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Original Research Article

Aspects of pharmacognostic evaluation of the herbs for a traditional antimalarial (Maloff-HB) powdered formulation in Ogbomoso, Nigeria

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ABSTRACT

Maloff-HB is a documented traditional oral powdered herbal drug in Ogbomoso, Nigeria whose botanical constituents, ascorbic acid and mineral elements composition have been quantified but there is inadequate information on the pharmacognostic properties of the nine herbal materials for its formulation. This study therefore sought to elucidate the bark and wood anatomy of eight of the nine herbs used in the formulation, and identify the diagnostic markers for their authentication. The conventional anatomical techniques of transverse sectioning (TS) and tissue maceration (TIM) were used to draw out 21 characteristics from the root barks of the eight woody species studied. In addition, 41 features of the wood in the roots of three of the species were drawn using TS, transverse longitudinal sectioning (TLS), radial longitudinal sectioning (RLS) and TIM. Following staining, mounting and microscopic examinations, the observed qualitative and quantitative features were taxonomically described in accordance with the provisions of International Association of Wood Anatomists, and their diagnostic values among the medicinal herbs were explored. Bark anatomical markers that are clearly diagnostic of the species studied included features of the secondary cortex, phloem rays, axial parenchyma, sclereids and resin ducts. In the wood, these included features of the vessels in the TS and variable ray characteristics in the TLS. The two artificial keys obtained from discontinuities in qualitative and quantitative features observed in the barks and the woods are useful tools for reliable identification of the herbal materials studied.

1. Introduction

Nature has been a source of medicinal agents for thousands of years. Plants in particular have been identified and used for therapeutic purpose since the start of human history, and an impressive number of modern drugs have been isolated from them and other natural sources.[1,2] Plant-based medicines have continued to play an essential role in health care, with about 80% of the world's human population relying mainly on traditional medicine for their primary health care.[3] Extensive use of herbal medicine calls for accurate and efficient means of authenticating herbal materials which are the ingredients of the drugs. This is necessary for two main reasons. Firstly, the growing market for herbal drugs worldwide has endangered many international trading companies and generated an increase in herbs of questionable quality. Secondly, herbal drugs are often taken as combinations which generate unique problems of authentication such as determining if there is species confusion of different herbs sharing one name or one herb using different names, and if correct herbs have been included in a particular proprietary medicine.[4]

Pharmacognosy is the science that deals with medicinal products of plant, animal, microbes or mineral origin in their crude or unprocessed state.[5] The subject matter of systematic pharmacognosy involves macroscopic and microscopic examination of herbal materials in order to recognize and identify unknown drugs. Microscopic evaluation of plant parts is particularly useful for assuring the identity of the material and as an initial screening test for impurities or adulterants.[6] These efforts which

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ensure botanical authentication are important aspects of herbal drug standardization.[7] Many poisoning incidents caused by misuse or confusion of herbal medicines have raised international concerns, and hence the necessity for authentication of the constituent herbs towards their safe and effective use.[4] Therefore, compilation of pharmacognostic parameters based on macroscopic observations, microscopic 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.[8]

Malaria fever is a major public health problem in Nigeria[9] and is responsible for over 70% of out-patient hospital visitation with a great toll on productivity.[10] The constant evolution of the malaria parasite has rendered the cheapest and most widely available antimalarial treatments ineffective, more so with the recent reports about the increasing resistance of *Plasmodium falciparum* to artemisin-based compounds.[11] Nowadays, anti-malarial drug resistance has become one of the most important challenges to malaria control efforts.[12,13] If we should rely on herbal drugs as saving grace in the management of this dreaded disease, then concerted efforts towards establishing an acceptable authentication of the herbal material are a must do. This is an area of focus in this study.

This study has laid emphasis on enumeration of bark and wood anatomical features for standardization and authentication of medicinal plant parts used for Maloff-HB, a documented oral powdered antimalarial herbal formulation in Ogbomoso, Nigeria. Although, nine herbal materials are used for Maloff-HB[13], one of them i.e. the vines of *Cassytha filiformis L.* is non-woody; so the study focused on the other eight. The objectives of this study were to elucidate the bark and wood anatomical composition of the herbal materials used for producing Maloff-HB in order to come up with microscopic markers for diagnosing its constituent herbs; and to generate bark and wood anatomy-based diagnostic keys which can be useful as handy tools for authenticating the identities of the constituents of the herbal formulation. These are with a view to preventing misidentification and misrepresentation of the herbal materials[14], and the attendant consequences.[15–18]

2. Materials and Methods

2.1. Collection and preparation of herbal materials

Eight of the nine herbal materials for the formulation of Maloff-HB powdered drug as listed in Table 1 were collected from medicinal herb sellers in Ogbomoso Nigeria, located around latitude 8.1333N and longitude 4.2567E. The materials were authenticated based on consultations with some experts in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Where applicable, further authentication was performed with the assistance of experienced traditional medicine practitioners from within and outside the town. The barks were prepared for sectioning[19] 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.[20] Wood samples were collected as short segments of roots, each of which was cut as discs of about 1cm to 2cm thickness after debarking; they were similarly rehydrated and fixed in FAA, ready for sectioning.[21]

