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Characterization and Utilization of *Sweitenia mycrophylla Exudate* Gum as an Excipient in Drug Formulation.

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Abstract: Recently, the development and utilization of polysaccharides isolated from natural sources have attracted increasing attention in biochemistry, pharmaceautical and food chemistry due to their sustainability, biodegradability and biosafety. *Sweitenia mycrophylla* gum tree is a plant growing naturally in the Nigeria forests unnoticed and unutilized. This gum is yet to be commercially exploited as the physicochemical properties of this gum are yet to be characterized. Due to advances in drug delivery technology, natural excipients are included in novel dosage form to fufil specific function. The present study sought to characterize and utilize *Sweitenia* gum (*S.mycrophylla*) as excipient in drug formulation. The acute toxicity test of the native gum on albino mice was determined using Lorkes method followed by histopathological study. Physicochemical properties such as moisture content, Ash content, Protein, pH, Viscosity, Refractive index, water holding capacity, Specific rotation, swelling index and emulsifying capacity were also evaluated using standard methods (AOAC). Scanning electron microscopy (SEM), X-ray powdered diffractometry (XRD), Fourier Transform Infrared (FTIR) and 1D and 2D Nuclear magnetic resonance (NMR) spectroscopy were used to characterized the native gum. The results of this research indicate that the LD_{50} of the gum in mice is greater than 5000mg/kg and the histopathological results shows no pathological lession on the organs tested. 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 suggests that the native gum has irregular particle size and the XRD pattern of the native gum indicate a completely armor lphous structure. FTIR spectrum showed band characteristics of O-H (3650-3500 cm^{-1}), C-O-C (1632 cm^{-1}), -COO (1429 cm^{-1}) groups. NMR spectroscopy (1D and 2D) indicate that the gum is a galactomannan type polysaccharide with mannose/galactose ratio of 2:1. The gum granules possessed good flow properties with Hausner ratio of 1.10 to 1.30 and Carr's index of 9.0 to 23.0%. Tablets prepared with 4-8% w/w gum had hardness ≥4kg while tablets prepared with 6-8% w/w binders had friability of <1% comparable to that prepared with 4% (w/w) *gelatin BP*. The disintegration time of the tablet was <15 min. The tablets had fast dissolution in aqueous media with >94% drug dissolution in 45 min.

Keywords: *Sweitenia mycrophylla gum, excipient, SEM, XRD, NMR, FTIR.*

1 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 biosafety [1]. Natural 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 bioadhensive drug delivery systems [9]. Polysaccharide gums used in the pharmaceutical and food

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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 was obtained from the back of the tree. *S. 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 physicochemical characteristics, structural studies and utilization of the gum from *S. mycrophylla* tree. Hence, this research aims at investigating the physicochemical, toxicological, structural studies and utilization as excipient of the gum from *S.mycrophylla* in order to evaluate its potential industrial applications, mostly in food and pharmaceutical. The results of this research is likely to highlight the physicochemical properties, structural information and the binding ability in tablet formulation of the gum in order to amplify the possibility of the gum applications.

2 Materials and Methods

2.1 Collection and preparation of gum

Gum was collected from the bark of *S. 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 forestry Department, Federal University of Technology, Akure.

Gum was tapped from the bark of the tree. The dried, cleaned gum sample was 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.

2.2 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 (UK) and later sieved using a bin (mesh size-250microns) kept in labeled plastic container for subsequent analysis.

2.3 Physicochemical Analysis of S. 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].

2.4 Preparation of Granules

Different batch of granules comprising of paracetamol (25.7% w/w), lactose (63.8% w/w), maize starch (8.2 w/w,) talc (2.0% w/w) and magnesium stearate (0.3% w/w) were prepared using the wet granulation technique. The powders, excluding talc and magnesium stearate were dry-mixed for 5 min in a planetary mixer (Model A120, Hobart Company, UK) and massed with the appropriate amount of binder solution (MG: 10% w/v, 20 % w/v, 30% w/v, 40% w/v; gelatin: 20 % w/v) equivalent to 2% w/w, 4% w/w, 6% w/w and 4% w/w gelatin in granules.

The damp mass was screened through 2.36 mm sieve and dried 60^0C for 1 h in a hot air oven. The dried granules were screened through 1.00mm sieve and lubricated with talc and magnesium stearate. The granules were stored in plastic containers for further evaluation and compression into tablets.

