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Characterization of *Ziziphus mauritiana LAM.* Seed (JUJUBE) Mucilage for Physicochemical and Mucoadhesive Properties.

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Abstract: Present investigation was focused on isolation of mucilage from seeds of ripe fruits of *Ziziphus mauritiana Lam.* Family Rhamnaceae. (Jujube). It was evaluated for various physicochemical and mucoadhesive properties. Isolated mucilage was shown positive results for molisch's test, felhing's test, ruthenium red test and ninhydrin test indicated presence of carbohydrate, reducing sugar, mucilage and amino acid. Results of physicochemical tests was indicated the suitability of mucilage for tablet dosage form due to its compressibility, flowability, weakly acidic pH, swelling and viscous nature.

Isolated mucilage was compared with carbopol 934P as standard mucoadhesive polymer. Mucilage was characterized by mucoadhesion models viz. wihelmy's method, falling sphere method, spring balance method and modified physical balance method to determine mucoadhesion strength. In wihelmy's method and falling sphere method, 3% w/v mucilage was shown comparative mucoadhesion strength with 1 % w/v Carbopol 934P. FTIR spectrograph showed presence of carbonyl and hydroxyl groups which are responsible for hydrogen bond formation between mucosa and the polymer. Mucilage isolated from *Ziziphus mauritiana Lam.* seed was found suitable excipient for design of oral mucoadhesive tablet.

Keywords: *Ziziphus mauritiana,* mucilage, mucoadhesion, wihelmy's method and swelling index.

Introduction:

Nature is a well-known potential source for pharmaceutical additives. Plants, animals, marine animals and algae, micro-organisms and inorganic clays are the rich sources of natural substances. Pharmaceutical additives are abundantly obtains from the natural sources.

Mucoadhesion is a relatively new and emerging concept in new drug delivery. Mucoadhesion enhances the delivery system adhering to the mucus membrane. Mucoadhesive drug delivery systems show various merits over conventional drug delivery systems (1, 2). Drug delivery using mucoadhesive dosage form via transmucosal route, bypasses hepato-gastrointestinal first pass metabolism associated with oral administration, increases gastric residence time and sustains or controls drug release thereby increases the bioavailability and produces longer therapeutic effect (3).

Mucilaginous substances have been reported as occurring plentiful in the Rhamnaceae family, including genus *Ziziphus*. Trees and shrubs are characteristically found in many semi arid regions of the world. Carbohydrates, starch, proteins, sugars and mucilages are abundantly available in *Ziziphus* (4).

Materials and Methods:

Materials:

In the present research work materials used were of analytical reagent grades. Ripe fruits of *Ziziphus mauritiana* (plant specimen was identified and submitted at botanical survey of India, Western regional centre, Koregaon Road, Pune, through BSI/WRC/Tech/2011/V. No. ABGZIM4, dated 06.09.2011), Carbopol-934P (Lobachemie), Acetone (Purechem), Ruthenium red (Purechem), Felhing's A and B solutions (Purechem). Molisch's reagent (Purechem), Sulphuric acid (Purechem), Ninhydrin (Lobachemie).

Methods:

Isolation and Purification of mucoadhesive substance from *Ziziphus mauritiana seed*:

Plant of *Ziziphus mauritiana* (Jujube)was authenticated from BSI, Pune. Ripe Jujube fruits were collected from the plant. Outer coverings were removed and seed part was allowed to soak in distilled water for overnight with mechanical stirring at 100 rpm. The sticky substance was released in distilled water. The material was pressed through muslin cloth. The clear filtrate was precipitated with its double quantity of acetone. It was washed three times with acetone. The obtained substance was dried in oven at 40ºC for 6 hours. It was reduced to fine powder and stored in closed container (5, 6).

