Some chemical characteristics of novel hemicellulosic Gums From seeds of *Afzelia africana* and *Prosopis africana*

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ABSTRACT

Background: Determination of chemical characteristics of a pharmaceutical excipient is important for establishing its compatibility with different drugs.

Objective: This study investigates some chemical characteristics of novel hemicellulosic gums extracted from seeds of *Afzelia africana* and *Prosopis africana*.

Methods: The afzelia gum (AFG), prosopis gum (PRG) and sodium carboxylmethylcellulose (SCMC) were subjected to ash value evaluation, pH determination, atomic absorption spectroscopy, fourier transform infrared analysis and X-ray powder diffraction.

Results: Afzelia seeds gave a yield of 17 % gum while prosopis seeds gave a yield of 18.2 %. There was no significant difference in the yield of hemicelluloses from the two seeds. AFG gave a significantly higher ash value compared to PRG signifying the presence of higher amount of inorganic substances. There was no significant difference in the pH of their dispersions. The two hemicelluloses contain significantly higher amount of potassium and magnesium ions compared to SCMC. The three polymers manifested similar X-ray diffraction patterns showing that they are closely related.

Conclusion: The two hemicellulosic gums can be differentiated from synthetic cellulose derivatives like SCMC on the basis of their potassium and magnesium content. However, being cellulose-related, they are all characterized by similar X-ray diffraction patterns. The type and level of the different metal ions present in the gums reflect their compatibility or otherwise with different types of drugs.

Keywords: afzelia gum, chemical characteristics, hemicelluloses, prosopis gum.

Certaines caractéristiques chimiques des gencives Roman hémicellulosiques à partir de graines de Afzelia africana et Prosopis africana

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RÉSUMÉ

Contexte: Détermination des caractéristiques chimiques d'un excipient pharmaceutique est important pour l'établissement de sa compatibilité avec différents médicaments.

Objectif: Cette étude examine certaines caractéristiques chimiques de nouvelles gommes hémicellulosiques extraits de graines de Afzelia africana et Prosopis africana.

Méthodes: La gomme de afzelia (AFG), prosopis gomme (PRG) et la carboxyméthylcellulose de sodium (SCMC) ont été soumis à l'évaluation de la valeur de la cendre, la détermination du pH, de la spectroscopie d'absorption atomique, transformée de Fourier analyse infrarouge et des rayons X de poudre de diffraction.

Résultats: graines Afzelia donné un rendement de 17% tandis que la gomme prosopis graines ont donné un rendement de 18,2%. Il n'y avait pas de différence significative dans le rendement de l'hémicellulose des deux graines. AFG a une valeur nettement plus élevée de cendres par rapport à PRG signifiant la présence d'une quantité élevée de substances inorganiques. Il n'y avait pas de différence significative dans le pH de leurs dispersions. Les deux contiennent des hémicelluloses quantité significativement plus élevée d'ions de potassium et de magnésium par rapport à SCMC. Les trois polymères manifestent motifs de diffraction des rayons X similaires montrant qu'ils sont étroitement liés.

Conclusion: Les deux gommes hémicellulosiques peuvent être différenciées à partir de dérivés de cellulose synthétiques comme SCMC sur la base de leur teneur en potassium et magnésium. Cependant, étant liés de la cellulose, ils sont tous caractérisés par des profils similaires de diffraction des rayons X. Le type et le niveau des différents ions métalliques présents dans les gencives reflètent leur compatibilité ou non avec différents types de médicaments.

Mots-clés: afzelia gomme, caractéristiques chimiques, hémicelluloses, prosopis gomme.

