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Establishment of Quality Parameters and Pharmacognostical Profiling of *Dialium guinneense* L. (Caesalpiniodeae)

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***Abstract*— The standardisation of the leaf, stem, and root of Dialium guinnense has been undertaken. The macroscopy, microscopy as well as the transverse section of these parts were studied. The preliminary phytochemical analysis, analytical standards and chemomicroscopical analysis of these parts were also determined. The macroscopical features of the morphological parts showed that the surface of the leaf is green with an entire margin and lanceolate-oblong shape and pubescent. The stem bark has a hard texture and is usually dark grey- brown to black in colour. The root which is light brown in colour consists of a long branched tapped root with a dense mass of superficial feeder roots. The result of the powdered microscopic analysis showed the presence of non glandular unicellular trichomes ,tracheid fibres, epidermal cells, prism of calcium oxalate, starch granules, paracytic stomata in the leaf; fibres with bordered pitted vessels, single fibre, prism of calcium oxalate crystals, cork cell in the stem and bundles of fibres with borderered pitted vessels, prism of calcium oxalate crystals, cork of thin walled cells, sclereids, unicellular non glandular trichome in the root. The transverse section of the leaf revealed the presence of cuticle, epidermis, palisade and spongy mesophyll, vascular bundle; the stem revealed the presence of cork cambium, cortex, vascular bundles, secondary xylem and pith and the root revealed the cork, cortex, vascular bundle and pith. The phyochemical analysis showed the presence of alkaloids, glycosides, tannins, terpenoids, reducing sugars, carbohydrates, saponins, flavaonoids and steroids. Tannins were found in the leaf and stem while resin was found in the leaf alone. Oil and acidic compounds were absent in all the plant parts tested. The percentage values obtained for analytical standard of the leaf were 7.50, 1.83, 1.32, 1.65, 10.00, 10.00, and 0.25 % for total ash, acid insoluble ash, water soluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and moisture content respectively; the stem values were, 4.75, 1.48, 1.40, 1.42, 20.00, 10.00, and 0.15 % for total ash, acid insoluble ash, water soluble ash, sulphated ash alcohol soluble extractive, water soluble extractive and moisture content respectively and the root were 5.00, 1.53, 1.60, 1.40, 10.00, 20.00 and 0.95 % for total ash, acid insoluble ash, water soluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and moisture content respectively. Chemomicroscopical analysis revealed the presence of tannins, calcium oxalate crystals, cellulose, starch, secretory cells and ducts, Suberized wall and fibres. The data obtained from this study can be used in standardisation of Dialium guineense L. and preparation of the monograph for its possible inclusion in the Pharmacopoeia.**

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***Index Terms*— *Dialium guineense*, Chemomicroscopy, microscopy, Standardization, Phytochemical analysis.**

I. INTRODUCTION

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process [1]. The Pharmacopeia and other official compendia have defined standards for crude drugs in terms of appearance, numerical constants and strength of active constituents. Generally, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective [2; 3; 4; 5; 6; 7].

Dialium guineense L. (Caesalpiniodeae) is a tree of 30m high, with a densely leafy crown, but often shrubby, bole without buttresses, bark is smooth, grey, slash reddish, yielding a little red gum. It *g*rows in dense savannah forests, shadowy canyons and gallery forests. It is found from Senegal to Sudan along the Southern border of the Sahel. *Dialium guineense* commonly known as black velvet and as Kedebe, Mako, Meko, mekohi (Fulani), Icheku (Igbo), Awin (Yoruba) is a multipurpose tree in West African region. The leaves and fruits are consumed during the dry season when farm produces are unavailable; they are sold in the market as refreshing drinks when mixed with water. In ethno medicine various parts of the plant have been used in the management of fever, diarrhea and palpation and as anti bacterial. *Dialium guineense* is used by traditional healers in some parts of Nigeria as antiulcer and vitamin supplement [8] and for the treatment of heart disease which might be attributed to the presence of tannins in the plant. Lawrence *et al.*, [9] reported that tannins possess excellent cardio protective qualities in addition to its antioxidant action. It's used as chewing stick and the teeth of the users are usually strong, clean, fresh and devoid of dental plaques and carries [10].

According to a research conducted by Akinpelu *et al*., [11] *D.guineense* forms part of the ingredients used in preparing decoction for treatment of some ailments and thus it is supposed to be safe in consumption and drugs formulated

from this plant may pose no dangers to the users. *Dialium guineense* the plant of interest has been found to possess anti-diarrhoea, anti-ulcer, anti-malarial effects and is used for the treatment of jaundice, severe cough, bronchitis, wound, stomach aches and haemorrhoids. These therapeutic effects were found residing in the leaves and stem bark of *Dialium guineense* [12]. The aim of this research is to establish standards for the leaf, stem, and root of *Dialium guineense* for the preparation of its monograph and possible inclusion in the African Pharmacopoeia.

