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FORMULATION AND *IN-VITRO* EVALUATION OF GLIPIZIDE LOADED MICROSPHERES PREPARED WITH PECTIN EXTRACTED FROM *DILLENIA INDICA & ABELMOSCHUS ESCULENTUS.*

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ABSTRACT

The purpose of this research was to formulate and evaluate mucoadhesive microspheres of glipizide by using pectin obtained from *Dillenia indica* & *Abelmoschus esculentu.*The Glipizide microspheres were prepared by w/o emulsion solvent evaporation method by using different variables like concentration of surfactant, polymer-to-drug ratio, and rotation speed of mechanical stirrer. The polymers like pectin obtained from *Dillenia indica* & *Abelmoschus esculentus* by acetone precipitation method. The prepared microspheres were characterized for the percentage yield, drug entrapment efficiency, FTIR, DSC, SEM analysis and *in-vitro* drug release studies. Microspheres were obtained are discrete, spherical, and free flowing. The microspheres exhibited good mucoadhesive property in the *in vitro* wash-off test and also showed high percentage drug entrapment efficiency (58.7 ± 1.2- 88.1 ± 0.9 %). The FTIR & DSC studies showed the stable character of glipizide in the microspheres. The SEM analysis revealed that the microspheres were spherical, non aggregating and they were nonporous in nature. The study concluded that that the pectin obtained from *Dillenia indica* & *Abelmoschus esculentus* can be used as a promising polymer for glipizide microspheres & giving opportunity to use in various novel drug delivery systems.

Keywords: Glipizide microspheres, *Dillenia indica, Abelmoschus esculentus.* Pectin, w/o emulsion solvent evaporation method, FTIR, DSC, SEM, Novel drug delivery systems.

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INTRODUCTION

The main objective of using mucoadhesive formulations orally would be to achieve a substantial increase in length of stay of the drug in the GI tract. Microsphere is one of the novel drug delivery system made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery[1]. Plant sources provide the largest amount of polysaccharides and therefore plant sources were selected for extraction of polysaccharides. In this study the plant parts were used like fruits of *Dillenia indica* L. (*Dilleniaceae*) (DI) and *Abelmoschus esculentus* (L.) Moench (*Malvaceae*) (AE) [2, 3, 4].Pectin is a very promising biopolymer to construct drug carriers for controlled drug delivery because of its gelling, film forming, binding properties, biocompatibility and stability towards acidic media, and non-toxicity[5, 6]. It would therefore be advantageous to have means of providing an intimate contact of the drug delivery system with absorbing membranes. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site. Pectin was selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive and biodegradable properties[7, 8].Glipizide is an oral rapid and short acting anti-diabetic drug from the second generation of sulfonylurea class[9]. Glipizide acts by partially blocking potassium channels among beta cells of pancreatic islets of Langerhans and resulting calcium influx encourages insulin release from beta cells & lowering the blood glucose level. Due to its short biological half-life (3 to 5 hours), it should be administered in 2 to 3 doses of 2.5 to 10 mg per day. Thus, an attempt was made in this investigation to use pectin as a mucoadhesive polymer and prepare sustained release microspheres[10].

MATERIALS AND METHODS

Materials

Glipizide was received as a gift sample from Aurobindo Pharmaceutical pvt. Ltd., Hyderabad. The fruit of *Dillenia indica* was collected from local market of Balasore (Odisha) and was confirmed by local people. *Abelmoschus esculentus* was procured from the local market around JNT University, Hyderabad. All other chemicals used in the study were of analytical grade and were procured commercially. They were used without testing and purification.

Methods

Extraction of pectin

At first the fruits of DI & AE were collected and were cut into small Pieces with knife. The chopped pieces of the fruits were kept in a beaker containing distilled water with ratio 1:1.5. The beaker was then placed on the heating mantle with temp. 60^0c for 5-6 hours. After about 6 hours the slurries were strained through a Buchner funnel and the filtrate was kept in the refrigerator in a beaker for overnight for sedimentation. The decanted filtrate was taken out of the refrigerator and the supernatant was poured into a clean and dry beaker of 1 L size. The supernatant was evaporated to $1/5^{th}$ of its volume by heating mantle at 60^0 c. The concentrated samples were washed with acetone and dried at 50 to 60^0 C in a hot air oven for 4 hours. On drying, the sample becomes hard and brownish in colour. The powdered samples were passed through sieve no 120 & were stored in desiccators under sealed conditions for further study[11, 12].

