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ANATOMICAL AND CHEMICAL COMPOSITION OF *Detarium senegalense J.F. Gmel* BARK

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ABSTRACT

The anatomical and chemical composition of Detarium senegalense bark was studied after grinding and fractioning into different particles sizes. The bark of D. senegalense mature trees was fractionized into fine and coarse granulometric particles using 0.150 mm and 4.0 mm mesh respectively. The bark showed the following compositions for fine and coarse bark granulometric respectively as ash (10.9 % and 12.1 %), total extractives (20.9 % and 16.4 %), suberin (0.97 % and 0.94 %) and lignin (36.5 % and 31.2 %). Ash elemental composition was different in both fractionized biomass. The suberin content in both fractions were as well low. Fine D. senegalense bark contained more extractives in relation to coarse granulometric D. senegalense bark. For the elemental compositional characterization, the values obtained for the fine and coarse bark solutes were almost in the same range for N (0.75 % and 0.72 %), P (0.095 % and 0.092 %), Mg (0.13 % and 0.14 %), Ca (6.6 % and 6.2 %), K (0.33 % and 0.31 %), Ni (1.81 mg/kg and 1.77 mg/kg), Cr (1.98 mg/kg and 1.93 mg/kg), Pb (2.02 mg/kg and 2.05 mg/kg). However, high concentration of Zn was found in fine granules of D. sengalense bark that is, 29 mg/kg compared to 13 mg/kg in the coarse bark. Extractives were present preferentially in the finest fraction in dichloromethane, ethanol and water soluble. D. senegalense bark had a high content of 36.5 and 31.2 cellulose and hemicelluloses especially in the coarser fraction. The rich lignin content of 39.5 and 20.9 in D. senegalense wood makes it a good potential resource for adhesives, chemical and biorefinery industries. The significant content of extractive in D. senegalense suggests conferment of natural durability to the wood. Particle size reduction by grinding is a unit operations that may be used as selective enrich solutes in soluble materials.

Keywords: Anatomical structure, Chemical composition, *Detarium senegalense*, fine granulometric fraction, coarse granulometric fraction

INTRODUCTION

Tree bark usually refers to all external tissues surrounding the vascular cambium of the tree. Generally, it takes up about 9-15 % of a typical log by volume Harkin and Rowe (1971). Bark has a similar chemical composition to wood, only that it contains more extractives, higher lignin content, and a smaller amount of holocellulose than wood. The bark chemical components can be separated into solutes with different polarity through sequential extraction using a series of organic solvents and hot water (Harkin and Rowe, 1971; Hon and Shiraishi, 2000).

Contrary to the fact that had been established that softwood are the most suitable specie for pulp production because of its long fibre, hardwood had also been confirmed as a suitable and potential source of pulp production in Nigeria Ogunwusi (2002) and Ndukwe *et al*. (2009). For pulp production process, debarking of logs is a major step in forest mill operations after bulking of the wood. Considerable large volumes of bark are

made available from log debarking, and are separated in the mill as residual, often abandoned or burned as fuel. In addition to being considered a valuable solid biofuel, bark is also scrutinized for more value-added products based on its specific potential chemical composition or properties (Demirbas, 2010).

Research and development efforts therefore can initiate to focus on transforming bark biomass to higher value, eco-friendly industrial products with large market potentials. These products can be used either as substitutes or replacement for petroleum-based products and help to mitigate climate change. In addition, innovative research in these areas will have great potentials to improving forest, chemical and medicinal use as well as resulting in higher economic and environmental benefits. In view of the above, among the properties that will qualify the bark of a wood as a potential raw material in biorefinery and chemical industries is the percentage bark abundance it possesses. Therefore, it is necessary to investigates into a number of wood species of high bark abundance so as to meet the ever increasing demand for petrochemical industries.

Among ten (10) tropical hard wood species studied for variation in their barks abundance within trees of the same species and those of different species, *D. senegalense* performed best (Ogunwusi, 2013). When bark percentages are too low, its use for production of bark chemicals may not be economical (Ogunwusi, 2013; Harkin and Rowe, 1971). It implies that, if the woods are only to be sawn and used for planks production, the lower the percentage of bark in the species the better because high bark percentage reduces the volume of usable wood and add to the transportation cost. Therefore high bark percentage in *D. senegalense* is an advantage since the interest is to explore it for chemical production. The objective of this study therefore, is to characterize the chemical composition of fractionized *Detarium senagalense* with a view to determining the variations in the barks chemical composition when fractionized.