II. MATERIALS AND METHODS

Collection and Identification

The plant materials were collected in June from University of Nigeria Nsukka, Enugu State, and were also authenticated by Mr. A.O Ozioko a taxonomist with International Centre For Ethnomedicine and Drug Development (inter CEDD) Nsukka. The voucher specimen deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka.

Preparation of plant materials

The leaves, stems and roots were carefully separated, washed and excess water allowed to drain off. Representative samples of the leaves stem and roots were kept for examination while the rest were dried under shade, until they were completely dried. They were then pulverised separately and stored in separate sample containers from were they were collected and used for analysis. Transverse sections were cut from the representative samples using sledge microtone. The sections were preserved in 70% ethanol until needed for studies.

Preliminary Phytochemical Test

The phytochemical tests were performed on the powdered leaf, stem and root samples in order to detect the presence or absence of major secondary plant metabolites of pharmacognostic importance which include; alkaloids, steroids tannins, saponins, flavonoids, oils e.t.c. following standard procedures [13; 14].

Standardization/Qualitative Analysis

Macroscopic analysis

The leaf, stem and root were examined visually. The macroscopic characters of the leaves which include the type of margin, petiole, veination, base and so on were observed and noted. Also macroscopic features such as size, shape, surface characters, fracture and texture of the roots were observed. Finally, the organoleptic properties like colour, odour and taste of both leaves and roots were observed and noted.

Microscopic Analysis

Microscopic examination of powdered materials

Little quantity of the powdered crude drug was placed on a slide and two drops of chloral hydrate was added to moisten the powdered drug. It was covered with the cover slip and passed across the flame of Bunsen burner repeatedly until bubbles occur. Then it was allowed to cool. Two drops of glycerine were added for clarity of structures and the slide was viewed under microscope to reveal microscopic characters which were observed and noted [15].

Microscopic examination of transverse sections

The staining method as described by Odoh *et al*., [15] was used. Sledge microtone was used for the sectioning of the specimen. The sections were transferred into staining jar and stained in safranin for 5 minutes. The safranin was drained off and sections were washed about three times with distilled water then 97% alcohol was used to wash the sections for two times each. The sections were counter stained in 1% past green for 5 minutes and washed with absolute alcohol for about three to four times. After that the sections were transferred into a staining jar containing 50/50 alcohol/xylene and washed until they became clear. Finally, the pure xylene was used to clear the sections and the Canada balsam moutant was used to mount the sections on the slide.

Determination of analytical standards

Ash values

The methods adopted for the determination of ash values follow the specification given by Odoh *et al*., [16]; British Pharmacopoeia [17]..

Total ash values

A tarred nickel crucible was ignited to a constant weight at a dull red heat, cooled and stored in a dessicator. 2g of the powdered materials was weighed into the nickel crucible and heated gently until all the moisture had been driven off and the material had been completely charred. The heat was increased until most of the carbon had been vaporized, after which the material was heated to about 450°C to make the residue carbon free. The residue was cooled and weighed. The heating and cooling were continued until a constant weight was achieved.

Acid insoluble ash

The total ash obtained from (a) above was transferred to abeaker containing 25ml dilute 30% hydrochloric acid heated to boil on a water bath for 5minutes and filtered with an ash less filter paper. The beaker and crucible were paper until it was free from acid. The filter paper was dried in the oven, folded into a narrow cone, inserted weighed a tarred nickel crucible and heated at 150°C until it was completely ashed. The residue was then heated more strongly and cooled in a dessicator after which the crucible was re-weighed.

Water soluble ash

A nickel crucible was ignited to a constant weight at 450°C and reweighed after 2g of the powdered material had been put into it. The crucible with the drug was ignited at low heat, initially to burn off the carbon content. The heat was gradually increased until all the carbon was burnt off. The crucible was cooled in a dessicator and reweighed, and heating was continued until a constant weight was obtained. The content of the crucible was transferred into a small beaker, 25ml of distilled water was added and the beaker contents were boiled for 5 minutes and filtered through an ashless filter paper. The filter paper together with the residue was dried in the oven and compressed into a small or narrow cone. This was then transferred into the crucible and heating was continued until the ashless filter paper was eliminated, acid the weight was noted.

Sulphated ash

A nickel crucible was ignited to a constant weight at a red dull heat in the oven. 2g of the powdered plant materials was spread over the bottom of the crucible and was re-weighed. The material was moistened with dilute sulphuric acid and ignited at low heat initially to burn off the carbon content. The crucible was cooled in a dessicator. More dilute sulphuric acid was added and heating continued to about 800°C with occasional cooling and reweighing until a constant weight was obtained.

Determination of extractive yields

The methods used were as described by Odoh *et al*., [15].

