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Evaluation of some pharmacognostic parameters of the herbs for a traditional hematinic (Haematol -B) powdered formulation in Ogbomoso, Nigeria

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ABSTRACT

The traditional hematinic oral powdered herbal drug (Haematol-B) from Ogbomoso, Nigeria had been characterized in terms of its botanical constituents, ascorbic acid and mineral elements composition, but there is inadequate information on the pharmacognostic properties of the ten herbal materials for its formulation. Therefore the objective of this study was to evaluatethe bark and wood anatomy (as appropriate) of seven of these herbs with a view to highlighting the diagnostic markers for their authentication. Following the conventional anatomical procedures of transverse sectioning (TS) and tissue maceration (TIM), a total of 21 characters were drawn from the barks (root or stem as appropriate) of the seven woody species studied, while 41 characteristics of the wood in the roots of three of the species were compiled using TS, transverse longitudinal sectioning (TLS), radial longitudinal sectioning (RLS) and TIM techniques. Staining, mounting and microscopic examinations followed, and the observed features were taxonomically described in accordance with the provisions of International Association of Wood Anatomists. Their diagnostic values among the medicinal herbs studied were also explored. The bark anatomical features that can be used to diagnose the species studied were those of the secondary cortex, phloem rays, axial parenchyma, sclereids and resin ducts. The wood anatomical markers included features of the vessels in the TS and variable ray characteristics in the TLS. The two artificial keys constructed from discontinuities in qualitative and quantitative anatomical features of the barks and the wood are useful tools for avoiding misidentification of the herbal materials studied.

1. Introduction

Mathur and Hoskins.[1] pointed out that about 80% of the world human population are dependent mainly on alternative medicines for their primary health care. There is therefore little wonder that there is a growing market for herbal drugs worldwide. This development has not only generated an increase in counterfeit herbs, but has encouraged trading in herbal drugs of questionable quality. Additionally, since these drugs are often taken as combinations of different medicinal herbs, there is the unique problem of authentication of their constituents, as one might not be sure if correct herbal material has been included in a given combination.[2,3]

Medicinal herb adulteration is a practice whereby the authentic crude drug is substituted partially or fully with other substances which are either free from, or inferior in therapeutic and chemical properties, or addition of low grade or spoiled drugs or entirely different drug similar to that of original material with an intention to enhance profit.[3,4] Adulteration or substitution of herbal drugs, be it intentional or otherwise, is a fraudulent practice which involves replacement or augmentation of original crude drug substance with spurious, inferior, defective, useless, and sometimes harmful substances, which do not conform to the official standard.[5] Such practice apart from being capable of causing serious health problems to herbal drug consumers, can as well paint the pharmaceutical industry in bad light and plunge them in legal battles.[6] Many poisoning

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incidents caused by confusion, misuse or misrepresentation of herbal material have raised international concern for safe and effective use of herbal drugs,[2] calling for accurate and efficient means of authenticating herbal materials which are the ingredients of the drugs.

Authentication of herbal material is a quality assurance process that ensures the correct plant species and plant parts are used as raw materials for herbal medicine. This process is very critical, basic and important to the safety and efficacy of herbal medicines. According to Kumar et al.[7], evaluation of a herbal drug involves determination of its identity, quality and purity, and detection of any form of adulterants. Evaluation is necessary because herbal drugs are usually mixtures of many plant materials, which by nature, are chemically and biologically variable due to the existing varieties and cultivars; and the fact that their sources and quality are variable.[8] The process of evaluation is also important due to possible deterioration arising from treatment and storage as well as substitution of herbal materials as a result of carelessness, ignorance or fraud.[9] As such, compilation of pharmacognostic parameters based on macroscopical observations, microscopical evaluation and physicochemical analyses of herbal materials is the first step towards establishing the identity and the degree of purity of crude drugs from wild sources.[10]

Hepatitis and anemia are two well- known diseased conditions of human blood.[11] In African traditional medical practice, anemia and blood impurities are taken seriously, perhaps because of the understanding by the traditional healers of the importance of state of health of blood on the general wellbeing of the body.[12] As such, advocacy for use of herbal drugs in ameliorating blood-related ailments is common from traditional medical practitioners in Nigeria, who also produce many remedies which are either of single or of multiple herbal combinations. Some of these herbal preparations are acclaimed to be hematinic or blood-forming, while others are blood- thinning, the latter serving to prevent blood clots[13] or remove blood impurities. In a report of the World Health Organization, iron deficiency is stated as the most common type of anemia estimated to affect approximately 2 billion people worldwide.[11]

The treatment of anemia depends on the confirmed diagnosis and severity of the disease. Since treating anemia is a matter of how much food we eat that aid in hemoglobin synthesis, the most pragmatic approach is to focus on foods such as fruits honey, meats. legumes and nuts. So, treatment usually includes iron therapy (oral and parenteral), iron poly-maltose complex, folic acid and vitamin B12 supplement[13], and sometimes administration of erythropoietin, and bone marrow transplantation.[14] The most prominent complications of iron therapy include gastrointestinal distress for the patients, allergic reactions, anaphylactic shock, abdominal pain, nausea, vomiting, and constipation, which often lead to non-compliance.[15] Arising from these challenges, the alternative form of therapy involving the use of herbal medicine appears to be a saving grace. However, if the use of hematinic herbal formulations will enjoy any form of acceptability, its standardization must be taken with much desired seriousness. Therefore, this study sought to enumerate the bark and wood anatomical characteristics that are diagnostic of the plant materials for producing Haematol-B, a documented hematinic powdered herbal formulation in Ogbomoso, Nigeria.

As reported by Ogunkunle et al.[16], Haematol-B, is formulated from ten botanical constituents, three of which are non-woody, namely; seed of *Garcinia kola* Haekel, fruit calyx of *Hibiscus sabdariffa* L (the red variety), and leaf sheath of *Sorghum bicolor* Moench., and are readily identifiable. This study therefore focused on the other seven herbal materials sold by vendors as roots, root barks and stem barks, whose identities are easily confused during visual examination.[17] Bark is the outermost layer of stems and roots of woody plants. It refers to all the tissues outside the vascular cambium, formed during the process of secondary growth of the stem or root. The cell types, arrangement and tissue types in the barks vary widely across different plant species, and these are useful in authentication of herbal materials with woody parts.[7] Microscopic examinations of the inner bark, the periderm and the rhytidome have also indicated the ability of various plant species to store different materials and chemical substances, some of which have not only made them medicinally useful, but also good for diagnostic purposes.[18]

Lying beneath the outer bark is the inner bark (or secondary phloem), which is the living plant tissue that transports nutrients from the leaves to the stem and roots. Underlying the secondary phloem is the living cambium tissue, the cells of which undergo divisions to produce new phloem and xylem, thereby creating growth rings of wood, and increasing the girth of the tree.[19] The wood or secondary xylem, which consists predominantly of non-living cells, is involved in the transport of nutrients and water from the roots to the leaves. Anatomical studies of wood can be used as a reliable aid for detecting adulteration, fraud or misrepresentation of herbal material.[20] Arising from the above account, the objectives of this study were to describe the bark and wood anatomical composition of the herbal materials used in formulating Haematol-B with a view to highlighting the microscopic markers for diagnosing them; and to generate bark and wood anatomy-based diagnostic keys for authenticating the identities of the recipes for the herbal formulation. With these handy tools, misidentification and misrepresentation of these herbal materials, and the attendant public health[21], social[22], environmental[23,24] and legal[6] consequences can be avoided.