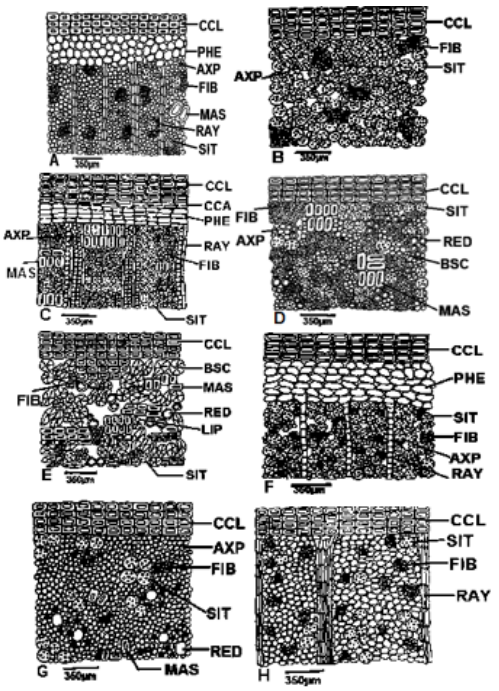


Fig. 1: Drawings showing morphology and arrangement of tissues in the transverse sections (TS) of the barks in A: stem of *Alstonia boonei*; B: root of *Calliandra haematocephala*; C: stem of *Enantia chlorantha*; D: stem of *Mangifera indica*; E: stem of *Okoubaka aubrevillei;* F: root of *Parquetina nigrescens;* G: stem of *Pterocarpus osun;* and H: root of *Sarcocephallus latifolius.* 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.

2.2. Tissue sectioning

Transverse sections of 15-20 micrometers thick were obtained from softened barks using hand-held microtome and the sections were transferred into FAA in labelled small specimen bottles for further treatment later. Smaller wood blocks of about $1cm^3$ each were cut across the circumference of the softened wood discs. From each block of wood, transverse, radial longitudinal and tangential longitudinal

Table 1: A list of the plant parts used for the preparation of Maloff-HB, a powdered anti malarial herbal formulation in Ogbomoso which were collected for anatomical evaluation

	Species name	Family name	Yoruba name	Parts used for the herbal formulation*	Parts evaluated
1	*Alstonia boonei* De Wild	Apocynaceae	*Ahun*	Stem bark	Bark
2	*Calliandra haematocephala* Hassk.	Fabaceae	*Tude*	Root	Bark and wood
3	*Cassytha filiformis* L.	Lauraceae	*Omonigelegele*	Vines	NE
4	*Enantia chlorantha* Oliv	Annonaceae	*Dokita igbo*	Stem bark	Bark
5	*Mangifera indica* L.	Anacardiaceae	*Mangoro*	Stem bark	Bark
6	*Okoubaka aubreviilei* Phelleg et Nomand	Santalaceae	*igi nla,*	Stem bark	Bark
7	*Parquetina nigerscens* (Afz.) Bullock	Periplocaceae	*Ogbo,*	Root bark	Bark and wood
8	*Pterocarpus osun* Craib.	Papilionaceae	*igi Osun,*	Stem bark	Bark
9	*Sarcocephalus latifolius* (J.E.Smith) E. A.Bruce	Rubiaceae	*Egbesi*	Root bark	Bark and wood

Source: Ogunkunle et al.[13], *NE*, not evaluated

Table 2: Some descriptive features in the wood bark of eight medicinal herbs studied

		ALBO	***CAHA***	***ENAC***	***MAIN***	***OKAU***	***PANI***	***PTOS***	***SALA***
	• A. Outer Bark								
Cork Cambium		-	-	+	-	-	-	-	-
	• B. Inner Bark								
Secondary Cortex (Pheloderm)		+	-	+	-	-	+	-	-
Secondary Phloem (Bast)	Fibers	+**	+**	+**	+**	+**	+**	+**	+**
	Axial parenchyma	+**	+**	+**	+**	+*	+**	+**	+**
	Sieve tubes	+**	+***	+*	+**	+*	+**	+**	+*
	Rays	+** (RPC)	-	+** (RPC)	-	-	+** (SQC;RPC)	-	+** (RPC)
	Sclereids	Brachy**; Macro*	-	Macro	Brachy*; Macro**	Brachy***; Macro**	-	Macro**	-
	Resin ducts	-	-	-	+	+	-	+	-

ALBO= Alstonia boonei;CAHA= Calliandra haematocephala;ENAC =Enantia chlorantha; MAIN = Mangifera indica; *OKAU* = Okoubaka aubrevellei; *PANI = Parquetina nigrescens*; *PTOS =Pterocarpus osun*; *SALA = Sarcocephalus latifolius*;; ***(frequent/averagelyobserved/high frequency i.e. 40-59% occurrence); **(less frequent/sometimesobserved/low frequency i.e. 10-39% occurrence); *(seldom frequent/rarelyobserved/very low frequency i.e. 1-9% occurrence); RPC = radially- procumbent cells; LPC = laterally-procumbent cells; SQC = square cells; +, Present; -, Absent/not observed.

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

	ALBO	***CAHA***	***ENAC***	***MAIN***	***OKAU***	***PANI***	***PTOS***	***SALA***
	• A. Cork Layer							
Number of rows	14c ±1.99	8ab ± 0.63	5a ± 0.16	22d ± 2.24	11bc ± 0.52	5a ± 0.13	6a ± 0.44	6a± 0.51
Thickness of layer (μm)	286.72c ± 46.15	115.76ab± 10.61	102.40 a ±3.41	399.36d ± 41.64	266.24b ± 16.44	119.81ab± 4.05	128. 00ab ± 6.70	121.78ab ± 9.82
Density of cells/mm^2	301ab ± 15.83	256 a ± 7.05	687 c ± 26.47	708c ± 37.80	521b ± 8.23	355b ± 7.69	357b ±13.44	348b ± 18.46
Cell width (μm)*	31.23b± 2.11	21.76ab ± 2.32	15.87a ± 1.98	13.82a ±2.01	33.79b± 3.12	26.37ab ± 3.22	30.21b± 3.06	48.89c ± 3.89
	• B. Cork Cambium Layer							
Number of rows	-	-	4 ± 0.38	-	-	-	-	-
Thickness of layer (μm)	-	-	39.94± 3.87	-	-	-	-	-