Alcohol soluble extractive

A 5g of the powdered plant materials was weighed accurately and placed in a 250ml stopper conical flask, and then 100ml of 90% alcohol was added. The stopper was firmly replaced and the contents of the flask were shaken mechanically for 6hours and allowed to macerate for a further 18 hr that is a total of 24 hours and then filtered. 20ml of the filtrate was evaporated to dryness in a 25ml beaker over a water bath. The residue was dried constant weight at 105°C and then weighed.

Water soluble extractive

A 5g of the powdered materials were weighed accurately and placed in a 25ml stopper conical flask. 100ml of distilled water was added and the stopper was replaced firmly. The contents of the flask were shaken mechanically for 6 hours and were allowed to mascerate for a further 18 hours that is a total of 24 hours and then filtered. 20ml of the filtrate was evaporated to dryness in a 25ml beaker over a water bath. The residue was dried to constant weight at 105°C.

Moisture content

A tarred evaporating dish was heated to a constant weight and stored in a dessicator. A 2g of the powdered plant was added to the dish and kept in an oven maintained at a temperature of 105°C. It was allowed to dry until a constant weight was achieved. The difference in weight of the evaporating dish was noted.

Chemomicroscopic Analysis

Test for cellulose, lignin, starch and suberized wall

A little quantity of the powdered drug was placed on a slide and two drops of iodinated zinc chloride solution (20 g of zinc chloride in 8.5 ml of water+ 1 g of potassium iodide and 0.5 g of iodine in 20 ml of water) was added to the slide and then viewed under a light microscope to observe the individual colour changes.

Test for secretory cells and ducts

A quantity of the powdered drug was mounted on a slide with two drops of sudan III solution (prepared with equal parts of glycerine and alcohol). The slide was then covered with a cover slip, and viewed under a light microscope and the colour was noted.

Test for fibres

A little quantity of the powdered drug was mounted with saturated aqueous solution of picric acid and allowed to stand for 5 minutes. The slide was irrigated with water and examined under a light microscope and colour noted.

Test for calcium oxalate crystals

A little quantity of the powdered drug was mounted cleared with chloral hydrate solution and observed under the microscope for calcium oxalate crystals. A few drop of concentrated 80% sulphuric acid was added and examined under a light microscope, the disappearance of the crystals confirms the presence of calcium oxalate.

Test for tannins

A little quantity of the powdered drug was mounted with ferric chloride solution and examined under a light microscope and the colour was noted.

III. RESULTS AND DISCUSSION

The identification and evaluation of the plant *Dialium guineense* have been carried out and the various characteristics and features associated with the plant dully determined by the various analysis and tests performed.

The result of phytochemical analysis of *Dialium guineense* shows that the plant is rich in chemical constituents. Also the degrees of the concentration of the constituents in the different morphological parts vary, explaining the variations in the degree of their pharmacological activities. The phytochemical tests revealed the presence of carbohydrates, reducing sugars, alkaloids, glycosides, saponins, flavonoids, proteins, steroids and terpenoids in the leaf, stem and root of *Dialium guineense* (Table 1). Tannins were found in the leaf and the stem while the leaf alone showed the presence of resins. Oils and acidic compounds were absent in all the plant parts tested. From the results of the phytochemical tests it was observed that the leaf has the highest concentration of constituents. These phytochemical compounds accounts for its relevance as a medicinal plant. Tannins are used for the treatment of diarrhoea and dysentery, haemorrhoids, inflamed or ulcerated tissues [18]. Mensah *et al*., [19] also reported the usefulness of tannins for the management of hypertension among Esan people of Edo state. These facts support the uses of *D. guineense* for the treatment of heart diseases. Clinton [20] investigated the use of plant tannins to treat ulcerative colitis caused by food allergens and microflora. This report serves as a pointer to the fact that a drug for the treatment of ulcerative colitis can also be developed from *D. guineense*. James and Friday [21] from their studies on wound healing effects of tannins supported the use of *D. guineense* as folklore remedies in treatment of wound infections. The phytochemical analysis revealed the presence of alkaloids which is known to possess pharmacological activities which include antihypertensive, antiarrythmic and anticancer effects. A number of alkaloids are used as antimalarial drug [22]. *D.guineense* is used as a folklore remedy for the treatment of malaria and this might be due to the presence of alkaloids in this plants.

The phytochemical analysis of the plant extract revealed some phenolic compounds which play major roles in health in addition to enhancing antimicrobial activities. This was revealed in the studies of Hermans *et al*., [23]. Ammar *et al*., [24] revealed in their study that flavonoids can inhibit dental plaque. Flavonoids one of the phytochemical compounds identified are natural products of high pharmacological potency which are widely distributed in plants. They possess antiallergic, antinflammatory, antiviral and antioxidant activities in addition to playing a good role as a

cardioprotective [25]. Schramm and German [26] suggested in their review article that if the total plasma flavonoids load exceeds a few micromoles per litre in vivo, flavonoids will protect humans against vascular disease. Saponins which are responsible for numerous pharmacological activities [27] also tested positive in *D. guineense* . Saponins possess biological activities and are used as folk medicines as well as intensively used in food, veterinary and pharmaceutical industries. Glycosides one of the phytochemical compounds detected in *D. guineense* are known to increase the force and contraction of the heart for most heart failure patients and thus found to be useful in treating congestive heart failure [28]. Glycosides have also been found useful in the treatment of cancer [29]. Steroids one of the phytochemical constituents in *D. guineense* have been found to possess numerous and diversified pharmacological and physiological effects on the functional capacities of the cardiovascular system [30].

