adaxial surfaces exhibits anomocytic stomatal type (stomatal accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells). Stomata occurrence or distribution is found to be greater in abaxial surface than adaxial surface. It has a stomata index of about 18.75% on the adaxial and 19.60% on the abaxial surface. Stomata density and size on the abaxial surface 26.00mm²and 19.60µm are respectively and on the adaxial surface24.00mm² and 91.30µm (Table 2; Figs. 1xlvii & 1xlviii). Trichomes are present on both surfaces with their sizes ranging from about 14.26- 40.55 mm².

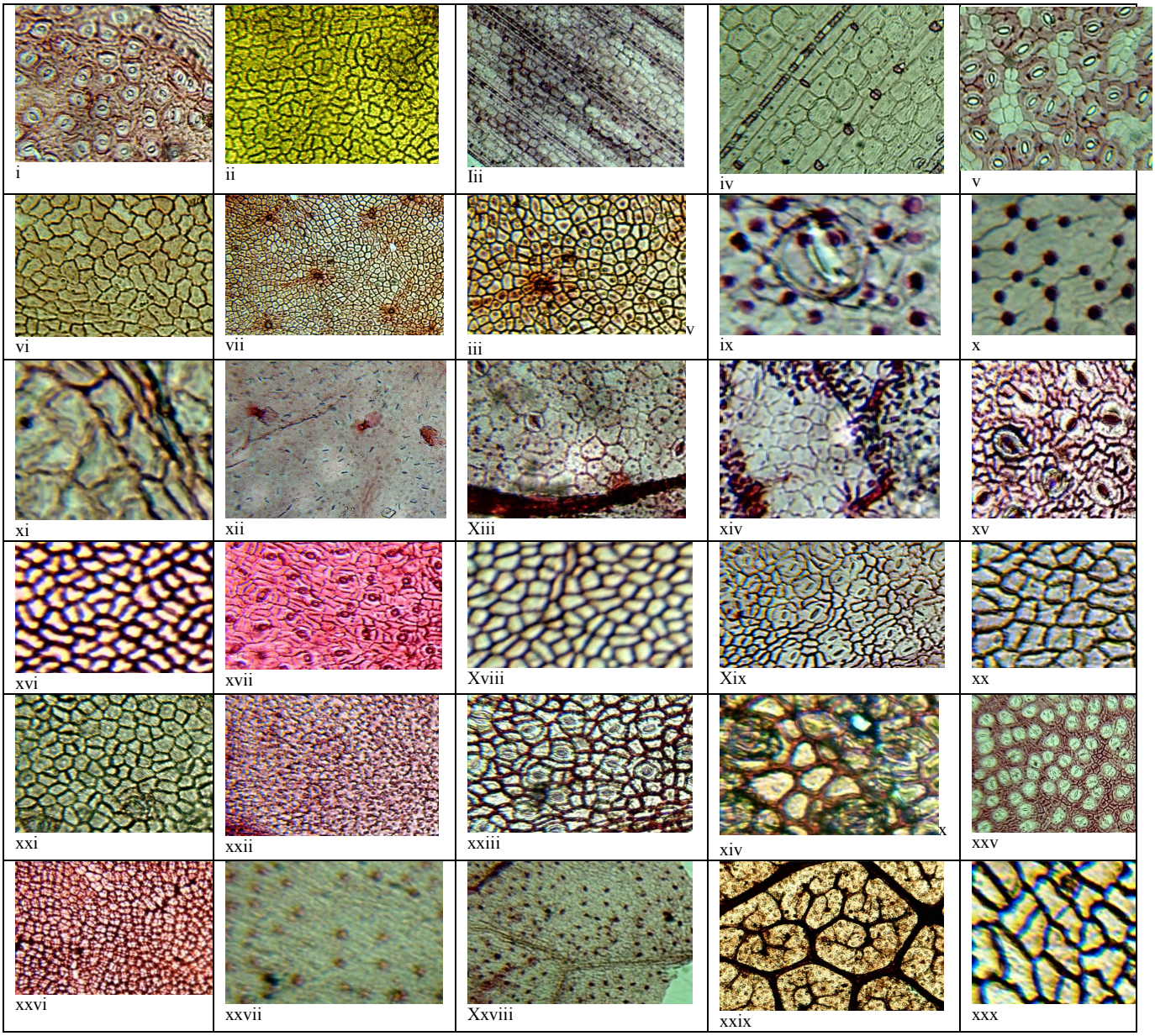
Vitellaria paradoxa. Possesses paracytic stomata on the abaxial surface while on the adaxial surface stomata are absent. Both surfaces i.e. abaxial and adaxial surface possesses pentagonal epidermal cell wall. Trichomes are absent on the adaxial surface but present on the abaxial surface. It has a stomatal index of about 19.84% and stomatal density of 27.58mm². Stomatal frequency is 100% and stomatal size is 61.58µm (Table 2; Figs. 1xlix & l).

