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Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

ISSN 2278-4136
ISSN 2349-8234
JPP 2013; 2 (4): 175-182

Received: 11-10-2013
Accepted: 26-10-2013

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Ethnobotanical survey and phyto-anatomical studies of some common plants used for the treatment of epilepsy in some rural areas of South west Nigeria

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ABSTRACT

Aim: To survey some areas in the southwest Nigeria where epilepsy is treated with plant extracts and determine the commonest plant species that are used, for pharmacognostic analysis.

Materials and Methods: Open-ended and semi-structured interviews were conducted for information from traditional medicine practitioners. Plant names given were authenticated in the herbarium and phytochemical and anatomical analyses were conducted on the plant parts that are used for treatment using standard approaches.

Results: Fifty practitioners were interviewed and they gave 17 different plants; out of these, the 5 commonest ones were investigated. The plants are either used individually or in combination with other plant(s). Services may be rendered freely or by collecting a token which is supernaturally believed to have negative consequences. The practitioners engaged in out-patient and in-patient services and patients are usually totally cured. There are also some inexplicable practices such as oracle visitation, incantations etc. A total of 30 patients have been cured in the last decade by each practitioner. Saponin, steroids, tannins, flavonoids, phlobatannins and alkaloids were isolated using three different media (methanol, petroleum ether and water) and diagnostic anatomical characters of the leaf epidermis, fruit wall, bark and root of the plants are presented in a single account for the first time in the country. The plants studied were *Newbouldia laevis, Securidaca longepedunculata, Tetrapleura tetraptera, Nicotiana tabacum* and *Senna occidentalis*.

Conclusion: The phytochemicals found are suggestive of the underlying medicinal potency of the plants and future work will help to establish the most active principles which will lead to development of drugs for treating the disease. The diagnostic anatomical characters can be employed in resolving doubts especially when the plant materials are in fragments. The least mentioned plant species can be saved from harvesting to conserve the rich flora diversity in the country.

Keywords: Epilepsy, Phytochemistry, Plant Anatomy, South-West Nigeria.

1. Introduction

It is well known that medicinal plants play a key role in the development and advancement of modern drugs [19, 21]. Approximately 25% of drugs used in modern Pharmacopoiea are derived from plants and many others are synthetic analogues built on prototype compounds isolated from plants. Epilepsy is a non-infectious neurological disease. It is a major public health issue especially in developing African countries. The etiologies and approach to management are significantly different in developed and developing countries. The patient with epilepsy is ostracized, stigmatized, misunderstood and likely to drop out of school, lose his job, finds it almost impossible to marry, loses his wife or her husband, and be tormented to the extent of becoming a vagabond [14]. A link between epilepsy and cognitive deterioration has also been observed. Ogunrin [14] noted that defective memory, especially for recent acquisitions and weakened capacity for attention are frequently encountered cognitive impairments in epileptic patients. These conditions necessitate the need to explore more sources for treating the disease. At a conservative estimate, 50 million people worldwide have epilepsy with an annual incidence ranging from 20 to 70 cases per 100,000 and around 40 million of them living in developing

countries[14, 20, 25].

Pharmacognostic methods have been used to investigate the medicinal properties of plants [5, 9]. In Nigeria, many plants are used for the cure and management of epilepsy, but a few of them that are common in most communities where the disease is often treated were considered for pharmacognostic investigation. The present work is a contribution to researches into medicinal plants and the University of Lagos, Nigeria is a frontier in the endeavour. We investigated *Newbouldia laevis*, *Securidaca longipedunculata*, *Tetrapleura tetraptera*, *Nicotiana tabacum* and *Senna occidentalis* the commonest plants used for treating epilepsy in the studied areas. However, these plants can be readily identified with their macroscopic features but when their parts are in fragments, identification becomes extremely difficult. Existence of drug plants in fragmentary condition is common in West African markets. Unfortunately, the basic anatomical data where generalized accounts were given on their families are insufficient for species identification. Similarly, reports on phytochemistry on the plants are on chemicals found in methanolic extract mostly [4, 8, 11, 12, 15, 16, 17].Therefore, the primary aim of the present work was to investigate the diagnostic anatomical features and phytochemical properties using three extractants (water, methanol and petroleum ether) in order to enhance ease of identification and document the pharmacological principles of these plants for future drug development. The study will also help to preserve local medical knowledge and contribute in the long-run to improve health care delivery system in the country.

