Original Research Article

Characterization of Mucilages from *Abelmoschus manihot* Linn., *Amaranthus spinosus Linn*. and *Talinum triangulare* (Jacq.) Willd. Leaves for Pharmaceutical Excipient Application

Aileen May Gabule Ang*, Inocencio Cañete Raman Jr

Department of Chemistry, College of Arts and Sciences, Central Mindanao University, Musuan, Bukidnon, PHILIPPINES.

Submission Date: 20-12-2018; Revision Date: 12-03-2019; Accepted Date: 17-04-2019

ABSTRACT

Introduction: The edible leaves of *Abelmoschus manihot* Linn., *Amaranthus spinosus* Linn. and *Talinum triangulare* (Jacq.) Willd. are exhibiting mucilaginous features and are utilized in making soupy dishes. However, the utilization of the said plant species as sources of mucilages for pharmaceutical excipient application has not yet been reported in literatures. **Objectives:** The study aimed to extract and partially characterize mucilages from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare*. for potential pharmaceutical excipient application. **Materials and Methods:** Mucilages were extracted from the leaves of A. *manihot*, *A. spinosus* and *T. triangulare*. Preliminary qualitative tests as well as determination of the physico-chemical properties, i.e. pH, solubility, swelling index, loss on drying, total sugar content and total uronic acid content, were conducted on the extracted mucilages. Identification of functional groups were also carried out through Fourier Transform Infrared (FTIR) spectroscopy. **Results and Discussion:** Results of the qualitative tests showed detection of carbohydrates, polysaccharides and mucilages among the mucilages extracted from *A. manihot, A. spinosus* and *T. triangulare*. The pH values of the extracted mucilage were nearly neutral indicating that they can be less irritating to the gastrointestinal tract when utilize for tablet formulations. Data obtained for the physico-chemical properties showed that mucilages of *A. manihot* gave statistically different values as compared to the mucilages derived from *A. spinosus* and *T. triangulare*. *A. manihot* mucilage exhibited significantly higher swelling index (357.78%) and total sugar content (273.65 mg glucose/g extract) and significantly lower loss on drying (10.72%) and total uronic acid content (105.20 mg glucuronic acid/g extract) than the other mucilages from *A. spinosus* and *T. triangulare*. Furthermore, FTIR spectral analysis showed typical peaks and characteristic bands of mucilage. **Conclusion:** *A. manihot* mucilage is promising for utilization as pharmaceutical excipient. However, further analysis is recommended.
Key words: *Abelmoschus manihot*, *Amaranthus spinosus*, *Talinum triangulare,* Mucilages.

Correspondence:
Aileen May Gabule Ang,
Department of Chemistry, College of Arts and Sciences, Central Mindanao University, Musuan, Bukidnon, PHILIPPINES.
Phone no.: +63 9753344190

Email: aisemg_ang@yahoo.com

INTRODUCTION

Pharmaceutical excipient is added intentionally to control the release of the active drug, improve the bioavailability and efficacy of the drug in the medicinal formulation and stabilize pharmaceutical dosage.[1,2]

SCAN QR CODE TO VIEW ONLINE	
	www.ajbls.com
	DOI : 10.5530/ajbls.2019.8.3

Pharmaceutical excipient can be animal, plant, mineral or of synthetic origin such as stearic acid, cellulose, calcium phosphate or polysorbates.[2] The plant-derived excipient, specifically the mucilage, is preferred over synthetic materials since it is nontoxic, non-irritating, easily available and low cost.[3]

Mucilage is a natural product of the normal metabolic processes of a plant cells. The binding, disintegrating, suspending, emulsifying and sustaining properties of mucilage guarantee its utilization in the development of desired pharmaceutical dosage forms.[4-6]

In the Philippine archipelago, the edible leaves of *Abelmoschus manihot* Linn. (lagikway), *Amaranthus spinosus* Linn. (kulitis) and *Talinum triangulare* (Jacq.) Willd. (talilong) are exhibiting mucilaginous features, i.e. slimy and gelatinous colloids when soaked in water and are used by the Filipinos in soupy dishes.[7]

In addition, it is confirmed scientifically that the *A. manihot* possesses anti-inflammatory,[8] anticonvulsant and antidepressant[9] and antiviral activities.[10] The *A. spinosus,* on the other hand, possesses antinociceptive,[11] antioxidant[12] and chemoprotective activities.[13] *T. triangulare* has been reported to exhibit antidiabetic[14] antioxidant and hepatoprotective activities.[15] However, the utilization of the said plant species as sources of pharmaceutical excipients is not yet been reported in literatures. Thus, this study was conducted.

MATERIALS AND METHOD

Sample Collection and Preparation

Healthy and mature leaves of *A. manihot*, *A. spinosus and T. triangulare* were collected from Lanise, Claveria, Misamis Oriental, Bangcud, Malaybalay City, Bukidnon and Poblacion, Santa Fe, Cebu, respectively. Plant samples were authenticated by the Botany section of the Central Mindanao University (CMU) Museum, Musuan, Bukidnon, Philippines. The collected samples were washed and rinsed with distilled water, air-dried and homogenized.

