Unilag Journal of Medicine, Science and Technology (UJMST) Vol 6. No. 2, 2018

CHARACTERIZATION OF *MANGIFERA INDICA* LINN (FAMILY: ANACARDIACEAE.) SEED ENDOSPERM GUM FOR POTENTIAL APPLICATION AS PHARMACEUTICAL EXCIPIENT.

***Ologunagba, M.O [1], Azubuike, C. P[1], Silva[1], B.O[1] and Ladanu, O. T[1]**
[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Idi-Araba, Lagos.

*Corresponding Author's Email: mologunagba@yahoo.com
Tel: +2348033953141

ABSTRACT
Natural products are increasingly used as excipients in formulation systems over the synthetics because of their comparative advantages. This study was aimed at the characterization of Mangifera indica seed gum (MisG) extracted from its endosperm using microwave-assisted technique. Physiochemical, pharmacognostic, microbial load were carried out using established methods and standard protocols. The yield of MisG was 15.63 ± 0.05% w/w. Physicochemical analysis revealed that MisG had particle size of 115.00 ± 0.12 µm, moisture content of 10.15 ± 0.11%, pH of 6.20 ±0.03, swelling index of 2.27 ± 0.09, water binding capacity 112.0 ± 0.1%, viscosity of 1.1 ± 0.2cP, flow rate of 3.01 ± 0.09 g/sec, bulk density of 0.51 ± 0.01 g/cm^3, tapped density of 0.63 ± 0.01 g/cm^3, Hausner's ratio of 1.23±0.03, compressibility index of 19.10 ±0.03 and angle of repose of 29.35°. Microbial evaluation revealed the absence of objectionable microorganisms while the total aerobic microbial and yeast and mould counts were 1.20x10^2 and 11.50 x10^1 respectively. These values did not exceed the microbial limits specified by the United States Pharmacopoeia. These desirable physicochemical, pharmacognostical and microbial properties suggest that MisG could have good excipient potentials in pharmaceutical formulations.

Keywords: *Mangifera indica* seed gum; Characterizations; Excipient potential.

INTRODUCTION

Excipients play important roles in development of drug formulations which makes it expedient that their selections are pharmaceutically acceptable with regards to compatibility, non-toxicity and microbial safety with active pharmaceutical ingredients (Airaksinen, *et al.,* 2005). There is an increasing global interest in the use of natural polymers and their derivatives as excipients in pharmaceutical formulations**.** Polymers are macromolecules characterized by biocompatibility, biodegradability, inertness and high stability. These properties make them appealing as pharmaceutical excipients (Anekant *et al*., 2007; Jain, *et al.*, 2007 and Liu, *et al.*, 2007).

Gums are natural polymers that are made up of translucent amorphous substances of a monosaccharide or mixed monosaccharides. Gums being hydrocolloids contain hydrophilic molecules that can combine with water to form viscous solutions or gels (Kipo, *et al.*, 2014). The structure, type and number of monosaccharides and their configuration, number and location of the linked groups that gives each gum its peculiar physicochemical characteristics. The degree of polymerization influences a gum's viscosity and hydration rate. Longer molecules tend to produce higher viscosities and take longer to hydrate than shorter ones (Kipo *et al*., 2014). A highly branched molecule takes up less space than a straight one with the same molecular weight and therefore provides less viscosity (Bhardwaj, *et al*., 2000). Gums can exist as exudates or extractives from barks and fruits or seeds of plants respectively. The search for cheaper, under-utilized, novel and renewable substances as sources of pharmaceutical excipients has led to several studies on utilization of different types of natural gums as excipients (Prajapati, *et al.*, 2013; Jani, *et al.*, 2009: Salamanca, *et al.*, 2018, Ologunagba, *et al*., 2017).

Mangifera indica Linn mango, family: *Anacardiaceae*) is a large evergreen tree that is capable of a growing to a height and crown width of about 100 feet and trunk circumference of more than twelve feet. The Mango fruit is a seasonal fruit that is popularly consumed in many countries worldwide. It is grown in the savannah areas of many African countries.

The average mango composition is water (83 g/100 g), carbohydrate (15.2 g/100 g), sugar (13.7 g/100 g), fibre (1.8 g/100 g), fats (0.38 g/100 g), proteins (0.510 g/100 g), vitamins (mainly vitamin A, 389 mg/100g, and vitamin C, 36 mg/100 g), and minerals (mainly potassium 168mg/100 g and phosphorous 14mg/100 g) (Pamplona and Roger , 2007).

