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Evaluation of fibre dimension and morphology of five Species of Malvaceae for Pulp and Paper Production

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ABSTRACT

Wood is a desirable construction material because the energy requirements of wood for producing a usable end-product are much lower than those of competitive materials, such as steel, concrete or plastic. The aim of this research was to evaluate the fibre dimension and morphology of individual plants species of Cola acuminata, C. nitida, Cochorus olitorius, Hibiscus cannabinus and Sida acuta. Standard analytical methods were applied in the evaluation of fibre dimension and morphology of individual plants. The results indicated that the fibre morphology assay showed significant differences (P < 0.05) among all the fibre characteristics of the woods. High variation was observed in the Runkle ratio (RR) among the five species ranging from 0.33 - 0.66 µm. The highest RR of $0.66 \pm 0.05 \mu m$ was recorded in C. nitida while the lowest RR value of $0.33 \pm$ 0.04µmwas observed in C. olitorius. Slenderness ratio (SR) was highest in C. acuminata with an average of $66.95 \pm 3.20 \mu m$ followed by that of *C. nitida* $(63.17 \pm 4.27 \mu m)$ and *H.* cannabinus (64.20 \pm 0.41 μ m). These values were however not significantly different from one another. Among the five species, flexibility coefficient (FC)ranged from 33.72 \pm 4.69 μ mto 68.79 \pm 4.25 μ m in the order of *C. olitorius > H. cannabinus > S. acuta > C.* acuminata > C. nitida. The results of this present investigation have shown that the wood of all the species would be suitable for paper making based on their fibre characteristics. Working with the principles that fibres with the lowest Runkel ratio and highest coefficient of flexibility make the strongest papers, the order of paper quality of the species studied is: *C. olitorius > H. cannabinus > S. acuta > C. acuminata > C. nitida*. Keywords: Fibre, dimension, morphology, plants species.

INTRODUCTION

Wood is an organic material, a natural composite of cellulose fibers which are strong in tension embedded in a matrix of lignin which resists compression [1]. Wood is also seen as a renewable resource with an exceptional strength to weight ratio. It is sometimes defined as only the secondary xylem in the stems Wood desirable trees. is material construction because energy requirements of wood producing a usable end-product are much lower than those of competitive materials, such as steel, concrete or plastic [2]. Wood differs from other construction materials because it is produced in a living tree [3]. Trees are categorized into two major classes which are hardwoods and softwoods. Hardwoods which are deciduous normally have broad leaves that are shed at the end of each growing season.

Softwoods have needle like leaves that normally remain green year [4]. The classification as hardwood or softwood has nothing or little to do with the comparative hardness of the wood, for instance, several species of softwoods are harder than many low to medium density hardwoods [5]. Looking at wood as a structural material, this can be considered at two levels: microstructure which can be examined only with the aid of a microscope, and macrostructure. which is normally visible to an unaided eve [6]. The primary structural building blocks of wood are the wood cells consisting of tracheid and/or fiber cells. When closely packed, these wood cells form a strong composite system that is compared to a bundle of drinking straws. Each individual cell has four distinct cell wall layers (primary, S₁, S₂,

[12].

strong

bundles

based

These

p-coumaryl

intermolecular

and S₂). Each layer is composed of a combination three chemical of polymers: cellulose, hemicellulose, and lignin.The cellulose and hemicellulose are linear polysaccharides (hydrophilic multiple sugar) and the lignin is an phenolic adhesive [7]. amorphous forms Cellulose long unbranched chains, and hemicellulose forms short branched chains. Lignin encrusts and stiffens the polymers [8].

Wood fibers are natural composite structures in which cellulose fibrils are held together by lignin hemicellulose. The major constituents of wood fibers are lignin, cellulose, hemicellulose, and extractivescontents Each of these components contributes to fiber properties, which ultimately impact product properties. These chemical constituents are made up of the structural and non-structural substances. Structural substances are carbohydrates. majorly and Carbohydrates contents of the wood include the cellulose and hemicellulose [10]. Non-structural substances also known as extraneous materials are mostly low-molecular mass compounds which are organic extractives, some water soluble organic substances and inorganic minerals (ash) mainly calcium, potassium, magnesium, besides manganese and silica. In a nutshell. wood is essentially composed cellulose, hemicellulose, lignin, and extractives [11]. Cellulose had been reported to be the world's most

