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Screening of polysaccharides from fruit pulp of *Ziziphus mauritiana* L. and *Artocarpus heterophyllus* L. as natural mucoadhesives

Priyanka Ray* , Sumana Chatterjee and Prerona Saha

Abstract

Background: Mucoadhesive polymers are applicable for improving the delivery of drug by prolonging the residence time and time of contact of the dosage form with the mucous membrane. Mucoadhesion may be defined as a process where the polymer substance gets adhered either to the biological substrate or synthetic or to a natural macromolecule, or to the mucus membrane. The natural polymers can be studied to determine whether they possess some mucoadhesive properties as several excipients derived from plants have proved their potential in the field of conventional or novel dosage form. The present work aims at determination of physical properties of polysaccharides from fruit pulp of *Ziziphus mauritiana* L. (ZM gum) and *Artocarpus heterophyllus* L. (AH gum), such as mucoadhesive strength (shear stress determination), swelling index, pH, viscosity, angle of repose, Carr's index, density, and its comparative study with synthetic polymers Carbopol 934 and HPMC and also to study its FTIR and ¹H-NMR spectra analysis.

Result: The most important properties such as mucoadhesive strength of ZM gum (3% w/v) and AH gum (3%) was found to be comparable with HPMC (3% w/v) and Carbopol 934 (3% w/v); also, the swelling index of the isolated gums were also found comparable with both HPMC and Carbopol 934. Falling sphere method is conducted in which the time taken by the sphere to move 50 divisions to the bottom for 3% w/v ZM gum solution was 10.14 s and for AH gum was 10.13 s which is comparable to HPMC and Carbopol 934. The FTIR & ¹H NMR spectra showed typical characteristic signals of polysaccharides and presence of typical sugar residues.

Conclusion: From the study, it can be concluded that ZM and AH gum has potential to be better than Carbopol 934 and HPMC in respect of mucoadhesive strength and also it has the potential to replace some synthetic mucoadhesive polymers and polysaccharides.

Keywords: Natural mucoadhesive, Natural gums, *Ziziphus mauritiana* gum, *Artocarpus heterophyllus* gum, Physicochemical properties

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Background

Controlled release drug delivery systems provide sustained therapeutic action along with its reproducibility and predictability of release of drug ingredients from the drug delivery system [1–3]. Mucoadhesion refers to a phenomenon based on the interface between two materials: one out of which is the mucus layer of mucosal tissue on which the drug is held together by the application of interfacial forces for extended period of time. Mucoadhesive drug delivery system aims at localization of drug to a particular site for improvement and increase in the bioavailability. The time of contact is also prolonged due to interaction between the polymers and the mucus lining of tissue for prolonged action [4]. The advancement in the polymer systems in controlled delivery maintains the release rate and the concentration in the biological system by enhancement of its localized effect and avoids the first-pass metabolism [5]. Different types of bioadhesive synthetic polymers such as Carbopol 934 and hydroxyl propyl methyl cellulose (HPMC) are used to prepare various mucoadhesive formulations [6]. However, the potency, bioavailability, and drug delivery efficiency of these devices can be enhanced by discovering more natural bioadhesive materials [7–10]. The biodegradability of the synthetic polymers is still a matter of concern at times; therefore, some natural mucoadhesive materials extracted from different natural sources show potent mucoadhesive properties and can be used for the purpose of mucoadhesive formulation [11]. These polysaccharides obtained from several plant sources have been used as potent pharmaceutical excipients in various novel drug formulations such as buccal gel, transdermal patches, ointments, nanoemulsions, liposomes, micro-particles, and dental molds [12, 13]. Therefore it becomes necessary to explore more polysaccharides from natural sources in order to meet the increasing industrial demands [14]. The excipients from plant sources are much in use nowadays because they are renewable sources of energy which will provide continuous supply if grown in a sustainable way. The present work aims at the isolation and screening of plant polysaccharides, namely, ZM gum and AH gum, and to find out whether these isolated polysaccharides has the potential to provide good mucoadhesive properties and can be applied further in formulation developments.

Methods

Materials

Ziziphus mauritiana L. (Indian jujube) and *Artocarpus heterophyllus* L. (jackfruit) plants were authenticated by the Botanical Survey of India, Central National Herbarium, AJC Bose Indian Botanic Garden, Howrah, West Bengal, India, and were allotted accession numbers PR-01 and PR-02, respectively. The fruits were obtained

from the local market in the month of November 2017. All other chemicals were of analytical grade and were purchased from E. Merck India.

Isolation of polysaccharides

Isolation of ZM gum

The ripe fruit of Indian jujube (250 g) was taken and washed properly. Thereafter, the seeds were removed and the fruit is crushed in a mortar and pestle to prepare the fruit pulp. Five hundred milliliters of distilled water was added to the fruit pulp to prepare the consistency like that of a slurry. Boiling is carried out at a temperature of 90–100 °C with continuous stirring till a viscous solution is obtained [15]. The slurry was subjected to filtration with the help of muslin cloth [16, 17]. Thereafter, a clear solution is obtained which was stand for overnight for settling of the different fibers, cell debris etc. The solution is further centrifuged for 20 min at 5000 rpm. The supernatant obtained is then separated and further treated with twice the volume of ethanol with continuous stirring. This will result in the precipitation of the ZM gum which is then separated and dried at 40–45 °C. The dried film-like material obtained was powdered. Then, it is allowed to pass through sieve no. 120. The final material obtained is stored in a desiccator [4].

