Pharmacognostic Profiling of A Valuable Medicinal Tree of Tropical Forest - *Persea americana* Mill (Lauraceae)

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Abstract: Pharmacognostic investigation of fresh, powdered and anatomical sections of leaf, stem and root of Persea americana Mill (Lauraceae), were carried out to determine its macroscopic, microscopic, chemomicroscopical features as well as the physicochemical and phytochemical profile. The macromorphological parts showed alternate, simple and broad with a lanceolate – oblong shape, swollen petiole at the base, margin is entire, slightly undulated and waxy, apex is acuminate with an acute leaf base. The leaf is glossy, pubescent and dark green with a slightly disagreeable smell. The stem has a hard texture, rough fracture, superficially cracked or inconspicuously fissured and peels off in thick pieces with a disagreeable smell. The root has a long and unbranched tapped root plus a dense mass of superficial feeder roots, light brown in colour with a disagreeable smell and rough fracture. The microscopy of the powdered leaf revealed the following features: Anisocytic stomata, epidermal cells, prisms of calcium oxalate, unicellular trichome, starch granules, fibres, bundle of fibres with calcium oxalate crystals, reticulate vessels. The powdered stem revealed the following; Cork cell in surface view, bundle of fibres, single fibre, prism of calcium oxalate crystals, sclereids and the powdered root revealed the following features: Bundle of fibres, cork cell in surface view, prism of calcium oxalate crystal, bundle of fibres with calcium oxalate crystals, single pitted fibre with lumen. Chemomicroscopical analysis or investigation revealed the presence of lignin, starch, suberized wall, fibres, calcium oxalate crystals, and tannins for leaf, stem and root. The result of the analytical standards of the powdered leaves, stem and root gave moisture content of 5.70, 7.70 and 6.20 %; total ash 10.55, 9.70 and 10.80 %; acid insoluble ash 1.78, 1.99 and 1.55 %; alcohol extractive of 0..75, 1.02 and 0.85 %, water extractive 0.83, 2.14 and 0.58 % and sulphated ash 7.15, 6.40 and 5.50 % respectively. The preliminary phytochemical analysis of the leaf, stem and root revealed the presence of carbohydrates, alkaloids, saponins, glycosides, resins, tannins steroids, oils, flavonoids and terpenoids. These findings are of importance in the establishment of diagnostic indices for the identification and standardization of the plant.

Keywords: Persea americana, Pharmacognostic analysis, Macroscopy, Chemomicroscopy, Analytical standards.

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I. Introduction

Natural products are the cornerstone of health care delivery especially in resource poor settings. Present estimates indicate that about eighty percent of the world's population relies on traditional medicine for health care delivery [1;2; 3]. This should be encouraged especially in countries where access to conventional treatment is inadequate, in as much as efficacy and safety are assured [4]. A number of studies have reported the toxic effects of herbal medicines [5; 6; 7; 8]. Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments. Ever since the dawn of civilization, man has used plants for his food, shelter and fodder for animals. Higher plants are employed as medicine by different people of both rural and urban areas all over the world who have been using them as sources of food and medicines since the dawn of civilization [9]. From ancient literature, it is evident that the various parts of the plants are used in Siddha, Ayurveda and Unani medicine for the treatment of disease of human beings [10]. The use of traditional medicine and medical plants in most developing countries, as a basis for the maintenance of good health, has been widely observed [11]. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs, chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [12].

However, besides all these, phytotherapy, though practiced by many countries like Egypt, Sri Lanka, China, and India and in the rural areas of Nigeria and some other countries, is still not recognized as an efficacious means of therapy by some of the elites. This is because of some of its obvious limitations like, imprecise dosing, unhygienic conditions for practice, lack of scientific proof of the efficiency of most of the plants used, and sometimes imprecise diagnosis (of stomach upset could mean an ulcer, diarrhoea, constipation etc.) hence the need for standardization of plants drugs . Nevertheless, phytotherapy still has some advantages like low toxicity, easy accessibility and cheapness.

Standardization of crude herbal drugs involves a thorough examination and evaluation of herbal drug that will enter the market. It attempts to establish some standards for the determination of the overall quality and strength of a given crude drug and adulterants. Public confidences and acceptance of herbal products will improve when control measures are put in place from the time a medicinal plant is collected to the time of development of the herbal finished product. Also, problems created by unskilled collectors who adulterate crude drugs for financial gains may reduce when detailed information is obtained. It is when the plant component of herbal medicine has been collected at the right time from a proper place, extracted by specific methods and contains the right active substances at the right concentrations, that specific doses of the herbal medicines can produce constants, reproducible pharmacological effects in most patient [9], and that each batch of the finished product will comply with the monograph.

