Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Isolation of the mucilages from *Hibiscus rosasinensis* linn. and Okra *(Abelmoschus esculentus* linn.*)* and studies of the binding effects of the mucilages

Ameena K*, Dilip C, Saraswathi R, Krishnan PN, Sankar C, Simi SP

Al Shifa College of Pharmacy, Kizhattur, Perinthalmanna, Kerala, 679325, India

ARTICLE INFO

Article history:
Received 15 April 2010
Received in revised form 27 May 2010
Accepted 1 June 2010
Available online 20 July 2010

Keywords:
Formulation
Hibiscus
Tablets

ABSTRACT

Objective: To isolate and evaluate comparatively the binding efficacy of the mucilages obtained from the plants of *Hibiscus rosasinensis* and Okra (*Abelmoschus esculentus*). **Methods:** Extraction of mucilages from the leaves of *Hibiscus* and pods of Okra (Ladies finger) was carried out by a cold maceration process. The extracted mucilages were subjected to various physicochemical properties for its suitability as an excipient in the formulation of tablet dosage form. Different concentrations (10, 8, 5, 2 and 1% w/v) of binder solutions of *Hibiscus* and Okra were used for the formulation of tablets and the formulated tablets were evaluated by studying the standard parameters like diameter, thickness, weight variation, hardness, friability, disintegration and *in vitro* dissolution. Stability studies of the formulated tablets were conducted for four weeks. **Results:** The formulated tablets prepared using the mucilages of both *Hibiscus* and Okra had good appearance. The *in vitro* drug release profile of the tablets prepared using Okra mucilage had an optimum of 90% at a mucilage concentration of 1% w/v concentration mucilage itself within 4 h. **Conclusions:** According to the observations, the lower concentration levels of Okra can be used as an alternative binder to starch. The higher concentration levels of Okra mucilage show a slow and sustained release, and can be considered as an alternative natural excipient in the modified drug delivery systems. At the same time, the above natural excipient of *Hibiscus* mucilage could be used as a platform for prolonged release if its binder concentrations are increased.

1. Introduction

Binders are pharmaceutical excipients that are commonly employed in tablet formulation to impact cohesion on powder mix and hence improve on the flow properties of the granules[1]. Binders act by causing aggregation of powders thereby forming granules through the process of granulation. They modify the cohesive properties of the granules by promoting the formation of strong cohesive bonds between such particles[2]. A majority of the investigations on natural excipients in drug delivery systems have centered on proteins and polysaccharides, due to their ability to produce a wide range of materials and properties according to molecular structural alterations[3] Mucilages are very often used in various industries. Vast application of plant mucilages and gums in various industries is because of low cost, ready availability and important properties which they confer on products[4]. In recent years, plant gums and mucilages have evoked tremendous interest due to their diverse pharmaceutical applications such as diluents, binders, disintegrants in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository[5], thus making them attractive substitutes for costly semi synthetic and synthetic excipients. India, due to its geographical and environmental positioning, has traditionally been a good source for such products among the Asian countries. [6] There are reports about the successful use of Ocimum gratissimum, *Butea monospermama*, *Albizia zygia* gum and *Leucaena leucocephala* seed gum as suspending agents[7-10].

Two plants are prominently used, and have been chosen for this investigation; these are the leaves of *Hibiscus rosasinensis* Linn and fruits of Okra (*Abelmoschus esculentus* Linn) which are belonging to the family *Malvaceae*. *Hibiscus* is widely grown as an ornamental plant throughout the tropics and subtropics. The plant is available in India in large quantities[11]. Its 250 species are widely distributed in tropical and subtropical regions of the world and

*Corresponding author: Miss Ameena k, Department of Pharmaceutics, Al Shifa College of Pharmacy, Kizhattur, Perinthalmanna.
E-mail: dillu7@rediffmail.com
Tel: +919947776902

are reported to possess various medicinal properties viz antitumor, antihypertensive, antioxidant etc[12]. About 40 species are found in India. *Hibiscus rosasinensis* Linn is a native of China and is a potent medicinal plant. It is a common Indian garden perennial shrub and often planted as a hedge or fence plant. Traditionally this drug is attributed to antifertility activity in Ayurvedic literature. Leaves and flowers also possess hypoglycemic activity. The mucilage of the leaf has antiinflammatory activity[13, 14]. The extracts of *Hibiscus rosasinensis* have also shown a protective effect against the tumor promotion stage of cancer development[15]. The anthocynidin from the petals of the plant have protective effect against carbon tetra chloride-induced acute liver damages[16].