2.5 Evaluation of Granules Properties

The bulk density (pB) was determined by slowly pouring the granules into a 10ml graduated glass cylinder and the excess granules leveled off with a spatula. The bulk density was obtained by dividing the weight of granules by the volume. The tapped density (pT) was determined by tapping a graduated glass cylinder containing a known weight of granules 50times from a height of 2.5 cm on a wooden bench top. The tapped density was obtained by dividing the weight of granules by the minimum volume attained after tapping. The Hausner ratio was calculated as the ratio of the tapped density to the bulk density (pT)/pB).

Hausner ratio values ~ 1.2 portrays low inter-particle friction and good granule flowability while values > 1.6 signifies cohesive properties and poor granule flowability. The Carr's index (C) is used to predict the compressibility and ease of flow of granules and was calculated as: C = (pT-pB /pTx 100). For Carr's index, values ≤ 16% indicates good flowability while values > 23% demonstrates poor flowability

2.6 Production of Tablets

The different batch of granules produced were compressed into tablets using a lubricated single punch tableting machine (DP30 tablet press, Pharmao industries Co. Ltd., China) fitted with a concave punch and die set. Tablets of weight ~ 400 mg and diameter ~ 11mm, containing ~ 100 mg paracetamol were prepared.

2.7 Determination of Tablet Properties

The mean tablet weight was determined by weighing twenty (20) randomly sampled tablets individually on a precision balance (Mettler Toledo, USA) and the average determined. The friability of the tablets was determined with a SOTAX F2 Friabilator USP (SOTAX AG, Switzerland). Twenty (20) tablets were randomly selected, dedusted and weighed on a precision balance. The tablets were placed into the transparent drums of the fraibilator and set to rotate at 100 revolutions. The tablets were de-dusted after the test, weighed and the difference in weight expressed as a percentage of the initial weight.

Tablet hardness which is the force required to diametrically cause a tablet to fracture was determined using a Dr. SchleunigerPharmatron tablet hardness tester (Modle 5Y, Switzerland). The test was repeated twice and the mean recorded. Disintegration test was carried out in distilled water with the ElectrolabDisintergrating Tester (USP) ED-2L (Electrolab, India), six tablets were placed in the cylindrical glass and the time taken for the tablets to disintegrate was recorded as disintegration time.

2.8 In-vitro Dissolution Tests

Dissolution tests were carried out with an Erweka Dissolution Apparatus (Type DT6, Ereweka GmbH, Heusenstamm, Germany). The dissolution test conditions used were: 900 ml 0.1 M HCL dissolution medium set at 37 ± 0.5^0C, and a paddle speed of 100rpm. 5 ml samples were withdrawn at 5, 10, 15, 45 and 60 min intervals and replaced with fresh medium pre-warSmed at 37± 0.5^0C.

Samples were filtered (0.45µm HA membrane filters), diluted and analysed by UV spectrophotometry (Model Cecil CE 8020, Cecil instruments UK) at a wavelength of 278nm, using a 1cm cell and 0.1M HCL as blank solution. The amount of drug released was determined from regression data (y=381.07 x + 0.0271, R^2= 0.9994) obtained from a calibration plot of paracetamol powder in 0.1M HCl.

2.9 Microstructure Studies by SEM

Morphological features of the gum were 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.

2.9.1 X -Ray Powder Diffraction (XRD).

X-ray diffraction patterns of the gum wasanalysed 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 5ºC to 40ºC, 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.

2.9.2 Fourier Transform Infrared (FTIR) 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 *S. mycrophylla* gum were recorded in an NMR (600 MHz) spectrometer (Agilent technologies, America). The sample (10mg) was dissolved in 700μL at 70^0C with continuous 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.

2.9.3 Determination of Acute Toxicity (LD_{50}) of S. 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.

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 acclimatize 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, 3000 and 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.

3 Results and Discussion

Fig 1: Swietenia mycrophylla crude exudate Gum.

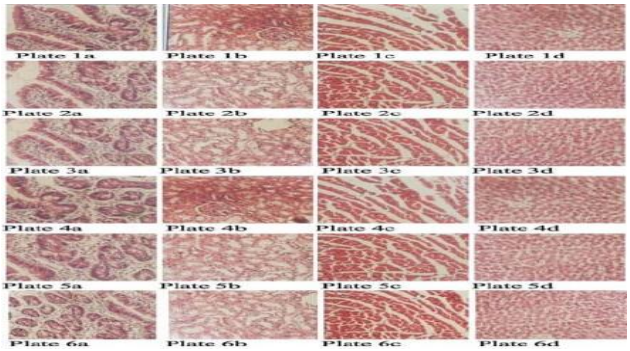
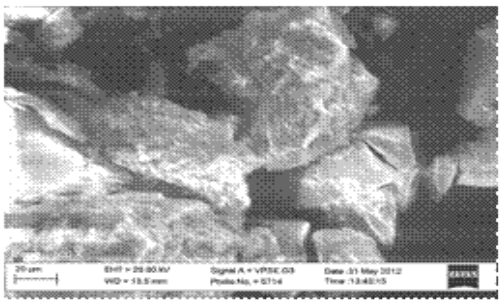