MATERIALS AND METHODS

Material Collection and Preparation

The bark of *Detarium senegalense* was obtained from Iyare sawmill, Old Garage, Ibadan, Oyo State. Reliable information from the sawmillers confirmed that the log was collected from Gambari Forest Reserve. Gambari Forest Reserve along Ibadan-Ijebu Ode Road, Oyo State, Nigeria located on latitude 7^o 7´ 60´´ N and longitude 3^o 49´ 60´´ E within the low land semi deciduous forest belt of Nigeria and covers a total land area of 17,984. The bulk bark samples were hammer-milled using New Holland grinder model 358, with 0.150 mm and 4.0 mm sieve size for particle reduction into fine and coarse granulometric solutes respectively (Plate 2). The weight of the fraction retained on each sieve was weighed and the corresponding percentage weight of solutes yields were determined using equation (1).
The yield of the solute fraction was quantified as:

$$\%\ Weight\ of\ solute\ fraction = \frac{Weight\ of\ solute\ fraction}{Weight\ of\ oven\text{-}dried\ raw\ D.senegalense\ Bark\ before\ fractionation} \times 100 \quad \text{------ (1)}$$

Microscopic observations of *Detarium senagalense* stem bark

The different granulometric bark samples were observed microscopically after cell dissociation by maceration in a 1:1 glacial acetic acid: hydrogen peroxide solution, and staining with astra blue.

Chemical characterization of *D. senegalense* bark fine and coarse solutes

Chemical characterization that was determined were ash, extractives soluble in ethanol, suberin, Klason and acid soluble lignin, holocellulose, and monomeric composition of polysaccharides. The granulometric solutes with particle size over 4.0 mesh were carefully ground prior to chemical analysis in order to obtain particles that passed through the 4.0 mesh sieve. Ash content was determined according to TAPPI Standard T 15 os-58 using 2.0 g samples as the residue of overnight

incineration at 450 –500 ◦C. Solvent extraction was performed in a Soxtec extractor using ethanol during 1.5 h and the soluble extractives was determined using the weight difference from the weight of the solid residue after drying at 105 ◦C and reported as a percentage of the original samples.

Suberin content was determined on 1.5 g of extractive-free material by refluxing with 100 mL of a 3% $NaOCH_3$ solution in CH_3OH during 3 h (Pereira, 1988a). The extractive free-sample was filtrated, washed with methanol, again refluxed with 100 ml CH_3OH for 15 min and filtrated. The combined filtrates were acidified to pH 6 with 2 mL of H_2SO_4 and evaporated to dryness. The residue was suspended in 50 mL water and the alcoholysis products recovered with dichloromethane in three successive extractions, each with 50 mL dichloromethane. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The suberin extract that include the fatty acid and fatty alcohol monomers of suberin were quantified gravimetrically, and the results expressed in percent of the initial dry weight.

Klason and acid-soluble lignin were determined on 0.35 g of extractive-free and desuberinized samples. Sulfuric acid (72 %, 3.0 mL) was added to the sample and the mixture placed in a water bath at 30 ˚C for 1 h after which the sample was diluted to a concentration of 3% H_2SO_4 and hydrolyzed for 1 h at 120 ˚C. The sample was vacuum-filtered through a crucible and washed with boiling purified water. Klason lignin was determined as the weight of the solid residue after drying at 105 ˚C and acid-soluble lignin was determined on the filtrate by measuring the absorbance at 206 nm using a UV/VIS spectrophotometer. Klason lignin and acid-soluble lignin were reported as percentage of the original sample and combined to give the total lignin content.

The polysaccharides were calculated based on the amount of the neutral sugar monomers released by total hydrolysis, after derivatization as alditol acetates and separation by gas chromatography with a method adapted from Tappi 249 cm-00.

