



Viscous Behaviour and Interactive Nature of Indigenous Legume Seed Galactomannans and Biodegradable Nanoparticles for Drug Delivery

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ABSTRACT

Background: Galactomannans are the seed polysaccharides also known as seed gums, mostly isolated from the legume seed endosperm. Galactomannans can easily mix with xanthans by physical association, exhibits synergistic effect and form complex mixtures due to their entangle nature in deferent ratios. Polysaccharides having these properties make them versatile materials which are useful in different biomedical and food applications as additives, thickening, gelling agents, emulsifiers and stiffeners. Biopolymer-based colloidal particles have potential application in drug and gene delivery.

Methods: Galactomannans extracted from indigenous legume seed endosperm from *Adenanthera pavonina* L and *Mimosa pudica* L. and investigated during 2015-2021 for different aspects *i.e.* viscous behaviour and interaction properties, complex formation with other polysaccharide like xanthan and preparation and physical characterisation of galactomannan-based nanoparticles obtained by covalent cross-linking with genipin.

Result: The intrinsic viscosity of *A. pavonina* and *M. pudica* measured also compared with the commercial polysaccharide LBG (Locust Bean gum). Prepared small spherical shaped nanoparticles of diameters ~33 to 67 nm and negative zeta potential of ~-9.2 - 19.0 mV were obtained. We suggest that the nanoparticles form due to the covalent crosslinking effect of genipin on the residual protein fraction of both galactomannans. The biodegradable nanoparticles offer to be a potential new platform for the oral delivery of drug and nutraceuticals.

Key words: Emulsifier, Endosperm, Galactomannan, Genipin, LBG, Xanthan.

INTRODUCTION

Galactomannans are the heterogeneous polysaccharides also known as seed gums, mostly isolated from the legume seed endosperm (Anderson, 1949; Kapoor, 1992; Srivastava and Kapoor, 2005). Because of their viscous behaviour in aqueous media, emulsifying and gelling property, they are preferred as hydrocolloids and they are cheap, eco-friendly and non-polluting while production and in several applications (Smith and Montgomery, 1959). They are also using in different ways for human consumption (Srivastava and Kapoor, 2005). Legumes (*sensu lato* Leguminosae = Fabaceae family) seed endosperm is the main source of galactomannans. The galactomannans distribution in the plant kingdom is limited (Bailey *et al.*, 1971).

Seed galactomannans have highly variable chemical structure in the nature. It includes wide variation in the degree of galactose and mannose ratio, which are commonly formed by a linear chain of β -1, 4-Dmannopyranosides branched with single unit of D galactopyranosides through α -1,6 linkages. Their mannose/galactose ratios depend on their source. It varies from species to species (Dea and Morrison, 1975; Dey, 1978; Winter *et al.*, 1984). The mannose to galactose ratio influences the solubility and the chain stiffness (Wu *et al.*, 2012). The galactomannans are considered as multipurpose macromolecules basically neutral polysaccharides (Reid and Bewley, 1979). The

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galactomannans accumulate moisture at the early stage of seed swelling and play an important role in seed imbibitions (Reid and Bewley, 1979), germination, when the galactomannans are catabolised and transferred to the embryo as a carbon and energy source and also in the protection of the seed.

The different chemical properties of these gums made them versatile materials which are used for many different applications, they are largely used in thermoplastic, rubber industries and preparation of food products additives ice creams in food industries, pharmaceutical industry as a drug carrier and emulsifier, cosmetics, pastes, toiletries industries, thickener in toothpastes, conditioner in shampoos and textile industries. Also used for the sizing, finishing in printing industries, in crude oil drilling and explosives (Schneider and Soster-Turk, 2003; Sharma *et al.*, 2008; Vendruscolo *et al.*, 2009; Williams and Phillips, 2003). Recently come to know that these are also used in the preparation of nanoparticles (Soumya *et al.*, 2010).

Nanoparticles are the ultrafine solid particles with sizes ranging from 1 to 100 nm and have gained attention as potential carriers for drugs, immune modulators, hormones, nucleic acids, proteins and antibodies (Akagi *et al.*, 2006). These nanoparticles can either be constituted by inorganic components (e.g. metals, silica) or by synthetic or natural polymers. Due to their physicochemical and bioactive properties of these materials have a broad variety of applications in various fields such as medicine, food, cosmetics and environment (Sathishkumar *et al.*, 2014; Chaudhry and Castle 2011; Borase *et al.*, 2013; Pant *et al.*, 2013; Zuas *et al.*, 2014). There are several methods used for the preparation of nanoparticles, namely by self-assembly by dialysis, nanoprecipitation, ionic or covalent crosslinking, spray drying, among other (Gaucher *et al.*, 2005). Guar gum is an industrial galactomannan of high degree of substitution (D-mannose/D-galactose molar ratio ~2.0) and it has been used in drug-delivery, because of its low cost and other desirable functions (Cheng *et al.*, 2002). The formation of nanoparticles depends on the molecular mass of the galactomannans, crosslinking agents, solvent and surfactant (Soumya *et al.*, 2010). Nano precipitation is one of the major technique for the preparation of nanoparticles (Becer *et al.*, 2009).