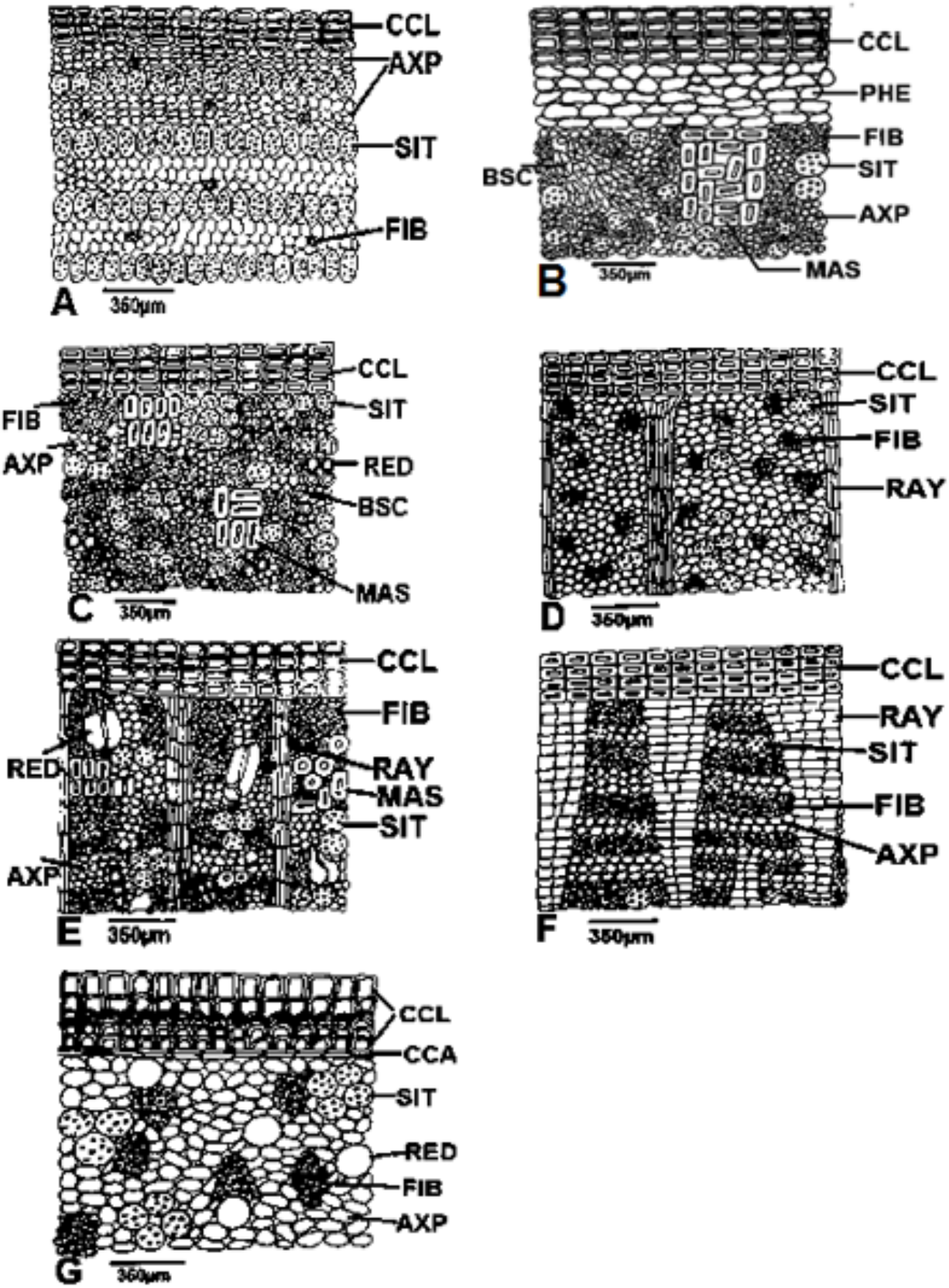


Fig. 1: Drawings showing morphology and arrangement of tissues in the transverse sections (TS) of the barks in A: root of *Aristolochia ringens*; B:stem of *Khaya senegalensis*; C: stemof *Mangifera indica*; D: root of *Sarcocephallus latifolius*; E: stem of *Theobroma cacao*; F: root of *Uvaria chamae*; and G: stem of *Zanthoxyllum zanthoxyloides*. CCL, cork cells layer; AXP, axial parenchyma; PHE, phelloderm/secondary cortex; RED, resin duct; SIT, sieve tube; RAY, ray parenchyma; FIB, fibers; BSC, brachysclereids; MAS, macrosclereid.

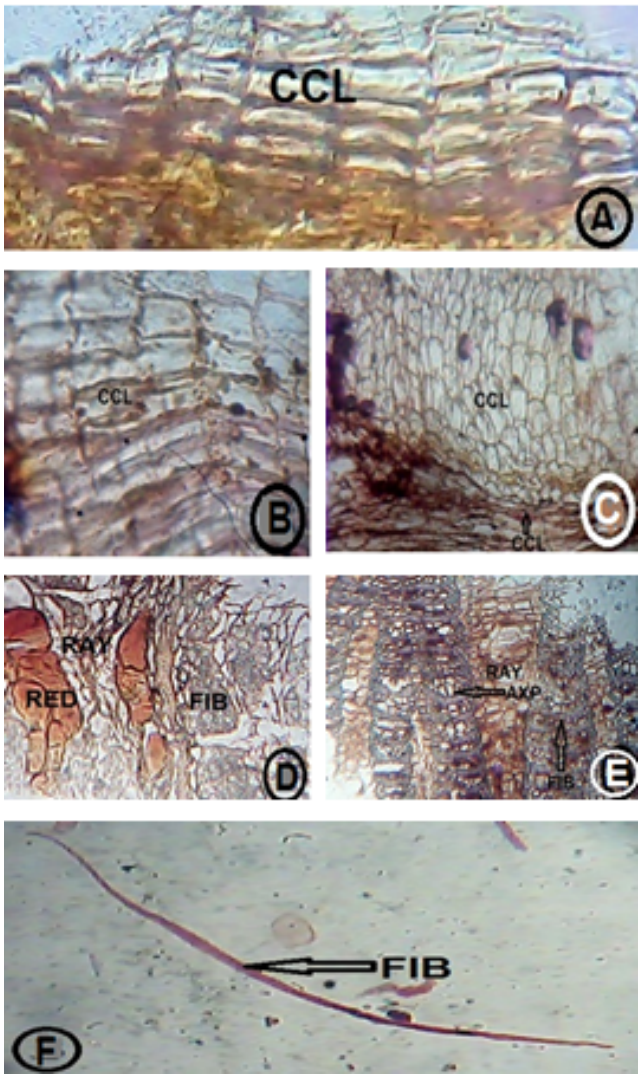


Fig. 2: Morphology of the cells in the barks of some medicinal herbs studied. A-C: Comparative morphology of cork cells (outer bark) of the stem of *Khaya senegalensis*(400X), sstem of *Theobroma cacao* (400X) and root of *Zanthoxylum zanthoxyloides*(100X) respectively; D-E: Cells in theinner barks of stem of *Theobroma cacao*(100X) and root of *Uvaria chamae*(100X);and F: Isolated secondary phloem fiber in the root bark of *Uvaria chamae* (100X).