ALBO = Alstonia boonei; *CAHA = Calliandra haematocephala*; *ENAC = Enantia chlorantha*; *MAIN = Mangifera indica;OKAU = Okoubaka aubrevellei;PANI = Parquetina nigrescens*; PTOS = *Pterocarpus osun; SALA = Sarcocephalus latifolius;*; -, not applicable; *Cellwidth = diameter of cork cell at the widest point. The mean values of data in arow with the same alphabets are not significantly different (p>=0.05)while those with different alphabets are significantly different(p<0.001).

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

	ALBO	***CAHA***	***ENAC***	***MAIN***	***OKAU***	***PANI***	***PTOS***	***SALA***
	• A. Secondary Cortex (Phelloderm)							
Number of layers	11b ± 0.88	-	5a ± 0.21	-	-	13c ± 0.45	-	-
Thickness (μm)	422.91b ± 25.09	-	154.62 a ±3.88	-	-	514.05c ± 6.07	-	-
	• B. Secondary Phloem (Bast)							
Fibres (%)	32.26c ±2.66	27.20c ±3.01	32.99c ± 3.11	37.18cd ±4.09	12.15a ±2.55	17.16b ± 2.43	38.83d ±4.33	32.55c ±4.78
Axial parenchyma (%)	20.55b ± 3.22	29.47bc ± 2.98	24.44b ± 2.99	25.88b ±3.22	6.41 a ± 0.54	23.30bc ± 3.11	27.28b ± 4.23	36.74c ± 4.89
Sieve tubes (%)	10.56 ab ±2.87	43.33d ±3.89	7.27ab ±1.21	14.25c ± 2.12	6.10 a ±2.03	38.03d ±4.47	11.55bc ±2.89	7.37ab ±1.98
Rays (%)	20.90a ± 4.20	-	32.99ab ± 4.97	-	-	21.51a ± 4.12	-	23.34ab ± 4.99
Sclereids (%)	15.73b ± 4.28	-	2.31 a ± 0.98	22.69b ± 5.22	75.34c ±11.46	-	22.33b ± 4.87	-

ALBO = Alstonia boonei;CAHA = Calliandra haematocephala;ENAC = Enantia chlorantha;MAIN = Mangifera indica; *OKAU = Okoubaka aubrevellei;PANI = Parquetina nigrescens; PTOS =Pterocarpus osun; SALA = Sarcocephalus latifolius;* (%) = percent composition or mean relative abundance of tissues; - = not applicable. The mean values of data ina row with the same alphabets are not significantly different (p>=0.05)while those with different alphabets are significantly different(p<0.001).

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

	Parameters	***CAHA***	***PANI***	***SALA***
	• A. Vessels			
1	Pore type	Diffuse-porous	Ring-porous	Diffuse-porous
2	Shape in TS	Round***; Oval***	Round***; Oval***	Round***; Oval***
3	Occurrence	Solitary**; Radial chains of 2-7****	Solitary***; Radial chains of 2-3***	Solitary
4	Pore size	Relatively narrow	Relatively wide	Relatively wide
5	Wall thickening pattern	Pitted	Pitted	Reticulate
6	Frequency/relative abundance	Very low*	High***	Low**
7	Vessel members	Fairly long	Fairly long	Short
8	End walls	Oblique***; Truncate****	Oblique**; Truncate****	Oblique***; Truncate***
9	Tylose	Absent	Present*	Present*
	• B. Fibers			
10	Occurrence	Aggregates and diffuse; Non-storied	Diffuse; Non-storied	Aggregate s; Non-storied
11	Frequency/relative abundance	high***	Low**	Low**
12	Morphology	Short	Short	Fairly-long
13	Lumen and tip	Non-septate; pointed	Non-septate; pointed	Non-septate; pointed & blunt

CAHA= Calliandra haematocephala; PANI= Parquetina nigrescens; *SALA= Sarcocephallus latifolius;*****(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	***CAHA***	***PANI***	***SALA***
	• A. Parenchyma Cells (PC)			
1	PC type (in TS)	Apotracheal (diffuse-aggregate)	Apotracheal (diffuse-aggregate)	Apotracheal (diffuse)
2	Frequency/relative abundance of PC	High***	Low**	Very low*
	• B. Rays (RY)			
3	RY cells (in TS)	Square**; procumbent****	Square***; procumbent***	Square**; Procumbent****
4	RY width (in TLS)	Uniseriate****; biseriate**; multiseriate*	Uniseriate****; biseriate**; multiseriate*	Uniseriate***; biseriate**; multiseriate**
5	RY composition (in TLS)	Homocellular****; heterocellular*	Homocellular****; heterocellular**	Heterocellular
6	RY general shape (in TLS)	Mono-convex**; bi-convex****; linear**	Mono-convex**; linear****; dumb-bell**	Bi-convex****; dumb-bell*

CAHA= Calliandra haematocephala; PANI= Parquetina nigrescens; *SALA= Sarcocephallus latifolius;* ****(very frequent/usually observed i.e. 60-99% occurrence);***(frequent/averagely observed/high frequency i.e. 40-59% occurrence); **(lessfrequent/sometimes observed/low frequency i.e. 10-39% occurrence); *(seldomfrequent/rarely observed/very low frequency i.e. 1-9% occurrence).

