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Research Article

1D AND 2D NMRCHARACTERIZATION OF *Sweitenia mycrophylla* GUM

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Abstract

Sweitenia mycrophyllagum (Mahoganygum) from Nigeria was characterized using standard procedures. Scanning electron microscopy (SEM), X-ray powdered diffractometry (XRDP), Fourier Transform Infrared (FTIR) and 1D and 2D Nuclear magnetic resonance (NMR) spectroscopy were used to characterized the gum from *S.mycrophylla*. SEM analysis suggested the gum has irregular particle size. The XRPD pattern of the gum showed no sharp peak which shows a completely armophous structure. FTIR spectrum showed band characteristics of O-H(3650-3500 cm^{-1}), C-O-C (1632 cm^{-1}), -COO (1429 cm^{-1}) groups that are present in the structure of polysaccharide. NMR spectroscopy revealed that the gum is a galactomannan type polysaccharide which contains α-D galactopyranosyl unit linked to β-D-mannopyranosyl Unit at 0-6 with mannose/galactose ratio of 2.60 :1.20. Due to its mannose/galactose ratio, *Sweitenia mycrophylla* galactomannan could be a useful polysaccharide for food and pharmaceutical industry.

Keywords: *Sweitenia mycrophylla* gum, polysaccharide, SEM, XRPD, NMR, FTIR.

Introduction

The polysaccharide gums represent one of the most abundant industrial raw materials and have been the subject of intensive research over comparable synthetic materials due to their sustainability, biodegradability and safety [1].Natural polysaccharide gums represent a group of polymer which swell to form highly viscous solutions or dispersions in aqueous medium.They have found wide application in pharmaceutical formulation such as polymer matrix in sustained release solid dosage form (2-6), binders in tablet (7), stabilizers or suspending agents in liquid dosage forms (8) and in bioadhesive drug delivery systems [9].Polysaccharide gums used in the pharmaceutical and food industries include guar gum, gum tragacanth, acacia gum and xanthan gum among others. They have the advantage of biocompatibility, low cost and relatively wide spread availability compared to their synthetic counterparts [9].The characterization of polysaccharide gums is an essential step in establishing their suitability as pharmaceutical excipients.

Sweitenia mycrophylla polysaccharide gum is obtained from the back of the tree. *Sweitenia mycrophylla* is a large tree, reaching a height of 30-40m and a girth of 3-4m, in favourite condition it can reach 60m high and 9m girth. It is popularly known as Mahogany [10,11].Gum is produced from bark of the tree for sales in markets in Bombay, India. It is marketed in pure form or mixed with other gums. An oil that might be of commercial value can be extracted from the seed kernels and various medicinal uses of the parts of the tree was reported from central America[12].Evidently, there are no sufficient studies that confirm the structural studies of the gum from *Sweitenia mycrophylla* tree.Hence, this research aims at investigating the structural studies of the gum from *Sweitenia mycrophylla* in order to evaluate its potential industrial applications, mostly in food and pharmaceutical. The results of this research is likely to highlight the structural information from the gum in order to amplify the possibility of the gum applications.

Materials and Methods

Collection and preparation of gum

Gum was collected from the bark of *Sweitenia mycrophylla* tree in Owena Forestry, Ondo State, Nigeria between November 2009 and February 2010. The plant was identified and authenticated at the herbarium of the Department of Plant Science Technology, University of Jos and department of Forestry and wood technology, Federal University of Technology, Akure. Gum was tapped from the bark of the tree. The crude samples of gum consist of a mixture of large and small nodules mixed with bark and organic debris. These were hand sorted to remove fragments of bark and other visible impurities and then were spread out in the sun to dry for one weeks. The dried cleaned gum samples were milled with a Kenwood blender and later sieved using a bin (mesh size-250microns) so as to obtain a fine and uniform sample, kept in labeled plastic container for subsequent analysis.