In traditional medicine, varied properties are attributed to different parts of the mango tree, both as food and medicine (Rajan, *et al*., 2011; Shan, *et al*., 2010). Singh *et al*., (2010) established the good binder property of a gum obtained from the stem bark *Mangifera indica* gum in a tablet system. However, to the best of our knowledge the evaluation of *Mangifera indica* seed gum (MisG) extracted from its endosperm using microwave-assisted technique as an excipient in tablet system has not been undertaken. Thus, this study, sought to characterize the physicochemical, microbial and toxicological profiles of MisG (BP, 2017; Aloko *et al*., 2017; Ologunagba *et al.*, 2017; Ogbonnia *et al*., 2010; Ibrahim *et al*., 2016).

MATERIALS AND METHODS

Materials

Ethanol (AnarR^R, Batch No: 1097, BDH Ltd. Poole, England), Glacial acetic acid (Merck KGaA, 64271 , Darmstadt, Germany), Ethyl acetate (K3673464623646, Merck KGaA, 64271, Darmstadt, Germany), Methanol (Lot No: 52BB257AV,CAS 67-56-1, Merck KGaA, 64271, Darmstadt, Germany), Sulphuric acid (Merck KGaA, 64271, Darmstadt, Germany), Chloroform (K48902365718, CAS:67,66-3, Merck KGaA, 64271, Darmstadt, Germany) Hydrochloric acid, Resorcinol , Petroleum ether (Merck KGaA, 64271, Darmstadt, Germany), Acetone (Product :100034Q, BDH Ltd. Poole, England) , Alpha naphthol (CAS No: 90-15-3, Labtech Chemicals), Ferric chloride, Lead acetate, Fehling's solution, Draggendoff's reagent (Pharmacognosy Dept., Faculty of Pharmacy, University of Lagos, Nigeria), Methyl red solution, Bromocresol green (0.1%) and all other reagents were of analytical grades and were used as received from suppliers.

The ripe fresh fruits of *Mangifera indica* were obtained from a local market in Mushin area of Mushin Local Government, Lagos State, Nigeria during its fruiting season**.** The plant was authenticated at the Department of Botany, Faculty of Science, University of Lagos, Voucher Specimen number being, LUH 8077. They were stored for two days at 27 ± ^{0} C and relative humidity (RH) 50 ± 2% prior to use.

Gum Extraction and Purification

The *M. indica* fruits were sorted, cleaned and split open manually. The seeds were sundried for one week and then cracked manually to expose the endosperm which was further sundried for five weeks. The dried endosperm was further subjected to hot air oven (Gallenhamp Oven 300 plus series, England) drying for five days at 40 ± 2 ^{o} C; RH 50 ± 2%. It was then finely powdered with the crushing and milling unit of the Kenwood blender (Model No: A524, 240V, 50Hz, 300W, Kenwood Mfg. Co. Ltd., England). The microwave assisted extraction, a cost effective technique as by indicated by Hong-Wu *et al*., 2010 was used for extraction as described in a previous study (Ologunagba, *et al.*, 2017).

Characterization of *Mangifera indica* Powder

Physiochemical Parameters

The organoleptic properties, moisture content, swelling capacity, hydration, solubility profiles, pH, bulk and tapped densities angle of repose and flow rate of *Mangifera indica* powder were determined using standard procedures (Ajala *et al*., 2016 and Ologunagba *et al.*, 2017). The viscosities of the dispersions of MisG

at 2, 5, 10, 15 and 20% w/v concentrations were determined using shear rates of 0.5, 1, 1.5, 2, 2.5 and 3 rpm with a Brookfield viscometer (spindle number 2) at 25°C .

Phytochemical screening

Phytochemical screening for MisG was carried using the established methods and specified phytonutrient tests in standard texts: carbohydrates (Molish test, Ruthenium), reducing sugars (Fehlings), alkaloids (Dragendorff's), steroids and terpenoids (Liebermann Burchard), tannin and phenolic compounds (Ferric chloride), flavonoids (Shinoda), amino acids (ninhydrin) (Kokate, 1994 and Khandelwal, 2004). The authentication of MisG as a gum was undertaken with the Ruthenium red (ammoniated ruthenium oxychloride) evaluation.

The probate analysis (ash value) was determined with the use of a furnace (Carbolite AAF1100, Parsons Lane, Hope Valley, S336RB, England) in accordance with established methods and as detailed by Ologunagba *et al*., 2017

Microbial load

Microbial load determination was carried out on MisG powder in accordance with the US Pharmacopoeia (2018) procedures for microbial limit test).

