



Pharmacognostic Profile of Leaf, Stem and Root of *Anthocleista djalonensis* A. Chev. (Loganiaceae)

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ABSTRACT

Pharmacognostic investigation of fresh, powdered and anatomical sections of leaf, stem and root of *Anthocleista djalonensis* (Loganiaceae) were carried out to determine its macromorphological, micromorphological, chemomicro-morphological, numerical and phytochemical profile. The macromorphological parts revealed a simple, glabrous and leathery leaf that is oblong – elliptical to obovate – elliptical with cordate base, rounded apex, with an entire margin. The root is light brown in colour with hard and brittle texture while the stem is cream in colour with grey hair bark and hard and brittle texture. The micromorphology of the powdered plant shows warty walled epidermal cells, paracytic stomata, tracheid fibres, pitted vessel, T – shaped hair, single fibre with moderately thickened walls and calcium oxalate crystals associated with lignified fibres were found in the leaf. Stellate trichome with multicellular arms, starch granules, single fibre with moderately thickened walls, unicellular trichome, parenchyma cells, fragments of cork cells and fibrous sclereids were found in the stem while that of root showed cork of thin walled cells, bundle of fibres with calcium oxalate crystals, starch grain and tracheid fibre. Chemomicroscopical analysis or investigation revealed the presence of cellulose, lignin, starch, suberized wall, fibres, secretory cells and ducts, calcium oxalate crystals, and tannin for leaf and stem while the root shows presence of cellulose, starch fibres, secretory cells and ducts and calcium oxalate crystals. The result of the analytical standards of the powdered leaves, stem and root gave moisture content of 8.50, 5.00 and 6.50 %; total ash 11.50, 8.50 and 7.00 %; acid insoluble ash 3.50, 2.50 and 2.00 %; alcohol extractive of 17.00, 15.00 and 7.00 % and sulphated ash 2.50, 2.00 and 0.10 % respectively. The preliminary phytochemical analysis of the leaf and stem revealed the presence of carbohydrate, alkaloid, saponins, glycosides, resins, tannins, steroids and terpenoids. While that of root shows presence of carbohydrate, alkaloids, saponins, glycosides, resins, steroids and terpenoids. These findings are of importance in the establishment of diagnostic indices for the identification and standardization of the plant.

Keywords: *Anthocleista djalonensis*, pharmacognostic analysis, macroscopy, chemomicroscopy, analytical standards.

INTRODUCTION

The use of medicinal plants constitutes an important part of traditional medicine which is a part of African heritage [1]. The use of plant materials as sources for remedies in health care delivery dates back to human history (Nigeria Natural Medicine Development Agency (NNMDA) [2]. Since ancient times, humanity has depended on the diversity of plant resources for food, clothing shelter, and traditional medicine to cure myriads of ailments [3]. Traditional medicine is undoubtedly a reliable alternative to health care delivery in the metropolis because it is cheap, easily accessible, and

efficacious [4]. Based on current research and financial investments, medicinal plants will seemingly, continue to play important role as health aid [5, 6]. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Many of today's synthetic drugs have their roots from plants. It has been reported that natural products represent over 50 % of all drugs in clinical use, in which natural products derived from higher plants represent about 25 % of the total [7]. The World Health Organization (WHO) estimated that over 80% of the people in developing countries



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Oboh et al

rely on traditional remedies such as herbs for their daily needs and about 855 traditional medicines include crude plant extracts [8]. This means that about 3.5 to 4 billion of the global population rely on plants resource for drugs [9].

Anthocheistadjalonenensis (Loganiaceae) the plant of interest in this research has been reported for medicinally efficacious within and outside the continent of Africa [10].