Genipin is a natural dye extracted from the plant *Gardenia jasminoides* from the family Rubiaceae. It is an intermediate of alkaloid synthesis, plays an important role in alkaloid synthesis. It is an excellent cross-linking agent for proteins, collagens and chitosan [Fujikawa *et al.*, 1987, 1988], also used for regulating agent in drug delivery [Zhang *et al.*, 2006; Djerassi *et al.*, 1961]. To the best of our knowledge, genipin has not so far been used to crosslink galactomannans as an strategy to obtain small nanoparticles.

In a recent accompanying paper we have documented the extraction, purification and characteristics of galactomannan sourced from *Adenanthera pavonia* (Ceraqueira *et al.*, 2009). Also, the galactomannan from *Mimosa pudica* L. and *Dichrostachys cineria* L has been studied in our laboratories (Harikrishna *et al.*, 2017; 2018) and these legume trees are indigenous to Southern India. In the present study we have investigated the potential applications of these new galactomannans as building blocks of emulsifier, nanoparticles with potential use in pharmacy and food industry.

MATERIALS AND METHODS

Galactomannans of *Adenanthera pavonia* (Ceraqueira *et al.*, 2009) and *Mimosa pudica* (MP) were extracted, purified and characterized as described in our previous paper (Harikrishna *et al.*, 2017). Work carried out in University of Hyderabad, University of Munster and MVRDL ESIC, Hyderabad, during 2015-2021. The galactomannan samples were designated as APG and MPG, respectively. Genipin was purchased from Challenge Bioproducts (Taiwan) and other chemicals were purchased from Sigma-Aldrich (Germany). Milli-Q water was used throughout. All reagents were of analytical grade.

Preparation of galactomannan solutions for viscosity measurements

The highest possible concentrations of gum solution was prepared, by dissolving in 5 mM NaCl, 5 mg/ml. Stirred under magnetic stirrer for overnight and the galactomannan solutions allowed to hydrate slowly in the minimum volume the solution. After overnight stirring the clear solution was obtained and the solution was further filtered through membrane (0.2 µm) filter. The solutions were stored at 4° for viscosity measurements.

All other reagents and solvents (Sigma-Aldrich) were used without further purification. Experiments were carried out with MilliQ water. The intrinsic viscosity $[\eta]$, of galactomannan solutions was measured as average values of four runs for each samples at 25°C using an AMVn automated rolling ball micro-viscometer (Anton paar, Ostfildern, Germany) with programmable tube angle based on the principle of rolling ball time (the time that a steel ball needs to roll through the mixture inside a calibrated 1.6 mm diameter capillary). This was expressed as η_{rel} with respect to water. All measurements were carried out at angles of 50° using the following formulae.

$$\eta_{rel} = \eta/\eta_s$$

$$\eta_{sp} = (\eta - \eta_s) = \eta_{rel} - 1$$

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \quad \text{Eq1}$$

$$(\ln \eta_{rel})/c = [\eta] + k''[\eta]^2 c \quad \text{Eq2}$$

$$[\eta] = \{2(\eta_{sp} - \ln \eta_{rel})\}^{1/2}/c \quad \text{Eq....3}$$

The intrinsic viscosities of galactomannan samples was determined in water from experimental measurements of the dynamic viscosity of solutions with different concentrations of each component by the joint extrapolation of the Huggins (Eq 1), Kraemer (Eq 2) and single point (Eq 3) standard relationships (Bohdanecky and Kovar, 1982). Where η and η_{ss} are the viscosities of the solution and the solvent, η_{srel} and η_{ssp} are parameters of relative and specific viscosity, c is concentration and k' and k'' are constants. Different ratios of polysaccharides and xanthan were taken and mixed for the interaction studies mentioned in the Table 1.

Nano particle preparation

Galactomannan nanoparticles were prepared by covalent crosslinking with genipin. To this end, galactomannan aqueous solutions of 2 to 12 mg/ml were made in milliQ

water under magnetic stirring. Solutions were filtered through 0.22 μm PES membranes (EMD Millipore, U.S.A.), then placed in a 5 mL glass vials. Genipin stock solution was made in ethanol and subsequently diluted in water (10 mg/mL). Aliquots of genipin aqueous solution of 0 to 48 μL were added to the galactomannan solutions and incubated for 48 h at 37°C under gentle shaking. Subsequently, the GMNPs were isolated by centrifugation at 10000 rpm, for 40 min, at 25°C in Eppendorf 1.5 mL vials containing a glycerol bed (~30 mL, the supernatant was removed cautiously with a pipette and the pellets were re-suspended in 100 μL of water. The optimal incubation conditions for formation of galactomannan-genipin nanoparticles were determined by visual inspection of turbidity and color formation in preliminary trials (results not shown).

Physical characterization of nanoparticles

The particle size distribution of the GMNPs was analysed in water by dynamic light scattering with non-invasive back scattering (DLS-NIBS) with an output 4 mW He/Ne laser beam operating at $\lambda = 633 \text{ nm}$ at an angle of 173° with automatic attenuator setting. The zeta potential was determined in 1 mM KCl by mixed laser Doppler electrophoresis and phase analysis light scattering (M3-PALS). Both measurements were

carried out in a Malvern Zetasizer NANO-ZS, (Malvern Instruments, Malvern, U.K.) at $25 \pm 0.2^\circ\text{C}$.

