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EXTRACTION AND EXVIVO CHARACTERIZATION OF VARIOUS NATURAL MUCOADHESIVE POLYMERS

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ABSTRACT

A stable, non-toxic, economical and compatible dosage form can be achieved with the right choice of the excipients. Use of natural polymers instead of synthetic polymers, aids in the development of a dosage form that is safer, with minimal side effects. The present study envisaged the extraction of mucoadhesive polymers from natural ingredients like Abelmoscusesculentus fruits, Hibiscus rosa-sinensis leaves, Artocarpusheterophyllus fruit, Linumusitatissimum seeds and Colocasiaesculenta corms. All the extracted mucoadhesives were preliminarily evaluated using Molisch's test, Iodine test, enzyme test and Ruthenium test. Exvivo evaluation of extracted mucoadhesive was executed using Wash off test performed using modified disintegration apparatus and Mucoadhesive bond strength test performed using modified digital balance apparatus. The results revealed that Linum seed gum (M4) has highest mucoadhesive bond strength of 0.091N/m^2 and a force of adhesion 1.601 N followed by Artocarpus fruit gum (M3). Furthermore the results conclude that the extracted mucoadhesive serve as a best alternative for the existing synthetic mucoadhesives in formulating safer and cost effective dosage forms.

KEYWORDS: Natural, Mucoadhesive, Extraction, Bond strength, Wash off test.

INTRODUCTION

Mucoadhesion is process which involves adherence of polymer/dosage form to the mucosal surface. The process involves various steps like wetting or hydration, adsorption, interpenetration of polymer chains.[1,2] Mucoadhesion is influenced by various polymer related factors like polymer chain length, extent of cross linking, presence of hydrophilic functional groups etc. Based on the nature of origin mucoadhesive polymers are classified into two types: Natural polysaccharides and Synthetic. Natural mucoadhesive have greater significance than synthetic polymers in terms of safety, availability and minimal excipient toxicity. Moreover these natural polysaccharides are economical, biodegradable, biocompatible and easily available to mankind. Most of the natural polysaccharides are certified as safe for human use by food industry.[3,4]

Natural polysaccharides are most widely included in designing controlled drug delivery systems like targeted drug delivery, pulsatile drug delivery etc. which can be achieved by including polysaccharides in tablets, granules, pellets.[5-7] etc. Various mechanisms like degradation of polysaccharides by colonic enzymes make these polysaccharides significantly useful in colon-targeted drug delivery systems.[8-10] Modified natural polysaccharides give a tough competition to the existing synthetic excipients. Hence in the near future Pharma industries show tremendous orientation towards these excipients of natural origin for making safer and successful formulations.

In the current work five natural sources namely Abelmoscusesculentus fruits.[11] (commonly known as Lady's finger), Hibiscus rosa-sinensis leaves.[12] (commonly known as China rose), Artocarpusheterophyllus fruit.[13] (commonly known as Jack fruit), Linumusitatissimum seeds.[14] (commonly known as Flax seeds) and Colocasiaesculenta corms.[15] (commonly known as Taro) were selected for extraction of respective mucoadhesive portions.

METHODOLOGY

Materials Used

Natural ingredients like Abelmoscusesculentus fruits (S1), Hibiscusrosa-sinensis leaves (S2), Artocarpusheterophyllus fruit (S3), Linumusitatissimum seeds (S4) and Colocasiaesculenta corms (S5) were procured from local store.