Isolation of Mucilage

150 gms of powdered leaf samples of *A. manihot*, *A. spinosus* and *T. triangualare* were separately soaked in 750 mL of distilled water, allowed to stand for 24 hrs, heated at 60°C and stirred for 1 hr. After an hour, the mucilaginous extracts were squeezed through muslin cloth. The supernatant was collected and added with equal volume of absolute ethanol to precipitate the mucilage. The mixture was kept in the refrigerator at 20°C for 24 hrs. The precipitate was filtered, washed with acetone and oven-dried at 40°C for 48 hrs. The dried sample was weighed and ground into a fine mucilage powder using a mortar pestle and stored in a glass container inside the refrigerator for further analysis.[16]

Preliminary Identification of Mucilage

The preliminary identification tests using Ruthenium Red test, Molisch's test, Benedict's test, Iodine test and Biuret test were conducted to confirm the nature of the extracted mucilages.[17]

Molisch's Test for Carbohydrates

In a 4 mL of 0.25 % (w/v) aqueous solution of mucilage extract, few drops of Molisch's reagent (5 gms of α-naphthol dissolved into 95% ethanol and diluted to 100 mL) and few drops of concentrated sulfuric acid were added from the side wall of the test tube. A formation of purple-colored ring at the junction of the 2 layers would indicate the presence of carbohydrates.

Benedict's Test for Monosaccharides

In a 4 mL of 0.25 % (w/v) aqueous solution of mucilage extract, 1 mL of Benedict's solution (1.73 gms of copper sulfate, 10 gms of sodium carbonate and 17.3 gms of sodium citrate were dissolved in distilled water and diluted to 100 mL) was added and heated almost to boiling in a water bath. The brick red precipitate would confirm the presence of monosaccharide.

Iodine Test for Polysaccharides

In a 4 mL 0.25 % (w/v) aqueous solution of mucilage extract, 1 mL of 0.2 N iodine solution (5.08 gms of potassium iodide and 2.54 gms of iodine crystals were dissolved in distilled water and diluted to 100 mL) was added. If no color was observed in the solution, result would indicate presence of polysaccharides and absence of starch. Formation of blue color, which disappears on heating and reappears on cooling, would indicate the presence of starch.

Biuret Test for Protein

In a 2 mL of 0.25 % (w/v) aqueous solution of mucilage extract, 2 mL of 10% sodium hydroxide solution and 2-3 drops of 1% $CuSO_4$ solution were added and mixed. The presence of violet or purple color would confirm the presence of protein.

Ruthenium Red Test for Mucilage

A 0.02 g of ruthenium red was dissolved in 2.5 mL of 10% solution of lead acetate. A very small quantity of 0.25 % (w/v) aqueous solution of mucilage extract was put into it. The mucilage stains to red or pink color.

Physico-chemical Properties of Mucilage

Solubility Determination

1 gm of solid mucilage was dissolved in four different solvents such as cold water, hot water, methanol and chloroform.[18] Results were then recorded.

Swelling Index Determination

1 gm of mucilage was accurately weighed and introduced into a 10 mL of water in a graduated cylinder. The initial volume occupied by mucilage in the graduated cylinder was recorded. The mixture was then shaken thoroughly

every 10 mins for 1 hr and was then allowed to stand for 24 hrs at room temperature. After 24 hrs, the volume occupied by mucilage was measured and the swelling index was calculated using Equation 1.[19]

$$\text{Swelling Index (SI), \%} = 100\,(X_f - X_i)/X_i \qquad \text{Eq.1}$$

where:

X_i = initial volume of mucilage in graduated cylinder, mL

X_f = final volume after hydration, mL

pH Determination

1 gm of mucilage was weighed and dissolved by shaking in distilled water for 30 mins to get a 1% (w/v) suspension of mucilage. The pH of the solution was determined using a pH meter (Eutech Cyberscan PC 300).[20]

Loss on Drying Determination

About 1 g of the mucilage was placed in a pre-weighed crucible. The sample was oven-dried at 105°C for 2 hrs. The oven dried sample was transferred into a desiccator and allowed to cool for 30 mins. The oven-drying was repeated until constant weight (± 0.0004 g) was obtained. The percentage LOD was calculated using Equation 2.[20]

$$\text{Loss on Drying (LOD), \%} = 100\,(Y_i - Y_f)/Y_i \qquad \text{Eq. 2}$$

where:

Y_i = initial weight of mucilage, g

Y_f = final weight after drying, g

Total Sugar Determination

Total sugar content in the mucilage was determined by phenol-sulfuric method.[21] The mucilage solution was prepared by dissolving about 10 mg of mucilage in 100 mL of distilled water to obtain 0.1 mg/mL sample test solution.

From 1000 µg/mL aqueous stock solution of D-glucose, various concentrations of working standards (0, 10, 25, 40, 55, 70, 85 and 100 µg/mL) were prepared for the calibration curve. 2 mL of the mucilage or standard solution was pipetted into a test tube and added with 0.05 mL of 80% phenol. A 5 mL of concentrated sulfuric acid was then rapidly added into the mixture. The stream of acid was directed against the liquid surface rather than against the side of the test tube in order to obtain good mixing. The tubes were allowed to stand for 10 mins, shaken and placed in a water bath for 10 to 20 mins at 25-30°C before reading was taken. The absorbance was measured at 490 nm using a UV spectrophotometer (Shimadzu UV-1800). Blank determination was also conducted.