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey test to evaluate significant differences between groups. Significant differences between control and experimental groups were assessed with student's t-test. Values of $p < 0.05$ were considered significant. All statistical analyses were carried out using the SPSS for Window XP Software Program (Version13.0).

RESULTS

Physicochemical and Pharmacognostic studies

The yield of 15.63% ± 0.08% MisG from the seed of *M. indica* was obtained. The results of the physicochemical and pharmacognostical studies are presented in Table 1. The MisG gum had fine powder particle size (110 ± 0.12µm) and the moisture content (11.73 % ± 0.11%). The insoluble matter (total ash) content of the extracted MisG was (1.743± 0.07%). MisG has reducing sugars, carbohydrates, fats and oils as its phytochemical constituents. MisG exhibited good solubility in both hot and cold water (Table 1). It had a very low viscosity (1.1± 0.20 cp).

***Table 1: Physiochemical and Pharmacognostical Properties of MisG Powder**

Powder Properties	Result
Organoleptic properties (Colour, Taste, Odour)	Light brown, Bland, Odourless
Particle size (μm)	110.00 ± 0.12
Moisture (%)	11.73± 0.11
pH	6.20± 0.03
Swelling Index (%)	2.27 ± 0.19
Water binding Capacity (%)	112.00± 0.10
Viscosity (Cp)	1.10± 0.30
Flow rate (min^{-1})	3.013±0.09
Bulk Density (g/cm^3)	0.51 ±0.01
Tapped Density (g/cm^3)	0.63 ±0.01
Area of Repose (Aº)	29.35°±0.02
Compressibility Index (%)	19.10± 0.03
Hausner's ratio	1.23 ±0.05
Total ash (%)	1.74 ± 0.07
Phytonutrients: (Reducing sugars ; Carbohydrates)	Positive
Phytonutrients: (Alkaloids, Flavonoids,Terpenoids, Saponins, Steroids,Anthraquinones, Cardiac Glycosides, Phenolics, Tannins, Amino acids)	Negative

± *SEM (n=3)*

Microbial Evaluation

Table 2 shows the microbial load of extracted MisG powder. Objectionable organisms were absent in MisG. The microbial profile of MisG was found to conform to the specifications of the US Pharmacopoeia (2017).

Table 2: Microbial Load of Extracted MisG Powder

Type of Microbial Group	Microbial Load of Extracted MisG	USP Values
Total Aerobic Microbial Count	12000	Not more than 10^5
Total combined Yeast and Mould	1150	Not more than 10^3
Bile Tolerant gram – ve	1500	Not more than 10^3
Salmonella spp	Absent	Absent
Shigella spp	Absent	Absent
Echericha coli	Absent	Absent
Klebsiella spp	Absent	Absent
Psedomonas spp	Absent	Absent
Proteus spp	Absent	Absent
Staphylococcus aereus	Absent	Absent

DISCUSSION

Physicochemical and Pharmacognostic studies

MisG was successfully extracted using the green technology, cost and energy effective microwave extraction technique,

The MisG gum had bland taste, agreeable odour and desirable flavour, which suggest the excipient potential of MisG for the pharmaceutical industry as the possessed organoleptic properties will impact desirable qualities to the final product. The MisG gum had fine powder particle size and the moisture content was within the maximum permissible limit (BP, 2017). The pH of 6.2 inferred its excipient usefulness to both acidic and basic active pharmaceutical ingredients.

Bulk and tapped densities, Hausner's ratio and Carr's compressibility index as well as angle of repose of a material are measures of compressibility and flow properties of powder materials employed as pharmaceutical excipients. The bulk and tapped density values showed there was a reduction in volume of the gum powder due to packing under applied pressure from tapping. The Hausner's ratio, Carr's index and the angle of repose values obtained showed that the gum powder had good flow and compressibility properties (United States Pharmacopoeia, 2018). Moreover, the low bulk and tapped densities of MisG would imply loose packing arrangement of its particles and this might explain its swelling and water binding capacity. This is because water rises by capillary action through pores between particles in a powder. This wicking movement of water through pores in gum powder activates swelling of its outer layer to form a hydrogel, hence the diffusion of the active ingredient from the formulation (Shoaib *et al*., 2006). This suggests both binding and disintegrant potentials of MisG.

Ash values reflect the level of adulteration or handling of a biomaterial, therefore proximate analysis is a measure of the total amount of extraneous substances present within biomaterials. The ash value of MisG fell within the stated Pharmacopoeia permissible maximum limit of 0.5% w/w (BP, 2017). This implied its low level of contamination.