Persea americana Mill (Lauraceae), one of the plants of great medicinal value is studied in other to standardize the leaf, stem and root for proper identification and preparation of its monograph for possible inclusion in the African Pharmacopeia.

Taxonomy and Normenclature

Kingdom: Plantae Subkingdom: Tracheobionta Division:Magnoliophyta Class: Magnoliopsida Subclass: Magnoliidae Order: Laurales Family: Lauraceae Genus: Persea Mill Species: Persea Americana Mill Common names: Avocado pear, Alligator pear Synonyms: Perse agratissima Gaertn. L Local names: Ube oyibo (Ibo), Pia (Yoruba), Eben(Efik)

Description

Persea american Mill is a tree native to Central Mexico, classified in the flowering plant family Lauraceae along with cinnamon, camphor and bay laurel. It originated in the state of Puebla, Mexico. The native, undomesticated variety is known as 'criollo', and is small, with dark-black skin, and contains a large seed. *P. americana* is a dense, evergreen tree, shedding many leaves in early spring. It is a much branched, medium-sized tree, to 60 ft (18m) or more in height. The little flowers are greenish and not at all showy. *P.americana* likes loose, decomposed granite or sandy-loam soil best. They will not survive in locations with poor drainage. The tree grows well on hillsides and should never be planted in stream beds. They are tolerant of acid or alkaline soil [13].



Fig.1: Picture of Persea americana tree



Fig. 2: Picture of Persea Americana fruit

Chemical Compostion, Ethnomedicinal uses and Previous Pharmacological Investigations of Persea americana

About 10 furanoids have been isolated and termed "avocadofurans" and subjected to literature review, primarily from the point of view of the effects of the structural modification on their resultant antibacterial, antifungal, and insecticidal activities (Rodriguez, 2000). Several flavonoids have also been isolated from the leaves and seeds of avocados, with most of these being common flavones of wide distribution in the plant kingdom, such as quercetin which showed virustatic effects by inhibiting HIV syncytium formation and viral p^{24} antigen formation [14].

The fruits are edible and used in many parts of Africa. It is used as juice and in making salad in Ethiopia [15]. In Cote D'Ivoire, the seed is used as an antidiabetic agent [16]. The fruit has antimicrobial effect

and the leaf infusion is used to lower blood cholesterol levels. The seeds and leaves are thought to have anticancer activity. The leaves and bark are used in treating hypertension.

There have been several reports of biological activity showed by the extracts prepared from plant parts of P. americana for which the active principles have not been characterized structurally; including anticonvulsant in mice using standard convulsant drugs to cause seizuires [17], antioxidant, "chondroprotective"-reducing degenerative changes in granulomatous tissue, rat skin lysyl oxidase inhibitory [18] and periodontal disease-related activities [19]. An ethanol-soluble extract of the dried leaves of avocado showed antiinflammatory activity in a carrageenan induced edema protocol, with 1,2,4-trihydroxyheptadec-16ene being obtained from the active fraction [20]. In addition, 1,2,4-trihydroxyheptadec-16-ene and related compounds purified from the seeds of P. americana, showed moderate activity against epimastigates and trypomastigotes [21]. Persin, a constituent of avocado leaves is regarded as a toxin for lactating livestock [22]. In addition, persin has been found to reduce the larval growth of beet armyworm, Spodopteraexigua, and is a known antifungal agent against colletotrichum gloeosporioides (Domergue, 2000). The glycosylated abscisic acid derivatives (1S, 6R)-8-hydroxy abscisic acid-d-glucoside and (1R,3R,5R,8S)-pi-dihydrophaseic acid-dglucoside activities were attributed to these compounds [23]. Aqueous extract of P. americana showed vasorelaxant effect due to the synthesis and release of endothelium derived relaxing factors (EDRFS), activativing PGI₂ or PGE₂ receptors and/or by inhibition of Ca2+ mobilisation through voltage-dependent channels [24]. Also the aqueous extract of *P.americana* leaves caused a significant and dose-dependent inhibition of the control writhes. This effect was similar to that produced by indomethacin in the same duration [25]. The intravenous administration of doses of *P.americana* leaf, aqueous and methanol extract ranging from 6.25 to 50mg/kg caused death of rats within 10 min of administration due to hypotension. Also, the duration of action appeared to be dose dependent [26]. Aqueous leaf extract of *P.americana* possesses anti-convulsant activity in mice. It was able to inhibit and/or attenuate pentylenetetrazole and picrotoxin induced convulsions and bicuculine-induced seizures [26]. The first extract was found to promote wound-healing [27]. The leaf extract was found to have antiulcer effect [28], antioxidant effect and hypoglycemic activity [29].