Gum of the Okra pods has been reported to have binder potential for tablet formulations[17]. The fresh fruit of Okra is a common component of Indian diet. In addition, the plant has been used medicinally in treatment of several disorders [18, 19]. Anti-cancer, anti-microbial and hypoglycemic activities of plant had been reported[20,21]. The anti-ulcer activity of fresh fruits is recently reported[22]. This is a coarse, erect, branched, more or less hairy, annual herb with 0.6 to 1.5 meters in height, grown widely in India. Okra mucilage binds cholesterol and bile acid carrying toxins dumped into it by filtering liver[23], thus might act as a hepatoprotective agent. Additionally, it apparently has antioxidant activity, which may also be beneficial in this liver pathology. It was reported that aqueous-ethanol extract of Okra has antioxidant property. It has shown free radical scavenging activity (FRSA) in an *in vitro* study which reported major antioxidants in lady's finger are due to quercetin compounds[23].

In the present study an effort was made to extract the mucilage from the leaves of Hibiscus rosa-sinensis Linn and pods of Okra and look at the possibility of using these mucilages as the binding or granulating agents and releasing retardant material in the formulation of solid dosage forms.

2. Materials snd methods

The leaves of *Hibiscus rosasinensis* Linn were collected from the local area and Okra fruits were purchased from a local market. Both the plants were authenticated at the Department of Botany of Calicut University in Calicut, India. Paracetamol was obtained as gift sample from Modern Pharmaceutical (Kerala, India). Lactose IP, Starch, Purified talc and Magnesium stearate were procured from SD Fine Chemicals Ltd. All other reagents used were analytical grade.

2.1. Isolation of the mucilages

The fresh leaves of *Hibiscus rosasinensis* Linn were collected, washed with water to remove dirt and debris and then dried. The powdered leaves were soaked in water for 5-6 h, boiled for 30 min and kept aside for 1 h for complete release of the mucilage in to the water. The material was squeezed from an eight fold muslin cloth bag to remove the marc from the solution. Acetone was added to the filtrate to precipitate the mucilage in a quantity of three times the volume of the total filtrate. The mucilage was separated, dried in an oven at a temperature < 50 ℃, collected, dried, powdered and passed through a sieve no: 80 and stored for further use in the desiccators.

About 1 kg of fresh immature fruits of Okra was purchased from local market. After removal of the seeds, the fresh immature fruits were sliced, homogenized and extracted with cold water containing 1% w/v of sodium metabisulphate. The crude mucilage was centrifuged at 3 000 rpm for 5 min. The gum was precipitated from the supernatant with acetone. The precipitated gum was washed several times with acetone. The obtained cream colored product was dried under vacuum in desiccators. A light brown colored powder was obtained after complete removal of moisture. The dried gum was pulverized using end runner mill and screened with a 0.25 mm stainless steel sieve. This was stored in a well closed amber colored specimen bottle till ready for use.

2.2. Phytochemical examination and physicochemical characterization of mucilages

The mucilage solutions were tested for the presence of carbohydrates by performing the preliminary standard tests, Molisch's test and Ruthenium red test respectively. Dried-powdered mucilages were observed for solubility, pH, weight loss on drying, swelling index, density and viscosity.

2.3. Drug excipient compatibility study

A potassium bromide disc of each of the dried purified mucilages was prepared, and the FTIR spectra recorded (Shimadzu FTIR 8400S, USA). Triplicate measurements were made and the spectrum with the clearest identifiable peaks was chosen.