Purification of gum sample

Dried crude gum (l0g) was stirred in cold distilled water (250ml) for 2 hours at room temperature. The supernatant was obtained by centrifugation and made up to 500ml and ethanol solution was added (1:4 v/v) to precipitate all the carbohydrate. The precipitated material was washed again with ethanol, followed by distilled water and dried at room temperature milled with Kenwood blender and later sieved using a bin (mesh size-250microns) kept in labeled plastic container for subsequent analysis.

Microstructure studies by SEM

Morphological features of the gum was studied with a JSM - 5600LV scanning electron microscope of JOEL (Tokyo, Japan). The dried sample was mounted on a metal stub and sputtered with gold in order to make the sample conductive, and the images were taken at an accelerating voltage of 10KV and at 500x magnification.

X -ray powder diffraction (XRD).

X-ray diffraction patterns of the gum was analysed using a siemens D5000 X-ray diffractometer (Siemens, Munich, Germany). Powder sample, packed in rectangular aluminum cells, was illuminated using Cuk& radiation (A = 1.54056 Ao) at 45KV and 40mA. Samples were scanned between diffraction angles of 5^oC to 40^oC, scan steps of 0.1 were used and the dwell time was 15.0 sec. A nickel filter was used to reduce the Kp contribution to the X-ray signal. Triplicate measurements were made at ambient temperature.

Fourier transform infrared (FT-IR) AND NMR spectroscopy

The FT-IR spectrum of the sample was recorded in an FTIR spectrometer (Nicolet Magna 4R 560. MN USA), using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr. ^{13}C-NMR, ^{13}C-DEPT and Solid State NMR of *Sweitenia mycrophylla* gum were recorded in an NMR (600 MHz) spectrometer (Agilent technologies, America). The sample (10mg) was dissolved in 700µL at 70^0C with continous stirring for 6hours followed by sonication for 10minutes. The sample was centrifuged and transferred to a 5mm NMR tube. Chemical shifts were reported in ppm relative to an internal standard TMSP (Tetramethylsilanepropoinic acid). Peak integra were performed using Agilent software, America. In the solid state NMR, sample was packed carefully inside the NMR rotor for solid state nmr analysis.

Results

Table 1: ^{13}C and ^{13}C-DEPT 135^0 NMR assignment of *Sweitenia mycrophylla* gum (10mg in 700µL D_20, 60^0C) Referenced to TMS

	Chemical shift (ppm)					
Residue	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**
α-D-Galactopyranosyl	98.87	71.90	73.00	74.80	76.00	63.50
B-D-Mannopyranosyl	102.1	77.20	73.50	77.10	75.20	62.50

