

## Evaluation of LD<sub>50</sub> of Cashew Gum and the Comparative Study of its Functionality in Cotrimoxazole Granule and Tablet Formulations

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**Abstract:** This study evaluated the level of contamination of cashew gum, its acute toxicity in rabbits and its functionality in cotrimoxazole granule and tablet formulations in comparison to three standard binders (polyvinylpyrrolidone, gelatin and corn starch BP). The level of contamination was determined using physical and microbiological tests, while the acute toxicity was studied using Lorke's method and the granules were formulated via wet granulation and characterized micromeritically. Subsequently, the granules were compacted using a force of 15 KN and the resulting tablets subjected to all the compendial and non-compendial quality control tests. The results indicated that the contaminants in cashew gum were safe and within acceptable levels and the gum was not toxic in rabbits even at the dose of 5000 mg/kg. Granules formulated with cashew gum exhibited the best micromeritic properties, in comparison to the standards between 2 and 4% w/w binder concentrations, respectively. Furthermore, all the formulated tablets, except those formulated with gelatin at 4 and 5% w/w met the compendial and non-compendial requirements for good quality conventional tablets. In all the tests conducted, there were no significant differences between the properties of granules and tablets formulated with cashew gum or polyvinylpyrrolidone ( $p > 0.05$ ). Finally, since cashew gum possessed very low level of inorganic and microbial contaminants and is non-toxic, it may be economically worthy to exploit it for pharmaceutical purposes.

**Keywords:** Cashew gum, functionality in cotrimoxazole granules, LD<sub>50</sub>, tablets

### INTRODUCTION

Cashew gum is an exudate polymer from the bark of the tree *Anacardium occidentale* Linn (family, Anacardiaceae). The fresh exudate is usually milky in colour but on contact with air, the colour changes to light or dark brown (Zakaria *et al.*, 1997). 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% glucuronic acid (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-Akoko *et al.*, 2007; Feitosa *et al.*, 2007). The

gum is abundant in many tropical and sub-tropical countries and have been reported in many scientific journals to be useful in the study, cosmetic, food and pharmaceutical industries (Smith and Montgomery, 1959; Onunkwo and Okoye, 1997; Lima *et al.*, 2002; Owusu *et al.*, 2005; Ofori-kwakye *et al.*, 2010). The gum has also been reported to have anticancer and antihypertensive activities (Mothe *et al.*, 2008, 2009).

The development of robust and economic oral solid dosage forms is always very attractive because of their obvious advantages (Mattsson, 2000; Armstrong, 2002; Bansal and Nachaegeri, 2004), more especially in developing countries where the income per capita is low. It is therefore pertinent that locally available raw materials for the pharmaceutical and other industries be refined and studied extensively in order to ensure their efficacy and safety when employed for these essential purposes. The objectives of this research are therefore to study the microbial load, level of inorganic elements content, acute toxicity and the comparative functionality of cashew gum in cotrimoxazole granule and tablet formulations.

## MATERIALS AND METHODS

Cashew gum extracted from exudates by method reported by Okoye *et al.* (2010), Mac Conkey agar (Sigma-Aldrich, Germany), Mueller Hinton agar (Fluka, Germany), Sabouraud dextrose agar (Fluka, Germany), Sulfamethoxazole powder (Oak-Faith Pharmaceuticals, Lagos), Trimethoprim powder (Oak-Faith Pharmaceuticals, Lagos), Gelatin (gel strength (bloom): 160) (Fluka, Germany), Povidone (polyvinylpyrrolidone) K15 (Fluka, USA), Corn starch BP (Sigma-Aldrich, USA), Pregelatinized starch, generated from Corn starch B.P. (Sigma-Aldrich, Germany) by BPC (1979) method, Magnesium stearate (BDH Chem. UK), Xylene of specific gravity, 0.879 g/mL (Sigma-Aldrich, Germany).

### Determination of ash value and acid insoluble ash:

Two grams of cashew gum was accurately weighed into each of three 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 the 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 r/min 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 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 vacuum pump. The electron gun was then used to finely 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 Cabinet, 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 (Mettmert 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 (LD<sub>50</sub>) of extracted cashew gum in rabbits:

The study carried out using modified Lorke (1983) method was conducted in the animal facilities of National Institute for 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 mg/kg and 1000 mg/g 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 behaviors they manifested when compared to those that did not receive any treatment. Thereafter, observations and weighing were carried on for 3 d. 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 3 days of initial monitoring in each phase, the animals were further observed for any form of abnormal behavior for another 14 days before the study was terminated.

