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Micromorphological Study of Plant Fragments in Some Powdered Medicinal Plants Commercially Sold in Enugu Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Powdered samples of 10 medicinal plant species purchased from different herbal medicine sellers in markets across Enugu metropolis and Iwollo market in Ezeagu Local Government Area Enugu state Nigeria 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 onto a labeled glass slide containing 1 - 2 drops of water and plant stains; 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, parenchyma cells, trichomes, sclereid tissue system and long, branching non-septate fibre as in *E. sonchifolia, T. terrestis, P. santalinoides*; stomata as in *C. odorata*; elaioplast/oil storing cells as in *C. pallida* and *H. surattensis*; and then sheath cells as in *U. chamae*. Two of the plant samples studied did not display structures clear enough to identify them; while one of the samples had some structures recovered but wasn't helpful enough to identify the plant. Thus, the study suggested that an examination by microscopy can provide a form of identification of plants from processed plant materials.

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1. INTRODUCTION

"Since time immemorial, medicinal plants have been utilized in almost all cultures as a source of medicine" [1]. "The widespread utilization of herbal remedies as those described in ancient texts such as the bible and the vedas, and obtained from commonly used traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties" [2].

"The use of medicinal plant in many developing countries on the basis of their standard for the maintenance of good health has been widely observed" [3]. "More so, an increasing dependence on the utilization of medicinal plants in the industrialized societies have been linked to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies" [4].

"In general, the safety and effectiveness of alternative medicine have not been scientifically proven and remain largely unknown" [5]. "However, beyond side effects from the herb itself, adulteration, inappropriate formulation or lack of understanding of plant and drug interactions have led to adverse reaction that are sometimes life threatening or lethal" [6,7].

"A major criticism of plant derived medicine is lack of standardization and proper quality control. The most important aspect of quality control when dealing with herbal medicine is correct identification and authentication of the plant species involved, whether in the fresh, dried or powdered state" [8]. "The wrong classification and authentication of plant species and the erroneous substitution is a real danger in the preparation and administration of herbal medicine" [9].

"Most plants look so familiar and similar to the untrained eye that they are often mistook for one another. The erroneous classification and substitution of Chinese herbs have also given rise to serious adverse affects" [10]. "Misidentification of Chinese herbal medicine (CHM) can also lead to erroneous explanations concerning their mode of action" [11,12].

"Subjecting medicinal plant materials to microscopic inspection is essential and inevitable for the proper identification of broken or powdered materials. Following the works of Metcalfe and Chalk" [13] and Metcalfe [14] "which today serve as standard examples to plant anatomy, the use of vegetative anatomical characters in taxonomy became an undisputed routine procedure. Microscopic characters such as stomata, trichomes and epidermal cells are useful systematic tools in plant taxonomy, phylogeny and their applications in the identification of species which has been well recognized" [15].

The aim of this study is to detect the presence of various plant ingredients in the powder of some commonly used herbal plants purchased from sellers in Enugu metropolis and also to determine the patterns of variation in tissue characteristics and to assess their value in species identification.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Ten different grinded plant samples were purchased from different herbal medicine vendors in markets across Enugu metropolis and Iwollo market in Ezeagu Local Government Area Enugu state. These samples were properly preserved by keeping in dry and airy chamber to avoid fungal infestation. The plant samples purchased were only known by their local names in Igbo and Yoruba at the time they were purchased and they include: Aka-agu, Amunimuye, Ewe-ayo, Nturukpa, Obiara ohuru, Birana, Ewe-emu, Uda-agu, Azi-ezi and Aka-ogu.

2.2 Micromorphological Screening

Moistened head of sterile needle was used to transfer samples unto a labeled glass slide containing 1 - 2 drops of water and plant stain. Safranine red and alcian blue were the stains used; they were diluted with water, safranine red was first used to stain the samples and then alcian blue was later used to counter stain. Alcohol was applied on the stained sample and left for some seconds before been covered with cover slip and warmed gently over a flame source 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 slide

preparation followed the classical method recommended by World Health Organization (1998) with some modifications. Micromorphology of the samples was studied using camera lucida under × 25 objective power of Leitz DIALUX research microscope in search of taxonomic characters. All plant names were according to the Flora of West Tropical Africa [16]. Illustrations of cells and tissues recovered from the powdered samples are shown in Figs. 1 - 8. In Table 1 which contains the 10 processed medicinal plants are compared based on their morphological characteristics possible resemblance between and among species.

3. RESULTS

Figs. 1-8 shows the characteristic tissues recovered from powdered medicinal plants studied.