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

	Parameters	***CAHA***	***PANI***	***SALA***
	• A. Vessels (VS)			
1.	Density/mm2	74c ± 2.73	10b ± 0.72	5a± 0.22
2.	Relative abundance/ frequency(%)	12.1	42.6	52.3
3.	% frequency of VS shapes in TS	Round(54); Oval(46)	Round(58); Oval(42)	Round(47); Oval(53)
4.	Frequency of tylose (%)	NA	7.0	7.0
5.	Diameter (μm)	63.81a ± 1.85	231.94c± 11.77	197.03b± 10.01
6.	Lumen width (μm)	53.25 a ± 1.76	208.13c ± 11.45	181.47b± 5.48
7.	Wall thickness (μm)	5.84a ± 0.39	11.90c ± 0.51	7.47b ± 0.32
8.	Length of VS member (μm)	605.01b± 39.40	804.52c ± 52.86	499.78a ± 24.26
	B. Fibers (FB)			
9	Density/mm^2	270c ± 23.11	63a ± 5.05	120b ± 6.03
10	Relative abundance/ frequency(%)	44.19	12.91	34.68
11	Diameter (μm)	14.59a ± 0.81	34.05b ± 0.92	31.57b ± 1.15
12	Lumen width (μm)	9.64a ± 0.67	26.03b ± 0.95	25.17b ± 0.89
13	Wall thickness (μm)	2.56a ± 0.14	3.97c ± 0.22	3.15b ± 0.21
14	FB length (μm)	856. 06b ± 40.63	604.50a ± 14.38	1091.58c ± 67.40

CAHA= Calliandra haematocephala;PANI= Parquetina nigrescens; SALA= Sarcocephallus latifolius. Themean values of data in a row with the same alphabets. are not significantlydifferent (p>=0.05) while those with different alphabets are significantlydifferent (p<0.001).

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

	Parameters	***CAHA***	***PANI***	***SALA***
	• A. Parenchyma Cells			
1.	Density/mm2 in TS	256c ± 19.64	197b ± 11.14	32a ± 1.57
2.	Relative abundance/ freq. (%)	41.89	40.37	9.25
	B. Rays (RY)			
3	Density/mm^2 in TLS	11a ± 0.43	20c ± 0.70	13b ± 0.37
4	Relative abundance/ freq (%)	1.80	4.10	3.76
5	Number of cells in RY width (TLS)	2a ± 0.09	2a ± 0.10	2a ± 0.14
6	RY thickness in TLS (μm)	29.02a ± 1.78	31.40a ± 1.55	55.30b ± 2.93
7	Number of cells in RY height (TLS)	13a ± 0.89	13a ± 1.63	25b ± 2.02
8	RY height in TLS (μm)	308.57a ± 19.93	435.20a ± 49.82	1022.50b ± 73.98

CAHA= Calliandra haematocephala;PANI= Parquetina nigrescens; SALA= Sarcocephallus latifolius. Themean values of data in a row with the same alphabets. are not significantlydifferent (p>=0.05) while those with different alphabets aresignificantly different (p<0.001).

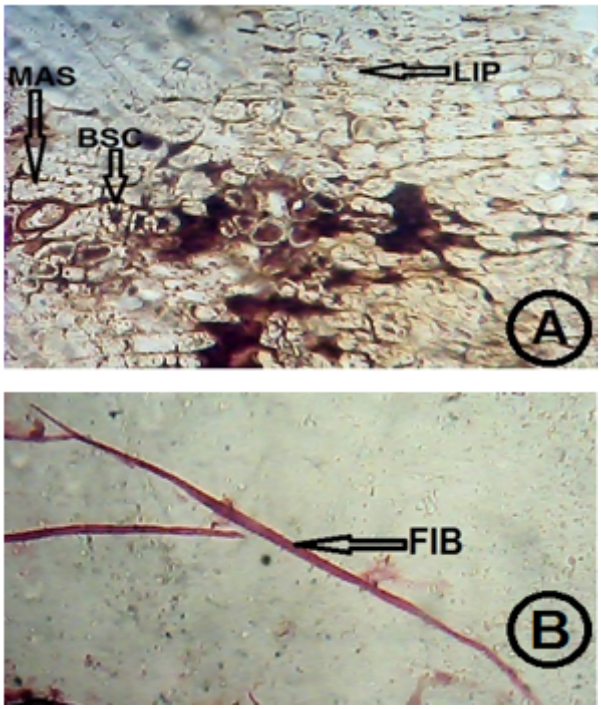


Fig. 2: Morphology of some types of cells (100×) in theinner barks of the stems of A: *Okoubaca aubreivellei* and B: *Enantia chlorantha.* BSC, brachysclereids; FIB,fiber; LIP, lignified parenchyma; MAS, macrosclereid

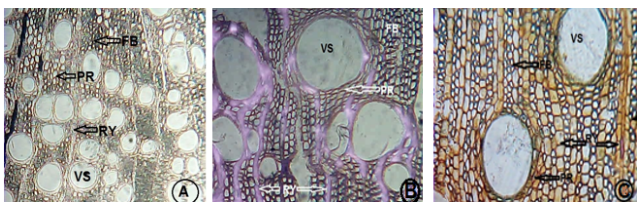


Fig. 3: Morphology and arrangement of cells in the wood TS (100×) of A: *Calliandra haematocephala;* B: *Parquetina nigrescens* and C: *Sarcocephallus latifolius.* FB, fibers; PR, axial parenchyma; RY, ray; VS, vessel.