**Preparation of cotrimoxazole granules:** Two hundred and fifty grams batches of a basic formulation of sulfamethoxazole powder (83% w/w) and trimethoprim powder (17% w/w) were dry-mixed for 10 min in a planetary mixer (Kenwood, model OWHM400020, Japan), 31.25 g of PGS was added and mixing continued for another 15 min. The powder mix was transferred into a mortar and moistened with water or the appropriate amount of binder (cashew gum, gelatin, PVP) solution or starch mucilage prepared according to the methods reported by previous researchers (Odeku and Itiola, 2002; Rudnic and Schwartz, 2006), except that the volume of the moistening agents was maintained at 25 mL equivalent to 2.0, 3.0, 4.0 and 5.0% w/w (cashew gum, PVP, gelatin), 5.0, 7.5 and 10.0% w/w (corn starch BP) in the final granules and granulated by wet massing with pestle, respectively. The homogeneous damp mass was then screened through a 1400  $\mu\text{m}$  sieve and the resulting granules dried in a hot air oven (Unitemp LTE Scientific Ltd Great Britain) at 50°C for 2 h. Thereafter, the dried granules were screened through a 600  $\mu\text{m}$  sieve in order to generate uniformly sized granules (Armstrong, 2002), further dried at 50°C for 1 h and stored in air tight containers over silica gel before subsequent tests and tableting were conducted.

#### Tests conducted on the granules:

**Particle size analysis:** Each sieve was tarred to the nearest 0.001 g. Thereafter, 20 g of each granule batch was carefully loaded on the coarsest sieve of the assembled stack (1000 to 150  $\mu\text{m}$ ) and the lid was replaced. The nest was subjected to mechanical vibration using the Shaker (AS 400 Retsch, Germany) for 25 at 5 min interval per shaking session. Thereafter, the sieves were carefully separated and each sieve was carefully reweighed 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, 2001a, b, 2002a, b) and the average diameter of the particles ( $d_{av}$ ) using the formula (Ansel *et al.*, 2007):

$$d_{av} = \frac{\sum(\% \text{ retained} \times \text{mean aperture size})}{100} \quad (1)$$

**Flow rate:** The time taken for 20 g of granules to pass through a stainless steel funnel with orifice diameter 1.1 cm clamped at a height of 4 cm from a flat bench was carefully determined in triplicate for each batch of the granules and the results presented as mean.

**Determination of bulk and tapped densities:** The bulk density of each granule sample was determined by pouring 20 g (M) of the granules into a 50 mL glass measuring cylinder slanted at an angle of about 15° and the bulk volume ( $V_0$ ) determined after gently returning it to the upright position. The bulk Density ( $D_B$ ) was then calculated from the relationship:

$$D_B = \frac{M}{V_0} \quad (2)$$

Triplicate determinations were made and the means reported (United States Pharmacopoeia, 2003).

The tapped density of each granule sample was subsequently determined using Stampf Volumeter (model STAV 2003, JEF Germany). The cylinder was subjected to 750 taps mechanically and the volume  $V_{750}$  of the powder column determined.  $V_{750}$  was used to calculate tapped Density ( $D_T$ ) using the relationship:

$$D_T = \frac{M}{V_{750}} \quad (3)$$

Triplicate determinations were made and the means reported (United States Pharmacopoeia, 2003).

**Determination of angle of repose:** The static angle of repose,  $\theta$ , was measured according to the British Pharmacopoeia (2009) fixed funnel and free standing cone method. A stainless steel funnel was clamped with its tip, of diameter 1.1 cm, at a given height ( $h = 0.02$  m) above a graph paper placed on a flat surface. Forty grams of granules sample was carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The diameter ( $d$ ) of the base of the cone was measured. This procedure was repeated three times for each granule batch and the means were used to calculate the angle of repose for each batch using the formula:

$$\tan \theta = \frac{2h}{d} \quad (4)$$

**Evaluation of Carr's index and Hausner's ratio:** Carr's Index (CI) and Hausner's Ratio (HR) were evaluated using equations 5 and 6, respectively:

$$CI = \frac{D_T - D_B}{D_T} \times \frac{100}{1} \quad (5)$$

$$HR = \frac{D_T}{D_B} \quad (6)$$

**Preparation of tablets:** Immediately before tableting, each batch of granules was mixed with 0.5% w/w of Magnesium stearate in a tumbler mixer (Karl kolb, D.

6072 Dreieich, Germany) for 5 min. Tableting was done with the 12 station tableting machine (JC-RT-24H, Jenn Chiang Machinery Co., LTD, Feng-Yuan, Taiwan) equipped with 0.013 m flat faced punches and compressed at a force of 15 KN. The resulting tablets were stored in air tight containers over silica gel for 7 days before relevant quality control tests were conducted on them.

#### Tests conducted on the tablets:

**Tablet weight uniformity:** Twenty tablets selected at random from each batch were individually weighed using the electronic balance (Mettler Toledo B154, Switzerland) to an accuracy of within  $\pm 1$  mg. The mean weight and percentage deviation from the mean for each tablet were calculated.