Table 1. Classification of plant species studied and number of tissues recovered from processed sample

S/N	Local name (language)	Number of recovered tissues	Botanical name	Family	Common name
1.	Aka-agu (Igbo)	4	-	-	-
2.	Amunimuye (Yoruba)	6	*Emilia sonchifolia* (L.) DC.	Asteraceae	Lilac tassel flower
3.	Ewe-ayo (Yoruba)	6	*Tribulus terrestris* L.	Zygophyllaceae	Puncture vine, devil's weed
4.	Nturukpa/Uturukpa (Igbo)	4	*Pterocarpus santalinoides* DC.	Fabaceae	
5.	Obiara ohuru (Igbo)	4	*Chromolena odrata* L.	Asteraceae	Siam weed
6.	Birana (Yoruba)	4	*Crotolaria pallida* Aiton.	Fabaceae	Smooth rattlebox
7.	Ewe-emu (Yoruba)	6	*Hibiscus surattensis* L.	Malvaceae	Bush sorrel
8.	Uda-agu (Igbo)	4	*Uvaria chamae* P. Beauv.	Annonaceae	Finger foot, bush banana
9.	Azi-ezi (Igbo)	0	-	-	-
10.	Aka-ogu (Igbo)	0	-	-	-

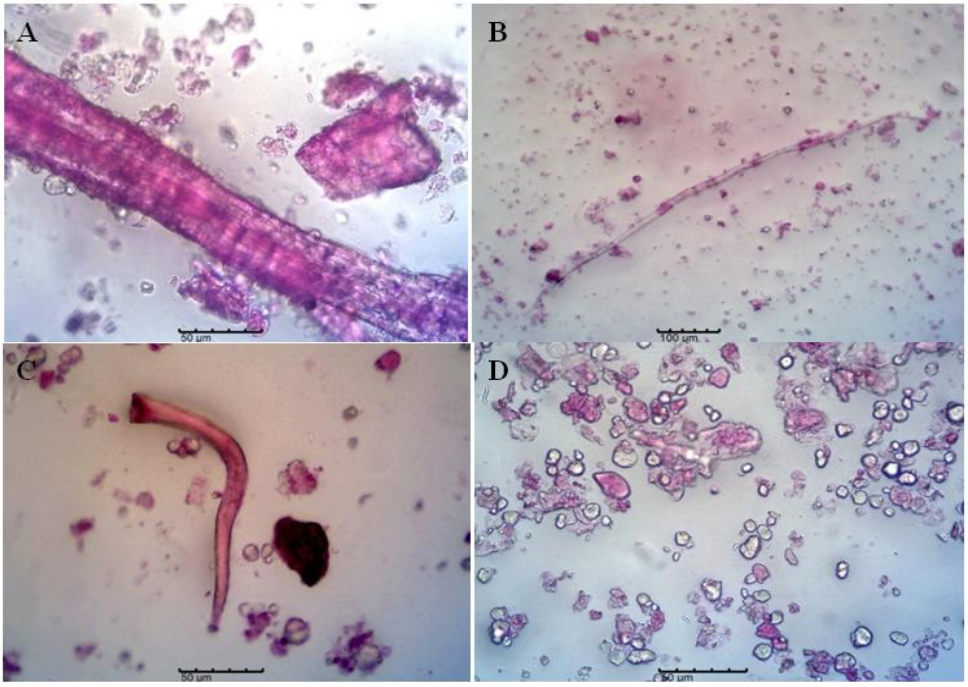


Fig. 1a-d. Tissues recovered from Aka-agu leave: (a) elongated parenchyma with pit-pairs (b) unbranched fibre (c) non-glandular trichome (d) isolated oxalate crystals