INTRODUCTION

Hemicelluloses are cell wall polysaccharides which are slightly less complex than cellulose in structure. They have a β-(1-4) linked backbone with a symmetrical configuration.[1] Unlike galactan gums which are water-soluble,[2] hemicellulosic gums are not soluble in water but can be solubilized by aqueous alkali.[3] They can be classified into: xyloglucans, xylans, mannans and glucomannans.[1] Afzelia gum (AFG) and prosopis gum (PRG) being closely related to guar gum had been classified as xyloglucan hemicellulosic gums.[3, 4] *Afzelia africana* and *Prosopis africana* are members of the plant family *Fabaceae.* Members of this plant family are notable for gum production; and their seeds contain substantial amount of gum.[5]

Afzelia africana belongs to the subdivision *Caesalpiniodea* of the Family *Fabaceae.* It is a perennial tree that grows up to a height of 10 - 20 m. It is a large tree that is widely distributed in the dense evergreen forests, savannah and the coastal forests of East and West Africa.[3] The fruit is a hard shell containing 6 - 10 black seeds with an orange-coloured aril. The seed is commonly used for soup and as a beverage thickener in the eastern part of Nigeria.[6]

Afzelia gum, a xyloglucan consists of a cellulose backbone of β-glucopyranosyl residues which are substituted at C-6 position.[3] Gas liquid chromatography (GLC) of the gum showed the presence of glucose, xylose, and galactose as the main monosaccharides. These sugars are present in the ratio 1.3 : 1 : 0.63 respectively. Small amounts of mannose, arabinose and uronic acid are also present in the gum.[6]

Prosopis africana is a flowering plant in the same pea family (*Fabaceae*). It grows in Tropical America, Africa, Western Asia and Southern Asia. It thrives in arid soil and it's resistant to drought.[7] It is the only *Prosopis specie* occurring from Senegal to Ethiopia. It is native to West, Central and East Africa. The wood is very hard, dense and durable.[8] The tree is a good source of firewood, the fruits are used as animal feed while the seeds are used to make 'locust bean' - a highly proteinous condiment.[7] The economic importance of the plant encourages its cultivation.

Prosopis gum consists of highly branched polysaccharides which consist of glucose, fructose, galactose and xylose as the main monosaccharide units.[9] Just like afzelia gum, it is extracted from the plant seed and it is a xyloglucan hemicelluloses.[4]

The major set-back of natural gums is their susceptibility to microbial attack.[10] This factor, coupled with the desire to improve their physicochemical properties has necessitated the production of semi-synthetic and synthetic gums. While synthetic gums are pure synthetic products (for example, polyvinyl pyrrolidone), semi-synthetic gums are produced by chemical modification of natural gums.[11] Cellulose derivatives can be made by etherification, esterification, nitration, cross-linking or graft copolymerization. Etherification produces derivatives like hydroxyl propylmethylcellulose and carboxylmethylcellulose; nitration produces cellulose nitrate while esterification produces derivatives like cellulose acetate and cellulose acetate phthalate.[12] Sodium carboxylmethylcellulose (SCMC) is the sodium salt of carboxylmethylcellulose. The major advantage of natural gums is that they are more compatible with drugs.[10]

Reported works on characterization of prosopis and afzelia seed gums dwelt more on their sugar composition and some physical characteristics.[3,4,6,9] Meanwhile, the suitability of these polymers as pharmaceutical excipients depends on both their physical and chemical characteristics.[10] The aim of this work is to investigate the chemical behaviour of these novel hemicelluloses and determine their metal ion composition. Some chemical characteristics of sodium carboxylmethylcellulose were examined for the purpose of comparison.

MATERIALS AND METHOD

Materials

Afzelia seeds collected from *Afzelia africana* tree and Prosopis seeds collected from *Prosopis africana* trees were purchased at Abuja, Nigeria and were authenticated by the taxonomist in the Department of Biological Sciences, University of Abuja, Abuja, Nigeria and issued with voucher numbers UNIABUJA I51 and UNIABUJA I53 respectively. Sodium carboxyl methylcellulose, ethanol and sodium metabisulphite (BDH Chemicals, Poole, England), acetone (Merck, Germany) and diethyl ether (Sigma–Aldrich, Germany) were used as obtained.

