Extraction and characterization of *Hybiscus Rosa-Sinensis* leaves mucilage for Pharmaceutical applications

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ABSTRACT

The present research was related to a process of production of crude, pharmaceutically useful dry water soluble mucilage/polysaccharide from leaves of *hybiscus rosa-sinensis*. Extraction of water soluble mucilage/polysaccharide from leaves was performed using water, phosphate buffer solutions of pH 4.0, pH6.8 and pH 9.2 as solvents for better yield. Physicochemical characterizations and other studies such as viscosity and effect of temperature on viscosity, pH, swelling in different physiological pH conditions were discussed. The dry water soluble mucilage/polysaccharide was then used as suspending agent and solubility enhancing agent to improve the bioavailability of some model drugs which are poorly soluble in water.

Highest percent yield of the dry water soluble mucilage/polysaccharide was obtained in distilled/demineralized water (14.7%) and phosphate buffer pH 9.2 (16.4%). Powder characterization study (26.32°, 0.54 g/mL, 0.73 g/mL, 22.62%, and 1.08 of angle of repose, lose bulk density, tapped bulk density, carr's index and hausner's ratio respectively) ensured the mucilage/polysaccharide suitable for Tablet formulation and higher viscosity and swelling index at distilled/demineralized water and phosphate buffer pH 7.4 respectively ensures the suitability for suspension formulation (1.0% solution gave 356cps).

Hence, the extracted dry water soluble mucilage/polysaccharide from leaves of *hybiscus rosa-sinensis* would be useful for pharmaceutical applications with economic, toxic less and biocompatible.

Keywords: Polysaccharide/Mucilage, Solubility enhancement, Suspending agent, Poorly soluble drugs, Viscosity, Swelling.

INTRODUCTION

Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility which is an important parameter to achieve desired concentration of drug in the right place for pharmacological response. [1,2] Plant excipients serve as an alternative to synthetic one because of economic, local accessibility, eco-friendly and toxic less to the human body.[3] Moreover, the plant gum and mucilage have been widely explored and used as pharmaceutical excipients[4] such as binding, thickening, suspending, emulsifying, disintegrating, gelling and stabilizing agents, etc and especially for solubility enhancement and targeting the drug release in controlled manner in drug delivery systems.[5-7]

Hybiscus rosa-sinensis or Chembarathai[8,9] (Family: Malvaceae, Genus: Hybiscus) a well known perennial plant and colloquially called as Chinese hibiscus, China rose and shoe flower. It is widely grown as an ornamental plant throughout the tropics and subtropics. All parts of the plant were studied in great detail by several investigators. Leaves are simple ovate or ovate lancolate and these are entire at the base and coarsely toothed at the apex and have abundance of mucilage/polysaccharide. It contain L-rhamnose, D-galactose, D-galactouronic acid, and D-glucuronic acid and it also contain moisture, protein, fat, carbohydrate, fibers, calcium, and phosphorus. The taste of the leaf is mucilagenous. The herb was commonly used and proved by many researchers that it has wide application in pharmaceutical and cosmetic technology and used in herbal shampoo/hair oil cosmetics, medicinal and beverages. Leaves were having emollient, aperient, anodyne and laxative properties, treat urinary complaints, good for healing ulcers, promoting growth and colour of hair, aphrodisiac, emmenagogue, and refrigerant.[10-12] The leaves were crushed well and squeezed to extract the mucilaginous content as juice. The juice is diluted with water or prepared as kudineer and given it for leucorrhoea. This juice was also used for head bath and known as thali traditionally. Its cool potency even makes it use to cure psychiatric ailments in siddha. The leaves of hibiscus rosa-sinensis or chembarathai are available more in india and majority of the leaves part are being treated as cattle feed and not used for any commercial purpose. All parts of this plant and their chemical constituents were proved by researchers as an anti-tumor, antifertility, antiovultory,

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antiimplantation, anti-inflammatory, analgesic, antiestrogenic, antipyretic, antispasmodic, antiviral, antifungal, antibacterial, hypoglycaemic, spasmolytic, CNS depressant, hypotensive and juvenoid activity. Especially, ethanolic and petroleum ether extract of the leaves shown Analgesic Activity, Antipyretic Activity, Antifungal Activity, Anti-inflammatory Activity, Hypoglycemic Activity, Hair Growth Activity.[13-22] But, the precipitate/solid waste during the extraction process i.e., water soluble mucilage/polysaccharide has been left as unidentified and waste.

