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Research Article

Physicochemical and structural characterization of *Sweitenia mycrophylla* gum

Adeyanju O*[1], Lajide L[2], Ajayi OO[3], Amoo IA[4], Plavec J[5]
[1]Department of Chemistry, University of Jos, Nigeria
[2,3,4]Department of Chemistry, Federal University of Technology, Akure, Nigeria
[5]Slovenia NMR Centre, National Institute of Chemistry, Hajdrihova, Ljubljana, Slovenia

***Corresponding author**
Adeyanju O
Email: adeyanju.olusola@yahoo.com

Abstract: *Sweitenia mycrophylla* gum (*Mahogany* gum) was characterized using standard procedure. The acute toxicity test of the gum on albino mice was determined using standard method. Physicochemical properties such as moisture content, ash content, protein, pH, viscosity, Refractive index, water holding capacity, Specific rotation, swelling index and emulsifying capacity were evaluated. Scanning electron microscopy (SEM), X-ray powdered diffractometry (XRDP), Fourier Transform Infrared (FTIR) and ^{13}C Nuclear magnetic resonance (NMR) spectroscopy were used to characterized the gum. Results revealed that the LD_{50} of the gum in mice is greater than 5000mg/kg. Physicochemical parameters shows moisture content to be 5.70±0.15%, ash content 6.8±0.52%, protein 1.43±0.51%, pH 7.80±0.5, viscosity 28.40±0.30, refractive index 1.34±0.01, water holding capacity 67.20±0.28%, specific rotation -42^0, swelling index 15.20±0.40 and emulsifying capacity 43.60±0.25%. SEM analysis suggested the gum has irregular particle size. The XRPD pattern of the gum showed no sharp peak confirming 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 with mannose/galactose ratio of 2.60. Due to its good physicochemical properties and 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, 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 the 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 (UK) 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 (lOg) 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 (UK) and later sieved using a bin (mesh size-250microns) kept in labeled plastic container for subsequent analysis.

Physicochemical analysis of *Sweitenia mycrophylla* gum

The moisture content was determined by drying to constant weight at 105^0C (in a muffle furnace) [13]. Nitrogen content of the gum was determined by kjeldah method [13] using Gerhadkjeldotherm and vapodest system (Germany). Crude protein was calculated from the nitrogen content using the conversion factor of 6.25. pH, relative viscosity, water holding capacity, emulsifying capacity, specific rotation and swelling index were measured according to [13].

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.

Determination Of Acute Toxicity (LD_{50}) of *Sweitenia mycrophylla* Gum.

The study was carried out using modified Lorke (1983) method. It was conducted in the animal facilities of the faculty of pharmaceutical sciences, department of pharmacology, University of Jos, Nigeria, following the principles of good laboratory practices and animal handling in the NIH guide for the care and use of laboratory animals (NIH, 1985). Thirteen mice (male and female) were utilized in the study. The animal weighted between 20.02 to 20.04kg at the beginning of the study. They were allowed to acclimatized after procurement for seven days before the test was commenced. The animals were fasted overnight before the test and had access to only water for the first 4hours and the administration of the extract. In the first phace, three groups, each consisting of three mice randomly selected was formed. The first, second and third groups received 10,100 and 1000mg/kg of gum dispersion in distilled water, respectively administered via intra-gastric cannula. They were observed frequently on the day of treatment. Hereafter, observations and weighing were carried on for 3 days. In the second phase, doses of 1500, 3000and 5,000mg/kg of the gum extract, respectively were administered to another three groups of one mice each. They were observed as above. After the three days of initial monitoring in each phase, the animals were further observed for any form of abnormal behavior for another 21 days before the study was terminated.