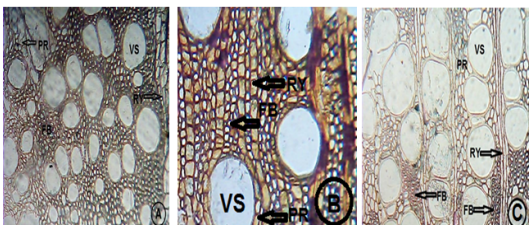


Fig. 3: Morphology and arrangement of cells in the wood TS (100X) of A: *Aristolochia ringens;* B: *Sarcocephallus latifolius* and C: *Zanthoxylum zanthoxyloides.* FB, fibres; PR, axial parenchyma; RY, ray; VS, vessel.

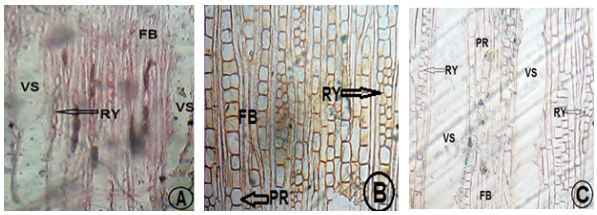


Fig. 4: Morphologyand arrangement of cells in the wood TLS (100X) of A: *Aristolochia ringens;* B: *Sarcocephallus latifolius* and C: *Zanthoxylum zanthoxyloides.* FB, fibres; PR, axial parenchyma; RY, ray; VS, vessel.

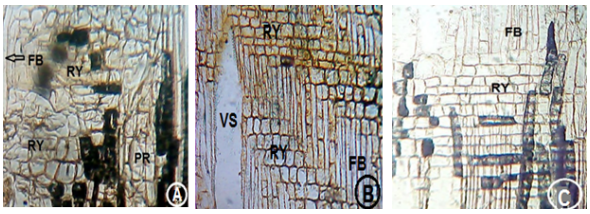


Fig. 5: Morphologyand arrangement of cells in the wood RLS (100X) of A: *Aristolochia ringens*; B: *Sarcocephallus latifolius* and C: *Zanthoxylum zanthoxyloides.* FB, fibers; PR, axial parenchyma; RY, ray; VS, vessel.

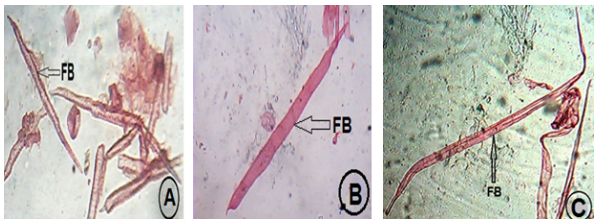


Fig. 6: Morphology of isolated wood fibres (100X) of A: *Aristolochia ringens;* B: *Sarcocephallus latifolius* and C: *Zanthoxylum zanthoxyloides.* FB, fiber.

2. Materials and Methods

2.1. Procurement, authentication and preparation of plant materials

Seven of the ten herbal materials for making Haematol-B powdered drug as listed in Table 1 were purchased from medicinal herb vendors in Ogbomoso Nigeria, located around latitude 8.1333N and longitude 4.2567E. Authentication of the herbs was carried out by consulting with some experts in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Further authentication as appropriate and necessary was performed with the assistance of experienced traditional medicine practitioners from within and outside the town.

The barks were prepared for sectioning[23] by cutting them into pieces of about 2cm × 2cm which were rehydrated by boiling in water for about 10 minutes. The re-hydrated

barks were fixed in Formal-Acetic-Alcohol (FAA) prepared in the v/v ratio 5:5:50:40 of formaldehyde, glacial acetic acid, 95% ethanol and distilled water respectively.[25] Wood samples were collected as short segments of roots, each of which was cut as discs of about 1- 2cm thickness after debarking; they were similarly rehydrated and fixed in FAA, in readiness for sectioning.[24]

2.2. Sectioning of plant tissues

Transverse sections, each 15-20 micrometers thick were obtained from the softened barks by means of a hand-held microtome, and transferred into FAA in labelled small specimen bottles for further treatment later. Small wood blocks of about $1cm^3$each were also cut across the circumference of the softened wood discs. From each block of wood, thin transverse, radial longitudinal and tangential longitudinal sections (TS, RLS and TLS) of similar thickness were also prepared and stored in FAA.

2.3. Treatment of sections for microscopic observation

After rinsing in water several times to remove the preservative, sections of root and stem barks from FAA-filled specimen bottles were stained in1% ethanol-based safranin for 10 minutes on microscope slides, and rinsed in several changes of drops of water until no excess stain came out of them. This was followed by counter-staining with fast green, and another round of distaining in water. Dehydration in 30%, 50%, 70%, 90% and absolute ethanol for 2 minutes each then followed. Staining of wood sections was also done with 1% ethanolic safranin for 10 minutes, distained, counter-stained with fast green, and dehydrated as earlier described. Following dehydration, sections of barks and woods were each cleared in pure xylene for 20 minutes[26] and mounting was done in few drops of Canada balsam.

2.4. Maceration of plant tissues

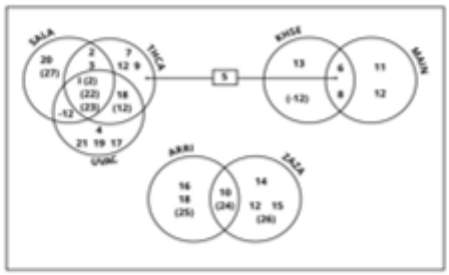
Using a modified form of Jeffrey's method, wood tissue maceration was carried out by boiling a small block of re-hydrated wood for 5 to 10 minutes in about 5ml of concentrated nitric acid to which a few crystals of potassium chlorate had been added.[27] The barks were however macerated with cold treatments i.e addition of concentrated nitric acid and few crystals of potassium chlorate without application of heat, and leaving the tissues inside the solvent overnight (i.e. between 10 and 12 hours) to soften.[28] Prior to the cold maceration process, the scaly part or rhytidome of the bark was peeled off by means of a knife, leaving only the secondary phloem part as the predominant tissue. Softened tissues of both the wood and the barks in concentrated nitric acid were rinsed in several changes of water and transferred, each onto a microscope slide in a few drops of water. With the bottom of a pair of forceps, the softened tissue was macerated by tapping it gently for some minutes and then teased out into its various components on the microscope slide by means of the pointed ends of the forceps.[29] Wood and bark macerates were stained with safranin for about 10 minutes and temporary mounting was done in a few drops of dilute glycerin.

