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ORIGINAL RESEARCH

Investigation on binding properties of *Grewia asiatica* mucilage in tablet formulations

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ABSTRACT

The binding property of *Grewia asiatica* mucilage was evaluated at 0.25-0.1% concentrations. The mucilage was extracted by cold maceration method and characterized for its swelling index, moisture absorption capacity, solubility, organoleptic characters and purity. The mucilage was also investigated for its toxicity, microbial growth and texture. Tablets were prepared by a wet granulation using paracetamol as a model drug. The granules were evaluated for micromeritics properties, percentage of fines and moisture content. The tablets were evaluated for appearance, weight variation, friability, thickness, hardness, disintegration time, drug content and *in vitro* drug release. It was observed that the friability percentage was decreased with an increase in mucilage concentration. Hardness of the tablet was increased with an increase in mucilage concentration and was found to be in the acceptable range. Disintegration time was found to be less than 15 min for all the four batches. Drug content was found to be in the range of 93.0- 98.08% for all the formulations. More than 80% of the drug was released within 30 min during the *in vitro* dissolution study.

Keywords: *Grewia asiatica*, excipients, mucilage, binder, Kitazawa.

INTRODUCTION

Excipients are pharmacologically inactive substances used as a carrier for the active ingredients in the manufacture of dosage forms (1). In general, the active substances may not be easily administered alone and they need to be converted into an appropriate form suitable for the administration, with the help of excipients. Excipients are also used to increase the bulk of formulation to allow for convenient and accurate dosage (2, 3). The natural polymers are cheap, easily available, less toxic and non irritant in nature. Mucilages are the high-molecular-weight (>200,000) metabolic byproduct of plant cell. These are sticky and gummy substances, acting as a membrane thickener and food reserve. The chief industrial sources of mucilages are Icelandic and Irish moss, slippery elm bark, linseed, locust bean and quince seeds. Mucilages are esters of sulphuric acid wherein an ester group is a polysaccharide compound (4, 5).

Binders are pharmaceutical excipients used in tablet manufacturing to impact cohesion and aggregation on the powder mix for the improved flow properties of the granules and to impart the structural strength to tablet. For a successful

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tablet formulation binder concentration must reach to form a tablet and finally disintegrate within a specified time period. Generally, increasing the binder concentration causes a corresponding increase in the disintegration time of the tablet. Binders are mostly employed in the tablets prepared by wet granulation method (6, 7).

Grewia asiatica is a shrub or small tree which can grow up to 12 feet high. Its bark is grayish-white or grayish-brown. Leaves with serrated margins vary from broadly heart-shaped to obliquely ovate. Bark infusion is used as demulcent, febrifuge and in the treatment for diarrhea. The root bark is used in treating rheumatism. The leaves are used for skin eruptions (8,9). However, a detailed study is needed for the identification and quantification of active constituents responsible for its medicinal activities. Betulin, β-amyrin, erythrodiol, friedelin, lupenone, lupeol, taraxerol, and β-sitosterol are some common compounds isolated from *Grewia asiatica* steam. Paracetamol is an analgesic and antipyretic used in the treatment of arthritic and rheumatic conditions involving musculoskeletal pain and in other painful disorders such as headache, dysmenorrhoea, myalgia and neuralgia and also used in diseases fever, common cold and other viral infections.

The aim of the present study was to investigate the purity, safety and tablet binding property of *Grewia asiatica* mucilage in the conventional tablet formulations. The mucilage was extracted by cold maceration technique and examined for toxicity and identification tests. Various physicochemical and pharmaceutical parameters were examined to determine the suitability as pharmaceutical excipient. The granules were prepared using wet granulation method and evaluated for various micromeritic properties. The tablets were evaluated for various official tests.

MATERIAL AND METHODS

Paracetamol was obtained as a gift sample from Embiotic Laboratories Pvt. Ltd., Bangalore, India. Starch and magnesium stearate were purchased from Yarrow Chem Product, Mumbai, India. Lactose was purchased from Merck Specification Pvt. Ltd., Mumbai, India. Talc was purchased from Central Drug House Ltd., New Delhi, India. Acetone was purchased from SD Fine Chem. Ltd., Mumbai, India.

Isolation of mucilage

The branches of *Grewia asiatica* plant were collected and washed. The bark was peeled off and dried under shade. The extraction of *Grewia asiatica* mucilage was carried out by cold maceration technique. The dried bark was cut into smaller pieces and soaked in fresh distilled water for 12 h and crushed in grinder. The grinded mass was passed through the eight folds of the muslin cloth. The mucilage was precipitated by adding acetone and separated by glass rod. The mucilage was dried at 30 to 35°C, crushed into powder, and passed through sieve # 80 and stored in well closed container for further use (9). The percentage yield of isolated mucilage was calculated.

Toxicity studies

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using Dulbecco's Minimum Essential Medium containing 10% Fetal Bovine Serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off; the monolayer was washed with medium. Test concentrations (100 µl) of mucilage were added on to the partial monolayer in microtitre plates. The plates were incubated at 37°C for 3 days in 5% CO_2 atmosphere. The microscopic examination was carried out and observations were noted every 24 h intervals. After 72 h, the sample solution from the wells was discarded and 50 µl of MTT in PBS was added to each well. The plates were shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 540 nm. The percentage growth inhibition was calculated using the following formula:

$$\text{Growth inhibition (\%)} = (100 - \frac{\text{Mean observed data of test group}}{\text{Mean observed data of control group}} \times 100)$$

The concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) was generated from the dose-response curves for each cell line (10,11).

Identification test for mucilage

Powdered polymer was treated with ruthenium red solution and observed for the appearance of pink color (10, 11).

Test for purity

Aqueous solution of the extracted mucilage was used to carry out the test for carbohydrates, monosaccharides, starch, proteins, amino acids, steroids, glycosides, saponins, alkaloids, tannins and flavonoids (Table 1) for the determination of purity of isolated mucilage (12).

Table 1. Test for purity of extracted mucilage.

