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ACETYLATION OF *SWEITENIA MYCROPHYLLA* GUM: SYNTHESIS AND CHARACTERIZATION.

[1]O. ADEYANJU, [2]A. O EDAH [3]M.F ADESEMUYI [4]D.A BALA AND [5]J. PLAVEC
[*1,2]Department of Chemistry, University of Jos, Nigeria,
[3]Department of Chemistry, Federal University of Technology, Akure, Nigeria.
[4]Department of Science Laboratory Technology,University of Jos,Nigeria
[5]Slovenia NMR Centre, National Institute of Chemistry, Hajdrihova, Ljubljana, Slovenia
*Corresponding Author: adeyanju.olusola@yahoo.com

Abstract

Isolation of non-conventional gums has increased in the last decade; chemical modifications of these gums may produce gums with improved physicochemical and functional properties that are not available from commercial gums.Sweitenia mycrophylla gum was obtained from mahogany tree. The modification discussed include the acetylation of gum with acetic anhydride in the presence of sodium hydroxide. The resulting product was characterized by SEM, XRD,EDX, FTIR and NMR spectroscopy. The degree of acetylatation was 0.24.Physicochemical characteristics such as solubility, viscosity and swelling index of the Sweitenia mycrophylla gum and acetylated gum were also determined. The results show that the acetylated gum had higher values of solubility, viscosity and swelling index as 93.40% at 80^{o}C, 65.4cs and 46.54% respectively while the native gum had solubility viscosity and swelling index as 30.10% at 80^{o}C, 28.40cs and 15.20% respectively. Chemical modification via acetylation increased the solubility, viscosity and swelling index of Sweitenia mycrophylla gum. The experimental work provides enough evidence to exploit this natural biopolymer in food, textile and pharmaceutical industry, especially as an efficient alternative approach for the oral delivery of hydrophilic macromolecules.

Keywords: *Sweitenia mycrophylla* gum, chemical modification, acetyl groups,FTIR,NMR.

1.0 Introduction

In recent years, the development and utilization of polysaccharides isolated from natural sources have attracted increasing attention in biochemistry, pharmacology and food chemistry, due to their sustainability, biodegradability and biosafety [1].*Sweitenia mycrophylla* gum polysaccharide extracted from the bark of *S.mycrophylla* tree, a large tree reaching a height of 30-40m. Gum is produced from cuts in the bark and sells at markets in Bombay, India [34]. Due to excellent properties of gums such as solubility, viscosity, thickening, binding, stabilizing and emulsifying, they are utilize in several multibillion-dollar industries such as adhesive, cosmetic, confectionaries, paint, paper, pharmaceutical and most importantly Food [3, 24-25]. Even if gum and its derivatives are well known for a wide range of applications, like other polysaccharides, there are evidence of some drawbacks, such as uncontrolled rate of hydration, pH-dependency, solubility, thermal decomposition, low shear stress resistance, high retrogradation and syneresis [1, 4].

Chemical modification provides an efficient route not only for removing such drawbacks but also for improving physicochemical properties such as solubility, viscosity and swelling index and to introduce new properties for different applications. A number of modifications via chemical treatment can be effected resulting in products suitable for specific purposes in the food and pharmaceutical industries [5].According to [2] chemical modification of *Anacardium occidentale* gum by oxidation increases the uronic acid content of the gum from 3.7% to 38% which further increases solubility and water holding capacity. Also according [6] oxidation of

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gum generally increases their hydrophilicity and solution clearity which make them more soluble in aqueous system. Chemical modification through acetylation generally increases the emulsifying capacity which further increases swelling index and solubility.[7].Nowadays, the development of new products in gum based industries are searching for gums with different or better physicochemical and functional properties such as viscosity, solubility, low retrogradation and syneresis tendency. In recent years, substantial progress have been made in obtaining polysaccharides from non-conventional botanical sources and studying their functional and physicochemical properties [8-11].

The acetylated gum is produced by the esterification of native gum with acetyl groups. The efficiency of the reaction is affected by factors such as reagent concentration reaction time, pH, presence of catalyst, gum source and amylose/amylopectin ratio. The functional properties of the gum acetate will depend on the number of acetyl group incorporated to the glucose unit of gum molecules [12 -14].