2.5. Microscopic examination of prepared slides and data collection

From each of the bark TS, wood TS, TLS, RLS and tissue macerations, four prepared slides were examined using an Olympus binocular microscope CH20i Model at 100X and 400X magnification.[30] In the bark TS, observations were focused on cell types, morphology and arrangement of cork cells in the outer bark and the inner secondary phloem. In wood sections, attention was focused on such structures as type, morphology and cellular composition of rays in the TLS, shape, occurrence and distribution of vessels, and types of axial parenchyma in the TS; presence or absence of tylose in the vessels, and cellular composition of the rays in the RLS.[31] In the macerated bark and wood tissues, attention was focused mainly on fiber morphology, but observations were also recorded on types of vessel members and sclereids. These observations were recorded in line with the descriptions of the international association of wood anatomists[32], and in drawings, or photomicrographs by means of Bresser microscope equipped with Photomizer SE Microcular camera attachement, 051012-VGA Model connected to a laptop computer.

Some cell and tissue dimensions in wood sections such as height and width of rays in TLS, diameter and lumen width of vessels in TS, fiber length and width in macerated samples etc. were determined in micrometers using a calibrated micrometer eyepiece accessory inserted in the ocular tube.[33] Making use of a calibrated ocular grid attached to the microscope, wood tissue composition in terms of density of fibers, vessels, and axial parenchyma per mm^2 area were calculated in the TS; and of wood rays in TLS. The relative abundance of these four tissue types were determined for each species by adding up the recorded mean values of their densities in a square millimeter area and computing the percentage of each in relation to the total.

In order to obtain frequency of vessels with tylose, 50 fields of microscope were randomly viewed in the wood TS and the occurrence of vessel tylose in each was noted with a tally. The frequency was thereafter computed as a percentage of those fields of view in which tylose was present in relation to the total number of observed fields, i.e. 50. The frequency of vessels with varying shapes in TS (namely, round or oval) was computed by first scoring each of the 50 microscope fields of view as present or absent with regards to each vessel shape. The number of views in which each shape was present was then calculated as a percentage



List of Characters/ character combinations

1. Rays, present in the inner bark
2. Rays, multiseriate
3. Ray cells are all radially procumbent/elongated
4. Ray cells are tangentially/laterally procumbent
5. Sclereids, present in the inner bark
6. Both macro-sclereids and brachy-sclereids are observable
7. Only macro-sclereids are found
8. Mean percent of sclereids by volume of bark relative to the other fundamental tissues of the inner bark (i.e. fibers, parenchyma and sieve tubes), about 20
9. Mean percent of sclereids by volume of bark relative to the other fundamental tissues of the inner bark (i.e. fibers, parenchyma and sieve tubes), less than 10
10. Mean axial parenchyma constitutes up to 50% by volume of inner bark (relative to the other fundamental tissues (i.e. fibers, parenchyma and sieve tubes)
11. Mean density of cork cells (outer bark), about 700/mm^2
12. Resin ducts, present in the inner bark
13. Phelloderm/secondary cortex, present
14. Cork cambium, observable
15. Sieve tubes, in small groups of 3-4 units
16. Sieve tubes, abundant (copious), occurring as tangential bands, alternating with those of axial parenchyma
17. Rays, wedge-shaped
18. Axial parenchyma, abundant (copious), occurring as narrow or wide tangential bands/tiers, alternating with those of sieve tubes, or fibers
19. Sieve tubes, scanty, occurring only as solitary units
20. Fibers, diffuse aggregates, widely distributed in small groups of 2-7 units
21. Fibers occur more or less in tiers , as aggregates/groups of short (squares) and long (rectangular) tangential bands, alternating with bands of axial parenchyma along with sieve tubes
22. Mean density of cork cells (outer bark), about 400/mm^2
23. Sieve tubes occur either as entirely solitary units or predominantly so, with groups of 2-4 units in addition
24. Mean density of cork cells (outer bark),less than 300/mm^2
25. Mean percent of fiber by volume, about 4; mean percent of sclereids, about 40
26. Mean percent of fiber by volume, about 40; mean percent of sclereids, about 10
27. Mean percent of axial parenchyma by volume, up to 36

Fig. 7: Bark-anatomy-based set diagram key for identifying seven medicinal herbs used for Haematol-B, a hematinic powdered herbal formulation in Ogbomoso, Nigeria. Characters in parentheses are regarded as being of secondary importance i.e. although they are, or may be diagnostic of a taxon or a cluster of taxa, such characters need not be observable for taxa recognition to occur

of the total of presence in the two.

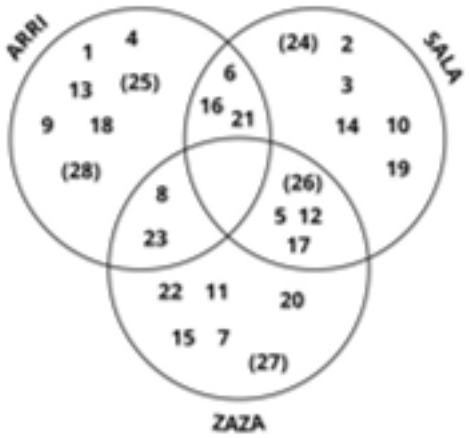
In the inner bark, anatomical features that were quantified in relative percentage as appropriate included fibers, axial parenchyma, sieve tubes, rays and sclereids following the procedure explained by. Ogunkunle et al.[34] Forty-one wood anatomical characters, consisting of 19 qualitative and 22 quantitative (in replicates of 30), and 21 bark anatomical characters, consisting of nine qualitative and 12 quantitative (in replicates of 4 for percent tissue composition and of 10 for others) were compiled to make a total of 62.

2.6. Statistical analysis

The replicated values of the 12 quantitative parameters drawn from the barks of the seven medicinal herbs studied were subjected to one-way analysis of variance (ANOVA) using the version 23.0 of the computer-based SPSS statistical package. The replicated values of those of 22 wood parameters from three of these medicinal herbs were also subjected to one way ANOVA, and the means in both cases were separated using multiple Duncan range test at $\alpha = 0.05$.[35]

3. Results

Figures 1-8, and Tables 2-8 show the results of the study.