Phytoconstituents	Test procedure
Carbohydrates	*Molisch's test:* To 2-3 ml aqueous dispersion of mucilage, few drops of alpha-naphthol solution in alcohol was added and shaken well. Concentrated sulphuric acid was added from sides of the test tube and formation of violet ring was observed at the junction of two liquids.
	Barfoed's test: Equal volume of Barfoed's reagent and test dispersion were mixed and heated for 1-2 min in boiling water bath. Formation of red color precipitate was observed.
Mucilage	*Ruthenium red test:* Mucilage powder was treated with ruthenium red solution and observed for the appearance of pink color.
Proteins	*Biuret test:* To 3 ml test dispersion, 4% sodium hydroxide and few drops of 1% copper sulphate solution were added, and reaction mixture was observed for violet or pink color.
	Million's test: To 3 ml test dispersion, 5 ml Million's reagent was added and observed for appearance of white precipitate. On warming precipitate should turns brick red or the precipitate dissolves giving red colored solution.
Starch	*Iodine test:* To 3 ml of test dispersion, few drops of dilute iodine solution was added and observed for the appearance of blue color. Blue color disappeared on boiling and reappeared on cooling.
Reducing sugar	*Fehling's test:* Equal volume of Fehling's A and Fehling's B solution were mixed together, and boiled for one minute. To the above solution equal volume of test dispersion was added and heated in boiling water bath for 5-10 min. The reaction mixture was observed for formation of first yellow and then brick red color precipitate.
	Benedict's test: Equal volume of Benedict's reagent and test dispersion were taken in test tube and heated in boiling water bath for 5 min. Reaction mixture was observed for the appearance of green, yellow or red color depending on amount of reducing sugar present in sample.
Alkaloids	Aqueous dispersion of mucilage was evaporated and residue was collected. To the residue dilute hydrochloric acid was added and filtered. Filtrate was collected and following tests were performed:
	Murexide test for purine alkaloids: To 3-4 ml test dispersion, 3-4 drops of concentrated sulphuric acid was added; and evaporated to dryness. Residue was cooled, two drops of ammonium hydroxide was added and observed for the appearance of purple color.
	Wagner's test: To2-3 ml filtrate, few drops of Wagner's reagent was added and observed for the appearance of reddish brown color precipitate.
	Hager's test: To2-3 ml filtrate, few drops of Hager's reagent was added and observed for the appearance of yellow color precipitate.
	Mayer's test: To2-3 ml filtrate, few drops of Mayer's reagent was added and observed for the appearance of precipitate.
	Dragendroff's test: To 2-3 ml of filtrate, few drops of Dragendroff's reagents was added and observed for the appearance of orange- brown precipitate
Glycosides	***Cardiac glycoside***
	Baljet's test: A dispersion of mucilage was observed for appearance of yellow to orange color with sodium picrate.
	Anthraquinone glycosides
	Borntrager's test: To 3 ml dispersion of mucilage, equal volume of dilute hydrochloric acid was added, boiled and filtered. To cold filtrate, equal volume of chloroform was added and shaken well. Then organic layer was separated and ammonia was added to it. Appearance of pink or red color in ammonia layer confirms the presence of glycosides.
	Saponin
	Foam test: Mucilage powder was shaken vigorously with distilled water in a test tube and observed for the appearance of foam.
Steroids	*Salkowski reaction*: To 2 ml of mucilage dispersion,chloroform (2 ml) and concentrated sulphuric acid (2 ml) were added and shaken well. Reaction mixture was observed for the separation of chloroform layer and greenish yellow fluorescence in acid layer.
Tannins and Phenolic compounds	*$FeCl_3$ (5%) solution*: To 2-3 ml of alcoholic dispersion of mucilage, few drops 5% ferric chloride solution was added, and reaction mixture was observed for the appearance of deep blue-black color.

Organoleptic evaluation of isolated mucilage

The isolated mucilage was characterized for organoleptic properties such as color, odor, taste and texture (13).

PHYSICOCHEMICAL CHARACTERIZATION OF MUCILAGE

Determination of swelling index

Accurately weighed (1 g) powdered mucilage was taken in a 25 ml measuring cylinder. Fresh distilled water (25 ml) was added and the mixture was shaken thoroughly every 10 min for 1 h and allowed to stand for 3 h at room temperature. Percentage swelling index was calculated using following formula (13):

$$\text{Swelling index} = \frac{Xt - Xo}{Xo} \times 100$$

where, X_0 and X_t are initial and final height of the powered mucilage, respectively.

Determination of water uptake capacity

Water uptake capacity was determined by placing a mucilage disc on the agar gel. Briefly, a known weight of powdered mucilage was compressed into the disc using IR press at 10 tons pressure. The initial weight of disc (W_1) was recorded and disc was placed on 2% w/v agar gel. At regular interval of 1 h, the disc was removed, excess water was removed using filter paper and the disc was weighed until a constant weight (W_2) was obtained. The percentage water uptake was calculated using following formula (10):

$$\text{Water uptake (\%)} = \frac{W1 - W2}{W1} \times 100$$

Determination of mucilage pH

The mucilage was weighed and dissolved in water separately to get a 1% w/v solution. The pH of solution was determined using a digital pH meter (LI120, Elico, India) (12).

Determination of moisture absorption

Mucilage powder (1 g) was uniformly spread on the petri-dish and placed in a desiccator containing saturated solution of aluminium chloride. After 3 days, the mucilage powder was taken out and weighed. The percentage of moisture absorption was calculated as (10):

$$\text{Moisture absorption (\%)} = \frac{\text{Inital weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Determination of loss on drying (LOD)

Accurately weighed 1 g of *Grewia asiatica* mucilage sample was heated at 105°C to get a constant weight in a hot air oven and percent loss of moisture on drying was calculated using following formula (10):

$$\text{LOD (\%)} = \frac{\text{Initial weight of the sample}}{\text{Weight of dry sample}} \times 100$$

Determination of solubility

One part of dry mucilage powder was shaken with different solvents such as methanol, ethanol, hot water and cold water for the determination of solubility behavior of the mucilage (10).

Determination of ash values

Ash value of the isolated mucilage was determined using following methods (14):

Total ash

The mucilage was accurately weighed and taken in a silica crucible, which was previously ignited and weighed. The powder was spread as a fine and even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to air dried sample.

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 min. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a silica crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried sample.

Water-soluble ash

The ash obtained as described for the determination of total ash was boiled for 5 min with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was then transferred into silica crucible, ignited for 15 min, and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air dried sample.

PHARMACEUTICAL CHARACTERIZATION OF MUCILAGE

Surface characterization

The morphology of *Grewia asiatica* mucilage was characterized using scanning electron microscope (SEM). The powdered *Grewia asiatica* was mounted on an aluminum stub, sputter-coated with a thin layer of silver by using a sputter coater (Polaron, UK) in an argon atmosphere, and examined using SEM (LEO1455 VP, UK).

Determination of particle size distribution

The particle size of mucilage was determined using a confocal optical microscope (7001-IMS Vaiseshika, Ambala, India). Small amount of mucilage was taken on slide and spread into thin layer. The size of 600 particles was measured using a calibrated eyepiece micrometer.