Aim of Study

The aim of this study is to evaluate fibre dimension and morphology of individual plants species studied (fibre

length, fibre diameter (FD), lumen width and cell wall thickness)

abundant and important biopolymer

homopolysaccharide consisting of B - D-

glucopyranose moieties linked together

by (1-4)-glycosidic bonds. Because of the

aggregate to microfibrils, which are

either highly ordered (crystalline) or

less ordered (armorphous). Microfibrils

are further aggregated to fibrils and

finally to cellulose fibers [13]. Beside cellulose, hemicelluloses are other

major naturally occurring carbohydrates

heteropolysaccharides and are clearly

less well defined than cellulose. The

building units of hemicelluloses are

hexoses, pentoses or deoxyhexoses.

Lignin is an armorphous polymer and

the chemical structure of lignin is

irregular in the sense that different

structural elements are not linked to

each other in any systematical order. In

general, lignin is roughly classified into

softwood lignin, hardwood lignin, and

grass lignin. Normal structural elements

of lignins are derived principally from

trans-conifervl alcohol (90%) with the

remaining consisting mainly of trans -

of trans- coniferyl alcohol and trans-

sinapyl alcohol in varying ratios (about

alcohol.

hardwood lignin is mainly

50%) for each alcohol [15,16].

exist

membered (pyranose) structures [14].

polymers,

units

tendency

of

It is a polydispersed linear

for

hvdrogen

cellulose

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which

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In

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composed

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MATERIALS AND METHODS

Source of Materials

The sampling of the five woody Malvaceae species (Cola acuminata, C. nitida. Cochorus olitorius, Hibiscus cannabinus and Sida acuta) was carried Nsukka, Nsukka in Local Government Area of Enugu State. Nsukka has an area of 1,810 km², on Latitude located 6.5124°N and Longitude 7.2345°E. Vegetation is characteristically derived savanna. Its

80%. Nsukka is located on an altitude of 400 m above sea level (m asl) and its climate is sub-humid tropical. The wet season extends from April to October, the dry season is from November to April and the annual rainfall ranges from 1,845 - 2000 mm. Annual temperature is between 25°C and 27°C [17,18].

relative humidity ranges between 70 and

Collection of Plant Samples

The five different species were randomly collected from their different natural habitat. The plants were thoroughly examined in their habitat and records were made on the field note book. Photographs of the samples were taken in the field with a Nikkon S500 digital camera. Proper identification and

authentication were carried out in the Herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka using [19] and other relevant literatures. The samples were carried in different plastic bags to the

Determination of lignin content

The determination of lignin content was carried out using a sample which had first been extracted with 1:2 (ethanol: toluene), in accordance with TAPPI standard method. Acid-insoluble lignin ("Klason Lignin")was determined byplacing 1 g of the ethanol- toluene extracted sample in 100 mL beaker, 15 ml of 72 % sulphuric acid was added gradually in small increments while stirring and macerating the sample with a glass rod. After the sample has dispersed, the beaker was covered with a watch glass and kept in a bath at about 20 °C for 2 h while stirring. At the end of the 2 h, the content was diluted to a total volume of 575 ml in a 1-liter flask

Accurately weighed 1 g of oven dried sample was placed in a 250 mL round bottom flask fitted with a reflux condenser. 15 mL of 80 % acetic acid and 1.5 mL of concentrated nitric acid (HNO₂) was added. The mixture was boiled for exactly 20 min. About 20 mL of 95 % cold ethanol was added. The resultant mixture was cooled

About 150 mL of Sodium Hydroxide (NaOH) solution (0.5 mol/L) was added to 1 g of extractive-free sample (sample was made extractive-free by washing with acetone at 90 °C). The temperature was kept at 80 °C using a hot plate for 3.5 h. After that, the sample was washed with deionised water until it was free

The method used for maceration in this study was that of Schulze's according to [20]. The bark of the air-dried stem discs was excised from the wood after which

the wood was cut into tiny bits of blocks measuring $0.1 - 0.3 \times 4$ cm. The tiny bits of wood blocks (woodchips) were then placed in a well labelled test tube to which 2 g of 5% potassium chlorate and 10 ml of nitric acids was added after which they were allowed to react in a fume cupboard until the lignin and the middle lamella of the chips dissolved. Since potassium chlorate is a strong

oxidizing agent, it accelerated instant

Plant Laboratory Anatomy in Department Plant of Science and Biotechnology, University of Nigeria, where they were dried, ground and sieved through the 200-mesh sieve (TAPPI- T 264 om-88).