2. Methodology

An ethnobotanical survey was conducted in some areas of southwest Nigeria (Fig. 1). We administered open-ended and semi-structured interviewing techniques for information from experienced traditional medicine practitioners as suggested by Martin [10]. The plant names were authenticated in the herbarium of University of Lagos where voucher specimens were deposited. Data matrix for responses is presented in Fig. 2.

Euclidean 2-way cluster analysis using paired group algorithm [6], was employed for determination of commonest species used in the studied areas for treating the disease (Fig. 3). Phytochemical method follows the approach of (Ajayi [3], Harborne [7]). For phytochemical examination, the plant materials were oven-dried at 40 °C for 3–4 days and ground to a coarse powder. Two hundred grams of powdered materials were extracted with petroleum ether and methanol in a soxhlet extractor to obtain their extracts. The extracts were concentrated with a rotary evaporator and amounts of 40 g (20%) were obtained. An aqueous extract was obtained by soaking the dried exhausted plant material for methanolic extraction in cold water for 48 hrs. The aqueous extracts were concentrated in a water bath, and weighed to obtain 21 g (10.5%). All extracts were stored in sterile bottles and kept in the refrigerator until use. Standard phytochemical screening methods were applied to each plant extracts, so as to test them for saponin, steroids, tannins, flavonoids, phlobatannins and alkaloids. The anatomical work was carried in the research laboratory of the Department of Botany while phytochemical study was done in the laboratory of Nigerian Natural Medicine Development Agency. The various parts of the plants that were used for the study are presented in Table 1. For anatomy, we adopted the approaches of Ajayi [3] and Kadiri [9]. Leaf epidermis was examined after acid treatment which involved cutting 2–5 cm² from the standard median part of the leaf lamina near the mid-rib. Dried leaves were boiled in water for thirty minutes and subsequently soaked in concentrated trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8–24 hrs. to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermal layers were separated and transferred into Petri dishes containing water for cleansing. Tissue debris was cleared off the epidermis with fine-hair brush and washed in several changes of water. Drops of different grades of ethanol, 50% – 100%, were added in turn to dehydrate the cells. The preparations were later stained with Safranin O in 50% alcohol for about five minutes before mounting in glycerine on glass slides. The epidermal layers were mounted on glass slides with the uppermost surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration.

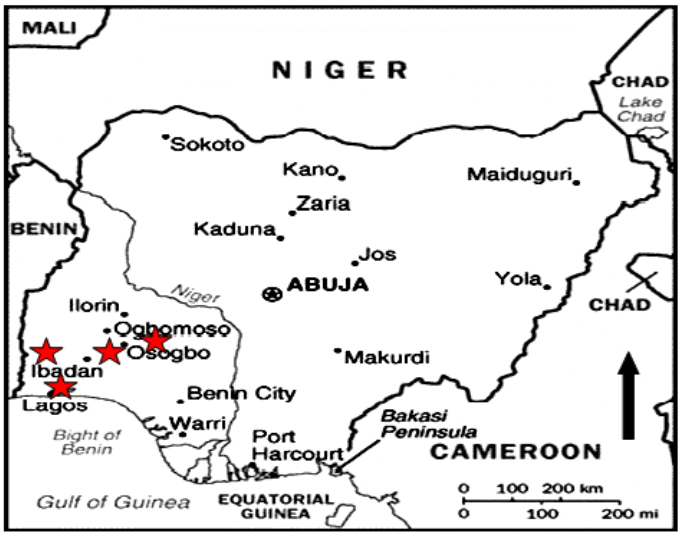


Fig 1: Locations where traditional medicine practitioners were interviewed.

For root and bark anatomy, the materials were dissected using the standard method of free-hand sectioning with the aid of dissecting blade. Thin sections were selected and few drops of ethyl alcohol were added, then two drops of safranin O and counter stained with Methylene blue. Prepared slides were covered with cover slip and ringed with nail varnish to prevent dehydration. All preparations

were observed with Olympus microscope and photographs were taken with digitized camera for microscope.