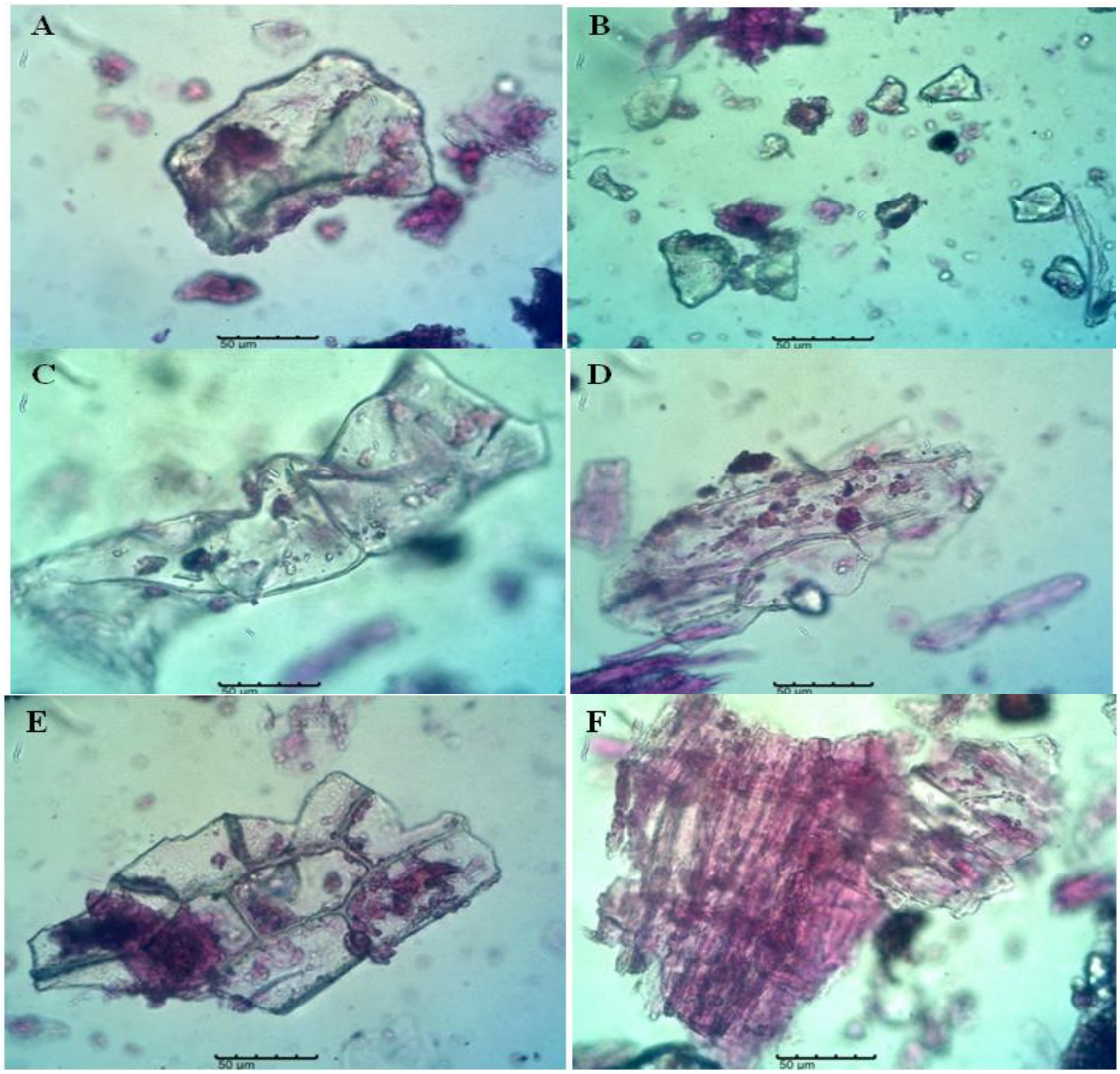
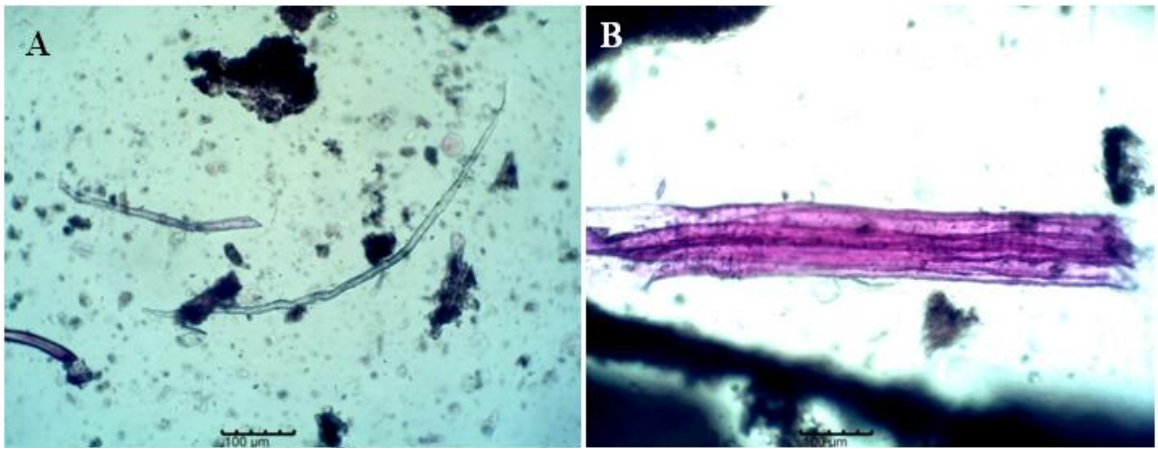


Fig. 2a-f. Tissues recovered from the leaves of *Emilia sonchifolia* (Amunimuye): (a) rectangular straite-wall epiderma, (b) scattered thin-walled parenchyma, (c) irregular shaped walled parenchyma. (d) elongated thin-walled parenchyma; (e) rectangular thick-walled parenchyma; (f) sclerieds



