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Full Length Research Paper

Micromorphological Keys to Identifying Plant Fragments in Powdered Herbal Drugs

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Oven dry powdered samples of 6 medicinal plant species were studied anatomically in search of micomorphological characters to identify the original plants used in the preparation. Moistened head of the needle was used to transfer samples unto a labeled glass slide containing 1 - 2 drops of water and glycerol/ethanolTS; covered with cover slip and warmed gently to remove air bubbles. Samples were observed under the microscope in search of intact whole tissues and cells which could be used to identify the species of plant. The main characteristics of the fragments recovered from the samples are, sclereid tissue system and long, branching non-septate fibre of *Teprapluera tetraptera* (Shum and Thonn.) Taubs; compound bundle sheath in *Cymbopon citratus* (DC.) Stapf; epidermal furrow and ridges and densely-rayed druses in *Hibiscus sabdarifa* Linn. ; spindle shaped epidermal cells in *Cajanu cajans* (Linn.). Millsp; sinous anticlinal wall in *Cucuma longa* L. and annular type tracheary elements in *Azadiracthta indica*. There is a suggestion that an examination by microscopy can provide a form of identification of herbal constituent from a prepared remedy.

Key words: Medicinal plant species, *Cymbopon citratus*, *Azadiracthta indica*.

INTRODUCTION

Approximately, 80% of the people in developing countries rely chiefly on traditional medicine for health care needs, of which a majority portion involves the use of plant ex-tracts or their active principles (Farnsworth, 1985). An es-timated 70% of South Africans regularly uses traditional plant medicines (Springfield et al., 2005). One of the criti-cisms of herbal medicine is lack of standardization and quality control profiles. Of central importance with respect to quality control is correct identification of the species concerned, whether in the fresh, dried or powdered state (Springfield, 2005). The misclassification of species and the mistaken substitution is a real danger in the prepara-tion and administration of herbal medicine (Opara, 2004). Some herbs look so familiar to the untrained eye that they are often mistaken for one another.

The misclassification of species and the mistaken sub-stitution of Chinese herbs have also given rise to serious adverse affects (Chan et al., 1993; Chan and Critchley, 1996). Misidentification of Chinese herbal medicine (CHM) can also lead to erroneous explanations concer-ning their mode of action. In correspondence concerning the anti-allergic properties of the CHM 'food allergy herbal formula-1' (FAHF-1), the incorrect identification of the chemical structure of one of its components (*G. luci-dum*) led to 'misleading conclusions' about the causes of FAHF-1's immunomodulatory action (Li, 2002; Fugh-Ber-man, 2000).

Microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials (WHO, 1998). Following the works of Metcalfe and Chalk (1950) and Metcalfe (1954) which today serve as standard references to plant anatomy, the use of ve-getative anatomical characters in taxonomy became a routine procedure.

The aim of this study is to detect the presence of va-rious plant ingredients in the powder of some comm.-only used herbal plants and also to determine the patterns of variation in tissue characteristics and to assess their value in species identification.

MATERIALS AND METHODS

6 species of commonly used medicinal plants were collected from the wild, sun-dried and ground into powder. The tip of the needle was moistened withy water before it was used to touch the sample. The sample that adhered to the needle was transferred on the la-beled glass slide containing 1 - 2 drops of water and glycerol/ etha-nol TS (ethanol Thin Stillage); covered with cover slip and warmed gently to remove air bubbles. This was repeated using phlorogluci-

Table 1. Some important tissues recovered from the herbal powder samples and their micromorphological characteristics.

Taxon and organ of plant commonly used	Family	Codes of the characters used													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A.).0 *A. indica* (Leave)	Meliaceae	-	-	+	+	-	-	-	-	-	-	-	-	+	-
B. *C. cajan* (Seed)	Fabaceae Papilionoideae	-	-	-	+	-	-	+	+	-	-	+	-	-	-
C. *C. longa* (Rhizome)	Zingiberaceae	-	-	-	-	-	-	-	-	-	-	+	-	-	+
D. *C. citrates*	Poaceae	-	+	-	-	+	+	-	-	-	-	-	-	-	+
E. *H. sabdariffa* (Calyx)	Malvaceae	-	-	-	-	-	-	-	+	-	-	-	+	+	+
F. *T. tetraptera* (Fruit)	Fabaceae-Mimosoideae	+	-	+	-	-	-	-	-	+	+	+	-	-	-