#### Tablet crushing strength and friability tests:

Crushing strengths of tablets were determined at room temperature by diametral compression using a hardness tester (Kal Kolb, Erweka Germany). Results were taken from tablets that split cleanly into two halves without any sign of lamination. All measurements were made in triplicates and their means reported. The percentage friabilities of ten tablets selected at random from each batch were determined using Roche Friabilator (Copley/Erweka, Type, TAR 20, GMBH Germany), operated at 25 r/min for 4 min and evaluated with equation 7:

$$\text{Friability} = \frac{W_i - W_f}{W_i} \times \frac{100}{1} \quad (7)$$

where,

$W_i$  = Initial weight of the tablets before test

$W_f$  = Final weight of the tablets after test

**Tablet disintegration time:** The disintegration times of the tablets were determined in distilled water at  $37 \pm 0.5^\circ\text{C}$  using the disintegration tester (Manesty, Model: MK 4, UK). Six tablets were selected at random from each batch and the machine operated till all the tablets disintegrated. The results reported are the means.

**Preparation of calibration curve:** The calibration curve was prepared with respect to sulfamethoxazole. One hundred milligrams of sulfamethoxazole were carefully weighed (Mettler Toledo B154, Switzerland) and dissolved with 10 mL of ethanol 96% in 100 mL volumetric flask. The solution was made up to 100 mL using 0.1N HCl and then filtered with Whatman no 1 filter paper. From the resulting filtrate, serial dilutions were made to generate 2, 4, 6, 10, 12, 14, 16, 18, 20,

22, 24, 26, 28, 30 and 32  $\mu\text{g/mL}$  solutions. The absorbance values of these solutions were determined at the  $\lambda_{\text{max}}$  (260 nm) of the sulfamethoxazole under study using as blank a 1:10 mixture of ethanol 96% and 0.1N HCL and the values used to construct the calibration curve.

**Content of sulfamethoxazole:** Ten tablets were selected at random and carefully pulverized using agate mortar and pestle. An amount of the resulting powder equivalent to 480 mg of cotrimoxazole was accurately weighed, dissolved with 25 mL of ethanol in a 250 mL volumetric flask and made up to volume with 0.1N HCl and shaken for 15 min using a Vortex Vibrator (Vortex Genie 2 scientific industries Inc Bohemia NY, Model C. 560 E). Thereafter, the mixture was filtered using Whatman no 1 filter paper; 1 mL of the resulting filtrate diluted to a theoretical value of 16  $\mu\text{g/mL}$  using a 1:10 mixture of ethanol 96% and 0.1N HCl and its true concentration evaluated by measuring its absorbance at 260 nm using the ethanol: 0.1N HCl mixture as blank and applying the calibration curve equation ( $y = 0.052x - 0.056$ ;  $r^2 = 0.992$ ).

**Tablet dissolution profile:** Tablet dissolution test was carried out using the USP XXIII basket method (Erweka Germany Type: DT 80) operated at 75 r/min for 60 min in 900 mL of 0.1N HCl maintained at  $37 \pm 0.5^\circ\text{C}$  (United States Pharmacopoeia, 2004). At 5 min intervals, 5 mL of dissolution fluid was withdrawn and replaced with 5 mL of fresh 0.1N HCl. Each withdrawn sample was diluted with the dissolution medium, filtered and the amount of sulfamethoxazole released (Mahmud *et al.*, 2010) determined using the UV-Visible spectrophotometer (UV-160A Shimadzu Corporation Japan), at 260 nm with 0.1N HCl as blank in conjunction with calibration curve equation:  $y = 0.052x - 0.056$ ;  $r^2 = 0.992$ .

**Statistical analysis:** The results were subjected to one way ANOVA where necessary using Excel 2007 package.

## RESULTS AND DISCUSSION

**Ash value and acid insoluble ash:** Ash value gives an indication of the level of inorganic contaminants in a biomaterial. The total ash and acid insoluble values of the extracted gum are 1.5 and 0.3%, respectively and these are much lower than the official requirement for acacia and tragacanth gums (British Pharmacopoeia, 2009). Total ash value includes physiological ash, which is derived from the plant tissues and non-physiological ash which is often from environmental