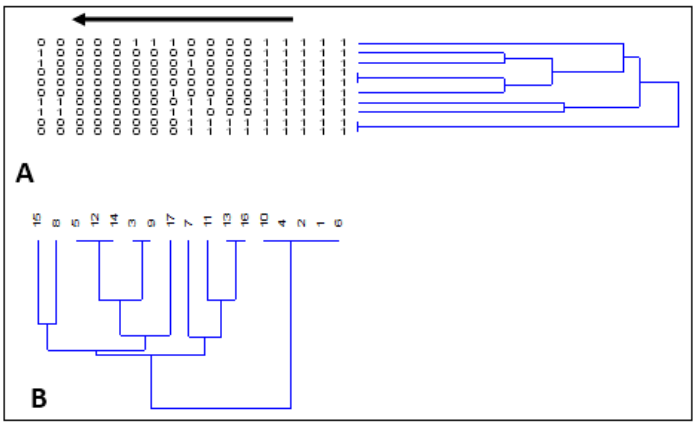


Fig 2: Euclidean 2-way cluster analysis of responses using paired group algorithm. **A**, shows the data matrix; from right to left, the figures are numbered 1-17. **B** is dendrogram showing plants used. 1= *Senna occidentalis* 2= *Nicotiana tabacum* 3= *Allium ascalonicum* 4= *Securidaca longepedunculata* 5= *Butyrospermum parkii* 6= *Tetrapleura tetraptera* 7= *Elaeis* guineensis 8= *Piper guineense* 9=*Capsicum frutescens* 10= *Newbouldia laevis* 11= *Lannea welwitschii* 12= *Raphia hookeri* 13= *Vigna unguiculata* 14= *Calotropis procera* 15= *Xylopia aethiopicum* 16= *Viscum album* 17= *Citrus sinensis*. 1= present, 0 =absent.

Table 1: Botanical information on the plants used for the study.

S. N	Species	Family	Local Name	Part used	Habitat	Morphology
1.	*Senna occidentalis* (L.) Link.	Fabaceae	Rere	Leaves	Waste area	A low branching perennial shrub. It has compound leaves with narrow linear-to-oval-shaped dark green leaflets. Flower is yellow in colour and fruits are brown containing brown seeds
2.	*Newbouldia laevis* (P. Beauv) Seem	Bignoniaceae	Akoko	Root bark	Wet and dry	A much-branched tree with ascending branches. The leaves are compound and the flowers are mauve – pink in colour. The fruits contain numerous thinly winged seeds
3.	*Nicotiana tabacum* L.	Solanaceae	Taba	Leaves	Farmland and waste area	It is a robust annual little branched herb with large green leaves which are oblong-elliptic in shape and long trumpet shaped white-pinkish flowers. All parts are sticky, covered with short viscid-glandular hairs, which exude a yellow secretion containing nicotine
4.	*Securidaca longepedunculata* Fresen.	Polygalaceae	Ipeta	Root bark	Woodland and savannah	A slender tree with pale grey, smooth bark. Leaves are variable in size and shape. Flowers are sweet scented having pink to purple colour. The fruit is round having a distinctive membranous wing that is purplish green when young and turn pale straw when mature
5.	*Tetrapleura tetraptera* (Schumach. And Thonn) Taub	Fabaceae	Aidan	Fruit pod	Wet	A robust, perennial tree with grey-brown and smooth-rough bark. Leaves are compound and the flower is yellow-pink in colour with dark brown, four winged fruits (pods) of about 12–25 x 3.5–6.5cm size. The fruit possesses a characteristic pungent aromatic odour

3. Results

A total of 17 plants were given out of which only five were commonly mentioned by the respondents. According to them, *Caspicum frutescens* and *Butyrospermum parkii* were used as stabilizing agents for the preparations. The plants that are in common use include *Newbouldia laevis*, *Securidaca longepedunculata*, *Tetrapleura tetraptera*, *Nicotiana tabacum* and *Senna occidentalis*; they were compounded and transformed into syrup, powder, tea, soup and cake for administration in different prescribed doses. From the questionnaire and interview conducted, we found out that the disease can affect people of all ages and a fee may be paid or treatment given freely to the patients but many supernatural beliefs were associated with the condition. For instance, the practitioners' children will be inflicted if a fee is collected for treatment and oracle may be contacted for divination. Treatment may be out-patient or in-patient and patients are not discharged until they have totally recuperated.

Most of the respondents are educated and they attend refreshers' courses (both formal and informal) from time to time so as to enhance service delivery. As presented by each respondent, a total of 30 patients have been fully treated within the last decade. However, all the practitioners solicited for incorporation into the national health care scheme and are willing to share ideas with government.