Acetylation was selected as a chemical means of attaching pendant acetic anhydride groups due to its technical simplicity, low cost of chemical reagents and wide range applications to produce acetylated gum. The result of this research is likely to highlight the effect of acetylation on the physicochemical properties of *S.mycrophylla* gum in order to amplify the possibilities of the gum applications in food and pharmaceuticals as an emulsifier, effective binder and suspending agent in drug formulation.

The objective of this research is to prepare and characterize an acetylated *Sweitenia mycrophylla* gum in order to improve on its physicochemical characteristics and amplify the possibilities of the gum applications.

2.0 Materials and Methods

Sweitenia mycrophylla gum was collected by tapping in March, 2010 from owena forestry Ondo - State ,Nigeria. Superficial incision was made at the bark of the tree and the bark was later stripped off. After five weeks, gum was manually collected. The gum samples were dried at room temperature, cleaned, milled with Kenwood blender [UK], sieved through a mesh-size 250 microns to obtain fine – size particles, kept in labeled plastic container and stored in the refrigerator for subsequent analysis.

2.1 Purification of gum sample

Dried crude gum [10g] was stirred in cold distilled water [250ml] for 3 hours at room temperature. The supernatant was obtained by centrifugation. The supernatant was made up to 500ml and treated with ethanol [1.4v/v] in order to precipitate the carbohydrate. The material was washed again with ethanol followed by distilled water and freeze-dried.

2.2 Preparation of acetylated gum

Acetylated gum was obtained using the method reported by [13]. In brief, gum (10g) was dispersed in 50cm^3 of distilled water and then constantly stirred for 30 minutes. The slurry was adjusted to pH 8.0 with 3% NaOH. Acetic anhydride (1.2g) was then added to the slurry. After the addition of the acetic anhydride, the reaction was allowed to proceed for another five minutes. The pH of the slurry was adjusted to 4.5 with 0.5MHCl and filtered through whatman No 1 filter paper. The residue obtained was washed four times with distilled water to remove completely some acids that may be present in the product and finally air dried at room temperature.

2.3 Solubility

The solubility of gum was determined according to a standard method reported by [15]. Gum sample (10g) was suspended in 40ml of distilled water. It was heated to the desired temperature (60^{0}c, 70^{0}c or 80^{0}c) for 30 minutes with continuous shaking. The mixtures were centrifuged at 1,000rpm for 15 minutes. An aliquot of supernatant (5ml) was evaporated at 130^{0}c and weighed. The percentage solubility of the gum was the ratio in mass (g) of the dried supernatant to the initial mass (g) of the dry gum expressed in percent.

2.4 Swelling and gel fraction

Swelling and gel fraction studies were carried out according to a standard method reported by [16] Samples weighing 0.01g of gum were placed in small dishes that were carefully inserted into glass flasks. Total volume of 60mL distilled water was slowly poured into each glass flasks. The samples were allowed to soak for 2 hours at room temperature, after which the excess solution was carefully removed and the galled sample remaining in the gelled bottle were weighed. The galled samples were lyophilized for three days and then weighed again. The swelling ratio and percentage of gel fraction were calculated. Using Equations (1) and (2)

Swelling ratio = W_{water}/W_{gel}----------(1)

Percentage fraction = W gel/W solid × 100 --------- (2)

W_{water} = weight of the sample after 2 hours soaking
W_{gel} = weight of the sample after lyophilization
W_{solid} = initial weight of the sample.

2.5 Viscosity

Apparent viscosity of gum was determined using a Brookfield Viscometer (Model RVF, Stoughton, MA). The gum slurry (5%) was placed in a boiling bath for

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15 minutes and then cooled to 22^{0}c. cold paste viscosity was determined using spindle at 25^{0}c.

2.6 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.

2.7 X -Ray Powder Diffraction Analysis (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^{o}C to 40^{o}C, 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

2.8 Fourier Transform Infra-red (FTIR) Sprctroscopy

FTIR spectra were obtained on a FTIR spectrometer [Shumadzu 8400s] using a KBr disc. The equipment was operated with a resolution of 4cm^{-1} and the scanning range from 4000 to 400cm^{-1}

2.9 Nuclear magnetic resonance (NMR) spectroscopy

^{13}C NMR spectra were recorded in an NMR (600MH$_Z$) spectrometer (Agilent Technology, America). The sample (10mg) was dissolved in 700µL at 70^0 C with continuous stirring for 6 hours followed by sonication for 10 minutes. The solution was centrifuged and transferred to a 5mm NMR tube. Chemical shift were reported in ppm relative to internal standard TMSP.