Extraction and purification of afzelia and prosopis gums

The method described by Builders *et al*. [6] was slightly modified and used for the extraction of the Afzelia gum. The seeds were washed and then soaked in water for 1 h to soften and remove the aril. It was thereafter boiled for 3 h to soften the seed coat which was later removed from the swollen tegument. A 500 g quantity of the hydrated tegument was chopped into small pieces with a knife. A 500 ml volume of distilled water was added and the mixture was homogenized using a laboratory blender (Christison, United Kingdom). The mixture was boiled with 100 ml of ethanol for 1 h to denature protein and inactivate enzymes and then treated with several portions of diethyl ether to remove lipids. The defatted granules were air-dried for 6 h to removes the residual diethyl ether. It was thereafter soaked in 1 L of distilled water with intermittent agitation for 18 h. The viscous material was filtered using muslin cloth and the gum was precipitated using double amount of acetone. The precipitated gum was air-dried for 3 h then in a hot air oven (Gallenkamp, Germany) at 50 ^{o}C for 24 h and pulverized using a laboratory blender (Christison, United Kimgdom).

The extraction and purification of the prosopis gum were done using the method described by Adikwu *et al*.[13] The seeds were soaked in water for 24 h and then cooked for 4 h. The softened seed coats were manually separated from the swollen tegument. A 500 g quantity of the swollen tegument was soaked in 1 L of aqueous solution of 0.1 % w/v sodium metabisulphite for 24 h. The material was homogenized using a laboratory blender (Christison, United Kingdom) and the highly viscous material was passed through a muslin cloth to remove gritty particles. The gum in the filtrate was precipitated using 500 ml of acetone. The material was air dried for 3 h then at 50 ^{o}C for 24 h after which it was pulverized using a laboratory blender (Christison, United Kingdom).

Determination of percentage yield of gum

The percentage yield of gum from the seeds was calculated as the percentage of the dried precipitated and purified gums relative to the initial weight of the seeds.

Determination of Ash value

This was determined by measuring the mass of ash left after combustion of 1 g sample of gum in a muffle furnace (Thomas Scientific Co., U.S.A.). The percentage of the ash left relative to the initial mass of the gum was thereafter calculated. The data were taken in triplicates.

Determination of pH

The pH of a 2 % w/v dispersion of the gum was determined 24 h after preparation using a pH meter (Hanna Instruments, U.S.A). The data were taken in triplicates.

Atomic absorption spectroscopy

The metal ion content of the gums was determined using a slightly modified form of the method described by Ofori-Kwakye *et al*.[5] One gram of the powdered material was transferred into a 250 ml beaker and 25 ml of concentrated nitric acid was added to it. The sample was digested on a hot plate in a fume cupboard followed by cooling at room temperature and addition of 1 ml of 70 % w/v perchloric acid and 30 ml of distilled water. The mixture was boiled for 10 min and filtered hot using a Whatman No. 4 filter paper. The filtrate was made to 100 ml mark with distilled water. The amount of divalent metals was determined using 1 ml of the digest. The ions: lead (II), magnesium (II), copper (II) and calcium (II) were respectively assayed at wavelengths of 283.30 nm, 285.21 nm, 324.75 nm and 422.67 nm using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 400, Perkin Elmer, USA) fitted with an acetylene flame. Also, 2 ml of the digest was used for the determination of monovalent metals (sodium and potassium) using a flame photometer (Jenway, United Kingdom) operated on methane gas.

Fourier transform infrared (FTIR) spectrophotometry

Samples of each gum were prepared in potassium bromide disks in a hydrostatic press at 6 - 8 tons pressure. FTIR spectra of these prepared samples were recorded over a scanning range of 350 to 5000 cm^{-1} using a spectrophotometer (Shimadzu Corporation, Koyto- Japan).

X-ray diffraction (XRD)

The X-ray diffraction study was carried out using an X-ray diffractometer (PANalytical Spectris Pvt. Ltd., Singapore). The XRD pattern was taken using a copper target at voltage of 40 KV and a current of 30 mA over a scanning range of 10 to 120 $^{o}2\Theta$ spacing.

Statistical analysis

Data obtained were expressed as mean value ± standard error of the mean. They were subjected to analysis of variance (ANOVA) using GraphPad Instat-3 software to determine the significance of differences and *p - values* less than 0.05 were taken to be significant.

RESULTS

Some physicochemical properties of the gums are shown in Table 1. There was a significant difference between the ash value of AFG and that of PRG ($P < 0.001$). The pH values of 2 % w/v dispersion of the three gums were significantly different; with SCMC having the highest value.