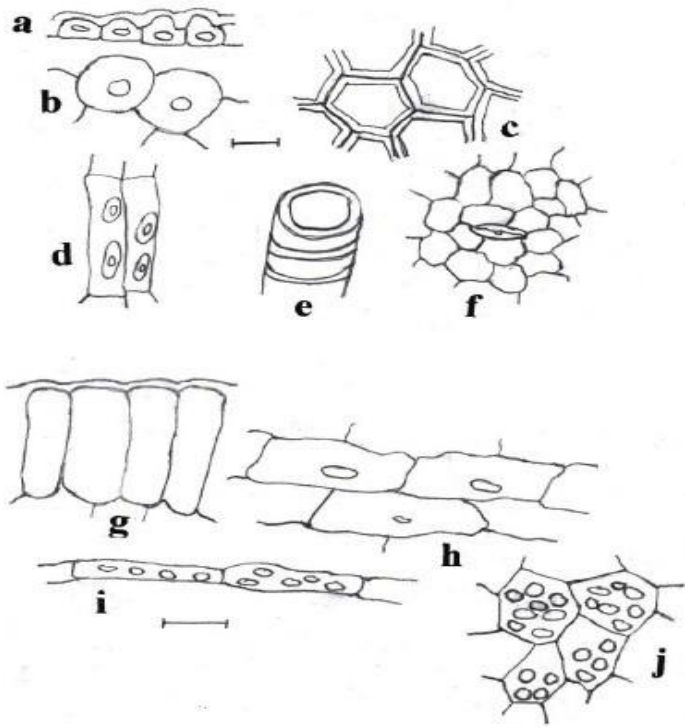
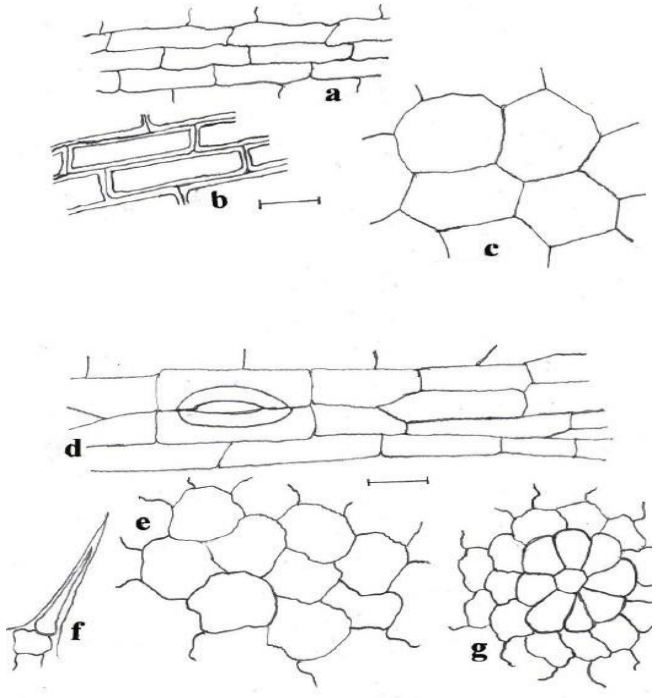


Figure 1 a - j. characteristic tissues recovered from powder medicinal plants.
1 a - f: Tissues recovered from *Azadirachta indica* leaves: (a) pilar-like epidermis tissue, (b) oil storing parenchyma, (c) thicken paren-chyma, (d) columnar cell, (e) tracheary element annular thic-kening,(f) anomocytic stomata. 1 g - j. Tissue recovery from *Caja-nus cajan* seeds: (g) columnar epidermal cells (h) sub-epidermal storage cell (i) oil-storing rectangular narrow cell (j) elaioplast.

nol TS to detect lignified cell wall; Sudan Red TS for suberized of cuticular cell walls and fats and oil. Slide preparation followed the classical method recommended by WHO (1998). Micromorphology of the samples was studied by camera lucida under × 25 objective power of Leitz DIALUX research microscope in search of taxonomic characters. Anatomical measurements were made in ocular units at
×40 objective power and converted to micrometers. All plant names were according to the Flora of West Tropical Africa (Hutchinson and Dalziel, 1958 - 1972).

RESULTS

The different tissues recovered from the powder of herbal



Figures 2 a-g. Characteristic tissues recovered from powdered medicinal plants.
2 a – c: Tissues recovered from the rhizome of Curcuma longa lea-ves: (a) rectangular straite-wall epiderma, (b) oil-storing thick-walled parenchyma, (c) cubiodal wavy-walled parenchyma. 2 d – g: Tis-sues recovered from *Cymbopogan citratus* leave, (d) epidermal tis-sue comprising paracytic stomata; (e) lignified striate-walled paren-chyma; (f) simplified trichome; (g) steath bundle.

plants are listed in Table 1. Illustrations of cells and tissues recovered from the powdered samples are shown in Figures 1 - 3. In Table 2 which is n x t table of matri-ces, plant species (n) are compared by their characters
(t) for possible resemblance between and among spe-cies. The coefficients of resemblance are listed in Table
3. The tissues recovered from the samples vary from species to species as described below.