Anthocheistadjalonenensis grows in dry localities, in savanna or thickets and is mainly tropical in distribution [11]. The plant is usually a small tree of up to 15m tall; bole up to 40cm in diameter; twigs sometimes with 2 erect spines or small cushions above the leaf axils [11]. Its use as anti-diurectic, purgative and for the treatment of jaundice has been reported [7]. The local uses of the root decoction include treatment of chest pain, for constipation, gonococci and regulation of menstruation [12]. The vapour bath of the leaves is used to treat leprosy, venereal diseases, oedema intestinal disorders, inflammations and boils on the skin [13]. In Mali, root maceration in water is taken alone or with honey to treat malaria and a root decoction or root powder in porridge is taken to treat abdominal pain, while in Nigeria, a decoction of the root with potash is taken orally to treat fungal skin and filarial worm infections [13]. A powder made from its bark mixed with roots of aloe is taken to treat hepatitis, jaundice and cirrhosis [14]. An alcoholic leaf extract showed in-vitro antispasmodic and smooth muscle relaxant activities [14]. It is also reported that *Anthocheistadjalonenensis* is used in the treatment of infertility [13]. The NNMDA [2] reported the use of the bark decoction to cure skin diseases, treat wounds, sores, ulcers, stomach problems, sexually transmitted diseases asthma, and diabetes. The phytochemicals found in *Anthocheistadjalonenensis* include fourteen alkanes with tetracosane and pentacosane being prominent [15]. A new monoterpenediol and a flavonoid (iridiodglucosidedjaloneside) were isolated from *Anthocheistadjalonenensis* by Onocha and Okorie [16]. The plant contains the quinoline alkaloid brucine and the monoterpene glycoside loganoside (Loganine). Loganoside plays an important role in the partial synthesis of alkaloids such as quinine, reserpine and strychnine [17]. Quinine has been efficacious in the treatment of malaria [18]. The root bark contains iribacholine, which tested positive for antifungal activity against the pathogens *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus Fumigatus* and *Trichophyton rubrum* [19]. The plant also contains triterpenes, monoterpene-

diol (djalonenol), dibenzo-pyrone (djalonenone), iridiodglucosides (sweroside or djalonenoside) and amplexione and xanthone (Lichexanthone) [16]. The stem bark contains the phthalide, djalonenin [20]. The root extract also showed pronounced activity against isolates from non-gonococcal urethritis [21]. The present investigation on the leaf, stem and root of *Anthocheistadjalonenensis* is therefore taken up to establish certain botanical and chemical standards which would help in identification as well as in checking adulteration, if any. Further, the study will greatly help in quality assurance of herbal products and for preparation of its monograph for inclusion in the Pharmacopoeia.

MATERIALS AND METHODS

Collection and identification of plant materials

The leaf, stem and root of *Anthocheistadjalonenensis* were collected in November, 2010 from Iheakpu-Awka, in Igbo-Eze South Local Government Area of Enugu State. It was authenticated by Mr. A.O. Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development (INTERCEDD), Nsukka and a voucher specimen (UNN/PCOG/010/405) deposited in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka.

Preparation of plant materials

The leaf, stem and root were carefully washed separately and excess water was allowed to drain off. Representative samples were kept for examination while the rest were dried. They were pulverized and the powdered samples stored in airtight containers for use in further studies. Transverse sections were cut from the representative samples using sledge microtome. The sections were preserved in 70 % ethanol until needed for studies.

Preliminary phytochemical tests

Chemical 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 using standard methods [22, 23]. These metabolites include; alkaloids, tannins, steroids, flavonoids, resins, saponins, oils, etc.

Macroscopical examination

The macroscopical features of the fresh leaf, stem and root were examined using the methods described by Evans [24]. The morphological data of the Leaf, stem and root examined include: type of

Oboh et al

margin, petiole, venation, base, size, shape, surface character fracture and texture. Also the organoleptic properties like colour, odour and taste were observed and noted.