The fruit epidermis of *Tetrapleura tetraptera* has polygonal epidermal cells where some endogenous substances were observed. The walls are unusually thickened with indications of some granular compounds within the cells. Transverse sections of the root of *Securidaca longepedunculata* evealed large parenchyma cells in the cortical area having some bulbous (glandular) endogenous substances. In *Nicotiana tabaccum*, the midrib in transverse section possesses glandular trichomes on both surfaces while the vascular bundle also has some endogenous chemicals. Scanty crystals and multicellular glandular trichomes are located within the epidermal cells and found on the upper surface of the leaf respectively. *Senna occidentalis* has chemical substances lodged around the stomata and cell lumen. Scalariform vessels of the mid-rib also have chemical materials within them. The root of

Newbouldia laevis has some chemical compounds within the thickened walls of the parenchyma cells and the vascular bundles.

Water extract of the plants showed presence of saponin, tannin and alkaloids in all the plants; but steroids, flavonoids and phlobatannins are absent in *Nicotiana tabacum* while flavonoids and phlobatannins are absent in *Newbouldia laevis* and *Senna occidentalis* respectively.

The analysis of the methanolic extract of the plants, indicated presence of saponin only in *Tetrapleura tetraptera*, absence of steroids in *Nicotiana tabacum* and lack of tannins in *Securidaca longepedunculata*. Flavonoids and alkaloids are present in all the species but none of the plants stores up phlobatannins.

In the petroleum ether extract, there is presence of saponin only in *Securidaca longepedunculata* and *Tetrapleura tetraptera*, and steroids in *Nicotiana tabacum* and *Cassia occidentalis*. Tannins, phlobatannins and flavonoids are absent but, alkaloids are present in all the plants.

Table 2: Features of the plants that can be used for diagnosis

Species	Anatomical Diagnosis			
	Leaf epidermis	**Parenchyma of root**	**Midrib**	**Vascular bundle**
Nicotiana tabacum	Presence of glandular trichomes (different types), crystals and lodgment of materials with cell lumen		Parenchyma cells are loaded with chemical substances and the cell lamellae have copious presence of chemical materials	
Securidaca longepedunculata		Large glandular parenchyma bearing endogenous substances		
Tetrapleura tetraptera	Lodgment of chemicals within cell lumen and cell wall			
Newbouldia laevis		Parenchyma is impregnated with chemicals. Lamellae of the cells are also lodged with chemicals		
Senna occidentalis	Presence of crystals in the cell lumen and presence of glandular trichomes			Scalariform vessels containing chemical substances

Table 3: Phytochemical characteristics of the plants studied.

Test	Species				
	Nicotiana tabacum	***Securidaca longepedunculata***	***Tetrapleura tetraptera***	***Newbouldia laevis***	***Senna occidentalis***
Saponin					
Water extract	+ve	++ve	+ve	+ve	+ve
Methanolic extract	-ve	-ve	+ve	-ve	-ve
Petroleum ether extract	-ve	+ve	+ve	-ve	-ve
Steroids					
Water extract	-ve	+ve	+ve	+ve	+ve
Methanolic extract	+ve	-ve	-ve	-ve	+ve
Petroleum ether extract	+ve	-ve	-ve	-ve	+ve
Tannins					
Water extract	+ve	+ve	+ve	+ve	+ve
Methanolic extract	+ve	-ve	+ve	+ve	+ve
Petroleum ether extract	-ve	-ve	-ve	-ve	-ve
Flavonoids					
Water extract	-ve	+ve	+ve	-ve	+ve
Methanolic extract	+ve	+ve	+ve	+ve	+ve
Petroleum ether extract	-ve	-ve	-ve	-ve	-ve
Phlobatannins					
Water extract	+ve	-ve	-ve	-ve	+ve
Methanolic extract	-ve	-ve	-ve	-ve	-ve
Petroleum ether extract	-ve	-ve	-ve	-ve	-ve
Alkaloids					
Water extract	+ve	+ve	+ve	+ve	+ve
Methanolic extract	+ve	+ve	+ve	+ve	+ve
Petroleum ether extract	+ve	+ve	+ve	+ve	+ve