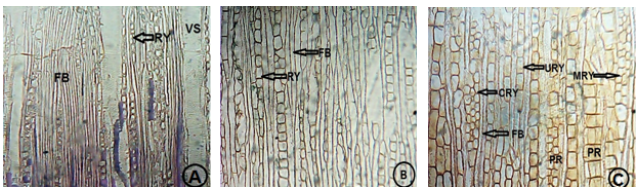


Fig. 4: Morphologyand arrangement of cells in the wood TLS (100×) of A: *Calliandra haematocephala;* B: *Parquetina nigrescens* and C: *Sarcocephallus latifolius.* FB, fibres; PR, *axial parenchyma;* RY, ray; VS, vessel; CRY, constricted ray; MRY, multiseriate ray; URY,uniseriate ray.

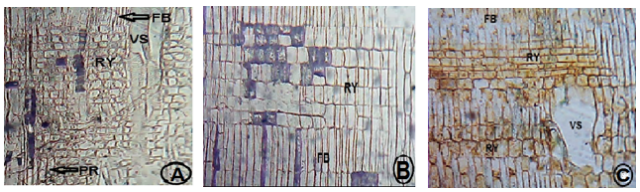


Fig. 5: Morphologyand arrangement of cells in the wood RLS (100×) of A: *Calliandra haematocephala;* B: *Parquetina nigrescens* and C: *Sarcocephallus latifolius.* FB, fibers; PR, *axial parenchyma;* RY, ray; VS, vessel.

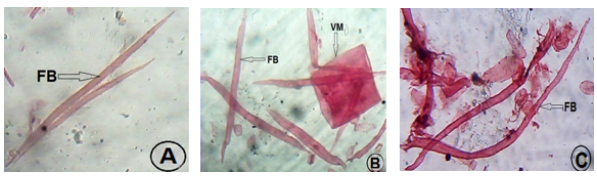


Fig. 6: Morphologyof isolated wood fibers (100×) of A: *Calliandra haematocephala;* B: *Parquetina nigrescens* and C: *Sarcocephallus latifolius.* FB, fibers; VM, vesselmember.

sections (TS, RLS and TLS) of similar thicknesses were cut.

2.3. Treatment of sections of barks and wood for microscopic observation

Each thin section of barks and woods were rinsed clean of FAA in several changes of water on a microscope slide. Sections of root and stem barks were stained in 1% ethanolic safranin for 10 minutes. Rinsing was done with water until no excess stain bled out of the sections. This was followed by counter-staining with fast green, and another round of destaining with water. The sections were then dehydrated in 30%, 50%, 70%, 90% and absolute ethanol for 2 minutes each. Staining of wood sections was also done with 1% ethanolic safranin for 10 minutes before de-staining, counter-staining and dehydration as earlier described. After dehydration, each tissue section, bark or wood was cleared in pure xylene for 20 minutes[22] and mounting was done in few drops of Canada balsam.

2.4. Tissue maceration

Wood tissue maceration was carried out using a modified form of Jeffrey's method i.e. 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.[23] Maceration of the barks was done with cold treatments i.e addition of concentrated nitric acid and crystals of potassium chlorate without application of heat. In doing this, the scaly part or rhytidome was first peeled off by means of a knife, leaving only the secondary phloem part as the predominant tissue for subsequent treatments. The tissues were left to stand in this solvent overnight (i.e. between 10 and 12 hours) to soften.[24] For both the woods and the barks, softened tissue in concentrated nitric acid was rinsed in several changes of water and transferred 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.[25] Wood and bark macerates were stained with safranin for 10 minutes and temporary mounting was carried out in a few drops of glycerin.

2.5. Microscopic examination and data collection

Four prepared slides from each of the TS and macerated barks as well as the TS, TLS, RLS and macerated wood tissue were examined using an Olympus binocular microscope CH20i Model at 100X and 400X magnifications.[26] In the TS of the barks, 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.[27] 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 accordance with the descriptions of the international association of wood anatomists[28], and in drawings or photographs by means of Bresser microscope equipped with Photomizer SE Microcular camera attachment, 051012-VGA Model connected to a laptop computer.

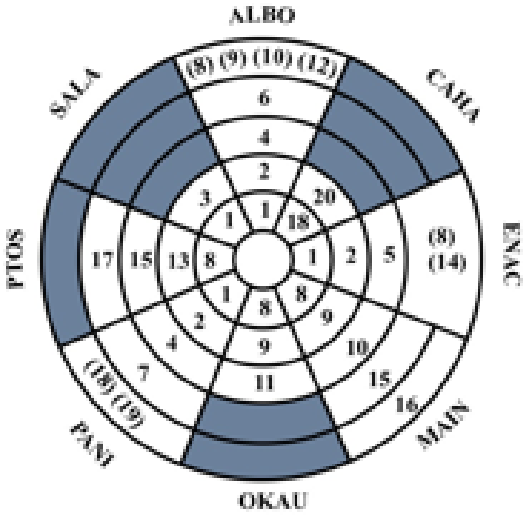
Some cell and tissue dimensions in wood sections such as height and width of rays in TLS, diameter and lumen width of vessels in TS, fiber length and width in macerated samples etc. were determined in micrometers using a calibrated eye piece micrometer accessory inserted in the ocular tube.[29] Making 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 summing up the recorded mean values of their densities in a square millimeter area and computing the percentage of each in relation to the total.

