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PHYTOCHEMICAL, TOXICOLOGICAL AND VISCOSITY STUDIES OF CASHEW GUM AND ITS DISPERSIONS

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Abstract
The exudate polysaccharide from *Anacardium occidentale* L. obtained in Nigeria were investigated for their phytochemical, safety and rheological properties. Acid hydrolysed samples were found to contain in various proportions galactose, glucose, xylose, rhamnose and ribose sugars. Swiss albino mice were used to investigate the safety or otherwise of using cashew gum (CG) in foods and systemic medicine. Results obtained showed the gum to be non-toxic up to an oral dose of 5 g/kg body weight. Continuous shear behaviours of the mucilages of CG were compared with those of acacia gum (AG). Results obtained showed both gums to exhibit shear-thinning non-Newtonian flow characteristics, with a trend of increasing viscosity with increase in concentration. Changes in pH were found to show little effect on the viscosity of both gums while a direct correlation was noticed between increase in temperature and decrease in viscosity.

Keywords: *Cashew gum*; *acacia gum*; *polysaccharide*; *safety*; *continuous shear*; *pH*; *temperature*; *Nigeria*

INTRODUCTION

Cashew (*Anacardium occidentale* L.) tree is known to exude a gummy substance that is similar to Gum Arabic and which may be used as a substitute in the pharmaceutical, cosmetic and in the food industry (Lima *et al*., 2002). Several findings of the gum's biological action and its use as tools in biochemistry have been reported (Lima *et al*., 2002; Marques and Xavier-Filho, 1991). Also the gum's rheological behaviour has been evaluated (Mothe and Rao, 1999). But, results of comparative studies among specimens of gums obtained from different geographical areas have been reported to show significant variations in properties associated with climatic conditions, such as the specific rotation and composition (Lima *et al*., 2002).

CG has been reported to be a low viscosity polysaccharide (Paula and Rodriques, 1995), and as polysaccharides are generally known to be non-toxic, toxicity screening of the gum should not have been necessary but for variations in nature and the fact that the gum is intended to be used as an excipient for products designed to be administered orally and possibly parenterally. It would be useful to know the toxicity or otherwise of many of the substances used (Cockton and Wynn, 1952). These, and the abundance of this gum in Nigeria, consequently, call for the investigation of some parameters of CG obtained in Nigeria.

This communication, therefore, reports the phytochemical, toxicological and rheological properties of CG obtained in Zaria, Northern Nigeria.

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MATERIALS AND METHODS

Purification of the gum

The method of purification of gums and mucilages by Karawya *et al.*, (1971) was adopted, with some modifications. The dried gummy exudates, collected, as hardened cakes from the stems of cashew trees available in the Ahmadu Bello University botanical garden in Zaria, Northern Nigeria, were size-reduced using pestle and mortar. Two hundred grams (200 g) of the gum powder was dissolved in 500 ml of hot water and the resultant solution strained free of insoluble matter by filtering using a vacuum filter through a piece of linen. The gum was precipitated from the aqueous medium by adding slowly while stirring, some aliquots of acetone instead of the traditional ethanol. Acetone is found to equally precipitate gums, it is cheaper and has lower boiling temperature, which permits easier solvent recovery. The precipitate obtained was washed several times with more acetone, oven dried at 40°C for 3 hours, pulverized with a ball mill and screened through 250 μm sieve mesh and thereafter stored in an air- tight container.

Preliminary Phytochemical Screening

Phytochemical analyses of both processed and unprocessed gum powders were carried out for secondary metabolites, viz: saponins, glycosides, alkaloids, anthraquinons, tannins, free reducing sugars, combined reducing sugars, resins, flavonoids and phenols (Brain and Turner, 1975; Trease and Evans, 1983; Sofowora, 1993).

Chromatographic Analysis

Acid Hydrolysis

Ten milliliters (10 ml) of 7%w/v aqueous Sulphuric acid (H_2SO_4) was used to reflux 1g of the CG powder already dissolved in 10 ml water, for 3 hours on a water bath. The resulting mixture was diluted with 5 ml water and then extracted with ethyl acetate. The aqueous layer containing the sulphate ions was mixed with powdered barium carbonate ($BaCO_3$) and filtered. The filtrate was concentrated using water bath to obtain a residue containing the sugar components, which was then dried over activated silica gel in a desiccator, weighed and stored for the chromatographic analysis.