The macroscopical examination of the plant reveals the physical appearance of the morphological parts, as can be seen with the naked eyes. The Leaf is finely hairy, with a common stalk 5-13 cm long, with an odd terminal leaflet and usually 2 pairs of opposite or alternate leaflets, the lower pair being somewhat smaller; leaflets mostly 3.5-10 x 2.5-5 cm, elliptic to broadly elliptic, it is slightly obovate; blunt at the apex or abruptly and shortly acuminate, symmetrical and rounded or slightly cuneate at the base; leathery, glabrous above and with the midrib slightly sunken, it is finely hairy beneath. The leaf is green in colour and the taste is bitter. The leaf has a fleshy texture which becomes brittle when dried. The stem has a hard texture and rough fracture. It is dark grey to black in colour, superficially cracked or inconspicuously fissured and peels off in thick pieces with a disagreeable smell. The root surface is light brown in color. It is hard and not brittle with ridges apparent in dried samples. The roots are branched with little rootlets. The root breaks with a fracture exposing a smooth transverse surface which is yellow in color. The root has a bitter taste. This however gives an insight on the plant and cannot be relied on solely for the identification of the plant.

The microscopical feature shows the characteristic features that could be found in the plant especially in its different morphological parts. The microscopy of powdered leaf revealed the non glandular unicellular trichomes, tracheid fibres in xylem , Epidermal cells, Fibres with epidermal cells, Prisms of calcium oxalate crystals, Paracytic stomata with two surrounding guard cells which are perpendicular and Starch granules (Fig.1). The microscopy of the powdered stem showed fibres with bordererd pitted vessels, cork thin walled cell in surface view, sclereids, prisms of calcium oxalate crystals and single fibre (Fig.2) and the microscopy of powdered root revealed prisms of calcium oxalate crystals, parenchyma in longitudinal section, fibers with bordered pitted vessels, bundle of fibers with calcium oxalate crystals, sclereids thickened with branched pits showing distinct striations and unicellular non glandular trichome (Fig.3). These characteristic features and their arrangements are usually not the same in all morphological parts but some features are peculiar to all or few of them. For example bundles of fibres, prisms of calcium oxalate crystals, unicellular trichomes and were found in the leaf, stem and root. Also the cork cells, and sclereids were found only in the root and stem, while the stomata occurred only in the leaf. The transverse section of the leaf revealed the presence of cuticle, epidermis, palisade and spongy mesophyll, vascular bundle, xylem, phloem, parenchyma, collenchymas and trichomes (Fig.4) ; the stem revealed the presence of cork cambium, cortex, vascular bundles, secondary xylem and pith (Fig.5) and the root revealed the cork, cortex, vascular bundle and pith (Fig. 6). The presence and arrangement of these features in the different morphological plants shows that the plant is a dicotyledonous plant.

The percentage values obtained for analytical standard of the leaf were 7.50, 1.83, 1.32, 1.65, 10.00, 10.00, and 0.25 % for total ash, acid insoluble ash, water soluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and moisture content respectively; the stem values were, 4.75, 1.48, 1.40, 1.42, 20.00, 10.00, and 0.15 % for total ash, acid insoluble ash, water soluble ash, sulphated ash alcohol soluble extractive, water soluble extractive and moisture content respectively and the root were 5.00, 1.53, 1.60, 1.40, 10.00, 20.00 and 0.95 % for total ash, acid insoluble ash, water soluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and moisture content respectively.The results of the ash values, are of tremendous importance in quality control especially for the determination of percentage yield and detection of adulteration. In a study done by Rao *et al.*, [31] Ash values were used to detect the presence of any siliceous contamination and presence of any water soluble salts. Alcohol and water soluble extractive values indicate the presence of adulterants, faulty processing and poor quality of the drug. The moisture content of *D. guineense* was low. This value was closely related to the values reported by Adepoju [32] and Achoba *et al*. [33]. The low moisture content was indicative of its high dry matter content, high resistance to enzymatic or microbial attack and indicative of long life. The British pharmacopoeia also specified using liquorice as standard that alcohol and water soluble extractives be greater than or equal to 4.5 and 26 % respectively. The result obtained for alcohol soluble extractive and water soluble extractive for the leaf, stem and roots were within the specified range.