Isolation of AH gum

AH gum was obtained from the fruits of jackfruit [15, 17]. About 250 g raw jackfruit was taken and washed properly and rinsed with distilled water. The seeds were removed properly and the fruit was cut into small pieces. The pieces of fruits were mashed with distilled water (fruits to water ratio, 1:3) using pestle and mortar to prepare a slurry. The slurry was subjected to centrifugation for 20 min at 3000 rpm at room temperature. The residue is collected and scraped off. It is then treated with 0.5 M sodium thiosulfate solution in the ratio of 1:1 (residue to solution) for around 24 h, during which it is stirred at regular intervals which will remove protein fractions. The filtrate was then centrifuged for 5 min at 2000 rpm. The residue obtained after centrifugation was subjected to neutralization by the addition of 0.1 M HCl. It is then washed with distilled water for two times. Thereafter, it is again washed with 50% ethanol for two times. Then, the collected material is then subjected to drying at 40–45 °C for overnight. The polysaccharide obtained was then powdered with the use of mortar and pestle. It is then allowed to pass through sieve (0.15-mm mesh size). The isolated AH gum powder was packed properly and stored in desiccators [18].

Table 1 Solubility studies of natural mucoadhesive agent

Solubility studies	ZM gum	AH gum
Cool water (8–25 °C)	a	a
Warm water (30–40 °C)	+	+
Ethanol	–	–
Methanol	–	–
Dichloromethane	–	–
Chloroform	–	–
Benzene	–	–
Ethyl acetate	–	–

+ soluble, – insoluble, ^apartially soluble**Determination of yield**

The weight of the raw material and isolated gum is taken. The yield was calculated by applying the formula [19, 20]:

$$\% \text{Yield} = \frac{\text{Weight of dried isolated polysaccharide}}{\text{Weight of the whole fresh crude material}} \times 100$$

Physicochemical characterization

The different properties of the isolated gum which includes the organoleptic characters, such as odour, colour, and taste, and physicochemical characters, such as solubility, pH of 1% w/v solution at 37 °C, and viscosity of 1% w/v solution at 37 °C, were determined. Since the polysaccharides are obtained in the powder form, so the various powder properties such as tapped density, bulk density, angle of repose, and Carr's index were also measured. The results of these parameters were compared with HPMC and Carbopol 934. The pH value of the 1% solution of isolated polysaccharide samples were measured using a digital pH meter [21, 22]. Ostwald's viscometer was used to calculate the viscosity of 1% solution of isolated polysaccharide samples [22].

Phytochemical tests

Various phytochemical tests were performed for the isolated polysaccharides [23] to detect if the samples

contain carbohydrates (Molisch's test), amino acids and proteins, mucilage (ruthenium red test), starch (iodine test), alkaloids (Dragendorff's test), glycosides (Keller-Killani test), tannins (ferric chloride test), and flavonoids (Shinoda test) [24].

Study of swelling property of mucoadhesive materials

Swelling characteristics of mucilage were tested in distilled water, simulated gastric fluid (0.1 N HCl at pH 1.2), and phosphate buffer (pH 7.4). The swelling index is defined as the volume in milliliter occupied by part (1 g) of the substance. The protocol mentioned in the British Pharmacopoeia is followed for the calculation of swelling index. The test for swelling index follows the procedure in which 1 g of the isolated mucilage was taken in a ground glass stoppered graduated cylinder. Thereafter, 50 ml of distilled water was added. It was then shaken vigorously at an interval of every 10 min, and this process is repeated till 1 h and then left undisturbed for 24 h. After the said time, the volume of the mucilage occupied was measured. The formula used for calculation of swelling index of mucilage powder is as follows:

$$S = V_2/V_1$$

where V₁ = volume occupied by the mucilage before hydration and V₂ = volume occupied by the mucilage after hydration [25].

Shear stress measurement

The shear stress is defined as the measuring of the force that is capable of causing a bioadhesive or mucoadhesive material to slide in accordance to the mucus layer in a direction which is completely parallel to their place of contact for adhesion [26, 27]. The test of shear stress took different concentrations of the mucoadhesive such as 1, 2, and 3% w/v for all the samples of Carbopol 934, HPMC, ZM gum, and AH gum [15]. The solution was prepared in different concentrations and spread on the glass slides. It was covered with another glass slide. A weight of 100 g was placed on the glass slides as it will also help to spread the polymer solution evenly in

Table 2 Physicochemical property of natural mucoadhesive agent

S. No	Parameter ^a	ZM gum	AH gum	HPMC	Carbopol 934
1	pH (1% w/v)	6.8 ± 0.023	6.6 ± 0.023	6.7 ± 0.023	6.2 ± 0.040
2	Viscosity (1% w/v)	1.156 ± 0.020	1.274 ± 0.033	1.133 ± 0.005	1.137 ± 0.004
3	Bulk density (g/ml)	0.623 ± 0.011	0.523 ± 0.009	0.462 ± 0.008	0.372 ± 0.004
4	Tapped density (g/ml)	0.720 ± 0.010	0.573 ± 0.11	0.791 ± 0.012	0.681 ± 0.018
5	Carr's index (%)	11.98 ± 0.054	15.98 ± 0.073	12.81 ± 0.080	11.23 ± 0.066
6	Angle of repose	24.72 ± 0.076	22.917 ± 0.023	22.56 ± 0.064	19.58 ± 0.080