RESULTS AND DISCUSSION

Table 1 shows the physicochemical parameters. The swelling capacity in water expressed in percent was 15.20$\pm$0.40 (Table-l).The result shows that the gum has a high swelling index compared to a standard gum arabic with swelling index of 8.5% [14], The gum may perform well as binder and matrix agent. The relatively high swelling index at pH = 7.8 implies

that the gum may be useful as a matrix former in controlled drug-release. Swelling is a primary mechanism in diffusion controlled release dosage form [15]. The pH measurement shows that the gum solution was slightly acidic. The pH value of 7.8±0.50 (Table 1) is in good agreement with reported pH values for gum arabic and *Anarcadiumoccidentale* .L (Cashew gum) by several authors [7, 16, 17]. The acidity of the plant gum is not unexpected since many of them are known to contain salts (Ca, Mg, K, Na and Fe) of acidic polysaccharides, the activity of which is due to uronic acids in their structure [18]. The pH of an exudate gum is an important parameter in determining its suitability in formulations since the stability and physiological activity of most preparations depend on pH [11, 19, 20]. Moisture content of the gum was 5.70±0.15 (Table 1) and compares favourably with the minimum standards (< 15%) for good quality gum according to European specification [14]. This suggests its suitability in formulations containing moisture sensitive drugs. Given suitable temperature moisture will lead to activation of enzymes and the proliferation of microorganisms, thereby affecting its shelf life. It is important to investigate the importance of an exudate gum, for industrial application lies not only on the cheap and easy availability of the material but the optimization of production processes such as drying, packaging and storing [21]. The total ash value of the gum was found to be 3.84±0.52 (w/w) (Table 1) this falls within the acceptable level of less than 4% for gum arabic reported by [22] for food and pharmaceuticals. Ash content is an important property considered as a purity parameter in gums. The very low values of ash show that *Sweitenia mycrophylla* exudates gum has a good quality of mineral content with low level of contamination [22]. Relative viscosity of gum solution at (30^{o}C) was found to depend on gum concentration [2], The relative viscosity of the gum was found to be 28.40±0.30 (Table 1). Molecular association in fluids greatly influences their rheological behaviors. Increase in viscosity with concentration is probably due to increase in the molecular weight of the gum.[23, 19].

The value for protein content obtained 1.43 (Table 1) fairly agrees with that of acacia gum (0.5 - 2.7%) [19].The moderate protein content in the gum sample is noteworthy. This is because protein content is known to have effects on the emulsifying behaviour of gum with the best emulsion capacity and stability found in gums with higher nitrogen content [24, 14], The specific rotation of the aqueous gum was found to be optically active (-25.46^{o}) (Table 1). This shows that the sugar present is laevorotatory. Emulsifying capacity was determined in form of Turbidity.

The emulsifying capacity was found to be 43.60±0.25 (Table 1). A higher turbidity is an indication of a better emulsion capacity. In, addition to protein content of gum, the typical molecular structure and high molecular weight are responsible for good emulsifying properties [25]. A similar correlation between molecular weight and emulsion stability of gum arabic was reported by [14].

Refractive index of the gum sample was found to be 1.34±0.01 (Table 1). This may prove to be a qualifying index for this gum. Water holding capacity of the gum was found to be 67.20±0.28. The water holding capacity of gum is the ability to hold water and does not only depend on the functional group of carbohydrate that are hydrophilic but also on the protein present in the gum, since they also contain functional groups that are able to bind with water molecule. Thus addition of other substance can be accommodated and this may improve texture of the overall product [26, 27]

In the toxicological studies of *Sweitenia mycrophylla* gum, no adverse sign of toxicity or death was observed at all the doses used for the study. The oral lethal Dose (LD_{50}) of *Sweitenia mycrophylla* gum in albino mice was thus estimated to be greater than 5000mg/kg body weight. The absence of adverse effects and death at the dose of up to 5000/mg/kg used for this study suggests that *Sweitenia mycrophylla* gum is practically non-toxic in mice orally.

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, 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, 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.

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, anomeric carbons 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-NMR 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 $\beta - D - mannose$.