From the calibration curve data, the milligram D-glucose per liter of the sample solution was determined using the linear regression equation of the line. Total D-glucose, expressed as mg glucose/g extract, was calculated using Equation 3.

$$\text{Total D-glucose, mg glucose/g extract} = A/C \qquad \text{Eq.3}$$

where:

A = concentration of glucose in the sample solution based on the calibration curve, mg/L

C = concentration of sample test solution, g/L

Total Uronic Acid Determination

The mucilage solution was prepared by dissolving about 10 mg of mucilage in 100 mL of distilled water to obtain 0.1 mg/mL sample test solution.

From 1000 µg/mL aqueous stock solution of glucuronic acid, working standards with various concentrations (0, 10, 25, 40, 55, 70, 85 and 100 µg/mL) were prepared. On the other hand, 0.0125 M sodium tetraborate reagent in concentrated sulfuric acid and 0.15% m-hydroxy-diphenyl reagent in 0.5% NaOH were prepared and kept in the refrigerator.

A 6 mL of sodium tetraborate reagent was added to 1 mL of the mucilage solution or standard solution in the test tube. The mixture was shaken, refrigerated in crushed ice, vortexed and heated in a water bath at 100°C for 5 mins. After 5 mins, the mixture was cooled in an ice-water bath and 100 µL of the m-hydroxydiphenyl reagent was added. The mixture was vortexed and, within 5 mins, absorbance measurements at 520 nm were recorded. The carbohydrates produced a pinkish chromogen with sulfuric acid at 100°C.[22]

From the calibration curve data, the milligram glucuronic acid per liter of sample solution was determined using linear regression equation of the line. Total D-glucuronic acid, expressed as mg glucuronic acid/g extract, was calculated using Equation 4.

$$\text{Total D-glucuronic acid, mg glucuronic acid/g extract} = A/C \qquad \text{Eq.4}$$

where:

A = concentration of glucuronic acid in sample solution based on the calibration curve, mg/L

C = concentration of sample test solution, g/L

FTIR Spectral Analysis of Functional Groups in Mucilage

The analyses of the diagnostic bands which are indicative of the functional groups present in the isolated mucilages were determined by FTIR Spectroscopy.

The mucilage powder was analyzed as powder in KBr using FTIR spectrophotometer (Shimadzu IRAffinity-1S). The mixture powder was placed in the sample holder and spectral scanning was taken at a resolution of 4 cm^{-1} with scan speed of 1 cm/s.[23]

Statistical Analysis of Data

The data gathered in the determination of the physico-chemical properties of the mucilages were subjected to One-Way Analysis of Variance (ANOVA) in Randomized Complete Block Design (RCBD) at 0.05 Level of Significance. Significant differences among the means were determined using Tukey's Test.

RESULTS

Percentage Yield

The *A. manihot*, *A. spinosus* and *T. triangulare* yielded yellowish brown, gray and brown amorphous mucilage powders (Figure 1), respectively.

The percentage yield of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare* were presented in Table 1.

Qualitative Tests for Mucilage

Mucilages are highly branched polymeric structure built from many different sugar units and uronic acid.[24] Table 2 summarizes the results of the qualitative tests conducted.

Physico-Chemical Properties

Solubility

The results of the solubility test are shown in Table 3.

pH, Swelling Index and Loss on Drying

The pH, percent loss on drying and swelling index of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare* are presented in Table 4.

Total Sugar and Uronic Acid Content

The total sugar (mg D-glucose/g extract) and uronic acid (mg D-glucuronic acid/g extract) content of the mucilage from *A. manihot*, *A. spinosus* and *T. triangulare* leaves is shown in Figure 2.

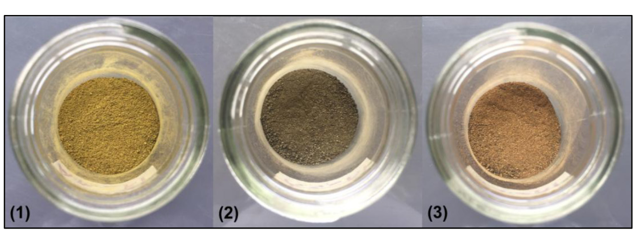

Figure 1: Mucilage powders from the leaves of (1) *A. manihot*, (2) *A. spinosus* and (3) *T. triangulare*.

Table 1: The percentage yield of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare*.

Plant	Yield, % (RSD)
A. manihot	8.76 (8.37)
A. spinosus	5.85 (4.49)
T. triangulare	5.30 (11.56)

Table 2: Results of the qualitative tests of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare*.

Identification Test	Plant		
	A. manihot	*A. spinosus*	*T. triangulare*
Test for Carbohydrates	+	+	+
Test for Monosaccharides	-	-	-
Test for Polysaccharides	+	+	+
Test for Starch	-	-	-
Test for Protein	-	+	+
Test for Mucilage	+	+	+

"+" – present; "-" – absent

Table 3: Solubility profile of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare*.

Plant	Solvent			
	Cold water (2°C)	Hot water (80°C)	Methanol	Chloroform
A. manihot	Slightly soluble (forms slimy mass)	Slightly soluble (forms slimy mass)	Insoluble	Insoluble
A. spinosus	Soluble	Soluble	Insoluble	Insoluble
T. triangulare	Soluble	Soluble	Insoluble	Insoluble

FTIR Spectra of the Mucilage

The FTIR spectra of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare* are presented in Figure 3.

DISCUSSION

Percentage Yield

The *A. manihot* leaves recorded the highest mucilage yield (8.76% ± 0.73) while 5.85% ± 0.26 and 5.30% ± 0.61 yield were obtained for *A. spinosus* and *T. triangulare*, respectively (Table 1).