Frequency of vessels with tylose was obtained from wood TS by randomly viewing 50 fields of microscope and noting the occurrence of vessel tylose in each with a tally. The frequency was then 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 (i.e. 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. Thereafter, the number of views in which each shape was present was calculated as a percentage of the total of presence for the two.

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 earlier explained.[30] Twenty-one bark anatomical characters, consisting of nine qualitative and 12 quantitative (in replicates of 4 for percent tissue composition and of 10 for others), and forty-one wood anatomical characters, consisting of 19 qualitative and 22 quantitative (in replicates of 30) were compiled, making a total of 62.

2.6. Statistical analysis

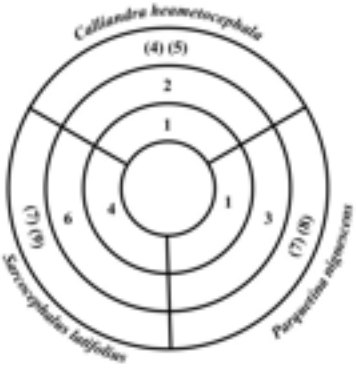
Using the version 23.0 of the computer-based SPSS statistical package, the replicated values of each of the 12 quantitative parameters drawn from the barks were subjected to one-way analysis of variance (ANOVA) across the eight medicinal herbs studied. The replicated values of those 22 wood parameters in respect of three of



List of Characters/Character Combinations

1. Rays, present in the inner bark; phellodeem or secondary cortex may also be observable
2. Phellodeem or secondary cortex, present, with varying thickness; mean percent axial parenchyma by volume, less than 25
3. Rays, multiseriate (5-6 cells in width), with radially procumbent cells; mean percent axial parenchyma up to 36 by volume; phellodeem not observable
4. Rays may be uniseriate or multiseriate; mean thickness of phellodeem above 500µm; mean density of cork cells, less than 400/mm^2
5. Cork cambium, present; rays, biseriate, with radially procumbent cells; mean thickness of phellodeem, less than 200 mm^2; mean density of cork cells, about 700/mm^2.
6. Rays, multiseriate, with radially procumbent cells; mean percent fiber by volume, up to 32;and of sieve tubes, about 10%.
7. Rays, uniseriate, with more or less square cells; mean percent fiber by volume, about 17; and of sieve tubes, up to 38%.
8. Sclereids, present in the inner bark.
9. Two types of sclereids, i.e. macro and brachy-sclereids are present
10. Mean percent sclereids by volume, never up to 25; mean percent axial parenchyma by volume, up to 20 or more.
11. Resin ducts, present; mean percent sclereids and axial parenchyma by volume , more than 70 and about 6 respectively.
12. Rays are present, multiseriate (3-7 cells in width), consisting predominantly of square cells, and sometimes of tangentially elongated cells; phellodeem, present; mean density of cork cells, less than 400/mm^2; sieve tubes occurring in solitary units and in pairs.
13. Only one type of sclereid, i.e. macro-sclereids are observable in the inner bark.
14. Sclereids, present in the inner bark; cork cambium, present; phellodeem or secondary cortex also present, with mean thickness of less than 200 µm; mean percent sclereids, about 2; and of cork cells, about 700.
15. Resin ducts, present; mean percent sclereids by volume, about 22; those of fibers, axial parenchyma and sieve tubes, about 38, 21 and 14 respectively.
16. Two types of sclereids, i.e. macro and brachy-sclereids are present; mean density of cork cells, about 700/mm^2.
17. Only one type of sclereid, i.e. macro-sclereids are observable in the inner bark; mean density of cork cells, less than 400/mm^2.
18. Sieve tubes, abundant/copious, usually in large groups or irregular arrangement.
19. Phellodeem/ secondary cortex, present; rays, observable in the inner bark; axial parenchyma, in solitary units and groups of 2 to 6 cells; sieve tubes, abundant/copious; irregular in arrangement.
20.Axial parenchyma, scanty; occurring in small groups; sieve tubes, abundant/copious; occurring in large proups; phellodeem and rays, not observable.

Fig. 7: Bark-anatomy-based circular diagnostic chart for identifying eight medicinal herbs used for Maloff-HB, an antimalarial powdered herbal formulation in Ogbomoso, Nigeria. Characters in parentheses are regarded as being of secondary importance i.e. although theyare, or may be diagnostic of a taxon or a cluster of taxa, such characters need not be observable for taxa recognition to occur.



List of characters/character combinations

1.Vessels occur in TS as both solitary units and in radial chains; secondary wall thickenings of the vessels, pitted; both homocellular and heterocellular rays, found in TLS; axial parenchyma, apotracheal of diffuse aggregate type; mean density of axial parenchyma, 195/mm^2 or more

2.Rays are of mono-convex, bi-convex and linear shapes in TLS; mean density of vessels in TS, above 70/mm^2; those of fibers and axial parenchyma are about 270 and 260/mm^2 respectively; vessels are narrow, with mean diameter of less than 70µm; mean length of vessel members and fibers, about 600 µm and 850 µm respectively

3. Rays are of mono-convex, dumb-bell and linear shapes in TLS; mean density of vessels in TS, about 10/mm^2; those of fibers and axial parenchyma are about 65 and 195/mm^2 respectively; vessels are wide, with mean diameter of about 230µm; vessel members, long, with mean length being about 800 µm;

4.Bi-convex ray, present in wood TLS; mean density of fibers, 120/mm^2 or more; mean length of vessel members, less than 650 µm; and of fibers, 850 µm or more