The leaves of *hibiscus* species have abundance of water soluble mucilage/polysaccharide that produces aqueous colloidal suspension when contact with water which may be considered for the suitability of the mucilage/polysaccharide as suspending agent. Traditionally and still being used this water soluble mucilage/polysaccharide for hair tonic/cleaning purpose.[23-24] Poorly soluble drugs are being loaded in this aqueous mucilage/polysaccharide brings them to contact with water very fast which facilitates the improvement of drugs solubility opting this water soluble mucilage/polysaccharide as solublizing agent. Further advantage of this system are the easy process, handling, ability to formulate suitable dosage form and biocompatible.

The main objective of the present investigation was to extract the dry crude, pharmaceutically useful powder of dry water soluble mucilage/polysaccharide from leaves of *hibiscus* species using different solvents by hot extraction technique followed by alcoholic precipitation and perform the physicochemical characterization of the derived product. Moreover, the objective has been extended to use the said product in pharmaceutical formulations as suspending agent, solubility enhancing agent for poorly soluble drugs (Ranolazine and Lumifenadrine) to improve bioavailability and to perform the stability studies of the formulations.

MATERIALS AND METHODS

The drugs such as Ranolazine and Lumefantrine were provided as gift samples from MSN Laboratory, Hydrabad, India. The leaves of hibiscus species were collected from the medicinal gardens of Karpagam College of Pharmacy, Coimbatore, India. The other chemicals and solvents used were of Analytical grade.

Process of production of dry water soluble mucilage/polysaccharide[25]

The matured leaves from *hibiscus* species were collected, washed, dried using tray dryer at 37°C for 24 h, crushed and soaked in different solvents such as water, hot-water, phosphate buffer solution (PBS) of pH 4.0, 6.8, 9.2, separately for 2-3h and heated up to 80-90°C for 30-45min for complete release of the water soluble mucilage/polysaccharide into the solvents. The mucilage/polysaccharide was then extracted by using a multi layer muslin/cheese cloth bag to remove the marc and concentrated viscous solution under reduced pressure at 60-70°C. Acidified ethanol (5% HCl in 75% Ethanol) was added to the concentrated viscous solution with constant stirring. The gel like precipitate was formed and separated by filtration. The precipitate was washed 2-3 times with Ethanol 75% and 95%. After complete washing of the precipitate with Ethanol 95%, creamy white powder was obtained. The powder was dried in an oven at 37°C, collected, grounded, passed through a # 80 sieve and stored in a desiccator till use. The dry white creamy powder was considered as water soluble polysaccharide for pharmaceutical use.

Physicochemical characterizations:

Percent yield

The percentage yield was calculated based on the amount of leaves of *hibiscus* species used for the extraction process and the amount of dry water soluble mucilage/polysaccharide obtained individually depends upon the solvents used. The percentage yield was calculated using the formula mentioned below,

$$\% \text{ yield} = \frac{\text{Wt. of dried mucilage obtained}}{\text{Wt. of Leaves powder taken}} \times 100$$

Chemical tests

Chemical identification tests of the dry water soluble mucilage/polysaccharide such as proteins, aminoacids, alkaloids, carbohydrates, tannins, glycosides, starch, terpinoids, etc were performed using conventional techniques.

Caliberation curve of Ranolazine and Lumefantrine

The stock solutions were prepared by dissolving 100 mg of drug 5ml of methanol and make up the volume to 100ml (1 mg/ml) using distilled/demineralized water for both the drugs. From the stock solutions, 10, 20, 30, 40, and 50 µg/ml dilutions and 2, 4, 6, 8 and 10 µg/ml dilutions were prepared using distilled/demineralized water for Ranolazine and Lumefantrine respectively**.** The λmax of the drugs were determined by scanning one of the dilutions between 200 and 400 nm using a UV-visible spectrophotometer. The wavelengths of λmax at 272 and λmax at 234 were used for Ranolazine and Lumefantrine respectively. The absorbance of all the other solutions was measured against blank.