Determination of elemental and ash composition of fine and coarse granulometric solutes of *D. senegalense* stem bark

Nitrogen was determined by the Kjeldahl method (Jackson, 1958) in a Tecator equipment (Auburn University, USA). After a hydrochloric digestion of the ash (Marti and Munoz, 1957), phosphorus was determined by molecular absorption spectrometry in Hitachi U-2000 VIS/UV equipment, and all the other minerals were determined by atomic absorption spectrophotometer in a Pye Unicam SP-9 apparatus.

Plate1: Raw stem bark of *D. senegalense* collected, oven-dried and packaged for chemical Analysis

Plate 2: Hammer-milling of *D. senegalense* bark with the sieve sizes

RESULTS

Bark fractioning

The weight yields obtained for *D. senegalense* bark was summarized in Table 1.

Table 1: Weight yields (% of total dry Weight) of the fine and coarse granulometric solutes after milling of barks.

Granulometric solutes (mm) (determined by sieve size)	Weight Yield (%) *D. senegalense*
0.150	16.2
4.0	56.4

Microscopic observations of *Detarium senagalense* stem bark

The microscopic features observed in the bark of *D. senegalense* were the secondary phloem (with an average thickness of 1.2 cm), the periderm and a narrow rhytidome that showed shedding of outermost periderms Plate (3) and (4)

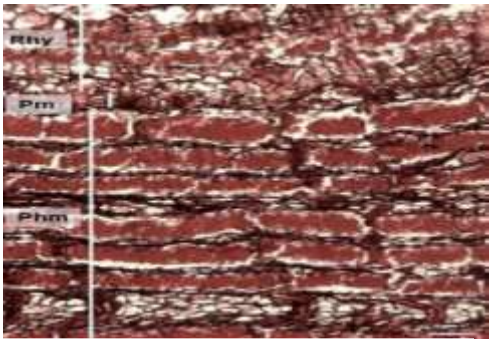

Plate 3: Bark of *D. senegalense*:

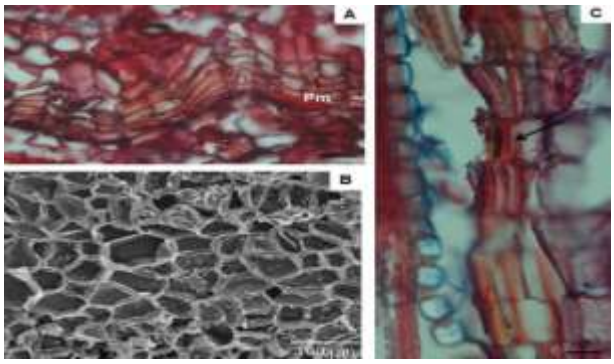

Plate 4: *Detarium senegalense* bark

(A) Granulometric solute with 4 mm particles, observed under the magnifying glass;
(B) Microscopic observations of dissociated cells obtained from granulometric solute.
(C) Conspicuous high amount of calcium oxalate crystals observed

Ash content and composition of fine and coarse granulometric of *D.senegalense* bark

The ash content of *D. senegalense* collected at the mill after milling and separation into the different granulometric solutes is presented in Table 2.

Table 2: Ash content (% of total dry weight of the fine and coarse granulometric solute of *D.senegalense* bark after milling

Granulometric solute (mm)	Mean ash % of total dry weight
0.150	10.9
4.0	12.1

Elemental composition of *Detarium senegalense* bark

The percentage composition and composition in (%) and (mg/1000g) of the granulometric solutes of *D. senegalense* bark is presented in Table 3.

The overall chemical composition of the oven dried granulometric solute of the fine and coarse *D. senegalense* bark is presented in Table 4

Table 3: Elemental and mineral concentrations of fine and coarse granulometric solute of *D.senegalense* bark.

Elemental concentration in % and mg/1000g	**Granulometric fine solute of *D. senegalense* bark (0.150 mm)**	**Granulometric coarse solute of *D. senegalense* bark (4.0 mm)**
N (%)	0.75 %	0.72 %
Ca (%)	6.2 %	6.6 %
Mg (%)	0.13%	0.14 %
K (%)	0.33 %	0.31 %
P (%)	0.095 %	0.092 %
(mg/1000g)		
Zn (mg/1000 g)	29 mg/kg	13 mg/kg
Ni (mg/1000 g)	1.81	1.77
Cr (mg/1000 g)	1.98	1.93
Pb (mg/1000 g)	2.02	2.05