Table 4: pH, swelling index (%) and loss on drying of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare*.

Plant	pH, (RSD)	Swelling Index, % (RSD)	LOD, % (RSD)
A. manihot	6.83 (0.00)	357.78 (8.81)[a]	10.72 (4.25)[c]
A. spinosus	7.11 (0.16)	120.51 (7.37)[c]	13.59 (1.98)[a]
T. triangulare	7.16 (0.00)	216.67 (1.90)[b]	11.48 (2.22)[b]

[a,b,c] – means of the same letter superscript within a column are not significantly different by Tukey's test at 0.05 level of significance

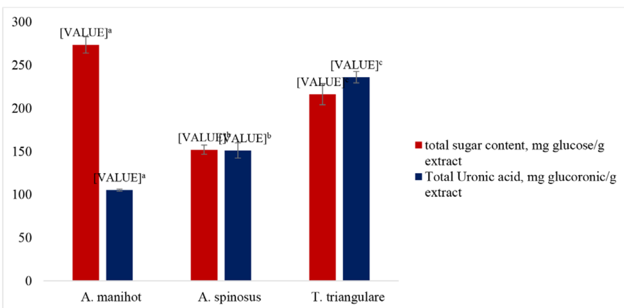

Figure 2: Total sugar (mg D-glucose/g extract) and uronic acid (mg D-glucuronic/g extract) content of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare*. ([a,b,c] – means of the same letter superscript are not significantly different by Tukey's test at 0.05 level of significance)

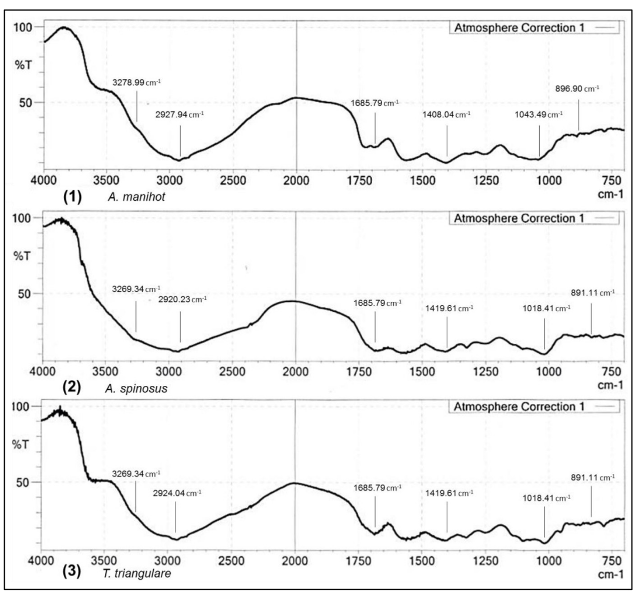

Figure 3: The FTIR spectra of the mucilage from the leaves of (1) *A. manihot*, (2) *A. spinosus* and (3) *T. triangulare*.

Qualitative Tests

The isolated mucilage from the leaves of *A. manihot, A. spinosus* and *T. triangulare* showed presence of carbohydrates, polysaccharides and mucilage. However, monosaccharides and starch were absent among the mucilages from the three plant species. Protein was, moreover, found present in *A. spinosus* and *T. triangulare* only, but not in *A. manihot*.

The results obtained are consistent with the results of previous studies. The confirmatory tests for mucilages from *Opuntia dillenii*,[25] *Coccinia indica*,[26] *Abelmoschus escalantus*[27] and *Trigonella foenum-graecum*[23] showed presence of carboyhrates, polysaccharides and mucilages, while absence of monosaccharides and starch. The results of qualitative tests can be used as preliminary proof for purity of the isolated mucilage.[27] Mucilage, being polysaccharide, should only give positive result to the test for carbohydrates. The detected protein in the mucilage of *A. spinosus* and *T. triangulare* can be considered as impurities.[28] Thus, based on the results of the qualitative tests, mucilage from *A. mahinot* can be considered relatively pure than the other two mucilage powders.

Physico-Chemical Properties

Solubility

Solubility is the property of a solute to dissolve in a solvent to form a homogeneous solution. The mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare* are insoluble in 100 mL methanol and chloroform. In 100 mL hot and cold water, only the mucilage from *A. spinosus* and *T. triangulare* are soluble. *A. mahinot* mucilage swells and forms slimy mass in both hot and cold water (Table 3).

Mucilages are insoluble in organic solvents like methanol and chloroform but dissolve or swell in water, forming slimy mass.[28] Moreover, mucilages being plant hydrocolloids contain hydrophilic molecule which combine with water to form viscous solution or gels.[29] Swellable polymers are either water insoluble hydrogels or water soluble hydrophilic polymers. Both could be used in designing sustained release dosage forms.[30]

The nature of the compound involved in the polysaccharide, like gum and mucilages, influences their properties.[28] *A. manihot* mucilage which forms gels more easily is speculated to be made up of branched polysaccharides. Accordingly, branched polysaccharides form gels more easily and are more stable.[28] In a previous study conducted, mucilage from *Hibiscus rosa-sinensis* leaves swells well when in contact with water in colon and release drug in sustained and controlled manner for a long time thereby preventing tablet burst as it comes in contact with water causing dose dumping that can pose a significant risk to patient, either due to safety issues or diminished efficacy or both.[31] Thus, among the mucilage powders, *A. manihot* can be potential for pharmaceutical application.

pH

The pH is an important factor in determining the suitability of the excipient in tablet formulations since the stability and physiological activity of most pharmaceutical preparations depends on the pH.[25]

The results, as shown in Table 4, indicate that the leaf mucilage solutions are nearly neutral. A neutral pH of an excipient is important since it denotes less irritation to the gastrointestinal tract when used in uncoated tablets. Excipient with the neutral pH may find useful application in formulation of acidic, basic and neutral drugs.[32,33] Thus, the three leaf mucilage samples may be potentially used for uncoated tablet formulations.