Microscopic examination of the powder

Little quantity of the powdered leaf, stem and root were placed separately on a glass slide and 2 drops of chloral hydrate was added to moisten the powdered drugs. It was covered with cover slip and passed over the flame of burner repeatedly until bubbles occurred. It was then allowed to cool and 2 drops of dilute glycerin were added for clarity of structures and the slide was viewed under microscope (monocular microscope Olympus, Tokyo, magnification x 40).

Microscopic examination of transverse section

Anatomical sections of the fresh leaf, stem and root were prepared for the microscopic studies. The staining was done using standard laboratory methods [24, 25]. The transverse sections were done by sectioning of the specimen using a sledge micrometer. They were transferred into a staining jar and stained in safranin for 5 minutes. The sections were washed with distilled water, followed with alcohol, and thereafter stained again with 1 % fast green for 5 minutes and washed with absolute alcohol. They were transferred into a jar containing 50/50 alcohol/xylene and washed until they became clear. The sections were cleared with chloral hydrate solution and mounted on a slide with dilute glycerin.

Chemomicroscopic examination

Chemomicroscopical analysis was carried out to detect the presence or absence of various chemical compounds such as starch, cellulose, tannins, and lignin, fat and oil, mucilage and calcium oxalate crystals [24]. Little quantity of the powdered drug was placed on a slide and two drops of iodinated zinc chloride solution (for cellulose, lignin, starch and suberized wall), sudan III solution (for secretory cells and ducts), aqueous solution of picric acid (for fibres), 80 % H_2SO_4 (for calcium oxalate crystals) and ferric chloride solution (for tannins) were added to each slide. A cover slip was used to cover the slide and then viewed under a light microscope (monocular microscope Olympus, Tokyo, magnification x 40) to observe the individual colour changes.

Analytical standards

Ash values

The methods adopted for the determination of ash values followed the specification given by British Pharmacopoeia [26].

Total ash

A nickel crucible was placed in a muffle furnace for 15 minutes at 35 °C and cooled in a desiccator and the weight recorded. A 2 g each of the powdered material (leaf, stem and root) was placed inside the crucible and heated gently until the moisture had been driven off and the plant materials had been completely charred. The temperature was slowly increased to a maximum of 450 °C, until the residue was free from carbon, then cooled and weighed. The percentage of the total ash was calculated with reference to the weight of the dry powdered plant material.

Acid insoluble ash

The total ash was boiled with 25 ml dilute 2 M HCl acid for 5 minutes. The mixture was then filtered through an ashless filter paper. The filter paper was then washed with hot water until the filtrate was neutral. After that, the paper was ignited at about 500 °C in a muffle furnace, then cooled and weighed. The percentage of the acid insoluble ash was calculated with reference to the weight of the dry powdered plant material.

Water soluble ash

The total ash was boiled with 25 ml of distilled water for 5 minutes and filtered through an ashless filter paper. The filter paper together with the residue was then ignited at about 450 °C in a muffle furnace, then cooled and weighed. The water soluble ash is the difference in weight between the total ash and the residue obtained after boiling the total ash in water. The percentage of water soluble ash is calculated with reference to the dry powdered plant material.

Sulphated ash

The total ash was moistened with 1 ml of dilute H_2SO_4 acid and ignited at low heat initially to burn off the carbon content. The crucible was cooled and more dilute H_2SO_4 acid was added and heated to about 800 °C with occasional cooling and re-weighing until a constant weight was obtained. The percentage of sulphated ash is calculated with reference to the dry powdered plant material.

Extractive values

The methods adopted for the determination of extractive are as described in the British Pharmacopoeia [26].

