Solid State Characterization of *Anacardium occidentale* Gum

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**Abstract:** This study sought to characterize *Anacardium occidentale* gum (cashew gum-CG) from Nigeria. Microbial load on the purified gum and acute toxicity of the gum on rabbits were determined using standard procedures. The elemental content of extracted gum was determined using Energy Dispersive Spectroscopy (EDS) and the amount of inorganic elements was evaluated by determining the total ash content and acid insoluble ash. Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), X-Ray Powder Diffraclometry (XRPD), Scanning Electron Microscopy (SEM) and swelling index test were employed to further characterize the gum. Results revealed that no enteric or fungal microbe was present in the extracted gum; and the LD50 of the gum in rabbits is greater than 5000 mg/Kg. The ash content and acid insoluble ash of the purified gum were 1.5 and 0.3% respectively, while EDS revealed only Ca as the inorganic element present in the gum. DSC revealed a melting point of 291.5ºC; XRPD showed no sharp peak; FTIR showed characteristic bands at 710 to 1040 per cm, 1643, 2901, 3208 and 3379 per cm; SEM revealed that the gum is amorphous; and the purified gum did not swell in water. It is therefore evident from this work that *Anacardium occidentale* gum of Nigerian origin possesses some characteristics that are markedly different from those of Brazilian or Ghanaian origin.

**Keywords:** Acute toxicity, cashew gum characterization, microbial load

**INTRODUCTION**

*Anacardium occidentale* Linn (family, Anarcardiaceae) or cashew is a native of West Indies, Central and South America but was one of the first fruit trees to be distributed throughout the tropics by early Spanish and Portuguese adventurers (Onunkwo and Okoye, 1997; Lima et al., 2002; Mothe et al., 2008). It is a species of woody green trees widely grown in many tropical and subtropical countries and very popular for its nuts used as a food ingredient especially in oriental delicacies. Under certain conditions the bark of cashew tree exudes a gummy material known in general as cashew gum (Zakaria et al., 1997). The milky exudates from the tree darken and thicken rapidly on exposure to air. Cashew gum is a complex polysaccharide of high molecular mass. Comparative studies among specimens of gums obtained from different geographical areas indicate that there are significant variations in properties associated with climatic conditions, such as specific rotation and composition. On hydrolysis, a sample of the gum from Brazil gave 70% galactose, 5% arabinose, 11% glucose, 4% rhamnose, 1% mannose and 6% glucoronic acid, respectively (De Paula et al., 1998; Mothe et al., 2008). The gum has a highly branched galactan framework comprising chains of (1→3)-linked β-D galactopyranosyl units interspersed with β-(1→6) linkages. The acid number has been found to vary from 13.4 to 22.7; the variation in acid number is influenced not only by the source of the sample, but also by its age (Lima et al., 2002; Gyedu-Akoto et al., 2007; Moura-Neto et al., 2011). The gum is similar to gum arabic and may be used as a substitute to liquid glue in paper industries, as an agglutinant for capsules and pills in the pharmaceutical industries, as stabilizer in the cosmetic and food industries and also in the production of cashew wine (Smith and Montgomery, 1959; Lima et al., 2002; Owusu et al., 2005). Previous researchers, Onunkwo and Okoye (1997) and Ofori-kwakye et al. (2010) reported that cashew gum has excellent binding properties in the formulation of tartrazine dye tablets and metronidazole tablets, respectively.

Solid state characterization of polymers involves the study of the physicochemical (e.g., glass transition temperature, melting point, ash value, acid insoluble ash, moisture content, constituent atoms or elements etc), mechanical, spectroscopic, diffraction and morphological characteristics of the polymers using different
instrumental techniques such as Scanning Electron Microscopy (SEM), particle size analysis (by analytical sieving or other methods), X-Ray Powder Diffraction (XRPD), Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Fourier Transform Infra Red (FTIR), Energy Dispersive Spectroscopy (EDS), Nuclear Magnetic Resonance spectroscopy (NMR) etc. Extensive literature search revealed that the instrumental solid state characterization techniques which cashew gum had been subjected to include-TGA, FTIR, DTG, DTA, ¹³C NMR of cashew gum from Brazil (Feitosa et al., 2007; Mothe et al., 2008), Atomic Absorption Spectroscopic (AAS) determination of elemental content in cashew gum from Ghana (Ofori-Kwakye et al., 2010). Currently, a lot of effort is being made to encourage manufacturers of food and pharmaceuticals to substitute gum arabic with cashew gum in their formulations in order to reduce dependence on the former and also to make the latter a cash crop for countries that produce it in abundance (Gyedu-Akoto et al., 2010). It is therefore relevant that more studies on cashew gum be conducted in order to further evaluate its properties preparatory to its projection as an excellent excipient in both pharmaceutical and food industries. The objectives of this work therefore, are to conduct some instrumental characterization of cashew gum from Nigeria, determine the microbial load on the purified gum and its acute toxicity in rabbits.