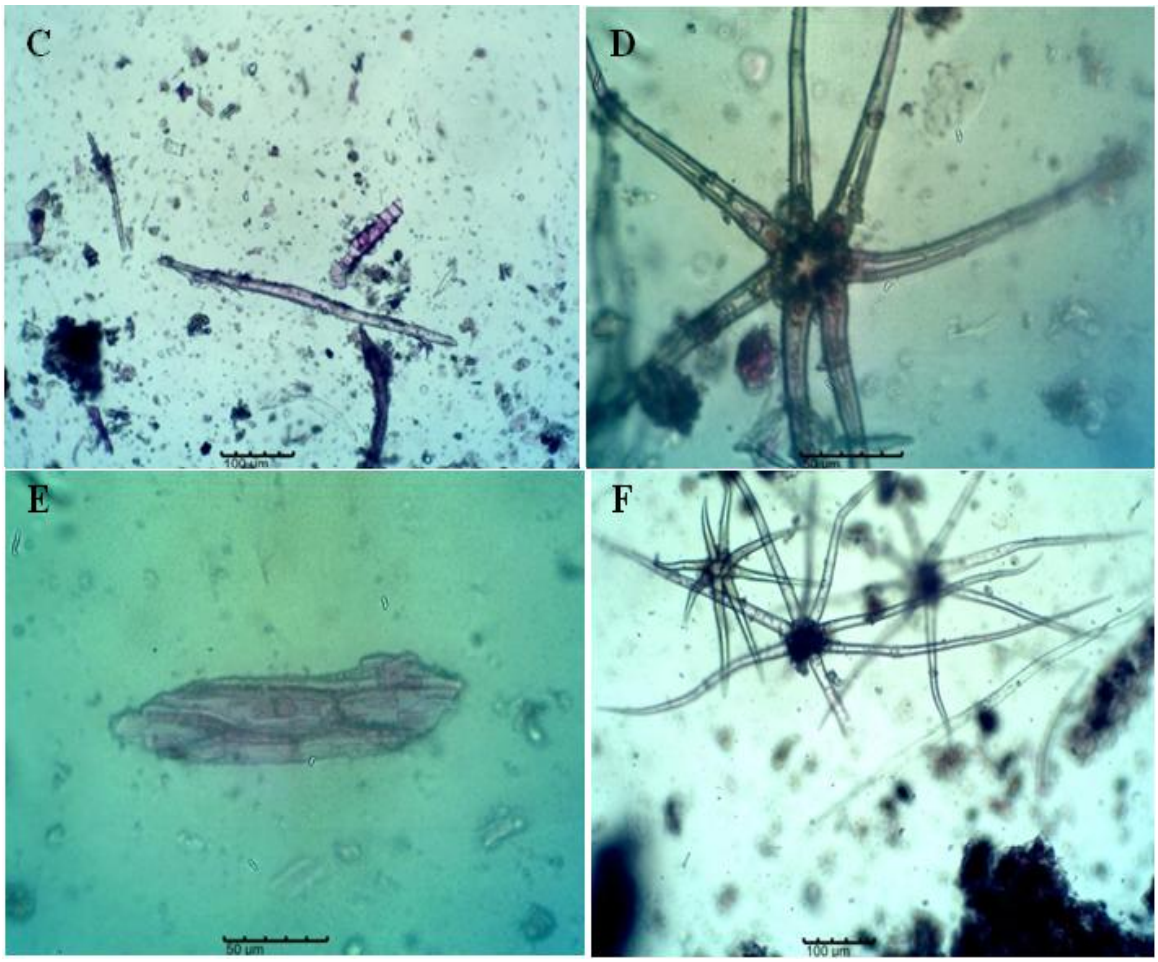
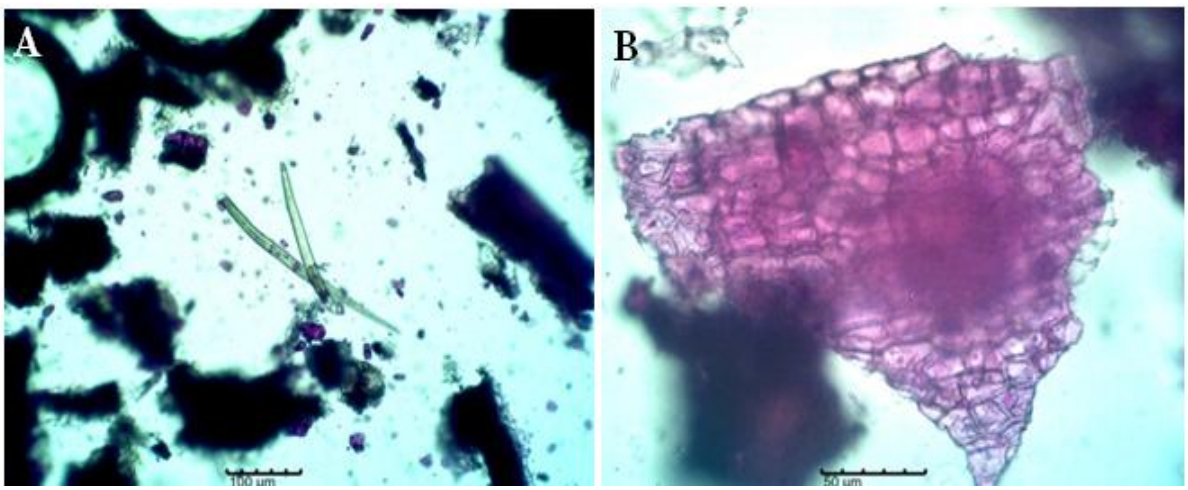