Table 1: Physicochemical characteristics of *Sweitenia mycrophylla* Gum

Moisture content (%)	5.70±0.15
Ash content (%)	3.84 ±0.52
Protein content (%)	1.43 ± 0.51
pH	7.80 ± 0.50
Relative viscosity	28.00± 0.30
Refractive index	1.34 ± 0.10
Water holding capacity (%)	67.20 ± 0.28
Specific rotation (^{0})	-42 ± 0.20
Swelling index (%)	15.20 ± 0.20
Emulsifying capacity (cm^{-1})	43.60 ± 0.25

Table 2: Stage I of Acute Toxicity Test of *Sweitenia mycrophylla* Gum

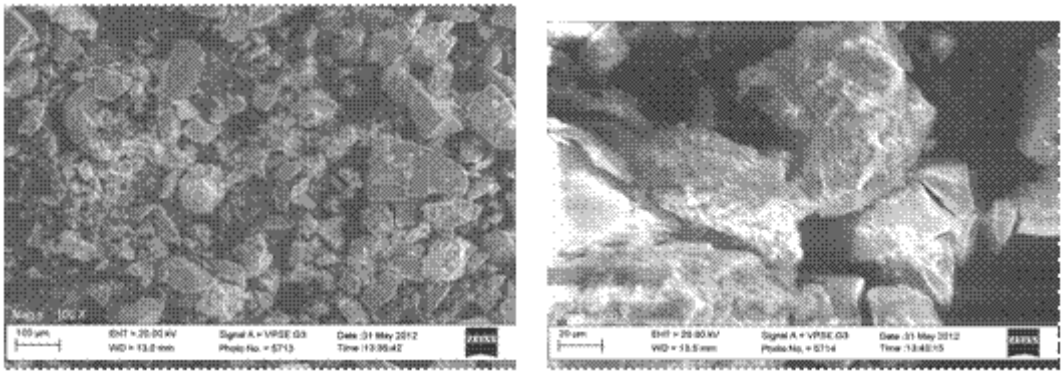
Dose (mg/kg)	Dose Administer (mg)	Stock Concentration (mg/ml)	Volume administered (ml)	Average weight of animals (g)
10	0.2	0.5	0.02	20.00±0.08
100	2	5	0.02	20.00±0.10
1000	20	50	0.02	20.00±0.07

Table 3: Stage II of Acute Toxicity Test of *Sweitenia mycrophylla* Gum

Dose (mg/kg)	Dose Administer (mg)	Stock Concentration (mg/ml)	Volume administered (ml)	Average weight of animals (g)
1500	30	250	0.12	22.00±0.10
3000	60	250	0.24	22.00±0.10
5000	100	250	0.40	22.00±0.8

Table 4: ^{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
β-D-Mannopyranosyl	102.1	77.20	73.50	77.10	75.20	62.50