II. Materials And Methods

Collection and Identification of plant materials

The plant materials were collected in June, from Orba, Nsukka Local Government Area of 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 Department of Pharmacognosy and Environmental Sciences, University of Nigeria, Nsukka.

Preparation of Plant Materials

The leaf, stem and root were carefully separated, washed and excess water allowed to drain off. Representative samples of the leaf, stem and root were kept for examination while the rest were dried under shade, until they were completely dried. They were then pulverized separately and stored in separate sample containers from where 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.

Phytochemical Analysis

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, flavonoids, resins, oils etc following standard procedures [30; 31].

Determination of structural standard

Structural standard parameters include: the macroscopic and microscopic characteristics. The macroscopic characteristics comprise organoleptic features (taste, odour and colour) and morphological characters (physical appearance-texture, fracture etc) of the crude drug. The microscopic characters include tissue and cellular components. In microscopy we have quantitative and qualitative microscopy.

Macroscopic examination of the leaf, stem and root

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 stem and roots were observed. Finally, the organoleptic properties like colour, odour and taste of the leaf, stem and root were observed and noted.

Microscopic examination of the leaf, stem and root

Microscopic examination of powdered materials

Little quantity of the powdered crude drug was placed on a slide and two drops of chloral hydrate were added to moisten the powdered drug. It was covered with 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 microscopical characters which were observed and noted.

Microscopical examination of transverse sections

The staining method described by Odoh *et al.*, [32] was used. Sledge microtone was used for sectioning of 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, 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 Canada balsam mountant was used to mount the sections on the slides.

Determination of Analytical Standards

Determination of ash values

The ash content of a crude drug is generally taken to be residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include organic matter added for the purpose of adulteration. An ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. The method adopted for the determination of values follow the specification given by Odoh *et al.*, [33].

Total ash

A total ash value represents the amount of the residual substance not volatilized on ignition at 450° C. It is used to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance. A tarred nickel crucible was ignited to a constant weight at a dull red head. Cooled and stored in a dessicator. 2 g 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 materials 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

This acid insoluble means the ash is insoluble in dilute hydrochloric acid and is often of more value than ash value. Majority of dugs contain calcium oxalate, but in variable amounts. A total ash varies within wide limits for specimens of genuine drug. Total ash is therefore useless in the detection of earthly matter(s) adherent to such a drug, since the calcium oxalate or carbonate yields by incineration is soluble in hydrochloric acid. One can therefore remove all the variable constituent of the ash by means of dilute hydrochloric acid and weighing the residue which is known as acid-insoluble ash. This way, one can obtain evidence of the presence of excessive earthly matter(s), which is likely to occur with leaves that are densely pubescent, clothed with abundant trichomes or those that tend to retain earthly matter(s) splashed on them during heavy rain fall. The total ash obtained from (a) above was transferred to a beaker containing 25 ml dilute 30% hydrochloric acid, heated to a boiling on a water bath for 5 minutes and filtered with on 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 starred nickel crucible and heated at 150 °C until it was re-weighed.

Water soluble ash

This is used to detect the presence of materials exhausted by water. The water soluble ash is subjected to a much greater reduction than the total ash and is therefore used as an important indication of the presence of exhausted materials substituted for the genuine article. A nickel crucible was ignited to a constant weight at a dull red heat in the oven. 2 g 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 dessicators. More dilute sulphuric acid was added and heating continued to about 80°C with occasional cooling and reweighing until a constant weight was obtained.

Sulphated ash

Sulphated ash provides a more consistent ash. In this method, all oxides and carbonates are converted to sulphates at the higher temperature used. A nickel crucible was ignited to a constant weight at a dull red heat in the oven. A 0.2 g of the powdered plant material was spread over the bottom of the crucible which was reweighed. 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 values

The determination of alcohol-soluble extractive and water soluble extractive is used as a means of evaluating drugs, the constituents of 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 methods of Odoh *et al.*, [33] were used.