and then boiled for 4 h at a constant volume by frequent addition of hot water. It was then left overnight. The insoluble material (lignin) was filtered filtrate and the kept for determination of acid-soluble lignin. The acid-insoluble lignin obtained was washed free of acid with hot water and dried to a constant weight at 105 °C in the oven. The experiment was done in triplicate. The acid-insoluble lignin was calculated as follows:

Klason lignin (%) = $Y/W \times 100$

Where:

Y = oven-dry weight of Klason lignin (g), W= oven-dry weight of initial specimen

Determination of cellulose content

filtered. The residue was then washed successively with hot benzene, hot alcohol, and diethyl ether. The residue was dried overnight to a constant weight and then ashed in a muffle furnace at about 500 °C for 5 h. The loss in weight upon ignition was taken as a measure of the cellulose content.

Determination of Hemicellulose Content

from Na+. The sample was dried in an oven at 105 - 110 °C until constant weight was obtained. The amount of hemicellulose was calculated as follows: Amount of Hemicellulose (g) = weight of extractive-free sample (g) - final weight of sample (g)

Wood Maceration

reaction with nitric acid (HNO) to effect the maceration while the presence of lignin was revealed by the reddishbrown color of the test tubes. A white tissue appeared at the base of the test tubes indicating that maceration had taken place. The residual acid was decanted from the test tubes into a reserve receptacle after which distilled water was added to the test tubes, the test tubes were then covered with caps or stoppers and shaken vigorously to allow the tissue dissolve and also stop further reactions; the test tubes were left in a standing position for 24 hours

and the supernatant was decanted thereafter. The macerated tissues were stored in micro bottles to which glycerine was added to remove air

Measurement of Wood Parameters

flexibility

light Olympus Tokyo (Japan No.271961) microscope to which an ocular diameter was fitted was used for the measurement of fibre length (FL), fibre diameter (FD), fibre diameter (FLD), fibre cell wall diameter (FCWD), vessel pore diameter (VPD), vessel length (VL). Ten random wood parameter measurements were taken for each plant sample. From the fibre measurements, the Runkle ratio (RR),

follows: Runkle 2w/l: ratio = coefficient = l/D x 100; slenderness ration = L/Dwhere, w = fibre wall thickness (µm) 1 = lumen diameter (µm) $D = fibre width (\mu m)$

 $L = fibre length (\mu m)$

Statistical Analyses

Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS version 20) was used to test significance (at $P \le 0.05$) of means of generated from the physicdata chemical and fibre morphological

variables, and Duncan's Multiple Range Test - DMRT (1955) was used for mean separation. Pearson's Correlation was conducted across the variables at P ≤ 0.05.

bubbles while formalin was also added

to prevent fungal growth or decay of the

tissue. Staining of the macerated tissues was done using 1% Safranin solution.

coefficient

slenderness ratio (SR) were calculated as

(FC)

and

flexibility

RESULTS

Comparative Fibre Morphology of the Wood of the Five Species of Malvaceae The result of the fibre morphology showed highly significant differences (P < 0.05) existed among all the fibre characteristics of the woods (Table 1). The fibre length (FL) ranged from 841.83 to $1610.97\mu m$ with H. cannabinus wood recording significantly the longest fibre while S. acuta had significantly the shortest fihres Whereas H. cannabinus also recorded significantly the widest fibre diameter (FD) with an average of $25.09 \pm 0.26 \mu m$ followed by 22.67 \pm 0.80 μm in C. olitorius, the FD of C. nitida, acuminata and S. acuta were the same smaller compared to and as Η. cannabinus.C. olitorius and cannabinus (Table 1). The fibre lumen diameter (FLD) in C. olitorius and H. cannabinus (15.73 \pm 1.55 μ m and 15.08 \pm 0.56 µm) were statistically similar and higher than the significantly observed in the other plant species. The results also showed that the Fiber Cell wall Diameter (FCWD) ranged from 3.73

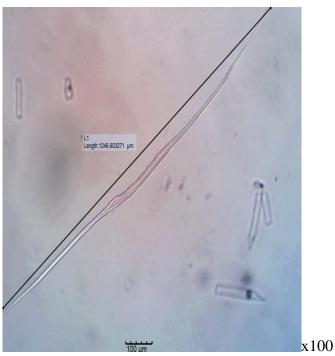
6.53 μ m, with *C. nitida* having significantly the highest value of 6.53 ± 0.26 µm and C. olitorius having the lowest value with an average of $3.73 \pm$ 0.32 μm (Table 1).