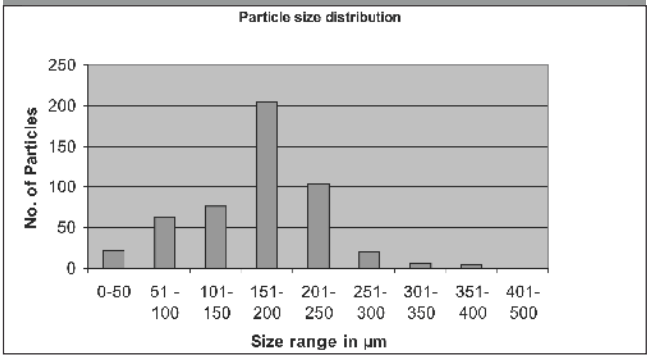
Powder characterization

The bulk characterization of the dry water soluble mucilage/polysaccharide such as Angle of repose, Loose and Tapped bulk density, Carr's index and Hausner's ratio were determined using conventional techniques.[6-7]

Particle size distribution

The particle size distribution of the mucilage/polysaccharide was done conventionally. The mucilage/polysaccharide powder was sprinkled on the glass slide containing glycerin. The particle size of the mucilage/polysaccharide was carried out using calibrated eye piece micrometer. About 500 particles size were counted in different fields. The particle size distribution of the mucilage/polysaccharide shown in Fig. 1.

Fig. I. Particle size distribution of the dry water soluble mucilage/polysaccharide.



Swelling ratio and pH

In this study, 1.0g of dry water soluble mucilage/polysaccharide was placed in a 100-mL stoppered graduated cylinder. The initial bulk volume of the dry mucilage/polysaccharide was measured. 2-mL of alcohol (95%) wais added for good dispersion and then distilled/demineralized water was added to sufficient quantity to yield 100-mL of uniform dispersion. The viscous solution was stored at room temperature and the sediment volume of the swollen mass was noted after 24hr. The swelling ratio was calculated by determining the ratio of the swollen volume to the initial bulk volume using the formula;

$$S = V_2 / V_1$$

Where;

S = Swelling index.

V_1 = Volume occupied by the gum prior to hydration

V_2 = Volume occupied by gum after hydration.

The same process was performed with other solvents such as simulated gastric fluid (0.1 N Hydrochloric acid), and PBS pH 6.8 and 7.4. The swelling study indicates the ability of the polymer to form matrix and dissolving capacity of the entrapped drug molecule in the biological pH system and the results of this study ensure the suitability of polymer for targeting dosage form development.

The pH of 1% w/v solution of the dry water soluble mucilage/polysaccharide in distilled/demineralized water was measured using a calibrated digital pH meter at room temperature. The study was conducted in triplicate to ensure the accuracy.

Rheological study and effect of temperature on viscosity

Rheological study (viscosity) of 1.0% solutions of the dry water soluble mucilage/polysaccharide in distilled/demineralized water was carried out using Brook field viscometer (LVDV-E) at room temperature. The spindle ($S6_1$) was given maximum torque (74%) at 6-10 rpm, therefore, the same conditions were set for the entire study. The same solutions were heated in different temperatures such as 40°C, 50°C, 60°C, 70°C, 80°C and performed the viscosity following the above said procedure. The viscosity of the mucilage/polysaccharide solutions in room temperature and the elevated temperatures are shown in Fig. 2. The study was done in triplicate to ensure the accuracy.