Swelling Index

Swelling index denotes the degree of granule hydration.[34] The swelling capacity of mucilage reflects the increase in its volume following water absorption.

Although, the three leaf mucilage powders exhibited high swelling index (Table 4), *A. manihot* registered the highest value while *A. spinosus* gave the lowest value. The results of the analysis of variance (ANOVA) at 0.05 level of significance, reveal significant differences in the percent swelling index among the leaf mucilages. The subsequent Post Hoc Tukey's Test indicates that the swelling index of mucilage from the leaves of *A. manihot* (357.78%) gave significantly higher value than those of *T. triangulare* (216.67%) and *A. spinosus* (120.51%).

Swelling capability of excipient is related to wetting properties. High swelling capability may be due to excellent wetting property, which is the ability to form more viscous mixtures.[13] Swelling could be a result of entanglement of the polysaccharide chains and development of intra-and inter-molecular hydrogen bonds between the polysaccharide and water causing more water to be entrapped within the macromolecular chains.[35]

The increase entrance of water may result in the disruption of H-bonds holding the molecules together thereby breaking up the tablet.[13] Previous studies showed that swelling properties of excipients correlated best with swelling of tablet formulation mixtures and consequently, enhances their efficiency in increasing drug dissolution.[36] Moreover, the swelling index is useful for modulating the drug release.[25] The increase in swelling increases the surface area, surface wettability and consequently, water penetration to form biofilm matrices with higher hydrophilic nature which could be easily biodegradable.[37] Hydrophilic polymers like mucilage have natural swelling properties and when in use as pharmaceutical excipient, the tablet weight would increase as the time increases when in contact with water.[38] High swelling index mucilage may perform well as a binder, disintegrant and matrixing agent.[39] Thus, the mucilage from the leaves of *A. manihot*, with a significantly high swelling index, may be utilized as a potential disintegrating and binding agent in drug formulation.[25,40]

Loss on Drying

Loss on drying (LOD) is used to determine the moisture content and the loss of volatile matter in a material. The % LOD of the mucilage increases in the order of *A. manihot* < *T. triangulare*< *A. spinousus* (Table 4). The data indicate hygroscopic nature of the extracted mucilage and, thus, needs to be stored in air-tight container.[27]

The analysis of variance (ANOVA) at 0.05 level of significance reveal significant differences among the percent LOD values of the mucilages. The subsequent Post Hoc Tukey's Test showed that the LOD of mucilage from *A. manihot* is significantly lower than the LOD values of the mucilages from *A. spinosus* and *T. triangulare.*

The determination of loss on drying of a material is of great importance in order to optimize the production process such as drying, packing and storage of an excipient for industrial application. Inherent moisture in pharmaceutical excipient could lead to the activation of enzymes and proliferation of microorganisms which affects the shelf life of tablet formulations[41,42] especially for tablet dosage containing moisture-sensitive drugs.[43] Although LOD values of the mucilages from *A. manihot, A. spinosus* and *T. triangulare* does not exceed the regulatory limit (15.0%) set by US Pharmacopeia, the mucilage from *A. manihot*, having the significantly lowest LOD, is the most potent for pharmaceutical excipient application.[44]

Total Sugar Content

Mucilages consist mainly of polysaccharides[45] that generally have a high water-binding due to high concentration of hydroxyl groups.[46] Polysaccharides, such as gums and mucilage, are very important as pharmaceutical excipient.[47] In the determination of the total sugar content in the leaf mucilages, the addition of a strong acid led to the hydrolysis of polysaccharide chain yielding various simple sugar units, i.e. glucose, arabinose, galactose, rhamnose and galacturonic acid among others.[48]

ANOVA at 0.05 level of significance reveal significant differences among the total sugar content of the mucilages (Figure 2). Subsequent Post Hoc Tukey's Test showed significantly higher total sugar content in the *A. manihot* mucilage than those of *T. triangulare* and *A. spinosus*.

The high glucose levels in mucilage resulting from the acid hydrolysis of *A. manihot* mucilage extract may the suggest the presence of abundant glycosidic polysaccharides. Moreover, the previously observed high swelling index of *A. manihot* is associated to its high total sugar content. High total sugar content indicates high concentration of hydroxyl groups.[46] With this, *A. manihot* mucilage have high water binding capacity and, consequently, high swelling index. Furthermore, sugar-based excipients are preferably used for oral tablets as alternative to conventional dosage forms to achieve patient-friendly dosage form of bitter drugs. The sugar-based excipients are degraded by the bacteria present in human colon which make them potentially useful for targeted drug delivery systems to the colon.[49,50] Thus, the mucilage from the leaves of *A. mahinot* can be potentially utilized for pharmaceutical application specifically as sugar-based excipient.

