Amination of a Natural Polymer, *Leucaena leucocephala* (Lam.) galactomannan: Synthesis, Optimization and Characterization

NEERAJ MITTAL AND GURPREET KAUR*

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, 147002, Punjab, India

ABSTRACT

Currently, biomedical, food, and pharmaceutical industries are exploring biocompatible, biodegradable, and readily available polymers as an alternative to synthetic polymers. The naturally occurring galactomannans comprising of galactopyranosyl (Gal) and mannopyranosyl (Man) residues are an attractive alternative to synthetic polymers due to their wide availability, and easily maniputable physical, and chemical properties. In the present study, a natural polymer, Leucaenea leucocephala galactomannan (LLG) was extracted from the seeds of Leucaenea leucocephala by an aqueous method, derivatized by reductive amination process using ethylene diamine as aminating agent to form aminated LLG (AMLLG). The conditions for amination were optimized by varying the concentration of aminating agent, temperature of the reaction mixture and time of reaction and their effect on degree of substitution was determined. The derivatized galactomannan was characterized by FTIR, DSC, XRD, SEM and zeta potential analysis. The texture properties (firmness, consistency, and cohesiveness) and bioadhesive strength of AMLLG was also determined. Furthermore, the antimicrobial potential of AMLLG was evaluated against Escherichia coli and Staphylococcus aureus suggesting better antibacterial activity of AMLLG than chitosan (standard). Overall, the study highlights that the amine derivative of LLG could be a promising candidate as preservative in food industry and as a drug delivery vehicle in pharmaceutical industry.

KEY WORDS : *Amination, bioadhesion strength, mechanical properties, modification, polysaccharides, thermal properties.*

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Correspondence author e-mail :kaurgpt@gmail.com
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1. INTRODUCTION

Galactomannans are neutral, naturally occurring heterogeneous polysaccharides, comprising of galactopyranosyl (Gal) and mannopyranosyl (Man) residues [1,2]. These are mostly obtained from endosperm of dicotyledonous seeds of the family leguminosae. The galactomannans usually vary in the mannose/galactose (M/G) ratio, distribution of galactose residues along the mannan backbone and in molecular weight. The molar ratio of galactose to mannose varies with plant origin but is typically in the range of 1:1, 1:2, 1:3, and 1:4 for fenugreek, guar, tara, and locust bean gum, respectively. These are widely employed in paper, textile, pharmaceutical, cosmetics, food and oil recovery industries, due to their good binding, gelling, emulsifying and suspending properties [3]. In food industry, guar gum and locust bean gum are extensively used as thickening and stabilizing agents and to improve the shelf-life. They also prevent creaming or settling in salad dressings, soft drinks, and fruit juices [4]. In pharmaceutical industry, galactomannans are used for the manufacturing of solid monolithic matrix system, implants, films, beads, microparticles, nanoparticles, inhalable and injectable systems as well as viscous liquid formulations [5,6]. Further, galactomannans are ideal polymeric matrix for mucoadhesive sustained release formulations because of their water absorption, swelling and gel forming ability [7].

Although galactomannans are widely employed in biomedical industry, they exhibit certain drawbacks such as uncontrolled hydration, pH dependent solubility and prone to microbial contamination. Chemical derivatization of galactomannans not only minimizes these disadvantages, but also enhances their functional properties (e.g. rheological properties, drug release characteristics, good antimicrobial properties) and makes them more suitable for use in various fields and drug delivery systems [8]. Recently, a large number of studies have been reported on galactomannans and their derivatives (amine, carboxymethyl, sulphate) for their prospective applications in drug delivery systems. Much work has been reported on galactomannans extracted from guar and fenugreek [8,9]. These galactomannans have been exploited as binding agents, disintegrants, suspending agents, stabilizing agents, and thickeners in drug delivery, however, not much work has been done on *Leucaenea leucocephala* galactomannan (LLG) as a pharmaceutical excipient.

Leucaena leucocephala (Lam.) plant is native to tropical America and belongs to family Fabaceae and subfamily Mimosoideae [10]. The plant has been widely used as firewood, fuel, organic fertilizer, and raw material for pulp and paper industry, in food and pharmaceutical industry [11]. It displays anti-cancer, anti-viral, anti-coagulant, anti-thrombotic, anti-inflammatory, anti-diabetic and immunostimulant properties [12,13]. The seeds majorly comprise of galactomannan gum, although oils (unsaturated linoleic and oleic fatty acids), tannins, oxalic acid and non protein substance mimosine are also present in minor amounts [14]. The galactomannan gum extracted from *Leucaena leucocephala* seed endosperm is composed of linear chains of β-(1-4)-D-mannose units substituted by single α-D-galactose units at O-6. The ratio of mannose to galactose of *Leucaena* gum is 1.3:1 [15].

Extensive research has been carried out to explore new pharmaceutical excipients for novel drug delivery systems. The exploration of new excipient is a ponderous, expensive, and time consuming process. However, exploring the new excipient is economic and safer when extracted from the abundantly available natural sources [16]. With the aim to explore novel excipient, in our earlier study, we reported the synthesis and characterization of carboxymethyl derivative of *Leucaenea leucocephala* galactomannan (CMLLG) [17].

In the present investigation, amine derivative of LLG was synthesized and characterized. The amination of LLG (AMLLG) was carried out by reductive amination process using ethylene diamine as aminating agent. Although amine derivatives of certain galactomannans have been carried out, no studies have been reported on the optimization of the method. Process variables play an important role in determining the degree of substitution as is evident from studies carried out in preparation of carboxymethylated gums [17,18]. In this study, the reaction conditions for amination were varied and the reaction conditions were optimized on the basis of degree of substitution. The substituted product was characterized and the texture properties (firmness, consistency, and cohesiveness) and bioadhesive strength of AMLLG were determined. Further, the antimicrobial potential of AMLLG was determined against *Escherichia coli* and *Staphylococcus aureus*.