Yield of production

The processing yield was determined by centrifuging ($10000 \times g$, 40 min, 25°C) accurately weighed aliquots of GMNPs in Eppendorf vials without added glycerol. The supernatants were carefully separated and the centrifuged pellets were freeze-dried for three days and subsequently weighed. The yield estimated relative to the total mass of added galactomannan and genipin ($n=3$).

Transmission electron microscopy (TEM)

The morphology of the GMNPs was characterized by TEM. The samples were prepared by depositing a small drop of pure nanoparticle solution on a carbon-coated copper grid and allowed to dry at room temperature with no subsequent staining. The samples were analysed using accelerating voltage 100 kV using FEI transmission electron microscope (Model Tecnai G2 S Twin 200 kV).

RESULTS AND DISCUSSION

Intrinsic viscosity studies

The intrinsic viscosity of the *Adenanthera pavonina* galactomannan (APG) and *Mimosa pudica* galactomannan by combined (MPG) were analyzed, Locust Bean Gum (LBG) has taken as reference. The data on intrinsic viscosities of APG, MPG and LBG were presented in Table 2 and Fig 1, 2 and 3 respectively.

Investigations of galactomannans of *A. pavonia* (AP) and *M. pudica* (MP) interaction with xanthan gum

In the investigations the mixed solutions were characterized by measurements of specific viscosity in dilute solution. The galactomannans of *A. pavonia* (APG) and *M. pudica* (MPG) forms gel with mixing xanthan gum. The specific viscosity of 0.2% solution of xanthan and APG, MPG respectively

Table 1: Showing different concentrations of galactomannan and xanthan gum.

Con in (ml)	Galactomannan in (ul)	Xanthan in (ul)
0	0	1000
0.2	25	975
0.3	42	958
0.4	65	935
0.6	140	860
0.8	333	667
1	1000	0

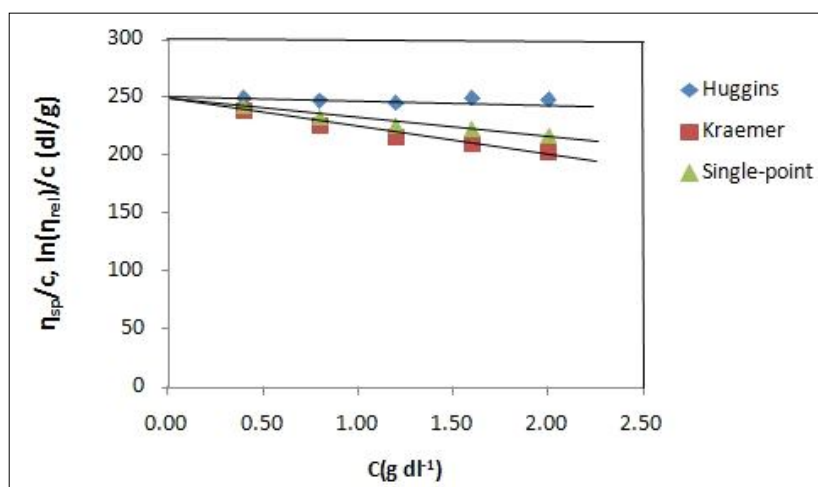
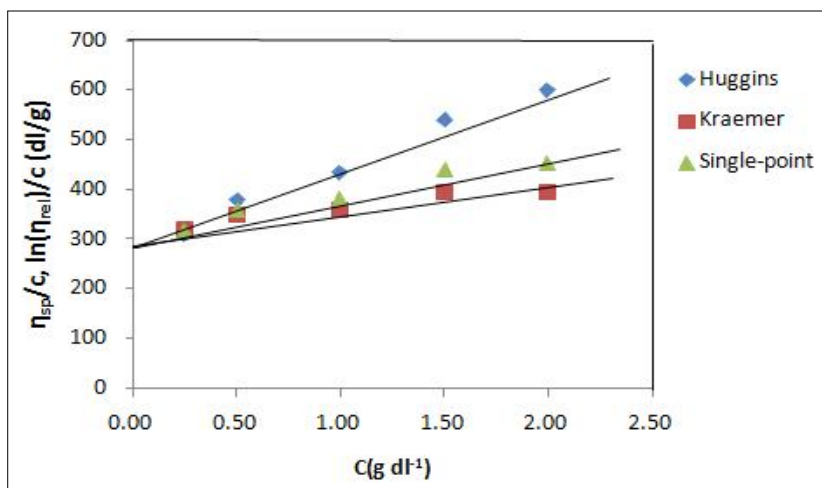
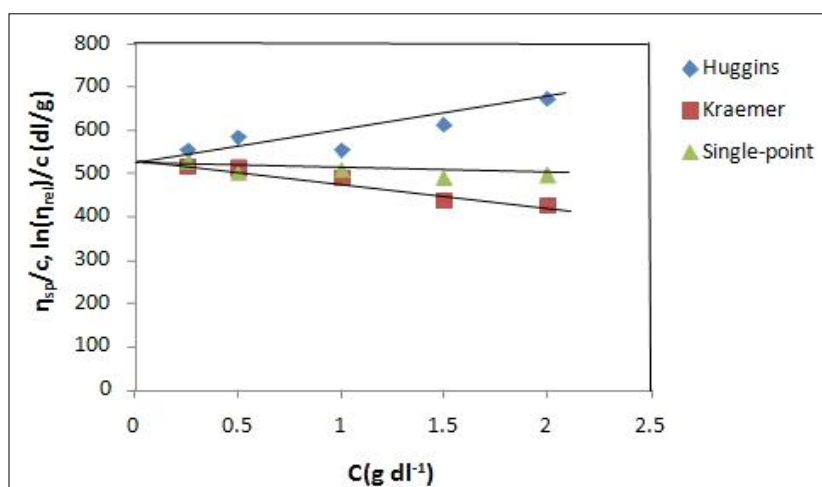