2.4. Preparation of tablets

Lactose and starch powder were passed through sieve No: 40. Paracetamol IP was homogeneously dry-mixed with lactose and starch powder. The granules prepared by wet granulation method used *Hibiscus* and Okra binders in concentrations of 10%, 8%, 5%, 2% and 1% (w/v). The moistened coherent mass was passed through sieve No: 16 and granules were dried at 50 ℃ for 30 min. The dried granules were re-sieved through sieve No: 20. Magnesium stearate and talc were mixed with prepared granules. This uniformly mixed blend was compressed into 450 mg tablets using flat face round tooling on a Rimek-10 STN rotary tablet machine. The tablets were stored in tightly closed glass container and evaluated for following parameters.

2.5. Evaluation of prepared tablets

Compressed tablets were then evaluated for shape, diameter and thickness, weight variation, disintegration, hardness, friability study. Diameter and thickness were measured by Vernier Caliper. For disintegration test, one tablet was placed in each tube of disintegration apparatus (Electrolab ED-2L, USP) and the test was carried out using

distilled water as a disintegrating media. Hardness was measured by Monsanto type hardness tester. Friability was determined in friabilator (Electrolab EF-2, USP).

2.6. In vitro dissolution study

In vitro dissolution studies of prepared tablets were performed using USP apparatus type II (Electrolab TDT-08L) at 50 rpm in pH 7.8 phosphate buffer (900 mL) medium at (37 ± 0.5) ℃. At predetermined intervals (1, 2, 3 and 4 hours) 5 mL of samples were withdrawn and filtered through Whatmann filter paper No.41. From this filtrate 1 mL was withdrawn in to 10 mL volumetric flask and the volume was made up to the mark. After removal of each sample, 5 mL of fresh dissolution medium was added to the vessel to maintain the constant volume. The samples were then analyzed at 249 nm using a Shimadzu-UV 1700 spectrometer (Shimadzu, Japan). The data presented here is for triplicate determinations (n=3). Concentration of drug in each sample was determined from the standard graph and percentage drug dissolved was calculated.

2.7. Stability studies

The stability of the formulated tablets of the mucilages was tested according to International Conference on Harmonization guidelines. It was carried out, on the storage of formulated mucilage tablets at room temperature, 40 ℃ and 5 ℃ for 4 weeks. Tablets were withdrawn at the end of 4 weeks and evaluated for change in hardness, disintegration and *in vitro* drug release.

3. Results

3.1. Physicochemical properties

The presence of carbohydrate and mucilage was substantiated for both *Hibiscus* and Okra, with the positive result upon the treatment with Molisch's Test (formation of purple color) and Ruthenium red test (formation of pink color on powdered particles), respectively.

The physical and chemical properties of both the plants have been examined by the study of their extracted mucilages. Both the extracted mucilages are slightly soluble in water and a dispersion of it yielded a brown, slimy solution and it was practically insoluble in ethanol, acetone and chloroform. A 1% w/v suspension of *Hibiscus* mucilage in water gave a pH of 6.5 and that of Okra gave 6.1; both of the mucilages were near neutral pH, which implied that when used in uncoated tablets, it may be less irritating to GIT. The weight loss on drying indicates the amount of moisture present in the material available to interact with other material. For the dried mucilages of *Hibiscus* and Okra, the weight loss on drying was 9.8% and 6.8%, respectively. The swelling index of ratio determined was 40 for *Hibiscus* and 30 for Okra. The density and viscosity was found to be 1.55 mg/cm^3 and 5.5 mPa for *Hibiscus* and 1.57 mg/cm^3 and 4.08 mPa for Okra, respectively.

3.2. Drug excipient compatibility study

The spectra showed that the high intense peak at 3325.39 cm^{-1} which can be considered as the characteristic strong bond for Para substituted phenol. The other peak at 3292.6 cm^{-1} showed the stretching secondary amine group. i.e. NH. Peak at 1654.98 cm^{-1} showed C=O amide keto. 1259.56 cm^{-1} and 1442.28 cm^{-1} showed that the characteristic strong band of stretching phenolic C-O. Finally 796.93 cm^{-1} and 713.69 cm^{-1} showed that Para substituted benzene.