Paper Chromatography

7% w/v aqueous solutions each of rhamnose, xylose, galactose, arabinose, mannose, glucose, ribose and fructose sugars were prepared and, along with same concentration of the hydrolysed sample, applied using fine capillary tubes on Whatman No.1 chromatographic paper. Using a solvent system n-butanol: Acetic acid: water in the ratio 4:1:5, the chromatogram was developed for 30 hours in a chromatographic tank, by the ascending technique. The chromatogram was then air dried, activated in an oven at 40^0 C and sprayed with Aniline phthalate solution. Respective positions and colours of the sugar spots were marked.

Toxicological Studies

Swiss albino mice obtained from Animal House, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria, were used for this study. The animals, apparently healthy, were preconditioned for two weeks before the commencement of the work. The animals' care and handling were conducted in compliance with the National Regulations for Animal Research. University Ethical Committee reviewed the protocols, which were consistent with International Animal Welfare Guidelines. The method of Lorke (1983) that described a procedure for the investigation of acute toxicity of an unknown chemical substance with an estimation of the LD_{50} was adopted. A stock solution of the CG in water was made and, appropriately calculated oral doses of the substance relative to body weight were administered to 3 groups of 3 animals each.

The 1st group (G_1) received a dose of 1000 mg/kg body weight, the 2nd group (G_2) was given 2000 mg/kg body weight while the last group was administered a dose of 3000 mg/kg body weight. Responses of the animals observed after the oral doses of the substance suggested for 2 more groups of 3 animals G_4 and G_5 to be made. These groups were administered oral doses of 4000 mg/kg and 5000 mg/kg body weight respectively. In each case, the animals were monitored on hourly basis for the 1st day and thereafter daily for 2 more days. Deaths or any visible sign of injury were observed.

Viscosity Determination

A Brookfield synchro-lectric viscometer (Model RVT) was used for the study. It is a rotating spindle instrument and comes with spindles of varying sizes, which makes it appropriate for the measurement of viscosities of various classes of fluids.

Hundred milliliter (100 ml) mucilages of the gum samples at 10%, 12.5%, 15% and 20%w/v concentrations were respectively prepared in beakers, appropriate enough to immerse the spindle. Viscosity measurements were carried out at varying revolutions per minute (r.p.m.) using spindle No. 3, at 30°C.

Temperature and pH have in so many instances been associated with causing changes in viscosities of some fluids. An Ostwald viscometer (size BS/U) was used to investigate the effects of these two parameters on the viscosity of CG and the reference AG mucilages, at 30°C. The pH of the mucilages of CG and AG were varied between the acidic and the alkaline (pH 1 to 14) levels, while 10°C fold rise in temperature up to 70°C was adopted and, respectively, the viscosities of the mucilages were determined.

RESULTS AND DISCUSSIONS

Phytochemical screening

Results of preliminary phytochemical screening showed the presence of resins and combined reducing sugars. As gums occur in the pure form as polysaccharides, the presence of resins is probably due to contamination.

Chromatographic Analysis

The results (Table 2) revealed the presence, in varying proportions, of glucose, galactose, rhamnose, ribose and xylose sugars. Hydrolysis of a sample of CG from Brazil has been reported to give the following composition: galactose, arabinose, glucose, rhamnose, mannose and glucuronic acid in varying proportions (Mothe and Rao, 1999). While a sample from Venezuela contained: galactose, arbinose, rhamnose, mannose and xylose sugars. This seems to support the claim that geographical location plays a role in causing variations in composition and even proportion of sugars contained in the CG. It can be seen that ribose sugar evidenced in Nigerian CG sample is not reported to be present in samples from either Brazil or Venezuela. Mannose sugar reported to be present in both samples from Brazil and Venezuela was not noticed in the Nigerian CG sample and this possibly caused some of the observed variations such as in viscosity.

Toxicity Studies

LD_{50} determination studies carried out using Swiss albino mice have shown CG to be non-toxic up to an oral dose of 5000 mg/kg body weight as no death of animal was noticed; LD_{50} values greater than 5000 mg/kg body weight are of no practical interest (Lorke, 1983). CG can thus be said to be non-toxic and therefore safe for use in foods and orally administered medications. The concentration of the gum required to stabilize most foods and orally administered medications, is quite insignificant, when compared with the adopted ceiling test dose.

Viscosity Determination

Viscosity considerations of CG mucilage alongside the standard AG mucilage showed both gums to exhibit shear-thinning non-Newtonian flow characteristics. Fig. 1 shows the viscosity characteristics of CG mucilage. The shear-thinning attribute of both gums is demonstrated. A decline in viscosity was observed with increase in shear rate. This behavior is found to be associated with most hydrophilic colloids, a category which, cashew, just like acacia seems to belong. Bean *et al.,*. (1964), Martin *et al*., (1983) and Marriott (1988) offered an explanation to this behaviour that, these materials consisting of long-chain molecules or other complex structures are disarranged and matted together at rest and remain so at low shear rates but tend to align themselves in the direction of flow at higher rates of shear. The molecules thus offer less resistance to flow and this together with the release of some of the entrapped water accounts for the lower viscosity.