Fig 1: Determination of intrinsic viscosity for AP galactomannan (APG) by combined Huggins (\diamond), Kraemer (\blacksquare) and single-point (\blacktriangle) extrapolation of respectively, η_{sp}/c , $\ln(\eta_{rel})/c$ and $\{2(\eta_{sp} - \ln \eta_{rel})\}^{1/2}/c$ to zero concentration.

Table 2: Explaining the intrinsic viscosities of different galactomannan samples.

Name of the species	Intrinsic viscosity huggins extrapolation (dL/g)	Intrinsic viscosity kraemers extrapolation (dL/g)	Intrinsic viscosity (average values) (dL/g)
<i>Adenenthera pavonina</i>	25.01	23.83	24.42
<i>Mimosa pudica</i>	29.76	27.39	28.57
LBG*	49.56	54.76	52.16

*Locust bean gum.

**Fig 2:** Determination of intrinsic viscosity for MP galactomannan by combined (MPG) Huggins (\diamond), Kraemer (\blacksquare) and single-point (\blacktriangle) extrapolation of respectively, η_{sp}/c , $\ln(\eta_{rel})/c$ and $\{2(\eta_{sp} - \ln \eta_{rel})\}^{1/2}/c$ to zero concentration.**Fig 3:** Determination of intrinsic viscosity for Locust Bean Gum (LBG) by combined Huggins (\diamond), Kraemer (\blacksquare) and single-point (\blacktriangle) extrapolation of respectively, η_{sp}/c , $\ln(\eta_{rel})/c$ and $\{2(\eta_{sp} - \ln \eta_{rel})\}^{1/2}/c$ to zero concentration.

(Table 1), mixed in different proportions is shown in Fig 4. APG exhibited considerable synergism with xanthan gum and the highest viscosity was reached for a proportion 20/80 (APG/xanthan). The synergistic action of APG is lower. This can be explained by the lower M/G ratio of APG and MPG. Galactomannans exhibits synergistic effect by mixing with xanthan gum, it is because of physical association between xanthan molecules and galactomannan side chain, which is widely useful in the food industries

(Fernandes, 1995). Synergistic effects depend on their structure and M/G ratio. Tara and guar gums are the best examples of this phenomenon. Galactomannans can interact with other polysaccharides, this behavior make them useful in industries (Milas *et al.*, 1990). It has already proved that LBG, tara and fenugreek gums forms gels even small additions of agarose, carrageenan and xanthan gum, which is expensive (Fernandes and Figueiredo, 1995). Interaction studies explained the formation of complex

mixtures with other polysaccharides like xanthan. It was concluded that galactomannans would work as a good thickening and gelling agent and modifier in food industries.

Preparation of galactomannan nanoparticles

The addition of genipin to galactomannan solutions and subsequent incubation during 48 h at 37°C, resulted in the change in colour of the solutions that turned slightly dark blue shown in Fig 5. This change was more intensified as the concentration of polysaccharide increased from 4 to 12 mg/mL (Fig 6). Also, in the case of the solutions of MPG galactomannan the solutions of 8 and 12 mg/mL seem to have developed turbidity. The solutions turned into a teneous blue colour, diagnostic evidence of the reaction of genipin

and galactomannan. Genipin is known to interact only with polymers that contain primary amino groups e.g. chitosan and gelatin (Sung *et al.*, 1998). To explain the development of blue coloration and turbidity in galactomannan solutions, we propose that it is the residual protein of the galactomannan sample what reacts with genipin. To the best of our knowledge, this is the best account of the use of genipin to crosslink galactomannans.

Isolation of the various solutions by centrifugation enabled to quantify the yield of production of the sedimentable fraction after genipin crosslinking. Fig 6 shows that when increasing concentrations of genipin were added to a fixed amount of galactomannan (8 mg/mL) the amount of sedimentable fraction increased with respect to the