High variation was observed in the Runkle ratio (RR) among the five species ranging from 0.33 - 0.66μm. The highest RR of $0.66 \pm 0.05 \mu m$ was recorded in C. nitida while the lowest RR value of 0.33 ± 0.04µmwas observed in *C. olitorius*. Slenderness ratio(SR) was highest in C. acuminata with an average of 66.95 ± 3.20µm followed by that of *C. nitida* $(63.17 \pm 4.27 \mu m)$ and *H. cannabinus* $(64.20 \pm 0.41 \mu m)$. These values were however not significantly different from one another. Among the five species, flexibility coefficient (FC)ranged from $33.72 \pm 4.69 \mu \text{mto } 68.79 \pm 4.25 \mu \text{m}$ in the order of C. olitorius > H. cannabinus > S. acuta > C. acuminata > C. nitida (Table 1). Fibre morphology of the five wood species were assessed and photomicrograph (Plates 1 - 5) showed variations in their features.

 ${\it Izundu}~\it et~\it al \\ {\it Table~1:Comparative~Wood~Fibre~characteristics~of~the~five~species~of~Malvaceae} \\$

(µm)	C. acuminata	C. nitida	C. olitorius	H. cannabinus	S. acuta	
Fibre length	1247.63 ± 23.17 ^b	1244.82 ± 28.43 ^b	765.42 ± 33.76°	1610.97 ± 20.88 ^a	841.83 ± 46.12°	
FD	18.84 ± 1.12°	19.95 ± 0.94°	22.67 ± 0.80 ^b	25.09 ± 0.26°	18.50 ± 0.55°	
FLD	9.21 ± 1.19 ^{bc}	6.83 ± 1.24°	15.73 ± 1.55ª	15.08 ± 0.56°	10.38 ± 0.54 ^b	
FCWD	5.03 ± 0.26 ^b	6.53 ± 0.26^{a}	$3.73 \pm 0.32^{\circ}$	5.07 ± 0.20 ^b	4.59 ± 0.17 ^b	
RR	0.54 ± 0.03 ^b	0.66 ± 0.05^{a}	0.33 ± 0.04^{d}	0.40 ± 0.01^{cd}	0.50 ± 0.02^{bc}	
SR	66.95 ± 3.20°	63.17 ± 4.27 ^a	33.86 ± 1.61°	64.20 ± 0.41 ^a	45.55 ± 2.28 ^b	
Flexibility Coefficient	48.18 ± 3.66°	33.72 ± 4.69 ^d	68.79 ± 4.25 ^a	60.15 ± 2.56^{ab}	56.04 ± 1.84 ^{bc}	

The results are presented with means \pm standard error. Means with different alphabets along each row represents significant differences using Duncan new multiple range test (DNMRT) at P < 0.05.



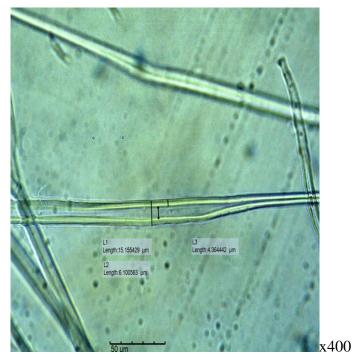
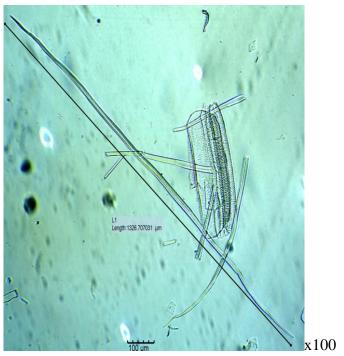