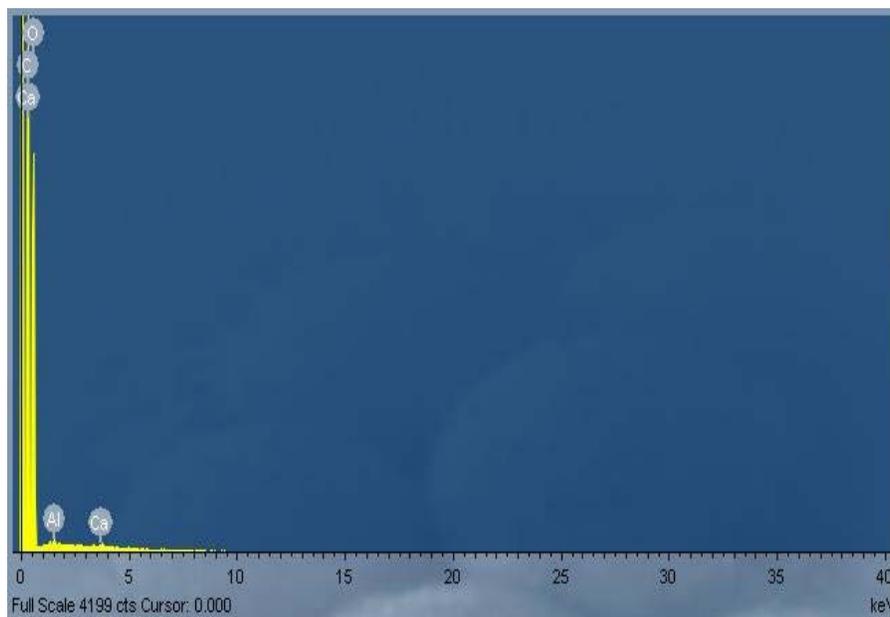


Fig. 1: EDS of cashew gum

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 reported in this work is an indication of low contamination with siliceous matter. The low level reported in this work is corroborated by the result from Energy Dispersive Spectroscopy (EDS) (Fig. 1). Gyedu-Akoto *et al.* (2008) and 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). The content of inorganic elements in a biomaterial is influenced by factors such as source/origin, post harvest handling, method of extraction and purification, post purification handling, etc., thus, one or a combination of any of these factors might be responsible for the observed difference in the inorganic elements in the cashew gum of this study in comparison to the one reported by previous researchers.

**Microbial load on the extracted cashew gum:** There was no growth on the MacConkey agar media. This implies that the *coliforms* and other enteric gram negative organisms which it 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 media for moulds and yeast (Mitchell, 2010). However, there were some growths on the Mueller Hinton agar. This media is a general purpose media 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-coliform* load on products to be administered orally (Martinez, 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 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.

**Micromeritic properties of cotrimoxazole granules:**

The average particle diameter (D<sub>av</sub>) of the granules ranged from 290 μm (X-granules formulated with no additional binder)-431 μm (PVP-5-granules

Table 1: Micromeritic properties of cotrimoxazole granules

| Binder   | D <sub>av</sub> (µm) | % <150 µm | ρ <sub>g</sub> (g/mL) | FR (g/sec) | BD (g/mL) | TD (g/mL) | HR   | CI (%) | AR (°)   |
|----------|----------------------|-----------|-----------------------|------------|-----------|-----------|------|--------|----------|
| X        | 290                  | 29.54     | 0.742±0.032           | 4.48±0.098 | 0.556     | 0.725     | 1.30 | 23.33  | 33±0.071 |
| CAG-2    | 388                  | 7.76      | 0.627±0.032           | 6.45±0.136 | 0.481     | 0.588     | 1.22 | 18.26  | 28±0.071 |
| PVP-2    | 378                  | 13.43     | 0.738±0.083           | 6.22±0.304 | 0.544     | 0.714     | 1.31 | 23.88  | 30±0.566 |
| GEL-2    | 309                  | 12.53     | 0.938±0.323           | 6.24±0.013 | 0.455     | 0.544     | 1.20 | 16.37  | 35±0.495 |
| CAG-3    | 398                  | 7.64      | 0.646±0.055           | 6.39±0.243 | 0.505     | 0.610     | 1.21 | 17.17  | 25±0.141 |
| PVP-3    | 349                  | 11.19     | 0.755±0.047           | 5.18±0.173 | 0.481     | 0.568     | 1.18 | 15.38  | 32±0.566 |
| GEL-3    | 332                  | 12.46     | 0.941±0.509           | 5.33±0.121 | 0.490     | 0.610     | 1.24 | 19.61  | 33±0.141 |
| CAG-4    | 401                  | 5.83      | 0.647±0.084           | 6.58±0.027 | 0.495     | 0.581     | 1.17 | 14.86  | 23±0.071 |
| PVP-4    | 378                  | 15.62     | 0.779±0.037           | 6.43±0.010 | 0.490     | 0.581     | 1.19 | 15.69  | 33±0.212 |
| GEL-4    | 378                  | 11.71     | 1.193±0.421           | 6.93±0.095 | 0.463     | 0.602     | 1.30 | 23.14  | 32±0.141 |
| CAG-5    | 399                  | 4.08      | 0.776±0.144           | 6.77±0.220 | 0.510     | 0.602     | 1.18 | 15.31  | 23±0.283 |
| PVP-5    | 431                  | 16.24     | 0.900±0.339           | 6.86±0.174 | 0.521     | 0.633     | 1.22 | 17.71  | 26±0.141 |
| GEL-5    | 385                  | 9.67      | 1.211±0.249           | 7.25±0.081 | 0.481     | 0.575     | 1.20 | 16.34  | 32±0.495 |
| CSBP-5   | 352                  | 13.48     | 0.901±0.106           | 5.06±0.009 | 0.455     | 0.538     | 1.18 | 15.46  | 30±0.283 |
| CSBP-7.5 | 336                  | 15.95     | 0.965±0.200           | 5.86±0.698 | 0.424     | 0.588     | 1.39 | 27.97  | 32±0.283 |
| CSBP-10  | 343                  | 14.03     | 1.174±0.094           | 5.88±0.499 | 0.439     | 0.610     | 1.39 | 28.07  | 33±0.071 |