List of Characters/Character combination

1. Vessels occur in wood TS as solitary units, radial chains of 2 and clusters of 3
2. Vessels occur in TS as solitary units
3. Vessels occur in TS as solitary units and radial chains of 2-8
4. Vessel wall thickenings, scalarifor
5. Vessel wall thickenings, reticulate
6. Axial (wood) parenchyma, apotracheal of diffuse type
7. Axial (wood) parenchyma, paratracheal of scanty, vasicentric and aliform types
8. Homocellular rays are observable in wood TLS, but may not be entirely so
9. Ray composition in TLS, only homocellular type
10. Ray composition in TLS, only heterocellular type
11. Ray composition in TLS, both homocellular and heterocellular types
12. Biconvex-shaped rays, present in wood TLS
13. Linear rays, present in wood TLS
14. Rays in TLS occur as bi-convex and dumb-bell shapes
15. Rays in TLS occur as mono-convex and bi-convex shapes
16. Uniseriate rays, present
17. Multi-seriate rays, present
18. Rays in TLS, all uniseriate (i.e. ray width/thickness is 1-cell)
19. Range of cells in ray thickness, 1-3
20. Range of cells in ray thickness, 2-3
21. Morphology of ray cells in TS, square and procumbent
22. Morphology of ray cells in TS, all procumbent
23. Mean density of vessels, and of wood parenchyma in wood TS, greater than 30/mm^2 and 50/mm^2 respectively
24. Mean density of vessels, and of wood parenchyma in wood TS, about 5/mm^2 and 30/mm^2 respectively
25. Mean density of rays in wood TLS, about 19/mm^{2}and mean fiber length, about 500µm
26. Mean length of vessel members, about 500µm; mean density of fibers, about 120/mm^2
27. Mean density of rays in wood TLS, about 6/mm^2
28. Mean length of vessel members, about 200µm; mean density of rays, about 20/mm^2

Fig. 8: Wood-anatomy-based set diagram key for identifying three medicinal herbs used for Haematol-B, a hematinic powdered herbal formulation in Ogbomoso, Nigeria. Characters in parentheses are regarded as being of secondary importance i.e. although they are, or may be diagnostic of a taxon or a cluster of taxa, such characters need not be observable for taxa recognition to occur.

Table 1: A list of the plant parts used for the preparation of Haematol-B, a powdered hematinic herbal formulation in Ogbomoso which were collected for anatomical evaluation*

	Species name	Family name	Yoruba name	Parts used for Hematinic herbal formulation*	Parts evaluated
1	*Aristolochia ringens* Vahl.	Aristolochiaceae	*Akogun*	Root	Bark and wood
2	*Garcinia kola* Heckel	Guttiferae	*Orogbo*	Seed	NE
3	Hibiscus sabdariffaL.(red variety)	Malvaceae	*Isapa pupa*	Fruit calyx	NE
4	*Khaya senegalensis* (Desr.) A.Juss	Meliaceae	*Agano*	Stem bark	Bark
5	*Mangifera indica* L.	Anacardiaceae	*Mangoro*	Stem bark	Bark
6	*Sarcocephalus latifolius* (J.E.Smith) E. A.Bruce	Rubiaceae	*Egbesi*	Root bark	Bark and wood
7	*Sorghum bicolor* Moench.	Poaceae	*Oka baba*	Leaf sheath	NE
8	*Theobroma Koko* L.	Sterculiaceae	*Koko*	Stem bark	Bark
9	*Uvaria chamae* P. Beauv.	Annonaceae	*Eruju*	Root bark	Bark
10	*Zanthoxylum zanthoxyloides* (Lam.)	Rutaceae	*Igi ata*	Root bark	Bark and wood

*NE, Not evaluated

Table 2: Some descriptive features in the wood bark of seven medicinal herbs studiedc

		ARRI	*KHSE*	*MAIN*	*SALA*	*THCA*	*UVAC*	*ZAZA*
• **A. Outer Bark**								
Cork Laye		Homo (oval)	Homo (rec)	Homo (rec)	Homo (rec)	Homo(rec)	Homo(rec)	Hetero(rec, squ)
Cork Cambium		-	-	-	-	-	-	+
• **B. Inner Bark**								
Secondary (Pheloderm)	Cortex	-	+	-	-	-	-	-
Secondary Phloem (Bast)	Fibers	+*	+***	+**	+**	+**	+**	+**
	Axial parenchyma	+***	+**	+**	+**	+**	+**	+***
	Sieve tubes	+***	+**	+**	+*	+*	+*	+**
	Rays	-	-	-	+** (RPC)	+** (RPC)	+** (LPC)	-
	Sclereids	-	Brachy**; Macro*	Brachy*; Macro**	-	Macro*	-	-
	Resin ducts	-	-	+	-	+	-	+

ARRI = Aristolochia ringens; *KHSE= Khaya senegalensis*; *MAIN =Mangifera indica*; *SALA =Sarcocephalus latifolius*;*THCA*= Theobroma cacao;*UVAC*= Uvaria chamae; *ZAZA=Zanthoxylum zanthoxyloides* ***(frequent/averagely observed/high frequencyi.e. 40-59% occurrence); **(less frequent/sometimes observed/low frequency i.e.10-39% occurrence); *(seldom frequent/rarely observed/very low frequency i.e.1-9% occurrence); *RPC* = radially-procumbent cells; *LPC* =laterally-procumbent cells; *SQC* =square cells; +, Present; -, Absent/not observed.

4. Discussion

4.1. Ethnopharmacological value of the plant species studied

The herbal materials of the seven species studied as components of Haematol-B, a traditional hematinic powdered formulation in Nigeria are listed in Table 1. According to the information in the table, barks and/or wood of *Theobroma cacao*, *Aristolochia ringens*, *Khaya senegalensis*, *Mangifera indica, Sarcocephalu slatifolius,Uvaria chamae and Zanthoxylum zanthoxyloides* are used along with the leaf sheath of *Sorghumbicolor* fruit calyx of *Hibiscus sabdariffa* and seed of *Garcinia kola* to formulate the blood-enriching traditional drug. According to WebMD[36], despite some safety concerns, bark of *Aristolochia* sp. is used to prevent seizures, increase sexual desire, boost the immune system and start menstruation. It is also used to treat snakebite, intestinal pain, gallbladder pain, arthritis, gout, achy joints (rheumatism), eczema, weight loss and wounds[36] The stem bark of *Khaya senegalensis* was mentioned by Takin et al.[37] to be variously indicated for cancer, diarrhea, fever caused by malaria, helminth infections, tripanosomiasis and diabetes among others.

According to Wauthoz et al.[38], the stem bark of *Mangifera indica* is ethnopharmacologically useful for its antioxidant, anti-inflamatory and immunomodulatory

Table 3: Mean quantitative characteristics of some types of tissue in the outer barks of seven medicinal herbs studied

	ARRI	*KHSE*	*MAIN*	*SALA*	*THCA*	*UVAC*	*ZAZA*
	• A. Cork Layer						
Number of rows	9bc± 0.64	3a ± 0.16	22f ± 2.24	6ab± 0.51	11cd ± 1.19	7ab ± 0.43	16e ± 2.09
Thickness of layer (μm)	181.25bc ± 12.68	62.46a ± 1.84	399.36 e ± 41.64	121.78ab ± 9.82	200.70d± 25.46	154.62b± 10.95	665.60 f ± 92.54
Density of cells/mm^2	286 a ± 4.01	664d ±20.97	708d ± 37.80	348ab ± 18.46	414ab ± 24.79	353b ± 7.70	354ab ± 4.43
Cell width (μm)*	21.25cd± 2.09	12.03a ± 1.87	13.82± 2.01	48.89h ± 3.89	23.81de± 2.76	19.97c ± 2.11	58.34i ± 3.98
Cell wall thickness (μm)	3.46bcd ± 0.20	3.58bcd ± 0.17	4.48ef ± 0.21	3.33bc± 0.28	3.20b ± 0.21	4.99f ± 0.23	4.10de± 0.17
	• B. Cork Cambium Layer						
Number of rows	-	-	-	-	-	-	5 ± 0.82
Thickness of layer (μm)	-	-	-	-	-	-	83.94 ± 13.11

ARRI = Aristolochia ringens; *KHSE= Khaya senegalensis*; *MAIN =Mangifera indica*; *SALA =Sarcocephalus latifolius*;*THCA= Theobroma cacao*;*UVAC= Uvaria chamae*; *ZAZA=Zanthoxylum zanthoxyloides*. —, not applicable; *Cell width = diameter of cork cell at the widest point.