Determination of bulk density

For the determination of bulk density of *Grewia asiatica* mucilage, accurately weighed powdered sample was placed into a graduated cylinder and the volume was recorded (15).

$$\text{Loose Bulk density} = \frac{\text{Weight of powder}}{\text{Volume occupied by powder}}\ \text{g/cm}^3$$

Determination of tapped density

The mucilage sample was taken into a 10ml graduated measuring cylinder and the volume was noted down. The measuring cylinder was tapped 50 times using USP bulk density apparatus (ETD1020, Electrolab, Mumbai, India). The bulk density and tapped density were determined using the following formula (15):

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Final volume after tapping}}\ \text{g/cm}^3$$

Determination of Carr's index

It is indirectly related to the relative flow rate, cohesiveness and particle size. It is simple, fast and popular method of predicting powder flow characteristics. It was calculated by following formula (15):

$$\text{Carr's index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Determination of Hausner's ratio

Hausner's ratio is an index of ease of powder flow; it was determined by using the following formula (15):

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Loose bulk density}}$$

Determination of true density

True density of *Grewia asiatica* mucilage was determined by the liquid displacement method. The weight of the clean, dry 15 ml density bottle was determined (W1). The bottle was filled with distilled water and the top of the bottle was dried with filter paper and weighed as (W2). The procedure was repeated using carbon tetrachloride to obtain the weight of the bottle plus carbon tetrachloride (W3). Carbon tetrachloride was used as the displacement liquid. *Grewia asiatica* mucilage powder (2 g) was transferred to same clean and dried density bottle and weighed as (W4). The bottle was filled with carbon tetrachloride and the weight was measured (W5). The density of the carbon tetrachloride was calculated using the following formula (13):

$$\text{Density of carbon tetrachloride } (\rho) = \frac{(W3 - W1)}{(W2 - W1)} \times 0.9971$$

(Density of distilled water at 25°C = 0.9971 g/cc)

The true density of *Grewia asiatica* mucilage powder was calculated using following formula:

$$\text{Density of sample} = \frac{(W4 - W1)}{[\{(W3 - W1)\}/\rho\} - \{W5 - W4\}/\rho]}$$

Determination of percentage porosity

Percentage porosity was calculated by using following formula (10):

$$\text{Porosity (\%)} = 1 - \frac{\text{Bulk density}}{\text{True density}} \times 100$$

Determination of angle of repose

Angle of repose was determined by the fixed funnel and free standing cone method. A funnel with a discharge spout opening of 0.74 cm and a capacity to hold approximately 150 g of powder, with the end of the stem cut perpendicular to its axis of symmetry, was fixed at a given height (h) above the graph paper placed on a flat horizontal surface. The mucilage powder was carefully poured through the funnel until the

apex of the conical pile just touched the tip of the funnel. The radius (r) of the base of the pile was determined and the tangent angle of repose (θ) was calculated by following formulae (15):

$$\tan \theta = \frac{h}{r}$$

Determination of microbial load

One gram of the mucilage powder was suspended in peptone water to produce 150 ml in a sterile conical flask. Later the conical flask was incubated at 37°C for 60 min and allowed the material to settle down to get the clear solution. Sterile nutrient agar and Sabouraud dextrose agar media plates were prepared as per standard procedure. Clear supernatant (0.1 ml) from the incubated tube was pipetted on the sterile nutrient agar and sabouraud dextrose agar media plates. Sample on the surface of the medium was spread using sterile autoclaved spreader uniformly maintaining aseptic conditions, in laminar air flow cabinet. Petri plates were kept in invert position and incubated for 24 h to 48 h at 37°C ± 1°C for bacteria and at 28°C ± 1°C for fungi, respectively. Plates were examined for microbial growth; the number of colonies were counted and expressed in terms of colony forming units per gram of the substance (cfu/g) (12).

Compatibility studies

Fourier transforms infrared spectroscopy (FTIR)

The possible molecular interaction between the drug and the mucilage was determined by FTIR peak matching method (IR-affinity-1, Shimadzu, Japan). The samples were gently triturated with KBr and compacted into the disc using IR press at 10 tons. The sample disk was placed into sample holder and scanned from 4000-400 cm^{-1} at a resolution of 4 cm^{-1} (14).

Thermal analysis by differential scanning calorimetry (DSC)

The DSC thermograms of pure drug, mucilage and physical mixture were recorded using differential scanning colorimeter (DSC822, Mettler Toledy, Switzerland). The instrument was calibrated using Indium (156°C), Tin (232°C) and Zinc (419°C) as internal standards. Each sample was placed into a 40 μL aluminium pan and sealed. The probes were heated from 25 to 400°C at a rate of 10 K/min under nitrogen atmosphere (14).

PREPARATION OF GRANULES

The binder solution was prepared using mucilage at different concentrations (0.25%, 0.5%, 0.75%, and 1.0% w/v) in freshly prepared and cooled distilled water. Paracetamol, starch and lactose were passed through sieve # 22. The mixture was taken in mortar and the granules were prepared by wet granulation technique using 0.25-1.0% w/v binder solutions (Table 2). The cohesive wet mass was passed through sieve # 10 and the granules were kept in a hot air oven for drying at 35°C. Talc and magnesium stearate were added to the dried granules. Finally, the dried granules were stored in airtight container for further use.

Table 2. Composition of formulations.

Ingredients	Formulation code			
	F 1	F2	F 3	F 4
Paracetamol (mg)	500.00	500.00	500.00	500.00
Mucilage as binder (%)	1.63	3.25	4.87	6.50
Starch (mg)	65.00	65.00	65.00	65.00
Lactose (mg)	70.38	68.75	67.13	65.50
Talc (mg)	6.50	6.50	6.5.00	6.50
Magnesium stearate (mg)	6.50	6.50	6.5.00	6.50

EVALUATION OF GRANULES

All the four batches of granules were evaluated for moisture content, flow properties and percentage of fines by using above mentioned procedures.

Percentage of fines

The percentage of fines was determined by passing granules through meshes 22/44. Granules retained on 44-mesh sieve were weighed as coarse granules and the granules passed through 44-mesh sieve were weighed as fines.

COMPRESSION OF GRANULES

The granules were compressed into tablets using 11 mm

diameter die cavity and flat faced punches using 10 station rotary tablet compression machine (M26 A12, Karnavati Engineering Limited, Ahmedabad. The weight of tablets was adjusted to 650 mg.