^aValues are mean ± SEM (n = 3)

Table 3 Phytochemical tests of natural mucoadhesive agent

Identification test	Name of the test	ZM gum	AH gum
Test for carbohydrates	Molisch test	+	+
Test for proteins and amino acids	Ninhydrin test	-	-
Test for mucilage	Ruthenium red test	+	+
Test for starch	Iodine test	+	+
Test for alkaloids	Dragendorff's test	-	-
Test for glycosides	Keller-Killani test	-	-
Test for tannins	Ferric chloride test	-	-
Test for flavonoids	Shinoda test	-	+

between the slides. It was kept for the allotted time for 15, 30, and 60 min. After this, one end of the glass slide was fixed hook and the other was collected to a twin passing over a pulley and at the end of pan was attached. Weight was placed in an increasing manner for the defined time till the plates which are attached to the polymer detach itself [28]. The weight at which just the polymer gets detached was noted, and the values were tabulated [13].

Falling sphere method

This method was used to characterize the mucoadhesive strength. In this process, 10% mucus solution is filled in a burette. It is then attached to a stainless steel tube. Mustard grains which can not pass through sieve size #12 were taken and poured in polymer solutions (Carbopol, HPMC, ZM gum, and AH gum) of different concentrations prepared such as 1.0, 2.0, and 3.0% w/v, and then each grain were slowly placed at the top of the mucus layer [28]. The time taken for the mustard grain

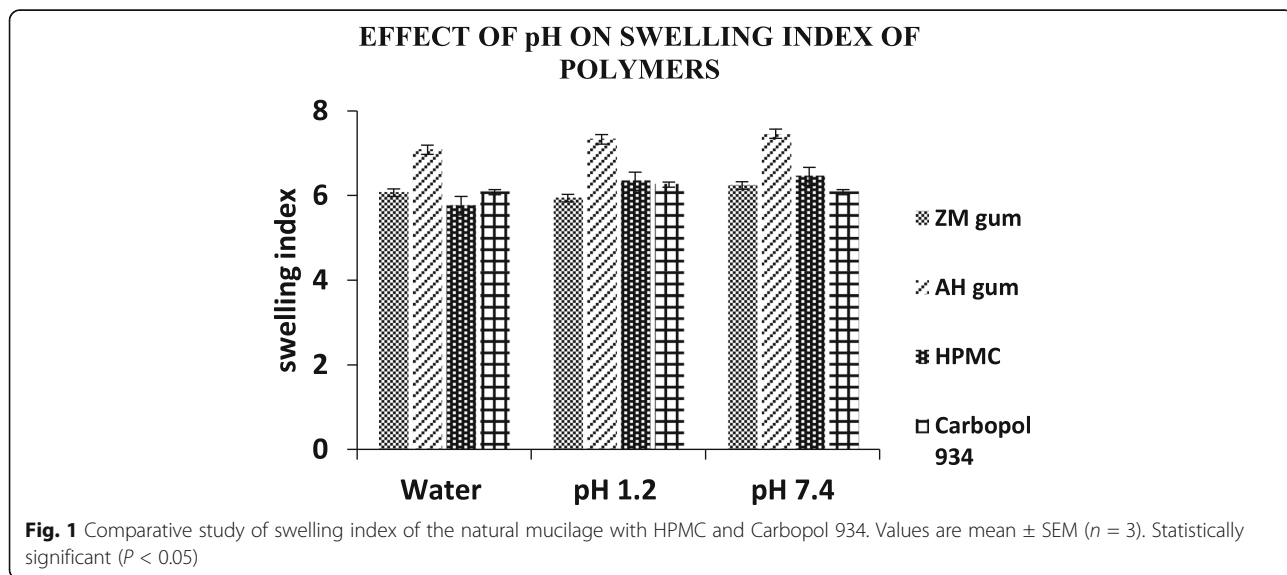
to fall about 50 divisions in the burette was noted, and the values obtained were tabulated [29, 30].

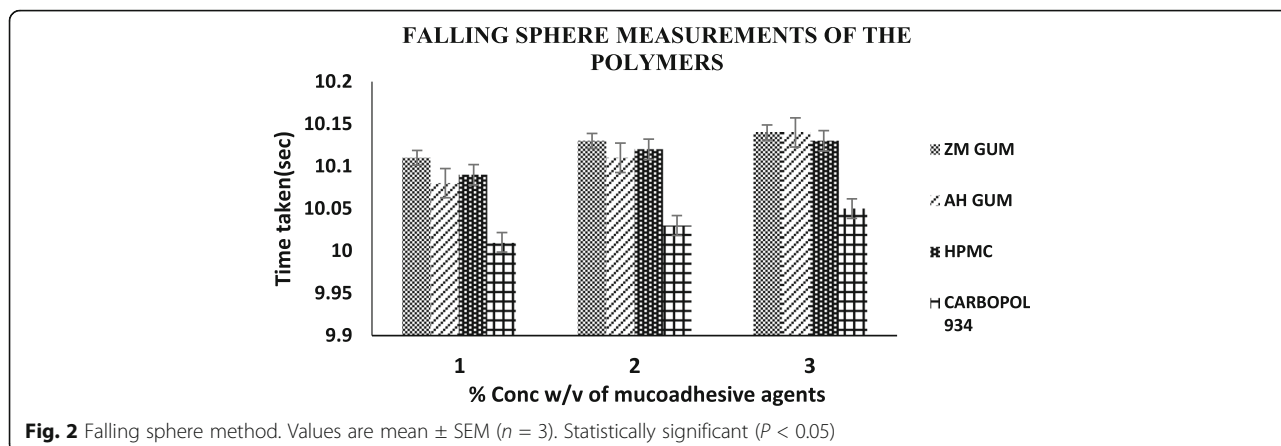
Fourier transform-infrared (FTIR) spectroscopy analysis