**MATERIALS AND METHODS**

**Materials:** Cashew exudates (Anacardium occidentale), supplied by the plant collector in National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja; ethanol 96% (Sigma-Aldrich, Germany), Mac Conkey agar (Sigma-Aldrich, Germany), Mueller Hinton agar (Fluka, Germany), Sabouraud dextrose agar (Fluka, Germany), Xylene of specific gravity, 0.879 g/mL (Sigma-Aldrich, Germany), distilled water, other reagents were of analytical grade.

**Methods:**

**Extraction and purification of cashew gum:** The gum was extracted from exudates by a modified method of that reported by Okoye et al. (2010). Briefly, clean cashew exudates were pulverized and screened through a 600 μm sieve. Thereafter, 100 g of the powder was soaked in 500 mL of distilled water at room temperature (32°C) with intermittent stirring for 24 h. At the end of the 24 h, the dispersion was strained through a muslin bag and the resulting mucilage was precipitated by mixing it with thrice its volume of 96% ethanol. The precipitated gum was filtered using a filter cloth and air dried. Further purification of the gum was carried out by dissolving it in fresh distilled water to yield 1.0% w/v solution. This solution was filtered using a 100% cotton cloth overlaid with 2 inch thick surgical cotton wool (Maimed GMBH, Germany) and the resulting filtrate mixed with thrice its volume of 96% ethanol to precipitate the gum. The precipitated gum was harvested and soaked in 96% ethanol for 18 h and finally air dried. In order to kill peroxidase enzymes present in the gum, it was further heated in a hot-air oven (Unitemp LTE scientific Ltd Great Britain, Greenfield Oldham OL37EN) at 65°C for 1 h, at the end of which it was pulverized and stored in air tight container over silica gel.

**Particle size analysis:** Each sieve was tarred to the nearest 0.001 g. Thereafter, 20 g of cashew gum powder was carefully loaded on the coarsest sieve of the assembled stack (1000 to 150 μm) and the lid was replaced. The nest was subjected to mechanical vibration using the Shaker (AS 400 Retsch, Germany) for 25 min at 5 min interval per shaking session. Thereafter, the sieves were carefully separated and each sieve was carefully weighed with its content. The weights of powder retained on each sieve and the collecting pan were determined by difference. The values were used to calculate the percent of sample retained on each sieve (Brittain, 2001 a, b; Brittain, 2002a, b) and the average diameter of the particles (d₅₀) using the formula (Ansel et al., 2007):

\[
Dav = \frac{\sum(p\text{ retained } \times \text{ mean aperture size})}{100} \tag{1}
\]

**Determination of average moisture loss on drying:** The method described in British Pharmacopoeia (2005) was adopted, using the oven (Lab. Oven Model No. DHG-9101. 1SA, Ceword Medical Equipment, England) at 105°C for 3 h. Triplicate determinations were made and the means reported.

**Determination of ash value and acid insoluble ash:** Two grams of cashew gum was accurately weighed in tarred crucibles. The crucibles with their contents were heated in a furnace (Nabertherm. Karl kolb, D-6072 Dreieich, West Germany, Model L3/P) to 450°C and allowed to stand at this temperature for 3 h. The temperature of the furnace was then allowed to drop to 100°C before the crucibles were removed from the furnace with a metal tong and cooled in a desiccator over silica gel. Thereafter, the crucibles with contents were weighed using an analytical balance (Mettler Toledo AB54 GmbH USA).The weights of the residues (carbon free ash) were determined and expressed as percentages of the initial materials. The mean of three determinations was recorded.

In order to determine the acid insoluble ash, the ash obtained from above was boiled with 25 mL of 2M HCl for 5 min. The insoluble residue was separated by centrifugation at 2000 rpm for 5 min using a centrifuge (80-3 Techmel and Techmel USA). The sediment was re-
suspended in a hot water and evaporated to dryness in a tarred crucible. The weight of the residue was expressed as a percentage of the initial weight of the material.

**Determination of pH of extracted cashew gum dispersion**: This was done by preparing a 1.0% w/v dispersion of cashew gum in distilled water and the pH measured using a pH meter (Corning, model 10 England). Triplicate determinations were carried out and the mean recorded.