2. MATERIALS AND METHODS

2.1. Materials

Leucaena leucocephala seeds were procured from Greenfield Agro Forestry Products, Madhya Pradesh. Isopropyl alcohol was obtained from Merck India Limited, Mumbai. Galactose and mannose were purchased from Tokyo Chemicals Co. Ltd, Japan. Microbial strains of *Escherichia coli* and *Staphylococcus aureus* were procured from Govt. Medical College, Patiala. All other chemicals and reagents employed in the study were of analytical grade.

2.2. Isolation and purification of *Leucaena leucocephala galactomannan* (LLG)

LLG was isolated by an aqueous extraction method from the seeds of *Leucaena leucocephala* by the method already described by our group [17]. Briefly, crushed seeds were soaked in water for 24 h and homogenized (Remi equipments, Mumbai) with double volume of distilled water. The extract was separated out with the help of muslin cloth followed by centrifugation (4000 rpm) and then precipitated with isopropyl alcohol (100 ml × 3). The precipitated gum was separated by filtration, purified by dialysis, dried by freeze drying (Allied Frost, Delhi) and stored in desiccator till further use.

2.3. Derivatization of LLG

2.3.1. Synthesis of aminated Leucaenea leucocephala galactomannan (AMLLG)

A homogenous solution of LLG was prepared by dispersing 1.0 g of LLG in distilled water. Different concentrations of aminating agent (Ethylene diamine; 20-80% v/v) were added to the above solution and the reaction was allowed to proceed under continuous stirring for 5-25 h at varying temperature (15-45 °C). Sodium borohydride (5% w/v, double volume of aminating agent) was added to the reaction mixture and stirring continued for another 2 h[9,19] followed by addition of isopropyl alcohol to precipitate AMLLG. The precipitated sample was dried by freeze drying and powdered to obtain uniform particles. Table 1 depicts the reaction parameters employed for preparing AMLLG and its effect on degree of substitution.

2.3.2. Determination of Degree of Substitution (DS)

DS of AMLLG was determined by a method reported by Bassi and Kaur [9]. The aminated gum (50 mg) was dispersed in 10 ml of acidic water (1.0% v/v acetic acid). 5 ml of the prepared solution was then treated with ninhydrin (1.0 ml) at 80 ± 5 °C in water bath for 5 min

under stirring. The absorption of the colored complex so obtained was determined spectrophotometrically at 570 nm (Shimadzu, Japan). The blank solution was composed of native LLG instead of AMLLG. The experiment was repeated thrice and DS was calculated using following equations:

$$DS = \frac{360 \times (\%\ \text{amino}/16)}{100 - (15/16 \times \%\ \text{amino group})} \quad (1)$$

$$\%\ \text{amino group} = \frac{\text{Absorbance of AMLLG} - \text{Absorbance of native LLG}}{\text{Absorbance of native LLG}} \quad (2)$$

where, 360 is the molecular weight of galactomannan in LLG, 16 is the molecular weight of NH_2 group and 15 is the molecular weight of NH group.

2.4. Characterization of derivatized galactomannan

2.4.1. Fourier-transform Infrared Spectroscopy (FTIR)

FTIR spectroscopic studies were performed using FTIR spectrophotometer (NICOLET iS50, Thermo Scientific). Powdered samples of LLG and AMLLG (1-2 mg) were compressed with KBr to form pellets. The spectrum was recorded over a frequency range of 4000-400 cm^{-1} [20].

TABLE 1. Conditions employed for synthesis of aminated LLG and their effect on degree of substitution (DS)

Batch code	Amount of ethylene diamine (ml)	Amount of sodium borohydride (ml)	Temperature (°C)	Time (h)	DS*
A1	20	40	25	5	0.401±0.025
A2	30	60	25	5	0.613±0.045
A3	40	80	25	5	0.522±0.035
A4	60	120	25	5	0.485±0.042
A5	80	160	25	5	0.394±0.033
A6	30	60	15	5	0.241±0.041
A7	30	60	35	5	0.323±0.016
A8	30	60	45	5	0.305±0.029
A9	30	60	25	10	0.511±0.051
A10	30	60	25	15	0.486±0.042
A11	30	60	25	20	0.469±0.048
A12	30	60	25	25	0.458±0.039

*All values are expressed as mean ± SD (n=3).

2.4.2. X-ray diffraction (XRD)

The crystallographic analysis of powdered gum samples (LLG and AMLLG) were performed by XRD. X-ray diffractogram was recorded on XPert PRO diffractometer system equipped with Cu Kα radiations (λ=1.5406 Å) generated at 45 kV and 40 mA. XRD diffractogram was determined with 2θ ranging from 10-80°, step size of 0.017°, scan step time of 30.36 s at room temperature 25 °C. The dried powdered samples were placed on sample stage (PW 3071/xxBracket) and were evaluated for diffraction patterns[21].

2.4.3. Scanning electron microscopy (SEM)

A morphological characterization of LLG and AMLLG was carried out by SEM (JEOL, JSM- 6510LV). Powdered gum samples were taken and coated with

gold (auto fine coater JFC-1600) to make the particles conductive. Images were taken at an acceleration voltage of 5-10 kV electron beam [22].

2.4.4. Differential scanning calorimetry (DSC)

The thermal properties of native and derivatized galactomannan were analyzed by DSC (EVO131, SETARAM, France). Powdered gum samples were heated in the range of 40-400 °C at a rate of 10 °C/min under nitrogen atmosphere [23].

2.4.5. Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance spectroscopy (^{1}H) of LLG and AMLLG was observed using NMR spectrometer (Bruker Avance III, 400 MHz) at 25 °C using D_2O as a solvent [24].

2.4.6. Rheological studies

A Brookfield Viscometer (Model LVDV-I) employing spindle no. S18 was used to determine the viscosity of different concentrations (1-7% w/v) of LLG and AMLLG solutions. The solutions were prepared in distilled water under continuous stirring for 24 h and then centrifuged to remove insoluble fractions. A temperature of 25 °C was maintained by circulating water bath throughout the study [25].