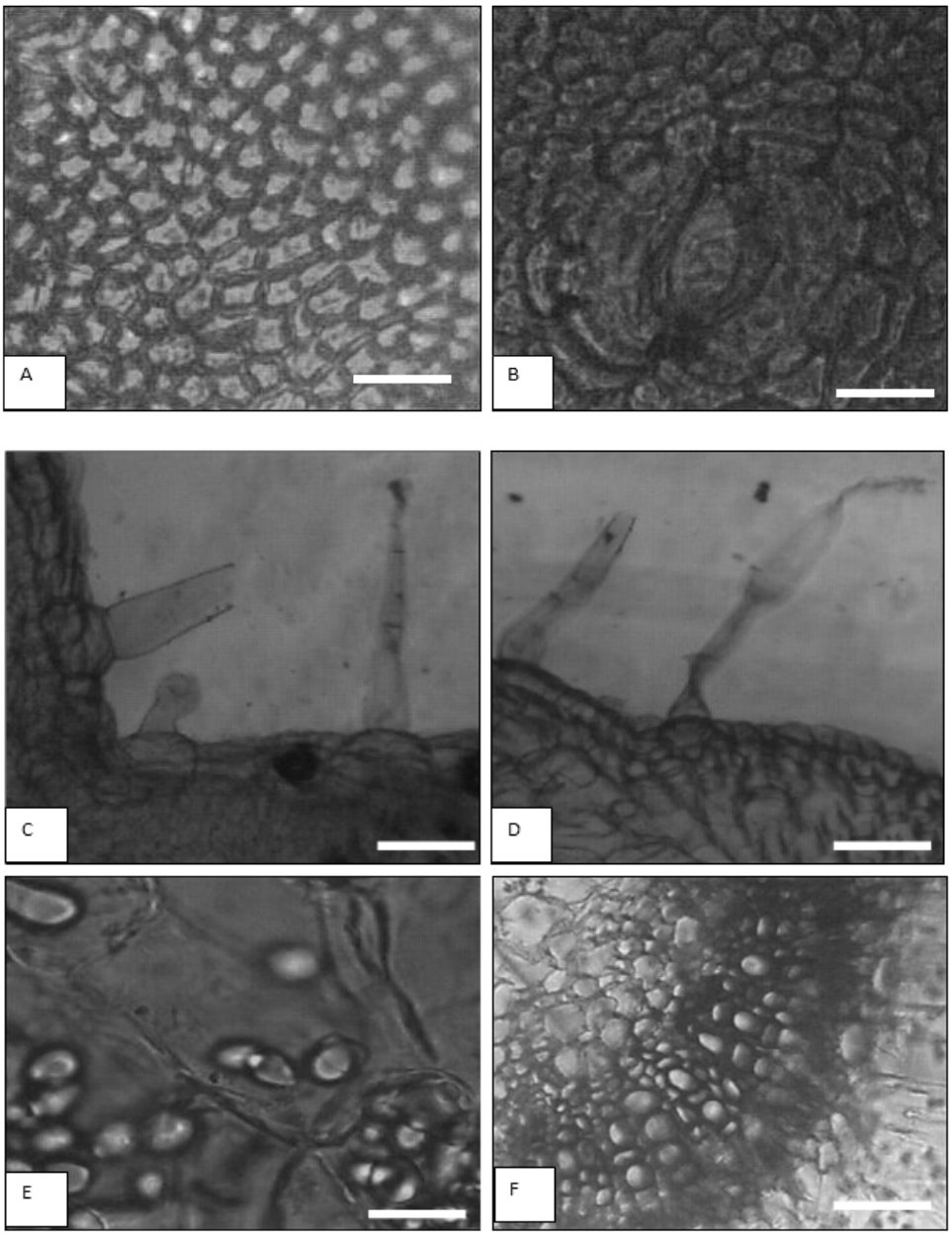
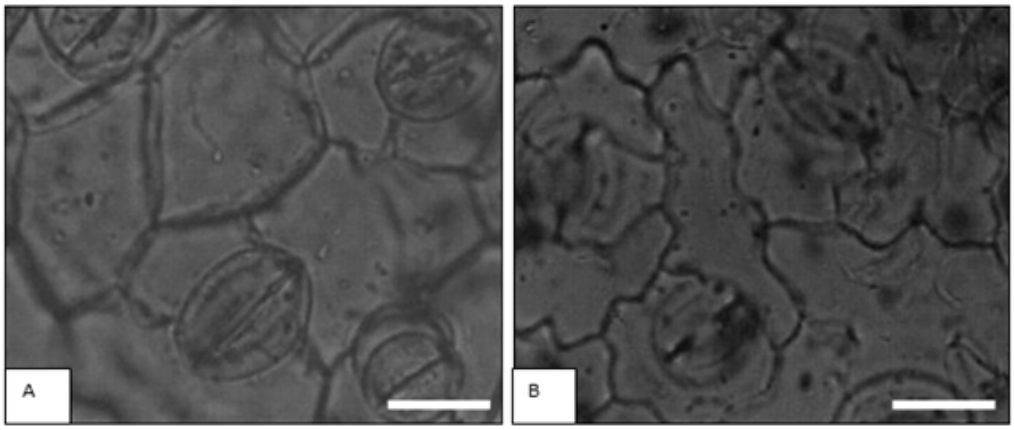