X: Granules containing no additional binder; CAG-2-5: Granules containing cashew gum as binder at 2-5% w/w; PVP-2-5: Granules containing povidone as binder at 2-5% w/w; GEL-2-5: Granules containing gelatin as binder at 2-5% w/w; CSBP-5-10: Granules containing mucilage of corn starch BP as binder at 5-10% w/w; D<sub>av</sub>: Average granule diameter; ρ<sub>g</sub>: Granule density; FR: Flow rate; BD: Bulk density; TD: Tapped density; HR: Hausner's ratio; CI: Carr's index; AR: Angle of repose

formulated with PVP 5% w/w (Table 1). The D<sub>av</sub> of granules formulated with cashew gum or gelatin increased with increase in binder concentration, while granules containing PVP or corn starch BP as binders displayed no particular order in their D<sub>av</sub> values. The implication is that cashew gum as well as gelatin formed stronger binding networks between cotrimoxazole powder particles than both PVP and corn starch BP as binder concentration was increased. The stronger inter-particle bonds present in granules containing cashew gum or gelatin was therefore responsible for their greater resistance to abrasion during dry screening, hence the increase in their D<sub>av</sub> with binder concentration. The comparative resistance to abrasion as depicted in the D<sub>av</sub> of the resulting granules is further corroborated by the percentage of granules passing through sieve of aperture size, 150 µm. The amount of these granules regarded as fines decreased consistently as binder concentration was increased from 2 to 5% w/w for CG and GEL (Table 1). High percentage of fines in capsule or tablet formulation can lead to high weight variations and consequently, non-uniformity of dosage units. It may therefore be stated that granules formulated with CG may display the least weight uniformity problems during encapsulation or compaction.

The granule flow indicators (Flow rate, Carr's index, Hausner's ratio and angle of repose) show that the granules differ in their flow characteristics. Granules formulated with cashew gum manifested the best flow characteristics as estimated using their angle of repose and flow rate values between 2 and 4% w/w concentration, while granules formulated with no additional binder (X) or with corn starch BP exhibited

the worst flow characteristics especially at 7.5 and 10% w/w concentrations (Table 1). This could be attributed to their particle size (Table 1) and greater affinity for moisture by granules containing corn starch BP.

**Weight uniformity and mechanical properties of cotrimoxazole tablets:** Table 2 shows that batches formulated with binders met the official recommendations for weight uniformity (British Pharmacopoeia, 2009), in that not more than two individual tablets displayed percentage errors greater than 5% and non deviated by 10%. Tablets formulated with no additional binder (X) however failed the test. This may be attributed to their relatively poor flow properties which invariably resulted to uneven filling of the dies during tableting.

The crushing strengths of the tablets ranged from 94 N (tablets formulated with cashew gum at 2% w/w)-174 N (tablets formulated with gelatin at 5% w/w). These values are higher than the minimum (40 N) required for conventional tablets (Oishi *et al.*, 2011). On the application of single factor ANOVA, a significant difference between the hardness values of the tablets was revealed (p<0.05) and post-hoc (LSD) showed that the difference resulted from the consistent low hardness value of tablets formulated with cashew gum at all binder concentrations in comparison to the other binders utilized in this study. The friability values of all the batches, except batch X (Table 2), were lower than the official specification, i.e., maximum 1.0% (United States Pharmacopoeia, 2004).

Tablets' disintegration times (Table 2) ranged from 10.13 min (batch X) - 29.85 min (tablets formulated

Table 2: Some physicochemical properties of cotrimoxazole tablets

| Binder type /conc. (% w/w) | Weight uniformity (no of tablets with % error >5%) | Crushing strength (N) | Friability (%) | Disintegration time (min) | Content of sulfamethoxazole (%) |
|----------------------------|--|-----------------------|----------------|---------------------------|---------------------------------|
| X                          | 4  | 124±2.607             | 1.33           | 10.13±1.459               | 101.7                           |
| CAG-2                      | 0  | 94±1.845              | 0.65           | 14.08±0.368               | 98.8                            |
| PVP-2                      | 1  | 101±0.757             | 0.30           | 11.23±2.138               | 96.1                            |
| GEL-2                      | 0  | 139±1.917             | 0.58           | 19.18±0.629               | 99.4                            |
| CAG-3                      | 1  | 109±3.301             | 0.32           | 14.72±0.405               | 103.5                           |
| PVP-3                      | 0  | 132±0.577             | 0.12           | 13.03±1.260               | 105.3                           |
| GEL-3                      | 0  | 145±0.912             | 0.50           | 24.72±0.431               | 94.7                            |
| CAG-4                      | 1  | 118±1.590             | 0.23           | 16.82±0.683               | 97.3                            |
| PVP-4                      | 2  | 144±1.743             | 0.08           | 15.13±1.624               | 101.8                           |
| GEL-4                      | 2  | 165±1.833             | 0.43           | 28.65±0.380               | 101.4                           |
| CAG-5                      | 2  | 125±1.537             | 0.28           | 18.03±0.023               | 99.1                            |
| PVP-5                      | 1  | 159±1.916             | 0.12           | 17.53±1.634               | 98.0                            |
| GEL-5                      | 2  | 174±0.912             | 0.25           | 29.85±0.605               | 95.4                            |
| CSBP-5                     | 2  | 124±2.607             | 0.55           | 16.53±0.606               | 97.2                            |
| CSBP-7.5                   | 0  | 143±3.099             | 0.63           | 18.57±0.034               | 100.7                           |
| CSBP-10                    | 0  | 151±1.603             | 0.53           | 19.60±0.426               | 94.9                            |