Leaf anatomical features have been employed by many anatomists to resolved some taxonomical problems (Esau, 1977; Davis & Heywood, 1963; Metcalf & Chalk, 1988; Ayodele & Olowokudejo, 2006; AbdulRahaman *et al*., 2011). Based on these evidences, the authors also deemed it fit to use these features in some selected dry and fresh samples of some medicinal plants. It was observed that stomatal index, density, size and stomatal complex types are in one way or the other differ between different species studied. But the same cannot be said within a species i.e. between dry and fresh samples.

Although anatomists such as Edeoga & Osawe (1996) and Mbagwe & Edeoga (2006) stressed that epidermal and cuticular traits of plant epidermal cells, types, and arrangement of stomata, size and shape of trichomes and number of vascular bundles could serve as vital tools in solving taxonomic problems in plants. The same principle is applicable in identifying plant materials contain in ground samples.

Table 2: Stomata, trichomes and epidermal cell features in some medicinal plants

Species	Leaf surface	Stomatal complex type	Frequency (%)	Stomatal density (mm²)	Stomatal size (µm)	Sstomatal index (%)	Epidermal cell wall	Trichome
Telfaria occidentalis	Adaxial	-	-	-	-	-	Polygonal	Absent
	Abaxial	Paracytic	100.00	136.10	108.80	21.00	Polygonal	Present
Vernonia amygdalina	Adaxial	Paracytic	100.00	120.30	91.30	24.00	Tetragonal	Present
	Abaxial	Paracytic	100.00	123.00	19.60	26.00	Tetragonal	Present
Citrus sinensis	Adaxial	-	-	-	-	-	Pentagonal	Absent
	Abaxial	Paracytic	100.00	36.00	26.40	92.60	Pentagonal	Absent
Citrus limon	Adaxial	-	-	-	-	-	Hexagonal	Absent
	Abaxial	Paracytic	100.00	26.55	30.30	90.10	Pentagonal	Absent
Citrus aurantifolia	Adaxial	-	-	-	-	-	Pentagonal	Absent
	Abaxial	Paracytic	100.00	45.76	19.69	94.60	Pentagonal	Absent
Citrus paradise	Adaxial	-	-	-	-	-	Pentagonal	Absent
	Abaxial	Paracytic	100.00	32.33	39.20	25.15	Pentagonal	Absent
Azardirachta indica	Adaxial	-	-	-	-	-	Hexagonal	Absent
	Abaxial	Anomocytic	100.00	30.36	52.24	29.65	Hexagonal	Absent
Mangifera indica	Adaxial	-	-	-	-	-	Wavy	Absent
	Abaxial	Paracytic	100.00	28.52	62.58	17.85	Hexagonal	Absent
Anacardium occidentale	Adaxial	-	-	-	-	-	Pentagonal	Absent
	Abaxial	Paracytic	100.00	25.55	74.75	7.99	Isodiametric	Absent