2.4.7. Zeta potential

Zeta potential of LLG and AMLLG solution (1% w/v) was determined at 25 °C using Beckman coulter Delsa™ Nano (Malvern Zeta sizer). The solutions of native LLG and derivatized LLG were prepared in triple distilled water and the dispersion of each sample was taken in zeta cell and zeta potential was measured [26].

2.4.8. Mechanical properties and bioadhesion strength

The mechanical properties and bioadhesive strength of native and derivatized galactomannan were analysed using Texture analyzer (TA-XT plus Texture Analyzer) ASTM D3039. The firmness and cohesiveness were determined by back extrusion employing a rig consisting of flat 35 mm diameter disc probe (model A/BE, stable Micro systems) which was driven into a large cylinder sample holder (50 mm diameter) to force down into the sample. The movement of the probe forced the sample to flow upward through the concentric annular space between the probe and the container. The probe was allowed to fall at pretest speed of 1.0 mm s^{-1}, test speed of 2.0 mm s^{-1} at a distance of 50 mm above the top of the sample to a depth of 10 mm, and returned back to the starting position. Firmness [g], consistency [g.s], and cohesiveness [g] and index of viscosity [g.s] were determined for six replicates of samples [27].

For determination of bioadhesive strength, a pellet of LLG or AMLLG was prepared by compressing their powders using single punch, hydraulic press. The pellets were attached to upper probe and dialysis membrane-50 (Hi-Media) hydrated with mucin solution (0.3% w/v) was attached to lower probe. Then upper probe was lowered at a rate of 0.1 mm/s until a contact was established with the cellophane membrane at a force of 0.25 N which was maintained for 3 min and then the upper probe was moved upwards at a rate of 0.1 mm/s [28,29]. The force required (bioadhesive strength) to withdraw the pellet from the dialysis membrane was determined employing Texture-Pro CT V1.3 Build14 software. All the measurements were carried out in triplicate.

2.5. Antimicrobial activity

The amine derivatives of gums have been reported to possess antimicrobial properties [30]. The agar diffusion method was employed to evaluate the antimicrobial potential of AMLLG. Nutrient agar media was prepared and sterilized in an autoclave. The media was cooled to a temperature range of 50-55 °C and inoculated with 24 h old culture (1% v/v) of *E. coli* (Gram negative) or *S. aureus* (Gram positive), separately. The media was then poured in petriplates and allowed to solidify. The wells were made in the solidified media with the help of a standard sterilized borer (1 cm diameter). A 200 µl of LLG, AMLLG and chitosan (as reference) were added into the wells and allowed to diffuse for 2 h. The petriplates were then incubated at 37 °C for the period of 24 h and the zone of inhibition was noted.

3. RESULTS AND DISCUSSION

3.1. Isolation of LLG

The yield of LLG extracted by aqueous method was found to be 20% (w/w). The reported yield of galactomannan is 25% (w/w) [31]. The average

molecular weight (M_V) of LLG was found to be 6.3 × 105 g/mol [17].

3.2. Derivatization of LLG

AMLLG was synthesized by reductive amination process in which –OH group of LLG is substituted by $–NH_2$ group upon addition of ethylene diamine ($C_2H_8N_2$) as aminating agent and sodium borohydride as reducing agent. Fig. 1 shows a diagrammatic representation of reactions involved in amination.

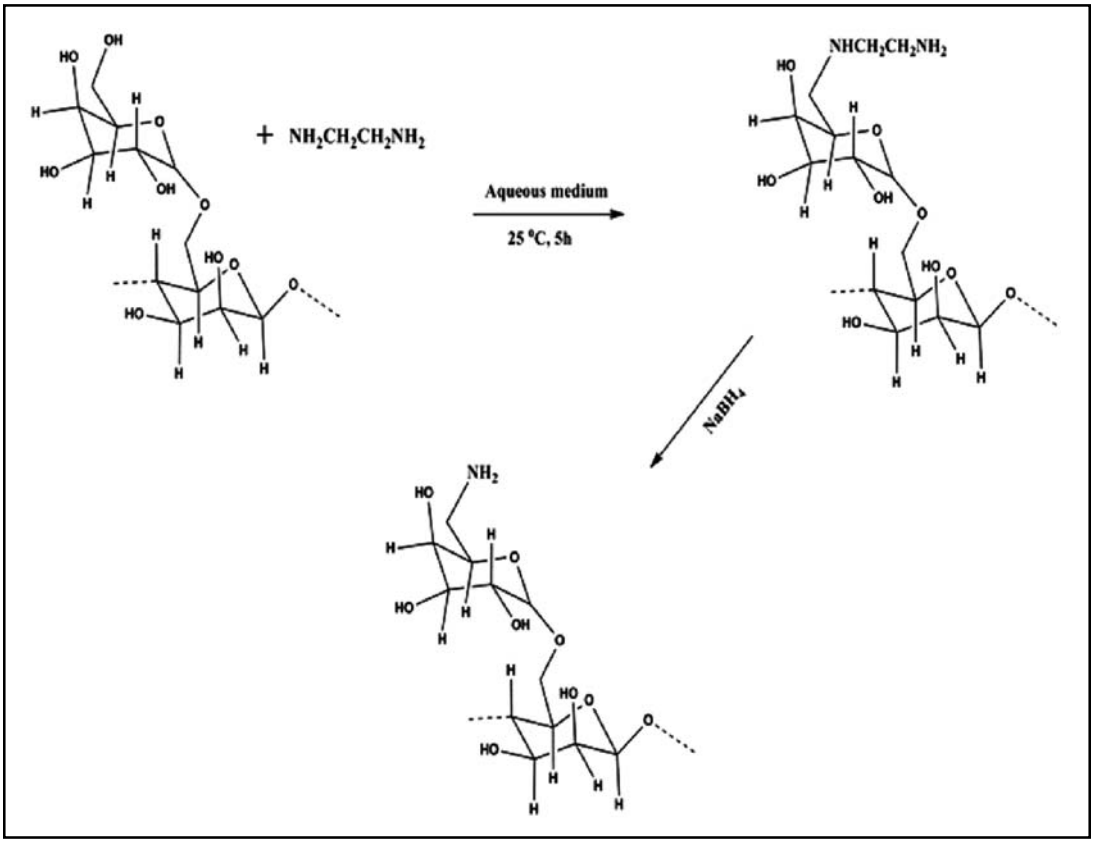

Fig. 1. Scheme of reductive amination of *Leucaena leucocephala* galactomannan.