Evaluation of tablets

Appearance

Tablets were examined for color, surface characteristics, picking, sticking and lamination.

Weight variation

Twenty tablets were weighed individually. Average weight was calculated from the total weight of all the tablets. The individual weights were compared with the average weight. The percentage deviation was calculated by using following formula (17):

$$\text{Percent deviation} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight}} \times 100$$

Friability

Twenty tablets were weighed collectively and placed in the chamber of the USP tablet friability apparatus (EF-2, Electro lab, Mumbai, India). After 100 rotations, the tablets were taken out and intact tablets were weighed collectively. Percentage friability was determined using the following formula (17):

$$\text{Friability} = \frac{W1 - W2}{W1} \times 100$$

where, W1and W2are the tablet weight before and after test, respectively

Hardness

The hardness of tablets was tested using Monsanto tablet hardness tester (Rolex, Ambala Cantt). The tablet was placed across the diameter between the spindle and the anvil. The knob was adjusted to hold the tablet in position. Reading of the pointer was adjusted to zero. The pressure was increased slowly to break the tablet. The pressure at which tablet break down was recorded as tablet hardness (17).

Thickness

The thickness of 20 tablets was measured using vernier caliper (Mitutoyo, Japan) (17).

Disintegration time

Tablets were placed in each of six tubes of the disintegration apparatus (ED-2SAPO, Electrolab, Mumbai, India). Distilled water at 37°C was used as disintegration medium. The discs were placed over each tablet to prevent the tablet coming outside of the tubes. The time required for the complete disintegration of all the six tablets was recorded (17).

Drug content

Six tablets were powdered, and powder equivalent to the weight of one tablet was transferred to a 100 ml volumetric flask. The powder was dissolved in 100 ml of phosphate buffer solution (pH 5.8) and shaken for 24 h. After 24 h, the dispersion was filtered, diluted and analyzed at 249 nm using UV spectrophotometer (Labindia UV3000$^+$, Mumbai, India).

Dissolution

In vitro dissolution study was carried out using USP- Type II dissolution apparatus, operated at 50 rpm (TDT-06T, Electro lab, Mumbai, India). The dissolution study was carried out in phosphate buffer solution (pH 5.8) maintained at 37±0.5°C. At specified time intervals, 5 ml of sample was withdrawn and immediately replaced with equal quantity of fresh buffer solution maintained at the same temperature to maintain the sink conditions. The samples were filtered and diluted suitably using the same buffer solution and analyzed at 243 nm using a UV spectrophotometer (Labindia UV3000$^+$, Mumbai, India).

RESULTS AND DISCUSSION

Yield (%)

The percentage yield of the mucilage obtained from the *Grewia asiatica* bark was found to be 15.89% w/w. The percentage yield of mucilage was found to be satisfactory.

Cytotoxicity

Cytotoxicity studies were carried out for *Grewia asiatica* mucilage against A-431 and Vero cell lines. The CTC_{50} (µg/ml) for *Grewia asiatica* was found to be more than 100 µg/ml. The results of cytotoxicity study are shown in Table 3. On the basis of CTC_{50} values, the extracted mucilage was considered as safe and non-toxic.

Table 3. Cytotoxic properties of *Grewia asiatica* mucilage against A-431 and Vero cell lines.

Grewia asiatica (µg/ml)	Percentage Cytotoxicity in A-431 cell line	Percentage Cytotoxicity in Vero cell line
1000	82.18±0.2	82.22±0.2
500	75.34±0.4	71.65±0.5
250	29.14±0.3	51.00±0.4
125	16.08±0.4	28.29±0.4
62.5	15.01±0.4	15.73±0.6
CTC_{50} (µg/ml)	550±0.00	480±0.00

Cytotoxicity studies were carried out on Vero and A-431 cell lines. The percentage growth inhibition was calculated and the concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) value was generated from the dose response curve for each cell line. On the basis of cytotoxicity study results, the extracted mucilage was found to be safe (non toxic) for using as a pharmaceutical excipient.

Identification of mucilage

The isolated and purified mucilage stained pink color with ruthenium red which confirmed it as mucilage.

Purity

The purity of mucilage was determined using reported phytochemical tests, which indicated the absence of alkaloids, steroids, flavonoids, saponins, tannins and phenols. Only carbohydrates were present, which confirms the purity of the extracted mucilage. The results of various phytochemical tests are shown as shown in Table 4.

Table 4. Results of test for purity of isolated mucilage.

Tests for Phytoconstituents	Results
Carbohydrate	+
Monosaccharides	_
Starch	_
Proteins	_
Amino acids	_
Steroids	_
Glycosides	_
Saponins	_
Alkaloids	_
Tannins	_
Flavonoids	_

+ Present; - Absent

Organoleptic properties

The mucilage was characterized for various organoleptic properties such as color, odor, taste, and texture as shown in Table 5.

Table 5. Organoleptic properties of *Grewia asiatica* mucilage.

Parameter	Results
Color	Creamish
Odor	Odorless
Taste	Tasteless
Texture	Rough

Physico-chemical properties of mucilage

Swelling index

Swelling index of the *Grewia asiatica* mucilage was found to be 148.6±%1.52. It showed high swelling index, which is an indication of good water absorption by the mucilage. Hence, the mucilage can form a hydrated three dimensional network, from which, the drug release might follow diffusion (18).

Water uptake studies

The swelling ability of any mucilage depends upon its water absorption capacity or water uptake capacity. The water uptake can give clue about the mucoadhesive nature of the excipient. The higher water uptake by a mucoadhesive polymer can help in establishing a quicker and stronger interpenetration of polymer chains into the mucus and improve its contact time. The water uptake is usually followed by swelling and formation of three-dimensional network structure, which may sustain the drug release (18). In the present study, the water uptake capacity was found to be 396.43±15.21% (Figure1 and Table 7) indicating good mucoadhesive nature of the mucilage.

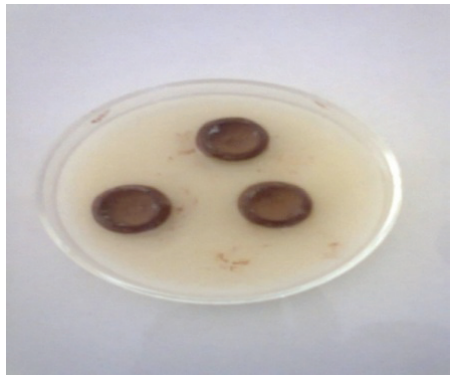

Figure 1. Water uptake study of *Grewia asiatica* mucilage.

pH

Biocompatibility and irritant nature of the excipient can be established on the basis of pH determination. The pH of 1% w/v *Grewia asiatica* mucilage was found to be 6.88 ± 0.123. It can be considered to be non irritant to the gastrointestinal tract and hence can be used as an excipient for the oral route of administration.