Fig. 3a-f. Tissues recovered from the leaves of *Tribulus terrestris* (Ewe-ayo): (a) Unbranched fibre, (b) rectangular straite-wall epiderma, (c) Non-glandular trichome, (d) Branched trichome, (e) thick walled pentagonal parenchyma, (f) cluster of branched trichome



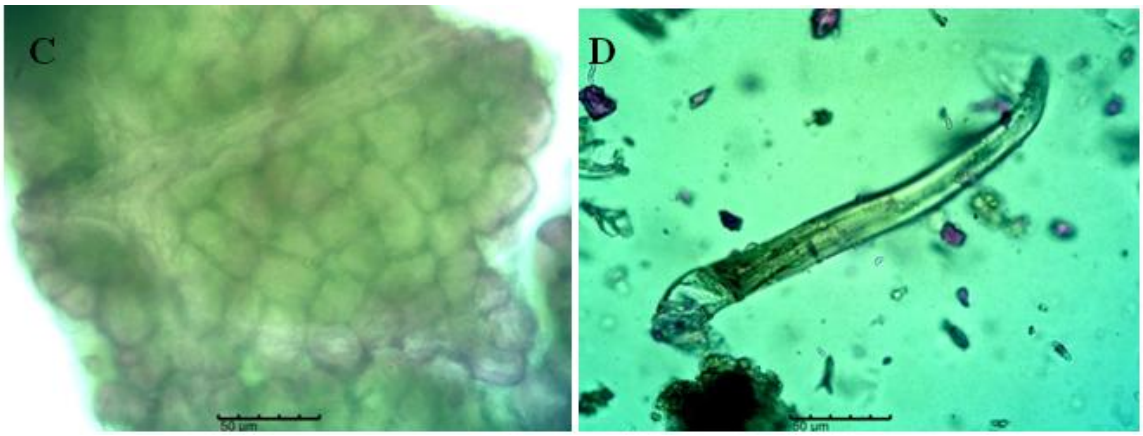


Fig. 4a-d. Tissues recovered from the leaves of *Pterocarpus santalinoides* (Nturukpa): (a) simple trichome, (b) densely packed parenchyma and rectangular striate walled epiderma, (c) irregular shaped parenchyma attached to rectangular shaped parenchyma, (d) Non-glandular trichome