Fig 3: Anatomical features that are useful for species' diagnosis. A, B: Fruit epidermis of *Tetrapleura tetraptera*. C, D: Glandular trichomes found on the midrib of *Nicotiana tabacum*. E: Cortical region of the root of *Securidaca longependuculata* showing parenchyma lodged with some substances. F: Vascular bundle of *Nicotiana tabacum* showing presence of chemical substances around the cells. Scale bar= 50µm.



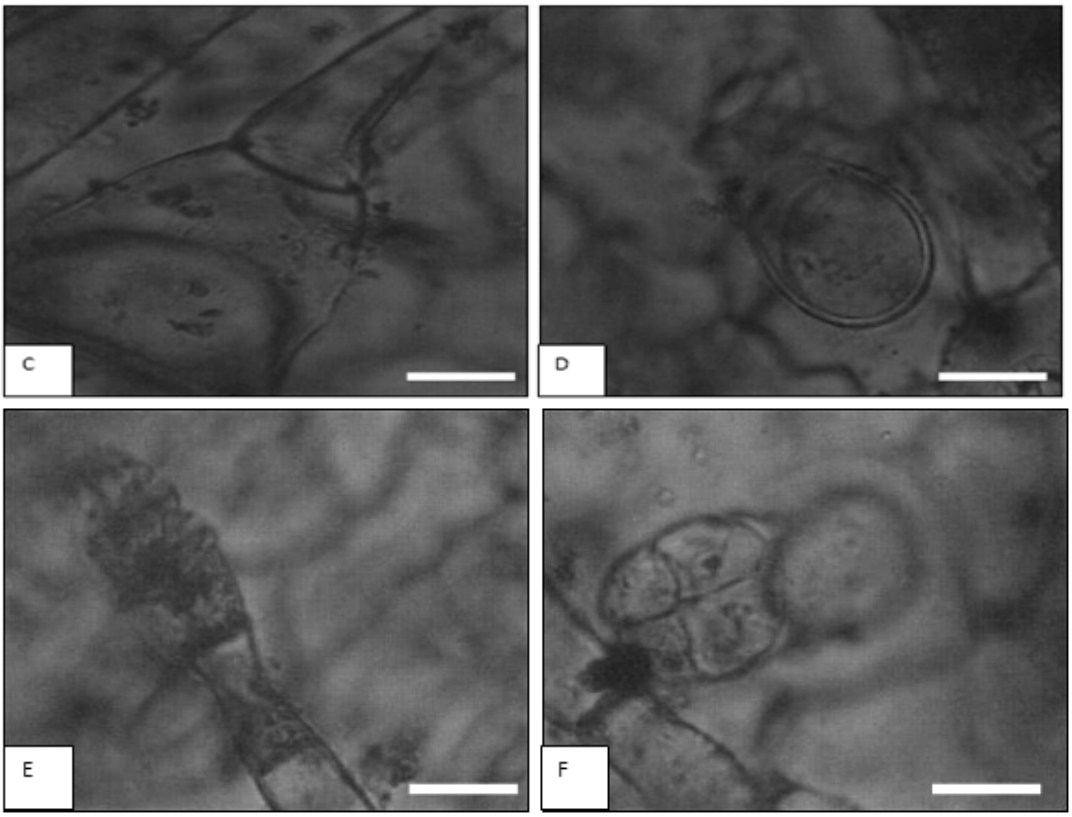
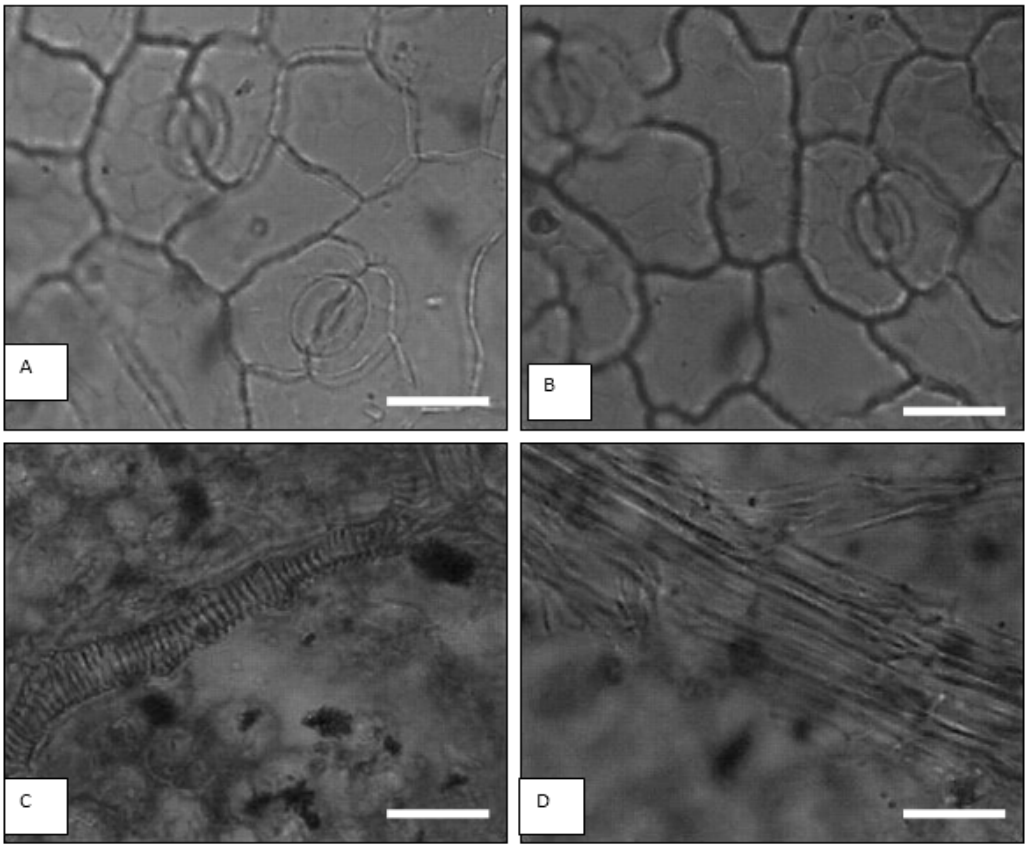