Moisture absorption

Moisture absorption study gives information about the hygroscopic nature of the mucilage. If the excipient is hygroscopic in nature, it can alter dosage form properties. Hence, it is necessary to determine the hygroscopic nature of the excipient and the amount of moisture that can be absorbed by the excipient. Hygroscopicity also influences the packaging and storage conditions (19). Moisture content of *Grewia asiatica* mucilage was found to be 0.75 ± 0.05%. The results suggest that *Grewia asiatica* mucilage can be used in pharmaceutical formulations.

Loss on drying

Loss on drying also gives information about the hygroscopic nature of the excipient. Loss on drying of *Grewia asiatica* was found to be 0.2 ± 0.01%. Based on the results it can be recommended that the formulations containing *Grewia asiatica* mucilage do not require special packaging of the finished product.

Solubility

The solubility behavior of the mucilage indicates that it is insoluble in acetone, ethanol and methanol, but swellable in cold water and warm water. The results of solubility study are presented in Table 6.

Table 6. Results of solubility study of isolated mucilage.

Solvent	Solubility behavior
Cold water	Insoluble but swellable
Warm water	Insoluble but swellable
Ethanol	Insoluble
Methanol	Insoluble
Acetone	Insoluble

Ash values

Different ash values such as total ash, acid insoluble, water-soluble and sulfated ashes should be determined for the excipient which can be used as standard value for determination of the quality of the excipient and batch-to-batch variations in quality. The sulphated ash tells about the degree of contamination of the material (18). The ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash of *Grewia asiatica* mucilage were found to be 9.9%, 0.4%, 8.11% and 1.47%, respectively.

Surface characterization

Scanning electron microscopy (SEM) gives an idea about the surface roughness (rugocity), particle size distribution, flow behavior and particle packing arrangements. It has been also reported that the drug release from the dosage form depends on surface characteristics of the excipient. If the surface is rough, drug release will be retarded because of the entrapment of drug particles in the pores and crevices. The SEM image of the mucilage revealed that the shape of the polymer was irregular and rough (Figure 2).

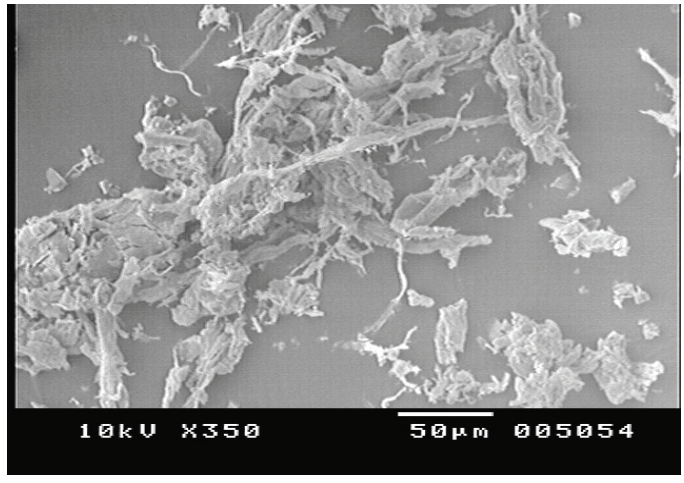

Figure 2. SEM photomicrograph of *Grewia asiatica* mucilage showing morphological characteristics.

Micromeritics

Micromeretic characterization is the characterization of powder properties such as bulk density, tapped density, true density, Carr's index, Hausner ratio, porosity and angle of repose. All these properties give hint about the packing behavior of the excipient powder - whether the excipient undergoes 'close' or 'loose' packing. Carr's index tells about the cohesiveness of the particles. A 15% Carr's

index value result in good to excellent flow properties and indicate desirable packing characteristics. Carr's index above 25% is often a source of poor tableting qualities. The values between these two indices may result in less than the optimum performance and modification of the particle size distribution could be advisable. The particle size distribution was found in the range of 30-90 µm (Figure 3). The results of bulk density, tapped density, true density, Carr's index, Hausner's ratio and porosity of *Grewia asiatica* mucilage are shown in Table 7. The results indicate that the *Grewia asiatica* mucilage has good flow properties and compressibility. Porosity influences the rate of disintegration and dissolution. Angle of repose gives clue about the mixing problems of the excipient in dosage forms due to poor flow. Angle of repose less than 30° indicates the free flowing nature of the material and values greater than 40° suggest poor flow nature of the material. In the present study, static angle of repose value for *Grewia asiatica* mucilage was found to be 40° ± 1.28 indicating passable flow properties of the mucilage.

Table 7. Results of physicochemical characteristics of the isolated *Grewia asiatica* mucilage. (Mean ± SD, n=3).

Properties	Results
Swelling index (%)	148.6 ± 1.52
Water uptake study (%)	396.4 ± 15.21
pH	6.88 ± 0.123
Moisture absorption study	0.75 ± 0.05
Loss on drying (%)	0.2 ± 0.01
Bulk density (g/cc)	0.124 ± 0 .001
Tapped density (g/cc)	0.141 ± 0.003
Compressibility (%)	12.3 ± 1.93
Hausner's ratio	1.12 ± 0.05
True density (g/cc)	1.23 ± 0.01
Porosity (%)	62.23 ± 1.65
Angle of repose (°)	40 ±1.28

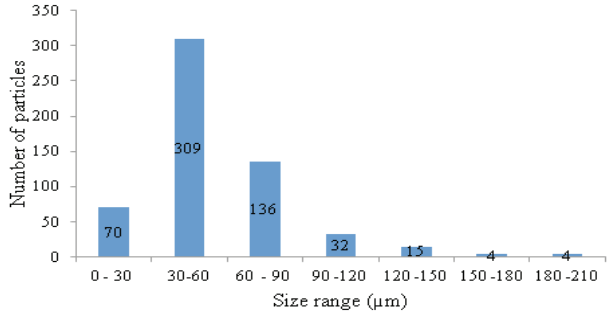

Figure 3. Results of particle size distribution of *Grewia asiatica* mucilage.

Microbial characterization

For natural excipients, the aerobic count should be below 1000 Cfu/g and the total fungal count should not exceed 100 cfu/g (18). The results of microbial studies did not show the presence of microbial contamination (Figure 4). Hence, tests for specific pathogens were not carried out.