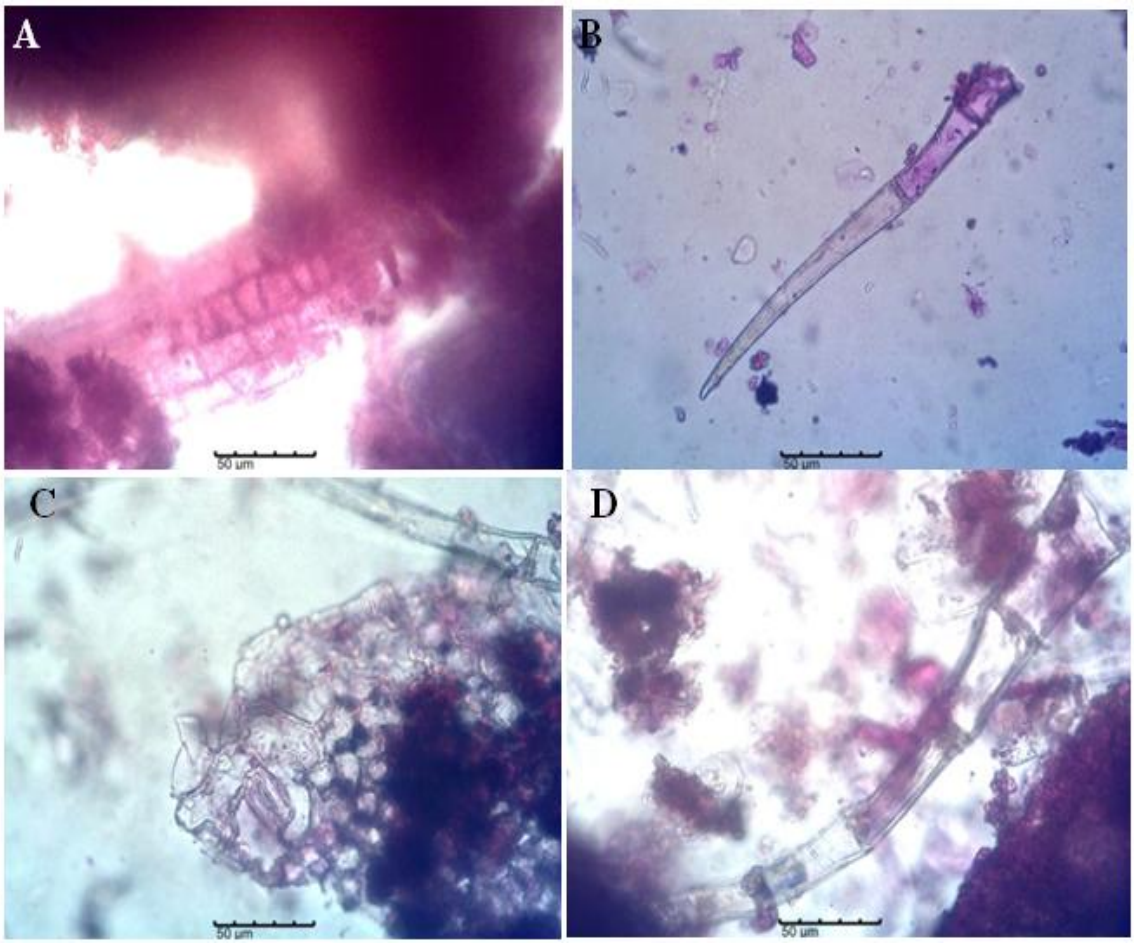


Fig. 5a-d. Tissues recovered from the leaves of *Chromolena odorata* (Obiara ohuru): (a) columnar epidermal cells and rectangular striate wall epiderma, (b) non-glandular trichome, (c) epidermal tissue comprising dicytic stomata, (d) glandular trichome

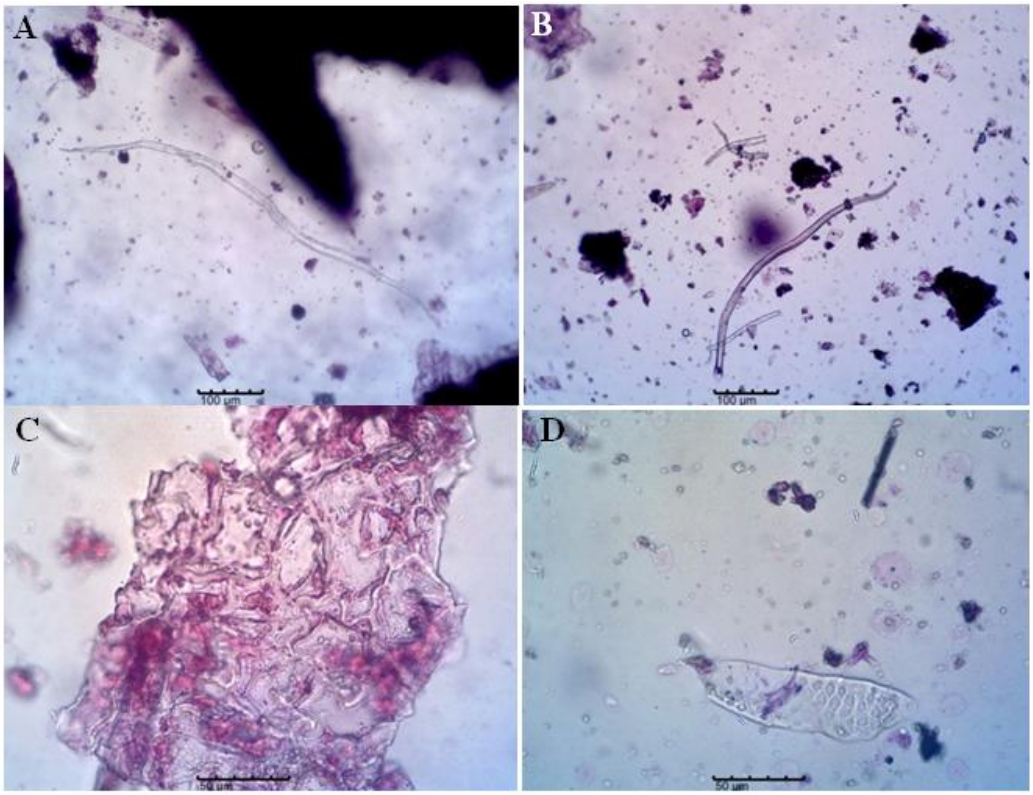
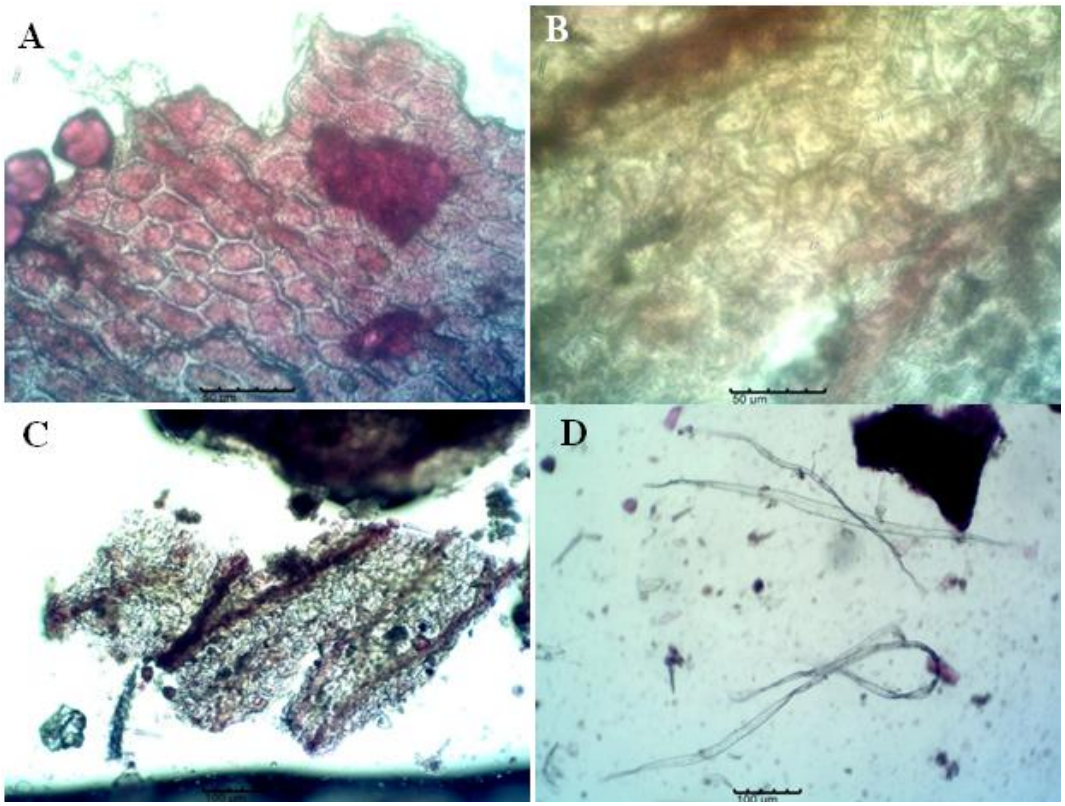