Procedure for Extraction of Mucoadhesive From Natural Sources

Processing of excipients

All the raw materials collected were processed to remove superficial impurities. The selected plant part was processed individually for extraction of mucoadhesive portion using the schematic procedure.[16-18] mentioned in **Figure 1.** Step by step extraction process was diagrammatically represented in **Figure 2.**

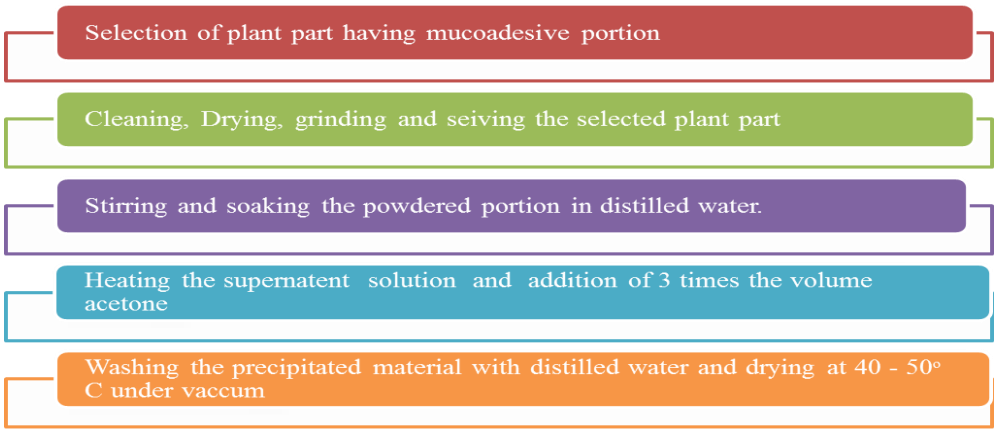

Figure 1: Schematic representation of general extraction procedure.