Fig 2: Histopathological Examination



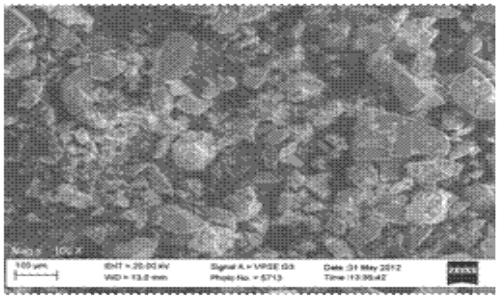


Fig 3: SEM of *Sweitenia mycrophylla* crude exudate gum at 100x and 500x

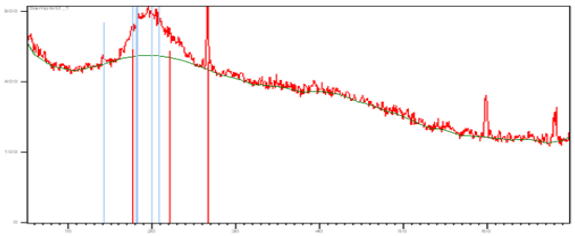


Fig 4: XRD of *Sweitenia mycrophylla* Gum

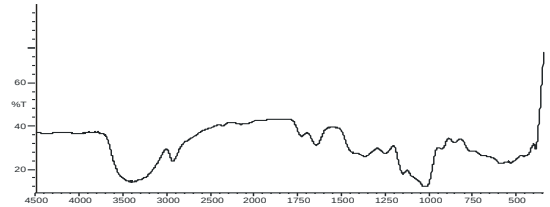


Fig 5: FTIR of *Sweitenia mycrophylla* Gum

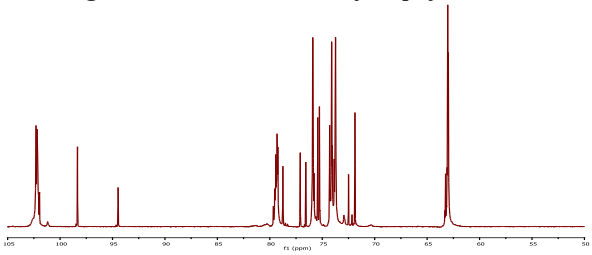


Fig 6: ^{13}C Spectrum of *Sweitenia mycrophylla* gum (10mg in 700μL D_20, 60^0c) Referenced to TMSP.