KBr pellets were prepared from the powdered materials in order to perform the Fourier transform-infrared (FTIR) spectroscope (PerkinElmer Spectrum UTR II). The prepared KBr pellet was positioned properly in the sample holder as per protocol, and spectral scanning was carried out with a scan speed of 1 cm/s at a resolution of 4 cm⁻¹.

1H nuclear magnetic resonance (1H NMR) spectroscopy analysis

1H NMR (600 MHz, 25 °C) spectra of sample in dimethyl sulfoxide (DMSO) were analyzed on a Bruker Avance TM III 500 spectrometer (Bruker Biospin GmbH, Germany) operating at 500.13 MHz using a 4-mm CP-MAS probe head.





Results

Yield

ZM gum and AH gum were isolated from Indian jujube (*Ziziphus mauritiana* L.) and jackfruit (*Artocarpus heterophyllus* L.) fruit pulp, respectively. The yields (%) of isolated ZM gum and AH gum were calculated as 38.56 and 29.16%, respectively.

Physicochemical characterization

The ZM gum was straw-colored, sweet, and acidic in taste with slight odor. It is soluble in water at room temperature, but it is more soluble in hot water and partially soluble in cold water as shown in Table 1.

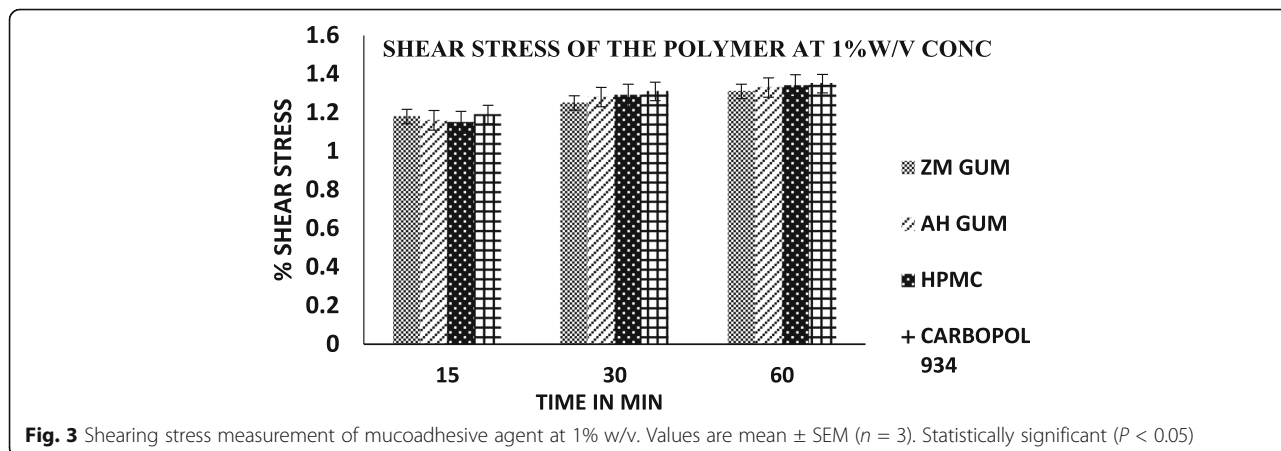
The AH gum was creamish white-colored, mucilaginous in taste, and possesses a characteristic odor. The gum was soluble in cold water and warm water but insoluble in other solvents as shown in Table 1. The different physicochemical properties like pH, viscosity, tapped density, bulk density, Carr’s index, and angle of repose were evaluated and reported in Table 2 [29].