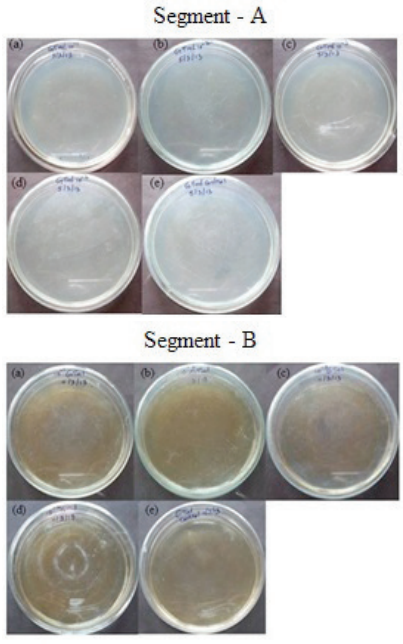

Figure 4. Microbial load study for *Grewia asiatica* mucilage in sabouraud dextrose agar media (segment A) and sterile nutrient agar media (segment B) at 10^{-1} concentration (a), at 10^{-2} concentration (b), at 10^{-3} concentration (c), at 10^{-4} concentration (d), Control (e).

Compatibility studies

Fourier transform infrared spectroscopy (FTIR)

This study was performed to check compatibility related

problems associated with drug and excipients used for the formulation of tablets. The drug and excipients must be compatible with each other to produce a product that is stable, efficacious, attractive and easy to administer and safe. If the excipients are new and not been used in formulations containing the active substance, the compatibility studies are of paramount importance. FTIR spectrum of drug, mucilage, and drug-polymer mixture are shown in Figure 5 to 7. Frequencies of functional groups of pure drug remained intact in a physical mixture containing *Grewia asiatica*, so it was concluded that there was no major interaction between the drug and mucilage.

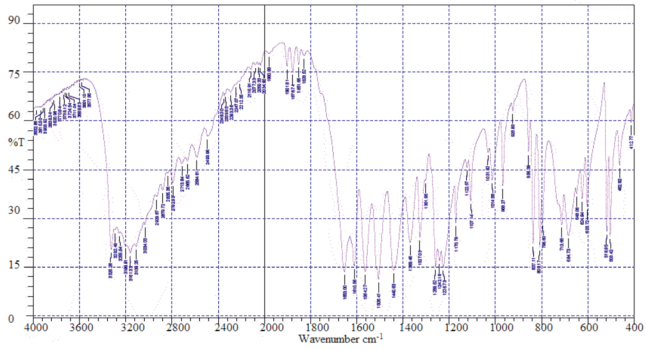

Figure 5. FTIR spectrum of pure paracetamol.

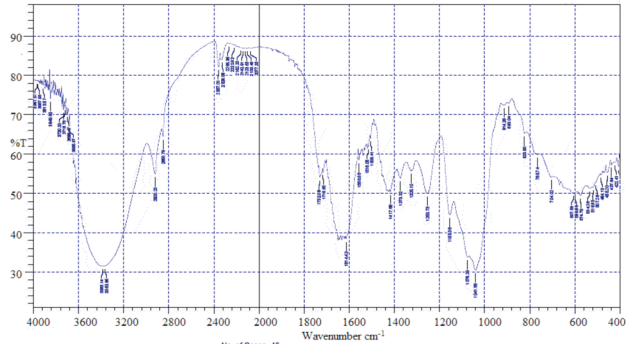

Figure 6. FTIR spectrum of isolated *Grewia asiatica* mucilage.

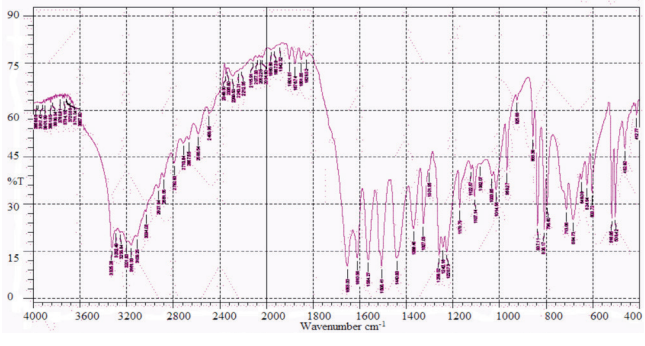

Figure 7. FTIR spectrum of physical mixture of paracetamol and isolated mucilage at 1:1 ratio.

Differential scanning calorimetry

DSC thermograms of paracetamol, mucilage and physical mixture are depicted in Figure 8. The thermogram of the pure drug exhibited exotherm peak at 170.8°C corresponding to its melting point. The thermogram of the mucilage exhibited exotherm peak at 158.5°C. The DSC thermogram of physical mixture showed the exotherm peak at 170.8°C. Since, there was no peak shifting or suppression, it was concluded that there was no interaction between the mucilage and selected drug. DSC results are also supported by the FTIR study results.

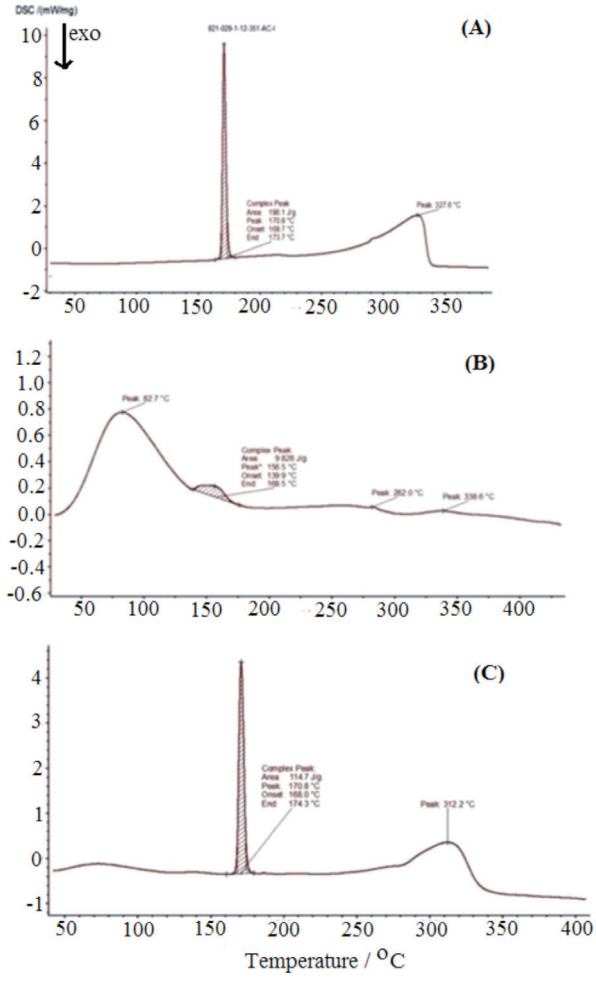

Figure 8. DSC thermogram of paracetamol (A), isolated *Grewia asiatica* mucilage (B), physical mixture of paracetamol and isolated mucilage at 1:1 ratio (C).