Total Uronic Acid Content

Sugars in mucilage contain uronic acid.[51] Uronic acid is a component in repeating unit of all acid mucopolysaccharide.[52] The total uronic acid content among the mucilages are significantly different by ANOVA at 0.05 level of significance (Figure 2). The subsequent Post Hoc Tukey's Test indicate that the total uronic acid contentin *A. manihot* mucilage is significantly lower than those of *A. spinosus* and *T. triangulare*.

The amount of uronic acid in the polysaccharide is related to the total sugar content in the mucilage. As have been reported, polymers containing uronic acid resist acid hydrolysis because the carboxylic acid moiety stabilizes the glycosidic linkage.[53]

The high total sugar content in *A. manihot* mucilage may be attributed to the structural composition of the polymeric mucilage. It is speculated that *A. manihot* mucilage, may contain the least number of uronic acid in its structure. Thus, resistance to acid hydrolysis is low, thereby, giving the highest concentration of simple sugars upon hydrolysis.

Amount of uronic acid play significant role in tablet formulations. The uronic acid residues can also alter the characteristics and modify the solubility of associated polysaccharide conjugates.[36] In the present study high water solubility of *T. triangulare* and *A. spinosus* can be accounted to its high uronic acid content. Thus, *T. triangulare* and *A. spinosus*, may be appropriate as pharmaceutical excipient for fast release drugs while mucilage from *A. manihot* is potential for sustained release therapeutics.[18]

FTIR Spectra of the Mucilages

The FTIR spectra (Figure 3) of the mucilage from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare* exhibit the typical characteristic peaks of mucilage. The diagnostic bands between 3400 and 3200 cm^{-1} can be assigned to –OH groups,[28] while peak at 1420 cm^{-1} is attributed to symmetrical deformation of the C-H and C-OH groups.[54] The band at 2920 cm^{-1} is associated to aliphatic C-H stretching.[55] The bands at 1150-1000 cm^{-1} may be attributed to carbohydrates.[56,57]

CONCLUSION

The present study showed the potential of utilizing *A. manihot* as source of mucilage for pharmaceutical excipient application. The preliminary results obtained serve only as baseline data. Further studies on the organoleptic properties of the mucilage as well as the pre- and post-compression parameters of tablet formulations using mucilage from *A. manihot* is recommended.

ACKNOWLEDGEMENT

The authors wish to thank the Chemistry Department, Natural Science Research Center and Natural Products Research and Development Center of Central Mindanao University, Musuan, Bukidnon for the assistance during the conduct of the study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in the subject matter or materials discussed in this manuscript.

ABBREVIATIONS

FTIR Spectroscopy: Fourier Transform Infrared Spectroscopy; **SI:** Swelling Index; **LOD:** Loss on Drying; **ANOVA:** Analysis of Variance; **RSD:** Relative Standard Deviation.

SUMMARY

This study aimed to extract and partially characterize mucilages from the leaves of *A. manihot*, *A. spinosus* and *T. triangulare*. for potential pharmaceutical excipient application. Preliminary qualitative tests as well as determination of the physico-chemical properties, i.e. pH, solubility, swelling index, loss on drying, total sugar content and total uronic acid content, were conducted

on the extracted mucilages. Identification of functional groups were also carried out through Fourier Transform Infrared (FTIR) spectroscopy. Results of the study highlights the potential of *A. manihot* mucilage for phameceurical excipient application. However, further studies on the organoleptic properties of the mucilage as well as the pre- and post-compression parameters of tablet formulations using mucilage from *A. manihot* is recommended.