2.9.1 Statistical analysis

The data obtained from the study were analyzed using the Statistical Analysis System (SAS) software and the means were separated by T-Test.

3.0 Results and Discussion

Table1: Physico-chemical characteristics of acetylated and native *Sweitenia mycrophylla* gum

	Solubility (%)		Swelling ratio (%)	Viscosity (cs)	Gel fraction (%)
Native gum	60^{0}c	3.90 ± 0.01			
	70^{0}c	23.10 ± 0.04			
			15. 20 ± 0.40	28.40 ± 0.30	60 ± 4.8
	80^{0}c	30.10 ± 0.03			
Act gum	60^{0}c	46.70 ± 6.05			
	70^{0}c	80.80 ± 5.14	46.16 ± 0.20	65.20 ± 1.40	34 ± 2.81
	80^{0}c	93.40 ± 7.20			

Mean ± S.D, n = 3, Act=Acetylated

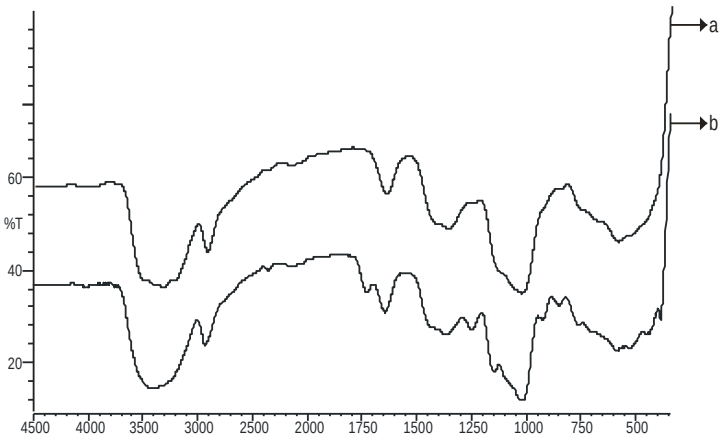

Fig 1: FTIR of (a)unmodified *S.mycrophylla* gum (b) acetylated *S. mycrophylla* gum

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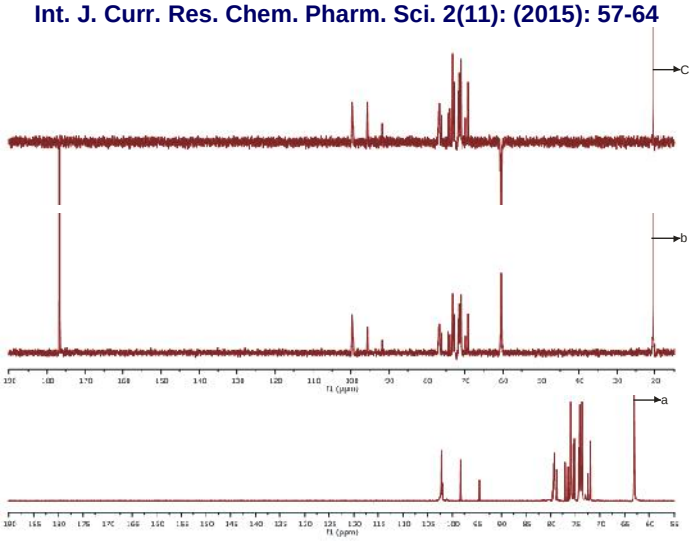

Fig 2:^{13}C NMR Spectra obtained for (a) Unmodified *S. mycrophylla* gum (b) acetylated *S. mycrophylla* gum (c).^{13}C-DEPT for acetylated *S. mycrophylla* gum

The viscosity value for the acetylated gum (65.20±1.40 cs) (Table1) was higher than the native gum (28.40 ±0.30cs). This higher value of viscosity could be explained by the increase in the swelling power and solubility of the chemically modified gum. According to [17] acetylation of gum generally increases the emulsifying capacity which further increases the viscosity and water holding capacity.