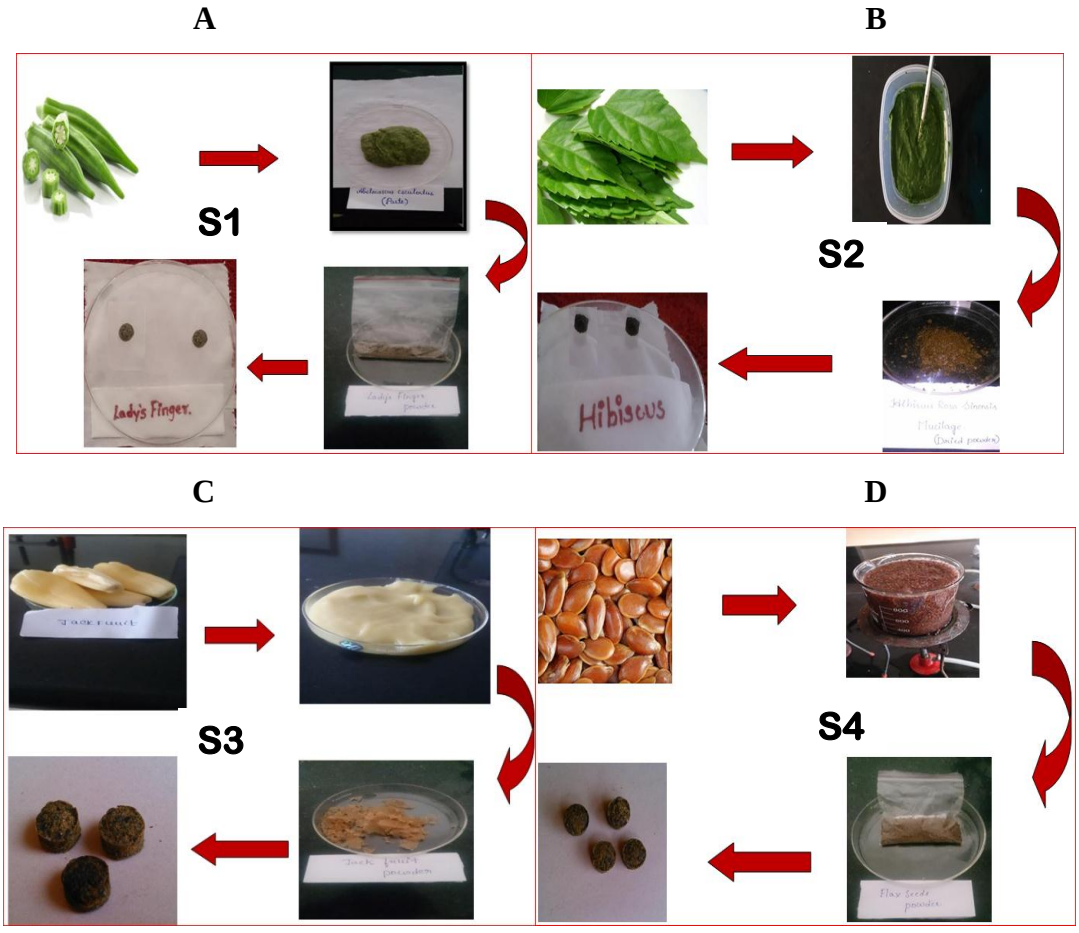

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Figure 2: Step by step process involved in preparation of mucoadhesive tablets from A) Abelmoscusesculentus fruits (S1) B) Hibiscus rosa-sinensis leaves(S2), C) Artocarpusheterophyllus fruit (S3), D) Linumusitatissimum seeds (S4) and E) Colocasiaesculentacorms (S5).

Characterization of Natural Mucoadhesive Polymers

Preliminary evaluationtests

All the extracted mucoadhesive portions from sources S1 – S5 were designated as M1 – M5. The mucoadhesives were subjected to preliminary evaluation tests like Molisch's test, Iodine test, enzyme test and Ruthenium test. The observations and inferences were shown in Table 1.

Physicochemical evaluation of extracted mucilages

Various physicochemical parameters like physical appearance (microscopic method), Solubility (as per BP specifications), swelling in water, Melting point (scientific, MP-DS TID 2001), ash values (as per IP), loss on drying (as per BP), Foreign matter, percentage yield and microbial test were performed for all the extracted mucilages.

Micromeritic evaluation of extracted mucilages

Micromeritic evaluation of extracted mucilages (M1-M5) was performed using various evaluation parameters like angle of repose (fixed funnel method), bulk density, tapped density (graduated cylinder tapping method) and compressibility index.

Formulation of mucoadhesive tablets

- 200 mg of extracted mucoadhesive powders were separately weighedaccurately.
- The weighed powders were transferred into the 9mm die and compressed using Elite - 10 station GMP model rotary press with round biconcave punches.

- Each mucoadhesive powder was compressed into tablets having a batch size of 30 – 40 tablets.

Evaluation of Prepared Mucoadhesive Tablets

Hardnesstest

Tablet hardness was measured using Monsanto hardnesstester. The force or load required to break the tablet longitudinally was measured in Kg/cm^2.

Weight variation

Twenty tablets were randomly selected from each batch and individually weighed. The average weight andstandard deviation of 20 tablets was calculated. The batch passes the test for weight variation test if not morethan two of the individual tablet weight deviates from the value shown in the below table and none deviate twice the percentage shown as perUSP.

Friabilitytest

Laboratory friability tester is known as the Rochefriabilator. The tablets were subjected to the combined effects of abrasion and shock by utilizing a plastic chamber that revolves at 25rpm for 4min or 100 revolutions.

% Loss = {1-(Wo/W)} ×100

Swellingstudies

Swelling studies[19] was performed to estimate molecular parameters of the extracted swellable polysaccharides S1-S5 using USP type I dissolution apparatus (DS 8000). The tablets were initially weighed using an Essae electronic balance having sensitivity 10mg. The tablets were added to dissolution basket. After 5 minutes, the tablets were removed and placed on a butter paper and the tablets were re-weighed. The swelling ratio was calculated from the followingformula.

Exvivo Evaluationtests

MucoadhesiveStrength

Mucoadhesive strength of the tablet was measured on the modified digital balance.[20] The design used for measuring the mucoadhesive strength was shown in **Figure 3a**. The apparatus consist of a modified electronic digital balance on which a rectangularly bent aluminum rod was affixed and at the bottom end of the rod an iron punch was fixed at the centre, to which the tablet was adhered as shown in the **Figure 3b**. The balance was tared to zero before

performing the experiment. At the lower end, a beaker containing a block for placing the mucosal layer is arranged and the up and down moments of the beaker was fabricated by using syringes assembly as shown in the **Figure3c**. The beaker was filled with Tyrodes solution for maintaining the consistency of the tissue. The other side (outer part of stomach) tissue was adhered to the support in thebeaker.