Plate 1: Fibre morphology of Cola acuminate



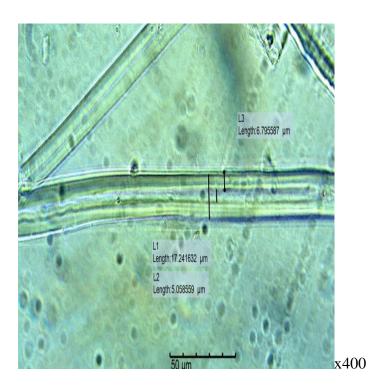
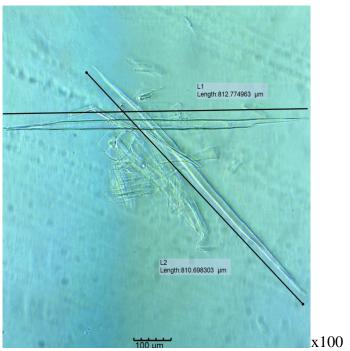


Plate 2: Fibre morphology of *Cola nitida*



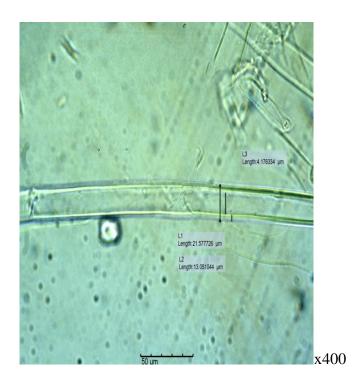
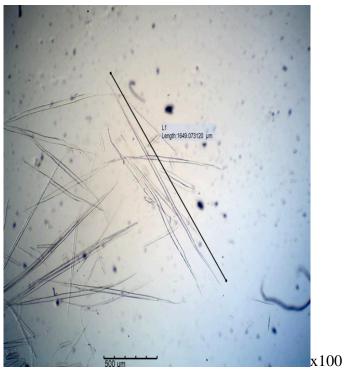


Plate 3: Fibre morphology of *Corchorus olitorius*



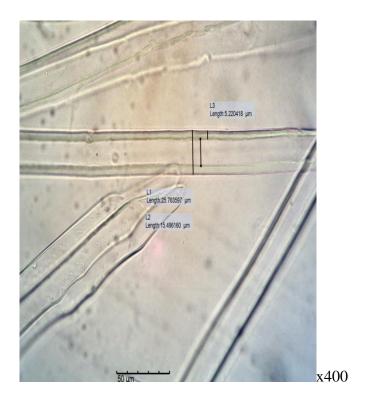
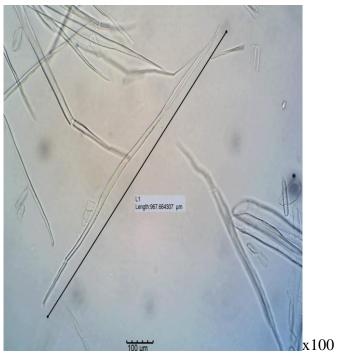


Plate 4: Fibre morphology of *Hibiscus cannabinus*



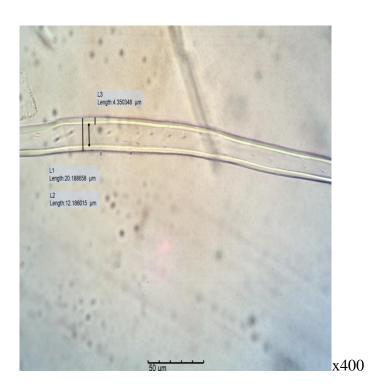


Plate 5: Fibre morphology of Sida acuta

Relationship among the chemical characters of the wood samples

relationships Strong positive exist between the chemical characters of the evaluated wood (Table 2), While Cold Extractives (CWE) correlated only Hot positively with Water Extractive (HWE) $(r^2 = 0.832)$ cellulose ($r^2 = 0.713$) at P < 0.01 and P < 0.05 respectively, HWE significantly correlated with all the traits except hemicellulose. Similarly, Alcohol benzene Extractives (ABE), Lignin, Inlignin and Moisture content (MC) correlated significantly with all the traits except CWE and hemicellulose (Table2).