X: Tablets containing no additional binder; CAG-2-5: Tablets containing cashew gum as binder at 2-5% w/w; PVP-2-5: Tablets containing povidone as binder at 2-5% w/w; GEL-2-5: Tablets containing gelatin as binder at 2-5% w/w; CSBP-5-10: Tablets containing mucilage of corn starch BP as binder at 5-10% w/w

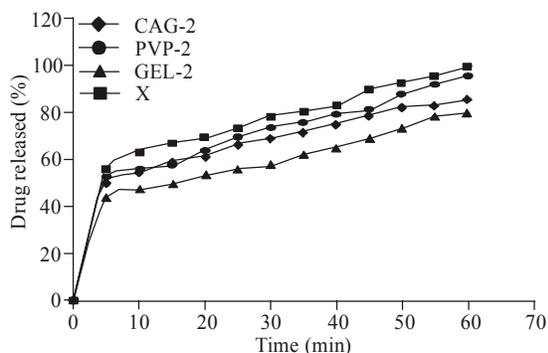


Fig. 2: Dissolution profile of sulfamethoxazole from cotrimoxazole tablet formulated with binders at 2% w/w or none (x)

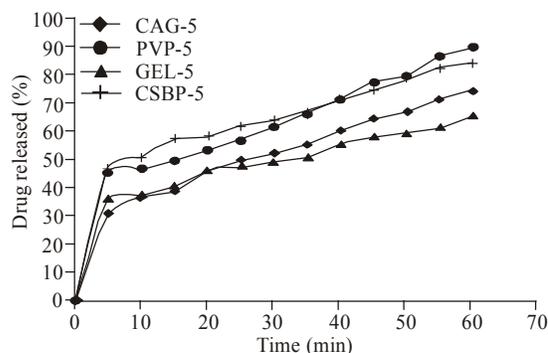


Fig. 4: Dissolution profile of sulfamethoxazole from cotrimoxazole tablet formulated with binders at 5% w/w

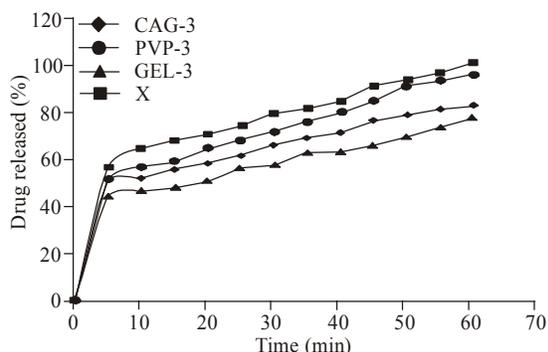


Fig. 3: Dissolution profile of sulfamethoxazole from cotrimoxazole tablet formulated with binders at 3% w/w or none (x)

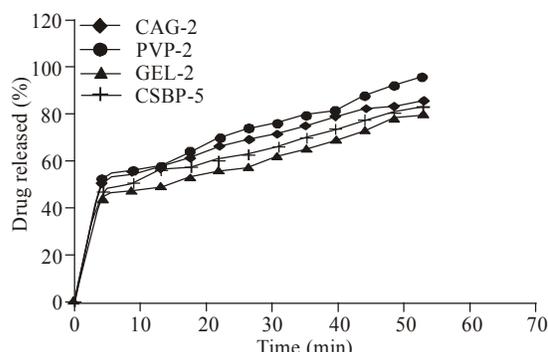


Fig. 5: Dissolution profile of sulfamethoxazole from cotrimoxazole tablet formulated with binders at 2 and 5% w/w (CSBP)

with gelatin at 5% w/w). The disintegration times of the tablets are significantly different ( $p < 0.05$ ) and tablets formulated with gelatin are responsible for the difference as revealed by LSD post-hoc analysis

( $p < 0.05$ ). It is obvious from Table 2, that although tablets formulated with gelatin or corn starch BP at all concentrations studied and those formulated with cashew gum and PVP at 4 and 5% w/w, failed the

(British Pharmacopoeial, 2009) test for disintegration ( $\leq 15.0$  min), they met the United State Pharmacopoeial specification ( $\leq 30.0$  min) (Oishi *et al.*, 2011).