Table 1: Some physicochemical properties of the gums

Parameter	AFG	PRG	SCMC
Yield (%)	17.0	18.2	ND
Ash value (%)	8.00±1.00	1.50±0.50	4.00±2.00
pH	4.50±0.01	4.84±0.01	5.29±0.01

ND = Not determined.

The distribution of metal ions in the different polymers is shown in Table 2. Sodium carboxyl methylcellulose had the highest level of sodium ion followed by PRG while AFG had the lowest level. AFG had the highest concentration of potassium ion followed by PRG while SCMC had the lowest level. The seed gums (AFG and PRG) have abundant levels of magnesium ion and the difference in the concentration of the ion in the two polymers is not significant. Sodium carboxyl methylcellulose contains significantly lower amount of magnesium ion. The distribution of calcium ion in the three cellulose-related polymers followed the same trend as that for potassium (AFG > PRG > SCMC). The concentration of copper ion in the polymers ranged from 0.02 to 20.90 mg/kg while lead ion was absent in the three polymers.

Table 2: Metal ion content of the gums

	Concentration (mg/kg)		
Ion	**AFG**	**PRG**	**SCMC**
Na^{+}	400±0.08	1,000±0.35	71,000±2.50
K^{+}	2,100±1.00	1,800±0.60	100±0.05
Mg^{2+}	2,045±0.07	2,359±0.30	268±0.34
Ca^{2+}	2,489±0.19	967±0.35	204±0.06
Cu^{2+}	20.90±0.08	0.02±0.1	2.1±0.03
Pb^{2+}	0.00±0.00	0.00±0.00	0.00±0.00

The FTIR spectra of PRG and SCMC were similar but different from that of AFG (Figure 1). AFG had peak of minimum intensity at 1390.72 cm^{-1} and that of maximum intensity at 4384.34 cm^{-1}. PRG had distinct absorptions at 1643.41cm^{-1} and 2176.74 cm^{-1} while SCMC had its own at 1611.58 cm^{-1} and 2160 cm^{-1}.

The XRD patterns of the different gums are shown in Figure 2.