**Determination of powder particle density and refractive index of cashew gum**: The fluid displacement method using xylene as the displacement fluid according the previous reports (Eichie et al., 2005; Ohwoavworhua et al., 2007) was adopted to determine the particle density. The refractive index determination was conducted using a 1 mg/mL solution of cashew gum, prepared at room temperature (32ºC) in distilled water. The refractive index of this solution was then measured with Abbe Refractometer (Leica Mark II). The procedure was repeated two more times and the mean of the three determinations recorded.

**Determination of the swelling index of extracted cashew gum**: Swelling index is the volume in mL occupied by 1 g of a hydrophilic material including any adhering mucilage after it has swollen in an aqueous liquid for 4 h. The method described in British Pharmacopoeia (2009) was utilized, with slight modification. Briefly, one gram of cashew gum powder of particle size less than 150 µm was accurately weighed and carefully transferred into a 50 mL measuring cylinder. It was moistened with 1ml of ethanol 96% and 25 mL of distilled water was added. The cylinder was firmly closed and shaken vigorously every 10 min for 1 h and then allowed to stand undisturbed for 3 h. The volume occupied by the material under test after the entire 4 h was measured. The mean of triplicate determinations was recorded as the swelling index.

**Determination of elements in extracted cashew gum**: The organic and inorganic elements present in the extracted cashew gum were determined using Energy Dispersive Spectroscopy (EDS) (EVO/MAIO. Carl Zeiss Germany). Briefly, about 5 mg of the cashew gum powder was placed on the sample holder and vacuum was created using the relevant pump. The electron gun was then used to focus the electron beam on the sample and the operating voltage maintained at 20 kV to generate the required spectrum. The elements present were then identified using energy level to wavelength conversion scale (Oxford Instruments, England).

**Determination of microbial load in the extracted cashew gum**: This was carried out using three different media-Mueller Hinton agar (a general purpose medium), Mac Conkey agar (specific for enteric bacteria) and Sabouraud dextrose agar (specific for fungal organisms). One thousand milligrams (1000 mg) of cashew gum was dissolved in 25 mL of sterile water using a Vortex Vibrator (Vortex Genie 2 scientific industries Inc Bohemia NY, Model C. 560 E). One millilitre (1 mL) of this stock solution was then used to inoculate 19 mL of the respective media inside a sterile cupboard (ESCO class II Biohazard Safety Carbinet, Dreieich, West Germany). The inoculated media were poured into different sterile petri dishes and allowed to solidify within the sterile cupboard. For each culture medium, three replicates and one control were prepared. The inoculated media and their controls were incubated at 37±2°C (Memmert Karl Kolb, D-6072, Dreieich, West Germany) for 24 h. At the end of which the media were examined for growth and further identification of the organisms was done.

**Determination of acute toxicity (LD₅₀) of extracted cashew gum in rabbits**: The study carried out using modified Lorke (1983) method was conducted in the animal facilities of National Institute of Pharmaceutical Research and Development (NIPRD) Idu, Abuja following the principles of Good Laboratory Practices and Animal handling based on the NIH guide for the care and use of laboratory animals (NIH, 1985). Thirteen rabbits (males and females) were utilized in the study. The animals weighed between 1.40 to 1.65 Kg 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 4 h after the administration of the extract. In the first phase, three groups, each consisting of three rabbits randomly selected was formed. The first, second and third groups received 10, 100 and 1000 mg/Kg of cashew gum dispersion in distilled water, respectively administered via intra-gastric cannula. They were observed frequently on the day of treatment during normal working hours to note if there were any abnormal behaviours they manifested when compared to those that did not receive any treatment. Thereafter, observations and weighing were carried on for 3 days. In the second phase, doses of 1600, 2900 and 5000 mg/Kg of the extract, respectively were administered to another three groups of one rabbit each. These were also observed as above. After the three days of initial monitoring in each phase, the animals were further observed for any form of abnormal behaviour for another 14 days before the study was terminated.

**Differential Scanning Calorimetric (DSC) analysis**: DSC characterization of extracted cashew gum was carried out using the apparatus Netzsch DSC 204 F1 Phoenix (Nietzsche Germany). Five milligrams (5 mg) was carefully weighed using the analytical balance (Mettler Toledo AB54, Switzerland) and sealed in
aluminium pan. Calibration of the calorimeter was done with indium and the purge gas was nitrogen. Heating of the sample was carried out at the rate of 10°C/min from 30 to 400°C under nitrogen flow rate of 20 mL/min, followed by cooling back to 30°C at the same rate.