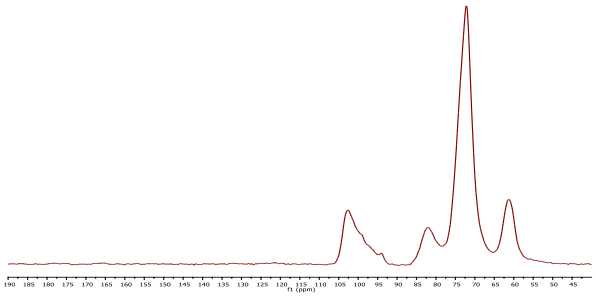


Fig 7: ^{13}C-solidstate NMR of *Sweitenia mycrophylla* gum

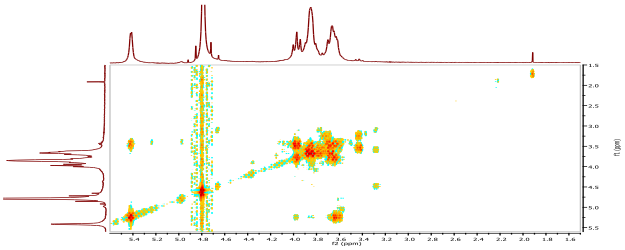


Fig 8:^{1}H-^{1}H COSY of Sweietenia mycrophylla gum

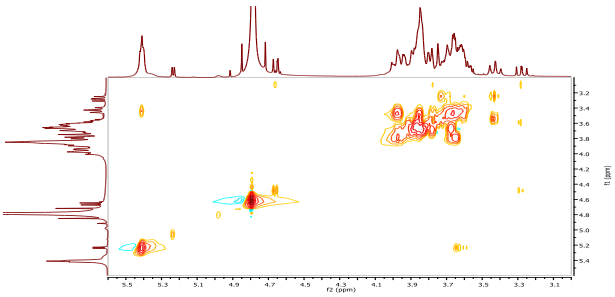


Fig 9:^{1}H-^{1}H TOCSY of Sweitenia mycrophylla gum

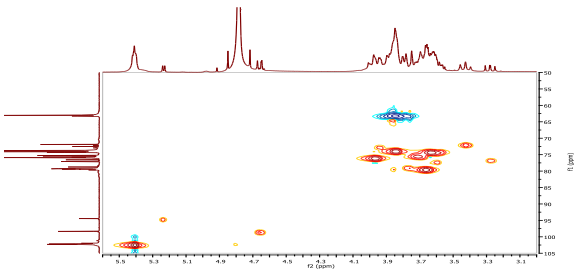


Fig 10:^{1}H-^{13}C HSQC of Sweitenia mycrophylla gum

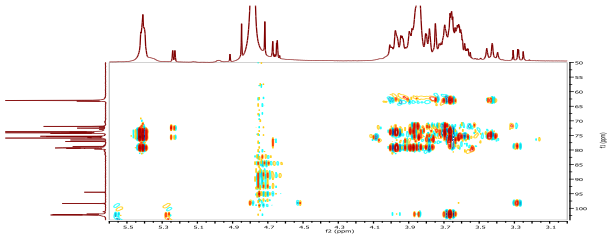


Fig 11:^{1}H-^{13}C HMBC of Sweitenia mycrophylla gum

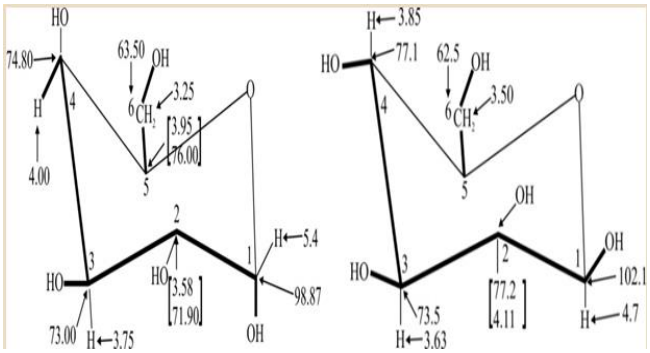


Fig. 12: Signal Assignment of α-D-*galactopyranosyl* and β-D-*Mannopyranosyl*Unit Residue Present in *S. mycrophylla*

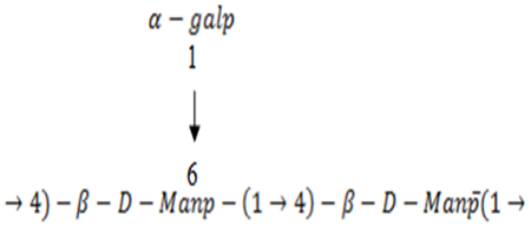


Fig 13: Skeleton of Chemical Structure of *S.mycrophylla*

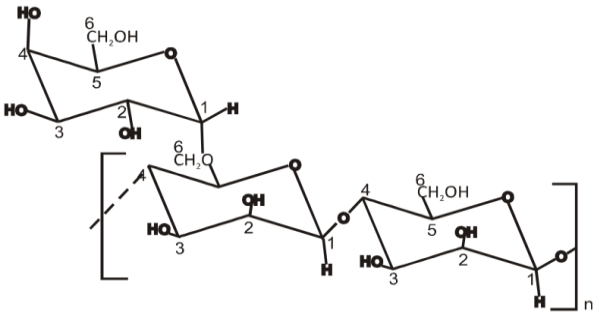


Fig. 14: Galactomannan from *S.mycrophylla* Gum

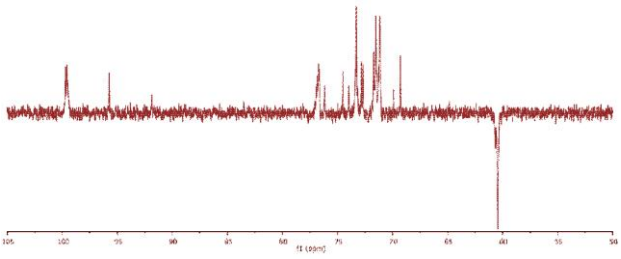


Fig15: ^{13}C -DEPT-135 Spectrum of *Sweitenia mycrophylla* gum (10mg in 700μL D_20, 60^{0}c) Referenced to TMSP

4 Discussion

Table 1 shows the physicochemical parameters. The swelling capacity in water expressed in percent was 15.20±0.40 (Table l).