100X **500X**

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

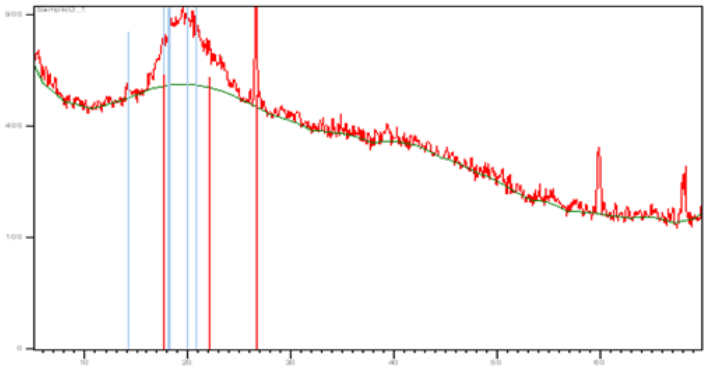


Fig 2: XRD of *Sweitenia mycrophylla* Gum

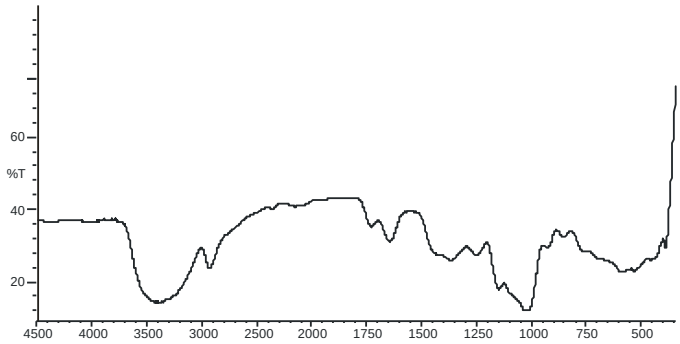


Fig 3: FTIR of *Sweitenia mycrophylla* Gum

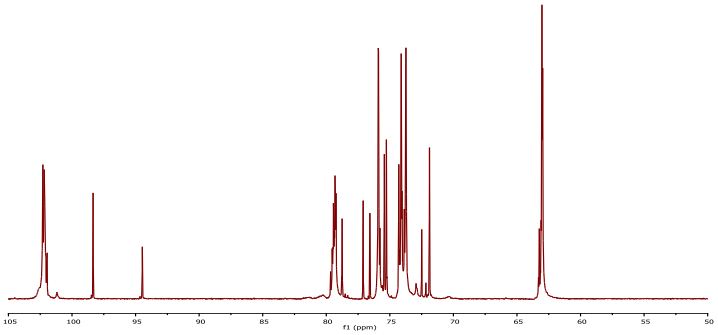


Fig 4: $^{1}D^{13}C$ NMR Spectrum (600MH$_Z$) of unmodified *Sweitenia mycrophylla* gum (10mg in 700μL D_20, 60^{0}c) Referenced to TMSP

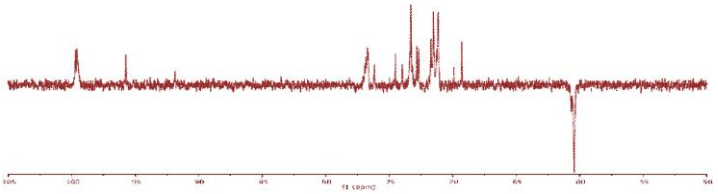


Fig 5: ^{13}CDEPT-135 Spectrum (600MH$_Z$) of unmodified *Sweitenia mycrophylla* gum S (10mg in 700μL D_20, 60^{0}c) Referenced to TMSP

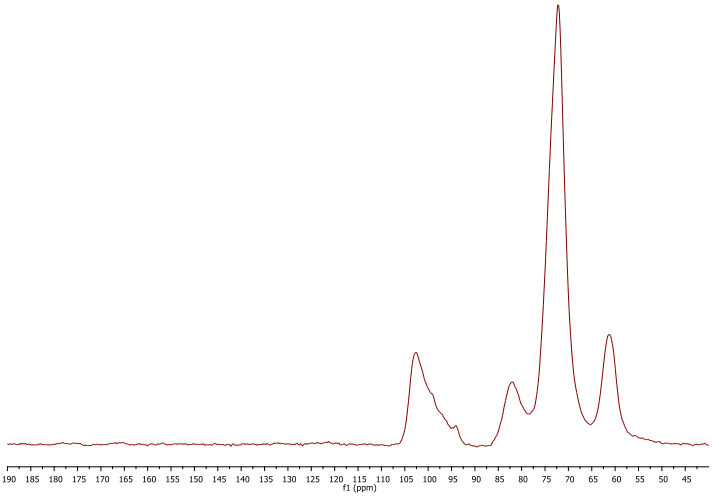


Fig. 6: ^{13}C-Solid state NMR of *Sweitenia mycrophylla* gum

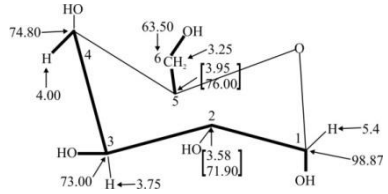


Fig-7 : Signal Assignment of α-D-galactopyranosyl Unit Residue Present in *S. mycrophylla*

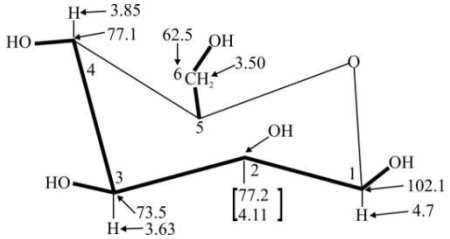


Fig-8 :Signal Assignment of β-D-mannopyranosyl Residue Unit Present in *S. Mycrophylla.*

CONCLUSION

Sweitenia mycrophylla(Mahogany) gum has been extracted from the bark of Mahogany tree. The gum shows good physicochemical characteristics during characterization. This was demonstrated using SEM,XRPD,FTIR and NMR. This properties could be employed in food, cosmetic and pharmaceutical industries. Materials with such 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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