Table 4: Mean quantitative characteristics of some types of tissue in the inner barks of seven medicinal herbs studied

	ARRI	*KHSE*	*MAIN*	*SALA*	*THECA*	*UVAC*	*ZAZA*
	• A. Secondary Cortex (Phelloderm)						
Number of layers/rows	-	6a± 0.27	-	-	-	-	-
Thickness (μm)	-	183.30 a± 5.39	-	-	-	-	-
	• B. Secondary Phloem (Bast)						
Fibers (%)	4.76 a ±0.88	42.65g ±3.56	37.18efg ±4.09	32.55def ±4.78	37.63def ±5.02	29.51d ±3.44	38.09g ±5.12
Axial parenchyma (%)	52.92e ± 3.89	27.15bc ± 3.12	25.88bc ±3.22	36.74d ± 4.89	23.42b ± 3.12	32.38cd ± 4.09	51.34e ± 4.89
Sieve tubes (%)	42.32c ±4.22	10.09 ab ±2.32	14.25b ± 2.12	7.37 a ±1.98	7.87 a ± 2.44	7.46 a ± 2.09	10.57ab ±2.89
Rays (%)	-	-	-	23.34a ± 4.99	24.71a ± 5.02	21.47a ± 4.79	-
Sclereids (%)	-	20.11b ± 5.21	22.69b ± 5.22	-	6.37a ± 2.01	-	-

ARRI = Aristolochia ringens; *KHSE= Khaya senegalensis*; *MAIN =Mangifera indica*; *SALA = Sarcocephaluslatifolius*;*THCA= Theobroma cacao*;*UVAC= Uvaria chamae*; *ZAZA= Zanthoxylum zanthoxyloides*.(%) = percent composition/ mean relative abundance of tissues; — = not applicable.
The mean values of data in a row with the same alphabets are not significantly different (p>=0.05)while those with different alphabets are significantly different (p<0.05).

properties. As such, it has been useful against gastric and dermatological disorders, AIDS, cancer and asthma.[39] Abbah et al.[40] have provided pharmacological evidence in favor of the use of root bark of *Sarcocephalus latifolius* in malaria ethnopharmacy, while ethanolic extracts of the roots of *Zanthoxylum zanthoxyloides* and its tooth paste have been reported by Orafidiya et al.[41] to have exhibited the highest antibacterial activities comparable at 2.5% w/w but considerably higher at 5.0% w/w of commercially available toothpaste used as positive control. These submissions are not only confirmatory of the wide application of the medicinal herbs studied, but also a pointer to the necessity to ensure their identity and purity. The qualitative and quantitative bark anatomical data obtained from this study have the potential of being used to establish these specific taxonomic categories. At the least, the barks and woods of the species examined can easily be distinguished from their adulterants.

4.2. The diagnostic value of bark anatomy in the species studied

Among the seven species examined, *Zanthoxylum zanthoxyloides* distinguishes itself by possession of cork cambium and heterocellular cork layer. Among the other

Table 5: Some diagnostic features of vessels and fibers in the wood of three medicinal herbs studied

	Parameters	***ARRI***	***SALA***	***ZAZA***
	• A. Parenchyma Cells (PC)			
1	Pore type	Diffuse-porous	Diffuse-porous	Diffuse-porous
2	Shape in TS	Round***; Oval***	Round***; Oval***	Round***; Oval***
3	Occurrence	Solitary***; Radial chains of 2***; Clusters of 3*	Solitary	Solitary*; Radial chains of 2-8****
4	Pore size	Relatively narrow	Relatively wide	Relatively wide
5	Wall thickening pattern	Scalariform	Reticulate	Reticulate
6	Frequency/relative abundance	Very low*	Low**	Low**
7	Vessel members	Short	Short	Short
8	End walls	Oblique	Oblique***; Truncate***	Oblique***; Truncate****
9	Tylose	Present**	Present*	Present*
	• B. Rays (RY)			
10	Occurrence	Aggregates	Aggregate s; Non-storied	Aggregates; Non-storied
11	Frequency/relative abundance	high***	Low**	Low**
12	Morphology	Short	Fairly-long	Fairly-long
13	Lumen and tip	Non-septate; pointed & blunt	Non-septate; pointed & blunt	Non-septate; pointed

ARRI= Aristolochia ringens; *SALA= Sarcocephallus latifolius*; *ZAZA= Zanthoxylum zanthoxyloides*. ****(very frequent/usually observed/very high frequency i.e. 60-99%occurrence); ***(frequent/averagely observed/high frequency i.e. 40-59%occurrence); **(less frequent/sometimes observed/low frequency i.e. 10-39%occurrence); *(seldom frequent/rarely observed/very low frequency i.e. 1-9%occurrence).

Table 6: Some diagnostic features of parenchyma and rays in the wood of three medicinal herbs studied

	Parameters	***ARRI***	***SALA***	***ZAZA***
	• A. Parenchyma Cells (PC)			
1	PC type (in TS)	Apotracheal (diffuse)	Apotracheal (diffuse)	Paratracheal (scanty**; vasicentric**; aliform**)
2	Frequency/relative abundance of PC	Low**	Very low*	Low**
	• B. Rays (RY)			
3	RY cells (in TS)	Square*; procumbent****	Square**; Procumbent****	Procumbent
4	RY width (in TLS)	Uniseriate	Uniseriate***; biseriate**; multiseriate**	Biseriate**; multiseriate****
5	RY composition (in TLS)	Homocellular	Heterocellular	Homocellular***; heterocellular***
6	RY general shape (in TLS)	Linear	Bi-convex****; dumb-bell*	Mono-convex**; bi-convex****

ARRI= Aristolochia ringens; *SALA= Sarcocephallus latifolius*; *ZAZA= Zanthoxylum zanthoxyloides*. ****(very frequent/usually observed/very highfrequency i.e. 60-99% occurrence); ***(frequent/averagely observed/highfrequency i.e. 40-59% occurrence); **(less frequent/sometimes observed/lowfrequency i.e. 10-39% occurrence); *(seldom frequent/rarely observed/very lowfrequency i.e. 1-9% occurrence).

six species, which lack these two features, *Sarcocephalus latifolius,Theobroma cacao* and *Uvaria chamae* are notable for possession of phloem rays in their inner barks. From these three, *T. cacao* is distinctive in having macro-sclereids in addition to the rays, while *S. latifolius* and *U.* chamae do not have sclereids. These two species are however distinguishable in that the mean width of cork cells in the former (i.e. 48.9μm) is significantly wider ($p<0.001$) than in the latter with 19.9μm (Table 3). Considering the last three species, namely: *Arristolochia ringens*, *Khaya senegalensis*, and *Mangifera indica,* both macro and brachy-sclereids are observable in the last two, while the first species in the list lacks these structures. So also, the relative composition of fibers in the secondary phloem of the two species (i.e. 42.65 and 32.55 % respectively) are significantly higher than 4.37% in *A. ringens.* On the other hand, the frequencies of axial parenchyma and sieve tubes in this species i.e.52.9 and 42.32% are significantly higher