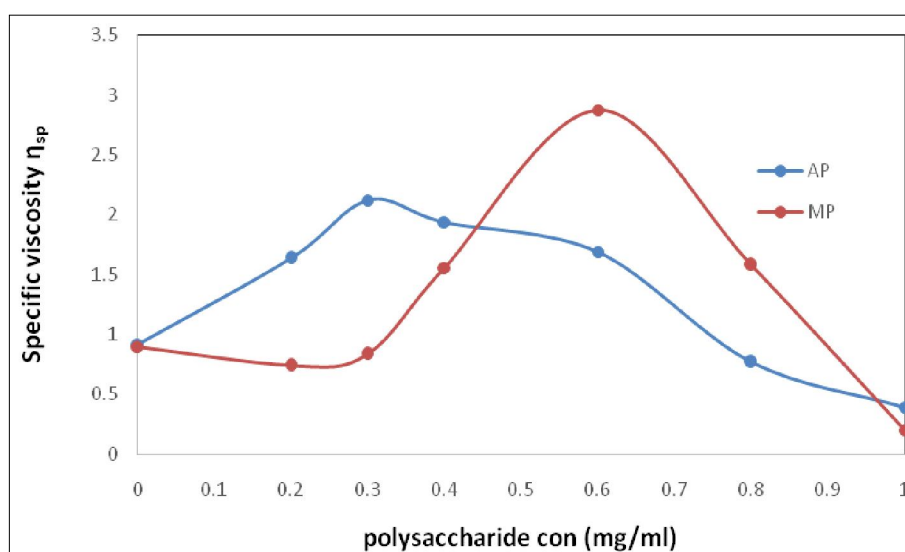


Fig 4: Specific viscosity (measured at 20°C) for dilute solution of AP and MP in combination with xanthan in 10 mM KCl; the concentrations (% w/w) of the individual polymer solutions were: 0.025 AP, 0.475 xanthan.

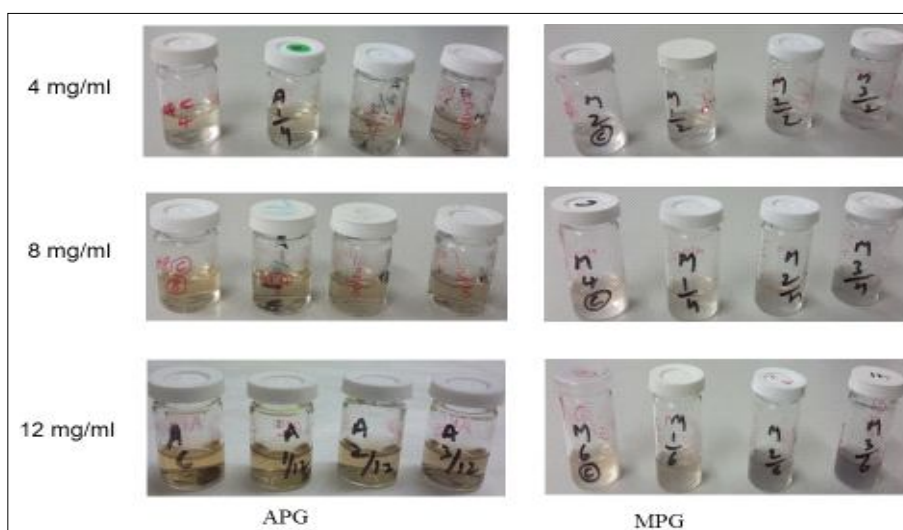


Fig 5: Appearance of galactomannan-genipin solutions of AP (left frames) and MP (right frames) at varying polysaccharide concentrations (as shown on labels) and genipin/ polysaccharide crosslinking ratios (increasing from left to right), upon incubation during 48 h at 37°C. Blueish coloration and turbidity are clearly observed at high crosslinking ratios.

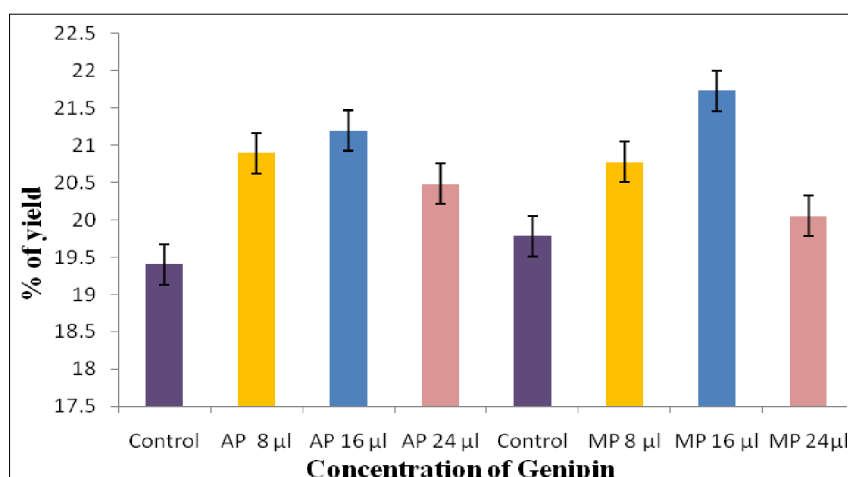


Fig 6: Yield of production of nanoparticles of galactomannan-genipin solutions of AP and MP (galactomannan concentration 8 mg/mL) at varying genipin/galactomannan crosslinking ratio (amount of genipin solution upon incubation during 48 h at 37°C ($n=3$)).