Preliminary Phytochemical and Physicochemical evaluation of JUJUBE Mucilage:

Phytochemical and physicochemical evaluation was subjected to identification of nature and to determine suitability of isolated mucilage as a tablet excipient. Phytochemical evaluation parameters for the study were Ruthenium red test for mucilage, Felhing's test for reducing sugars, Molisch's Test for carbohydrates, Ninhydrin test for amino acids, Iodine test for polysaccharide, Biuret test for protein, Salkowski test for steroid, Baljet's test for glycoside, Shinoda test for flavonoids and Wagner's test for alkaloids. Physicochemical parameters for the study were Loss on Drying, Viscosity, P^H Determination, Swelling Index, Bulk Density, Tapped density, Carr's compressibility index and Angle of repose (7, 8, 9, 10).

In-vitro Mucoadhesion Studies on isolated substances:

WIHLMY'S Method:

In this method, mucoadhesion strength was determined by following procedure. Small glass plate of dimension 2×5cm was coated by dipping into 1%, 2 % and 3% w/v of isolated mucilage and 1% w/v Carbopol 934P. It was allowed to dry at 40ºC for 30 min. They were uniformly coated to small glass plates respectively. The mucus gel was scrubbed from goat intestine and kept at 20-25ºC. Nylon thread was attached at one end of coated glass plate. Other side of thread was passed through pulley and attached to small plastic bag. Weight was added into plastic bag. Weight in gram required to pull out the glass plate from the mucus gel represents the force required to break the mucus-polymer bond against adhesion. Weight of coated glass plate was minimized to obtain actual weight in gram. Six plates each were tested and average weights required were calculated (11, 12).

Falling Sphere Method:

Mucilage powder was characterized to determine mucoadhesive strength by using reported falling sphere method. Clean burettes were filled with 1 %, 2%, 3% w/v solutions of isolated mucilage and 1 % w/v Carbopol 934P. Mustard grain of size 1.5-1.6 mm were taken and added in solution of isolated substance and Carbopol 934P at the top of burette respectively. Each grain was slowly placed at the top solution layer. Time in seconds taken by the grain to fall at the bottom reading level in the burette was noted (13, 14).

Spring Balance Method:

This mucoadhesion model was designed to determine mucoadhesion strength of pharmaceutical polymers. Laboratory based model was developed by using previously calibrated spring balance, and goat gastric mucus membrane. Goat stomach mucosa was collected from slaughter house and stored in Tyrode's solution.

The sliding stage was fitted with the clamp on the burette stand. Upper side of spring balance was vertically attached to lower end of the sliding stage. To the lower side of balance, plastic closure was attached using thin metal wire. Plastic bottle was filled with sand and closed with plastic closure. Base of plastic bottle was attached to the bottom of 500 ml plastic beaker by cynoacrylate adhesive. The freshly excised goat gastric mucosa was cleaned and cut to closure size. The mucosa was attached to plane surface of upper plastic closure by using cynoacrylate adhesive. The mucosa stick closure was attached to the lower end of spring balance.

Tablets of thickness 1mm and diameter 8.75 mm of test substance were prepared on tablet press. Tablet was stick to the bottle closure by cynoacrylate adhesive. The tablet was allowed to wet in slow dripping of 0.1 N HCl. After 5 minute of wetting, previously calibrated the spring balance was vertically aligned. Mucosa attached closure was kept over thin mucilage tablet and slightly pressed and allowed to

form a bond between mucosa and polymer for 10 minute.
With the help of sliding stage, the spring balance was very slowly raised till the mucosa get detached from the tablet surface. Simultaneously reading was observed on the balance scale. The weight required to detach the disc was measured in grams and noted as mucoadhesion strength. Mucoadhesion strength was calculated as force of adhesion in kgm/s^2. (15)

The force of Adhesion was calculated by,
Force of Adhesion (kgm/s^2)
= Weight in kg x acceleration.