**Content of active ingredient and dissolution profile of cotrimoxazole tablets:** The amount of sulfamethoxazole content in each batch (Table 2) ranged from 94.7% (tablets formulated with gelatin at 3% w/w) -105.3% (tablets formulated with PVP at 3% w/w). These values fall within the range specified for sulfamethoxazole tablets (93.0 to 107.0%) in the United States Pharmacopoeia (2004).

The dissolution profiles (Fig. 2 to 5), reveal that the amount of sulfamethoxazole released decreased as the binders concentrations increased from 2 to 5% w/w. This effect resulted from the increase in bond strength between the compressed granules whose plasticity was enhanced by increasing the concentration of binders in the formulations, consequently, the amount of drug released from the tablet decreased with increase in binder concentration. Tablets formulated with no additional binder (batch X), since pregelatinized starch incorporated as disintegrant has binding property, displayed the best profile (Fig. 2 and 3), however, they failed friability test (Table 2). At all concentrations evaluated, tablets formulated with PVP released the highest amount of drug in comparison to tablets containing the other binders. The amount of drug yielded by tablets formulated with cashew gum was higher than those from tablets containing gelatin or corn starch BP and consistently followed closely that of PVP containing tablets. These results are consistent with earlier reports on cashew gum (Onunkwo and Okoye, 1997; Ofori-kwakye *et al.*, 2010) and may be explained based on its solubility in aqueous medium. PVP is very soluble in both hot and cold water while cashew gum dissolves much more slowly in cold water, whereas gelatin and corn starch BP are practically insoluble in cold water. At  $37 \pm 0.5^\circ\text{C}$  therefore, drug release from tablets containing them was determined by the rate and extent of the binders' solubility in the aqueous medium (0.1N HCl). In the course of this work, the researchers discovered that cashew gum did not swell when subjected to swelling index test, but dissolved slowly at room temperature ( $32^\circ\text{C}$ ). This may therefore further explain the observed difference in the amount of drug released from tablets formulated with cashew gum in comparison to those formulated with corn starch BP (Fig. 5). It is also noteworthy that apart from tablets formulated with gelatin at 4 and 5% w/w, others met the compendial requirement of releasing a minimum of 70% of sulfamethoxazole in 60 min (United States Pharmacopoeia, 2004).

## CONCLUSION

From the preceding results and discussion, it is obvious that cashew gum possessed good properties that imparted positively on both the granule and tablet formulations of cotrimoxazole. It compared excellently well to the standard binders and in some cases outshined them. Finally, since cashew gum possessed very low level of inorganic contaminants and is non-toxic, it may be economically worthy to exploit it for pharmaceutical purposes.

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## REFERENCES

- Ansel, H.C., L.V. Allen Jr. and N.G. Poporich, 2007. Powders and granules. Ansel's Pharmaceutical Dosage forms and Drug Delivery Systems. 8th Edn., Lippincott Williams and Wilkins, New Delhi, pp: 189.
- Armstrong, N.A., 2002. Tablet Manufacture. In: James S. and C.B. James (Eds.), Encyclopedia of Pharmaceutical Technology, 2nd Edn., Marcel Dekker Inc., New York, 3: 2713-2732.
- Bansal, A.K. and S.K. Nachaegeri, 2004. Coprocessed excipients for solid dosage forms. Pharm. Tech., 28(1): 52.
- British Pharmacopoeia, 2009. Her Majesty Stationery Office. London, 1: 37, A281, A313, A449.
- Brittain, H.G., 2001a. What is the "correct" method to use for particle size determination? Pharm. Tech., 96-98.
- Brittain, H.G., 2001b. Particle size distribution, part I: Representation of particle shape, size and distribution. Pharm. Tech., 38-45.
- Brittain, H.G., 2002a. Particle size distribution II: The problem of sampling powdered solids. Pharm. Tech., 26(7): 67-73.
- Brittain, H.G., 2002b. Particle size distribution III: Determination by analytical sieving. Pharm. Tech., 56-64.
- De Paula, R.C.M., F. Heatley and P.M. Budd, 1998. Characterization of anacardium occidentale exudates polysaccharide. Polym. Int., 45(1): 27-35.