Fig 4: Anatomical features which are useful for species' diagnosis. Leaf epidermal characteristics and trichome types found to lodge substances in the globular head. A: lower surface, B: upper surface of the leaf of *Nicotiana tabacum* showing scanty deposits of crystals within cell lumen. D-F: Glandular unicellular and multicellular trichomes found on the leaf of *Nicotiana tabacum*. Scale bar= 50µm.



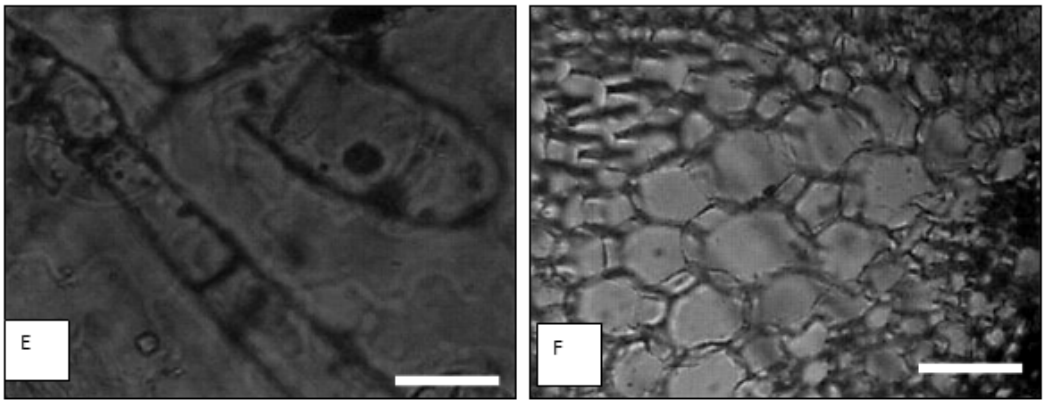


Fig 5: Anatomical features that are useful for species' diagnosis. A-D: Features of the leaf of *Senna occidentalis* C, Scalariform vessels containing chemical substances and D, bundle of vessels. E: A unicellular glandular trichome on the leaf of *Nicotiana tabacum*. F: Cortical region of the root of *Newbouldia laevis* surrounded with some chemical substances. Scale bar= 50µm