Newboudia leavis	Adaxial	-	-	-	-	-	Irregular	Absent
	Abaxial	Anomocytic	100.00	39.50	25.00	15.90	Irregular	Present
Psidium guajava	Adaxial	-	-	-	-	-	Polygonal	Absent
	Abaxial	Paracytic	100.00	35.00	17.50	10.90	Irregular	Absent
Morinda lucida	Adaxial	-	-	-	-	-	Irregular	Absent
	Abaxial	Paracytic	100.00	20.50	165.00	5.54	Tetragonal	Absent
Morus messosygia	Adaxial	-	-	-	-	-	Tetragonal	Absent
	Abaxial	Paracytic	100.00	35.50	14.00	15.60	Tetragonal	Absent
Gliricidium sepium	Adaxial	-	-	-	-	-	Tetragonal	Absent
	Abaxial	Paracytic	100.00	32.35	15.00	11.30	Hexagonal	Absent
Ravoulvia vomitora	Adaxial	-	-	-	-	-	Tetragonal	Absent
	Abaxial	Tetracytic		73.71	46.30	6.98	Pentagonal	Absent
Jatropha curcas	Adaxial	Brachyparacytic	100.00	25.00	122.30	39.21	Tetragonal	Absent
	Abaxial	Paracytic	100.00	35.00	142.30	57.56	Hexagonal	Absent
Petivera alliaceae	Adaxial	Brachyparacytic	100.00	30.20	23.25	12.25	Tetragonal	Absent
	Abaxial	Paracytic	100.00	56.80	73.00	19.50	Irregular	Absent
Baphia nitida	Adaxial	Paracytic	100.00	36.04	53.30	6.23	Tetragonal	Absent
	Abaxial	Paracytic Diacytic	70.50 29.5	66.54	43.30	9.45	Polygonal	Absent
Ocimum grathicimum	Adaxial	Paracytic Diacytic	60.50 39.50	13.26	50.58	25.11	Tetragonal	Absent
	Abaxial	Actinocytic Diacytic Paracytic	11.10 56.50 32.40	17.72	62.96	36.40	Irregular	Absent
Chromoleana odorata	Adaxial	-	-	-	-	-	Tetragonal	Absent
	Abaxial	Anomocytic Anisocytic	60.40 39.60	48.58	68.20	6.32	Tetragonal	Absent
Nicotiana tabbacum	Adaxial	Brachyparacytic Paracytic	40.00 100.00	12.60	70.00		Rectangular	Absent
	Abaxial	Paracytic	100.00	9.80	106.60	26.00	Hexagonal	Present
Vitellaria paradoxa	Adaxial	-	-	-	-	-	Pentagonal	Absent
	Abaxial	Paracytic	100.00	27.58	61.58	19.84	Pentagonal	Absent
Cymbopogon citrates	Adaxial	Tetracytic	100.00	36.00	80.60	10.50	Tetragonal	Present
	Abaxial	Tetracytic	100.00	72.50	73.30	16.30	Tetragonal	Present
Polyathia longifolia	Adaxial	Anomocytic Anisocytic	70.00 30.00	40.90	8.50	7.59	Polygonal	Absent
	Abaxial	Anomotetracytic	60.00	36.20	10.50	3.26	Polygonal	Absent
Terminalia catappa	Adaxial	Tetracytic	100.00	9.00	66.17	6.23	Wavy	Absent
	Abaxial	Anisocytic Tetracytic Laterocytic	15.40 50.05 34.55	12.03	47.50	10.59	Wavy	Absent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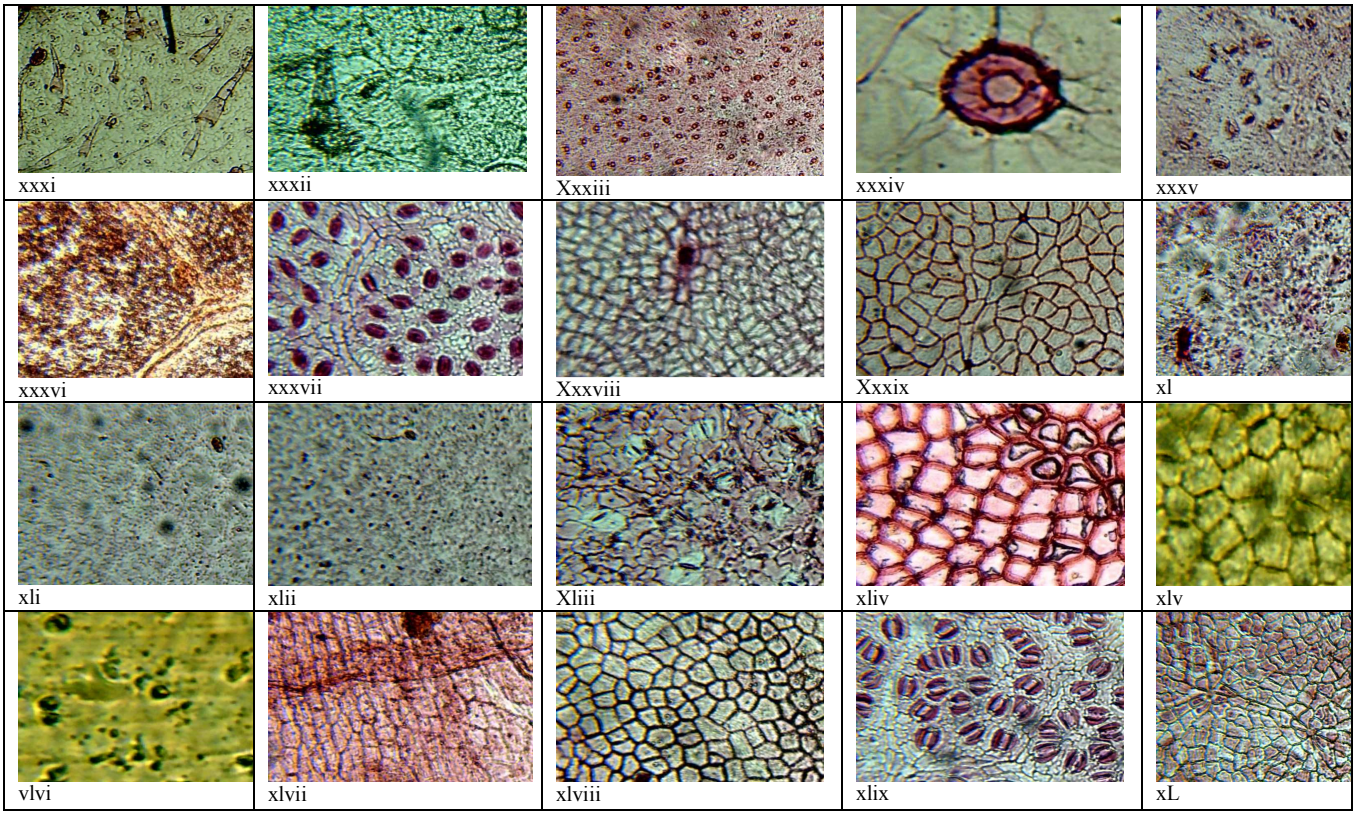