Pork stomach mucosa was used as a model membrane since it has higher similarities with that of human stomach mucosal layer. The pork stomach mucosa was kept in Tyrodes buffer solution during transportation.

After the arrangement of the assembly was done, the one side of the tablet was fixed to the punch with the aid of Cyanoacrylate gum and the beaker containing mucosal layer was raised slowly until contact between the goat mucosa and the mucoadhesive tablet was established. A preload of 10 mg was placed on the punch for 5 min (preload time) to establish adhesion bonding between mucoadhesive tablet and pig stomach mucosa. The preload and preload time were kept constant for all formulations. After completion of preload time, preload was removed from the punch and the beaker assembly was lowered slowly. The weight at which the tablet was detached from the mucoadhesive layer was noted as mucoadhesive strength in grams. From the mucoadhesive strength following parameter was calculated.

Force of adhesion (N) = (Mucoadhesive strength × 9.81) / 1000

Bond strength (N/m^2) = Force of adhesion (N) / Surface area of tablet (m^2)

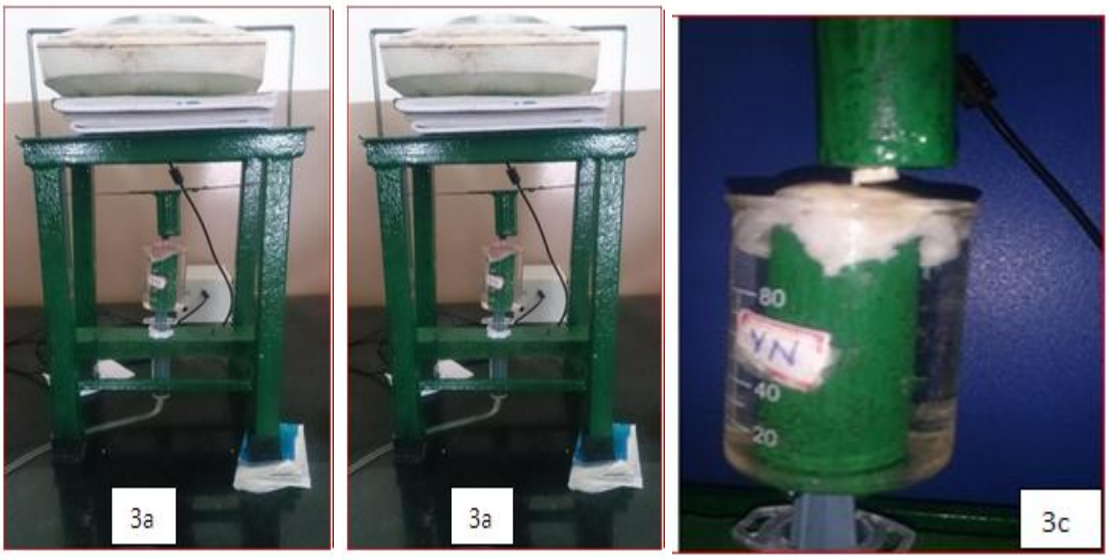

Figure 3: Modified Digital balance apparatus for performing Mucoadhesive bond strength test.

Wash offtest

The mucoadhesive properties of the tablets were evaluated by an *in vitro*, wash-off method using modified USP tablet disintegration apparatus [20] shown in **Figure 4c**. Freshly collected pork stomach as shown in **Figure 4a** was sliced to the required size as shown in **Figure 4b.** Pieces of stomach mucosa of pig were mounted on the glass slides provided with suitable support as shown in **Figure 4d**. The slides were fixed to the arm of disintegration apparatus such that height from the bottom is 25mm at the down stroke and at the highest point the slide is 15 mm below the liquid surface.