Alcohol soluble extractive

A 5 g quantity of each of the powdered plant materials was macerated in 100 ml of 90 % ethanol in a 250 ml stoppered conical flask for 24 hours. The stopper was frequently shaken during the first 6 hours and allowed to stand for 18 hours. The extract was then rapidly filtered. The filtrate was evaporated to dry and dried at 105 °C to a constant weight. The percentage of the alcohol-soluble extractive was calculated with reference to the weight of the dry powdered plant material

Water soluble extractive

A 5 g quantity of each of the powdered plant materials was macerated in 100 ml of water in a 250 ml stoppered conical flask for 24 hours. The stopper was frequently shaken during the first 6 hours and allowed to stand for 18 hours. The extract was then rapidly filtered. The filtrate was evaporated to dry and dried at 105 °C to a constant weight. The percentage of the water-soluble extractive was calculated with reference to the weight of the dry powdered plant material.

Moisture content

A 5 g quantity of each of the powdered plant materials was added to a tarred evaporating dish previously ignited. The dish was heated 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 dish and powdered drug before heating and weight of the dish and powdered drug after heating is the moisture content. The percentage of the moisture content was calculated with reference to the weight of the dry powdered plant material.

RESULTS AND DISCUSSION

The results of the phytochemical screening of leaf, stem and root powders are shown in Table 1.

The macroscopic examination of the shows a simple leaf, glabrous, leathery and large with opposite arrangement. The margin is entire. They are auricled, with petiole 1 – 9 cm long and 2 cm wide. The blade is oblong-elliptical to obovate-elliptical with cordate base and rounded or cuneate apex. The arrangement of the stalk is dilated and sometimes more or less winged. It has a fleshy

texture which becomes brittle when dried. The colour is green and taste is sour and has a pipe tobacco odour. The stem surface is cream in colour with grey hair bark, hard and brittle. The stem breaks with a short fracture exposing a smooth transverse surface which is cream in colour. It has a pipe tobacco odour and is tasteless. The root surface is light brown in colour. It is hard and brittle, with longitudinal ridges apparent in the dried samples. The roots are branched with little or no rootlets and breaks with a short fracture exposing a smooth transverse surface which is cream in colour. It has a sour taste and odourless.

The microscopic examination of the powdered leaf revealed the following features; lignified trichome, tracheid fibres in xylem with smaller pits, thicker walls and tapering ends, epidermal cells with paracytic stomata, prisms of calcium oxalate crystals, bordered pitted vessel and single fibre with moderately thickened walls (Fig 1). The stem shows thick walled and fibrous sclereids, single fibre with moderately thickened walls, unicellular trichome, starch granules, bordered pitted vessel, bundle of fibres, parenchyma and prism of calcium oxalate crystals, fragments of cork cells in surface view and fibre with parenchyma cells (Fig. 2). For the root was cork of thin walled cells, polygonal and elongated in surface view, bundle of fibres with calcium oxalate crystals, phloem parenchyma cells, prisms of calcium oxalate crystals, starch granules, tracheid fibre and bordered pitted vessel (Fig. 3).

The transverse section of the leaf shows a thin waxy cuticle (outermost cover), followed by a layer of epidermal tissue. The palisade layer comes next followed by a wide zone of spongy mesophyll which has a lot of inter air spaces. Within the spongy mesophyll lies the vascular bundle of the midrib and the bundle is made up of xylem to the upper surface and phloem to the lower surface (Fig. 4). The section of the stem shows single layer epidermis, cortex - a wide zone of storage parenchyma tissue, phloem and the vascular cambium. To the inside of vascular cambium lies the xylem tissue which is made up of vessels, visible ray lines and the pith (Fig. 5). The root outermost cover is made up of the thick multiple layered cork followed by cortex, phloem and the cambium. The parenchyma tissues consists xylem tissue which is made up of vessels (pores), visible medullary rays and pith (Fig. 6).

The analytical standardization such as moisture content, total ash, acid insoluble ash, water soluble

Oboh et al

ash, and extractive values of the dried leaf, stem and root powder were calculated in terms of air dried sample as shown in Table 2. Ash values are an indication purity of drug, extractive values are representing the presence of polar or non polar compounds and moisture content also indicates that drug is safe regarding any growth of bacteria, fungi and yeast.