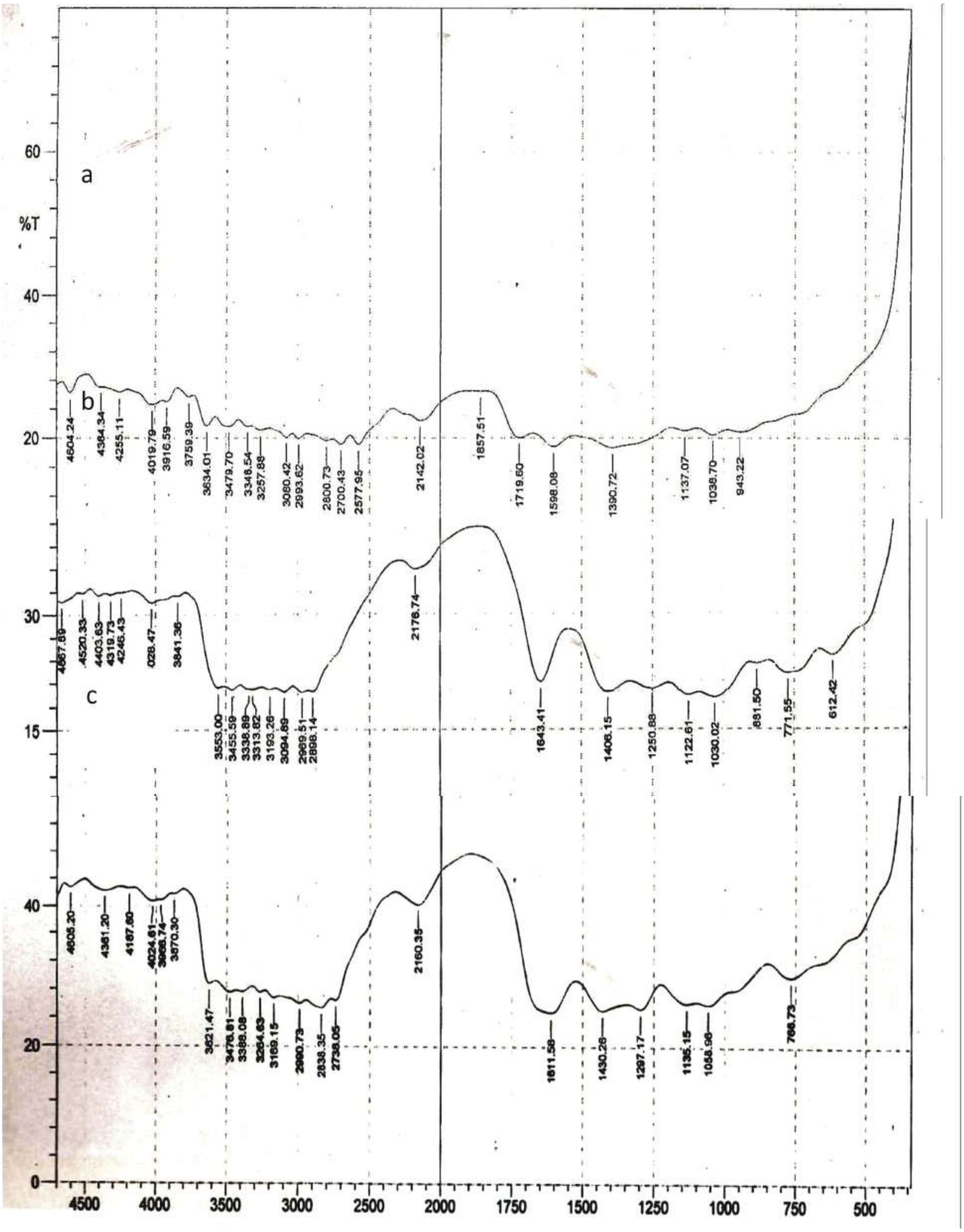

Figure 1. Fourier transform infrared spectra of (a) afzelia gum (b) prosopis gum (c) sodium carboxyl methylcellulose.