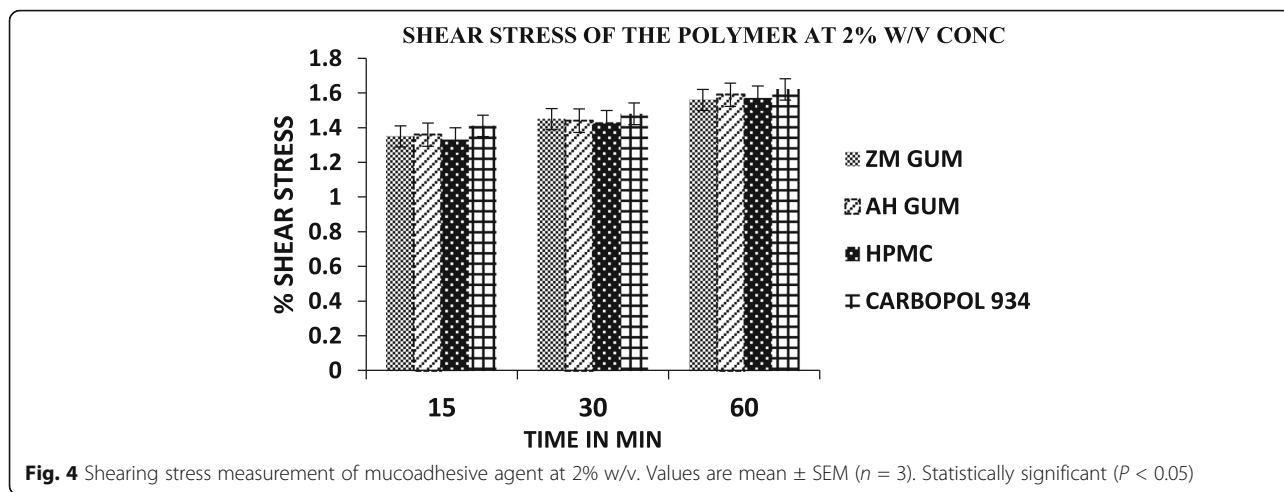
Phytochemical characterization

Both the isolated mucilage produced positive result for ruthenium red test, Molisch’s test, and Fehling’s test, and test with iodine indicated presence of mucilage, carbohydrate, reducing sugar, and polysaccharide. The ZM gum also showed positive results for Shinoda test indicating presence of flavonoids. Alkaloid, glycosides, and tannins were found absent. The result is illustrated in Table 3.

Swelling property of the isolated mucilages

The swelling index of the dry mucilages was performed in different solvents such as distilled water, 0.1 N hydrochloric acid, and pH 7.4 phosphate buffer as shown in Fig. 1. The swelling capacity of mucilages ZM gum and AH gum was found to be 6.069 and 7.081 in distilled water, 5.94 and 7.33 in simulated gastric fluid (0.1 N HCl), and 6.24 and 7.46 in phosphate buffer (pH 7.4), respectively [31]. This proves that the swelling of mucilage is not dependent on the pH. The swelling index of both the isolated mucilage was similar to that of Carbopol 934 and HPMC;





hence, the nature of the said mucilage ensures the suitability to use for mucoadhesive drug delivery system.

Falling sphere method

This test showed that with the increase in the concentration of mucilage, resistance to movement of the mustard grain towards the bottom has increased. The time (in seconds) required to move the mustard grain from the top of the burette towards the bottom in 3% w/v ZM gum solution was found to be 10.14 s and for AH gum 10.13 s. The results are comparable to HPMC and Carbopol 934 as shown in Fig. 2 [29].

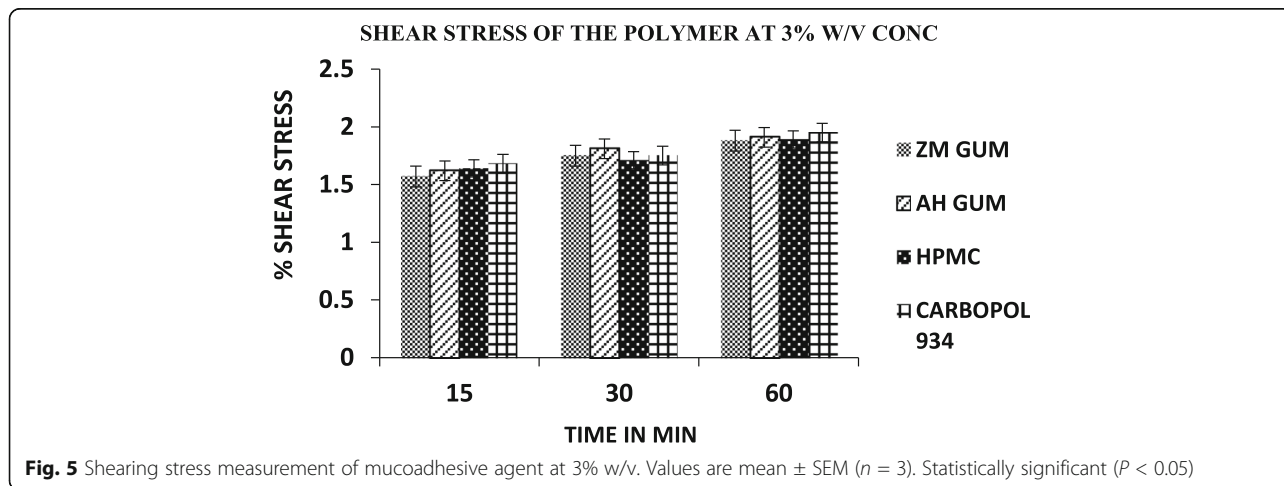
Shear stress measurement

Shear stress studies of the isolated mucilage was carried out and compared with HPMC and Carbopol 934. The solutions of 1, 2, and 3% of ZM gum, AH

gum, HPMC, and Carbopol 934 were prepared, and the shear stress study was carried out (Figs. 3 and 4). The analysis of shear stress is shown graphically in Fig. 5 for different concentrations. It is seen that the shear stress at 15, 30, and 60 min of both the isolated mucilage is comparable to that of HPMC and Carbopol 934.