Table 4: **Summative chemical composition (% o. d. material) of the fine and coarse granulometric solutes of *D. senegalense* bark calculated as mass weighted average**

Chemical composition	**Granulometric fine solute of *D. senegalense* bark (%)**	**Granulometric coarse solute of *D. senegalense* bark (%)**
Ash	10.9	12.1
Total Extractives	20.9	16.4
Dichloromethane	5.1	4.25
Ethanol	5.5	4.64
Water	5.2	4.23
Suberin	0.97	0.94
Lignin	32.0	29.1
Klason	25.2	22.7
Acid soluble	6.8	6.4
Hollocellulose	36.5	31.2

DISCUSSION

The average yield of fine solute was low, 16.2.9 % was recovered for particles under 0.150 mm, and the major solute of 56.4 % was recovered for the larger particles under 4 mm sieve Table 1.This may be attributed to the fact that the milling process applies differently to different barks and the bark structural features influence the grinding behaviour. Bark are made up of different tissues – phloem, periderm and rhytidome – in varying extent and cellular characteristics.This grinding behavior with little recovery of fines was also found for conifer barks of *Pinus pinaster* (Vázquez *et al*., 1987), *P. sylvestris* and *P. abies* (Miranda *et al*., 2012).

Anatomical characterization

The result of the anatomical characterization of *D. senegalense* bark revealed that the bark was bulky (on average 4.0 cm) with a chocolate brown colour, lightly streaked longitudinally, with a shedding of long and fibrous strips (Plate 3). The bark constituents were the secondary phloem (with an average thickness of 1.7 cm), the periderm and a narrow rhytidome which showed shedding of outermost periderms (Plate 3). The rhytidome constitutes the periderms and the in-between dead phloem with bulky quantities of fibres, resulting in a fibrous and brittle bark structure; this was tracked macroscopically in the 4 mm fraction since it had a clear fibrous aspect unlike the fine solute which showed no clear or obvious fibrous aspect (Plate 4a).

When carefully observing the types of cells present in the solute after dissociation an abundant content in crystals were observed in the phloem of the bark (Plate 4b). *D. senegalense* bark has a conspicuous high amount of calcium oxalate crystals (Plate 4c), due to the content in crystals observed in the phloem which was also confirmed through the

SEM-EDX analysis that clearly observed the calcium composition (Plate 4c).

D. senegalense bark have a small proportion of phellem (average thickness of 1.7 cm) in their periderms and therefore, their content of suberin is low (Table 4). Suberin is a typical structural component of bark periderms where its presence is specific to the wall of phellem cells for which it is a chemical fingerprint (Ferreira *et al* 2015; Pereira (2007); Pereira (2015). Therefore when the proportion of phellem in a bark is small, the content in suberin will also be small as well. This has been shown for several species e.g. *Pinus sylvestris* and *Picea abies* (Miranda *et al* 2012; Baptista *et al* 2013).

Elemental composition of *Detarium senegalense* bark

The result of the elemental composition of *D.senegalense* bark solute presented in Table 3 showed a slightly high mineral content in the fine and coarse solute N: (0.75 % and 0.72 %), Ca: (6.2 % and 6. 6 %), Mg: (0.13 % and 0.14), K: (0.33 % and 0.31 %, P: (0.095 % and 0.092 %) This showed that the bark obtained in the mill contained considerable contamination with soil particles and other extraneous fine material. The amount of sand granules was obvious by a direct macroscopic observation of the sample. Due to their small size, these particles were retained in the finer granulometric solutes after the milling and screening. A similar occurrence of substantial accumulation of minerals was also found for pine bark obtained at the mill (Miranda *et al*., 2012). This clearly shows the importance of bark cleaning during field and mill handling in order to avoid unsuitable contaminations during subsequent bark valorization steps. *D. senegalense* is a "calcicolous" tree species that may requires a relative large amount of calcium for its growth development and could takes up Ca rapidly. A similar value was also reported a high content of minerals in teak bark Kumar *et al*. (2009).

Ash

The result of the ash content for the fine and coarse granulometric fraction of *D. senegalense* bark was 10.9% and 12.1 % respectively as determined by the weight average of two solutes taken. (Table 3). It is known that ash tends to accumulate in the finer sized solute during biomass processing due to the small size and brittleness of inorganic material (Bridgeman *et al*., 2007; Liu and Bi, 2011). However the extent of mineral accumulation in bark solute depends on the species (Miranda *et al*., 2012).