The results chemomicroscopic analysis indicated the presence of lignin, starch, cellulose, suberized walls, secretory cells and ducts, calcium oxalate crystals and tannins.as shown in Table 3. Histochemical localization of certain important compounds enables to get a preliminary idea of type of compounds and their accumulation in the plant tissues. Based on this study, one can choose the organ or tissue where the required compounds are located.

Standardization is very necessary in determination of quality, purity and percentage strength of the crude drug as well as the nature of adulterants if any. In the last three decades, scientists have been keen and sincere to evaluate many plant drugs used in traditional medicines due to their specific healing properties, healthy action and non-toxic effects. *Anthodeistadjalonensis* is currently being used in the treatment of various diseases without standardization. In traditional medicine, the healers use this plant to treat jaundice, chest pains, leprosy, venereal diseases, abdominal pain etc, but no scientific parameters to identify the true plant material to ensure its quality have been established. The evaluation of crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal Pharmacopoeia, pharmacognostical parameters and standards must be established. Therefore some diagnostic features and physicochemical standards have been evolved to identify and to differentiate the *Anthodeistadjalonensis* from other crude drugs and adulterants. These findings are in agreement with the literature of the family Loganiaceae and the specie *A. djalonensis* according to Keay [14]. In this regard the important microscopic features of the leaf have been documented. The presence of abundant prisms of crystals of calcium oxalate salt of oxalic acid that is present usually at about 1.0 % in plants [27], the covering trichomes and paracytic stomata can be used as identifying characters of the plant. Studies on the physicochemical constants can serve as a valuable source of information and provide suitable standards to determine the quality

of this plant. The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration [28]. An ash determination furnishes a basis for judging the cleanliness of a drug and gives information relative to its adulteration with inorganic matter. The determination of ethanol-soluble extractive and water soluble extractive is used as a means of evaluating drug constituent which are not readily estimated by other means. In some cases, the amount of a drug soluble in a given solvent is an index of its purity. The moisture content of the crude drug is not too high (falls within the limit of the general requirement of 8 – 14 %), indicating less probability of microbial degradation. Excess moisture in crude drugs may lead to the breakdown of important constituent and the growth of microorganisms especially during storage of drug. The presence of tannins and other phenolic compounds which are known to have antimicrobial activity were revealed in the chemomicroscopy. This justifies the use of the plant in the traditional treatment of intestinal disorders, boils, fungal infections and worms [10, 12 - 14]. In this respect, the information obtained from this study can serve in the identification and preparation of monograph of *Anthodeistadjalonensis* for possible inclusion in the pharmacopoeia.