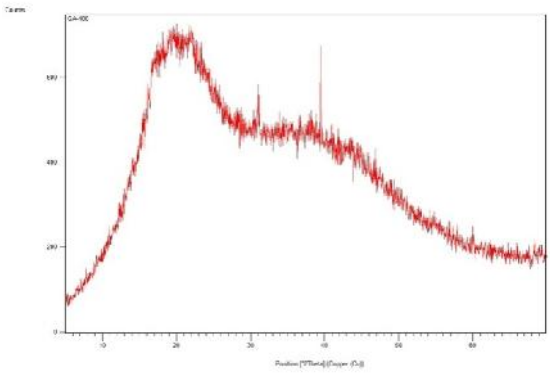

Fig 3:XRD of acetylated *S.mycrophylla* gum

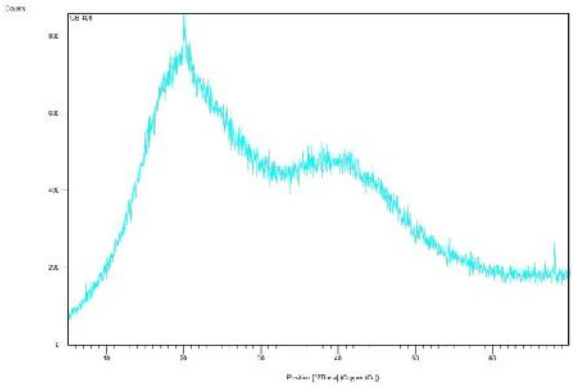

Fig 4: XRD of native *S.mycrophylla* gum

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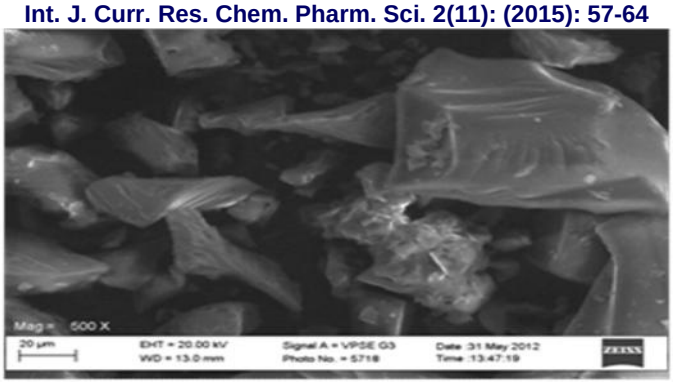