The presence of carbohydrates and reducing sugars suggested the presence of polysaccharides which was established as a gum with the positive outcome on Ruthenium test. The moisture content of any material is an important attribute and could be an inference index for the stability and susceptibility to microbial contamination of a pharmaceutical material. The British Pharmacopoeia specified limit of moisture content for conventionally dried substances is 15% or less. The moisture content of 10.15% for MisG was found to be within the acceptable

range, this therefore implies that MisG would be less prone to microbial contamination and spoilage.

The swelling capacity of the gum demonstrates its hydrophilic nature and also its ability to swell into gel in aqueous media to release the embedded drug. It therefore gives an idea about the viscous nature and binding character of MisG as well as its disintegrant property. The hydration capacity, on the other hand, was suggestive of the water penetrative and retention ability of MisG. Hydration properties could also be linked to the chain length or degree of polymerization of the gum as longer molecules tend to take longer to hydrate than shorter ones. Hence, the shorter chains or highly branched molecules and ultimately less viscous gum.

MisG exhibited good solubility in both hot and cold water (Table 1). This infers that MisG may not have the presence of insoluble metallic compounds. Most salts of calcium and magnesium (Group 2 elements) are usually insoluble in water, and therefore the solubility profile exhibited by MisG may be due to the absence of these salts. MisG had a very low viscosity (0.60 ± 0.30 cp) and would be expected to exhibit the Newtonian pattern of flow.

Microbial Evaluation

The total aerobic microbial counts and total combined yeast and mold count did not exceed limits specified by (USP, 2018). Objectionable organisms (*Salmonella spp.*, *Shigella spp*., *Fscherichia coli*, *Klebsiella spp.,* and *Pseudomonas aeruginosa*) were absent in MisG. The acceptable microbial profile obtained for MisG as a fresh extract was also observed when it was re-evaluated on this parameter after 6 and 12 months of storage. This implies stability of this biomaterial which could be due to its low moisture content that would not encourage microbial contamination and spoilage.

CONCLUSIONS

The endosperm gum of *Mangifera indica* seed has been successfully extracted by microwave assisted extraction technique and characterized. It has been established that MisG has good and desirable physicochemical, pharmacognostical, rheological and microbiological profiles.

These desirable pharmaceutical material attributes infer and suggest the excipient potential usefulness of MisG as either a diluent, disintegrant, binder of low viscosity and emollient in solid and semi-solid pharmaceutical formulations.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the kind assistance and technical support provided by technologists in the University of Lagos in the persons of Mr. Abdul R Usman of the Department of Pharmaceutics & Pharmaceutical Technology and Mr. Solomon Julius of the Department of Pharmacognosy both of the Faculty of Pharmacy as well as Mr. Simeon I. Dike of the Department of Physiology, Faculty of Basic Medical Sciences, University of Lagos.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

REFERENCES

Airaksinen, S., Karjalainen, M., Kivikero, N., Westermarck, S., Shevchenko, A., Rantanen, J., &Yliruusi, J. (2005). Excipient selection can significantly affect solid-state phase transformation in formulation during wet granulation. *AAPs Pharm Scitech,* **6** (2), E311-E322.

Ajala, T. O., Akin-Ajani, O. D., Ihuoma-Chidi, C., and Odeku, O. A. (2016). *Chrysophyllum albidum* mucilage as a binding agent in paracetamol tablet formulations. *Journal of Pharmaceutical Investigation*, **46** (6): 565-573.

Aloko, S., Azubuike, C.P. and Coker, H.A.B. (2017). Physicochemical properties and lubricant potentials of *Blighia sapida* Sapindaceaeae seed oil in solid dosage formulations. *Trop J Pharm Res,* **16** (2):305-311.

Anekant, J., Yashwant, G. and Sanjay, K. J. (2007). Perspectives of Biodegradable Natural Polysaccharides for Site-Specific Drug Delivery to the Colon. *J Pharm Pharmaceutical Sci*., **10** (1): 86-128.

Bhardwaj, T. R., Kanwar, M., Lal, R., & Gupta, A. (2000). Natural gums and modified natural gums as sustained-release carriers. *Drug Development and Industrial Pharmacy*, **26** (10): 1025-1038.

British Pharmacopoeia, (2017). Vol.VI, Appendix XII A. Department of Health, London.

Hong-Wu, W., Yan-Qing, L., Shou-Lian, W., Zi-Jun, Y. and Kuan, L. (2010). Comparison of Microwave-Assisted and Conventional Hydrodistillation in the Extraction of Essential Oils from Mango (*Mangifera indica* L.)