3.3. Formulation and evaluation of tablets

Tablets using both Hibiscus and Okra were prepared by wet granulation method. The formula is shown in Table 1. The physical tests for all the formulated tablets were shown in Table 2. All the batches of tablets exhibited the diameter and thickness, uniformity in weight, hardness and friability values were within the pharmacopoeial limits. The mucilage had given increase in disintegration time with increase in its concentration.

3.4. In vitro release study

The *in vitro* dissolution profile of different concentrations of Hibiscus and Okra is shown in Figure 1 & 2. It was found that the rate of drug release from the tablets prepared using Okra mucilage at 1% w/v concentration was more than 90% in 4 h. The mucilage had given a decreased release rate with increase in concentration. That is at 10% w/v concentration the drug release was 65%. At the same time the cumulative drug release of the tablets using Hibiscus mucilage was 75% at 1% w/v concentration and was found to be 21% at 10% w/v concentration. The drug release profile of both the mucilages is shown in Table 3.

Table 1

Paracetamol tablet formulations containing *Hibiscus* mucilage as binder(mg).

SL.NO	Ingredients	Product code				
		H1 10%	H2 8%	H3 5%	H4 2%	H5 1%
1	Paracetamol IP	250.0	250.0	250.0	250.0	250.0
2	*Hibiscus* mucilage	45.0	36.0	22.5	9.0	4.5
3	Lactose	98.5	107.5	12.0	134.5	139.0
4	Starch (8%)	36.5	36.5	36.5	36.5	36.5
5	Talc	10.0	10.0	10.0	10.0	10.0
6	Magnesium	10.0	10.0	10.0	10.0	10.0

Total weight of each tablet was 450 mg, formulations of O1-O5 containing Okra mucilage as binder were exactly the same.

Table 2

Comparison of evaluation of formulated paracetamol tablets using *Hibiscus* and Okra mucilage binders.

Formulation code	Diameter (mm)	Thickness (mm)	Weight variation (mg)	Disintegration time (min)	Hardness (kg/cm^2)	Friability %
H1	9.000±0.160	4.000±0.178	451.400±0.019	46	5.000±0.670	0.560±0.464
H2	8.000±0.091	4.000±0.187	454.600±0.050	36	5.000±0.352	0.800±0.327
H3	9.000±0.165	4.000±0.081	452.800±0.013	22	4.000±0.091	0.480±0.044
H4	9.000±0.151	4.000±0.191	450.300±0.014	15	3.000±0.192	0.930±0.030
H5	8.000±0.143	4.000±0.092	448.800±0.015	16	4.000±0.312	0.790±0.088
O1	9.000±0.153	4.000±0.179	455.700±0.012	41	4.000±0.167	0.810±0.036
O2	9.000±0.147	4.000±0.098	449.600±0.015	30	4.000±0.261	0.280±0.032
O3	9.000±0.132	4.000±0.171	453.100±0.016	33	5.000±0.291	0.920±0.065
O4	9.000±0.081	4.000±0.281	450..600±0.018	24	4.000±0.156	0.400±0.028
O5	9.000±0.158	4.000±0.152	452.100±0.017	20	5.000±0.197	0.940±0.171

Data show as mean±SD

Table 3

Comparative effects of the type and concentration of binding agents on the release rate of paracetamol tablets.

Time in hours	Products									
	H5 (1%)	O5 (1%)	H4 (2%)	O4 (2%)	H3 (5%)	O3 (5%)	H2 (8%)	O1 (8%)	H1 (10%)	O1 (10%)
1	39.52	22.82	28.51	21.52	13.39	19.65	13.89	21.31	10.22	13.39
2	69.19	51.19	42.40	44.92	18.14	34.77	20.59	33.76	14.18	25.63
3	72.79	71.71	48.31	62.06	33.91	53.56	22.24	52.20	16.27	58.82
4	75.38	92.08	58.89	77.83	37.15	72.36	27.21	69.69	21.52	65.44

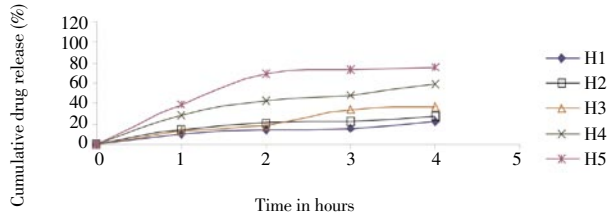

Figure 1. Dissolution profile of paracetamol tablets made with different concentrations of *Hibiscus* mucilage .