The CG mucilage was found to be acidic in nature with a pH in the range 4-5, a range that seem to agree with the 4.2- 4.7 pH range of CG mucilage determined by Mothe and Rao (1999). Hydrocolloids may be linear or branched, and they may have acidic, basic or neutral characteristics. Differences in gum source seem to influence the effect of pH on the viscosity of respective gum mucilages. Zakaria and Rahman (1996) found that when the pH of dispersions of CG sample from Malaysia was raised above 5.5, the viscosity of the dispersion dropped sharply; while Lima *et al*., (2002) working on CG samples from Northeast Coast of Brazil concluded that, cashew tree-gum sols are sensitive to alkali and attain their maximum viscosity at pH 8.5, above which the sols tend to become stringy.

Results obtained (Fig.2) from this study show that pH does not seem to significantly affect the viscosity of CG and the reference AG. Highest viscosity for CG mucilage was attained at PH range 5-7 after which the viscosity dropped a little as the pH is raised.

Geographical location causing some variations in gum composition possibly varies with the viscosity of gum samples obtained from different sources. For example the maximum viscosity of the Venezuelan gums are reported to be lower than those observed for species obtained from Papua and India (De Pinto *et al*., 1995).

Temperature is another important factor that alters viscosity of fluids. Gomez and Navaza (2002) found that, type of hydrocolloid; temperature and concentration have meaningful influences on the rheological behaviour of hydrocolloid dispersions. It is thus pertinent to investigate how temperature may affect the viscosity of CG dispersions. Fig. 3 represents a behaviour shown by the CG and AG mucilages when the viscosity was measured at increasing temperatures. It was found that the viscosity tends to decrease as the temperature increases, with AG mucilages appearing affected, the more. Increase in temperature is associated with increase in kinetic energy of a system, which in turn leads to increased particle movement. A possibility of solvent expansion leading to decrease in solvent density and consequently decrease in the viscosity of the material may also not be ruled out.

Denaturing of the gum as a result of increase in temperature up to a value of 70^{o}C may not be connected with the decrease in viscosity observed, because thermo gravimetric curves of CG in sausage showed that major decomposition of the gum occurred at temperatures of about 260^{o}C (Mothe and Rao, 1999).

Table 1: Identified Sugar Monomers of 7%w/v Sample of Hydrolysed Cashew (*Anacardium occidentale* L.) Gum

Reference Sugars	Result
Rhamnose	Present
Xylose	Present
Galactose	Present
Arabinose	Absent
Mannose	Absent
Glucose	Present
Ribose	Present

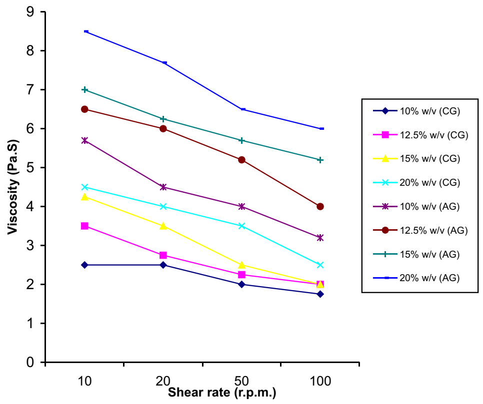

Fig.1: viscosity (Pa.s) Vs Shear rate (rpm) for different concentrations of cashew and acacia gum dispersions in water at 30°C