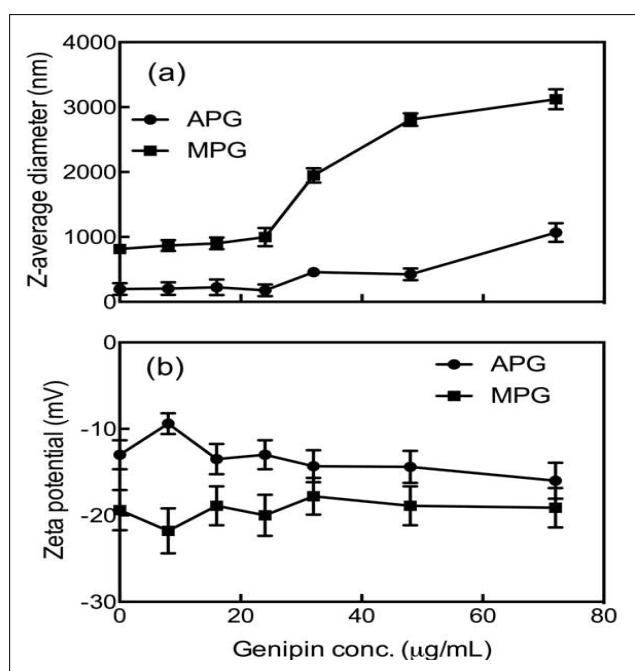


Fig 7: Variation of (a) Z-average diameter (mean \pm SD; $n=3$) and (b) z-potential (mean values \pm minimum and maximum, $n=2$) of AP and MP galactomannan-genipin nanoparticles on the concentration of genipin (48 h, 37°C).

corresponding controls (*i.e.*, the solutions without added genipin) up to a genipin concentration of 24 $\mu\text{g/mL}$. These experiments revealed that even in the absence of added genipin, both galactomannans contain a fraction of sedimentable polysaccharide of ~19.5%. Interestingly, when the concentration of genipin was greater than 16 $\mu\text{g/mL}$, the yield values decreased in both galactomannans. The initial increase in yield of the sedimentable fraction of putative nanoparticles upon addition of genipin, can be taken as direct evidence of the crosslinking effect. A possible

offered explanation to account for decrease in yield at the highest concentration could be that in this case, a tenuous gel network starts to be formed. This would increase the viscosity of the solution and hence prevent the fraction of particles to sediment during the centrifugation process. The results, therefore, indicate that there is an optimal degree of crosslinking that maximizes the yield of nanoparticles production (hence, the greatest sedimentable fraction) after addition of genipin to the galactomannan aqueous solutions.

Fig 7a shows the results of the Z-average hydrodynamic diameter and z-potential of the isolated nanoparticles, as a function of the concentration of added genipin to the aqueous solutions of both systems 8-24 $\mu\text{g/mL}$, after incubation for 48 h at 37°C. Notice that even in the absence of genipin, there is a fraction of particles of average diameter 900 or 250 nm for MPG and APG, respectively. Upon addition of genipin, the particle size of APG increases almost linearly up to 24.0 $\mu\text{g/mL}$, however, beyond this concentration, the particle size rises exponentially up to ~3000 nm. Overall, smaller sizes were recorded for the particles of MPG galactomannan. In this case, also an increase in size was evident as the concentration of genipin increases beyond 24.0 $\mu\text{g/mL}$, albeit much less pronounced as for APG galactomannan particles. The accompanying data for z-potential for these same systems are shown in Fig 7b. Notice that for APG galactomannan particles, the addition of genipin resulted in a slight increase in z-potential, ranging from an initial value of ~-12 up to ~-16 mV. By contrast, MPG galactomannan particles attained overall greater z-potential values ~-20 mV. However, in this case, within experimental error, the values did not change with the addition of genipin. Table 3 summarizes the characteristics of selected nanoparticle systems from both galactomannans of the smallest size in each case. The corresponding size distribution curves revealed bimodal distributions. The presence of more than one size distribution peak, can be explained as the consequence of the crosslinking reaction

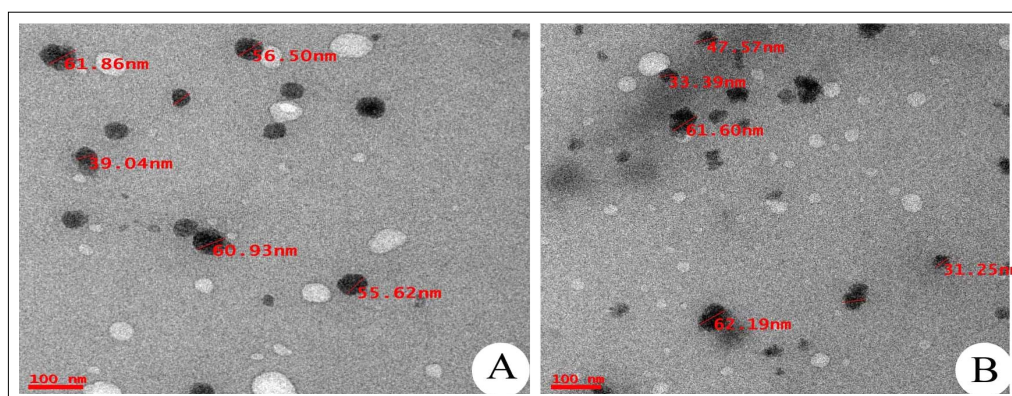


Fig 8: Transmission electron microscopic images of galactomannan-genipin nanoparticles (galactomannan concentration 8 mg/mL; genipin 16 μ g/mL): A) AP and B) MP.

Table 3: Characteristics of *Adenanthera pavonina* and *Mimosa pudica* galactomannans nanoparticles by genipin crosslinking (Mean values \pm SD; n=3).