After fixing 2 tablets to this glass slide, it was tied to the arm of USP tablet disintegration test apparatus and was run at 37^{O}C. Time of detachment of both tablets was noted down as wash-off period.

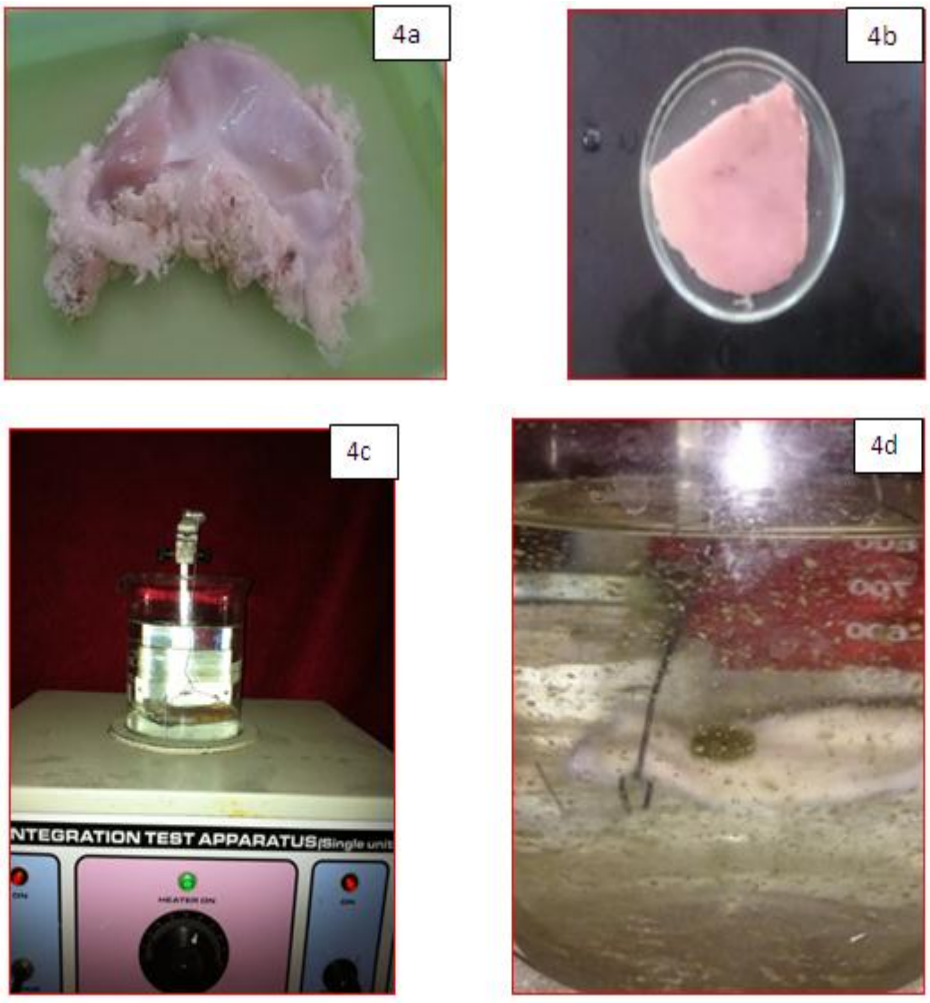

Figure 4: Modified Disintegration apparatus for performing wash-off period test.

RESULTS AND DISCUSSION

Preliminary phytochemical evaluationtests

The preliminary phytochemical evaluation of all the extracted mucilages gave a positive result for carbohydrates, polysaccharides and mucilage and a negative test for enzymes. All the results were mentioned in **Table 1.**

Table 1: Preliminary evaluation tests for dried mucilages.