3.2.1. Determination of DS by UV spectrophotometer

The DS of the AMLLG was determined spectrophotometrically by ninhydrin method. Table 1 and Fig. 1 depict the effect of various process variables on DS of derivatized galactomannan.

3.2.1.1. Effect of temperature

The effect of temperature on degree of substitution was elucidated by carrying out

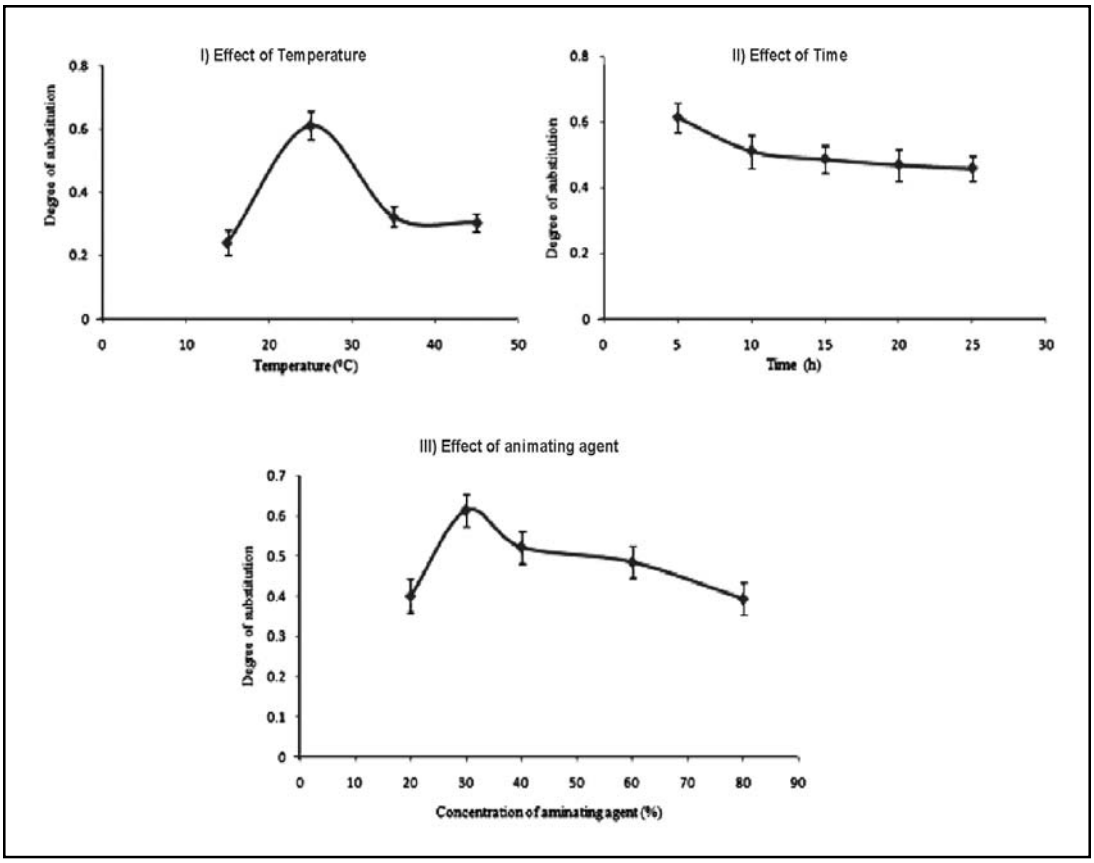

Fig. 2. Effect of (a) temperature, (b) time, and (c) concentration of aminating agent on degree of substitution.

reaction at temperature (15-45 °C) [Table 1, Fig. 2a]. The DS was initially found to increase with an increase in reaction temperature and reached a maximum value of 0.613±0.045 at reaction temperature of 25°C. An increase in temperature increases the solubility of the etherifying agent which facilitated both the swelling of the galactomannan and the diffusion of the reactants. In addition the proportion of molecules with higher energy than the activation energy increases leading to a faster rate of reaction. However, a further increase in temperature decreased the DS value. This might be due to the favorable effect of temperature on the optimum swellability of galactomannan for amination to occur. An increase in temperature also inhibits reaction due to the exothermic reaction system [32,33]. The effective concentration of ethylene diamine decreases with an increase in the temperature because of volatility of the reactant.

3.2.1.2. Effect of reaction time

The effect of reaction time (5-25 h) on DS is shown in Fig. 2b. A maximum value of DS was obtained at 5 h. This could have been

due to swelling of gum. The swelling of AMLLG leads to the rapid adsorption and diffusion of reactants followed by better contact between aminating agent and LLG. However, after 5 h no further increase in DS was recorded which can speculate that the accessibility of etherifying agent have a maximum value no matter how long it takes. It may be reasonable to keep the reaction at 5 h to save time [18,33].

3.2.1.3. Effect of concentration of aminating agent

In order to investigate the effect of concentration of aminating agent on DS, a series of reactions were carried out at 25 °C for 5 h with concentration of aminating agent increasing from 20-80% (v/v). The DS was found to increase with increase in concentration of aminating agent up to 30%, followed by a leveling phase with no effect on DS (Fig. 2c). It probably may be due to the absence of available position in LLG backbone for amination which showed that DS value can be simply controlled by adjusting the weight ratio of aminating agent to LLG [34].

The findings suggested that the maximum DS was obtained when 30% v/v of aminating agent was used and the reaction was carried out at a temperature of 25 °C for 5 h.