Table 2: The correlation coefficients among the chemical characters

	CWE	HWE	ABE	AC	CELLULOSE	LIGIN	INLIG	HEMICE	MC
CWE	1								
HWE	.832**	1							
ABE	.447	.817**	1						
AC	.259	.740*	.866**	1					
CELLULOSE	.713*	.873**	.874**	.705*	1				
LIGIN	.229	.709*	.916**	.902**	.632	1			
INLIG	.215	.682*	.788**	.962**	.676*	.813**	1		
HEMICE	451	456	374	307	596	128	363	1	
MC	.378	.822**	.955**	.968**	.799**	.951**	.917**	350	1

^{*.} Correlation is significant at the 0.05 level (2-tailed).; **. Correlation is significant at the 0.01 level (2-tailed).

Relationship among the fibre characters of the wood samples

The relationships between the fibre characters of the evaluated wood were also tested and the result presented in Table 3. It was observed that strong positive and negative relationships exist among the traits. Fibre length correlated significantly positively with FCWD ($r^2 = 0.471$) and SR ($r^2 = 0.817$) at P < 0.05 and P < 0.01 respectively. FD correlated positively with FLD ($r^2 = 0.801$) and FC

 $(r^2 = 0.563)$ and negatively with RR $(r^2 = 0.678)$ and RC $(r^2 = -0.678)$ at P < 0.01 respectively. Apart from FD, FLD also significantly correlated positively with FC and negatively with FCWT, RR and SR (Table 3). Similarly, SR correlated significantly positive with FD and FLD and negatively with FCWD and RR (Table 3).

Table 3: The correlation coefficients among the fibre characters

	ruble 5: The correlation coefficients among the fibre characters								
	Fibre length	FD	FLD	FCWT	RR	SR	FC		
Fibre length	1								
FD	.390	1							
FLD	011	.801**	1						
FCWT	.471*	219	741**	1					
RR	.166	678**	959**	.857**	1				
SR	.817**	195	484*	.598**	.574**	1			
FC	241	.563**	.941**	897**	964**	591**	1		

^{*.} Correlation is significant at the 0.05 level (2-tailed).; **. Correlation is significant at the 0.01 level (2-tailed).

Relationship among the chemical, elements, and fibre characters of the wood samples. Result showed that out of all the fibre traits evaluated, only

FCWD significantly correlated (P < 0.05) negatively with the physical characters (ABE, Lignin and MC) (Table 4).

Table 4: The correlation coefficients among the chemical and fibre characters

	Fibre length	FD	FLD	FCWT	RR	SR	FC
CWE	512	192	053	347	263	021	.061
HWE	209	136	.265	590	505	.038	.406
ABE	023	275	.305	708*	511	.256	.497
AC	.269	013	.484	568	525	.096	.590
CELLULOSE	268	280	.102	550	396	.145	.277
LIGIN	.232	133	.473	674*	537	.220	.621
INLIG	.279	.080	.464	421	454	009	.535
HEMICE	.523	337	361	.366	.611	.511	344
MC	.139	088	.456	651*	565	.125	.596

^{*.} Correlation is significant at the 0.05 level (2-tailed).; **. Correlation is significant at the 0.01 level (2-tailed).

From our observations, the extractive content of H. cannabinus was lowest among the three methods used (cold water, hot water and acid). This species may be most advantageous in the pulp and paper making process because high extractive content lowers pulp yield, impacts on the brightness of unbleached pulp and increases chemical demand of pulping and bleaching chemicals [7]. Generally, the presence of extractives in materials increases consumption of pulp reagent and reduces yields. For this reason, material with little or no extractive content is desirable [9]. More so, the range of alcohol benzene extractive content obtained in this study was in agreement with the report of [13] in mixed tropical hardwood species.

The cellulose content recorded from all the plant species were satisfactory for pulp and paper production. [3] had reported that plant with cellulose close to or above 40% as satisfies the need for pulp and paper production. Generally, there are positive relationship between pulp quality and cellulose content. However. the cellulose contents observed in this study among the species were lower than that reported by [6] in plum [9]. Some important properties of cellulosic fibers are high tensile strength, water insoluble. hydrophilic, wide range of dimensions, ability to absorb modifying additives and being chemically stable. Therefore, it is expected that the pulp yield and strength will be high in paper made from species with high cellulose content such as C. nitida.