IV. CONCLUSION

The phytochemical compounds identified in *Dialium guineense* are known to be biologically active and their presence further confirmed its medicinal uses in folklore remedies for treatment of various infections. Further investigations on this plant may lead to the development of drugs of natural origin that may help to combat different infections.

The study also emphasises the significance of standardisation in the determination of adulterants, if adulterated. Since the results obtained from the determination of analytical standards conform to the BP specifications, it can be concluded that the methods used were adequate within the limits of experimental error. The information obtained

from this study can be used to build up a monograph of *Dialium` guineense* for its possible inclusion in the Pharmacopoeia.

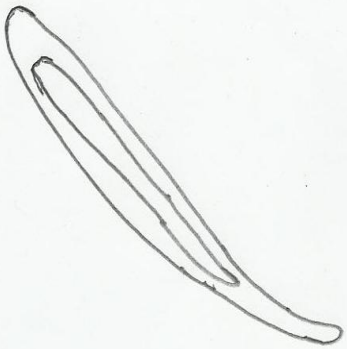
Table 1: Results of the preliminary phytochemical analysis of *Dialium guineense*

Constituent	Inference		
	Morphological parts		
	Leaf	Stem	Roots
Carbohydrates	+++	++	+
Reducing sugars	+	+	+++
Alkaloids	+++	++	+++
Glycoside	++	++	+
Saponins	++	++	+
Tannins	++	+	-
Flavonoids	+++	++	+
Resin	+++	-	-
Proteins	+++	++	+
Oil	-	-	-
Steroids	++	++	++
Terpenoids	++	++	+++
Acidic compounds	-	-	-

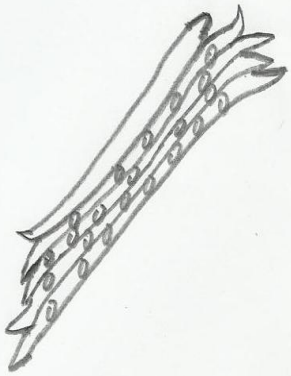
Key: + = Slightly present, ++ = Moderately present, +++ = Highly present, - = Absent

Table 2: Results of analytical standards

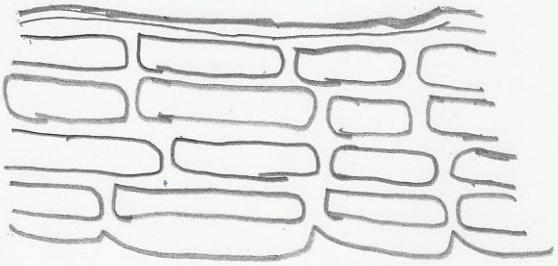
Analytical standards	**Composition (%)**		
	Leaf	**Stem**	**Root**
Total ash	7.50	4.75	5.00
Acid insoluble ash	1.83	1.48	1.53
Water soluble ash	1.32	1.40	1.60
Sulphated ash	1.65	1.42	1.40
Alcohol soluble extractive	10.0	20.0	10.0
Water soluble extractive	10.0	10.0	20.0
Moisture content	0.25	0.15	0.95



Trichome

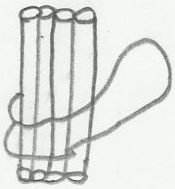


Tracheid fibres in xylem

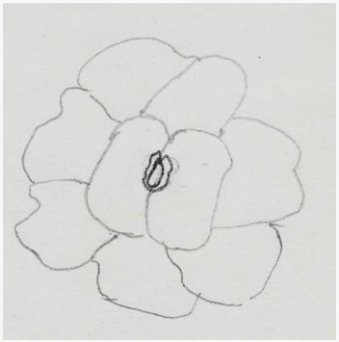


Epidermal Cells

Prisms of calcium oxalate crystals



Fibres with epidermal cells



Paracytic stomata

Starch granules

Fig.1: Microscopy of powdered Leaf **(**MAG X 100)