Evaluation of granules

All the four batches of granules were evaluated for moisture content, loss on drying, flow properties (bulk density, tapped density, compressibility) and percentage of fines and results are shown in Table 8.

Table 8. Micromeretic properties of granules prepared using *Grewia asiatica* mucilage (Mean ± SD, n=3).

Parameters	F1	F 2	F 3	F 4
Bulk density (g/cc)	0.34 ± 0.04	0.29 ± 0.05	0.28 ± 0.01	0.27 ± 0.20
Tapped density (g/cc)	0.43 ± 0.05	0.35 ± 0.005	0.36 ± 0.17	0.33 ± 0.02
Compressibility (%)	19.37 ± 0.88	21.26 ± 3.23	19.54 ± 2.0	18.86 ± 1.96
Hausner's ratio	1.23 ± 0.015	1.24 ± 0.005	1.23 ± 0.032	1.15 ± 0.08
Angle of repose (°)	26.0 ± 1.28	26.81 ± 1.20	28.21 ± 1.45	30.0 ± 1.22
Fines (%)	1.17 ± 0.52	1.05 ± 0.49	1.03 ± 0.71	1.0 ± 0.28

The bulk density granule was found to decrease slightly with an increase in concentration of binder. This might be due to the formation of larger agglomerates and the decrease in fines in the granules as increasing mucilage concentration provides better binding to the granules.

The flow properties of paracetamol granules were expressed in terms of Hausner's ratio and angle of repose. The Hausner's ratio and angle of repose decreased with increased concentration of mucilage indicated the improvement in flow. Carr's index was found to be in the range of 18.86-21.26%. Based on the results granules were considered to have good flow properties. From Table 7, it can be easily concluded that the percentage of fines decreased with increase in binder concentration. This may be due to better cohesion of solid mass at increased mucilage concentration. Low percentages of fines indicated the effectiveness of binder.

Evaluation of tablets

The color of tablet was creamish or off white. The tablets were free from cracks and depressions (Figure 9). Weight variation is the major test to be checked frequently. Any variation in the weight of the tablet leads to either under medication or overdose. The average weight of all the formulations is given in Table 9.

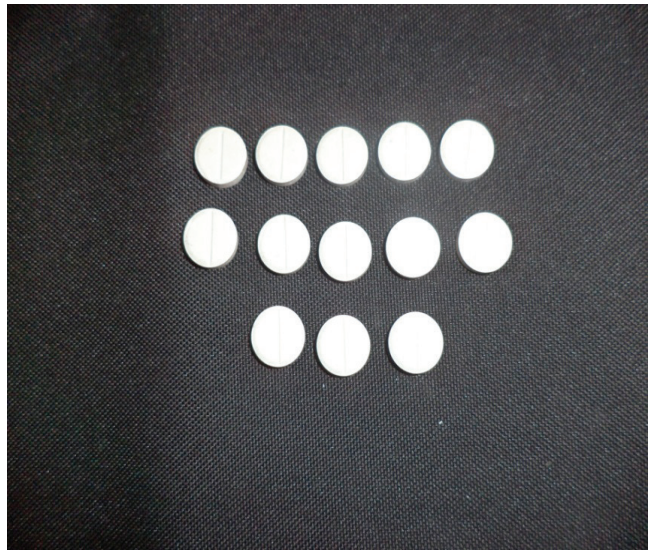

Figure 9. Tablets of paracetamol using *Grewia asiatica* mucilage as a binder.

The effect of binder concentration on tablet friability is shown in Table 9. Friability decreased as the binder concentration increased. An increase in binder concentration will enhance the formation of stronger inter-particulate bonds between the granules during compression in a tabulating machine. This means that the tablets would offer greater resistance to shock and abrasion since there is a stronger adhesive bonding of the granules at high binder concentrations. In general, the F3 and F4 showed good friability profiles, both batches had friability values of less than 1.0%.

Table 9. Evaluation of Paracetamol tablets containing *Grewia asiatica mucilage* as a binder.

Formulation code	Weight variation (mg)n=20	Hardness (Kg/cm^2) n=20	Friability (%) n=20	Thickness (mm) n=20	Disintegration time(min) n=6
F1	648.00 ± 1.5	3.8 ± 0.41	1.02 ± 0.04	4.00 ± 0.22	5 ± 0.17
F2	649.66 ± 2.57	3.9 ± 0.44	1.0 ± 0.02	3.98 ± 0.09	8 ± 0.26
F3	649.66 ± 2.57	5.56 ± 0.20	0.75 ± 0.10	3.98 ± 0.06	10 ± 0.48
F4	649.67 ± 0.97	5.68 ± 0.12	0.63 ± 0.09	3.99 ± 0.14	11 ± 0.52

The hardness of tablets is an indication of its strength. The tablet should be stable to mechanical stress during handling and transportation. The effect of binder concentration on tablet hardness is shown in Table 9. An increase in binder concentration increased the hardness of the tablets. The hardness of batches F1, F2, F3 and F4 was 3.8 ± 0.41, 3.9 ± 0.44, 5.56 ± 0.20 and 5.68 ± 0.12, respectively. From the results, the hardness of F1, F2 was found to be less where as F2, F3 had good hardness values. The tablets should have uniform thickness and these values are checked and are used to adjust the initial stages of compression. The thickness of the F1, F2, F3, and F4 are given in Table 9. The thickness of all the four formulations was found to be uniform. The results showed that the disintegration time of tablets was increased from 5 ± 0.17 to 11 ± 0.52 min as the binder concentration increased from 0.25 to 1.0%, respectively (Table 9). The drug content was found to be in the range of 93.0% - 98.08%.

The results of *in vitro* dissolution study are shown in Figure 10. It was found that the drug release was decreased with an increase in mucilage concentration. This study showed that the drug release from the tablets was more than 80% within 30 min. All the formulated tablets met the official specifications for conventional tablets, which states that at least 80% of the drug should be released in 30 min.