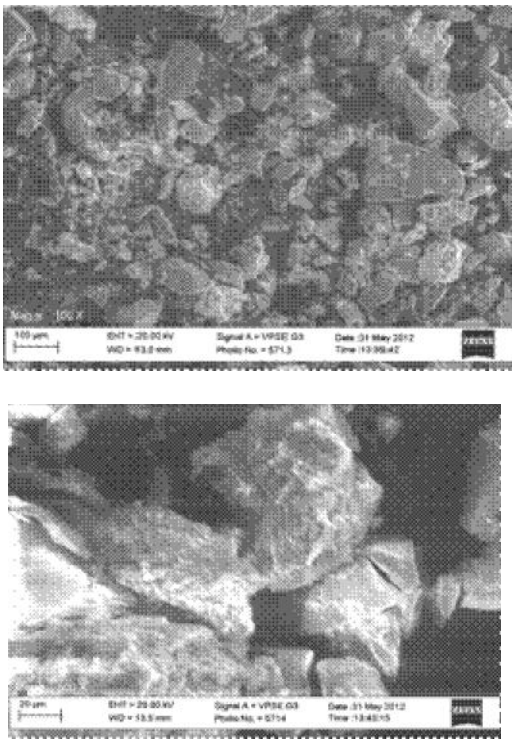


Fig 1: SEM of *Sweitenia mycrophylla* crude exudate gum at 100x and 500x

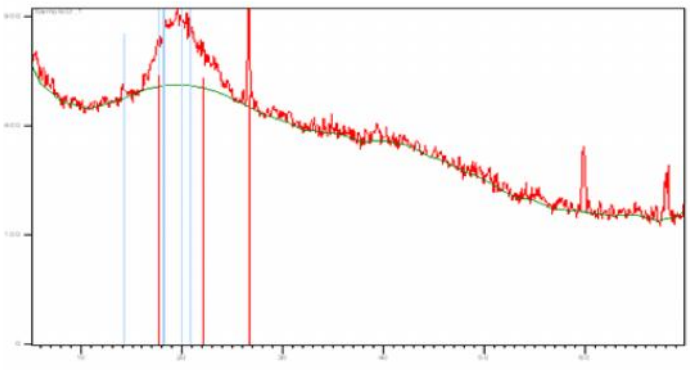


Fig 2: XRD of *Sweitenia mycrophylla* Gum

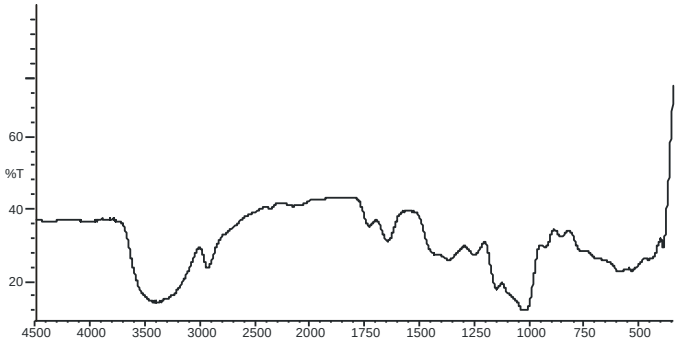


Fig 3: FTIR of *Sweitenia mycrophylla* Gum

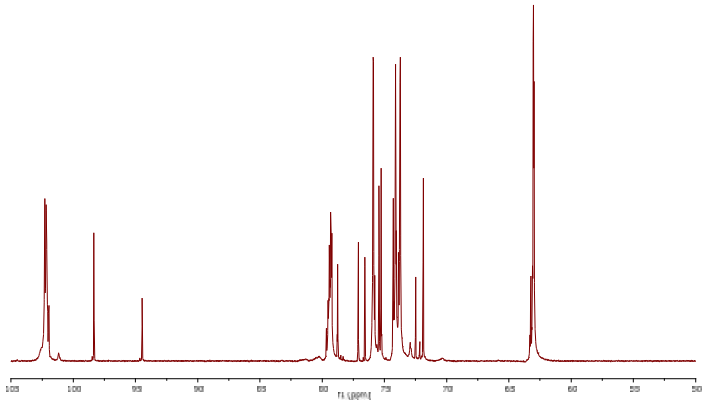


Fig 4: $^{1}D^{13}C$ NMR Spectrum ($600MH_Z$) of unmodified *Sweitenia mycrophylla* gum (10mg in 700µL D_20, 60^0c) Referenced to TMSP

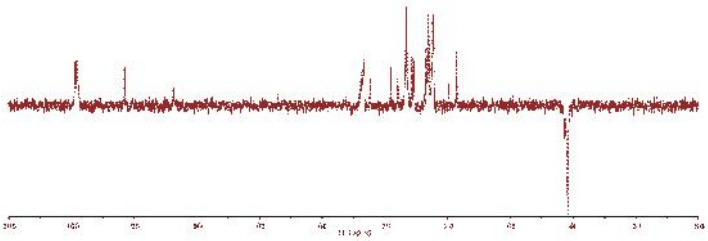


Fig 5: ^{13}CDEPT-135 Spectrum ($600MH_Z$) of unmodified *Sweitenia mycrophylla* gum S (10mg in 700µL D_20, 60^0c) Referenced to TMSP