**X-Ray Powder Diffraction (XRPD) analysis:** X-ray diffraction pattern test was carried out on extracted cashew gum using the diffractometer-XRD PANalytical (X' Pert PRO, Netherlands). Cashew gum powder sample was finely ground and thinly spread in the sample holder of diameter 10 mm. The latter with its content was then placed on the stage of the instrument and scanned continuously at a step time of 10.16 sec, temperature of 25°C, using an anode material of Cu with Kα1 and Kα2 equal to 1.54060Å and 1.54443Å, respectively at 40 kV and 10 mA. Scanning of samples was initiated at 5.0251º2 and ended at 119.9751º2 at step size of 0.05º2. A nickel filter was used to reduce the Kβ contribution to the X-ray signal.

**Fourier Transform Infra Red spectrophotometric (FTIR) analysis:** The FTIR analysis of extracted cashew gum was carried out using the apparatus FTIR-8400S Spectrophotometer (Shimadzu, Japan). Two milligrams (2 mg) of cashew gum and 200 mg KBr were powdered with an agate mortar and pestle and then compressed into a pellet using the pellet press. The resulting pellet was mounted on the sample holder and the system was purged with nitrogen gas. Scanning was conducted in the range of 400 to 4000 per cm with a resolution of 1 per cm. Duplicate measurements were made and the spectrum with the clearer peaks was chosen.

**Morphological study of extracted cashew gum powder:** This was conducted using the scanning electron microscope (EVO/MAIO. Carl Zeiss Germany). Briefly, about 5 mg of cashew gum powder, uncoated, was placed on the sample holder and vacuum was created using the relevant pump. In order to eliminate electrostatic charging arising because the powder was uncoated, a variable pressure aperture for the electrons was used. The electron gun was then aligned to finely focus the electron beam on the sample and different magnifications (x100, x200, x500 and x1000) were employed to examine the sample. The magnification that gave the best resolution was selected and the image saved. The operating voltage was limited to 5kV since at higher voltage, charging effect would negatively affect the resolution of the image because the sample was not coated with any metal.

### RESULTS AND DISCUSSION

**Gum yield and some of its physical characteristics:** Table 1 shows some of the physical properties of purified cashew gum. The gum recovered from the precipitation and purification processes appeared crystalline in nature, off-white in colour, odourless, tasteless and the percentage yield was 52.35%. The results are within compendial specifications for acacia (United States Pharmacopoeia/National Formulary, 2004; British Pharmacopoeia, 2009) and compare well with some previous reports (Kumar et al., 2009; Ofori-Kwakye et al., 2010), except that there are some variations which might have resulted from the source or origin of the crude gum, methods of extraction of the gum and/or the level of purification given to the gum. Total ash value includes physiological ash, which is derived from the plant tissues and non-physiological ash which is often from environmental contaminations. Upon treatment with dilute acid and ignition, the residue from total ash, which is the acid insoluble ash includes silica materials and siliceous earth (Xiang and Rao, 2009), therefore the low level of acid insoluble ash in this work is an indication of low contamination with siliceous matter. This low value is corroborated by the result from Energy Dispersive Spectroscopy (EDS) (Fig. 1). Ofori-kwakye et al. (2010), reported the presence of Ca, Fe, Mg, K, Na and Zn, whereas in this study, only Ca was identified while the Al present is suspected to have been contributed by the sample holder in the EDS employed (Fig. 1). It is also noteworthy that the swelling index of this cashew gum is 0.5, which contradicts previous report (Kumar et al., 2009). In this study, cashew gum was found to dissolve slowly rather than swell in water at room temperature (32°C) when subjected to the BP test for swelling index. This character may be a pointer to the acetyl group and polyvalent cations contents of Nigerian cashew gum.

### Table 1: Some physical characteristics of cashew gum

<table>
<thead>
<tr>
<th>Property tested</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>9.05%</td>
</tr>
<tr>
<td>Ash value</td>
<td>1.50%</td>
</tr>
<tr>
<td>Swelling index</td>
<td>0.5</td>
</tr>
<tr>
<td>pH</td>
<td>4.7</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.3%</td>
</tr>
<tr>
<td>Particle density</td>
<td>1.887 g/mL</td>
</tr>
<tr>
<td>Average particle diameter (dav)</td>
<td>245 µm</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.3323</td>
</tr>
</tbody>
</table>

![Fig. 1: EDS of cashew gum](image-url)
since previous reports indicated that high percentage of both favour the formation of viscous gels by cashew gum (Jefferies et al., 1977; Zakaria et al., 1997).