Fourier Transform Infra Red (FTIR)

The FTIR study of the dry water soluble mucilage/polysaccharide obtained from different solvents were performed to ensure the stability while using different solvents for extraction. Study recorded using Simadzu 8400S model from 4000 – 400 as scanning range between wave number (cm-1) and % Transmittance. Samples were prepared in KBr discs (2mg sample in 200mg KBr) with a hydrostatic press at a force of 5t cm-2 for 5min and the resolution was 4cm-1. Experiments were duplicated to check the reproducibility. The spectra of the dry water soluble mucilage/ polysaccharide are shown in Fig. 3.

Fig. 2:Rheological study of the mucilage/polysaccharide solution in Distilled/Demineralised water at room and elevated temperatures.

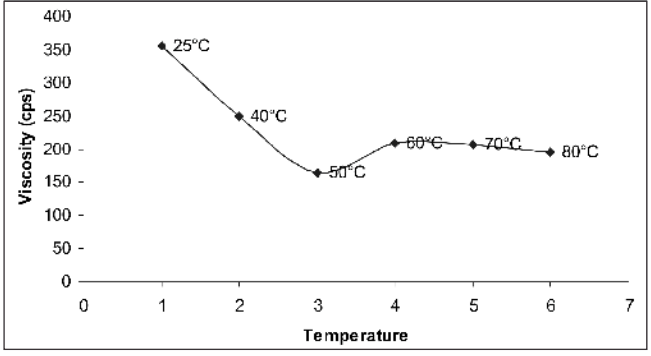
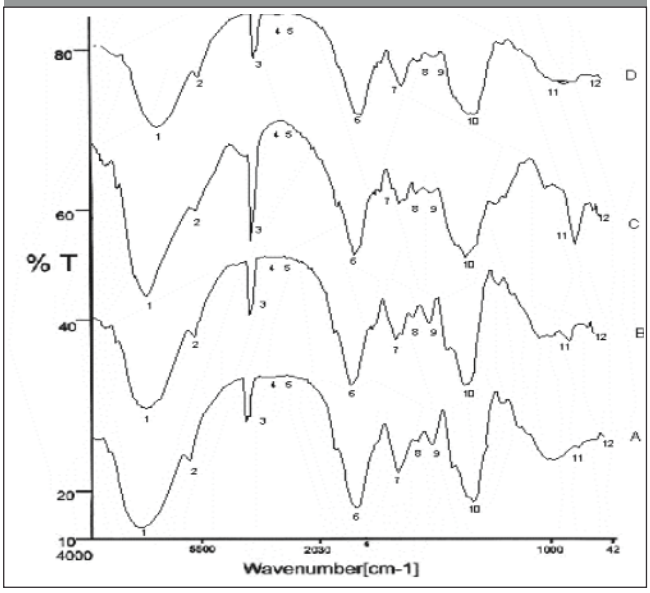


Fig. 3:The spectra of the dry water soluble mucilage/ polysaccharide (A) Extracted by using Distilled/Demineralised water, (B) Extracted by using PBS pH 4.0, (C) Extracted by using PBS pH 6.8, (D) Extracted by using PBS pH 9.2.



Pharmaceutical use as suspending agent

The slightly soluble model drug (Satranidazole) in suspension system comprises of the dry water soluble mucilage/polysaccharide from leaves of hibiscus species was formulated to precisely define the use of this mucilage/polysaccharide as suspending agent. Several batches were formulated using different concentration of the mucilage/polysaccharide. The formula of the suspension formulations is shown in Table 1.

Table 1: Formulas of the satranidazole suspensions.

S.N	Materials	Compositions			
		F1	F2	F3	F4
1	Satranidazole (mg/5-mL)	150	150	150	150
2	Leaves mucilage/polysaccharide (%)	0.5	1.0	1.5	2.0
3	Simple syrup (mL)	25	25	25	25
4	Aspartame (%)	1.0	1.0	1.0	1.0
5	Methyl and Propyl paraben (4:1 ratio)	qs	qs	qs	qs
6	Distilled/Demineralized water	qs	qs	qs	qs

In this study, water was divided into 2 parts. In one part, the dry water soluble mucilage/polysaccharide was added, mixed well and set aside for 2hr at room temperature for complete swelling. In the another part, aspartame and simple syrup was added and it is poured into the first part and mixed well using magnetic stirrer until get uniform viscous solution. The drug was placed in a clean glass mortar and the viscous solution was poured into it while continuous stirring using pestle till get uniform distribution of the drug into the viscous solution. The final product was said to be suspension. The suspension characteristics were evaluated after 24hr to ensure the suspending capability of the mucilage/polysaccharide. The characteristics evaluated on the suspensions are shown in the Table 2.