FTIR analysis

The study of FTIR spectrum for ZM gum is shown in Fig. 6, and it shows a typical absorption band at 3435 cm⁻¹ which shows the presence of an OH group which is hydrogen-bonded [32]. The bands at 292 cm⁻¹ shows typical polysaccharide sugars, arabinose, rhamnose, and galactose and shows aldehyde C-H stretch and alkane C-H stretch. Apart from these characteristic band of amide, NH bend, C-C stretch, NO₂ from both aliphatic and aromatic galactoproteins, and amino acids are shown at around



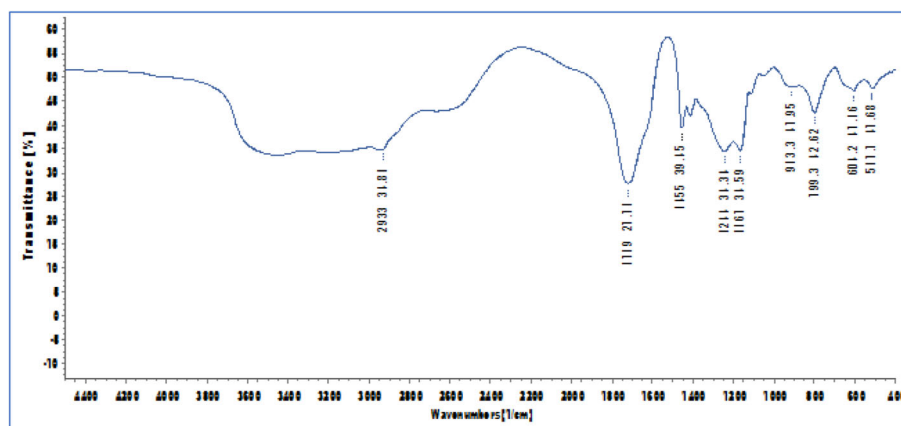


Fig. 6 FTIR spectrum of isolated ZM gum

1618 cm^{-1} [33]. The peak at 1427 cm^{-1} shows the presence of ether linkage. At 1074 cm^{-1} shows the alkene C–H bend which is usually present in the polysaccharides of all gums [34].

The study of FTIR spectrum for AH gum is shown in Fig. 7, and absorption band at 3401 cm^{-1} is visible depicting the presence of hydrogen-bonded OH group. Typical bands are shown at 2926 cm^{-1} which depicts the presence of rhamnose, galactose, and arabinose, also the presence of aldehyde C–H stretch and alkane C–H stretch similar to ZM gum. The band at 1610 cm^{-1} shows amino acids [33]. Here also, the peak at 1420 cm^{-1} shows the presence of ether linkage and at 1074 cm^{-1} represents alkene C–H bend [15].

^1H NMR studies

^1H NMR spectra of isolated ZM gum and AH gum is represented in Figs. 8 and 9, respectively. ^1H NMR spectra of AH gum shows marked signals of polysaccharides, which are prominent in the region around 3–5 ppm [35, 36]. The ^1H NMR spectra of ZM gum shows signal in the region between 5 and 6.5 ppm indicating presence of α -glucopyranose. At 7.265 ppm, a marked peak depicts the presence of polyphenolic compounds in ZM gum [37].

Discussion

In the present study, two gums were isolated from plant sources, viz., ZM gum and AH gum, from fruits of Indian jujube (*Ziziphus mauritiana* L.) and