**Microbial load on the extracted cashew gum:** There was no growth on the MacConkey agar medium. This implies that the *coli* forms and other enteric gram negative organisms which its supports and differentiates were absent in the extracted cashew gum. Examples of these organisms are *E. coli*, *Salmonella* spp., *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Proteus* spp. and *Shigella* spp (Winn et al., 2006). There was also no growth on Sabouraud agar, a selective medium for moulds and yeast (Mitchell, 2010). However, there were some growths on the Mueller Hinton agar. This medium is a general purpose medium that supports the growth of different types of bacteria. When the growths were subjected to gram stain, gram positive rods (*Bacillus* spp.) were observed. The result of the enumeration of the colonies showed that there were 66 colony forming units per gram of the extracted cashew gum. This value is far below the accepted limit (10000 cfu/g) for non-*coli* load on products to be administered orally (Martínez, 2002).

**Acute toxicity test (LD<sub>50</sub>) of cashew gum in rabbits:** No adverse sign of toxicity, or death was observed at all the doses used for the study. The oral Lethal Dose (LD<sub>50</sub>) of cashew gum in rabbits was thus estimated to be greater than 5000 mg/kg body weight. The absence of adverse effects and death at the dose of up to 5000 mg/kg used for the study suggests that cashew gum is practically non-toxic in rabbits orally. Previous researchers, Gyedu-Akoto et al. (2008) and Kumar et al. (2009) reported that cashew gum was safe in rats. The present finding using rabbits has therefore confirmed their reports.

**Differential Scanning Calorimetric (DSC) analysis of cashew gum:** The thermogram of cashew gum has broad peaks (Fig. 2). This is however not an indication of impurity but the amorphous nature of the biomaterial. Cashew gum thermogram shows no kind of glass transition or crystallization points. The first relatively broad endothermic peak in the thermogram (at about 75ºC) correspond to the desolvation temperature (Gong, 2009), while the broader endothermic point is the melting point (291.5ºC) of cashew gum. Mothe et al. (2008), using DTA reported three stages of decomposition of Brazilian cashew gum at 60, 260 and 310ºC corresponding to desolvation, melting and complete decomposition of the gum respectively. The broad nature of cashew gum’s thermogram shows that it is amorphous in nature even though it looks crystalline to unaided eyes.

**X-Ray Powder Diffraction (XRPD) analysis of cashew gum:** The diffraction pattern of cashew gum shows halo peak (Fig. 3) which is indicative of the amorphous nature of this excipient (Danjo et al., 1999; Najafabadi et al., 2006; Hulse et al., 2009). Many natural gums have also been reported to exhibit similar diffraction patterns, an indication of their amorphous nature (Malik et al., 2002; Murali-Mohan et al., 2002; Reddy and Shekharam, 2004; Odeku et al., 2010). This result further confirms that of differential scanning calorimetry.

**Fourier Transform Infra Red spectroscopy (FTIR) of cashew gum:** Figure 4 shows the FTIR spectrum of
The fingerprint region for cashew gum lies between 710 and 1040 per cm. It is within this region that the characteristic functional groups (alcohol OH out-of-plane bend, hydrogen bonded OH out-of-plane bend and C-O stretch) are located (Coates, 2000). The spectrum further reveals that the alcohols OH in-plane bend lies at 1379 per cm. This is followed by the peaks: aldehyde C = O stretch at 1643 per cm and aldehyde C-H asymmetric stretch at 2901 per cm. The above mentioned bands are all narrow. The broad band which is diagnostic for alcohols
and water is visible between 3208 and 3379 cm. The water identified may be accounted for as the residual water in the biomaterial.

**Scanning electron micrographs of cashew gum powder:** The scanning electron micrographs of cashew gum powder are shown in Fig. 5 and 6. They show distinct particles of fairly defined shape. The shape must have been influenced by the type of milling operation employed during its production. The micrograph of cashew gum at x 200 magnification (Fig. 6) shows some wing-shaped particles which points to the amorphous nature of the powder. Thus this micrograph further confirms the results from DSC and XRPD analyses.

**CONCLUSION**

It is evident from the results of this research that *Anacardium occidentale* gum from Nigeria differs in some of its physicochemical properties from those of Ghanaian and Brazilian origins. Its LD$_{50}$ in rabbits is greater 5000 mg/Kg.

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