Table1: Physicochemical characteristics of *S. 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

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 *Anarcadium occidentale*.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 favorably 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

Table 2: Flow properties of paracetamol granules prepared with S.mycrophylla gum as binding agent.

Concentration of Gum (% $^w/_w$)						
Parameter					**Acacia Gum**	**Gelatin BP**
	2	**4**	**6**	**8**	**4**	**4**
Bulk density (g/cm^3)	0.581±0.05	0.581±0.09	0.598±0.03	0.590±0.07	0.575±0.02	0.567±0.06
Tapped density (g/cm^3)	0.755±0.09	0.692±0.02	0.657±0.05	0.654±0.04	0.657±0.08	0.705±0.03
Hausner ratio	1.299±0.06	1.191±0.07	1.099±0.02	1.108±0.01	1.143±0.09	1.243±0.02
Carr's index (%)	23.000±0.08	16.000±0.03	9.000±0.06	9.800±0.05	12.481±0.07	19.574±0.08

Table 3: Stage 1& 2 of Acute Toxicity Test.

Dose (mg/kg)	Dose Administered (mg)	Stock concentration (mg/ml)	Volume administered (ml)	Number of animal Used	Number of death	Number of Survival	Average weight of animals (g)
10	0.2	0.5	0.02	3	0	3	20.00±0.1
100	2	5	0.02	3	0	3	20.00±0.1
1000	20	50	0.02	3	0	3	20.00±0.1
1500	30	250	0.12	3	0	3	20.00±0.1
3000	60	250	0.24	3	0	3	20.00±0.1
5000	100	250	0.40	3	0	3	20.00±0.1

low level of contamination [22]. Relative viscosity of gum solution at (30°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°) (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].

Table 4: Physical properties of paracetamol tablets formulated with *S.mycrophylla* gum as binder.

Parameter	Concentration of Gum (% $^{w}/_{w}$)					
					Acacia Gum	Gelatin BP
	2	4	6	8	4	4
Tablet Weight (mg)	360±1.5	400±2.4	412±4.00	415±3.1	418±3.1	416±5.0
Hardness (kg)	2.4±0.5	4.0±0.5	4.3±0.5	6.5±0.4	5.0±0.3	4.7±0.2
Friability (%)	3.0±0.3	2.35±0.6	2.1±0.5	2.1±0.2	2.03±0.02	2.2±0.1
Disintegration time (Min)	1.5±0.1	1.3±0.2	3.2±0.4	9.2±0.3	9.7±0.3	8.8±0.5
Dissolution time, d_{45} (Min)	110±3.4	85.6±2.2	97±4.3	95±3.6	91.7±2.6	90.0±3.2

Refractive index of the gum sample was found to be 1.34±0.01 (Table 1). This may prove to be a 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 (Table 3). 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.This was also comfirmed in the histopathological studies in figure 2 as there were no pathological changes in the organs tested(kidney,liver,heart and intestine).

Scanning Electron Microphotographs (SEM) of the gum sample is depicted in Figure 3 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 shows some wing-shaped particles which points to the amorphous nature of the powder. Thus this micrograph further confirms the result from XRPD analyses (fig. 4). 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 4) with weak peaks, confirming its almost complete amorphous nature. The diffraction pattern of *S. mycrophylla* shows halo peak (fig 4) 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 5. The finger print region of the spectrum consists of two characteristic peaks between 700 and 1316 cm^{-1}, attributed to the C-O 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 indicates 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. 7. The spectrum give line widths which are typical of an amorphous 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 suggests 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 *S. mycrophylla* gum (fig 6), signals from anomeric carbons appear in the 90 to 105ppm regions while the non anomeric 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 *S. 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 (fig15).

The resonances of the carbon atoms were well resolved (fig 12) 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 (α-D-Galactose). The facts are almost identical with gums of other origin. [34,36,37]. The ^{13}C-DEPT NMR 135^0 spectrum (fig 15) 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 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 8).