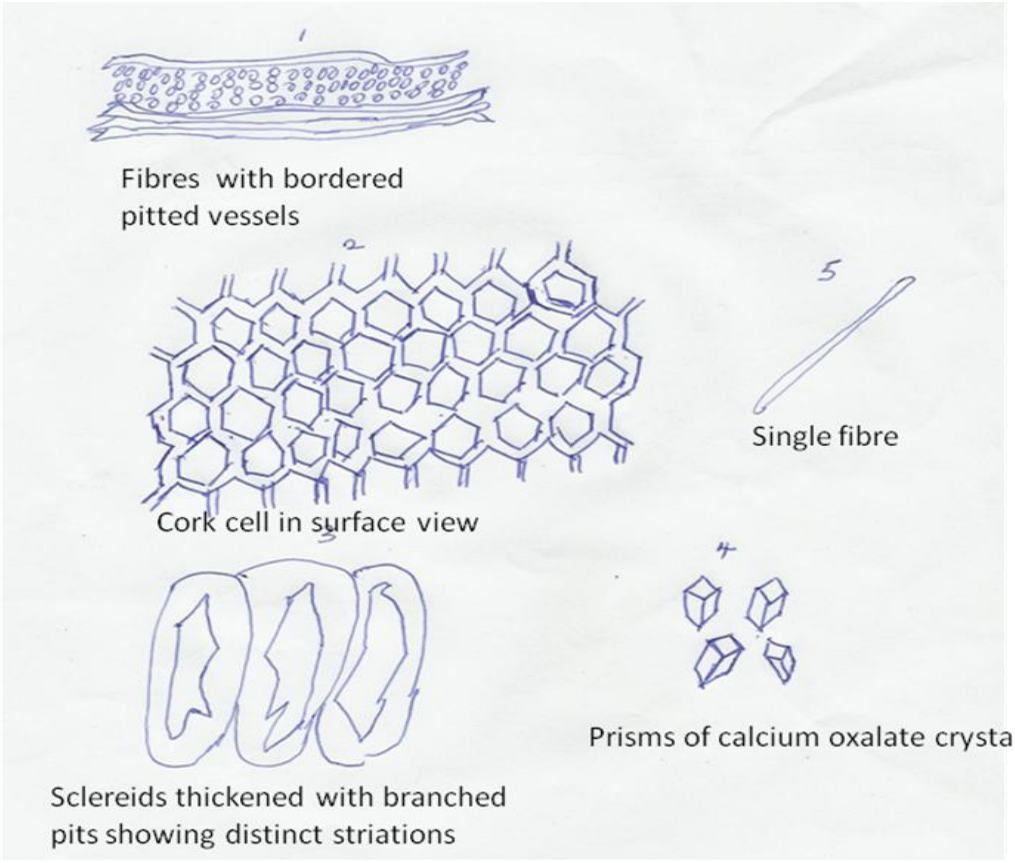


Fig. 2: Microscopy of powdered stem (MAG X 100)

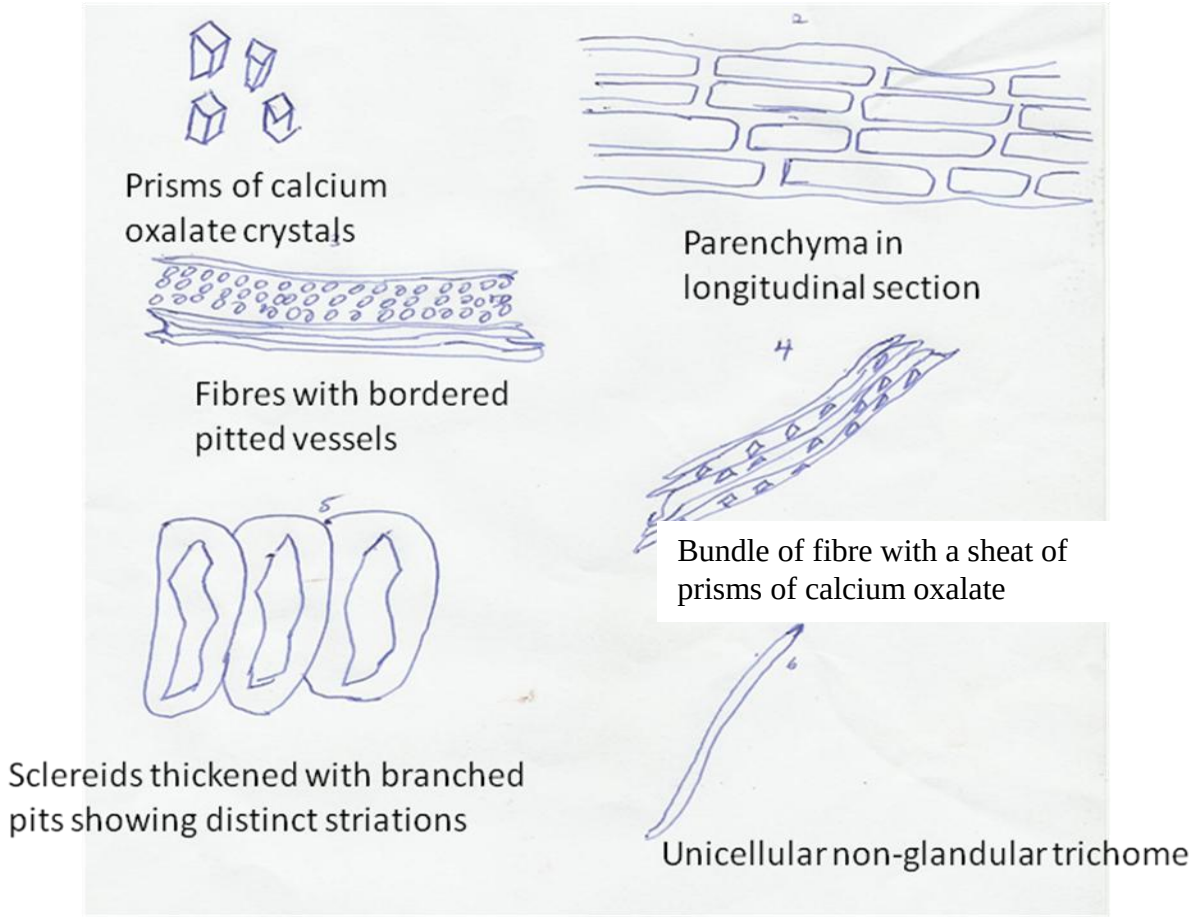


Fig. 3: Microscopy of powdered Root (MAG X 100)