5. Vessels occur in TS as both solitary units and in radial chains of 2-7; secondary wall thickenings of the vessels, pitted; rays are of homocellular and heterocellular types in TLS, the shapes being mono-convex, bi-convex and linear types; axial parenchyma, apotracheal of diffuse aggregate type;

6.Vessels occur in TS only as solitary units; secondary wall thickenings of the vessels, reticulate; axial parenchyma, apotracheal of diffuse type; rays, only heterocellular types, the shapes being bi-convex and dumb-bell/or constricted;

7. Constricted or dumb-bell shaped rays, present; mean density of vessels, not up to 15/mm^2; mean vessel diameter, about 200 µm or more;

8. Vessels occur in TS both as solitary units and in radial chains of 2-3; rays, both homocellular and heterocellular types, the shapes being dumb-bell(or constricted) and linear;

9. Vessels occur in TS only as solitary units; rays, all heterocellular typein TLS, the shapes being bi-convex and dumb-bell (or constricted); mean density of vessels, 5/mm^2, those of fibers, axial parenchyma cells and rays being 120/ mm^2, 32/ mm^2 and 13/ mm^2 respectively.

Fig. 8: Wood-anatomy-based circular diagnostic chart for identifying three of the medicinal herbs used for Maloff-HB, anantimalarial 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.

these medicinal herbs were equally subjected to one way ANOVA, while the means in both cases were separated using multiple Duncan range test at α= 0.05.[31]

3. Results

The results of the study are shown in Figure 1-8, and Tables 2-8

4. Discussion

4.1. Ethnopharmacological applications of the plant materials studied

Barks and wood of trees are very complex in structure and have the potential of storing many primary and secondary metabolites, some of which are useful in the drug industry.[32] For this reason, these items fall in the category of crude drugs, whose complex structure is also useful for their botanical identification to ensure their quality and purity.[33]Table 1 gives a highlight of the herbal materials (barks and/or woods) of the eight species studied as components of Maloff-HB, a traditional oral powdered drug for malaria fever in Nigeria. Researchers in other parts of the world have also listed some of these items as portions of herbal remedies for a wide range of health conditions: e.g. *Mangifera indica.*[34,35], and *Sarcocephalus latifolius*.[36] While stem and root barks are frequently sold and used directly as medicinal herbs, wood is seldom so used unless as chewing sticks.[37,38] More commonly however, wood is medicinally used as whole roots or stem twigs i.e. in combination with its bark.[39] This account is not only confirmatory of wide application of the plant species studied as medicinal herbs, it is also a pointer to the necessity to ensure their identity and purity. The qualitative and quantitative bark and wood anatomical data obtained from this study are potentially useful to establish specific and higher taxonomic categories among these herbal materials. At the least, the barks of the species studied on the one hand, and their woods on the other hand can easily be distinguished from their adulterants.

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

The eight plant species studied can be separated using their bark anatomy characteristics. Four of the species namely, *Alstonia boonei*, *Enantia chlorantha, Parquetina nigrescens* and *Sarcocephalus latifolius* have rays in their inner barks, while rays are not observable in the other four species. The first three of the four species mentioned here have secondary cortex /phelloderm in their inner barks but the fourth, i.e. *S. latifolius* lacks the tissue. The three species are however, distinguishable in that the number of layers and thickness of phelloderm in the three showed significant variation at p< 0.001(Table 4) Moreover, *E. chlorantha* is the only species among those studied in which cork cambium was visible; *P. nigrescens* lacks sclereids/stone cells, while *A. boonei* and *E. chlorantha*, both of which have sclereids in them differ in that the former possesses the brachy- and *macro-sclereids,* with significantly higher percentage of these strengthening cells, but the latter, only *macro-sclereids*, with significantly lower percentage by volume at p <0.001(Table 4)The other four species examined, which lack the rays, also do not possess phelloderm; the species are *Calliandra haematocephala, Mangifera indica, Okoubaka aubrevelle*i and Pterocarpus osun. The last three of these species all have sclereids and resin ducts but the first, i.e. *C. haematocephala* does not. These three species can however be distinguished by the fact that only macrosclereids are observable in *P. osun,* while both brachy- and macro-sclereids are found in *M. indica* and *O. aubrevellei.* Furthermore, these two species can be separated on account of their significantly wide variation (at p <0.001) in relative percentage composition by volume of fibers, axial parenchyma, sieve tubes and sclereids (Table 4).

Ohemu et al.[40] conducted organoleptic, macroscopic and microscopic evaluation of stem bark of *E. chlorantha.* Their observations on powdered bark revealed the presence of brachy-sclereids, cork cells, bundles of phloem fibers, polygon-shaped parenchyma cells and numerous prism-shaped calcium oxalate crystals. The results of the present study are in part, similar to those of Ohemu et al.[40] in terms of presence of sclereids in the bark of *E. chlorantha.* However, only macro-sclereids were those observed, and no crystals were found. Alam and Saqib[41] carried out a microscopic examination of powdered bark of *Gaultheria trichophylla* to reveal 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 barks and generated 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.

Babu et al.[33] examined the macroscopic, microscopic and HPTLC profiles of the barks of four species of *Ficus* sold as medicinal herbs in Indian markets. Similar to the exercise carried out in this 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 namely, *F. racemosa, F.*

virens, F. religiosa and *F. benghalensis.* Kumar et al.[43] also evaluated the pharmacognostic characters of the root bark of *Holoptelea integrifolia,* an important medicinal plant in India. They, not only found the qualitative and quantitative anatomical features of the bark distinctive enough to identify and decide the authenticity of this drug, but in addition, recommended the inclusion of these diagnostic features as microscopic standards in Indian herbal pharmacopeia.