Galactomannan	Galactomannan conc (mg/mL)	Genipin conc. (μ g/mL)	Size (d.nm)	PDI	Zeta potential (mV)	Yield (%)
APG	8.0	16	33 \pm 3	0.376	-12 \pm 2.3	21.19 \pm 1.3
MPG	8.0	16	67 \pm 5	0.433	-9 \pm 1.6	21.72 \pm 1.1

that occurs at varying extent dictated by the polydispersity of the galactomannan polymers themselves. The furnished nanoparticles had an average size, polydispersity index (PDI) and zeta potential concordant with those found in previous studies that documented the formation of nanoparticles of chitosan/galactomannan, in which the M/G ratio (1.1-5.3) of the seed polysaccharide was similar to APG and MPG (Il'ina *et al.*, 2011).

Representative TEM images of the APG and MPG nanoparticles are shown in Fig 8. The figure reveals the morphology of the nanoparticles is spherical and in the size range of ~30 - ~60 nm, with few larger particles and clusters in the size range of ~100~300 nm, which can be attributed to aggregates.

CONCLUSION

The Intrinsic viscosity values of the galactomannans extracted from *Adenanthera pavonia* and *Mimosa pudica* were similar with commercial gums which are available in the market (Locust bean gum and Tara gum) and natural galactomannan *Dichrostachys cineria* Galactomannans can be easily interacts by physical attachments with xanthan in different ratios and form complex mixtures with xanthans. The combination of the polysaccharide with xanthan could be effective gelling, thickening agent, stiffener and modifiers in food industries and it is a very good alternative for different applications in various fields, this behaviour make them useful in industrial applications.

Galactomannans were used to prepare nanoparticles in the size range of ~30 to 60 nm by covalent crosslinking using genipin. The two galactomannans, having a 3-5% of residual proteins, react with genipin via the well-known

reaction of primary amino groups of the protein and genipin, as evidenced by the formation of blue colouration, in a similar way as it occurs with other biopolymers (e.g. chitosan). The potential of these unprecedented systems as drug delivery carriers will be explored in future studies that could exploit the presence of galactose substituents of the galactomannan, for example, for targeting hepatic asialoglycoprotein receptors.

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Conflict of interest: None.

REFERENCES

- Akagi, T., Higashi, M., Kaneko, T., and Kida, M.T. (2006). Akashi, Hydrolytic and enzymatic degradation of Nanoparticles based on Amphiphilic poly (γ -glutamic acid)-graft-L-phenylalanine Copolymers. *Biomacromol.* 7: 297-303.
- Anderson, E. (1949). Endosperm mucilages of legume. *Ind. Eng. Chem. Res.* 41: 2887-2890.
- Bailey, R.W. Harborne, J.B., Boulter, D. and Turner, B.L. (1971). Polysaccharides in the Leguminosae. In: *Chemotaxonomy of the Leguminosae*. [Harborne, J.B., Boulter, D. Turner, B.L. (eds.)], Academic press, New York, London. Pp: 503-541.
- Becer, K.C.R., Babiuch, D., Pilz, S., Hornig, T., Heinze, M., Gottschaldt and Schubert, U.S. (2009). Clicking pentafluorostyrene copolymers: Synthesis, nanoprecipitation and glycosylation. *Macromolecules.* 42: 2387-2394.