Fig. 6a-d. Tissues recovered from the leaves of *Crotolaria pallida* (Birana): (a) Unbranched fibre, (b) Trichome, (c) Epidermal tissue comprising subsidiary cells, (d) elaioplast/oil storing cells



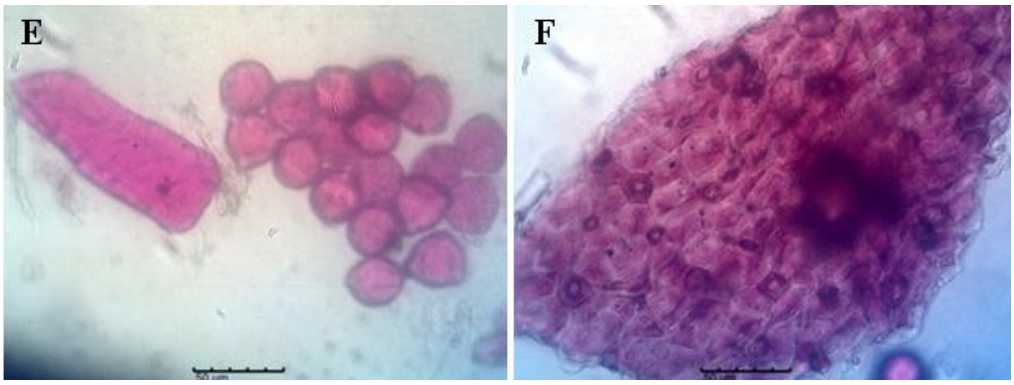


Fig. 7a-f. Tissues recovered from the leaves of *Hibiscus surattensis* (Ewe-emu): (a) Epidermal tissues, (b) Irregular shaped parenchyma, (c) Oil storing parenchyma, (d) Elaioplast/oil storing cells, (e) Tissues, (f) Cell morphology