The argument so far has pointed to two important facts. Firstly, that all the plant species examined for their bark anatomical characteristics in this study are well-known for their diverse medicinal value worldwide, and secondly, that the complex nature of stem/root bark anatomy lends itself to applications in pharmacognostic studies of herbal materials. While empirical data on bark anatomy have been successfully employed to diagnose a long list of medicinal plants (e.g.[8];[33];[42]; and[44]), available literature appears to be deficient in pharmacognostic studies of these medicinal plants from Ogbomoso Nigeria from the point of view of their bark anatomy. The dearth of information from this direction is yet another justification for this study. The results of bark anatomy obtained in this study are diagnostic enough to establish the species identities of the eight 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.

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

The results of this study have clearly resolved the three medicinal plant species studied as follows: In *Sarcocephalus latifolius,* the vessels occur as only solitary units; rays in TLS are all heterocellular with bi-convex and constricted (i.e. dumb-bell) shapes. Additionally, no linear rays, while the wood fibers are significantly ($P<0.001$) longer (>1000μm) than in the other two species i.e. *Calliandra haematocephala* and *Parquentina nigrescens (*Table 7). In these two species however, vessels, apart from occurring in solitary units in TS, are also found in groups/radial chains; both homocellular and heterocellular rays occur in the TLS, whose general shapes include, but not limited to mono-convex and linear; and mean fiber length is significantly ($P<0.001$) shorter (<900μm). These two species can however, be diagnosed in that *C. haematocephala* has significantly narrower vessels (mean diameter of about 60μm), and less predominant (below 40%) in linear ray composition. These are against the mean vessel diameter of 200μm or more (Table 7)and more predominant (>60%) linear rays (Table 8) in *P. nigrescens.* In addition, there are bi-convex shaped rays, but no vessel tylose in *C. haematocephala*, while the reverse is the case in *P. nigrescens.*

Pandey[45] stated that among others, qualitative and quantitative features of wood vessels, parenchyma, rays and fibers are reliable diagnostic and phylogenetic indices. The findings from the present study are in consonance with Pandey's 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.[46] In the eight woody species of *Hypericum* studied by Peronne et al.[47], the number and mean diameter of vessels showed interspecific differences. So also the difficulty posed by the identification of *Cola acuminata* and *C. nitida* when not in fruit was reported by Jensen et al.[48] to be resolvable using wood anatomical features. In particular, wood fiber composition, types and amount of crystals have proved very strongly of diagnostic value in the classification and delimitation in the *Cola* species studied. Some of the features reported by these authors to be useful diagnostic markers were also found to be so useful in the current study.

Marques et al.[49] reported homogeneous wood anatomical features in the genus *Psychotria* but the statistical analyses based on qualitative and quantitative features allowed the separation of the nine species they studied as distinct taxa. According to these authors, the woods of *Psychotria* were characterized by slightly distinct growth rings, diffuse porosity, solitary vessels or in radial multiples of 2-6 or clusters of 3-5 vessel elements with terminal and lateral simple perforation plates, septate fiber-tracheids and rare axial parenchyma. In the present study, similar observations were made on the woods of the medicinal plants examined, with a number of the features found to be diagnostic after showing significant statistical differences.

So far, two main facts can be pointed out from the three medicinal plant species examined for their wood anatomical charactersistics in this study. The first is that these species are well-known for their diverse medicinal value, and secondly, that the complex nature of wood anatomy can be reliably used in pharmacognostic studies of these herbal materials. While empirical data on wood anatomy have been successfully used to diagnose many medicinal and non-medicinal plants (e.g.[47–50]), available literature seems to be poor in pharmacognostic studies of these three medicinal plants from Ogbomoso Nigeria from the point of view of their wood anatomy. By and large, the results of this study are confirmatory of the view that qualitative and quantitative characteristics of wood anatomy are reliable in pharmacognostic evaluation of medicinal plants. 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 identify any of the medicinal herbs studied , the user enters each of the keys in Figure 7 and 8 at the center and proceeds centrifugally (toward the circumference) by selecting the characters applicable to the unknown specimen at the successive rings of compartments. This process progressively narrows down on the choice of the possible identities (i.e. sectors) of the unknown specimen until only one choice is achieved, which represents its identity. These keys, which follow the single- access format[51] can also be used to authenticate any of the medicinal herbs suspected or supplied under a given name. As an illustration, if a user in applying the key in Figure 7 suspects the identity of a plant to be *Enantia chlorantha,* confirmation is done by evaluating the specimen based on characters 1, 2, and 5, and if desirable, 8 and 14 in addition. Thus, if given a key, and the assurance that a suspected taxon is included in that key, the first step 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. Going by the easy mode of navigation, and the possibility for confirmation of suspected identity, it is clear that the functionality attributes of the keys generated from this study outweigh those of the dichotomous key, the most widely used tool for plant identification.[52]

5. Conclusion

The salient bark anatomical characteristics which were diagnostic of the eight plant species studied 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 notable wood anatomical features that are clearly diagnostic of the three species studied were in the TS (namely: occurrence and diameter of vessels, and abundance of wood parenchyma); and the TLS (namely: cellular composition, width, height, thickness and morphology of wood rays). Discontinuities in qualitative and quantitative features of the bark and wood anatomical observations yielded two taxonomic keys whose clarity, simplicity and diagnostic value are evident in avoiding misidentification of these medicinal herbs.

6. Source of Funding

None.

7. Conflict of Interest

None.

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