- Bohdanecky, M. and Kovar, J. (1982). Viscosity of polymer solutions. Amsterdam, The Borase, H.P.C.D., Patil, R.K., Suryawanshi and Patil, S.V. (2013). *Ficus carica* latex-mediated synthesis of silver nanoparticles and its application as a chemophotoprotective agent. Appl. Biochem. Biotechnol. 171: 676-688.
- Ceraqueira, M.A., Pinheiro, A.C., Souza, B.W.S., Lima, A.M.P., Ribeiro, C. and Miranda, C. *et al.* (2009). Extraction, Purification and characterization of Galactomannans from non-traditional sources. Carbohydr Polym. 75: 408-414.
- Chaudhry, Q. and Castle, L. (2011). Food applications of nanotechnologies: An overview of opportunities and challenges for developing countries. Trends in Food Sci. Technol. 22: 595-603.
- Cheng, K.M. and Brown, R.K. (2002). Prud'homme, Characterization and intermolecular interactions of hydroxypropyl guar solutions. Biomacromol. 3: 456-461.
- Dea, I.C.M. and Morrison, A. (1975). Chemistry and interactions of seed galactomannans. Adv. Carbohydr. Chem. Biochem. 35: 241-312.
- Dey, P.M. (1978). Biochemistry of plant galactomannans. Adv. Carbohydr. Chem. Biochem. 35: 341-376.
- Djerassi, T.C., Nakano, A.N., James, L.H., Zalkow, E.J., Eisenbraun and Shoolery, J.N. (1961). Terpenoid. XLVII. The structure of genipin. J. Org. Chem. 26: 1192-1206.
- Fernandes, P.B. and Figueiredo, A.A. (1995). Effect of mesquite seed gum addition to kappa-carrageenan or xanthan gum mixed systems. Food Hydrocolloids. 9: 251-255.
- Fujikawa, S., Fukui, Y. and Koga, K. (1987). Structure of genipocyanin G1, a spontaneous reaction product between genipin and glycine. Tetrahedron Lett. 28: 4699-4700.
- Fujikawa, S., Nakamura, S.K. and Koga, K. (1988). Genipin, a new type of protein crosslinking reagent from Gardenia fruits. Agric. Biol. Chem. 52: 869-870.
- Gaucher, G.M.H., Dufresne, V.P., Sant, N., Kang, D., Maysinger and Leroux, J.C. (2005). Block copolymer micelles: Preparation, characterization and application in drug delivery. J. Controlled Release. 109: 169-188.
- Harikrishna, N.K.L., Seshagirirao, and Goycoolea, F.M. (2017). Extraction, purification and characterization of water soluble galactomannans from Mimosa pudica seeds. The Eurobiotech. J. 1(4): 303-309.
- Harikrishna, N.L.K. Seshagirirao and Goycoolea, F.M. (2018). Extraction and physicochemical characterization of galactomannans from *Dichrostachys cinerea* seeds. Food Hydrocolloids. 82: 451-456.
- Il'ina, N.M., Mestechkina, D.V., Kurek, A.N., Levov, P.I., Semenyuk, V.N., Orlov, V.D. Shcherbukhin and Varlamov, V.P. (2011). Preparing, studying and prospects of using nanoparticles based on chitosan and galactomannan. Nanotech. Russia. 6: 1-2.
- Kapoor, V.P. (1992). Rheological properties of seed galactomannan from *Cassia nodosa Buchhem*. Carbohydr. Polym. 25: 79-84.
- Milas, M., Rinaudo, M., Knipper, J.L. and Schuppiser, J.L. (1990). Flow and viscoelastic Netherlands: Elsevier. of xanthan/galactomannan mixtures. J. Food Eng. 24: 269-283.
- Pant, G., Nayak, N. and Prasuna, R.G. (2013). Enhancement of antidandruff activity of shampoo by biosynthesized silver nanoparticles from *Solanum trilobatum* plant leaf. Appl. Nanosci. 3: 431-439.
- Reid, J.S.G. and Bewley, J.D. (1979). A dual role for the endosperm and its galactomannan reserves in the germinative physiology of fenugreek (*Trigonella foenum-graecum* L.), an endospermic leguminous seed. Planta. 147: 145-50.
- Sathishkumar, C. Gobinath, A., Wilson, and Sivaramakrishnan, S. (2014). *Dendrophthoe falcata* (L.f) Ettingsh (Neem mistletoe): A potent bioresource to fabricate silver nanoparticles for anticancer effect against human breast cancer cells (MCF-7). Spectrochimica acta part A, Mol. Biomol. Spectrosc. 128: 285-290.
- Schneider, R. and Sostar-Turk, S. (2003). Good quality printing with reactive dyes using guar gum and biodegradable additives. Dyes and Pigments. 57(1): 7-14.
- Sharma, B.R., Dhuldhoya, N.C., Merchant, S.U. and Merchant, U.C. (2008). A Glimpse of Galactomannans. Sci. Tech Enterpr.
- Smith, F. and Montgomery, R. (1959). The chemistry of plant gums and mucilages and some related polysaccharides. Reinhold Pub. Corp., New York.
- Soumya, R.S. Swapankumar, G. and Abraham, E.T. (2010). Preparation and characterization guar gum nanoparticles. Int. J. Biol. Macromolec. 46: 267-269.
- Soumya, R.S., Swapankumar, G., Abraham, E.T. (2009). Preparation and characterization guar gum nanoparticles. Int. J. Biol. Macromolec. 46: 267-269.
- Srivastava, M. and Kapoor, V.P. (2005). Seed galactomannans: An overview. Chem. Biodivers. 2: 295-317.
- Sung, H.W.R.N., Huang, L.L.H., Huang, C.C., Tsai and Chiu, C.T. (1998). Feasibility study of a natural crosslinking reagent for biological tissue fixation. J. Biomed Mater Res. 42: 560-567.
- Vendruscolo, C.W., Ferrero, C., Pineda, E.A.G., Silveira, J.L.M., Freitas, R.A. and Jimenez- Castellanos, M.R. (2009). Physicochemical and mechanical characterization of galactomannan from *Mimosa scabrella*; effect of dying method. Carbohydr. Polym. 76: 86-93.
- Williams, P.A. and Phillips, G.O. (2003). Introduction to food hydrocolloids. In: Handbook of Hydrocolloids. [Phillips, G.O. and Williams, P.A. (eds.)], CRC Press, New York. pp. 1-19.
- Winter, W.T., Chien, Y.Y. and Bouckris, H. (1984). Structural Aspects of Food Galactomannans. In: Gum and Stabilisers for the Food Industry, [Phillips, G.O., Wedlock, D.J. and Williams, P.A. (eds.)], Pergamon, Oxford, U.K. Vol. 2. pp. 535-539.
- Wu, Y., Li, W., Cui, S.W. and Goff, H.D. (2012). A molecular modeling approach to understand conformation-functionality relationships of galactomannans with different mannose/galactose ratios. Food Hydrocolloids. 26: 359-364.
- Zhang, L.E.C.Y., Parton, C.P., Ye, S., Krauss, R., Shen, C.T., Lin, J.A., Porco, Lowell and Jr, B.B. (2006). Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced β cell dysfunction in isolated pancreatic islets. Cell Metabolism. 3: 417-27.
- Zuas, O., Hamim, N. and Sampora, Y. (2014). Bio-synthesis of silver nanoparticles using water extract of *Myrmecodia pendans* (Sarang Semutplant). Mater. Lett. 123: 156-159.