Table 2: Physicochemical characterization on the formulated suspensions.

S.N	Parameters*	Formulations			
		F1	F2	F3	F4
1.	Drug content (%)	98.4	93.2	97.7	98.1
2.	Sedimentation volume	0.96	0.98	0.93	0.92
3.	Dispersibility	2 stroke	2 stroke	3 stroke	3 stroke
4.	Viscosity	422 cps	422 cps	422 cps	422 cps
5	Dissolution after 1h (%)	87.6	87.6	87.6	87.6

* Tests were carried out in triplicate and the average value is presented.

Drug content

A 5ml suspension was placed in 100ml standard volumetric flask and diluted with saline phosphate buffer pH 7.4 up to the mark, shaken well, filtered, made suitable dilution with the same solvent and the drug content was measured using UV spectrophotometer (Shimadzu-Pharmaspec UV-1700) at λ_{max} 318 nm. The blank was made with the suspension additives with out addition of drug and suitable dilution made as per the above said procedure. All the observations were determined in duplicate.

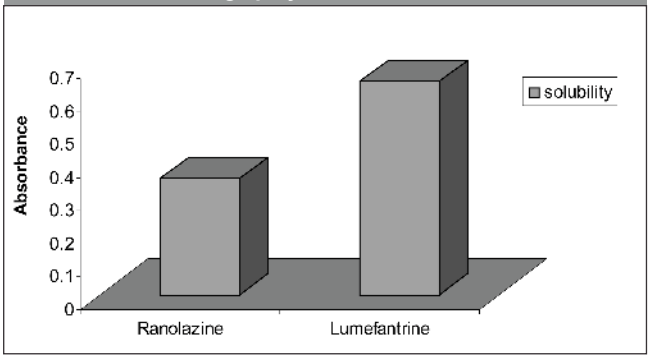
Pharmaceutical use as solubility enhancing agent

The poorly soluble model drugs such as Ranolazine and Lumefantrine are in solubility/bioavailability enhancing system contain dry water soluble mucilage/polysaccharide from leaves of hibiscus species used in different concentration to precisely define the use of this mucilage/polysaccharide as solubility enhancing agent.

In this study, six clean, dry 100-mL volumetric flasks were taken and known concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) of the dry water soluble mucilage/polysaccharide were placed in five flasks and one flask was considered as blank (without mucilage/polysaccharide). Little amount of water was added in the flasks contain mucilage/polysaccharide and allowed it for an hour to swell, some more water was added and shaken well to get almost clear solution. Then, water (approximately 50%) was added in all the flasks and excess amount of the model drug was added into it and intermittently shaken the flasks for 6hr. Set aside the flasks for 24hr at room temperature, after that the flasks were shaken for 5min and filtered through whatman filterpaper (pore size of 0.4mm). From the filtered samples, 1-mL of the solution was taken, suitably diluted, estimated the amount of drug present

and compared the solubility of the drug in blank (distilled/demineralized water). Using same procedure, the solubility of other drug was also performed. The solubility of the drugs in 1% solution of dry water soluble mucilage/polysaccharide is shown in Fig. 4.

Fig. 4: Solubility study of the drugs in 1% solution of dry water soluble mucilage/polysaccharide.



Oral acute toxicity study

The acute toxicity studies were performed by following OECD guidelines (423). Mice used in the toxicity studies were sanctioned by the Institutional Animal Ethics Committee (KU\IAEC\M.PHARM\017\2010). The male albino female mice weighing 25-30gm were divided into four different groups comprising of three animals each. The control group received distilled water and the other groups received 500, 1000 and 2000 mg/kg of mucilage/polysaccharide suspension in distilled water orally. The animals were under observation continuously for the behavioral changes for first 4hr, followed by regular observations every day and then observed for mortality until 14^{th} day.