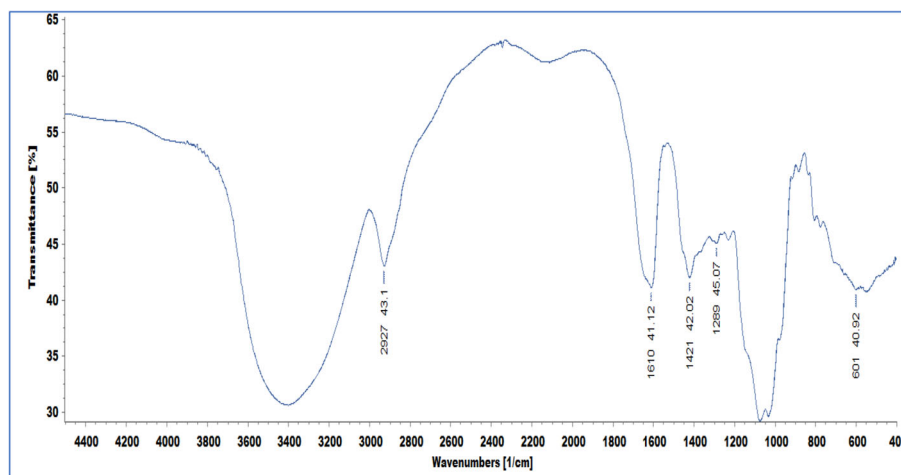
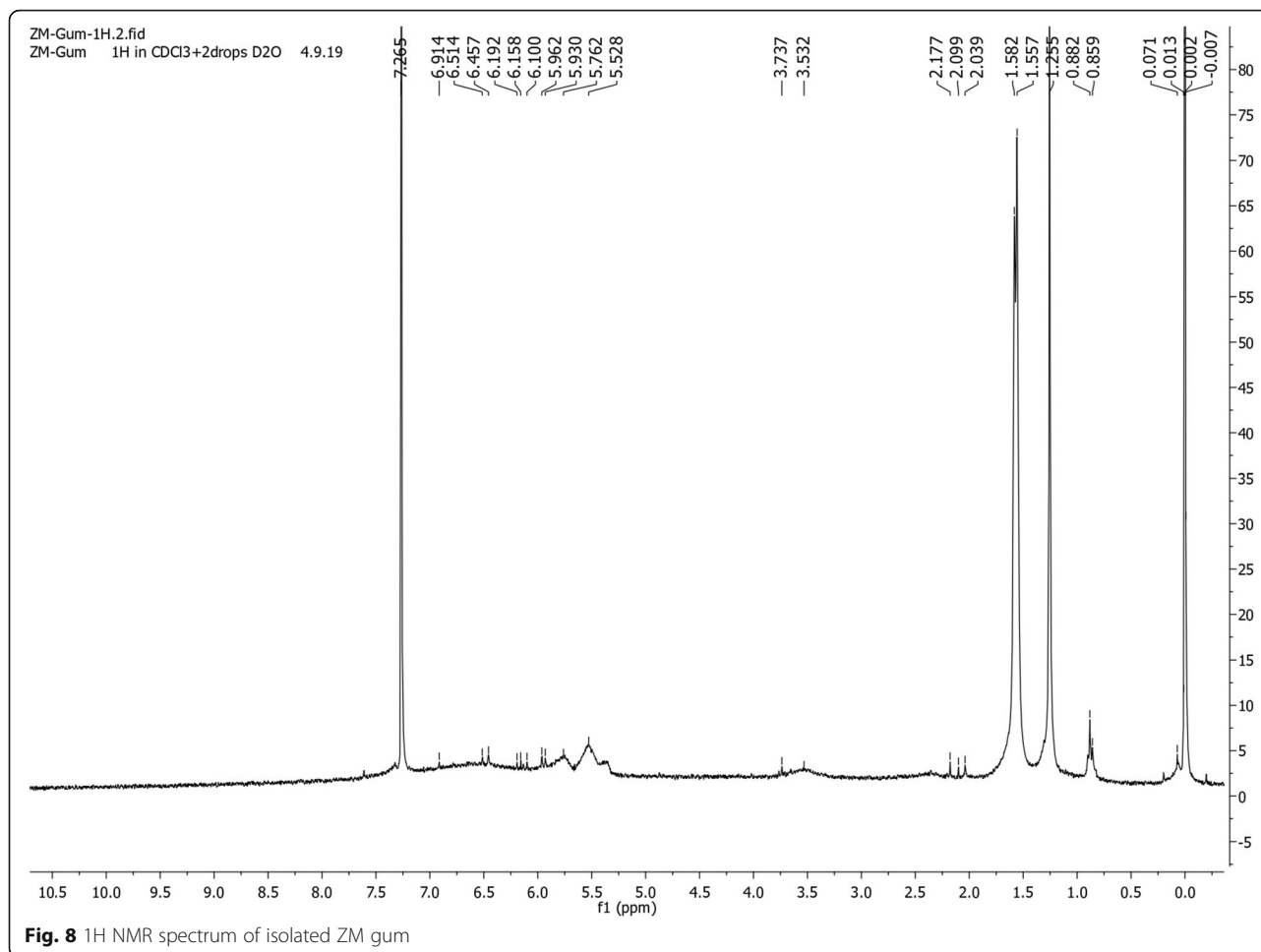