Alcohol soluble extractive

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

Water soluble extractive

A 5 g of the powdered plant material was weighed accurately and placed in a 25 ml stoppered 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 macerate for further 18 hours that is a total of 24 hours and then filtered. 20 ml of the filtrate was evaporated to dryness in a 25 ml beaker over a water bath. The residue was dried to a constant weight at 105° C.

Determination of moisture content

A tarred evaporating dish was heated to a constant weight and stored in dessicators. 2 g of 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.

Chemomicroscopy/Microscopical 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. A cover slip was used to cover the slide and then viewed under a light microscope to observe the individual colour changes.

Test for secretory cells and ducts

A little quantity if 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 covered with a cover slip, viewed under a light microscope and 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 with 80% H₂S04 and examined under a light microscope and the colour was noted.

Test for tannins

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

III. Results

Result of Phytochemical Analysis

The result of the phytochemical analysis of *Persea americana* showed the presence of important constituents (Table 1).

Result of Structural Standard

Macroscopic Examinations

Leaf

The leaves are evergreen, alternate, simple and broad with a lanceolate – oblong shape. The petiole is swollen at the base. The margin is entire, slightly undulated and waxy. The apex of the leaf is acuminate with an acute leaf base. The leaf is glossy, public entire, and darkgreen with a slightly disagreeable smell.

Stem

The stem of *Persea americana* has a hard texture and rough fracture. The stem is superficially cracked or inconspicuously fissured and peels off in thick pieces with a disagreeable smell.

Root

The tree of *Perseaamericana* forms a long and unbranched tapped root plus a dense mass of superficial feeder roots. It is light brown in colour with a disagreeable smell. It has a rough fracture.

Microscopic Examinations

Microscopic analysis of the powdered plant materials

The microscopy of the powdered leaf revealed the following features: Anisocytic stomata, epidermal cells, prisms of calcium oxalate, unicellular trichome, starch granules, fibres, bundle of fibres with calcium oxalate crystals, reticulate vessels (Fig. 3). The powdered stem revealed the following; Cork cell in surface view, bundle of fibres, single fibre, prism of calcium oxalate crystals, sclereids (Fig. 4) and the powdered root revealed the following features: Bundle of fibres, cork cell in surface view, prism of calcium oxalate crystals, single pitted fibre with lumen.

Transverse sections of the plant part of Persea americana

Microscopical examinations of the transverse section of the leaf of *Persea americana* shows waxy cuticle and covering trichomes densely occurring on the upper surface. The epidermis which is the first layer was seen and the next layer which is the palisade mesophyll followed by the spongy mesophyll. The next layer showed presence of the vascular bundles which consist of xylem and phloem vessels. The phloem vessel was seen on the outer part while the xylem vessel is located in the innermost part. Trichomes were present on the mid rib (Fig. 6).

The transverse section of the stem revealed the presence of epidermis. The cork and cork cambium was seen next. On the outer part of the vascular bundle, the phloem tissue is seen while the xylem vessels are on the inner part. The pith and the vascular rays were also observed (Fig. 7).

Microscopical examination of the transverse section revealed the presence of epidermal layer containing no trichomes. The cortex was seen next. The vascular bundles are arranged in a circular manner with the cambium in between the xylem and the phloem. The pith was also observed (Fig. 8).

IV. Discussion

Identification, examination and evaluation of the plant *Persea americana* and the various features and characteristics associated with the plant was determined by the various analysis and tests performed. Preliminary phytochemical analysis revealed that the leaf, stem and root of the plant possessed the phytoconstituents such as alkaloids, glycosides, saponins, tannins, flavonoids, steroids, terpenoids, acidic compounds (low concentration), proteins, reducing sugars, carbohydrate and resins which have been found to exert a effect on the body. Resin was found only in the bark and none of the morphological part contained oil

The macroscopical examination of the plant revealed the physical appearance of the morphological parts, which can be seen with the naked eyes and cannot be relied solely for the identification of the plant since other plants could possess similar morphology but it gives you an idea of plant morphology. The microscopic investigation gave hints about the characteristic features that could be found in the plant especially in its different morphological part. Usually, the diagnostic features and their arrangement in each morphological part

are different, but there are some features that are peculiar to all or few of the morphological parts. For example, the result of the microscopy of the powdered leaf showed the presence of prism of calcium oxalate crystals and bundles of fibres which can be found in all the morphological parts while the cork cells can be found in the root and stem and the anisocytic type of stomata occurred only in the leaf. Another important diagnostic tool is the presence of sclereids in the stem of *Persea americana* which confers hardness and mechanical protection to the plant.