REFERENCES

1. Elder DP, Kuentz M, Holm R. Pharmaceutical excipients - Quality, regulatory and biopharmaceutical considerations. Eur J Pharm Sci. 2016;87:88-99.
2. Pifferi G, Restani P. The safety of pharmaceutical excipients. Farmaco. 2003;58(8):541-50.
3. Kulkarni GT, Gowthamarajan K, Rao B, Suresh B. Evaluation of binding properties of selected natural mucilages. J Sci Ind Res India. 2002;61:529-32.
4. Prajapati DV, Jani KG, Moradiya GN, Randeria PN. Pharmaceutical applications of various natural gums, mucilages and their modified forms. Carbohydr Polym. 2014;92(2):1685-99.
5. Sengkhamparn N, Bakx EJ, Verhoef R, Schols HA, Sajjaanantakul T, Vora-Gen AGJ. Okra pectin contains an unusual substitution of its rham- nosyl residues with acetyl and alpha-linked galactosyl groups. Carbohydr Res. 2009;344(14):1842-51.
6. Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR. Farmacognosia: da planta ao medicamento (6th ed.). Porto Alegre: UFRGS Editora, Florianópolis: Editora da UFSC. 2007.
7. Agbonon A, Eklu-Gadegbeku K, Aklikokou K, Gbeassor M, Akpagana K, Tam TW, *et al*. *In vitro* inhibitory effect of West African medicinal and food plants on human cytochrome P450 3A subfamily. J Ethnopharmacol. 2009;128(2):390-4.
8. Jain PS, Bari SB. Evaluation of wound healing effect of *Abelmoschus manihot* in rats. Rev Bras Farmacogn. 2010;20(5):756-61.
9. Guo J, Xue C, Duan JA, Qian D, Tang Y, You Y. Anticonvulsant, antidepressant-like activity of *Abelmoschus manihot* ethanol extract and its potential active components *in vivo*. Phytomedicine. 2011;18(14):1250-4.
10. Lin-Lin WU, Xin-Bo Y, Zheng-Ming H, He-Zhi L, Guang-Xia WU. *In vivo* and *in vitro* antiviral activity of hyperoside extracted from *Abelmoschus manihot* (L.). Med Acta Pharmacol Sin. 2007;28(3):404-9.
11. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective and antioxidant activity of *Amaranthus spinosus* against CCl_4 induced toxicity. J Ethnopharmacol. 2009;125(2):364-66.
12. Odhavo B, Beekrum S, Akula US, Baijnath H. Preliminary assessment of nutritional value of traditional leafy vegetables in Kwazulu-Natal, South Africa. J Food Compos Anal. 2007;20(5):430-5.
13. Kumar P, Kulkarni GT. Characterization of mucilage from *Grewia optiva* as pharmaceutical excipient. J Chronother Drug Deliv. 2013;3(2):55-67.
14. Emaleku SA, Omueti OD, Emaleku GO. *Talinum triangulare* Whole wheat meal fortified with soy flour consumed with *Talinum triangulare* (gbure) soup glycemic index and the test human subjects' lipid profiles. Diabetes Metab Syndr. 2018;12(6):831.
15. Liang D, Zhou Q, Gong W, Wang Y, Nie Z, He H. Studies on the antioxidant and hepatoprotective activities of polysaccharides from *Talinum triangulare*. J Ethnopharmacol. 2011;136(2):316-21.
16. Deshmukh SS, Katare YS, Shyale SS, Bhujbal SS, Kadam SD, Landge DA, Shah DV, *et al*. Isolation and evaluation of mucilage of *Adansonia digitata* Linn as a suspending Agent. J Pharm. 2013;2013:1-4.
17. Ahmad S. Introduction of Plant Constituents and their Tests. Department of Pharmacognosy and Phytochemistry. Nagar, New Delhi. 2007;2-40.
18. Malviya R. Extraction characterization and evaluation of selected mucilage as pharmaceutical excipient. Polim Med. 2011;41(3):39-44.
19. Deeksha BE, Rishabha MA, Pramod K, Sharma F. Extraction and characterization of *Aegle marmelos* derived polymer as a pharmaceutical excipient. Polim Med. 2014;44(3):141-6.
20. Bhat V, Nayak R, Praveena MB. Isolation and evaluation of disintegrating properties of *Basella alba* Linn. leaf mucilage in tablet formulations. J Biomed Pharm Re. 2015;4(2):29-42.
21. Dubois M, Gilles KA, Hamilton JK, Rebers AP, Smith F. Calorimetric method for determination of sugars and related substance. Am J Analyt Chem. 1956;28(3):350-6.
22. Blumenkrantz N, Asboe-Hansen G. New method for quantitative de- termination of uronic acids. Anal Biochem. 1973;54(2):484-9.
23. Nayak AK, Pal D, Santra K. Screening of polysaccharides from tamarind, fenugreek and jackfruit seeds as pharmaceutical excipients. Int J Biol Macromol. 2015;79:756-60.
24. Elli M, Cattivelli D, Soldi S. Evaluation of prebiotic potential of refined psyllium (*Plantago ovata*) fiber in healthy women. J Clin Gastroenterol. 2008;42:S174-6.
25. Kalegowda P, Chauhan AS, Nanjaraj USM. *Opuntia dillenii* (Ker-Gawl) Haw cladode mucilage: Physico-chemical, rheological and functional behavior. Carbohydr Polym. 2017;157:1057-64.
26. Motiwala MN, Dumore MN, Rokde VV, Bodhe MM, Gupta RA, Dumore NG, *et al*. Characterization and antioxidant potential of *Coccinia indica* fruit mucilage: Evaluation of its binding properties. Bioact Carbohydr Dietary Fibre. 2015;6(2):69-74.
27. Farooq U, Malviya R, Sharma PK. Extraction and characterization of okra mucilage as pharmaceutical excipient. Acad J of Plant Sci. 2013;6(4):168-72.
28. Jana S, Saha A, Nayak AK, Sen KK, Basu SK. Aceclofenac-loaded chitosan-tamarind seed polysaccharide interpenetrating polymeric network microprarticles. Colloid Surf B Biointerf. 2012;105:303-9.
29. Troncoso OP, Zamora B, Torres FG. Thermal and Rheological Properties of the Mucilage from the Fruit of *Cordia lutea*. Polym Renewable Resour. 2017;8(3):79-90.
30. Kolhe S, Kasar T, Dhole SN, Upadhye M. Extraction of mucilage and its comparative evaluation as a binder. Adv Drug Deliv Rev. 2014:2(3):330-43.
31. Jayapirakasam SV, Prabakaran L, Donthireddy BR. Formulation development and characterization of *Hibiscus rosa-sinesis* dry leaves mucilage as smart polymer for pharmaceutical use. Int J Appl Res Nat Prod. 2015;8(2):28-36.
32. Suvakanta D, Narsimha MP, Pulak D, Joshabir C, Biswajit D. Optimization and characterization of purified polysaccharide from *Musa sapientum* L: As a pharmaceutical excipient. Food Chem. 2014;149:76-83.
33. Shittu AO, Oyi A, Isah AB, Kareem SO, Ibrahim MA. Formulation and evaluation of microcrystalline tapioca starch as a filler–binder for direct compression. Int J Pharm Sci Res. 2012;3(7):2180-0.
34. Reyes EA, Navas HC, Rivera RG, Guerrero VV, Ramire JA, Alonso CP. Functional properties and physicochemical characteristics of tamarind (*Tamarindus indica* L.) seed mucilage powder as novel hydrocolloid. Int J Food Eng. 2007;209:68-75.
35. Azubuike CP, Alfa MA, Oseni BA. Characterization and Evaluation of the Suspending Potentials of *Corchorus olitorius* Mucilage in Pharmaceutical Suspensions. Trop J Nat Prod Res. 2017;1(1):39-46.
36. Chen H, Zhang M, Xie B. Quantification of uronic acids in tea polysaccharide conjugates and their antioxidant properties. J Agric *Food* Chem. 2004;52(11):3333-6.
37. Archana G, Sabina K, Babuskin S, Radhakrishnan K, Fayidh MA, Azhagu SBP, *et al*. Preparation and characterization of mucilage polysaccharide for biomedical applications. Carbohydr Polym. 2013;98(1):89-94.
38. Kshirsagar RV, Jain V, Wattamwar S. Effect of different viscocity grade hpmc polymers on gastroretantive drug delivery of metformin HCL. Int J Appl Biol Pharm. 2009;1(1):44-50.
39. Nep E, Conway BR. Grewia Gum 1: Some mechanical and swelling properties of compact and film. Trop J Nat Prod Res. 2010;10(4):385-92.
40. Ahuja M, Kumar A, Yadav P, Singh K. *Mimosa pudica* seed mucilage: Isolation; characterization and evaluation as tablet disintegrant and binder. Int J Biol Macromol. 2013;57:105-10.
41. Singh, AK, Shingala VK, Selvam RP, Sivakumar T. Evaluation of *Mangifera indica* gum as tablet binder. Int J Pharmtech Res. 2010;2(3):2098-100.
42. Martins E, Omoyeme I, Christiana I, Ofoefule S, Olobayo K. Isolation, characterzation and compaction properties of *Afzelia africana* gum exudates in hydrochlorothiazide tablet formulations. Afr J Pharm Pharmacol. 2009;3(5):265-72.