Preparation of microspheres

Microspheres were prepared by w/o emulsion solvent evaporation method. The polymers, sodium alginate and pectin (extracted from DI & AE) were dissolved in 50 ml of water and stirred with magnetic stirrer & kept overnight for complete solubility of polymers. The drug was dissolved in the polymeric solution .The oil phase liquid paraffin (100 ml) is taken and the emulsifying agent (span 80) was added with different concentrations .The aqueous phase emulsified into oily phase by stirring the system in a 500ml beaker and kept above the hot plate which is maintained at 38 ± 2^0C.The stirring and heating are continued for 2.5 to 4 hrs until the microspheres are formed. The oily phase was decanted and the microspheres were washed with n-hexane (10ml) 2 to 3 times for complete removal of oil phase and they were dried and kept in the dessicator[13].

Table 1
Preliminary Trial Batches of glipizide mucoadhesive microsphere using pectin obtained from DI & AE.

Formulation code	AE (g)	DI(g)	SA(g)	Drug (mg)	Conc. of surfactants (%)	Stirring speed	Sphericity of microspheres
GLP1	1	1	-	500	0.5	1000	Very Irregular
GLP2	1	-	0.5	500	0.5	1000	Slightly Irregular
GLP3	1	-	0.5	500	0.7	1000	Spherical
GLP4	1.5	-	0.5	500	0.7	1000	Spherical
GLP5	2	-	0.5	500	0.7	1000	Spherical
GLP6	-	1	0.5	500	0.7	1000	Spherical
GLP7	-	1.5	0.5	500	0.7	1000	Spherical
GLP8	-	2	0.5	500	0.7	1000	Spherical
GLP9	1.5	1.5	0.5	500	0.7	1000	Spherical
GLP10	1.5	1.5	0.5	500	0.7	1500	Irregular

AE-Abelmoschus esculentus, DI-Dillenia indica, SA-Sodium alginate.

Evaluation of microspheres

Particle size

The microspheres were mounted in light liquid paraffin, and the diameters of 100 particles were measured using an optical microscope provided with a standardized ocular and stage micrometer. The mean diameter was determined[14, 15].

Drug entrapment efficiency

Drug entrapment efficiencies of dried microspheres were calculated by mashing dried microspheres and extracting the drug into phosphate buffer of pH 7.4 by shaking with a magnetic stirrer for 24 h; the drug content was then estimated. Entrapment efficiency of microspheres was calculated[14, 15].

Swelling index

Swelling indices of different batches of microspheres were analysed by determining the percentage of water retained by the microspheres after 12 hours. About 25 mg of microspheres were placed on an electronic balance and weighed. The microspheres were then dispersed in 20 mL of distilled water, pH 1.2 and also phosphate buffer of pH 7.4 at a temperature of 37 ± 1 °C. After 12 hours, the microspheres were taken out from their respective media, air dried and weighed. The swelling index was determined by following equation.

$$S_{wt} = [(W_t - W_0)/ W_0] 100$$

Where W_t and W_0 are weight of sample swollen at time t (12hrs) and weight of the original sample respectively[14, 15].

In-Vitro Wash-off test for Microspheres

The mucoadhesive properties of the microspheres are evaluated by in-vitro wash-off test reported by Lehr et al. A 1cm by 1cm piece of rat stomach mucosa was tied onto a glass slide (3inch by 1inch) using thread. Microspheres are spread onto the wet rinsed tissue specimen, and the prepared slide is hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus is operated such that the tissue specimen was given regular up and down movements in a beaker containing the phosphate buffer (pH 7.4). At the end of 10 hours, the number of microspheres still adhering onto the tissue is counted[14, 15].

Determination of Flow Properties[15, 16]

The flow properties of prepared microspheres were determined by angle of repose, bulk density, tapped density, Carr's index & Hausner's ratio. The following equations were used to calculate the flow properties.