Table 7: Somequantitative characteristics of vessels and fibers in the wood of three medicinal herbs studied

	Parameters	*ARRI*	*SALA*	*ZALA*
	• A. Vessels (VS)			
1.	Density/mm2	37c ± 1.20	5a± 0.22	31b ± 1.06
2.	Relative abundance/ frequency(%)	9.16	52.3	38.1
3.	% frequency of VS shapes in TS	Round(50); Oval(50)	Round(47); Oval(53)	Round(53); Oval(47)
4.	Frequency of tylose (%)	17.0	7.0	3.0
5.	Diameter (μm)	101.97a ± 5.54	197.03c ± 10.01	146.86 b ± 3.65
6.	Lumen width (μm)	89.51a ± 5.34	181.47c± 5.48	132.95b± 3.73
7.	Wall thickness (μm)	6.23a ± 0.24	7.47b ± 0.32	6.95ab ± 0.41
8.	Length of VS member (μm)	194.25a± 7.47	499.78b ± 24.26	528.04b ± 11.84
	B. Fibers (FB)			
9	Density/mm^2	289b± 14.76	120a ± 6.03	127a ± 11.09
10	Relative abundance/ frequency(%)	71.53	34.68	36.39
11	Diameter (μm)	20.82a± 1.07	31.57c ± 1.15	25.43b± 1.09
12	Lumen width (μm)	11.95a ± 0.99	25.17c ± 0.89	20.14b ± 1.09
13	Wall thickness (μm)	4.44c ± 0.18	3.15b ± 0.21	2.65a ± 0.09
14	FB length (μm)	514.72a ± 17.45	1091.58b ± 67.40	1059.02b ± 31.31

ARRI= Aristolochia ringens;*SALA= Sarcocephallus latifolius*;*ZAZA= Zanthoxylum zanthoxyloides*. ****(very frequent/usually observed/very high frequency i.e. 60-99%occurrence); ***(frequent/averagely observed/high frequency i.e. 40-59%occurrence); **(less frequent/sometimes observed/low frequency i.e. 10-39%occurrence); *(seldom frequent/rarely observed/very low frequency i.e. 1-9%occurrence).

The mean values of data in a row with the same alphabets are not significantly different (p>=0.05)while those with different alphabets are significantly different (p<0.05).

Table 8: Some quantitative characteristics of parenchyma cells and rays in the wood of three medicinal herbs studied

	Parameters	*ARRI*	*SALA*	*ZAZA*
	• A. Parenchyma Cells			
1.	Density/mm2 in TS	59b ± 4.05	32a ± 1.57	83b ± 7.25
2.	Relative abundance/ freq. (%)	14.60	9.25	23.78
	.B. Rays (RY)			
3	Density/mm^2 in TLS	19d± 0.48	13c ± 0.37	6a ± 0.27
4	Relative abundance/ freq (%)	4.70	3.76	1.72
5	Number of cells in RY width (TLS)	1a± 0.00	2b ± 0.14	3c ± 0.07
6	RY thickness in TLS (μm)	12.97a ± 0.84	55.30a ± 2.93	86.02a ± 2.89
7	Number of cells in RY height (TLS)	6a ± 0.30	25c ± 2.02	34d ± 1.71
8	RY height in TLS (μm)	187.39a ± 6.21	1022.50c ± 73.98	882.35c ± 42.87

ARRI= Aristolochia ringens; *SALA= Sarcocephallus latifolius*; *ZAZA= Zanthoxylum zanthoxyloides*.; NA= not applicable.The mean values of data in a row with the same super scripts are notsignificantly different (p>=0.05) while those with different superscriptsare significantly different (p<0.05).

than those of the other two species, being less than 28 % for parenchyma and 15% for sieve tubes (Table 4).

In agreement with the finding that bark anatomical features could be reliably applied for species diagnosis among the medicinal plants studied, Alam and Najum[10] carried out a microscopic examination of powdered bark of *Gaultheria trichophylla* which revealed useful diagnostic features of the species. Additionally, these researchers examined the fluorescence properties of the powdered bark in different reagents, and conducted organoleptic evaluation of the whole and powdered bark to generate information usable for diagnosing the medicinal plant. In consonance with the observations made from the current study, Kotina et al.[42] examined the anatomy of the leaf and bark of *Warburgia salutaris,* and reported combinations of anatomical characters to be of diagnostic value for this important medicinal plant from South Africa. Such characters observed by these authors in the bark included scattered large druses and numerous small ones, secretory cells, thin-walled fiber-like sclereids, and sieve tubes having compound sieve plates on the lateral and oblique cross walls. Macroscopic, microscopic and HPTLC profiles of the barks of four species of *Ficus* sold as medicinal herbs in Indian markets were examined by Babu et al.[43] As carried out in the present study, these authors described and used the qualitative and quantitative anatomical features of the outer and inner barks to distinguish between the four species of *Ficus* studied i.e., *F. racemosa*, *F. virens*, *F. religiosa* and *F. benghalensis*. The pharmacognostic characters of the root bark of *Holoptelea integrifolia*, an important medicinal plant in India was also evaluated by Kumar et al.[7], with the result that the qualitative and quantitative anatomical features of the bark were distinctive enough to identify and decide the authenticity of this crude drug. The authors thereafter recommended the inclusion of these diagnostic features as microscopic standards in Indian herbal pharmacopeia. These submissions point to the fact that empirical data on bark anatomy have been successfully employed to diagnose a long list of medicinal plants. However, available literature appears to be deficient in pharmacognostic studies of the seven medicinal plants studied from the point of view of their bark anatomy. The dearth of information from this direction is yet another justification for this study.

On the contrary, there are a number of publications, which have provided pharmacognostic information on the plant species. Some of these include Mahmood et al.[44], which, among other studies examined the wood anatomy of *Zanthoxylum alatum* used as chewing stick in Pakistan; Orafidiya et al.[41], which examined the effectiveness of the root of *Zanthoxylum zanthoxyloides* formulated as toothpastes in Nigeria; Nwokonkwo and Okeke[45], which evaluated the chemical constituents and biological activities of stem bark extract of *Theobroma cacao*; and Ghorbani et al.[46], which adopted DNA bar coding technique to conduct a diagnostic study on three species of *Aristolochia* collected from Thailand. On the whole, the results of bark anatomy obtained in this study are clear enough to establish the species identities of the seven medicinal herbs studied. Qualitative and quantitative information, especially on the elements of the secondary phloem can be employed as suitable quality control measures to ensure purity, safety and efficacy of these drugs. Figure 7 is confirmatory of the diagnostic value of these features, being a key for unambiguous authentication of these important medicinal herbs in Ogbomoso.