S. No.	Tests	Observation	Inference
1.	**Molisch's Test** 100mg dried Mucilage + Molisch reagent + conc. *H*2S*O*4 on sides of test tube	Violet green color observed at junction of two layers.	Carbohydrates presents
2.	**Iodine test** 100mg powder +1ml 0.2N *I*2 solution	No color observed in solution	Polysaccharides present (starch absent)
3.	**Enzyme test** Dissolve 100mg power in 20ml distilled water add 0.5ml of Benzedrine in alcohol (90%) shake and allow to stand for few minuets	No blue color produced	Enzyme absent (distinction between dried mucilage and acacia)
4.	**Ruthenium test** Take a small quantity of dried mucilage powder, mount it on a slide with ruthenium red solution, and observe it under microscope.	Pink color developed	Mucilage present

Table 2: Physicochemical characteristics of extracted mucilages.

S. No.	Test	M1	M2	M3	M4	M5
1	Physical appearance	Greenish brown color	Greenish brown color	Wheatish color powder	Light brown powder	Off white powder
2	Solubility	Disperses in water forming colloidal solution	Soluble in luke warm to hot water forming colloidal solution	Disperses in water forming colloidal solution	Colloidal solution is formed in luke warm water	Soluble in luke warm water. Forms a colloidal solution.
3	Swelling in water	Moderate	Moderate to high	High swelling	High swelling	Very less
4	Melting point range	> 2050^{o}C	> 1800^{o}C	> 1500^{o}C	> 2000^{o}C	> 1550^{o}C
5	pH (1%w/v)	6.0	6.1	6.3	6.2	6.2
6	Total ash %	0.29	0.36	0.41	0.39	0.37
7	Loss on drying	9.35%	10.21%	11.03%	11.16%	10.73%
8	Foreign matter	NMT 0.1%	NMT 0.1%	NMT 0.1%	NMT 0.1%	NMT 0.1%
9	% yield value	10.43% w/w	11.91% w/w	15.15% w/w	13.63% w/w	9.26% w/w
10	Microbial test	Passes	Passes	Passes	Passes	Passes

Table 3: Micromeritic properties of extracted powders.

S. No.	Parameter	M1	M2	M3	M4	M5
1	Angle of repose (^{o})	30.47	25.72	28.69	27.96	26.11
2	Tapped density (gm/ml)	0.574	0.621	0.482	0.611	0.585
3	Bulk density (gm/ml)	0.362	0.586	0.373	0.452	0.397
4	Compressibility index	21.61	25.63	21.54	24.09	23.74

Table 4: Various evaluation parameters for the compressed mucoadhesive tablets

Tests	M1	M2	M3	M4	M5
Weight Variation (mg)	500 ± 9.2	500 ± 5.9	500 ± 10.1	500 ± 8.3	500 ± 7.6
Hardness (kg/cm^2)	3.17 ± 0.5	3.14 ± 0.5	3.11 ± 0.5	3.09 ± 0.5	3.1 ± 0.5
Friability (%)	0.34 ± 0.001	0.25 ± 0.001	0.23 ± 0.0017	0.91 ± 0.0018	0.87 ± 0.001

Table 5: Swelling Ratio of various Mucoadhesive portions.

Formulation	Swelling Index
M1	28.611 ± 3.027
M2	31.944 ± 4.164
M3	39.436 ± 1.931
M4	42.535 ± 4.832
M5	23.169 ± 2.869

Table 6: Mucoadhesive strength, force of adhesion and Bond strength values of various mucoadhesive portions.

Formulation	Mucoadhesive Strength (gm)	Force of Adhesion (N)	Bond strength (N/m^2)
M1	21 ± 0.73	0.20601	455.7743
M2	22.4 ± 0.92	0.219744	486.1593
M3	23 ± 0.53	0.22563	499.1814
M4	24 ± 0.395	0.23544	520.885
M5	20 ± 1.13	0.1962	434.071

Table 7: Wash – off period of various mucoadhesive portions.