Fig 5:SEM of unmodified *S.mycrophylla*

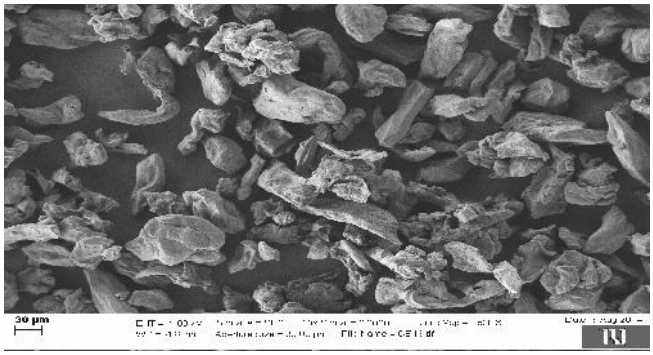

Fig 6: SEM of acetylated *S.mycrophylla*

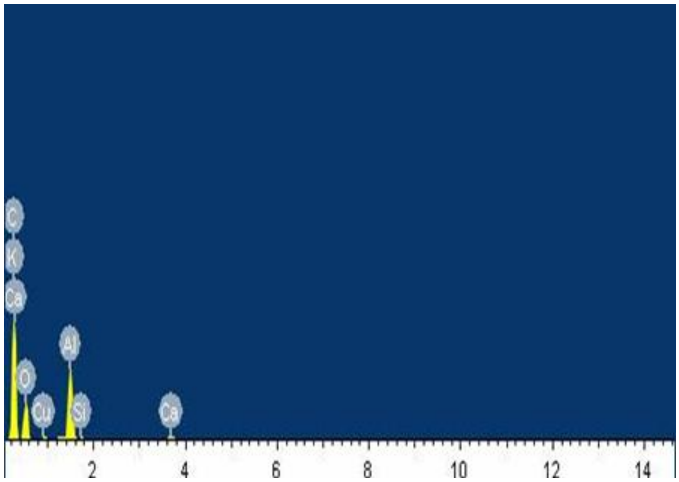

Fig 7: EDX of native *S.mycrophylla* gum

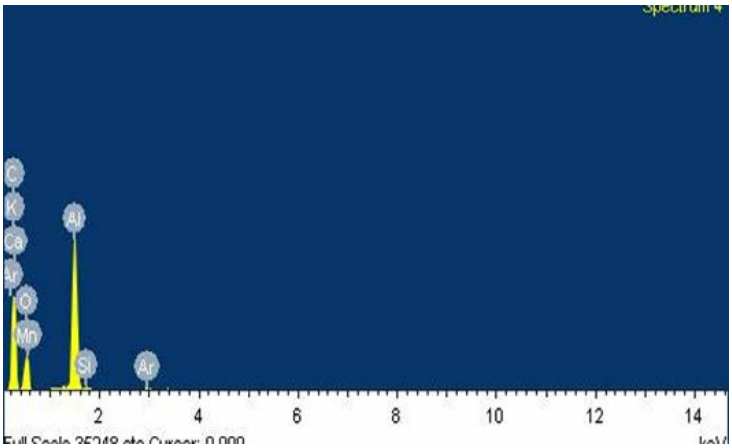

Fig 8: EDX of acetylated *S.mycrophylla* gum

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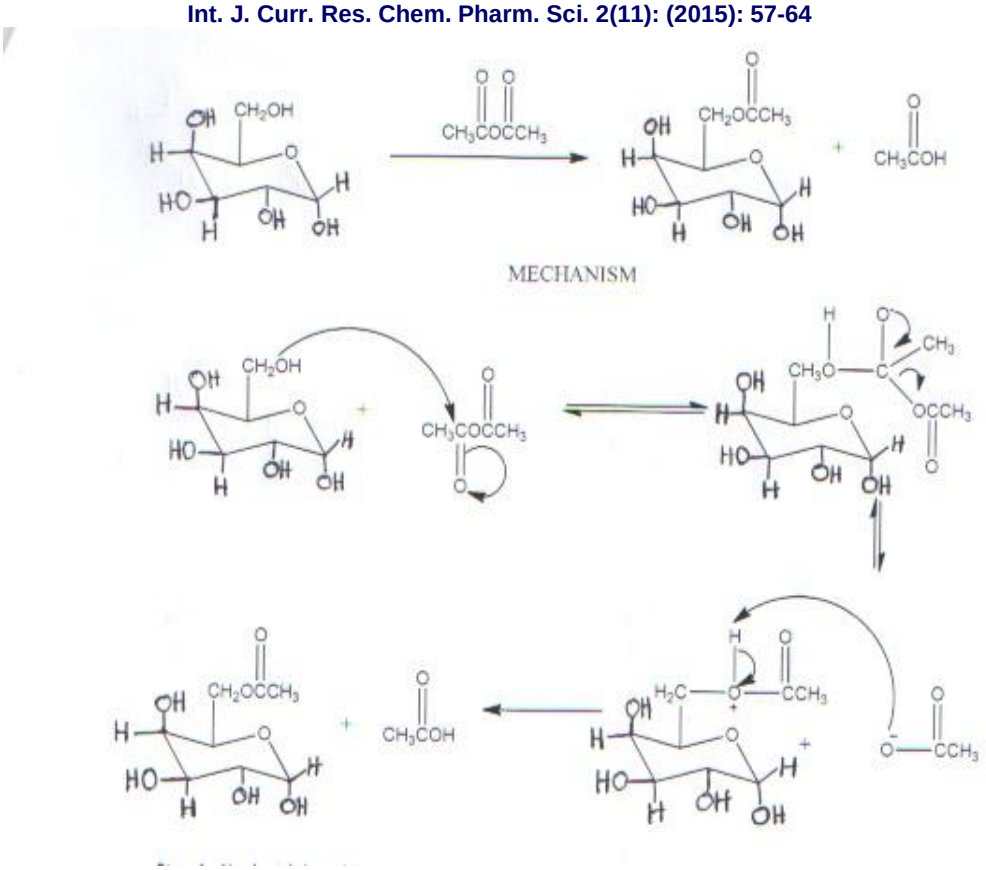

Fig 8: Mechanism of C-6 acetylation of free α-galactopyranose unit of *S.mycrophylla* gum