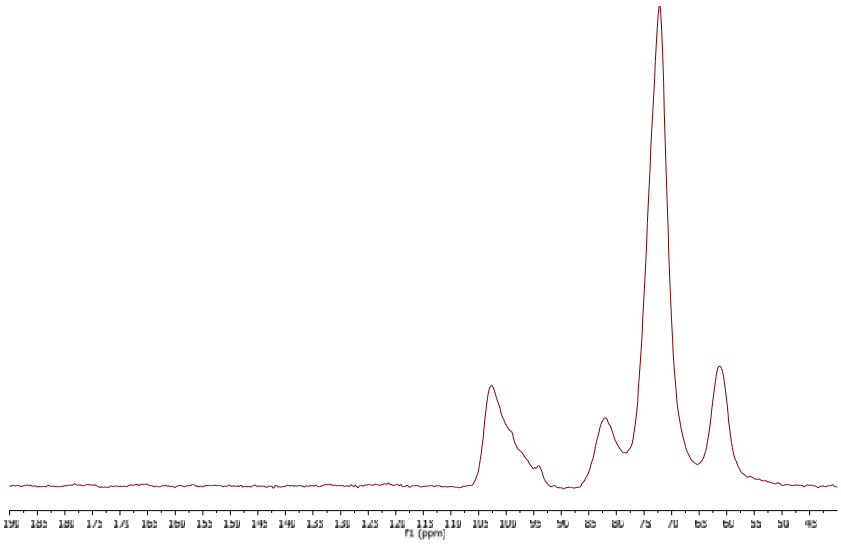


Fig. 6: ^{13}C-Solid state NMR of *Sweitenia mycrophylla* gum

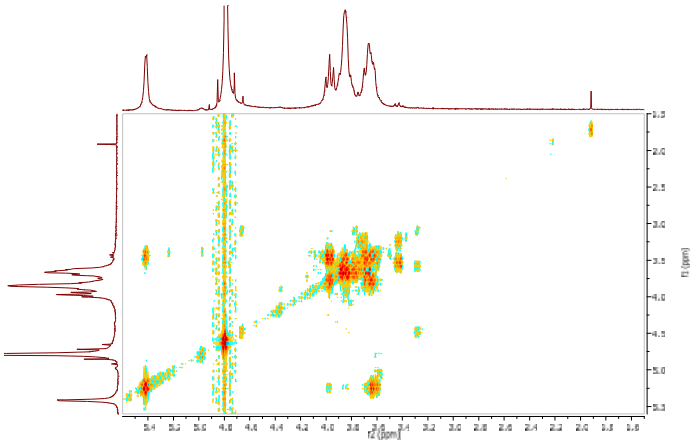


Fig 7: ^{1}H-^{1}H COSY of *Sweietenia mycrophylla* gum

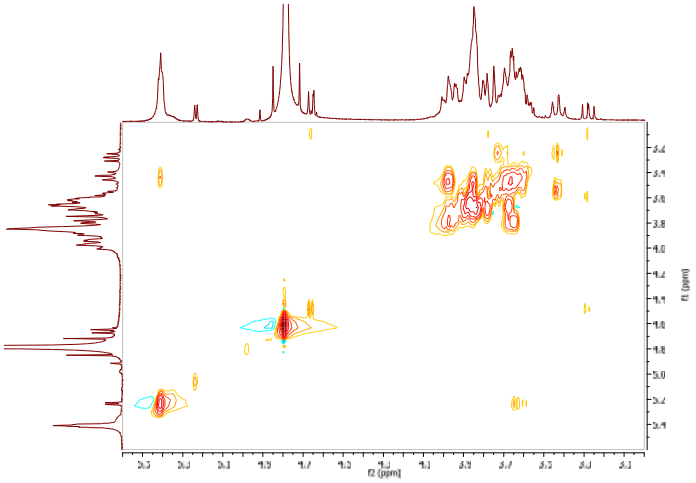


Fig 8: ^{1}H-^{1}H TOCSYof *Sweitenia mycrophylla* gum

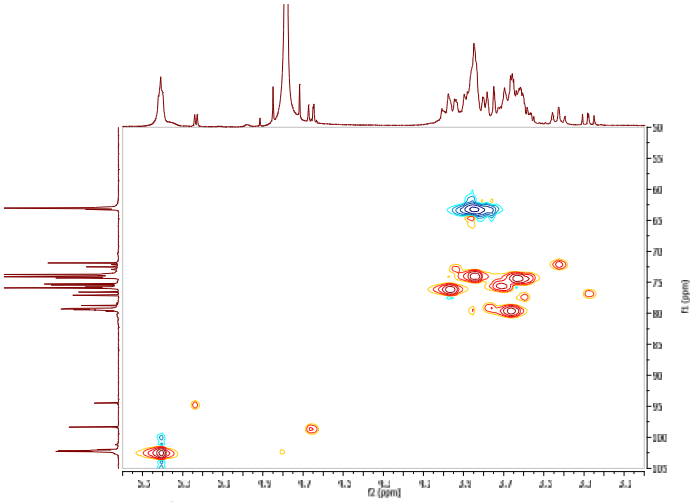


Fig 9: ^{1}H-^{13}C HSQC of *Sweitenia mycrophylla* gum

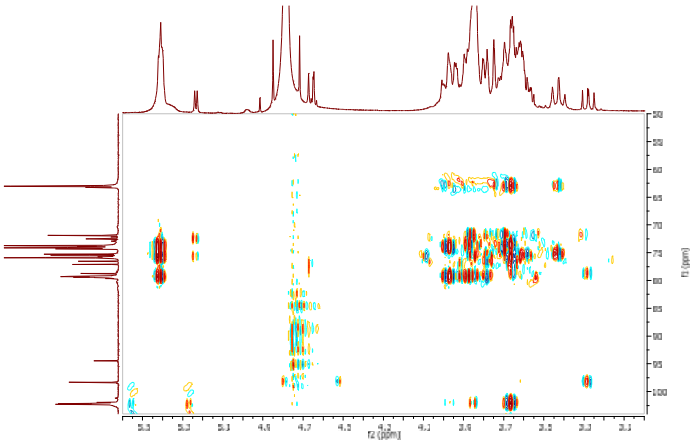


Fig 10: ^{1}H-^{13}C HMBC of Sweitenia mycrophylla gum

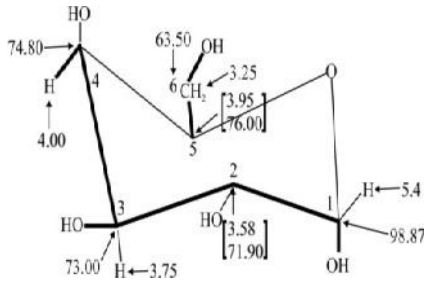


Fig 1: Signal Assignment of α-D-galactopyranosyl Unit Residue Present in *S. Mycrophylla*

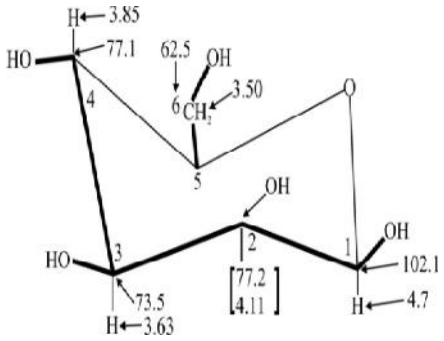


Fig 2: Signal Assignment of β-D-mannopyranosyl Residue Unit Present in *S. Mycrophylla*

Discussion

Scanning electron microphotographs (SEM) of the gum sample is depicted in Fig 1 at 100x and 500x magnification and 50m scale. It exhibit fibrous long non-distinct shaped large fibres. These properties could be of importance when considering applications based on surface characteristics. It is clear from the plate that the gum has irregular particle size. It has been reported that particle size and specific surface area influence the hydration behaviour of gums, which in turn influence their intrinsic viscosity and molecular mass [15,17,18,20]. Earlier studies carried out on guar gum - a galactomannam rich tree gum, established that particle size influenced the hydration kinetics and its molecular mass [2,22,23]. The micrograph of *Sweitenia mycrophylla* at X500 magnification (Fig 1) shows some wing-shaped particled which points to the amorphous nature of the powder. Thus this micrograph further confirms the result from XRPD analyses(fig. 2). Scanning electron microscopic studies (SEM) are used to examine the characteristic distinct crystalline morphology of some commercial gums at magnification from (Xl00) to (X6000).