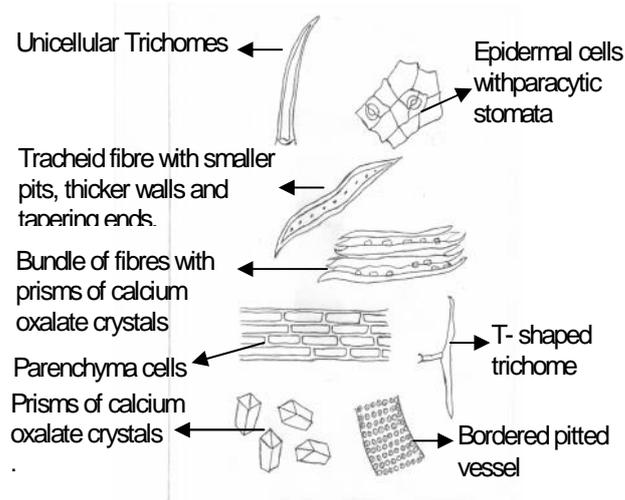


Fig 1: Microscopy of the leaf powder

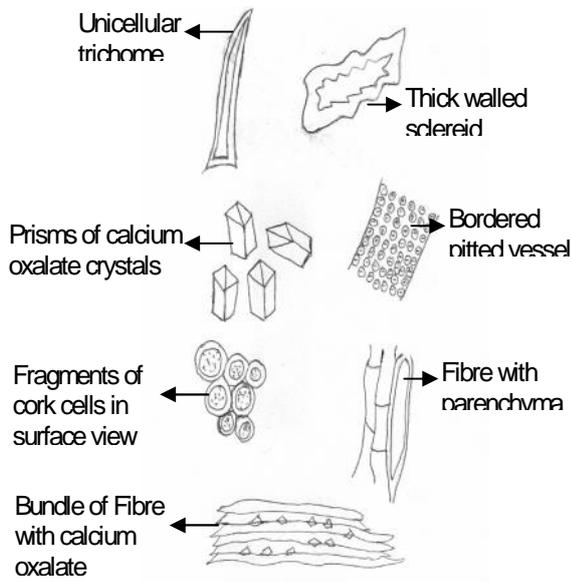


Fig 2: Microscopy of stem powder

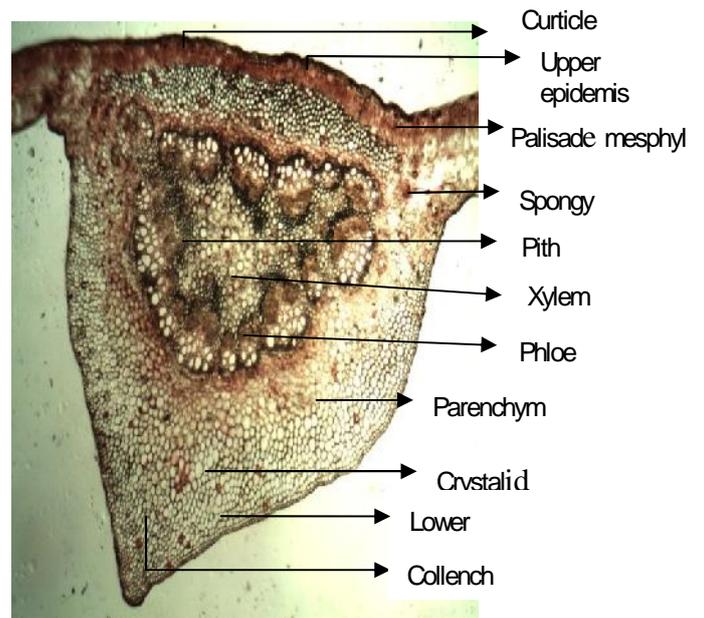


Fig. 4: Transverse Section of the Leaf x 40

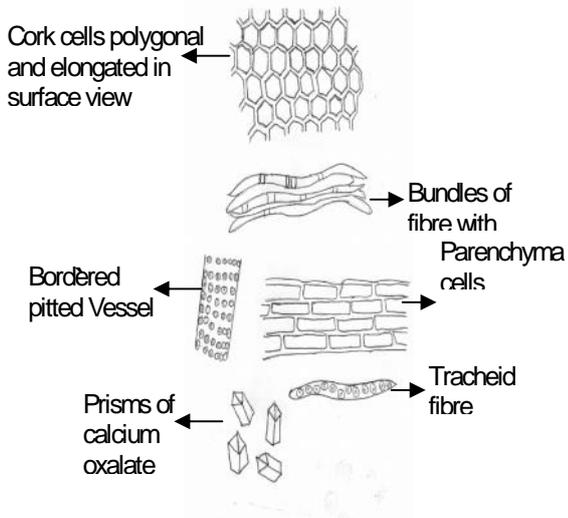


Fig 3: Microscopy of root powder

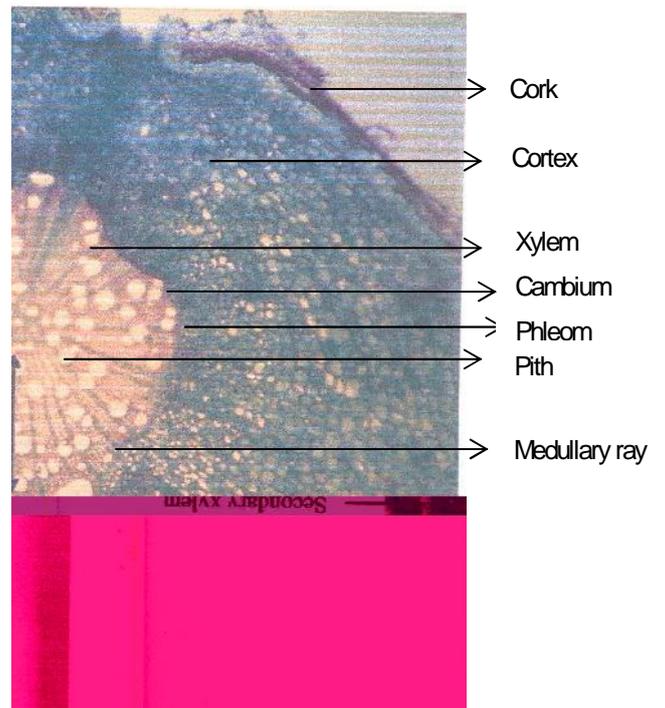


Fig. 5: Transverse Section of the Stem x 40

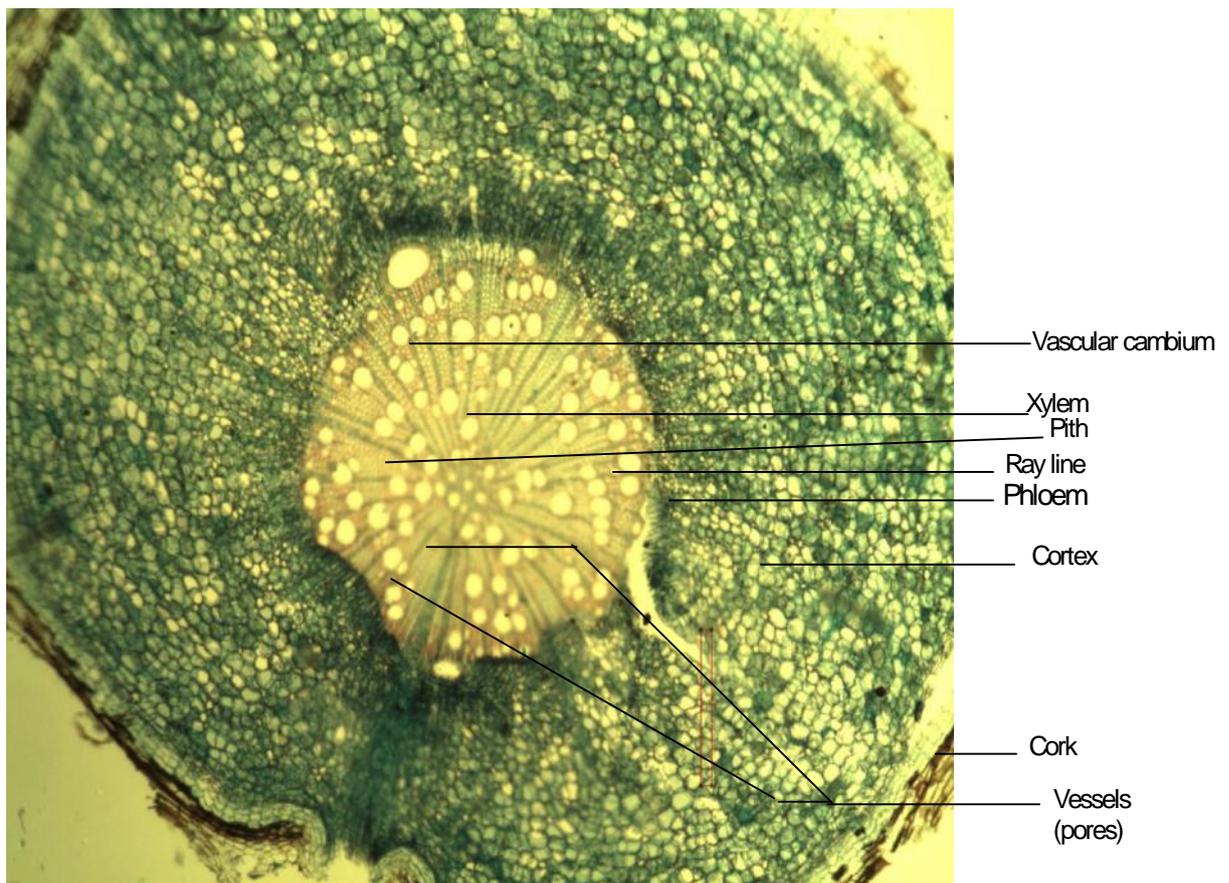


FIG. 6: Transverse Section of the root X 40

Table 1: Preliminary phytochemical test

Test	Inference		
	Morphological part		
	Leaf	Stem	root
Carbohydrate	+	+	++
Reducing sugars	+	+	-
Alkaloids	+++	+	+
Saponins	++	+	+
Glycoside	++	++	+
Tannins	+++	+	-
Flavonoids	-	-	-
Proteins	-	-	-
Resins	++	++	++
Steroids	++	+	+
Terpenoids	++	+	+
Fat and oils	+	-	-
Acidic compounds	-	-	-

Key: + (slightly present), ++ (moderately present), +++ (highly present) and - (absence) of metabolite.