On the basis of dissolution data, all the four batches of *Grewia asiatica* had good dissolution profiles, but the hardness and friability of batch F1 and F2 was found to be poor. Batch F3 and F4 had good hardness and friability properties. So, formulation F3 and F4 were considered as optimized formulations. Also, the friability for F1, F2 was more than 1%. The dissolution data of optimized batches were analyzed using Kitazawa plots. The Kitazawa equation has wide application in the analysis of dissolution profiles of various drug substances.

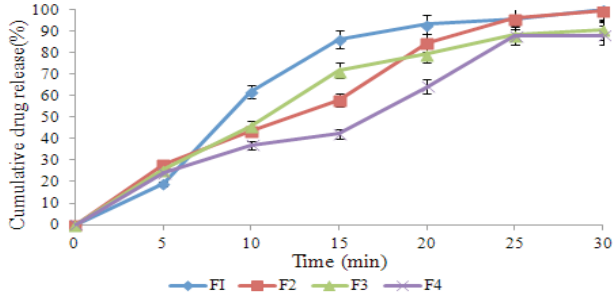

Figure 10. *In vitro* dissolution profile of paracetamol from tablet containing *Grewia asiatica* as a binder in phosphate buffer solution (pH 5.8) maintained at 37±0.5°C (mean ± SD, n=3).

The Kitazawa equation uses an ultimate amount of drug released (W_{inf}) in the analysis of dissolution profile. The simplified form of Kitazawa equation is:

$$\ln [W_{inf} / (W_{inf}-W)] = kt$$

Where, W_{inf} is the amount of drug released at infinite time (i.e., the total amount that could be released), W is amount released at various time interval t, k is the release rate constant, a first order rate constant that decreases with the amount of drug remaining in the dosage form, and 'ln' is the natural logarithm.

Usually, the plots of $\ln [W_{inf}/ (W_{inf}-W)]$ versus time generate multiple regression lines intersecting at various times. The times correspond to the phases in which the dosage form changes its physical form from solid through granules to fine particles. The slope of each line gives a dissolution rate constant (k) for that particular phase of release. The results of dissolution data of batch F3 and F4 were analyzed using Kitazawa plots, which produced linear graphs with two segments corresponding to the phases of drug dissolution from the tablet surface and granules, and from disaggregated (*i.e.*, fine) particles (Table 10). The time T represents the time when the tablet disintegrated during the dissolution process (Figure 11). There was a difference between the disintegration time estimated in disintegration test (DT) and in the dissolution profile (T_D) (Table 11). This might be because of the milder conditions prevalent in the dissolution apparatus (19, 20).

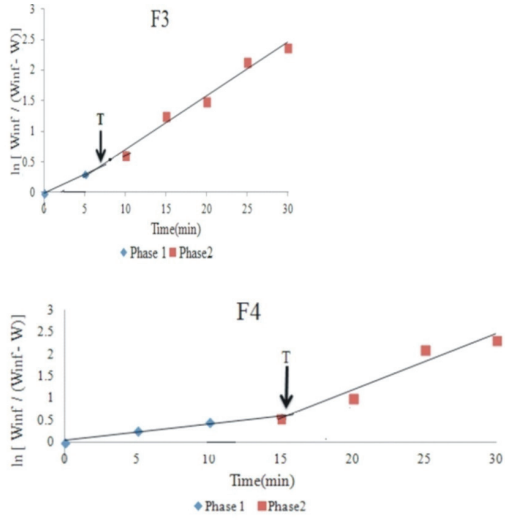

Figure 11. Kitazawa plots for the kinetics of dissolution of paracetamol from tablet containing *Grewia asiatica* as a binder.

Table 10. Parameters of Kitazawa plots for paracetamol dissolution from tablets containing *Grewia asiatica* as a binder.

Formulation code	Kitazawa plot parameters			
	k_1 (min^{-1})	k_2 (min^{-1})	r_1	r_2
F3	0.0613	0.08802	0.9980	0.9866
F4	0.0461	0.12812	0.9951	0.9698

r_1, r_2 represent correlation coefficients of the two segments of the Kitazawa plot

Table 11. $T_{80\%}$ and T_D of formulation F3 and F4.

Formulation code	$T_{80\%}$ (min)	T_D (min)
F3	25	8
F4	25	15

T_{80}% is the time required for the release of 80% of the drug from the tablet during dissolution study; T_D is the time taken by the tablet to disintegrate during dissolution study.

CONCLUSION

Tablets formulated with 0.75% w/w and above concentrations of *Grewia asiatica* mucilage exhibited satisfactory results. This study concludes that the *Grewia asiatica* mucilage can be safely used as a binder in the conventional tablet formulations at 0.75 - 1.0% concentration. The results of test for purity showed the presence of carbohydrates. The results of microbial load showed absence of microbial growth. From the above data, it was concluded that *Grewia asiatica* mucilage can be used as an excipient in pharmaceutical formulations.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

***Grewia asiatica* bitkisinden elde edilen müsilajın tablet formülasyonlarında bağlayıcı özelliklerinin araştırılması**

ÖZ

Bu çalışmada *Grewia asiatica* bitkisinden elde edilen müsilajın % 0,25-0,1 derişimlerdeki bağlayıcı özellikleri değerlendirilmiştir. Müsilaj soğukta maserasyon yöntemiyle ekstre edilmiş ve şişme indisi, nem absorblama kapasitesi, çözünürlük, organoleptik özellikleri ve saflık yönünden karakterize edilmiştir. Müsilajın ayrıca toksisitesi, mikrobiyal üreme ve dokusu incelenmiştir. Çalışmamızda, parasetamol model ilaç olarak kullanılmak suretiyle yaş granülasyon tekniğiyle tabletler hazırlanmıştır. Granüllerin mikromeritik özellikleri, ince partikül yüzdesi ve nem içerikleri değerlendirilmiştir. Tabletlerin görünümleri, ağırlık değişimleri, ufalanabilme özellikleri (friabilite), kalınlıkları, dağılma zamanları, ilaç içerikleri ve in vitro ilaç salım özellikleri değerlendirilmiştir. Formülasyondaki müsilaj yüzdesi arttıkça ufalanabilme yüzdesinin düştüğü gözlenmiştir. Tablet sertliğinin yükselen müsilaj oranıyla arttığı ve kabul edilebilir sınırlarda olduğu görülmüştür. Bütün serilerde dağılma süresi 15 dakikadan kısa bulunmuştur. Bütün formülasyonlarda ilaç içeriği % 93,0- 98,08 aralığında bulunmuştur.

Anahtar kelimeler: *Grewia asiatica*, yardımcı maddeler, müsilaj, bağlayıcı, Kitazawa.

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