Fig. 7 FTIR spectrum of isolated AH gum



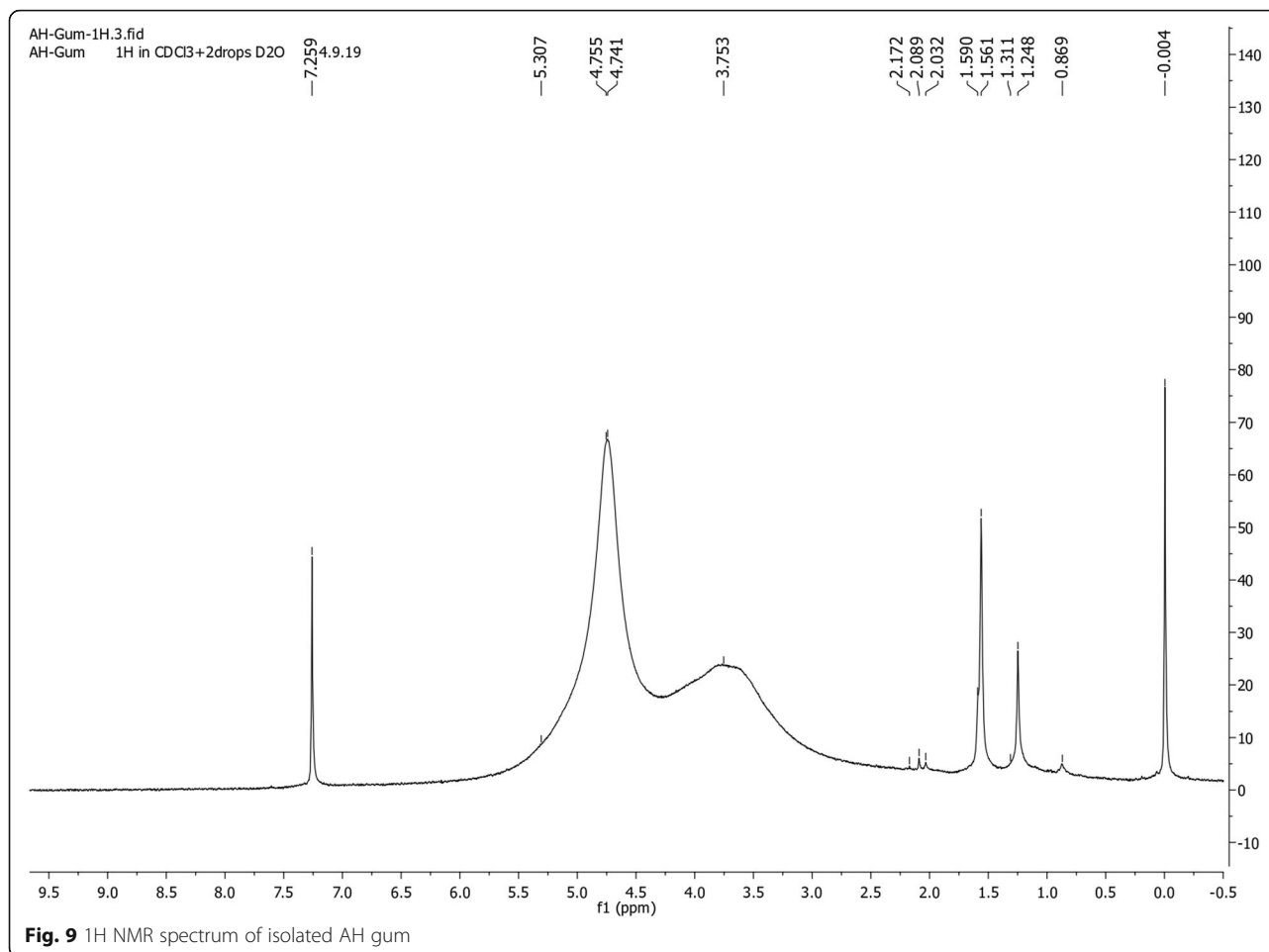
jackfruit (*Artocarpus heterophyllus* L.) [38, 39]. The yields of ZM gum and AH gum were 38.56 and 29.16%, respectively. The different organoleptic properties like color, odor, and taste and physicochemical properties like pH, solubility in water, and viscosity of these isolated plant polysaccharides were evaluated. ZM gum was straw-colored, and AH gum was creamish white in color [15]. The isolated plant polysaccharide from ZM gum was having slight odor, whereas the AH gum was having a characteristic odor. The taste of ZM gum was a little bit sweet and sour. However, AH gum was mucilaginous and bland in taste. These isolated gums were soluble in hot water and partially soluble in cold water. The pH of 1% isolated ZM gum and AH gum solution at 37 °C were measured as 6.8 ± 0.023 and 6.6 ± 0.023 , respectively, whereas the viscosity of the ZM gum and AH gum solution at 37 °C were found to be 1.156 ± 0.020 poise and 1.274 ± 0.033 poise, respectively. The bulk density and tapped density of the ZM gum were found to be 0.623 ± 0.011 gm/ml and 0.720 ± 0.010 gm/ml, respectively, and those of AH

gum were found to be 0.523 ± 0.009 gm/ml and 0.573 ± 0.11 , respectively. The powder flow property such as Carr's index and angle of repose for ZM gum were found to be 11.98 ± 0.054 and 24.72 ± 0.076 , respectively, and for AH gum 15.98 ± 0.073 and 22.917 ± 0.023 [40].

The result of shearing stress and falling sphere analysis were found comparable to HPMC and Carbopol 934. The FTIR and 1H NMR study confirms sugar residues in the isolated ZM gum and AH gum polysaccharides and shows the presence of polyphenolic compound in the ZM gum [41].

Conclusion

The study shows that ZM gum and AH gum have shown potent mucoadhesive properties as compared to HPMC and Carbopol 934 when compared on the basis of various parameters such as mucoadhesive strength, shearing stress, swelling index, and various physicochemical properties. The FTIR and 1H NMR spectra analysis have also led to the conclusion that it contains sugar residues which is usually present in



various polysaccharides. Therefore, it can be concluded that ZM and AH gum have the potential to be better than Carbopol 934 and HPMC in respect of mucoadhesive strength, and also, they can be further taken into account for replacing synthetic mucoadhesive polymers and polysaccharides.

Abbreviations

ZM: *Ziziphus Mauritiana*; AH: *Artocarpus heterophyllus*; NMR: Nuclear magnetic resonance; HPMC: Hydroxy propyl methyl cellulose; FTIR: Fourier transform infrared; NMR: Nuclear magnetic resonance

Acknowledgements

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Plant authentication

The plants were authenticated by Botanical Survey of India, Central National Herbarium, AJC Bose Indian Botanic Garden, Howrah, West Bengal, India, and were allotted the accession numbers PR-01 and PR-02, respectively.

Authors' contributions

PR conducted the experimental part of the research work under the guidance of SC and PS. PR prepared the initial draft of the manuscript. SC

and PS made critical revisions and approved the final version of the paper. All authors reviewed and approved the final manuscript.

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Availability of data and materials

Data and material are available upon request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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