Tetrapleura tetraptera (Shum and Thonn.) Taubs (Mimosaceae.)

The cuticle is relatively thick and glossy, overlaying the ir-

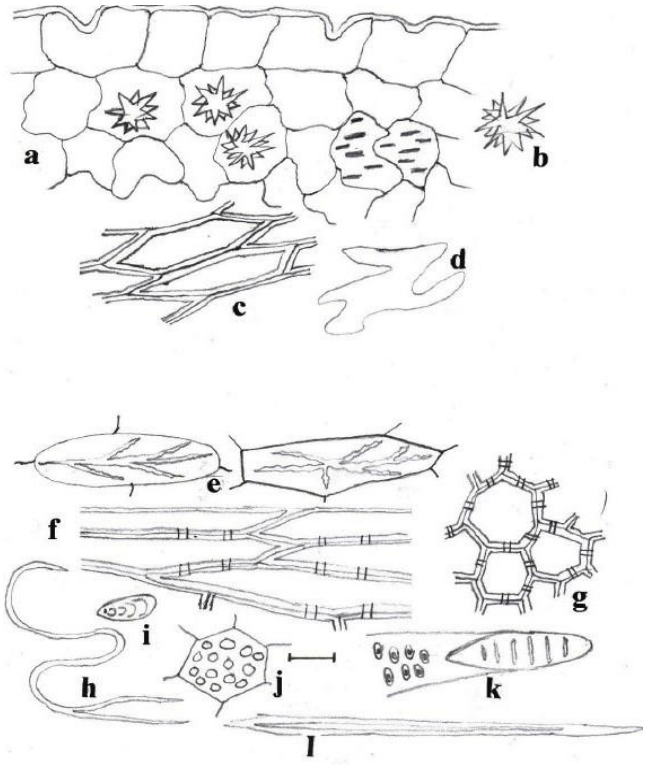


Figure 3 a – k. Characteristic tissues recovered from powdered medicinal plant. 3 a – c: Tissues recovered from the rhizome of *Habiscus sabdariffa* calyx: (a) tissues comprising furrowed epider-mis druses, dark- coloured pigment. (b) isolated oxalate crytal, (c) thick walled pentagonal parenchyma. (d) irregularly shaped paren-chyma cell. 3 e – k: Tissues recovered from *Tetrapluera tetraplue-ra* fruit: (e) scleriod, (f) elongated parenchyma with pit-pairs (g) cu-biodal parenchyma with pit-pairs: (h) branching fibre. (i) oil storing parenchyma. (j) vessel element with scalariform eforation plate, (k) unbranched fibre. Figure 3 a – k. Characteristic tissues recovered from powdered medicinal plant

regularly shaped epidermal cells; anticlinal cell is straite; cells densely filled with ergastic substances, notably oil droplets and copious starch grains. Fibres are character-istically branching and non-septate, scleroidal idiolasts characterized by narrow ramifying canals, simple perfora-tion plate of scalariform type present, parenchyma cell wall thickened and pitted. The presence of simple pit-pair is diagnostic.

Azadirachta indica A. Juss (Meliaceae)

Columnar parenchyma cells are characteristic, probably part of the epidermis, in addition rectangular oil-filled cells present, anomocytic stomata were observed as well as tracheary elements of the annular kind.

Cymbopogon citratus (DC.) Stapf (Poaceae)

Fragment of intact epidermis shows undulating single la-yered pigmented cells with a thick cuticle, prickle hairs

Table 2. n x t table of 14 characters by 6 medicinal plant species.

Character codes	Species used in the study					
	A	B	C	D	E	F
1	-	-	-	-	-	+
2	-	-	-	+	-	-
3	+	-	-	-	-	+
4	+	+	-	-	-	-
5	-	-	-	+	-	-
6	-	-	-	+	-	-
7	-	+	-	-	-	-
8	-	+	-	-	+	-
9	-	-	-	-	-	+
10	-	-	-	-	-	+
11	-	+	+	-	-	+
12	-	-	-	-	+	-
13	+	-	-	-	+	-
14	-	-	+	+	+	-

1 = scleridal idioblasts, 2 = sheath cells. 3 = tra-cheary elements. 4 = columnar cell. 5 = non-glandu-lar trichomes, 6 = silica bodies, 7 = starch grains, 8 = undulating/furrowed epidermis, 9 = scalariform per-foration plate, 10 = branched/non-septate fibres, 11 = oil bodies, 12 = druses, 13 = annomocytic stomata, 14
= anticlinal cell wall sinuous.

Table 3. n x t matrix showing the coefficient of resemblance among the species.