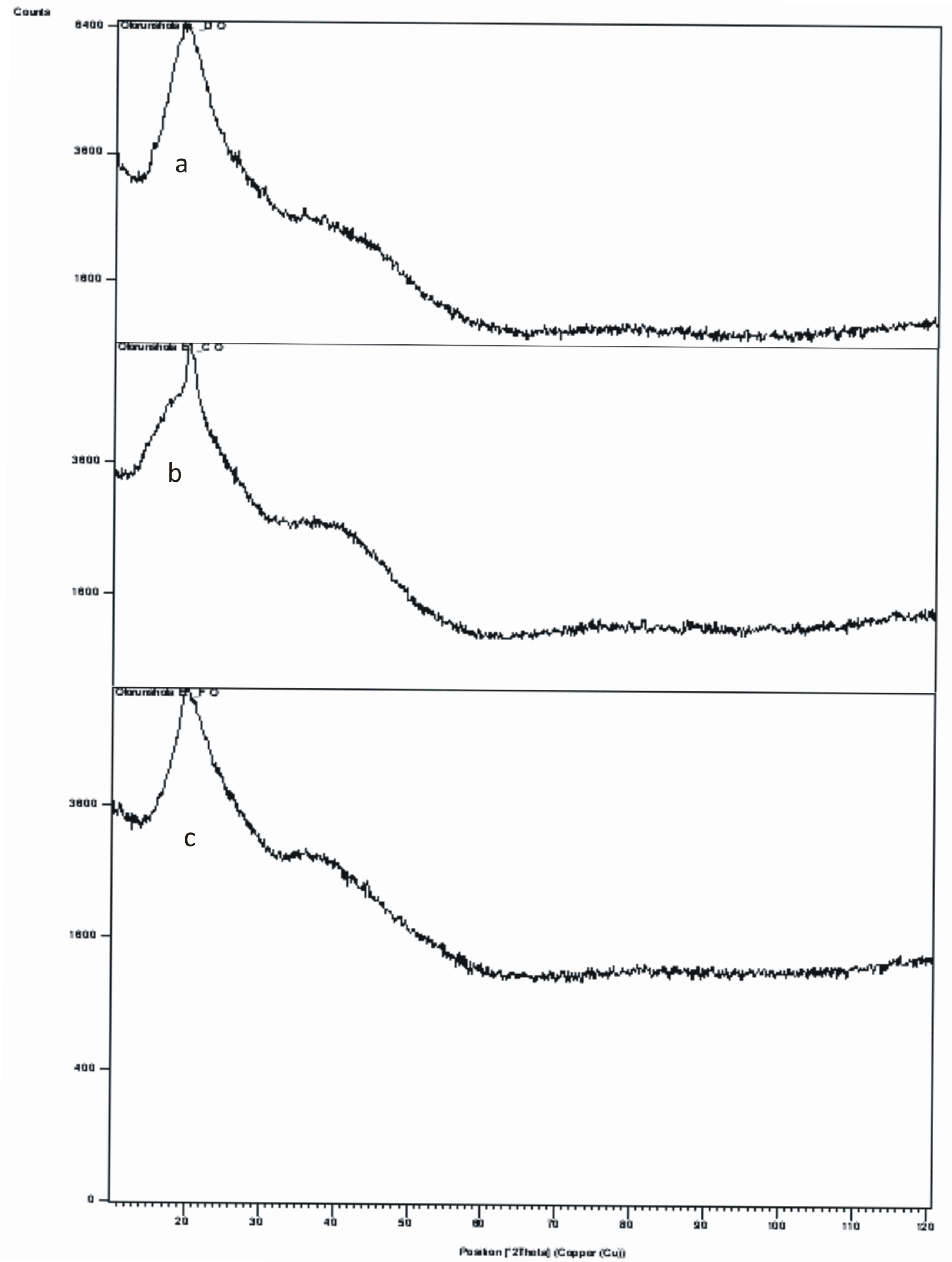

Figure 2.XRD patterns of (a) afzelia gum (b) prosopis gum (c) sodium carboxyl methylcellulose

DISCUSSION

The two seeds gave a lower yield of gum compared to plant exudates. Ofori-Kwakye *et al.*[5] have reported a gum yield of 78.5 % from cashew tree exudates. Afzelia and Prosopis seeds are rich in protein and they also contain substantial amount of fat.[6,13] Removal of protein and lipid during the extraction and purification processes lowered the yield of gum from these seeds.

Ash value is a measure of the relative amount of inorganic substances in the gum. Therefore, the ranking of the total amount of inorganic substances in the gums is AFG > SCMC > PRG. The pH of Afzelia gum is not significantly different from the 4.58 that was reported by Ibezim *et al*.[14] The pH of SCMC is significantly higher than those of the two seed gums and may be of less pharmaceutical importance in terms of pH but may possess better physicochemical properties being a semi-synthetic product. The three polymers have pH ranging from 4.50 to 5.28 (acidic region) and are likely to be pharmaceutically useful. Afzelia gum with the lowest pH is the most suitable for formulations targeting major absorption in the stomach while SCMC with the highest pH is the most suitable for formulations targeting absorption in the intestine. The knowledge of pH of polymer dispersion is important especially when they are used for formulation of emulsions. It is established that changes in pH of emulsions causes product instability.[10] Interactions between acidic drugs and basic excipients and vice versa have been reported by many researchers. Interactions between drugs and excipients based on their ionic composition have also been reported. For instance, according to Zhou,[15] indomethacin is incompatible with sodium bicarbonate, ibuprofen is incompatible with magnesium oxide while norfloxacin is incompatible with magnesium stearate. He also stated that lansoprazole is stabilized by basic excipients.