Angle of repose (θ) =$\tan^{-1}$h/r, Where h- height of the heap in cm, r- radius of the pile in cm.
Where h- height of the heap in cm, r- radius of the pile in cm
Bulk density=W/V_0, Tapped density= W/V_t, Where W-Weight of the formulation, V_0-Bulk volume, V_t-Tapped volume
Carr's index= Tapped density - Bulk density × 100/ Tapped density, Hausner's ratio=Tapped density/ Bulk density.

Drug polymer compatibility studies
FTIR Studies
The drug-Polymer interaction were studied by FTIR spectrometer, shimadzu 8400S 2% w/w of the sample with respect to a potassium Bromide (KBr) was mixed with microspheres. The mixture was mixed into a fine powder using mortar and then compressed into a KBr discs in a hydraulic press at a pressure of 10,000 PSI.The pellets were scanned in the range of 4000 – 600 cm-1for 20 times[16].

DSC Studies
The DSC thermograms of the pure drug & optimized formulation were obtained using the Perkin Elmer JADE DSC system, to identify any interaction between the components of dug and natural polymers (pectin) obtained from DI & AE[16].

Shape and surface morphology
In order to examine the surface morphology, the formulations were viewed under scanning electron microscope. The samples for SEM were prepared by lightly sprinkling the microspheres powder on a double adhesive tape, which was stuck on an aluminum stub. The stubs were then coated with gold to thickness of about 300Å using a sputter coater. The photomicrographs were taken with the help of SEM[16].

In -vitro drug release study
The use of several methods has been described in the literature , but in the present study , the standard eight stations USP basket (apparatus 1) method was used .The microspheres were placed in a basket having mesh size lower than microspheres to avoid the escape of any microspheres. The dissolution medium was 0.1 N HCl as simulated gastric fluid (SGF) (900 ml, pH 1.2) for 2 hr, followed by phosphate buffer as simulated intestinal fluid (SIF) (900 ml, pH 6.8) for the rest of 22 hr . 1 ml samples were withdrawn at specified time intervals (0-24 hr) and equal volume of fresh medium was replaced immediately .The samples were filtered through Whattman filter paper no 41. After a suitable dilution (10 times), samples were analyzed by UV spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a standard curve[16].

RESULTS & DISCUSSION

The Glipizide microspheres were prepared by the w/o emulsion solvent evaporation techniques with different proportion of pectin extracted from DI & AE described in methods. The used pectin was found to be insoluble in organic solvents due to the presence of the acetone insoluble precipitate. They formed colloidal mucilaginous dispersion in water, saturated saline and showed a good swelling property in phosphate buffer of pH 7.4. The pH of 1 % aqueous dispersion of DI and AE was found to be 5.4 and 6.5, respectively, which indicated that it was compatible to the alkaline pH of the intestine. These properties were significant for its use in controlled drug delivery.

Particle size analysis
Mean particle sizes of different batches of prepared microspheres were found to be within the range from 471.23 ± 1.5 to 1042.05 ± µm, as shown in Table 2. The microspheres were found to be uniformly sized and spherical. The results also indicated a more uniform distribution of particle size and shape at 1000 rpm while increased rpm caused non-uniform distribution. Variation in the concentration of pectin influenced particle size and shape. The results also shown that the formulation GLP 9 containing equal proportion of pectin extracted from DI & AE, smoothening the surface of prepared microspheres.

Drug entrapment efficiency
The percentage drug entrapment efficiency of microcapsules in all the formulations was found to be in the range of 58.7 ± 1.2- 88.1 ± 0.9 % shown in Table 2. The formulation GLP 9 which showed maximum drug entrapment efficiency of 88.1 ± 0.9 %.

Table 2

Particle size, Entrapment efficiency, Swelling studies & % Mucoadhesion of Glipizide microspheres.