Formulation	Wash off period*
M1	1hr 38min ± 6min
M2	2hrs ± 5min
M3	2hr 45min ± 11 min
M4	3hr 4min ± 5 min
M5	1hr 10min ± 4 min

Physicochemical characterization of extracted mucilages

The results obtained for physicochemical evaluation of extracted mucilages were mentioned in **Table 2**.The yield value was found to be in the range of 9 – 15% w/w. The pH of 1% w/v solutions of extracted mucilages was found to be in the range of 6.0– 6.3pH. The total ash

value was found to be NMT 1%. All the extracted mucilages pass the microbial test and thus proved to be safe for administration.

Micromeritic properties

The results obtained for Micromeritic properties were shown in **Table3**. Angle repose values were found to be in the range of 25.72 – 30.47^{o}. Bulk density values were found to be in the range of 0.362 -0.586 gm/ml. Tapped density values were found to be in the range of 0.482 – 0.621 gm/ml. Compressibility index valueswere found to be in the range of 21.54 – 25.63. Based on the results obtained from the Micromeritic evaluation tests the flow behavior all the mucoadhesive powders was found to be average/satisfactory.

Evaluation of compressed mucoadhesive tablets

The extracted mucoadhesive portions were compressed into 9mm tablets using Elite 10 station rotary tablet compression machine. The results of the evaluation tests were mentioned in **Table 4**. The hardness of all the formed tablets were between 3-5 kg, weight variation was found to be less than 2.5 %, the weight loss after friability test was found to be less than 1%. All the results obtained are found to be within limits as per USP.

All the obtained mucilages have a property to absorb and swell in the presence of water instead of dissolving in water. The results obtained were shown in **Table 5.** Swelling index was found to be at a range of 23.16 – 39.44. Maximum swelling index was observed in linum seed gum (39.44). Higher swelling index values indicate that the extracted mucilages can be used in controlled release dosage forms and can act as binder, disintegrant.

Exvivo evaluationtests

Exvivo evaluation tests like mucoadhesive bond strength and wash-off test were performed using modified digital balance apparatus and modified USP disintegration apparatus respectively. The force required to detach mucoadhesive dosage form from the mucoadhesive tissue. The mucoadhesive bond strength values as shown in **Table 6** reveal that all the mucoadhesives (M1 – M5) extracted were found to be in the range of 434 – 520 N/m^2. Linum seed gum was found to have maximum bond strength of 520.89 N/m^2. The time required to detach the mucoadhesive dosage form from the mucoadhesive tissues is noted as wash-off test. The wash off test period values as shown in **Table 7** was found to be in the range of 70 - 184 min. Linum seed gum was found to have maximum wash off period of 3 hr 4 min.

CONCLUSION

Natural excipients in the near future have great chances to emerge as a potential alternative to the existing synthetic excipients as the former have wide availability, economical, biodegradable, inert and non-toxic. The current study aimed at selecting natural ingredients as raw materials for extraction of gums or mucilages which can act as competent excipients in the pharmaceutical formulations. Sources namely Abelmoscusesculentus fruits (S1), Hibiscusrosa-sinensis leaves (S2), Artocarpusheterophyllus fruit (S3), Linumusitatissimum seeds (S4) and Colocasiaesculenta corms (S5) were selected for extraction of respective mucilages namely Bhendi gum (M1), hibiscus leaf gum (M2), jack fruit gum (M3), linum seed gum (M4), colocasia corm gum (M5) respectively. The results obtained in preliminary evaluation, phytochemical screening, micromeritic characterization, swelling studies establish fundamental characteristics of the extracted mucilages. The tablets prepared with the extracted gums/ mucilage show sufficient hardness and friability indicating their significant application as binders. Results obtained for exvivo evaluation studies like mucoadhesive bond strength, wash off period explored the potential mucoadhesive ability of the extracted mucilages. Further investigation will explore insights to the other applications of extracted material in the pharmaceutical industries.

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CONFLICT OF INTEREST

The authors exhibit no conflict of interest.