	Species used in the study					
	A	B	C	D	E	F
A	-					
B	0.2	-				
C	0	0.2	-			
D	0	0.0	0.2	-		
E	0.2	0.1	0.2	0.1	-	
F	0.1	0.1	0.2	0	0	-

and silica bodies. Grasses are known to possess a thick, diagnostic cuticle that is resistant to bacteria and fungal attack and can persist in the environment for a long time. Fragments of grass cuticles dating back to the late qua-ternary have been found preserved in east African lake sediments (Wooler et al., 2000). Other tissues observed include parenchyma cells with straite cell wall, including paracytic stomata and characteristic sheathed vascular bundles.

Hibiscus sabdariffa Linn. (Malvaceae)

Epidermal cell wall undulating and characteristically furro-wed and ridged, numerous oxalate crystals in the form of

druses occurred in cells. The parenchyma cells are of va-rying irregular shapes, some deeply invaginated and others forming gig-saw. Secretory cells are filled with brown pigment. Nwachukwu (2007) reported the occur-rence of dark stained oxalate crystals in the leaves of *Ha-biscus rosa-sinensis*, although the study did not say pre-cisely which oxalate crystals was encountered.

Cajanus cajan (Linn.). Mill sp. (Papilionaceae)

Some elongated cells which are closely packed were ob-served. There were other parenchyma cells ranging from pentagonal to hexagonal in shape and filled with copious starch grains. Rectangular parenchyma cells all filled with copious oil droplets were seen.

Curcuma longa L. (Zingiberaceae)

The tissues of *Curcuma* are relatively less differentiated unlike in the other monocotyledons, *Cymbopon*. The cuti-cle is devoid of thick characteristic cuticle of the *Cymbo-pogon.* The cells observed all parenchymatous, with anti-clinal cell wall generally straite and closely packed. Other parenchyma cells are relatively thick-walled and filled with copious oil droplets. Occurrence of essential oils has been reported in *Curcuma* by some workers (Uechi et al., 2000; Raina et al., 2002; Mau et al., 2003).

Relative abundance of different cells in the residues plants

A greater proportion of the tissues recovered from the samples consisted of lignified, thick walled cells, where-as, thin walled, unlignified tissues were not common and when present, they often lack the diagnostic status of the lignified tissues. From Table 1, it can be seen that *Tetra-ptera tetrapleura* retained more characters in its powder form than other species with *Curcuma longa* retaining the least. The herbal species show very little resemblance on the basis of these characters as suggested by the preva-lence of low resemblance coefficients among them in Table 3.

DISCUSSION

The characters available in the powder are much fewer than the potentially available characters in whole speci-mens. The difference is atributable to the damage of the plant cell wall during preparation, causing distortion in tis-sue arrangements and patterns normally found in the un-treated plant samples. This aspect of micromorphology of medicinal plants is yet to be studied keenly; hence litera-ture is very scanty on it. But there is abundant literature on aspects of the cell wall not related to medicinal uses such as cell wall expansion (Cosgrove, 2000); cell wall stress (Delly, 1999) and pectin structure of cell wall (Ridley et al., 2001).

The characters available in the powder form of the spe-cimens are potentially useful for distinguishing the sam-ples even in a mixture. While some herbal samples such as *T. tetraptera*, *C. citratus*, *H. sabdariffa, A. indica* and *C. cajan* may be readily detectable in powdered form, *C. longa* does not lend itself readily to histological identifica-tion and will require additional method for confirmation. The paucity of characters obtainable from *C. longa* sug-gests the inadequacy of histological technique in detec-ting the presence of this species while in mixture with other plants. Several herbal products in the market can-not be identified using morphological and histological dif-ferences. Springfield et al. (2005) suggested random am-plified polymorphic DNA (RAPD) technique in cases where botanical identification is impossible, as high per-formance liquid chromatography (HPLC) with diode array detection (DAD), offers an alternative qualitative profile and is being increasingly used for the authentication of crude drugs or their extracts. Fennel et al. (2004) repor-ted that where poisoning from traditional medicine has been reported, it is usually because the plants have been misidentified in the form in which they are sold. There-fore, the issue of quality control need be addressed with all resources and energy. The use of botanical identifica-tion in herbal medicine is cheaper and more rapid but where it is not efficient, other methods should be used. An examination by microscopy alone cannot always pro-vide complete identification, though when used in asso-ciation with other analytical methods; it can frequently supply invaluable evidence.

There is a suggestion that herbal substances should be subjected to the same stringent scrutiny and controls as common drugs before their release on the market. In the cases where micromorphology does not work as in the *Curcuma* experience, it is important to apply other techni-ques. There is the need to extend this study to all medi-cinal plants in order to provide a broad basis for compa-rison.

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