$$= \frac{\text{Weight in g}}{1000} \times 9.81$$

Modified Physical Balance Method:
Tablets of thickness 1mm and diameter 8.75 mm of test substance were prepared on tablet press. Goat stomach mucosa was colleted from slaughter house and stored in Tyrode's solution. Mucosa was placed on glass slide and tightly tied with thread. The glass slide with the mucosa was affixed on one side of modified physical balance. Prepared tablet was stacked to plastic bottle closure with cynoacrylate adhesive and attached the one arm of modified physical balance with nylon thread, below which glass slide was affixed. On the opposite side of balance, small plastic bag was attached. Now balance was calibrated. Glass slide with mucosa was raised to tablet surface. The mucosal and tablet surface was wetted with few drops of 0.1 N HCl and tablet was slightly pressed on mucosa for 10 minute to allow mucoadhesion. In plastic bag small increments of water was added to detach the surfaces. The weight in gram required to detach the surfaces was noted.(11, 16)

Fourier Transform InfraRed Spectroscopy Studies (FTIR):
(Kalsi PS. 2010; Ansari SH. 2006) FTIR spectrum was taken using Alpha FTIR spectrometer, Bruker optics. Thin film of test substance was prepared and placed on spectrometer lens. FTIR spectrum of sample was obtained. (17)

Differential Scanning Calorimetric Studies (DSC):
(Beckett AH. et. al., 2004; Singh SK. et.al. 2010) DSC curve was obtained using Mettler Toledo DSC 821, Switzerland. Test substance was weighed into aluminum crucible and sample was analyzed by heating at scanning rate of 10°C/ min over a temperature range 20-300°C under nitrogen flow of 40 ml/min. (18)