3.3. Characterization of AMLLG

3.3.1. FTIR spectroscopy

The FTIR spectrum of AMLLG is depicted in Fig. 3. A broad band at 3399 cm^{-1} could be ascribed to the stretching vibrations of hydroxyl (–OH) group of LLG. The band for –CH stretching vibration appeared at 2926 cm^{-1} (Fig. 3a) [17]. The bending vibrations of ether (C-O-C) groups were observed at 1024 cm^{-1}. The bands at 815 and 870 cm^{-1} could be attributed to α-D-galactopyranosyl units and β-D-mannopyranosyl units, respectively. The presence of characteristic bands in the FTIR spectra between 800 and 1200 cm^{-1} represented the highly coupled C-C-O, C-OH and C-O-C stretching modes of the polymer backbone [35,36,37]. The derivatized gum exhibited a sharp characteristic $–NH_2$ band between 3300-3500 cm^{-1} indicating introduction of amine group. The wide –OH band at 3399 cm^{-1} in LLG was found to be replaced with a sharp band at 3350 cm^{-1} (Fig. 3b). This shift reveals the substitution of –OH groups of native gum with $–NH_2$ groups [19]. The peaks of 2927, 1383 and 1077 cm^{-1} referenced as stretching vibration peaks of $–CH_2–$ groups. The intense peak at 1646 cm^{-1} is attributed to the in-plane deformation of the water molecule [38]. Further, amination of LLG was confirmed by the appearance of the NH–stretching peak at 1574 cm^{-1} that was absent in native LLG [19].

3.3.2. XRD

X-ray diffraction technique was employed to determine the changes in the crystalline lattice of gum. Crystallinity is an important parameter for determining the availability of the hydroxyl groups for interaction with the reagents and solvent molecules [39]. Generally in the crystalline state the intermolecular interactions are more intense and the arrangements are more ordered while in amorphous state these interactions are less intense. Fig. 4 depicts the XRD pattern of LLG and AMLLG. The XRD of LLG showed intense peak at 2θ value of 20° which shifted to 18° in AMLLG. The remaining peaks either became broad or disappear in AMLLG diffractogram. This could be attributed

to the replacement of –OH group with $–NH_2$ group upon reaction with ethylene diamine which indicates reduction in crystallinity of LLG after derivatization [40].

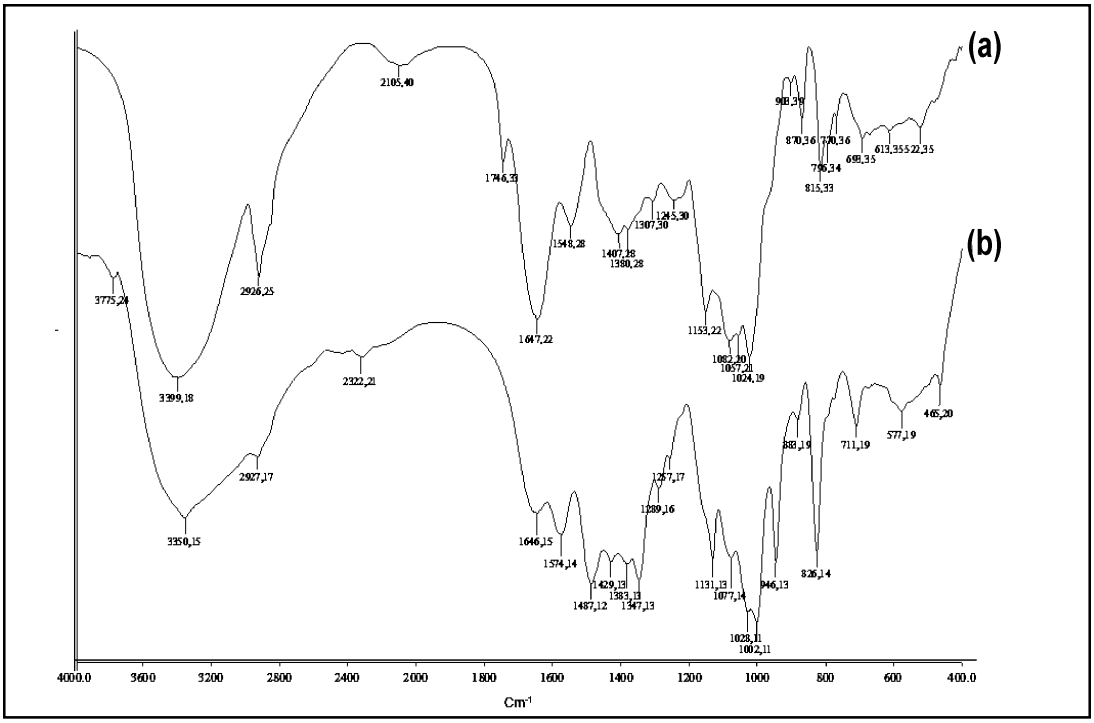

Fig. 3. FTIR spectra of (a) LLG, and (b) AMLLG.

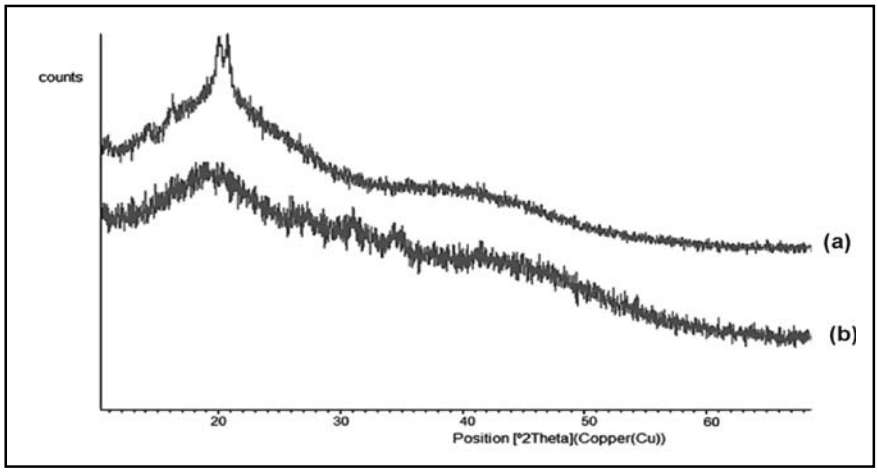

Fig. 4. XRD analysis of (a) LLG, and (b) AMLLG.