RESULTS AND DISCUSSION

The powdered dry water soluble mucilage/polysaccharide was extracted from hibiscus species for pharmaceutical use. The product was white creamy powder, acceptable and characteristic odour, mucilaginous taste, and coarse powder. The powder was swells and soluble in water and not soluble in acetone and alcohol.

The mucilage/polysaccharide was extracted using solvents such as distilled/demineralized water, PBS pH 4.0, pH 6.8 and pH 9.2 and the yield of the dry water soluble mucilage/polysaccharide was varied depends up on the solvents used. Percent yield of the dry water soluble mucilage/polysaccharide was 14.7%, 9.3%, 22.8% and 16.4% in distilled/demineralized water, PBS pH 4.0, PBS pH 6.8, and PBS pH 9.2 respectively. The solvents like distilled/demineralized water, phosphate buffer pH 9.2 could be used for extraction for better yield.

Chemical tests were performed on the extracted dry water soluble mucilage/polysaccharide for the identification of molecules such as starch, proteins, flavonoids, tannins, glycosides, terpenoids and alkaloids, etc, which ensured the presence of starch and proteins and absence of flavonoids, tannins, glycosides, terpenoids and alkaloids.

In powder characterization studies, Angle of repose, loose bulk, tapped bulk, corr's index and Housner ratio were performed and the results of the product were 26.32°, 0.54 g/mL, 0.73 g/mL, 22.62%, and 1.08 respectively. The values of bulk characterizations of the dry water soluble mucilage/polysaccharide ensured the suitability of the product to formulate a solid oral dosage forms, i.e., Tablets.

The swelling index of the dry mucilage/polysaccharide was performed in different solvents such as distilled/demineralized water, simulated gastric fluid (0.1 N Hydrochloric acid), and PBS pH 7.4 and results were found to be 82.17%, 57.02%, and 85.14% respectively. The higher swelling index of the dry water soluble mucilage/polysaccharide was in PBS pH 7.4.

The pH of 1% w/v solution of the dry water soluble mucilage/polysaccharide in distilled/demineralized water was 4.82 which indicate the product is acidic in nature.

In Rheological study, the average viscosity of 1.0% solution of the dry water soluble mucilage/polysaccharide was found to be 356cps in distilled/demineralized water at room temperature. In the effect of temperature on viscosity study, as the temperature increases gradually from 40°C to 80°C in the polymer solution, there was great change between 40°C (250cps) and 50°C (164cps) but as the temperature increases from 60°C to 80°C, there was no significant change in the viscosity of the polymer solution (i.e. 200-210cps) was observed.

FTIR study of the dry water soluble mucilage/polysaccharide extracted from different solvents such as distilled water, phosphate buffer pH 4.0, pH 6.8 and pH 9.2 are performed.

The product extracted from distilled water was showed the characteristic peaks (Figure A) with respect to their chemical groups such as 3417 cm^{-1} - OH stretching, intermolecular, dimeric hydroxyl compounds; 2330 cm^{-1} - N-H stretching, 3° amine salt; 1627 cm^{-1} - N-H in plane bending, 1° amides; 1419 cm^{-1} - symmetric -CH_2 bending in (CH_2)-C=O and C-H bending in alkenes; 1249 cm^{-1} - C-O-C stretching in aralkyl

ethers; 1041 cm^{-1} - C-O stretch in dialkyl ethers or saturated cyclic ethers; 640 cm^{-1} - amide OCN deformation.

The product extracted from phosphate buffer pH 4.0 was showed the characteristic peaks (Figure B) with respect to their chemical groups such as 3425 cm^{-1} - OH stretching, polymeric hydroxyl compounds; 2330 cm^{-1}- N-H stretching, 3° amine salt; 1651 cm^{-1} - C=C stretching; 1419 cm^{-1} - symmetric -CH_2 bending in (CH_2)-C=O and C-H bending in alkenes; 1242 cm^{-1} - C-O-C stretching in aralkyl ethers; 1064 cm^{-1} - C-O stretch in dialkyl ethers or saturated cyclic ethers; 516 cm^{-1} - S-S stretching, disulphides.