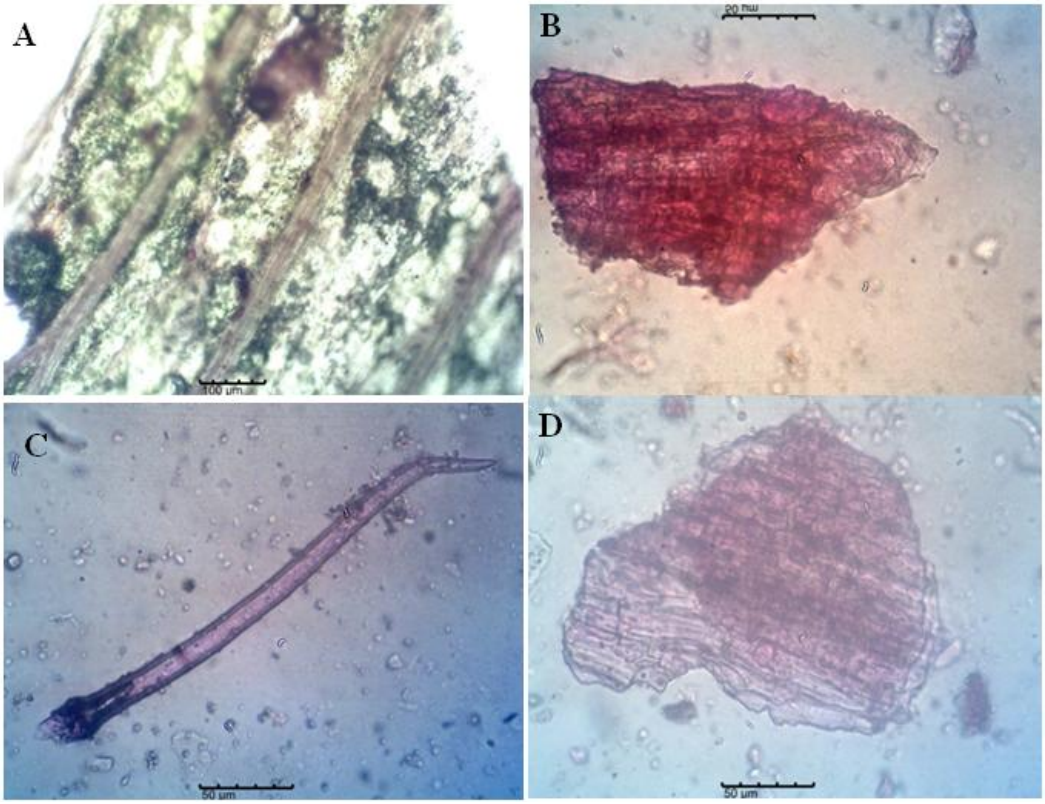


Fig. 8. Tissues recovered from the leaves of *Uvaria chamae* (Uda-agu): (a) columnar epidermal cells and rectangular striate wall epiderma, (b) Sheath cells, (c) Non-glandular trichome, (d) tightly parked rectangular parenchyma

4. DISCUSSION

The characters available in the powdered medicinal plant samples are much fewer than the potentially available characters in whole specimens. Medicinal plants are sources of therapeutic drugs for the treatment of various ailments and diseases. However, one of the major problems in herbal drugs is the misidentification of species and substitution of the plants with closely related species; this further poses the problem of adulteration which could be fatal to the consumers [17]. However, the difficulty encountered in the plant samples studied can be attributed to the destruction of the various tissues and plant cell wall during preparation, bringing about distortion in tissue architecture and normal arrangements found in

the whole plant section. This area of micromorphological make up of medicinal plants is yet to undergo detailed studies; and as such, there are fewer literatures to buttress finding.

The characters available in the powdered specimens were potentially useful for distinguishing the samples. While some herbal samples such as ewe-emu, birana, obiara ohuru, nturukpa, ewe-ayo, amunimuye, uda-agu and aka-agu may be readily detectable in powdered form, azi-ezi and aka-ogu was not easily detectable, thus, making it hard for histological identification and will require additional method for confirmation. More so, the difficulty encountered in the identification of Aka-agu, even though some tissues were recovered could be attributed to the issue of not having a uniform local name for a particular plant, because local names also helps in identification of plant sample. Most indigenous people, who share geographical locations not far from each other, tend to have different local names to which they use for a particular plant. This also may lead to misidentification of plant species by untrained users of these plants who may know a plant by a different local name other than the local name the plant is known within the location he/she finds the plant.

Also, the scantiness of characters obtainable from azi-ezi and aka-ogu raises concern on the inability of histological technique to detect the presence of this species while in mixture with other plants. Hence, several herbal products in the market cannot be identified by using only micro-morphological differences. As a result, Springfield et al. [8] suggested random amplified polymorphic DNA (RAPD) technique in cases where botanical identification is impossible, as high performance 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. [18] observed that when poisoning from traditional medicine occurs, it is often as a result of erroneous identification of the plant species in the form in which they are sold. Therefore, it is a matter of extreme necessity that the issue of quality control and assurance be addressed notwithstanding the limited resources and energy available. More so, the application of botanical identification in herbal medicine portends cheaper and more rapid measures, but where it proves inefficient, other efficient methods should be employed.

5. CONCLUSION

The assessment of plant materials by only microscopy may not readily provide complete and thorough identification as evident in the present study, although when combined with other analytical methods; it may certainly provide quite valuable evidence. There are suggestions and campaigns that herbal substances should be subjected to the same stringent scrutiny and quality controls as orthodox drugs before sending them to the public space and markets; this will help improve the quality and safety of herbal medicines and in turn eliminate the possibility of lethal adverse effect and thereby saving lives of potential users.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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