The quantities of sodium and potassium ions in these cellulose-related gums are inversely related. The two hemicelluloses have significantly higher amount of potassium ion (> 1500 mg/kg) compared to the cellulose-derived SCMC (100 mg/kg). Sodium is the predominant monovalent ion in SCMC while potassium is the predominant in AFG and PRG. This feature distinguishes SCMC from the two hemicelluloses. The two seed gums (hemicelluloses) can be distinguished by the predominant divalent ion. While magnesium is the predominant divalent ion in PRG, calcium is the predominant in AFG. The two hemicelluloses contain high amount of magnesium ion, and caution must be taken in utilizing them for formulation of acidic drugs like ibuprofen and norfloxacin which interact with magnesium salts.[15] They are however, appropriate for formulation of basic drugs like lanzoprazole that are stabilized by basic salts.[15]

The U.S.P. 31 – N.F. 26 [16] specifies the permitted daily exposure (PDE) to copper as 2.5 mg. Afzelia gum has the highest copper concentration; and a 500 mg quantity of the gum will need to be consumed to reach this level of 2.5 mg copper. Obviously, no daily dose of medication is likely to contain this amount of gum as pharmaceutical excipient. Therefore, copper ion is within the acceptable limits in all the polymers. Afzelia gum contains the highest amount of the ion and may have the highest tendency of inducing oxidation of susceptible medicaments when used in their formulations. The acceptable limits of copper and lead ions justify the three gums as being non-toxic polymers [2] and validate their suitability for pharmaceutical use.

The prominent peaks of Afzelia gum at 1038.70 and 1137.07 cm^{-1} can be assigned to 0-H bending vibration and C-O stretching vibration of ethers and alcohol. The peaks at 2993.62 to 3257.88 cm^{-1} can be assigned to C-H stretching vibration of the aromatic hydrocarbon. The peak at 3479.70 cm^{-1} can be assigned to O-H stretching of bonded hydroxyl group while 3634.01 cm^{-1} can be assigned to stretching of free O-H based on specifications given by Coutts.[17]

In the spectrum of prosopis gum, the peak at 771.55 cm^{-1} can be attributed to bending vibration of C-H in substituted aromatic hydrocarbon while those at 2898.14 to 3193.26 cm^{-1} can be assigned to C-H stretching of the aromatic hydrocarbon. The peaks at 1122.81 and 1250.88 cm^{-1} can be assigned to O-H bending and C-O stretching vibrations of ether and alcohol. The peaks at 3455.59 and 3553.00 cm^{-1} can be assigned to stretching vibrations of bonded O-H.[17]

The peak at 766.75 cm^{-1} in the FTIR spectrum of sodium carboxyl methylcellulose can be assigned to C-H bending of substituted aromatic hydrocarbon while those at 2990.73 – 3264.63 cm^{-1} can be attributed to C-H stretching of the aromatic hydrocarbon. The three peaks at 1058.98 - 1297.17 cm^{-1} can be assigned to O-H bending and C-O stretching of ether and alcohol, 3476.81 cm^{-1} can be assigned to O-H stretching of bonded O-H while 3621.47 cm^{-1} can be assigned to O-H stretching of free hydroxyl group based on specifications given by Coutts.[17]

The presence of a single peak of the same height (6400 counts) about the same point in the XRD pattern of AFG, PRG and SCMS is an indication that they are structurally related. This is a study limitation as it did not show the arrangement of the functional groups.

SCMC is sodium salt of carboxyl methylcellulose while AFG and PRG have been described as xyloglucan hemicelluloses.[6] The two hemicelluloses could be utilized for the formulation of suspensions and emulsions of different types of drugs in order to validate the characteristics observed above and to evaluate their suitability as suspending and emulsifying agents.

CONCLUSION

Seeds of *Afzelia africana* and *Prosopis africana* contain comparable amount of hemicellulosic gums. The gums are characterized by high concentrations of potassium and magnesium ions (> 1500 mg/kg and > 2000 mg/kg respectively); and can be differentiated from synthetic cellulose derivatives with these features. They might be very suitable for formulation of basic drugs like lanzoprazole that are stabilized by basic salts and be less suitable for formulation of acidic drugs like ibuprofen and norfloxacin which interact with magnesium salts. The three polymers (AFG, PRG and SCMC) have similar XRD patterns showing that they are structurally related – all having same backbone of cellulose.

ACKNOWLEDGEMENT

The authors are grateful to Dr. O.S. Odusanya of Sheda Science and Technology Complex, Abuja for facilitating part of the instrumentation.

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