Table 2: Result of analytical standards

Analytical standard	Composition (%) w/w		
	Leaf	Stem	Root
Total ash	11.50 ± 1.31	8.50 ± 1.05	7.00 ± 2.58
Acid insoluble ash	3.50 ± 1.40	2.50 ± 1.20	2.00 ± 0.11
Water soluble ash	4.01 ± 0.46	3.00 ± 3.15	1.50 ± 0.74
Sulphated ash	2.50 ± 2.08	2.00 ± 0.63	0.10 ± 0.27
Alcohol soluble extractive	17.00 ± 1.17	15.00 ± 0.22	7.00 ± 2.75
Water soluble extractive	25.00 ± 1.81	10.00 ± 3.09	11.00 ± 0.61
Moisture content	8.50 ± 0.92	5.00 ± 1.03	6.50 ± 1.71

Values are mean of 3 determinations.

Table 3: Result of Chemomicroscopy of *Anthocleista djalonensis*

Test	Observation	Inference		
		Morphological Part		
		Leaf	Stem	Root
Iodinated zinc chloride solution	Blue colour observed on epidermal cells	Cellulose (+)	Cellulose (+)	Cellulose (+)
Iodinated zinc chloride solution	Yellow colour observed	Lignin (+)	Lignin (+)	Lignin (-)
Iodinated zinc chloride solution	Blue-black colour observed	Starch (+)	Starch (+)	Starch (+)
Iodinated zinc chloride solution	Brown colour observed	Suberized wall (+)	Suberized wall (+)	Suberized wall (-)
Sudan III solution	Pink-red colour observed	Fibres (+)	Fibres (+)	Fibres (+)
Picric acid solution	Yellow colour observed	Secretary cells and ducts (+)	Secretary cells and ducts (+)	Secretary cells and ducts (+)
With 80% H ₂ SO ₄	Crystals of calcium oxalate dissolved	Calcium oxalate crystals (+)	Calcium oxalate crystals (+)	Calcium oxalate crystals (+)
With Ferric chloride solution	Greenish colour Observed	Tannins (+)	Tannins (+)	Tannins (-)

Key: - (absent - colour not observed) and + (present - colour observed).

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Oboh et al

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Odoh et al