The product extracted from phosphate buffer pH 6.8 was showed the characteristic peaks (Figure C) with respect to their chemical groups such as 3441 cm^{-1} - OH stretching, polymeric hydroxyl compounds; 2360 cm^{-1} - N-H stretching, 3° amine salt; 1651 cm^{-1} - C=C stretching; 1419 cm^{-1} - symmetric -CH_2 bending in (CH_2)-C=O and C-H bending in alkenes; 1064 cm^{-1} - C-O stretch in dialkyl ethers or saturated cyclic ethers.

The product extracted from phosphate buffer pH 9.2 was showed the characteristic peaks (Figure D) with respect to their chemical groups such as 3425 cm^{-1} - OH stretching, polymeric hydroxyl compounds; 2337 cm^{-1} - N-H stretching, 3° amine salt; 1620 cm^{-1} - N-H in plane bending, 1° amides; 1419 cm^{-1} - symmetric -CH_2 bending in (CH_2)-C=O and C-H bending in alkenes; 1041 cm^{-1} - C-O stretch in dialkyl ethers or saturated cyclic ethers are present.

In the FTIR analysis, products extracted from different solvents were given same characteristic peaks and chemical groups with out major change which ensures the dry water soluble mucilage/polysaccharides stability in all the solvents. Moreover, the mucilage/polysaccharide were contain major functional and structural groups such as polymeric hydroxyl groups (3500-3200cm^{-1}), alkanes (3000-2850cm^{-1}), primary amine groups (1650-1580cm^{-1}), and aromatic ring (1500-1400cm^{-1}) ensures the presence of polymeric chain and the mucilage/polysaccharide could be taken for further chemical modification to achieve the desired purpose.

High swelling in alkaline pH, presence of aromatic and primary amine for azo reaction and an acidic nature of the said mucilage/polysaccharide ensures the suitability to use for colon targeted drug delivery system.

In solubility study, Ranolazine showed 10-fold increased solubility and Lumifenadrine showed 20-fold increased solubility in 1.0% solution of the dry water soluble mucilage/polysaccharide when compared with the drug solubility in blank which ensures the mucilage/polysaccharide to use as a solubility/bioavailability enhancing agent in pharmaceutical formulations.

In suspension formulation study**,** suspension containing 1.0 % to 1.5% of the dry water soluble mucilage/polysaccharide was showed uniform distribution of drug, stable and easy redispersible and good flow from the narrow mouth bottle ensured the suitability of the product to use as suspending agent.

In oral acute toxicity study, no mortality and toxic manifestations were observed with maximum dose (2000mg/kg) and behavioural pattern was unaffected. Therefore, the mucilage/polysaccharide extracted from the leaves of *Hybiscus* could be opted as an additive for any dosage form for human use.

CONCLUSION

Overall results of the study, the crude, pharmaceutically useful, dry water soluble natural mucilage/polysaccharide extracted from leaves of hibiscus species which is an agriculture waste but as a potential source of a mucilage/polysaccharide in order to use as a multiple purpose excipient for pharmaceutical formulations. The mucilage/polysaccharide could be used to enhance the solubility/bioavailability of poorly soluble drugs and as a suspending, thickening agent for pharmaceutical suspension formulations due to the high viscosity and swelling characteristics. The bulk powder characterizations ensures that the mucilage/polysaccharide could be considered as an alternative to disintegrant, matrix former, binder in solid oral formulations with economic, toxicity less and biocompatible. Since, the mucilage/polysaccharide is an acidic and shown good swelling capability at alkaline pHs, having polymeric hydroxyl and aromatic amine groups as per the FTIR study, it could also be considered for colon targeted delivery system as an drug carrier or coating polymer. In our future study, it is planned to extract the mucilage/polysaccharide without proteins. Moreover, the mucilage/polysaccharide could be chemically modified to use as a drug carrier/coating polymer for colon drug delivery systems.

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