Fig. 1: Leaf epidermis surfaces (Abaxial – left, Adaxial – right) of *Anacardium occidentale* (i & ii), *Annona senegalensis* (iii & iv), *Azadirachta indica* (v & vi), *Baphia nitida* (vii & viii), *Chromolena odorata* (ix & x), *Citrus aurantifolia* (xi & xii), *Citrus limon* (xiii & xiv), *Citrus paradisi* (xv & xvi), *Citrus sinensis* (xvii & xviii), *Cymbopogon citratus* (xix & xx), *Gliciridium sepium* (xxi & xxii), *Jatropha curcas* (xxiii & xxiv), *Morinda lucida* (xxv & xxvi), *Morus messosygia* (xxvii & xxviii), *Newboudia laevis* (xxix & xxx), *Nicotiana tabaccum* (xxxi & xxxii), *Ocimum gratissimum* (xxxiii & xxxiv), *Mangifera indica* (xxxv & xxxvi), *Polyalthia longifolia* (xxxvii & xxxviii), *Psidium guajava* (xxxix & xl), *Terminalia catappa* (xli & xlii), *Ravoulvia vomitara* (xliii & xliv), *Telfaria occidentalis* (xlv & xlvi), *Vernonia amygdalina* (xlvii & xlviii) and *Vitellaria paradoxa* (xlix & L) showing stomata, trichomes and epidermal cells x600

Stomatal features such as its density, index and size are one common measure of plant response to rising atmospheric CO_2 concentration, climate change and water availability (Ianovici *et al,* 2009; Gan *et al,* 2010; Fanourakis *et al,* 2011). These features can also be used in taxonomy to delimit plant taxa. The features along with trichomes and ordinary epidermal cells are also used to authenticate the originality of ground medicinal plant materials. The results of this study indicate that there was no significant difference between both samples used (fresh and dry). Nevertheless, future observations should consider potential differences in shrinkage among other drying methods, including critical point drying (dehydration in ethanol and liquid co_2), and freeze drying. If these drying methods are put into considerations, stomatal densities measured from the dried sample may closely represent fresh leaves if the samples

were revived before counting. Generally, most of the members of Rubiaceae have paracytic stomata only restricted on the lower epidermis. These results indicate that changes in leaf structure should be considered when comparing stomatal densities obtained from more than one method, particularly if maceration techniques are used. Stomatal index is also a feature used; it could serve as a parameter for comparison between fresh and dry plant species. The stomatal index is independent of the environmental influence and portion of leaf surface, size of intervening epidermal cells (Metcalfe & Chalk, 1988). The epidermal walls found in all the dry plants used are thick, this occurs as a result of pressing or dehydration. The thickness gives them their rigidity and prevents moulds from growing on them thus preserving the plants. Trichomes occurrence varies in the plants used. Some plants have high percentage occurrence of trichomes such as *Nicotiana tobaccum* and *Azardiracta indica* while others such as *Psidium guajava* lacks trichomes.

CONCLUSIONS

It can be concluded that the present study of the anatomy of leaves of medicinal plants listed above can serve as an important maker or source of information to ascertain the identity of the plants used in drug plants. The anatomical studies of these plants can also be used in taxonomic studies. The approach can as well be put to use in herbal medicine and forensic science where, identification and authentication of plant specimens are essential.

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