Values above this magnification lead to decaying of sugar particles. The observation recorded has revealed that SEM studies of various polysaccharides could be used to find out the purity of substance e.g. in food and medicinal applications[13].

The x-ray diffractogram of the gum shows presence of numerous halves (Fig 2) with weak peaks, confirming its almost complete amorphous nature. The diffraction pattern of *Sweitenia mycrophylla* shows halo peak (fig 2) which is indicative of the amorphous nature of this excipient [27, 28, 29]. Many natural gums have also been reported to exhibits similar diffraction patterns, an indication of their amorphous nature [30, 31, 32, 33].The result of (XRD) confirms that the gum exhibits only an amorphous portion.

The FTIR spectrum is shown in Figure 3. The finger print region of the spectrum consists of two characteristic peaks between 700 and 1316 cm-1, attributed to the C-0 bond stretching [5], The band at 1604 cm-1 was assigned to the O-H bending of water [26]. Contribution from carbonyl stretches in the 1700 cm-1 region indicate the presence of ester linkages. The broad band at 3286cm-1 is due to hydrogen-bonded hydroxyl groups that contributes to the complex vibration stretches associated with free inter and intra-molecular bound hydroxyl groups which make up the gross structure of carbohydrate [26]. These are all consistent with a polysaccharide structure that is neither starch nor cellulose, but has some peptide cross-links and some amino sugars. [5].

^{13}C solid-state NMR spectrum of the gum sample is shown in fig. 6. The spectrum give line widths which are typical of an armorphous natural polymer with broad band signal between 64 and 90ppm arising from the bulk of the ring, C-OH. The C-4 carbon account for the high frequency shoulder while C-1, anomericcarbons give the signal between 90 and 110ppm. The shape of this band suggest it is composed of multiple signals but the low resolution suggest the contrary. The low intensity at about 62ppm is attributable to the $–CH_2OH$ belonging to galactose.

In the ^{13}C spectrum of *Sweitenia mycrophylla* gum (fig 4), signals from anomeric carbons appear in the 90 to 105ppm regions while the nonanomeric carbons are between 60 and 85ppm.The anomeric C-1 carbons are the most diagnostic; thus from C-1 alone one can often determine the different types of sequences present and their relative proportions [34]. The resonances of C-2 to C-5 can be found at 65-78ppm. The primary hydroxyl group (-OH) (C-6) resonate at 60-70ppm. [35, 34].The carbon anomeric region of ^{13}C NMR of *Sweitenia mycrophylla* gum showed two major signals which were assigned as C-1 of $\alpha - D - sugar$ residue A at 98.87ppm and C-1 of $\beta - D - sugar$ residue B at 102.1ppm. The spectrum region of anomeric carbons (102.1 and 98.87ppm) and the methylene carbons (62.50 and 63.50) are well depicted (fig 4 and 5).

The resonances of the carbon atoms were well resolved (fig 4) and identified as the resonances of C-2, C-3, C-4 and C-5 of sugar residue B and C-2, C-3, C-4 and C-5 of residue A (Table 4). The facts are almost identical with gums of other origin. [34, 36, 37].The ^{13}C-DEPT NMR 135^0 spectrum (fig 5) showed at a high field two inverted signals (62.45 and 63.65ppm) assigned to methylene carbon (C-6) of the sugar residues. The ^{13}C-DEPT NMR experiment was used to identify the methylene (CH_2) group signals of the carbon atoms bearing two protons which have opposite amplitude to the CH and CH_3. Resonances were assigned with the aid of literature data [38, 39, 40]. Based on the monosaccharide composition and NMR spectroscopy, residue A was assigned $\alpha - D - galactose$ and B was assigned $\beta - D - mannose$.