Formulation code	Particle Size (µm)	Drug Entrapment Efficiency (%)	Swelling studies (%)			% Mucoadhesion
			Water	pH 1.2	pH 7.4	
GLP1	1002.11 ± 1.2	-	-	-	-	-
GLP2	1042.05 ± 1	-	-	-	-	-
GLP3	625.6 ± 1.5	69.5 ± 1.11	25	17	35	40 ± 1.52
GLP4	678.81 ± 1.5	72.1 ± 1.58	28	19	42	42 ± 2
GLP5	708.21 ± 1.2	78.4 ± 1.31	30	20	50	43 ± 3.05
GLP6	597.61 ± 0.6	58.7 ± 1.2	20	12	28	33 ± 3.51
GLP7	626.18 ± 2.3	63.2 ± 1.4	24	16	32	35 ± 1.15
GLP8	640.11 ± 2.6	67.3 ± 0.5	26	18	40	38 ± 4.72
GLP9	471.23 ± 1.5	88.1 ± 0.9	40	21	60	54 ± 1.52
GLP10	999.31 ± 1	-	-	-	-	-

Determination of Flow Properties

The flow properties of prepared microspheres were determined by angle of repose, bulk density, tapped density, Carr's index & Hausner's ratio and shown in Table No: 3.

Table 3

Flow properties of microspheres.

Formulation code	Angle of repose	Bulk density	Tapped density	Carr's index	Hausner's ratio
GLP3	37.65 ± 0.754	0.497 ±0.057	0.614±0.031	19.06±0.04	1.23±0.024
GLP4	36.16 ± 0.529	0.5 ±0.045	0.597±0.012	16.25±0.032	1.19±0.03
GLP5	37.98 ± 1.149	0.503 ±0.065	0.632±0.031	20.41±0.021	1.25±0.07
GLP6	36.01 ± 0.543	0.493 ±0.012	0.581±0.016	15.15±0.12	1.17±0.191
GLP7	37.72 ± 0.688	0.510 ±0.056	0.638±0.05	20.06±0.031	1.25±0.22
GLP8	37.51 ± 1.350	0.495 ±0.041	0.605±0.021	18.18±0.09	1.22±0.23
GLP9	27.25 ± 0.166	0.498 ±0.009	0.543±0.006	8.28±0.031	1.09±0.06

Drug polymer compatibility studies

FTIR Studies

The IR spectrum of glipizide is shown in (Fig 1) and the following characteristic bands were observed 3324.5(N-H stretching), 1690 (- C=O, Amide), 1649.9 (- C=O, Urea), 1531 (Ar-CH, stretching), 1444 (Ar-CH, bending), and 1333.1 and 1159.2 cm-1 (- SO2NH). The IR spectrum of optimized formulation showed the presence of characteristic bands as that of glipizide shown in (Fig 2). Thus, any change in the structure of glipizide was ruled out, and it was concluded that there is no chemical incompatibility between glipizide and excipients used in formulations.

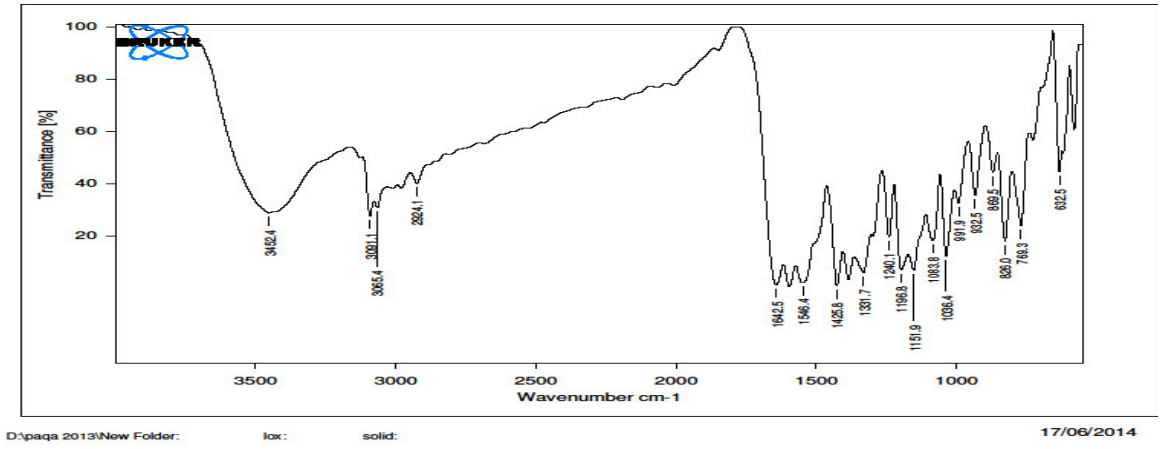

Figure 1

FTIR of pure drug (Glipizide)