Elemental composition of *Detarium senegalense* bark

D. *senegalense* bark had high content of inorganic material (Zn, Ni, Cr and Pb) Table 3. A similar value was reported of a high content of minerals in teak bark Kumar *et al*. (2009). However, the concentrations of all other major elements were almost the same for the two fraction of *D.senegalense* bark. High concentration of Zn (29 mg/kg) was found in the ashes of Granulometric fine Solute of *D.senegalense* bark with in comparison with 13 mg/kg in coarse solute. There were only small differences in the mineral composition of both solutes. (Table 3) with the exception of Ca concentration which decreased substantially in the fine fraction of *D. senegalense* bark. The values are in the range of those reported by Pereira (1988b) and Damin da Silva *et al.* (1983) for eucalypt barks, an exotic hardwood specie

Chemical composition of *Detarium senegalense* bark

The chemical characterization and composition calculated as a mass weighed average of the granulometric solute of D. senegalense as shown in Table 1 revealed that there was a slight differences between the fine and coarse granulometric solute of the *D. senegalense* barks in relation to extractives, suberin and holocellulose contents. Fine granulometric solutes of *D. senegalense* bark contains more extractives than the coarse granulometric solutes of *D. senegalense* bark, but less cellulose and hemicelluloses expressed as holocellulose.

However, No appreciable differences was observed in the content of suberin.in the fine and coarse granulometric of *D. senegalense* bark. This may be due to the similarity observed in the anatomical structure of both barks, with the absence of a rhytidome with suberized phellem tissues in both fine and coarse granulometric fraction of D. senegalense bark. Both granulometric showed similar cell wall lignification but there was a striking chemical difference between both barks in

relation to holocellulose content: coarse granulometric fraction of *D. senegalense* bark had much higher holocellulose (34.9% vs. 55.4%).

There are few published references on the chemical composition of hardwood barks. Harkin and Rowe (1971) reported, respectively, total extractives, 22.4 %, and lignin 37.8 % for *Eucalyptus globulus* bark: a typical tropical hard wood species, Yadav *et al.* (2002) reported 7.2 % alcohol extractives, 15.5% water extractives, 28.0 % lignin and 62.2% total carbohydrates for same wood bark. Vázquez *et al.* (2008) reported 19.2% lignin and Sakai (2001) 18.6% lignin, 43.2 % cellulose and 19.6 % pentosans for same wood bark.

Effect of particle size on chemical composition of stem bark

Particle size effect was observed to affect the content and composition of extractives of *D.senegalense* bark solute. Extractives were present preferentially in the finest solutes due to enrichment or evenly diffusion of the biomass in non- polar and polar compounds. The extractives content was highest in the fine solute i.e. 20.9% in comparison with 16.4 % for the coarser solute. For the structural components, the observed differences were either of small magnitude or without a consistent pattern of variation in *D. senegalense* bark. For instance, suberin content was similar for the two bark solutes. However, consideration of the chemical composition based on extractive-free material was not considered.

The lignin content was highest in the fine solutes 36.5 % in comparison with 20.9 % for the coarser fraction while holocellulose content was highest in the coarse solutes (55.4%). The differences in the chemical composition of the bark solutes are due to the differences in the condition of the distribution of sizes after grinding (Vázquez *et al.,* 2001). Bridgeman *et al.* (2007) reported that cellulose, hemicelluloses and lignin tend to remain in the larger particle sized fraction. Tamaki and Mazza, (2010); Chundawat *et al.* (2007) also reported compositional changes with particle size: with increasing particle size extractives content tend to decrease and hemicelluloses and glucan content to increase while lignin content did not show clear trends.

CONCLUSIONS

The results showed that particle size reduction by grinding is a unit operation that may be used to selectively enrich solutes in soluble materials. Coarser solutes have higher holocellulose content that might be suitable for carbohydrate related uses. Extractives were present preferentially in the finest solutes, the lignin content was highest in the fine solutes, high concentration of Zn was found in the ashes of granulometric fine solute, *D. senegalense* bark have a small proportion of phellem, hence, small content in suberin was present in both solutes.

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