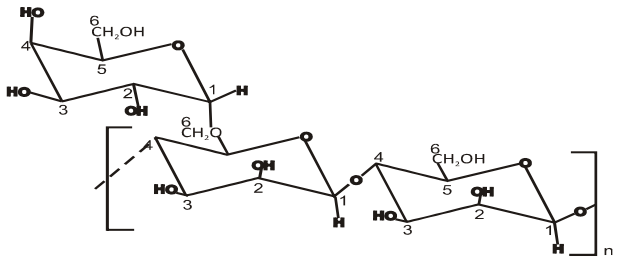
Presence of both α –and β-linked residues was identical with the result from FTIR. The intensive ^1H and ^{13}C anomeric signals of residue A at 5.40ppm (fig 7) and 98.8ppm (fig 4) corresponds to anα –linked sugar residue. The proton assignment of residue A (from H-1 to H-5; 5.42, 3.58, 3.75, 4.00 and 3.95ppm) was obtained from ^1H-^1H COSY spectrum (Fig 7, Table 1). The cross peak δ5.4/3.58 and δ3.58/3.75 were detected in ^1H-^1H COSY (correlated spectroscopy) spectrum. Since δ5.4 corresponded to H-1 of residue A, δ3.58 and δ3.75 were assigned H-2 and H-3 of residue A respectively. This assignment was also supported by the well resolved peaks in ^1H-^1H TOCSY (Total correlated spec) spectrum (fig 8). The anomeric proton and carbon signal of sugar residue B centered at 4.7ppm (fig 7) and 101.98ppm (fig 4) corresponding to a β –linked sugar residue. The proton assignment of residue B was achieved in ^1H-^1H COSY spectrum (fig 7). The chemical shits of H-1, H-2, H-3, H-4 and H-5/H-5^1 were 4.7, 3.42, 3.63, 3.79 and 4.1/3.44ppm respectively. All the assignment matched perfectly with ^1H-^1H TOCSY spectrum (Fig 8, Table 1) which showed well resolved cross peak of residue B (4.71, 4.10, 3.62, 3.80 and 3.75ppm).[36]. The corresponding chemical shift of ^{13}C were assigned in the ^1H-^{13}C HSQC (Heteronuclear single quantum correlation) spectrum starting with the residue A unequivocal ^{13}Cassignment (fig 9, Table 1) which were assigned as 98.7, 71.9, 73.0, 74.8 and 76.0ppm for C-1, C-2, C-3, C-4 and C-5 respectively. The H-1 of