The ash values can be used to detect foreign organic matters and adulteration of sand or earth [34]. The British Pharmacopoeia [35] also specified using liquorices as standard that alcohol and water-soluble extractives are greater than or equal to 4.5 and 26.0 % respectively. The results obtained were within the specified range. 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 [36]. 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 [36].

The chemomicroscopy of *Persea americana* leaf, stem and root revealed the presence of lignins, tannins, calcium oxalates, fibres and suberized wall.

V. Conclusion

It can be seen from the study that *Persea Americana* possesses phytoconstituents which account for its usefulness as a medicinal plant. Also the degree of their concentration varies according to different morphological parts explaining the variations in the degree of physiological and pharmacological activities. The study also showed that standardization is very necessary in determining the quality, purity and strength of the crude drug as well as the nature of adulterants if any, since the results obtained from the determination of analytical standards conform to BP specifications, it can be concluded that the methods used were adequate within limits of experimental error. Finally, the results obtained from this work can serve in the identification and preparation of a monograph of *Persea americana* from the family Lauraceae and its possible inclusion in Official Books.

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Tab le 1: Result of phytochemical analysis of Persea americana

Constituents	Inference		
	Leaf	Stem	Root
Carbohydrates	+++	+++	++
Reducing sugars	+	++	++
Alkaloids	+++	+++	+++
Glycosides	++	+	+
Saponins	++	+	+
Tannins	++	+++	+++
Flavonoids	++	++	+++
Resins	+	++	+
Proteins	++	+++	++
Oils	+	+	+
Steroids	+	++	++
Terpenoids	++	+++	+++

Key: + = slightly present, ++ = moderately present, +++ = highly present - =absence of metabolite

Standard	Value (% w/w)			
	Leaf	Stem	Root	
Total ash	10.55	9.70	10.08	
Acid –insoluble ash	1.78	1.99	1.55	
Sulphated ash	7.15	6.40	5.50	
Water -soluble ash	1.80	1.85	3.80	
Moisture content	5.7	7.7	6.2	
Extractive yields				
Alcohol-soluble	0.75	1.02	0.85	
Water-soluble	0.83	2.14	0.58	

Values are \pm SEM, n = 3

Table 3: Result of Chemomicroscopy of the Leaf, Stem and Root of Persea americana.

Test reagent	Observation	Leaf	Stem	Root
Iodinated zinc chloride	Blue colour observed on	Cellulose -	Cellulose -	Cellulose -
solution	epidermal cells.			
Iodinated zinc chloride	Yellow colouration observed in	Lignin +	Lignin +	Lignin +

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solution. Iodinated zinc chloride solution.	xylem vessels. Blue-black colouration observed on few grains on parenchyma cells.	Starch +	Starch +	Starch +
Iodinated zinc chloride solution.	Brown colouration observed.	Suberized wall +	Suberized wall +	Suberized wall +
Sudan III solution	Pink-red observation.	Fibres -	Fibres +	Fibres +
Picric acid solution	Yellow colouration observed.	Secretory cells and ducts -	Secretory cells and ducts -	Secretory cells and ducts -
80% H ₂ SO ₄	Crystals of calcium oxalate observed.	Calcium oxalate crystals +	Calcium oxalate crystals +	Calcium oxalate crystals +
Ferric chloride solution	No greenish colour in some parenchyma cells	Tannins +	Tannins +	Tannins +

Key: + = Present, - = absent



Reticulate vessel

Fig. 3: Microscopy of powdered leaf



Cork cell in surface view



Bundle of fibres

Single fibres



Bordered pitted vessels

Fig 4: Microscopy of powdered stem





Prisms of calcium oxalate crystals



Sclereids



Bundle of fibres



Prisms of calcium oxalate





Bundle of fibres with calcium oxalate

Cork cells in surface view



Single pitted fibre with lumen

Fig. 5: Microscopy of powdered root



Fig. 6: Transverse section of the Leaf of Persea americana (X 100)



Fig. 7: Transverse section of the Stem of Persea americana (X 100)



Fig. 8: Transverse section of the Root of Persea americana (X 100)

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