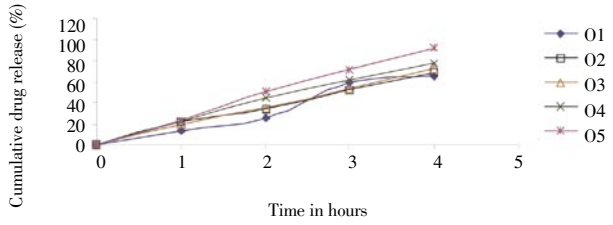

Figure 2. Dissolution profile of paracetamol tablets made with different concentrations of Okra mucilage.

3.5. Stability studies

In order to determine any changes on storage, stability of the formulated tablets was carried out at room temperature, 40 ℃ and 50 ℃ for 4 weeks. Tablets were withdrawn at the end of 4th week and evaluated for changes in hardness, disintegration and *in vitro* drug release. An appreciable change in the hardness, friability and *in vitro* drug release from the formulated tablets was not observed after the stability study.

4. Discussion

The mucilages obtained from both *Hibiscus* and Okra after drying were found to be amorphous free flowing powder. Total yield of *Hibiscus* and Okra was 8.3% w/w and 11.2% w/w, respectively. Both the mucilages exhibited good solubility in water and gave viscous solution on standing, and insoluble in other organic solvents. All other physicochemical properties of the mucilages indicated that both of them can be used as good excipients in formulation of tablets. The drug-excipient compatibility study indicated there is no significant interactions. Standard tests of the tablets showed that all the formulations of both mucilages were within the acceptable limits for diameter, thickness, weight variation, hardness and friability. The disintegration time of the mucilages was found to be increasing on increasing the concentration of the mucilages. The pattern of drug release from the prepared tablets of H1 and O1 formulations, which is having higher (10% w/v) concentrations of mucilage, shows a sustained release pattern. O5 formulations (1% w/v) of the formulated tablets show an optimum drug release of more than 90%, where H5 was shown at least 75% drug release

within 4 h. An appreciable change in hardness, friability and *in vitro* drug release from the formulated tablets was not observed after stability study. With the comparison between the corresponding concentrations of the two mucilages, Okra mucilage was found more effective than Hibiscus mucilage at low concentration (1% w/v) itself. At the same time, the *Hibiscus* plant sources could be used as a platform for prolonged release if their binder concentrations are hiked. In context, the present investigation shows both the mucilages displayed good tableting characteristics and have high potentials for substitution for other more expensive binders. Further studies can be done to explore its role in drug delivery systems including its release retardant properties and mucoadhesive nature.

Conflict of interest statement

The authors have no conflicts of interest.

References

[1]Vinod Doharey, Nisha Sharma. The permutation role of fenugreek seeds starch and Gunda glue as a binder in Paracetamol tablets. *J Pharm Sci & Res* 2010;**2**(2):64-8.

[2]Ebere I Okoye, Anthony O Onyekweli, Olobayo O Kunle. Brittle fracture index (BFI) as a tool in the classification, grouping and ranking of some binders used in tablet formulation: Lactose tablets. *Sci Res Essays* 2010; **4**(5):500-6.

[3]Lateef G Bakre, Kolawole T Jaiyeoba. Evaluation of a new tablet disintegrant from dried pods of *Abelmoschus esculentus* Linn (Okra). *Asian J Pharm Clin Res* 2009; **2**(3):55-8.

[4]Okoye EI, Onyekweli AO, Ohwoavworhua FO, Kunle OO. Comparative study of some mechanical and release properties of paracetamol tablets formulated with cashew tree gum, povidone and gelatin as binders. *Afr J Biotechnol* 2009;**8**(16):3970-3.

[5]Kuldeep Singh, Ashok Kumar, Naresh Langyan. Evaluation of *Mimosa pudica* Seed mucilage as sustained-release excipient. *AAPS Pharm Sci Tech* 2009 **10**(4):44-9.