The cross peak δ 5.4/3.58 and δ 3.58/3.75 were detected in ^1H-^1H COSY spectrum. Since δ 5.4 corresponded to H-1 of residue A, δ 3.58 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 spectrum (fig 9). The anomeric proton and carbon signal of sugar residue B centered at 4.7ppm (fig 8) and 101.98ppm (fig 6) corresponding to a β −linked sugar residue. The proton assignment of residue B was achieved in ^1H-^1H COSY spectrum (fig 8). 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 9) 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 spectrum starting with the residue A unequivocal ^{13}C assignment (fig 9) 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 [17,18,19,20,21,22].The cross-peak at 5.40/4.7ppm and 3.62/4.01 were detected in ^1H-^1H COSY spectrum (fig 8) since 5.40ppm corresponded to H-1 of residue A, 3.58ppm, and 3.75 ppm were assigned to H-2 and H-3 of residue A respectively[36,37].Similarly, along with ^1H-^1H TOCSY spectrum (fig 9), 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 ^1H-^{13}C HSQC spectrum (fig 10) were the characteristics of α −D-galactose, indicating that residue A might be galactopyranosyl[3,4,13,14,16].

C-4 of residue B has been found to contain two resolved signals corresponding with the H-1 signal of B (fig 10) 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 contiguous 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 ^1H-^{13}C HMBC spectrum (fig 11), 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 11). The ^1H-^{13}C HMBC experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity [36].

In HMBC unambiguous glycoside linkages and sequences of the sugar residues were established through long range C-H correlations as shown in fig 12. In the HMBC spectrum (fig 11), 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*[16] 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[24].

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 cross peaks 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 $\beta - 1 \rightarrow 4$ which carried alternatively $\alpha - D - galactopyranosyl$ 1 residue at 0-6 of a mannose unit. The following structure was proposed.. 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 $\beta - D - mannose$.

$$\alpha - galp$$

1

↓

6

$$\rightarrow 4) - \beta - D - Manp - (1 \rightarrow 4) - \beta - D - Man\bar{p}(1 \rightarrow$$

The flow characteristics of granules produced with different concentration of gum is shown in (Table 2). The different batch of the granules exhibited good flow properties with Hausner ratio and Carr's index values of 1.10-1.30 and 9.0-23% respectively. There was however, no direct correlation between the flow properties of granules and the gum concentration used. Table 4 provides details on the physical properties of the paracetamol tablets prepared using *S.mycrophylla* gum as binder compared to *Acacia* and gelatin. All the tablets prepared has uniform tablet weight. Tablet hardness increases with increase gum concentration. Table 4. The hardness of tablets containing 2% w/w gum were <4kg while that containing 4-8% w/w were ≥4kg.

The friability of the tablets decrease with increase in gum concentration. Tablets prepared with 2-4% w/w the gum had friability values >1% while that of 6-8% w/w had friability <1%. Thus tablets prepared with 4-8% w/w gum passed the BP tablet hardness test (British pharmacopoeia, 2007). The disintegration time of the tablets were <15 min and increase with increase in gum concentrations. All the tablets exhibited fast dissolution in aqueous media with>94% of the drug released in 45 min. The dissolution rate of the tablets decrease with increase in modified gum concentration. The fast disintegration and dissolution rate exhibited by the tablets have shown these gum are suitable for use as a binder in conventional tablets intended for fast disintegration and release in the gastrointestinal tract.

The increase in binder concentration increased the hardness and disintegration time and decrease friability values of the tablets. These findings may be attributed to gell forming properties of the gum in the tablet matrix and this is in line with the study of [3].

The higher friability values observed for paracetamol tablets compressed at lower compression forces could be due to moisture content of the granules. Report indicate that granules having optimum moisture content would result in tablets of less friable less capping tendency and good hardness and tensile strength[6].

The lower hardness value observed for paracetamol tablets could also be attributed to the same problem mentioned for tablet friability. Tablet hardness may also affect the friability due to moisture content of the gum granules.

Tablets formulation with the standard gums displayed gradual increase in disintegration with increase in binder concentration, unlike those formulated with gum. Tables 4 show the percentage of paracetamol released by all tablets formulations over 30 min intervals. Tablet formulated with the gum generally released the highest amount of drug from 5 to 25 min interval.

5 Conclusion

Sweitenia mycrophylla gum has been extracted from the bark of the tree. The gum shows good physicochemical characteristics during characterization. This was demonstrated using SEM, XRD, FTIR and NMR.The gum at 6% w/v exhibited good binding properties comparable to 4% Acacia gum and gelatin BP.This gum can be used as binders in preparation of tablets. Materials with such properties have therefore been used as stabilizer and suspending agents in food, 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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