- Feitosa, P.A., L.R. Pabhyana, S.M. Jeanny, M.R. Sierakowski and M.C. De Paula, 2007. Oxidation of Cashew gum exudates polysaccharide with TEMPO reagent. J. Braz. Chem. Soc., 18(1): 1-18.
- Gyedu-Akoto, E., I. Oduro, F.M. Amoah, J.H. Oldham, W.O. Ellis and K. Opoku-Ameyaw, 2007. Rheological properties of aqueous cashew tree gum solutions. Sci. Res. Essay., 2(10): 458-461.
- Gyedu-Akoto, E., I. Oduro, F.M. Amoah, J.H. Oldham, W.O. Ellis, K. Opoku-Ameyaw, F. Asante and S. Bediako, 2008. Quality estimation of cashew gum in the production of chocolate pebbles. Afr. J. Food Sci., 2: 016-020.
- Kumar, R., M.B. Patil, R.S. Patil and M.S. Paschapur, 2009. Evaluation of *Anacardium occidentale* gum as gelling agent in aceclofenac gel. Int. J. Pharm. Tech. Res., 1(3): 695-704.
- Lima, R.S.N., R.J. Lima, R.C. De Salis and R.A. Moreira, 2002. Cashew tree (*Anacardium occidentale* L.) exudates gum: A novel bioligand tool. Biotechnol. Appl. Biochem., 35: 45-53.
- Lorke, D., 1983. A new approach to acute toxicity testing. Arch. Toxicol., 54: 275-287.
- Mahmud, H.S., A.R. Oyi, T.S. Allagh and M.S. Gwarzo, 2010. Evaluation of the suspending property of *Khaya snegalensis* gum in cotrimoxazole suspensions. Res. J. App. Sci. Eng. Tech., 2(1): 50-55.
- Martinez, J.E., 2002. Microbial Bio-burden on oral solid dosage forms. Pharm. Tech., 58-70.
- Mattsson, S., 2000. Pharmaceutical binders and their function in directly compressed tablets. Ph.D. Thesis, Faculty of Pharmacy, Uppsala University, Sweden, pp: 7-54.
- Mitchell, T.G., 2010. Medical Mycology. In: Brooks, G.F., K.C. Carroll, J.S. Butel, T.A. Mietzner and S.A. Morse (Eds.), Jawetz, Melnick and Adelberg's Medical Microbiology. 25th Edn., Mc Graw-Hill Co. Inc., USA, pp: 630.
- Mothe, C.G., I.A. De Souza and G.M. Calazans, 2008. Antitumor activity of cashew gum from *Anacardium occidentale* L. Agro Food Ind. Hi-Tech., 19(6):50-52.
- Mothe, C.G., C. Tatiana and M.B. Aguila, 2009. The effects of cashew gum as anti-hypertensive agent: Thermo analytical investigation and micrographs of heart samples of spontaneously hypertensive rats (SHR). J. Therm. Anal. Calori., 97(2): 717-720.
- NIH, 1985. Publication No. 85-23, Revised, Department of health, Education and Welfare, U.S.
- Odeku, O.A. and O.A. Itiola, 2002. Characterization of khaya gum as a binder in a paracetamol tablet formulation. Drug Dev. Ind. Pharm., 28(3): 329-337.
- Ofori-kwakye, K., Y. Asantewaa and S.I. Kipo, 2010. Physicochemical and binding properties of cashew tree gum in metronidazole tablet formulations. Int. J. Pharm. Pharm. Sci., 2(4): 105-109.
- Oishi, T.S., M.A. Haque, I. Dewan and S.M.A. Islam, 2011. Comparative *in vitro* dissolution study of some ciprofloxacin generic tablets under biowaiver conditions by RP-HPLC. Int. J. Pharm. Sci. Res., 2(12): 3129-3135.
- Okoye, E.I., A.O. Onyekweli, O.O. Kunle and M.I. Arhewoh, 2010. Brittle Fracture Index (BFI) as a tool in the classification, grouping and ranking of some binders used in tablet formulation: Lactose tablets. Sci. Res. Essays, 5(5): 500-506.
- Onunkwo, G.C. and J. Okoye, 1997. Evaluation of *Anacardium occidentale* gum as a binder in lactose based tablet formulations. Boll. Chim. Farmaceutico. Anno., 136(9): 569-574.
- Owusu, J., J.H. Oldham, I. Oduro, W.O. Ellis and J. Barimah, 2005. Viscosity studies of cashew gum. Trop. Sci., 45: 86-89.
- Rudnic, E.M. and J.D. Schwartz, 2006. Oral Solid Dosage Forms. In: Gennaro, A.R. (Eds.), Remington the Science and Practice of Pharmacy. 20th Edn., Lippincott Williams and Wilkins Inc. Philadelphia, 1: 858-893.
- Smith, F. and R. Montgomery, 1959. The Chemistry of Plant Gums and Mucilages. Reinhold Publishing Corporation, New York, pp: 1-30.
- United States Pharmacopoeia/National Formulary, 2003. US Pharmacopoeial Convention Inc., Rockville, pp: 2125-2126.
- United States Pharmacopoeia National Formulary, 2004. US Pharmacopoeial Convention Inc., Rockville, pp: 2621.
- Winn, J., W., J. Allen, W. Janda, E. Koneman, G. Procop, P. Schreckenberger and G. Woods, 2006. The *Enterobacteriaceae*. Koneman's Colour Atlas and Textbook of Diagnostic Microbiology. 6th Edn., Lippincott Williams and Wilkins, New York, pp: 219.
- Xiang, B. and Y. Rao, 2009. Determination of total ash and acid insoluble ash of Chinese herbal medicine *Prunellae spica* by near infrared spectroscopy. Yakugaku Zasshi, 129(7): 881-886.
- Zakaria, M.B., Z.A. Rahman and N.N. Mahmood, 1997. Solution properties of polysaccharides from *Anacardium occidentale*. Pertanika J. Sci. Tech., 5(1): 69-76.