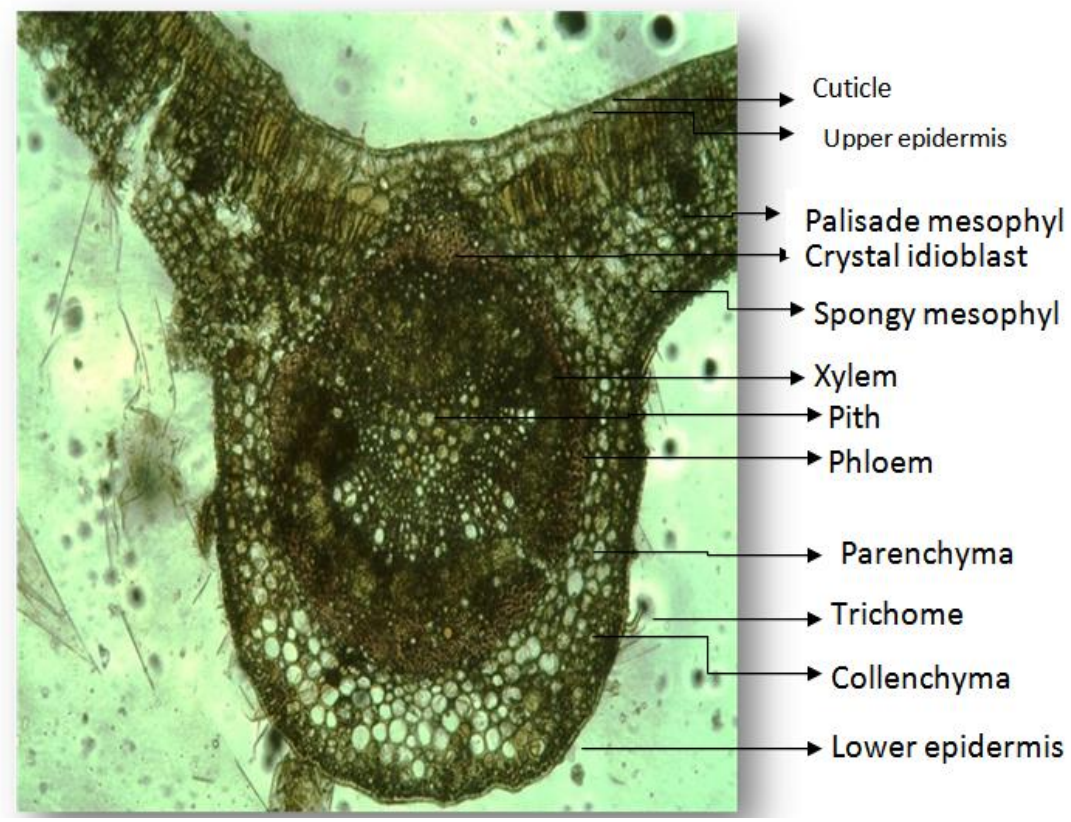


Fig. 4: Transverse Section of the Leaf X 100 JPG

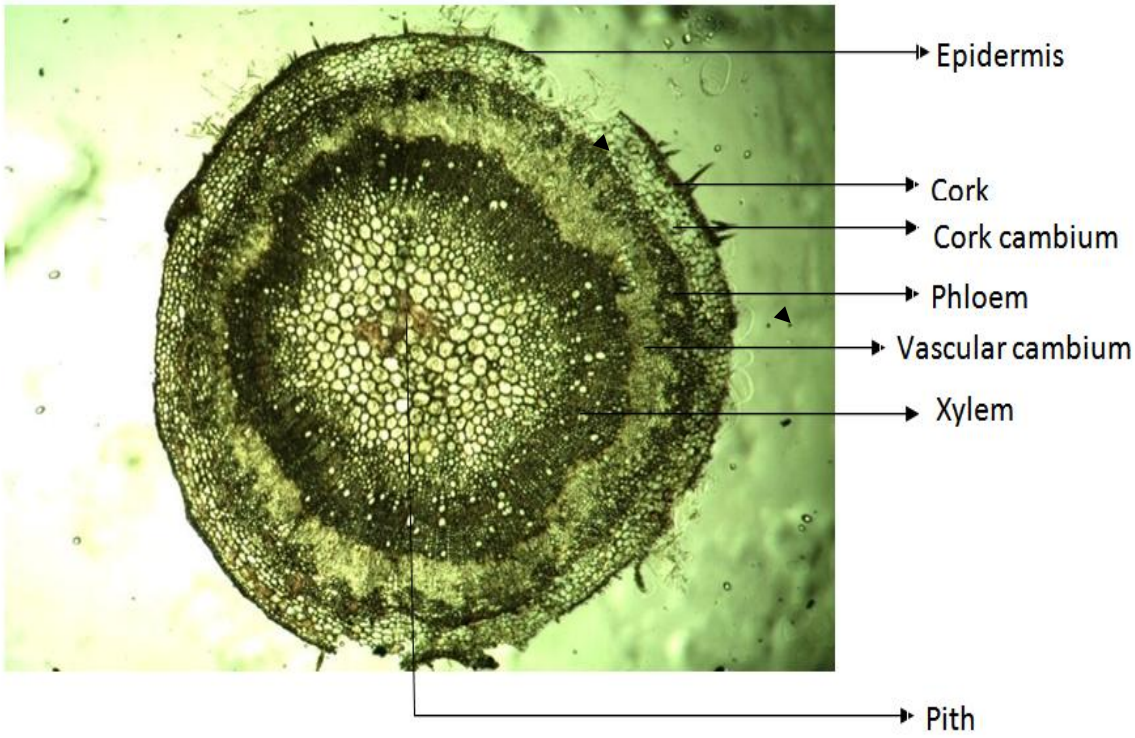


Fig. 5: Transverse Section of the Stem X 100 JPG

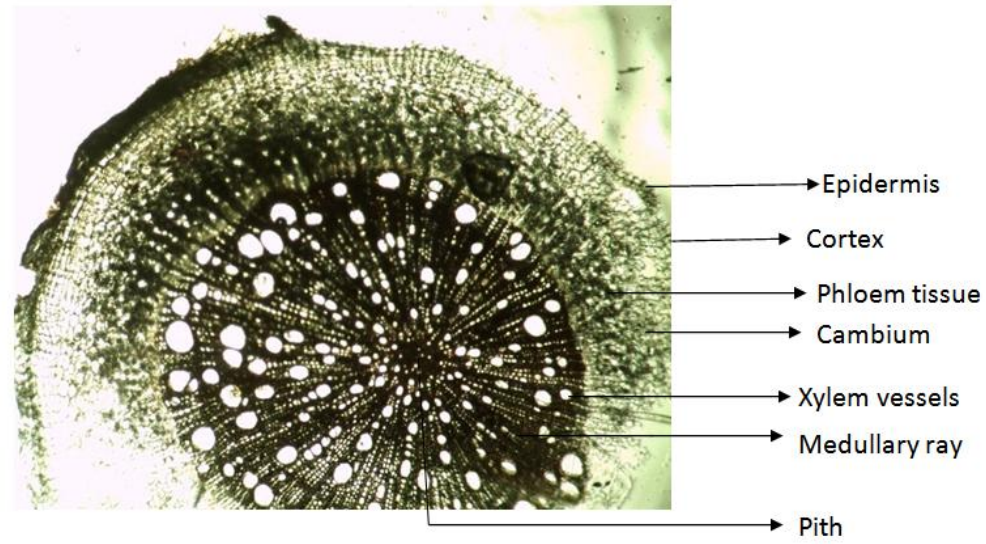


Fig. 6: Transverse Section of the Root X 100 JPG

Table 3: Results of the chemomicroscopy of the leaf, stem and root of *Dialium guineense*

Test reagent	**Observation**	**Inference**		
		Leaf	**Stem**	**Root**
Iodinated zinc chloride solution	Blue colours observed on epidermal cells	Cellulose(+)	Cellulose(+)	Cellulose (+)
Iodinated zinc chloride solution	Yellow colouration observed in the xylem vessels	Lignin (-)	Lignin (-)	Lignin (-)
Iodinated zinc chloride solution	Blue black colouration observed on few grains in the parenchyma cells	Starch(-)	Starch(+)	Starch (+)
Iodinated zinc chloride solution	Brown coluration observed	Suberized wall (+)	Suberized wall (+)	Suberized wall (+)
Sudan iii solution	Pink-red colouration observed.	Fibres (+)	Fibres (+)	Fibres (+)
Picric acid solution	Yellow colouration observed.	Secretory cells and ducts (+)	Secretory cells and ducts (+)	Secretory cells and ducts (+)
80% H_2SO_4	Crystals of calcium oxalate dissolved	Calcium oxalate crystals (+)	Calcium oxalate crystals(+)	Calcium oxalate crystals (+)
Ferric chloride solution	No greenish colour in some parenchyma cells	Tannins (+)	Tannins (+)	Tannins (+)

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