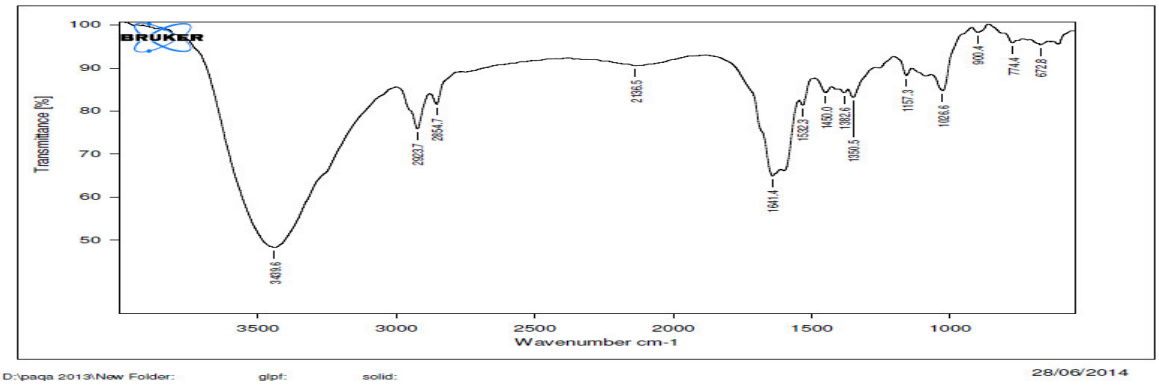

Figure 2
FTIR of optimized formulation.

DSC studies

The DSC thermogram of glipizide (Fig 3) showed a short endothermic peak at 215.3ºC the physical mixtures showed an endothermic peak of drug at 260^0C indicating a slight change in terms of shifting towards the higher temperature. It has been reported from the graphs that the quantity of material used affects the peak shape and enthalpy. Thus these minor changes in the melting endotherm in the drug could be due to the mixing of the drug and excipients which lower the purity of each component in the mixture and may not necessarily indicate potential incompatibility.

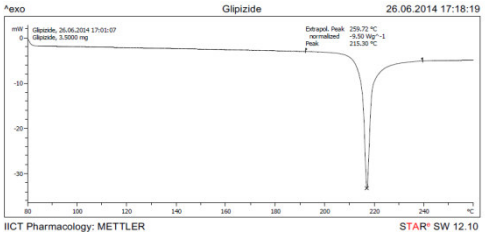

Figure3a
DSC of Glipizide

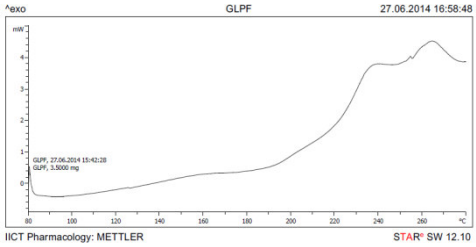

Figure3b
DSC of optimized formulation

Shape and surface morphology

The surface morphology of the prepared microspheres was investigated by Scanning Electron Microscopy. Glipizide loaded microspheres prepared by w/o emulsion solvent evaporation method to be spherical, smooth, homogeneously distributed without evidence of collapsed particle. The prepared microspheres showed smooth surface and exhibited regular spherical geometry. The SEM photograph did not show any aggregation of microspheres and there was no grafting of polymer in microspheres shown in figure 4.