4. Discussion

Inexplicable practices, belief in supernatural power, lack of standard dosage and concealment of information which are known to be problems of traditional medicine were established in the study again. We succeeded in the information gathering interview due to our mode of approach which included display of respectful disposition; need to appreciate the work for academic purpose, speaking the language of the respondents, paying of a token in appreciation among others, as suggested by Martin (1995). Seventeen plant species were given by 50 respondents and only 5 of these were commonly mentioned by them, hence we focused on these common ones. However, the present account will assist in future examination of the plants for drug development and unambiguous identification of the species. Reports on methanolic extract of some of these plants have been presented and a few of the phytochemicals were given [4, 8, 11, 12, 15, 16, 17]. In the present work, most phytochemicals of the plants extractable in three different media namely methanol, water and petroleum ether are presented for comparison. The six major chemicals that were extracted are thus suggested to underlie the medicinal potency of the plants. Alkaloids can be extracted easily from the plants with all extractants, methanol extracted flavonoids easily from the plants than other extractants and, tannins and saponins were better extracted with water in all plants. Saponin is quite abundant in all the plants and it was isolated in all the extracts.

Interestingly, the petroleum ether extract indicated presence of saponin only in *Securidaca longepedunculata* and *Tetrapleura tetraptera*, and steroids in *Nicotiana tabacum* and *Senna occidentalis*; as well as absence of tannins, phlobatannins and flavonoids in all plants. These isolated substances have been reported to have action on the biological membranes [12, 15, 16, 17] reported that methanol extract of *Securidaca longepedunculata* at 50-250 mg/ml inhibited or abolished contractions of the frog rectus abdominis muscle preparations induced by acetycholine (Ach), carbachol, or nicotine (0.1-10 mg/ml) in a dose dependent manner. In other studies, 1,7-dimethoxyl-2-hydroxyxanthone and 1,4 dihydroxyl-7-methoxy-xanthone, saponine aglycones presenegin elymoclavine, sinapic acid and 3,4,5-O-caffeoyl-quinic acid, securinine methylsalicylate and sugars due to hydrolysis of saponines, have been found in the plant; moreso, securidacaxanthone B and C, xanthones have been reported in the plant [8, 11, 12, 17], reported that nicotine found in *N. tabacum* binds stereospecifically to acetylcholine receptors at the autonomic ganglia, adrenal medulla, neuromuscular junctions and the brain where electrical stimulation-evoked neurotransmitters from sympathetic nerves is released. Saponin glycoside with an oleanolic acid aglycone, a monodesmosidic diglycoside of the rare sapogenin 27-hydroxyolean-12 (13)-en-28-oic acid has been reported in the methanolic extract of *Tetrapleura tetraptera* [1, 2, 4]. These compounds are anticonvulsant which has membrane stabilizing property [15, 16]. *Newbouldia laevis* has also been reported to be analgesic and anti-convulsant due to its pharmacological compounds [22, 23]. *Senna* as a genus, has been reported to have potency for managing muscle dysfunction and degeneration although toxicity has been reported in lower animals by *Senna occidentalis*. In this plant, anthraquinones and their derivatives, and alkaloids have been reported [24]. However, the anatomical characteristics of these plants; hitherto, are insufficient and this will make their identification difficult when their materials are in fragments. Metcalfe & Chalk provided some anatomical characteristics which are known for the families based on a few representative species and they are insufficient for identification of the species used. However, the epidermal characteristics (anticlinal wall pattern, stomatal type, epidermal cell shape and presence or absence of trichomes) recorded in the studied plants are in partial agreement with the work of Metcalfe & Chalk. The distinctive features that were now identified in the species will immensely assist in their identification especially when one is in doubt.

Conclusion: The phytochemicals found underlie the medicinal potency of the plants and future work will help to establish the most active principles which will lead to development of drugs for treating the disease. The diagnostic anatomical characters can be employed in resolving doubts especially when the plant materials are in fragments. The least mentioned plant species can be saved from harvesting to conserve the rich flora diversity in the country.

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