REFERENCES

1. Rohit Rajendra Bhosale, Riyaz Ali M Osmani, AfrasimMoin. Natural gums and mucilages: A review on multifaceted excipients in pharmaceutical science and research. International Journal of Pharmacognosy and Phytochemical Research, 2014; 6(4): 901-12.
2. Tomsmasina C and Matricadi P. Polysaccaride hydrogels for modified release formulations. Journal of Controlled release, 2007; 119: 5-24.
3. Guo J, Skinner GW and Harcum WW: Pharmaceutical application of naturally occurring water soluble polymers. Pharmaceutical Science and Technology Today, 1998; 1:

254-261.

4. Stephen AM and Churms SC: Food polysaccharides and their applications. Taylor and Francies, CRC Press, New York, Edited by Stephen AM, Phillips GO and Williams PA, 2006; 1-24.
5. Shirwaikar A and Prabhu SL: Herbal excipients in novel drug delivery systems. Indian Journal of Pharmaceutical Science, 2008; 70: 415-422.
6. G. K. Jani, D. P. Shah, V. D. Prajapatia, and V. C. Jain. Gums and mucilages: versatile excipients for pharmaceutical formulations. *Asian Journal of Pharmaceutical Sciences*, 2009; 4(5): 309–323.
7. Raymond CR, Paul JS and Marian EQ: Handbook of pharmaceutical excipients. Pharmaceutical Press, Edition, 2009: 6: 298.
8. Kokate CK, Purohit AP and Gokhale SB: A Textbook of Pharmacognosy. NiraliPrakashan, Pune, Edition, 2003; 22: 136-139.
9. Evans WC, Trease and Evans: Pharmacognosy. Harcourt Brace & Co., Asia Pvt. Ltd, Singapore, Edition, 1996: 196-208-213-215.
10. Raymond CR, Paul JS and Marian EQ: Handbook of pharmaceutical excipients. Pharmaceutical Press, Edition, 2009; 6: 298.
11. Ofoefule SI and Chukwu A: Application of Abelmoschus esculents gum as a mini-matrix for furosemide and diclofenac sodium tablets. Indian Journal of Pharmaceutical Science, 2001; 63: 532-535.
12. Mulchande A Shende and Rajendra P Marathe. Extraction of mucilages and its comparative mucoadhesive studies from hibiscus plant species. Word Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(3): 900-924.
13. Deepikagupta. Phytochemical, nutritional and antioxidant activity evaluation of seeds of Jackfruit (artocarpousheterolphyllus lam). Int J of Pharm Bio Sci., 2011; 2(4): 336-345.
14. ShyamoshreeBasu, SubrataChakrabatorty, Andamal K. Bandyopadhyay. Development and evaluation of a mucoadhesive nasal gel of Midazolam prepared with Linumusitassimum L. seed mucilage. Sci. Pharm, 2009; 77(4): 899-910.
15. RakeshPrajapati. Colocasiaesculenta: A potent indigenous plant. International Journal of Nutrition Pharmacology Neurological Diseases, 2016; 1(2): 90-95.
16. PranjalSikia, Bhanu P Sahu, S K Dash. Isolation and characterization of some natural polysaccharides as natural excipients. International Journal of PharmTech Research, 2013; 5(3): 1196-1206.

17. Geetha B and Shivalinge Gowda KP: Microwave assisted fast extraction of mucilages and pectins. Indian Journal of Pharmaceutical Education and Research, 2009; 43: 260-265.
18. Kulkarni GT: Microwave assisted fast extraction of mucilages and pectins. Indian Journal of Pharmaceutical Education and Research, 2009; 43: 260-265.
19. Sasa Baumgartner, JulijanaKristl and Nicholas A. Peppas. Network Structure of Cellulose Ethers During Swelling. Pharmaceutical research, 2001; 19: 34-42.
20. Pallavi K, Srinivasa Babu P and Kishore Babu G. Characterization and optimization of various polymers based on mucoadhesive strength and wash off period for mucoadhesive drug delivery system. Der Pharmacia Lettre, 2016; 8(4): 246-54.