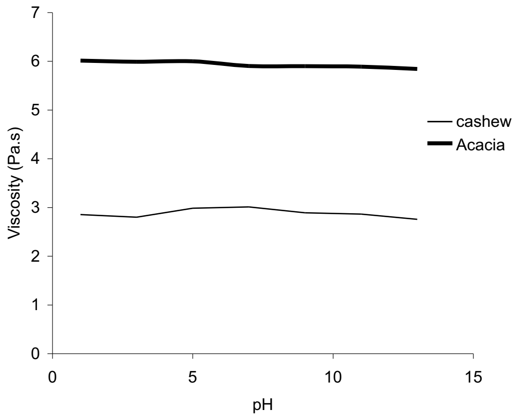

Fig. 2: Viscosity (Pa.s) Vs pH for 10% w/v dispertions of Cashew and Acacia Gums

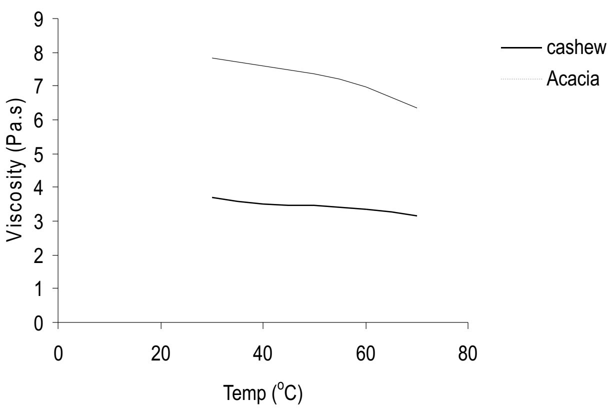

Fig. 3: Viscosity (Pas) Vs Temperature (°C) for 15% w/v aqueous dispersions of cashew and acacia gums

Conclusion

The cashew gum was found to give odourless, colourless and tasteless mucilages in water. The flow curves obtained for both cashew gum and acacia gum, sheared at varying rates showed both gums to exhibit shear-thinning non-Newtonian flow characteristics, a behaviour associated with most hydrophilic colloids. Permissible pH and temperature influences may not necessarily affect the suitability of using preparations made with the gum. Extrapolations from toxicity studies carried out using Swiss albino mice have shown CG to be non-toxic, thereby permitting the possible use of the gum at higher concentrations, to enhance performance. These and the abundance of the CG in a developing economy like Nigeria is a welcome development as it may, conveniently serve as a suitable substitute.

References

Bean, H. S., Beckett, A. H. and Carless, J. E. (1967): Advances in Pharmaceutical Sciences. Vol. 1, Pp 1 81, Academic Press, London and New York.

Brain, K. R. and Turner, T. W. (1975): The practical evaluation of phytopharmaceutical. Wright Scentechnical Bristol, U. K. Pp 98

Cockton J. R. and Wynn J. B. (1952): The use of surface active agents in pharmaceutical preparations. The evaluation of emulsifying powers. *J. Pharm. and Pharmacol*. **4:** 959-971.

De Pinto, G., Martinez, M., Mendoza, J. A., Ocando, E. and Rivas, C. (1995): Biochem. Systems. *Ecol*. 23: 151-156.

Gomez-Diaz, D. and Navaza, J. M. (2002): Rheological Characterisation of Aqueous Solutions of the Food Additive Carboximethylcellulose. *Electron. J. Environ. Agric. Food Chem*. 1 (1)

Karawya, M. S., Balbaa, S. I. and Afofi, M. S. A. (1971): Investigation of the carbohydrate contents of certain mucilaginous plants. *Planta Medica*. 20**:** 14- 23.

Lima, R. da S. N., Lima, J. R., Celio, R. de S. and Moreira, R. de A. (2002): Cashew Tree (*Anacardium occidentale* L.) Exudate Gum: a novel bioligand tool. *Biotechnol. Appl. Biochem*. 35: 45-53.

Lorke, D. (1983): A new approach to practical acute toxicity testing. *Arch. Toxicol*. 54: 275-287.

Marques, M. R. and Xavier-Filho, J., (1991): Enzymatic and inhibitory activities of cashew tree gum exudates. *Phytochem.* 30: 1431-1433

Marriot, C. (1988): Rheology and the flow of fluids. In Pharmaceutics, the science of dosage form design. Edited by Aulton, M E. Pp 17- 37

Martin, A., Swarbrick, J., and Cammarata, A., (1983): 3rd Edition; Physical pharmacy, Lea and Febiger, Philadelphia. pp.522-544.

Mothe, C. G. and Rao, M. A. (1999): Rheological behavior of aqueous dispersions of cashew gum and gum Arabic; effects of concentration and blending. *Food Hydrocoll.* 13: 501-506.

Paula, R. C. M. and Rodrigues, J. F. (1995): Composition and rheological properties of cashew tree gum, the exudates polysaccharide from *Anacardium occidentale* L. *Carbohyd. Polym*. 26 (3): 177-181

Sofowora, A. (1993): Medicinal plants and Traditional Medicine in Africa. Spectrum Book Limited, Lagos, Nigeria. Pp 100-108.

Trease, G. E. and Evans, W. C. (1982): Pharmacognosy. 12th edition, Balliere-Tindal, London.

Zakaria, M. B. and Rahman, Z. A. (1996): Rheological properties of cashew gum; *Carbohyd. Polym.* 29 (1): 25-27.