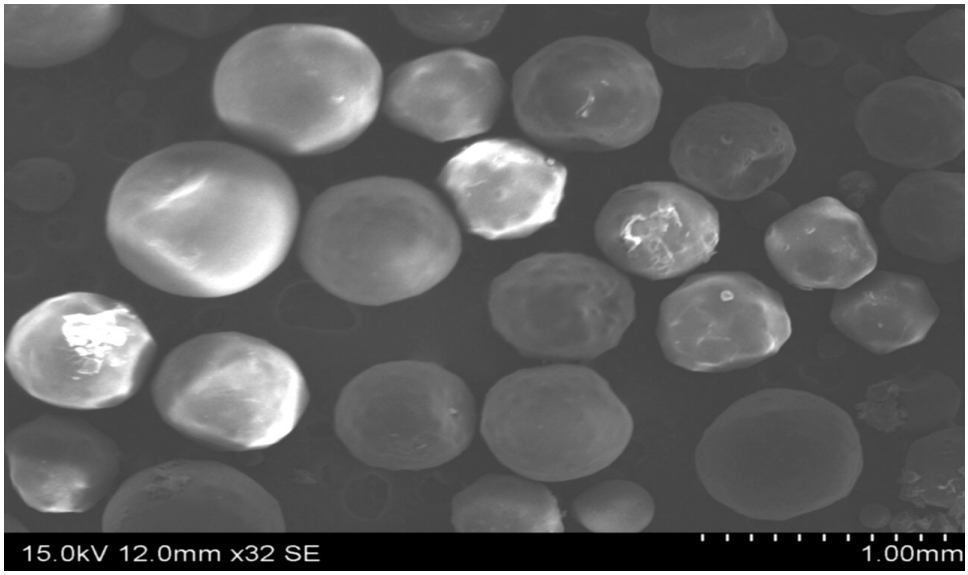

Figure4
SEM of optimized formulation

In-vitro drug release study

The release profile of glipizide from the prepared microsphere & the marketed product was studied in 0.1 N HCl as simulated gastric fluid (SGF) (900 ml, pH 1.2) for 2 hr, followed by phosphate buffer as simulated intestinal fluid (SIF) (900 ml, pH 6.8) for the rest of 22 hr. It was evident that the prepared formula (GLP 9) effectively controlled the release of glipizide compared to the market product. The release of drug was shown in different formulations and was found that GLP 9 formulation retarded the drug release up to 24 hours similar to that of marketed product i.e 100.6%.

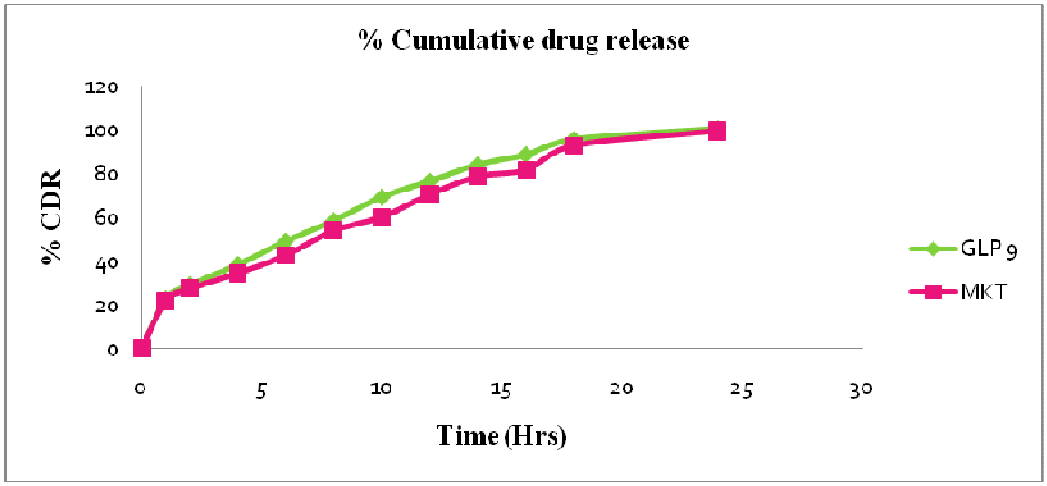

Figure 5
% Cumulative drug release with Marketed formulation.

CONCLUSION

It can be concluded from the proposed study that the w/o emulsion solvent evaporation method can be efficiently used to formulate microspheres of glipizide with blends of pectin obtained from DI & AE to obtain controlled release characteristics. The FTIR and DSC studies also revealed no interaction between the drug and polymer used. Natural mucilaginous polysaccharides (pectin) from edible sources possessed good swelling properties in the intestinal pH and also exhibited good mucoadhesivity. Besides controlled release formulation, due to the presence of pectous polysaccharides the extract may be considered, for colonic delivery of drugs.

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