3.3.3. SEM

The microscopic analysis of native LLG (Fig. 5Ia) showed discrete, irregular granular structure, however, the process of amination altered this structure and an amorphous character was observed in AMLLG (Fig. 5Ib). The substitution of –OH group with $–NH_2$ group altered inter and intramolecular interactions present in the material leading to disruption of the crystalline structure present in LLG [40]. These results corroborated the changes in structure morphology revealed by XRD.

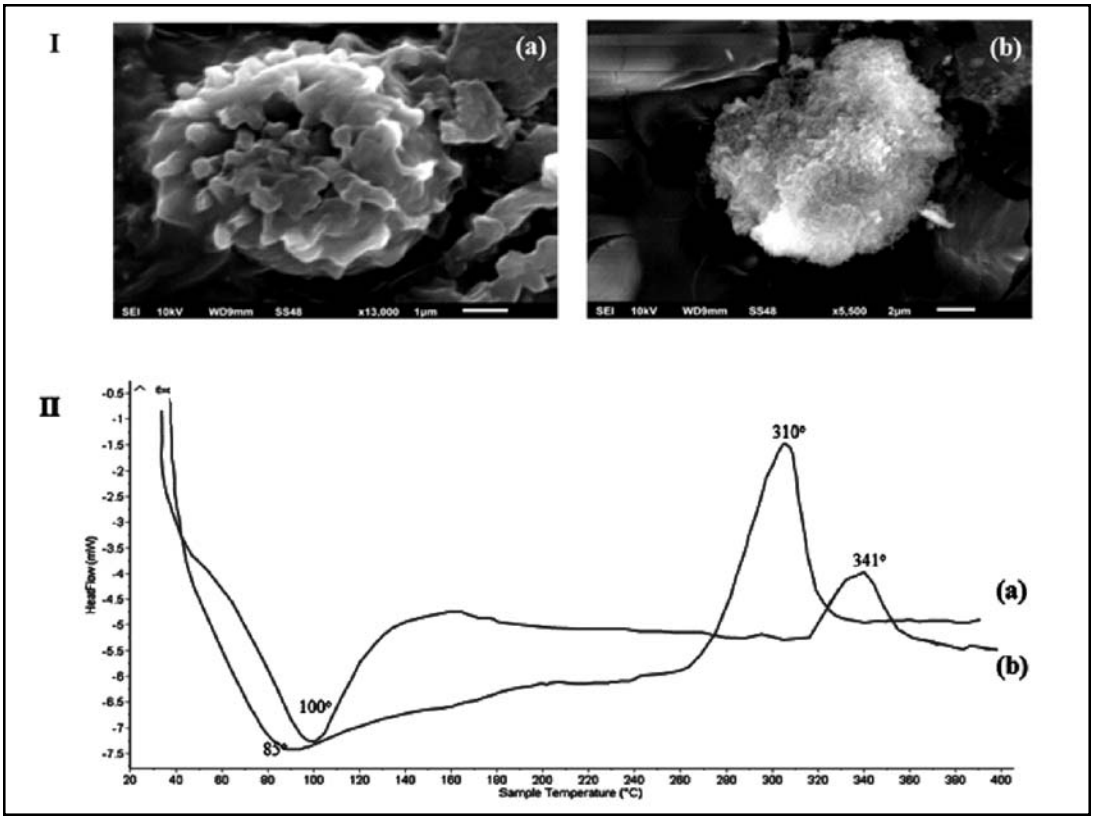

Fig. 5. SEM (I) and DSC thermograms (II) of (a) LLG, and (b) AMLLG.

3.3.4. DSC studies

The thermal properties of LLG and AMLLG were evaluated by DSC (Fig. 5II). The thermogram of LLG showed a broad endotherm and a sharp exotherm at 85 °C and 310 °C, respectively. The AMLLG thermogram revealed a sharp endothermic peak at 100°C and exotherm at 341 °C. The endothermic peak observed could be attributed to the loss of absorbed and hydrogen bonded water from the polymer while the exothermic peak could be attributed to polymer degradation. The results were in consonance with the thermal transitions of the other reported galactomannan gums [41]. This

respective shift and sharpening in the endothermic and exothermic peak validate the successful modification of LLG [19,29].

3.3.5. NMR

^{1}H NMR spectra of LLG revealed peaks between δ 1.0 to 5.0 as demonstrated in our previous study [17]. Similar peaks attributing to galactopyranosyl and mannopyranosyl units have been reported in literature for galactomannans [42]. The NMR spectra of AMLLG showed the occurrence of peaks between δ 0.5 to 5.0 ppm that could be associated to amines [43]. The peaks description for LLG and AMLLG is depicted in Table 2.

TABLE 2. Chemical shifts (in ppm) and the corresponding groups for ^{1}H NMR of LLG and AMLLG

LLG	Chemical shift (ppm)	Corresponding groups
	4.935	H-1 (α) (α -D- galactopyranose unit)
	4.701	H-2 (β) (β-D- mannopyranose unit)
	3.977	H_2 to H_6
	3.869	
	3.750	
AMLLG	0.5-4.0	R-N-H
	2.079-2.978	-CH-N-
	3.006-4.538	 -N-H

3.3.6. Rheological studies

Rheological properties of AMLLG solutions at different concentrations (1-7 % w/v) were evaluated by plotting a graph between shear rate and viscosity as shown in Fig. 6 and for LLG, these were described in our previous publication [17]. The derivatized galactomannan showed lower viscosity as compared to native LLG. It is well documented that hydroxyl-hydroxyl polar interactions are stronger than corresponding –NH_2 interactions which is further evident from the boiling points of ethylene glycol and ethylene diamine i.e. 197.6°C and 116 °C, respectively [44]. The rheological data obtained clearly revealed that the gum and derivatized gum solutions were exhibiting shear thinning behavior at increasing shear rate due to chain entanglements. The higher viscosities with increasing concentrations were attributed to an enhanced entanglement of galactomannan chains [45]. This could be attributed to interaction between –NH_2 groups of amine derivative and water molecules resulting in formation of NH_3^+–OH complex, which holds the water molecules inside the matrix of aminated gum [19].