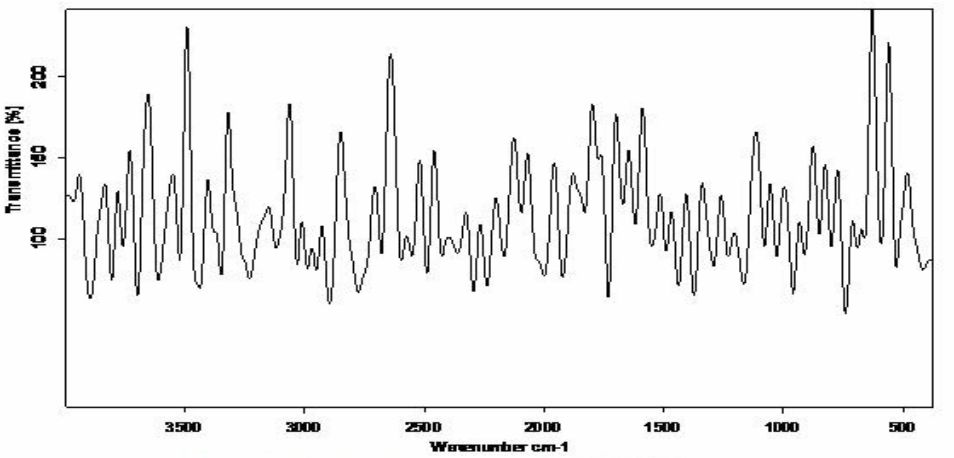

Figure 1 FTIR Spectrum of Jujube seed mucilage

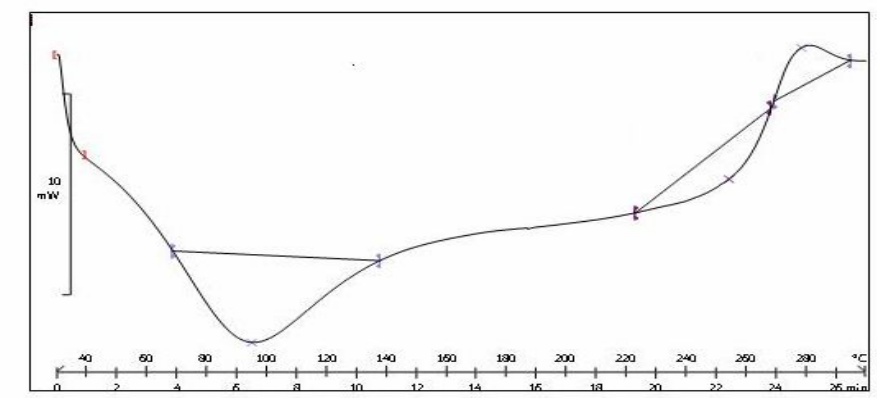

Figure 2 DSC curve of Jujube seed mucilage

Table 1: Physicochemical characterization of isolated substances:

Sr. No.	Property	Observation
1	Color	White
2	Odor	Characteristic
3	Mouth feel	Mucilaginous
4	Ruthenium red test	Positive
5	Molisch's test	Positive
6	Felhing's test	Positive
7	Ninhydrin test	Positive
8	Test with Iodine	Positive
9	Biuret test	Positive
10	Salkowski test	Negative
11	Baljet's test	Negative
12	Shinoda test	Negative
13	Wagner's test	Negative
14	Solubility in water (1 % w/v)	Soluble
15	Viscosity (1 % w/v) poise	2.324± 0.061
16	Swelling index in distilled water	3.7 ± 0.421
17	pH (1% w/v in water)	5.25 ± 0.124
18	Loss on drying % w/w	4.407± 0.234
19	Bulk density g/ml	0.690 ± 0.025
20	Tapped density g/ml	0.725 ± 0.029
21	Angle of repose (°)	31.14 ± 0.234
22	% Carr's Compressibility Index	4.85

Table 2: Wihelmy's method and Falling sphere method

Mucoadhesive substance		Wihelmy's method Weight in gram	Falling sphere method Time in sec.
Jujube seed mucilage	1 % w/v	1.112 ± 0.1	4.197 ± 0.546
	2% w/v	2.168 ± 0.161	9.767 ± 0.325
	3% w/v	3.237 ± 0.077	13.353 ± 0.347
Carbopol 934P	1% w/v	4.033 ± 0.145	17.63 ± 0.297

Table 3. Modified Physical Balance and Spring Balance method:

Tablet	Modified Physical balance		Spring balance	
	Weight in gm	Mucoadhesive strength (kgm/s^2)	Weight in gm	Mucoadhesive strength (kgm/s^2)
Jujube mucilage	30.504 ± 1.065	0.299	27.66 ± 1.505	0.271
Carbopol-934P	83.138 ± 1.68	0.814	80.833 ± 1.471	0.792

Result and Discussion:

Physicochemical Characterization of JUJUBE Seed Mucilage:

Results of chemical tests and physicochemical properties are shown in table 1. White colored, mucilaginous and characteristic odor substance was obtained. Jujube fruit mucilage was found viscous at 1 % w/v concentration with viscosity 2.324± 0.061 poise.

It was produced about 3.7 ± 0.421 times swelling in distilled water and may be useful as sustained or controlled release polymer. Generally mucilage exhibit high degree of swelling. P^H of isolated mucilage was found to be 5.25 ± 0.124, very weakly acidic and thus can be useful orally, as it will not produce irritation to gastric mucosa. Isolated mucilage was produced positive results for ruthenium red test, molish's test, felhing's test, ninhydrin test, Biuret test and test with iodine indicated presence of mucilage, carbohydrate, reducing sugar, amino acid, protein and polysaccharide respectively. Flavonoids, alkaloid, steroid and tannins were found absent. Bulk density of isolated mucilage was found to be 0.690 ± 0.025 g/ml and it's tapped density was found to be 0.725 ± 0.029 g/ml. It was observed that tapped density was increased after tapping indicated that mucilage have compressibility. Percentage Carr's compressibility index value was found to be 4.85. It indicated that isolated mucilage was flowable. In general compressibility index values upto 15% result in good to excellent flow properties. Angle of repose for the mucilage was found to be 31.14 ± 0.234°. It was indicated that mucilage have good flow property makes it suitable for direct compression.