residue A at 5.4ppm showed a cross peak at C-1 of residue A at 98.7ppm. Also, based on the proton chemical shift, the ^{13}Cchemical shift of residue B were completely assigned as 102.1, 77.2, 73.5, 77.1 and 75.2ppm for C-1, C-2, C-3, C-4 and C-5 respectively.[19,20,36].The cross-peak at 5.40/4.7ppm and 3.62/4.01 were detected in ^{1}H-^{1}H COSY spectrum (fig 7) since 5.40ppm corresponded to H-1 of residue A, 3.58ppm, and 3.75ppm were assigned to H-2 and H-3 of residue A respectively[36,37].Similarly, along with ^{1}H-^{1}H TOCSY spectrum (fig 8), the resonance at 4.0, 3.72 and 3.25ppm were assigned to H-4, H-5 and H-6 of residue A. The strong cross-peaks at 98.87/5.40ppm in ^{1}H-^{13}C HSQC spectrum (fig 9) were the characteristics of α –D-galactose, indicating that residue A might be galactopyranosyl.[4,14,19,20].C-4 of residue B has been found to contain two resolved signals corresponding with the H-1 signal of B (fig 7) showing two signal at 4.65 and 4.68ppm which suggest about the attachment pattern of the side residue A chains. It shows that the gum has a higher proportion of contigious residue B with one residue A unit. The resonance of residue B units is observed as doublet at 62.5ppm. These results were further confirmed by the inter and intra-correlation cross peaks of residue A and B in ^{1}H-^{13}C HMBC (Heteronuclear multiple bond corrrelation)spectrum (fig 10), in which the cross peak of H-1 with C-2, C-2 with H-3, C-3 with H-2, C-3 with H-4 and H-3 with C-4 were all tagged (fig 10). The ^{1}H-^{13}C HMBC experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity[36]. In HMBC unambigous glycoside linkages and sequences of the sugar residues were established through long range C-H correlations as shown in fig 22. In the HMBC spectrum (fig 10), two cross-peaks were observed for residue A at C-1 (98.7ppm), H-1 (5.4ppm) and C-1 (102.1ppm) and H-1 (4.7ppm) for residue B. Tischer*et al*[40] concluded that C-1 of galactose was linked to C-6 of mannose, which guided us to infer that the resonance at 63.5 and 3.75 might belong to C-6 and H-6 of galactopyranosyl unit which was further verified by comparing with other previous published data[36].There is spectra evidence of galactopranosyl unit in the structure of the polysaccharide. The C-6 (63.25ppm) is linked to the proton that appears at 3.70ppm and is also related with three linkages, to the proton at 4.21ppm which is linked directly to C-4 (68.23ppm). Accordingly, the H-1 of residue A at 5.40ppm is coupled to ^{13}C resonance at 62.5ppm (C-6 of residue B). While the H-4 residue B at 3.85ppm is coupled to the resonance at 102.1ppm (C-1 of residue B). This is also supported by another cross peak at 3.48 (H-6 of residue B) which is coupled to the ^{13}C resonance at 98.89ppm (C-1 of residue A).

Examining the crosspeaks of both anomeric protons and carbons of each sugar residues in ^{1}H and ^{13}C, 1D and 2D spectra, both inter-and intra-residual connectivities were evident. Cross peak between H-1 (4.7ppm) of residue B and C-4 (77.1ppm) of residue B; H-1 (5.4ppm) of residue A and C-6 (63.6ppm) of residue B were observed, indicating $\alpha-D-$galactopyranosyl unit is linked to a $\beta-D$-mannopyranosyl units linked to each other through 1,4-0-glycosidic bond as the main chain in the structure. Based on the results of the monosaccharide composition and NMR spectroscopy, residue A was assigned $\alpha-D-$galactose while residue B was assigned$\beta-D$-mannose. The result obtained by 1D and 2D NMR analysis indicated that the polysaccharide was a galactomanan with a chain of D-mannopyranosyl residues linked $\beta-1\rightarrow4$ which carried alternatively $\alpha-D-galactopyranosyl$ residue at 0-6 of a mannose unit. The following structure was proposed.

$$\begin{array}{c} \alpha-galp \\ 1 \quad\quad \downarrow \\ 6 \end{array}$$
$$\rightarrow 4)-\beta-D-Manp-(1\rightarrow 4)-\beta-D-Manp(1\rightarrow$$

The sugar residues ratio were directly from the 600MHz ^{1}H NMR relative areas of the signals for ^{1}H ($\alpha-galactose$) and ^{1}H ($\beta-Mannose$) and resulted in a Man/Gal = 2.65:1.00, being such value closely related to the value of 2.1:1.00 obtained by vieiro*et al*[38] for other galactomannan gums collected in a different environment.



Conclusion

Sweitenia mycrophylla(Mahogany) gum has been extractedfrom the bark of Mahogany tree and characterized. This was demonstrated using SEM,XRPD,FTIR and NMR. This galactomannan could be employed in food,, cosmetic and pharmaceutical industries. Materials with such structural properties have therefore been used as stabilizer and suspending agents in food s ,cosmetics

and in liquid or solid dosage forms.The relative abundance and easy availability of *Sweitenia mycrophylla* gum may reduce cost and save foreign exchange in Nigeria.

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