3.3.7. Zeta Potential

A negative zeta value of -2.58 was observed for LLG while AMLLG showed a positive value of +9.25. This could be attributed to the effect of incorporation of positively charged moiety (NH_3^+) onto the galactomannan structure on

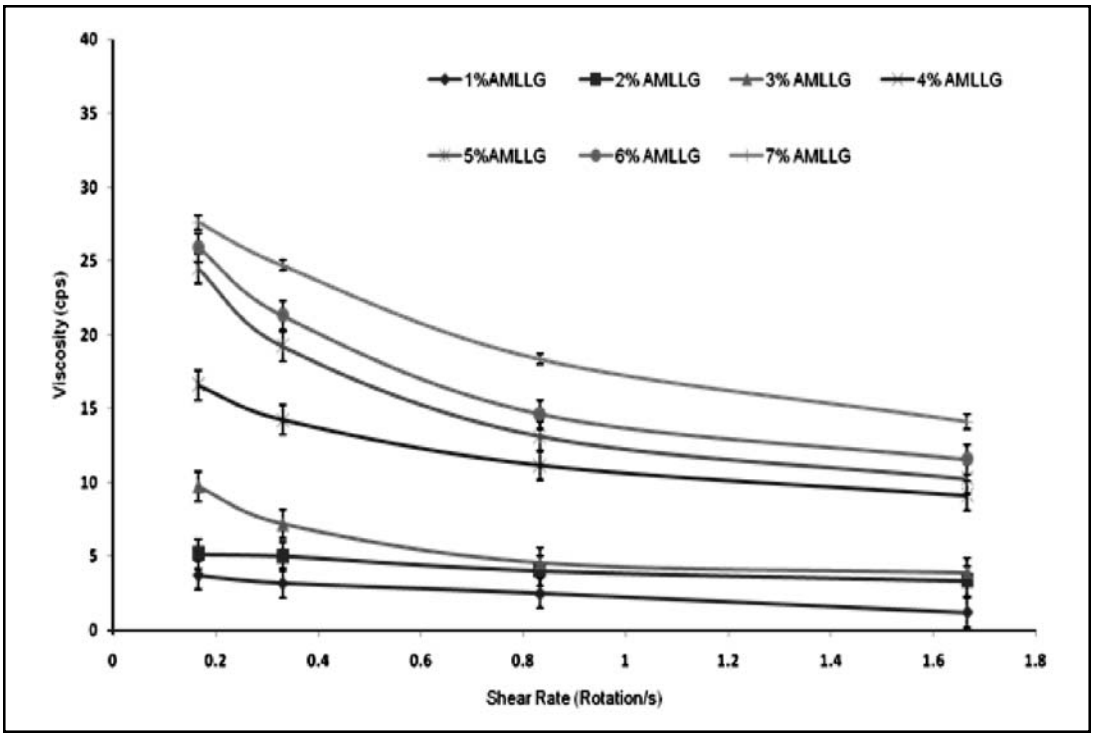

Fig. 6. Rheological profile of AMLLG

amination of LLG, further affirming the successful derivatization of LLG.

3.3.8. Mechanical properties and bioadhesion strength

The maximum force required to extrude the sample from concentric annular space between the plunger and the container shows its firmness and cohesiveness. It is an index that shows how well the product withstands a second deformation relative to its behavior under the first deformation. Fig. 7 depicts the mechanical properties of the LLG and AMLLG. These texture properties (firmness, consistency, and cohesiveness) are important indicators for use as viscosifying agents in food and pharmaceuticals. The bioadhesive strength of LLG and AMLLG was found to be 137.93 ± 1.9 g and 340.15 ± 2.3 g, respectively, indicating their potential applications in formulations of bioadhesive drug delivery systems. The aminated galactomannan showed higher bioadhesive strength as compared to native galactomannan. This may be attributed to greater binding of amine groups (positive charge) of AMLLG with negatively charged mucin chains through electrostatic interaction/hydrogen bonding resulting in stronger adhesion. The neutral bioadhesive polymers i.e. LLG can be physically interact by diffusion, interpenetration followed by physical entanglement of polymer with mucin resulting in weak bioadhesive strength [9,46].