4.3. The diagnostic value of wood anatomy in the medicinal herbs studied

The species boundaries of three of the medicinal herbs studied have been clearly resolved by the results of wood anatomy as follows: In *Sarcocephalus latifolius*, the vessels occur as only solitary units; and rays in TLS are all heterocellular with bi-convex and constricted (i.e. dumb-bell) shapes. Additionally, the vessels are significantly ($P<0.001$) wider (about 200μm) than in the other two species i.e. *Arristolochia ringens* and *Zanthoxylum zanthoxyloides* (Table 7). *Zanthoxylum zanthoxyloides* can be distinguished by lack of uniseriate rays in the TLS. In *Aristolochia ringens*, only uniseriate rays are observable, all of them being linear in shape and homocellular in composition.

Among others, the qualitative and quantitative features of wood vessels, parenchyma, rays and fibers have been acknowledged to be reliable diagnostic and phylogenetic indices.[47]The findings from the present study are in agreement with this position, and have further confirmed that these features have the potential for diagnosing the three herbal materials studied, which may otherwise be impossible using only morphological characterization.[48] Perrone et al.[49] studied eight woody species of *Hypericum*; secondary xylem was observed to be ring-porous in six and diffuse porous in two species. This feature as well as the number and mean diameter of vessels showed interspecific differences among *H. perforatum, H. perfoliatum, H. pubescens, H. tetrapterum, H. triquetrifolium, H. androsaemum, H. hircinum and H. aegypticum.* The difficulty posed by the identification of *Cola acuminata and C. nitida* when not in fruit was also resolved by Jensen et al. using wood anatomical features such as wood fiber composition, types and amount of crystals. Some of the features reported by these authors to be useful diagnostic markers were also found to be so useful in the current study.

It can be implied from the above account that, while empirical data on wood anatomy provided by authors such as Perrone et al.[49], Akinloye et al.[50] and Marques and Callado[50] have been successfully used to diagnose many medicinal and non-medicinal plants, the story has not been

the same for the three woody species reported in this study. This is a further justification for the present effort, with the results that qualitative and quantitative characteristics of wood anatomy are reliable in evaluating pharmacognostic parameters of the species. In particular, they are diagnostic of the three plant species examined as revealed by the entries in Figure 8.

4.4. Applicability of the diagnostic keys for authentication of the medicinal herbs studied

In order to apply the key in Figure 7 to identify any of the constituent medicinal herbs, the user should follow a number of steps:

1. **Step I:** Enter the key through the 'node' with character number 5(i.e. the node, connecting one taxon, *Theobroma cacao* on the left and two taxa, *Khaya senegalensis* and *Mangifera indica* on the right), and evaluate the unknown plant specimen with regards to this character;
2. **Step II:** If the result of the specimen evaluation in 'Step I' agrees with the point of entry in the key, proceed first, to the node's left point of contact; re-evaluate the specimen for characters 7, 9 and 12; and if the results are in consonance with these three characters, decide that the identity of the unknown specimen is *T. cacao*; else (i.e. if the results are in disagreement with the three characters), proceed to the node's right-hand point of contact and re-evaluate the specimen for characters 6 and 8; if the outcomes of the evaluation are in consonance with these two characters, consider character 13 to diagnose *Khaya senegalensis* on the one hand, and characters 11 and 12 to diagnose *Mangifera indica* on the other hand;
3. **Step III:** If the result of specimen evaluation in 'Step I' above does not agree with the condition of the point of entry into the key (i.e. if sclereids are not observable in the inner bark of the unknown plant specimen), omit 'step II' above i.e. exit the key; and re-enter into the key through the center of the three interlocked sets/ circles and evaluate the specimen with regards to character number 1; thereafter, proceed in centrifugal direction (i.e. towards the outside of the cluster of taxa), evaluating the specimen in the hand and systematically selecting taxa as probable identities of the unknown specimen as the exercise proceeds. At this point, it is advisable for user to try out all the three available alternative routes before a final choice of one taxon name is made;
4. **Step IV:** If the result of evaluation of the specimen along with character 1 in 'Step III' above is not workable, or if at any point in navigating the three-taxa cluster, the procedure is aborted or identification of the species is not possible due to disagreement between the listed features in the key and observations on the specimen, exit the key, and re-enter into it at the centre of the two-taxa cluster and repeat the systematic character comparison exercises to effect identification as either *Arristolochia ringens* or *Zanthoxylum zanthoxtloides.*

Application of the key in Figure 8 follows a procedure similar to the earlier described. The user enters the three-taxa cluster at the center and tries out the three available alternative routes as described in 'Step III' above. Both of the keys presented can also be used to authenticate any of the constituent medicinal herbs suspected or supplied under a given name. For the purpose of illustration, if a user in applying the key in Figure 7 suspects the identity of a stem bark to be *Theobroma cacao*, confirmation is carried out by evaluating the features of the specimen and comparing with those in the key in three alternative ways: firstly, considering only characters 1, 2, 3, 7, 9 and 12; secondly, only characters 1, 18, 7, 9 and 12; and thirdly, only characters 5, 7, 9 and 12. In summary, if given a key, and the assurance that a suspected taxon is included in that key, the first step to confirm is to locate the position of the taxon in the key and then work on that key along the established route of identifying the taxon, paying particular attention to only those characters leading to the taxon name, and ensuring that all such, rather than most of the statements are in agreement with the observable features of the specimen in the hand.

The set diagram format in which the two keys from this study are presented is characterized by a fixed route of navigation, i.e. the order of couplets/choices is defined by the author of the key, so that there is a single path to be followed by the user. This is a feature of single-access identification tools such as the dichotomous keys, which, by implication, are associated with some inadequacies, including the restriction to only one point of entry, and the problems of 'unanswerable couplet'[51], 'dead ends', and 'momentary distractions' that can cause a user to forget his position in a key.[52] Although the two keys generated are single-access in the strict sense, their functionality attributes have enabled them to substantially overcome the enumerated challenges by the fact that a user is free to exit if necessary, and re-enter the key at other points without losing focus. The possibility for authentication/confirmation of suspected identity is another laudable attribute of the set diagram key. This mission is not easily achievable with the dichotomous key, the most widely used diagnostic tool for plant identification.[52–55]

5. Conclusion

The notable bark anatomical characteristics which were diagnostic of the seven plant species examined included occurrence of secondary cortex (or phelloderm);

presence/absence, types, distribution and relative abundance (%) of sclereids; presence/absence, cellular composition and shape of phloem rays; arrangement, types and abundance (%) of axial parenchyma; and presence/absence of resin ducts. On the other hand, the salient wood anatomical features that can be used to authenticate three of the species studied were in the TS (i.e. occurrence and diameter of vessels, and abundance of parenchyma); and the TLS (i.e. cellular composition, width, height, thickness and morphology of rays). Two diagnostic keys have been generated from discontinuities in qualitative and quantitative features of the barks and wood anatomical observations. For the sake of avoiding species confusion and misrepresentation of these medicinal herbs, the simplicity, flexibility and diagnostic value of the keys are not in doubt.

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None.

7. Conflict of Interest

None.

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