***In-vitro* Mucoadhesion Studies:**

Mucilage powder was characterized for mucoadhesion properties and was compared with Carbopol 934P. 1% w/v, 2% w/v and 3% w/v solutions of Jujube seed mucilage and 1 % w/v Carbopol 934P were prepared. These were characterized for mucoadhesion using Wihelmy's method and Falling sphere method. Results are shown in table 2. In Wihelmy's method weight in grams required to pull out the plate from mucus gel was measured. It was observed that mucoadhesive bond formation between isolated substance and mucus gel resist plate to move upward and thus was required more weight while pull out the coated plates of higher concentrations of the isolated mucilage. Carbopol 934P was shown better mucoadhesion force than the Jujube seed mucilage powder. Force required to detach the glass plate coated with 3 % w/w mucilage was found to be 3.237 ± 0.077 g which is less than 4.033 ± 0.145 g of 1 % w/v Carbopol 934P.

In Falling sphere method small size mustard grains of size 1.5-1.6 mm were added into 1% w/v, 2% w/v and 3% w/v solutions of isolated substance and 1 % w/v Carbopol 934P respectively. Time in seconds required to move to bottom level in burette was measured as measure of mucoadhesion. It was observed that as concentration of the mucilage was increased, resistance in terms of time to move bottom side was also increased. Time in seconds required to move the mustard grain from top to bottom in 3 %w/v mucilage solution was found to be 13.353 ± 0.347 Seconds. It was found less than 17.63 ± 0.297 Seconds of 1 % w/v Carbopol 934P.

Modified Physical balance and spring balance were used to compare the mucoadhesive strength between isolated substance and Carbopol 934P. Both methods are used to determine mucoadhesive strength tablet of Jujube seed mucilage and Carbopol 934P.

Spring balance method was shown following results. Jujube seed mucilage tablet was shown 27.66 ± 1.505 g and 0.271 kgm/s^2 mucoadhesion strength. Carbopol 934P tablet was shown 80.833 ± 1.471 g and 0.792 kgm/s^2 mucoadhesion strength. Carbopol 934P was shown 2.922 times mucoadhesion strength than Jujube seed mucilage.

Modified physical balance method was shown following results. Jujube fruit mucilage tablet was shown 30.504 ± 1.065 g and 0.299 kgm/s^2 mucoadhesion strength. Carbopol 934P tablet was shown 83.138±1.68 g and 0.814 kgm/s^2 mucoadhesion strength. Carbopol 934P was shown 2.725 times mucoadhesion strength than Jujube fruit mucilage.

From the figure 5, it was observed, modified physical balance method was shown better mucoadhesion strength results than spring balance method.

Fourier Transform InfraRed Spectroscopy Studies (FTIR):

FTIR spectrum is shown in figure 1. Characteristic absorption occurs at various peaks (functional groups) 1080cm^{-1} and 1166.32cm^{-1} (R-OH strech), 1292.18cm^{-1} (C-O strech), 1374.47cm^{-1} and 1438.95cm^{-1} (C-H strech) 1731.67cm^{-1} (C=O Strech) , 2776.49cm^{-1} and 2951.97cm^{-1} (C-H strech), 3230.98cm^{-1} (O-H strech), 3439.16cm^{-1} (O-H strech) indicated that isolated mucilage may forms hydrogen bond with mucosa for mucoadhesion purpose.

Differential Scanning Calorimetric Studies (DSC):

The DSC thermogram of jujube seed mucilage is shown in figure 2. It was shown three broad endothermic peaks at temperature 94.55 °C, 254.12 °C and exothermic peak at temperature 278.49 °C.

Conclusion:

Jujube seed mucilage was found useful as mucoadhesive excipient for oral drug delivery systems.

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