43. Patel DM, Prajapati DG, Patel NM. Seed mucilage from *Ocimum americanum* linn. as disintegrant in tablets: Separation and evaluation. Indian J Pharm Sci. 2007;69(3):431.
44. US Pharmacopeia National Formulary. USP 38 NF 33 through Second Supplement. 2015.
45. Müller BM, Kraus J, Franz G. Chemical structure and biological activity of water soluble polysaccharides from *Cassia angustifolia* leaves. Planta Medica. 1989;55(6):536-9.
46. Clifford SC, Arndt SK, Popp M, Jones HG. Mucilages and polysaccharides in *Ziziphus* species (Rhamnaceae): localization, composition and physiological roles during drought-stress. J Exp Bot. 2005;53(366):131-8.
47. Bahadur S, Sahu U, Sahu D, Sahu G, Roy A. Review on natural gums and mucilage and their application as excipient. J Appl Pharm Res. 2017;5(4):13-21.
48. Ucar G, Balaban M. Hydrolysis of Polysaccharides with 77% Sulfuric acid for Quantitative Saccharification. Turk J Agric For. 2003;27(6):361-5.
49. Armando YP, Schramm SG, Silva M, Kano EK, Koono EEM, Porta V, *et al*. Bioequivalence assay between orally disintegrating and conventional tablet formulations in healthy volunteers. Int J Pharm. 2009;336(1-2):149-53.
50. Sinha VR, Rachna K. Polysaccharides in colon specific drug delivery. Int J Pharm. 2001;224(1-2):19-38.
51. Moody SF, Clarke AE, Bacic A. Structural analysis of secreted slime from wheat and cowpea roots. Phytochemistry. 1988;27(9):2857-61.
52. Madjdoub H, Roudesli S, Picton L, Le Cerf D, Muller G, Grisel M. Pricky pear nopals pectin from *Opuntia ficus indica*. Physico-chemical study in dilute and semidilute solutions. Carbohydr Polym. 2001;46(1):69-79.
53. Fazio SA, Uhlinger DJ, Parker JH, White DC. Estimation of uronic acids as quantitative measures of extracellular and cell wall polysaccharide polymers from environmental samples. Appl Environ Microbiol. 1981;43(5):1151-9.
54. Wang J, Somasundaran P. Mechanisms of ethyl (hydroxyethyl) cellulose-solid interaction: Influence of hydrophobic modification. J Colloid Interface Sci. 2006;293(2):322-32.
55. Dutta R, Bandyopadhyay AK. Development of a new nasal drug delivery system of diazepam with natural mucoadhesive agent from *Trigonella foenum-graecum* L. J Sci Ind Res. 2005;64:973-7.
56. Faccio C, Machado RAF, De Souza LM, Zoldan SR, Quadria MGN. Characterization of the mucilage extracted from jaracatiá (*Carica quercifolia* (A. St. Hil.) Hieron). Carbohydr Polym. 2015;131:370-6.
57. Lin H, Huang AS. Chemical composition and some physical properties of a water-soluble gum in taro (*Colocasia esculenta*). Food Chem. 1993;48(4):403-9.

Cite this article: Ang AMG, Raman IC. Characterization of Mucilages from *Abelmoschus manihot* Linn., *Amaranthus spinosus Linn*. and *Talinum triangulare* (Jacq.) Willd. Leaves for Pharmaceutical Excipient Application. AJBLS. 2019;8(1):16-24.