The hemicellulose content observed in this work also corroborated the report of [9] in woody materials. The main function of hemicellulose is to increase fibre-to-fibre bonding but at a higher amount, tends to lower the strength properties of paper. Starch is often added to pulp to accelerate the strength of paper with about similar mechanisms of effect as the hemicelluloses [7]. Hemicelluloses increase the strength of paper, especially tensile, burst and fold and the pulp yield [9]. So it is expected that the pulp yield, tensile and burst strength will be high in paper from species that had high hemicelluloses content like *C.* acuminata. Some

industries need raw materials with high hemicelluloses while others need low hemicelluloses and fortunately hemicelluloses content in raw materials can be controlled by silvicultural treatments as stated by [8,9]. Similarly, cellulose/hemicellulose ratio of lignocellulosic residues could be a measure of the pulp and paper quality of wood. This ratio affects mechanical properties of paper, where [8] reported thattheincreasedcellulose/hemicellulose increased the tear toughness and folding endurance, while the tensile index decreased. In line with this report, one expects that handsheet formed from C. olitorius pulps will be high in tear index and folding but it will be low in tensile index based on its cellulose/hemicellulose ratio.

Lignin is an undesirable polymer, and its removal during pulping requires high amounts of energy and chemicals. The average lignin content among the plant species were lower than the lignin content in many plant species already being used in pulping and paper industries such as the branch and stem of plum wood reported by [7], from coconut fibers [12] and Prunus americana wood [9]. Generally, it can be seen that lignin content in *C. olitorius* and S. acuta were significantly low and the pulp from the wood of these species will be light brown compared to other materials. Also, it is expected that pulp yield will decrease with C. acuminata and C. nitida because the more lignin the less pulp yield. Again, the color of pulp yield will be dark brown. This expected outcome is in accordance with who found that lignin content contributes to the brown color of the pulp and decrease the pulp yield.

According to [5] and [7], greater fibre length conforms to higher tearing of resistance paper, which attributed to stress dissipation; the longer the fibre, the larger the area over which the stress was dissipated. However, longer fibres tend to give a more open and less uniform sheet structure [11].This observation indicated that papers produced from wood fibres of *H. cannabinus* is likely to have high tear resistance than those from the other wood samples studied. Plants with wider fibre diameter

similarly recorded higher lumen This had similarly been diameter. reported by [15,16]. [2] also reported that fibre with large diameter and broad diameter ensures collapsibility and therefore provides enough bonding surface during paper production. This implies that cannabinuswith the largest diameter will provide better inter-fibre bonding while C. nitida will provides the least. Alternatively, C. olitorius would also produce stronger paper due to its thin cell walls. [5] pointed out that the thinner the cell wall thickness the better the fibre for papermaking, as fibres with thin walls collapse easily and provide effective bonding surface during papermaking.

The derived fibre values are usually applied as parameters for the assessment of hardwood fibres [5,8,14]. These values included Runkel ratio (RR), Slenderness Ratio (SR), and Coefficient of flexibility (CF). The lower the Runkel ratio especially when it is less than 1 the better for paper making [7,9,10,13,16]. Based on this, all the plants are considered fit for paper-making. [9] pointed out that papers made from fibre

The results of this present investigation have shown that the wood of all the species would be suitable for paper making based on their fibre characteristics. Working with the principles that fibres with the lowest

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with high Runkel ratio are porous and stiff. The slenderness ratio observed across the study plants were far higher than that reported by [5] for G. arborea which is a well-established plant for pulp and paper making. Similarly if the postulate by [8] as reported by [11] that the higher the slenderness ratio, the greater the tear resistance of the paper is true, then it is reasonable to say that from our data all the plant species evaluated will show the good tear resistance if used for pulp and paper making. Correlation analysis is liable to measure the level of association [8]. between two traits Biological characters are naturally correlated as a result of pleiotropy and linkage. It is obvious from the study that the fibre characters were almost unrelated with chemical and both the elemental characters of the plants. The importance of this relationship simply implies that the traits are not influenced by the same genes in the same direction Therefore, paper making qualities of these plants could be dependent on the fibre characters which are species dependent.

CONCLUSION

Runkel ratio and highest coefficient of flexibility make the strongest papers, the order of paper quality of the species studied is: *C. olitorius* > *H. cannabinus* > *S. acuta* > *C. acuminata* > *C. nitida*.

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