Flowers. *Molecules* **15**: 7715-7723; doi: 10.3390/molecules15117715, ISSN 1420-304 www.mdpi.com/journal/molecules

Ibrahim, M.B., Sowemimo, A.A., Sofidiya, M.O., Badmos, K.B., Fageyinbo, M.S., AbdulKareem, F.B .and Odukoya, O.A., (2016).Subacute and chronic toxicity profiles of *Murkhamia tomentosa* ethanolic leaf extract in rats. *J. Ethnopharmacology*. **193**:68-75.

Jain, A., Gupta, Y., & Jain, S. K. (2007). Perspectives of biodegradable natural polysaccharides for site-specific drug delivery to the colon. *J Pharm Pharm Sci*, **10** (1): 86-128.

Jani, G. K., Shah, D. P., Prajapati, V. D., & Jain, V. C. (2009). Gums and mucilages: versatile excipients for pharmaceutical formulations. *Asian J Pharm Sci***, 4** (5): 309-323.

Kipo, S. L., Oppong, E. E., &Ofori-Kwakye, K. (2014). Physicochemical evaluation and tablet formulation properties of shea tree gum. *Asian J Pharm Sci*, **7**:121-127.

Kokate, C.K. (1994). *Practical Pharmacognosy*, (4th ed.) Delhi: Vallaph Prakashan.107-111.

Khandelwal, K.R. (2004). *Practical Pharmacognosy* (11th ed.).Pune, India: Nirali Prakashan.149-153 and 157-159.

Liu, M., Fan, J., Wang, K., & He, Z. (2007). Synthesis, characterization, and evaluation of phosphated cross-linked konjac glucomannan hydrogels for colon-targeted drug delivery. *Drug Delivery*, **14** (6): 397-402.

Ogbonnia, S.O., Mbaka, G.O., Anyika,E.N. ,Osegbo, O.M.and Igbokwe, N.H. (2010).Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Chromolaena odorata* (L.) King and Robinson (Fam: Asteraceae) in rats; *Agriculture and Biology Journal of North America* **1** (5): 859-865.

Ologunagba, M. O., Azubuike, C. P., Silva, B. O., & Sadiku, O. R. (2017). Characterization of *Chrysophyllum albidum* Linn (family: Sapotaceae) endosperm seed gum for potential application as pharmaceutical excipient. *Trop J Nat Prod Res*, **1** (5): 217-222.

Ologunagba, M. O., Jain, S., Thanki, K., Suresh, S., Furtado, S., Azubuike, C. P., Silva, B. O. (2017). Extraction and Characterization of the Gum Exudate of *Anacardium occidentale* for its potential as an Excipient in Drug Delivery Systems. *Trop J Nat Prod Res***. 1**:76-83.

Pamplona, G. and Roger, M. D. (2007). *Encyclopedia of Foods and Their Healing Power*, vol. 2, S. L Safeliz, Madrid, Spain, 2007.

Prajapati, V. D., Jani, G. K., Moradiya, N. G., &Randeria, N. P. (2013). Pharmaceutical applications of various natural gums, mucilages and their modified forms. *Carbohydrate polymers*, **92** (2): 1685-1699.

Rajan, S., Thirunalasundari, T., & Jeeva, S. (2011). Anti—enteric bacterial activity and phytochemical analysis of the seed kernel extract of *Mangifera indica* Linnaeus against Shigella dysenteriae (Shiga, corrig.) Castellani and Chalmers. *Asian Pacific Journal of Tropical Medicine*, **4**(4): 294-300.

Salamanca, C. H., Yarce, C. J., Moreno, R. A., Prieto, V., &Recalde, J. (2018). Natural gum-type biopolymers as potential modified nonpolar drug release systems. *Carbohydrate polymers*, **189**: 31-38.

Shah, K. A., M. B. Patel, R. J. Patel, and P. K. Parmar. (2010). "*Mangiferaindica* (mango)." *Pharmacogn Rev.* **4**(7): *42–48. doi: 10.4103/0973-7847.65325*

Shoaib, M. H., Tazeen, J., Merchant, H. A., &Yousuf, R. I. (2006). Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. Pakistan Journal of Pharmaceutical Sciences, **19** (2): 119-124.

Singh, A. K., Shingala, V. K., Selvam, R. P., &Sivakumar, T. (2010). Evaluation of *Mangiferaindica*gum as tablet binder. *Inter J Pharm Tech Res*, ***2*:**2098-2100.

United States Pharmacopoeia, (2018). *United States Pharmacopoeia National Formulary* (USP 41 NF 36). Rockville, Md: United States Pharmacopoeial Convention Inc.