During the acetylation process, the gum-gum interactions in the granules are weakened by the introduction of acetyl groups, this makes the gum to be more attracted towards water molecules.[18-20, 26]. Also the swelling index and solubility of acetylated gum were higher than the native gum (Table1).Hovers and susilki.[21] reported that the introduction of acetyl groups during the acetylation process reduces the bond strength between gum molecules (amylose/ amylopectin) and thereby increases the swelling power and solubility of the gum granules. This facilitates access of water to amorphous areas, enhancing the water holding capacity of the gum matrix and developing a more organized structure, leading to a higher resistance to deformation and achieving a higher peak viscosity. The solubility of modified and unmodified gum profoundly increased with increase temperature (Table 1).At temperature above 90^0c, more than 100% modified gum were dissolved. This is due to introduction of acetyl groups which is bulkier than hydroxyl groups and capable of obstructing chain association. The superior solubility and swelling index of acetylated gum compared with the native gum may be due to the presence of hydrophilic substituting groups ($CH_3C=O$) which allow the retention of water molecules because of their ability to form hydrogen bonds. [22-23]. However, the swelling ratio of the acetylated carbohydrate increases while the gelling properties reduces, which is the main reason for studying the gel fraction percentage. The FTIR spectra of the native and acetylated gum is shown in Figure 1. The polysaccharide unit of glucose with hydroxyl group [-OH] as the major functional groups appear in the region [3650 to 3200cm^{-1}] and disappeared when the gum was acetylated, there was introduction of acetic groups and the spectra now processed peaks around [1750cm^{-1} to 1735cm^{-1}] attributed to C=O stretching, indicating the presence of the acetyl group. This peak was seen to decrease and in the case of native gum the peak was not resolved from that of the C=O group. The peak at 3300-3400cm^{-1} caused by -OH stretching was also seen to decrease with an increase in acetyl content. Thus, the FTIR and NMR spectrum confirms the acetylation of *Sweeitenia mycrophylla* gum.The carbon anomeric region shows two major signals assigned as C-1 of α-D-galactose (residue A) at 98.87ppm and C-1 of β-D-mannose (residue B) at 102.1ppm. The resonances of the carbon atoms were well resolved (Fig 2) and identified as the resonances of C-2, C-3, C-4 and C-5 (77.2, 73.5, 77.1, 75.2 ppm) respectively for residue B and C-2, C-3, C-4, and C-5 (71.90, 73.0, 74.80, 76.00ppm) for residue A. This facts are almost identical with gums of other origin [31, 32, 33].Spectrum for the acetylated gum (Fig. 2b) shows some differences in relations to unmodified gum. The anomeric signals decrease considerably due to sugar residues, probably because of chain degradation (31). A new signal at 179.4ppm and 21.37ppm were observed for acetylated gum in comparison with the

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unmodified gum. In ^{13}C DEPT NMR spectrum (fig 2c), the signal at 62.5ppm (CH_2) appeared with the opposite amplitude to those of CH_3 and CH, which can be attributed to the acetylation of CH_2 primary carbons (C-6). The presence of acetylated group causes an increase in the ^{13}C chemical shift. The absence of signal inversion at 20ppm of the acetylated gum spectrum in figure 2b demonstrated the correct signal assignment of the CH_3 of acetyl group introduced while the inverted signal at 179.4ppm (figure2c) shows the presence of –coo groups with no hydrogen atoms [31, 32, 33]. Furthermore, the shift of the peaks of the C-6 to 61.18ppm from 60.44 and 60.92ppm indicated the position on the carbohydrate ring where substitution occurred, however, the peaks were not sufficiently resolved to show separate peaks for the acetylation at position of C-6 of residue A and B.

The SEM analysis of the native gum shows irregular particle sizes (fig 5) while the SEM of the acetylated gum (fig 6) was found to be fibrilar, indicating loss in particulate morphology that was observed in the native form of the gum, suggesting acetyl groups are essential in the structural integrity of the gum for particulate appearance in the native form. The X-Ray diffraction analysis of the native gum (fig 4) shows numerous halves with weak peaks confirming the amorphous nature of the gum while the XRD of the acetylated gum shows more regular pattern with few sharp peaks confirming the level of crystallinity of the acetylated gum.(fig 3).The EDX of the native gum sample (fig 7) shows that the gum sample contain various elements such as carbon, potassium, calcium, oxygen, copper, aluminium, silicon, calcium and manganese. The high ratio of carbon to oxygen indicate the presence of a sugar polymer. Though, virtually all the elements present in the native gum were also present in the acetylated (fig 8) gum but the concentration of these elements increased for the acetylated gum.This may be as a result of the purification of the native gum sample.

4.0 Conclusion

The study confirms that purification by extraction and acetylation may improve physicochemical properties of *crude Sweitenia mycrophylla* gum. The feasibility of the procedure was demonstrated by SEM, XRD, FTIR and NMR spectroscopy. Furthermore, the obtained product can have wider biological application as drug delivery carriers by grafting/cross linking compounds of interest.

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