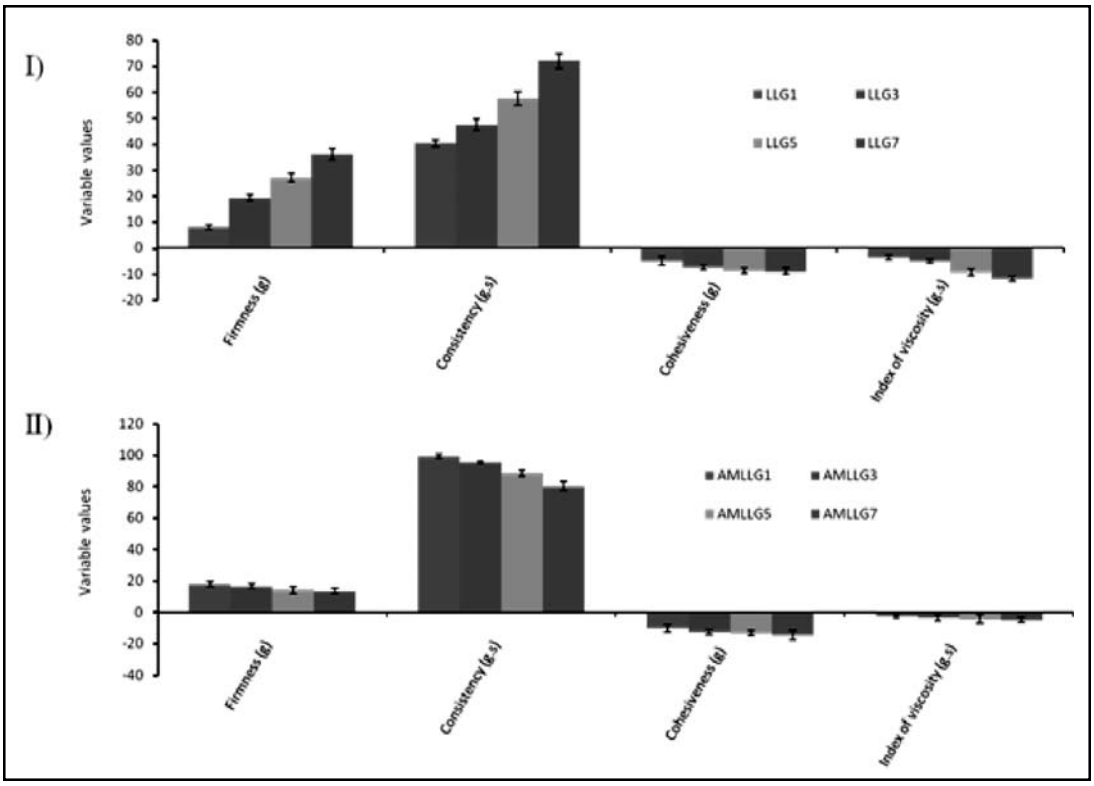

Fig. 7. Mechanical properties of (I) LLG and (II) AMLLG at different concentrations

3.4. Antimicrobial effect

Antimicrobial effects of LLG and AMLLG samples against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) were evaluated by agar diffusion assay and were compared with that of chitosan (Fig. 8). Native LLG showed no inhibitory effects on both the microbial strains, whereas AMLLG significantly inhibited the growth of *E. coli* (ZOI of AMLLG and chitosan was found to be 2.7 ± 0.5 cm and 2.4 ±0.4 cm, respectively, against *E. coli*) with no effect over *S. aureus*. The electrostatic interactions between the positively charged polymers and negatively charged microbial surfaces change the membrane permeability of cells and consequently inhibit the microbial growth. The considerably thick cell wall (around 20–80 nm) could have prevented the cellular lysis by the polymers [47,48,49]. Similar results have already been reported by Shin, *et al.,* [30] who demonstrated antimicrobial properties of β-glucan against *E.coli* only.

4. CONCLUSION

Natural polymers are extensively being explored for the drug delivery because they are safe (GRAS), cheap, freely available, biodegradable and biocompatible. These polymers contain a large number of functional groups (hydroxyl, carboxyl, etc.) that can be

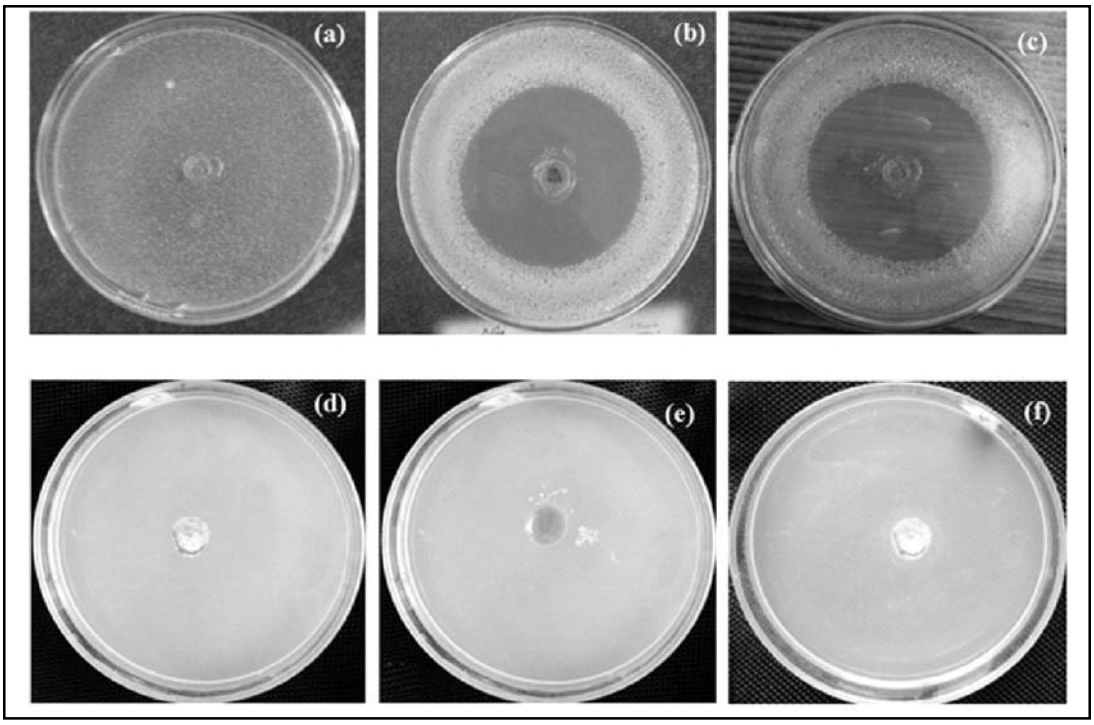

Fig. 8. Antimicrobial properties of (a) LLG (*E. coli*), (b) aminated LLG(*E. coli*), (c) chitosan (*E. coli*), (d) LLG (*S. aureus*), (e) AMLLG (*S. aureus*), and (f) chitosan (*S. aureus*).

easily tailored to introduce new functional moieties. The introduction of amine groups on polymers has been reported to enhance the commercial value of the natural polymers. In this study amine derivative of LLG was successfully prepared, the reaction parameters were optimized and AMLLG was characterized by FTIR, DSC, XRD and NMR studies. The aminated galactomannan was found to possess considerably higher bioadhesive strength as compared to native galactomannan. The antimicrobial studies revealed antibacterial activity (against *E.coli)* of AMLLG better than chitosan (standard). The above properties of AMLLG suggest its potential use as a drug delivery vehicle (as bioadhesive polymer) in pharmaceutical industry and as preservative in food industry.

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