[6]Kulkarni PK, Shant Reddy. Evaluation of sericin as a binder in tablet formulation. *Ind J Pharm Educ Res* 2009; **43**(4):370-4.

[7]Ravi Kumar, Patil MB, Sachin R Patil, Mahesh S Paschapur. Evaluation of disintegrating properties of Abelmoschus esculentus mucilage. *Inter J Pharm Tech Res* 2009;**1**(2):241-6.

[8]Ravi Kumar, Patil MB, Sachin R Patil, Mahesh S Paschapur. Isolation and evaluation of disintegrating properties of *Salicornia fruticosa* (L.) mucilage. *Inter J Pharm Tech Res* 2009;**1**(3):537- 43.

[9]Martins Emeje, Phyllis Nwabunike, Christina Isime, Joseph Fortunak. Isolation, characterization and formulation properties of a new plant gum obtained from Cissus refescence. *Ind J G Pharm* 2009;**3**(1):16-23.

[10]Oyi AR, Allagh TS, Olayemi OJ. Comparative binding effects of wheat, rice and maize starches in chloroquine phosphate tablet. *Formul Res J Appl Sci, Eng Technol* 2009;**1**(2): 77-80.

[11]Shirsand SB, Sarasija Suresh, Para MS, Swamy PV, D Nagendra Kumar. Plantago ovata mucilage in the design of fast disintegrating tablets. *Ind J Pharm Sci* 2009;**71**:41-5.

[12]Patel DM, Prajapati DG, Patel NM. Seed mucilage from *Ocimum americanum* Linn. as fisintegrant in tablets: Separation and evaluation. *Ind J Pharm Sci* 2007;**69**:431-5.

[13]Amelia Avachat, Gujar K N, Kotwal VB, Sonali Patil. Isolation and evaluation of fenugreek seed as a granulating agent. *Ind J Pharm Sci* 2007;**69**(5):676-9.

[14]Ghule BV, Darwhekar GD, Jain DK, Yeole PG. Evaluation of binding properties of Eulophia campestris wall mucilage. *Ind J Pharm Sci* 2006;**68**(5):566-9.

[15]Girish K Jani, Dhiren P Shah, Vineet C Jain, Manish J Patel, Disha A. Vithalani. evaluating mucilage from aloe barbadensis miller as a pharmaceutical excipient for sustained - release matrix tablets. *Pharm Technol* 2007;**31**:90-8.

[16]Ofoefule SI, Chukwu AN, anayakoha A, Ebebe IM. Application of Abelmoschus esculentus in solid dosage forms 1: use as binder for poorly water soluble drug. *Indian J Pharm Sci* 2001;**63**: 234-8.

[17]Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian medicinal plants*. New Delhi: Council of Industrial and scientific research;1956, p.1-133.

[18]Jha PK, Choudhary RS, Choudhary SK. Studies of medicinal plants of palamau (Bihar). (2nd part). *Biojournal* 1997; **9**: 21-38.

[19]Pal S, Chakrborty SK, Banerjee A, Mukharji B. Search of anticancer drugs from Indian medicinal plants (Ayurvedic, Unani, etc). *Ind J Med Res* 1968;**56**: 445-55.

[20]Tomoda M, Shiniza N, Oshima Y, Takahashi M, Murakami M, Hikino H. Hypoglycemic activity of twenty plant mucilages and three modified products. *Planta Med* 1987; **53**: 8-12.

[21]Gurbuz I, Ustun O, Yesilada E, Sezik E, Akyurek N. *In vivo* gastro protective effects of five Turkish folk remedies against ethanol-induced lesions. *J Ethnopharmacol* 2003; **83**:241-4.

[22]Pieroni A, Ansari NM. Antioxidant activity of five vegetables traditionally consumed by South Asian migrants ib Bradford, Yorkshire, UK. *Phytotherapy Res* 2005;**19**:907-11.

[23]Guanghov Shui, Leong Lai Peng. An improved method for the analysis of major antioxidants of *Hibiscus esculentus* Linn. *J Chromatogra* A 2004:**1048**: 17-24.