Study of raft forming anti-reflux preparation of mucilage from *Basella alba* L.

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Abstract

Ceylon spinach (*Basella alba*) is a plant in the Basellaceae family. The arial part of the plant contains a large amount of mucilage, consisting of polyuronides that may have raft-forming property. The objectives of this research were to study the physico-chemical properties of mucilage isolated from Ceylon spinach, to formulate suspension of the mucilage and to evaluate the raft-forming effect of the suspension via in vitro. The results showed that the isolated mucilage had a swelling index of 10.07 ± 0.03, pH 6.61 ± 0.11, and loss on drying of 8.95% ± 0.51. The chemical composition of the mucilage was determined by thermal hydrolysis and analyzed by thin layer chromatography. TLC chromatogram revealed bands corresponded to glucose and galactose. Suspensions were prepared using the isolated mucilage at the concentrations of 6%. Different types and concentrations of suspending agents, hydroxypropyl methylcellulose (HPMC) at 0.75% and 1.00% and carboxymethylcellulose sodium (Na CMC) at 0.50% and 0.75% were used. It was found that the formulation with the best physico-chemical properties was the one containing Na CMC at 0.50%. The percentage of sedimentation volume of the suspension was 44%. It was easily redispersed by 10 times of human shaking. However, raft-forming performed under simulated gastric acid conditions revealed that no raft was formed in all formulations.

Keywords : Basella alba, mucilage, raft forming, heartburn, swelling index

1. Introduction

Heartburn and acid reflux are primary symptoms of gastro-oesophageal reflux disease (GERD). Reflux of gastric acid into oesophagus most often occurs after meals. A variety of non-prescription products are used for the symptomatic treatment of heartburn, acid indigestion, and acid reflux disorders such as antacids, bismuth-containing products, and alginate rafting products. Alginate rafting products provide rapid onset and longer duration of action by a physical rather than a chemical or pharmacological mechanisms. Alginates isolated from brown seaweed are natural polysaccharide polymers composed of L-guluronic and D-mannuronic acid. Alginates and alginic acids rapidly form viscous gel with low density in the acid environment of stomach. Molecular weight and the ratio of L-guluronic and D-mannuronic acid residues of alginate affect the strength of the gel. In addition, calcium shows ability to cross-link alginic acid polymers to form raft and increase the raft strength. The alginate raft was proved to provide sufficient viscosity to reduce gastric acid reflux disorders (Mandel, 2000, p.669–690, Yaswantrao, 2015, p.178-192).

Basella alba L. is a perennial succulent vine which belongs to family Basellaceae. It is commonly known as Ceylon spinach, Malabar spinach, vine spinach, or Puk Pang Kao (Thai). The fresh leaf, thick heart-shaped with mucilaginous texture is used as vegetable. Several phytochemical compounds such as proteins, alkaloids, polysaccharides, phenols, flavonoids, and carotenoids were found in the plant (Kumar, 2013, p.53-58, Deshmukh, 2014, p.153-165). Mucilage isolated from *B. alba* is composed of D-galactose as a major component. It is viscous with low swelling capacity and exhibits suitable pH for skin (5.3 - 5.4). The mucilage was reported to use as gelling agent in cosmetics and provided a good stability (Palanuvej, 2009, p.837-850, Haneefa, 2012, p.1642-1648). Additionally, mucilage can be used for pharmaceutical aid as thickener, water-retention agent, suspending agent, and film former (Jani, 2007, p.90-98, Tosif, 2021, p.1-24). The present study was to isolate mucilage from stems and leaves of *B. alba* and the isolated mucilage was subjected to formulate the raft forming anti-reflux preparations. As well as physical properties of the preparations were studied.

2. Objectives

The objectives of the study are to formulate raft forming anti-reflux preparations using isolated mucilage from stems and leaves of *B*. alba and to evaluate physical properties of the preparations.

3. Materials and methods

3.1 Chemicals

Glucose, galactose, fructose, and arabinose were purchased from Sigma-Aldrich. Excipients used for preparation of suspension were hydroxypropyl methylcellulose (HPMC; from Dow company, USA), sodium carboxymethyl cellulose (Na CMC; from Shandong Yulong chemical technology, China), sodium bicarbonate (from Tokuyama corporation, Japan), calcium carbonate (from Konoshima chemical, Japan), and saccharin sodium (from S. Tong chemicals, Thailand). All other chemicals and reagents used were of analytical grade. Deionized water was used throughout the experiments.

3.2 Plant material

The fresh stems and leaves of *Basella alba* L. planted in Huachiew Chalermprakiet University, Bang Phli district, Samut Prakan province, Thailand were used in this study.

3.3 Extraction and isolation of mucilage from *Basella alba* L.

The fresh stems and leaves were rinsed with tap water and dried at 45°c using hot air oven before grounded into coarse powder by blender. The plant powder (1.543 kg) was defatted by soaking in petroleum ether (4 L) overnight. The defatted material was sent to extract by boiling in deionized water (18 L) for 30 minutes. After the extract was cool to room temperature, it was filtered through eight-fold of muslin cloth. Ethanol was poured into the filtrate in the ratio of (1:1) and gently mixed. For complete precipitation, the mixture was kept in an airtight container at 2°c for 2 days. Then precipitate was separated and washed with cold ethanol for three times before dried at 40°c. The dry precipitate was ground and passed through a 100-mesh sieve.

3.4 Evaluation of physicochemical properties of the isolated mucilage

Physical appearance of the isolated mucilage was evaluated by observing its color, odor, and consistency.

3.4.1 pH measurement

The isolated mucilage (0.25 g) was dispersed in 25 mL deionized water and sent to measure pH value using pH meter at room temperature.

3.4.2 Swelling index determination

The initial volume of the isolated mucilage (1 g, accurately weighed) was measured using a 25 mL graduated cylinder. Ethanol (1 mL) was added to moisten the extract and 25 mL deionized water was added. The mixture was shaken vigorously every 10 minutes for 1 hour then allowed to stand for 24 hours at room temperature. After 24 hours, the sediment volume was measured, and the swelling index was calculated by taking the ratio of swollen volume to the initial volume of 1 g.

3.4.3 Weight loss on drying

One gram of the isolated mucilage, accurately weighed, was used to determine weight loss on drying. Sample was oven-dried at 105°c for 2 hours until constant weight was obtained (± 0.0005 g). The percentage loss on drying was calculated by the equation as shown below.

$$\% \text{ Loss on drying} = 100 (W_1\text{-}W_2)/W_1$$

Where: W_1 = initial weight of sample, g
W_2 = final weight after drying, g

3.4.4 Thin layer chromatography analysis

The isolated mucilage (30 mg) was hydrolyzed with 3 mL of 10% hydrochloric acid at 100°c for 2 hours and concentrated before analyzing by thin layer chromatography. Glucose, galactose, fructose, and arabinose (10 mg/mL) were used as reference standards.

Chromatography was performed on 10 x 10 cm aluminum plates precoated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Germany). Sample and standard solutions, each 1 µL, were applied on the plates as 2 mm wide bands, positioned 10 mm from lower edge of the plate. The mobile phase was acetonitrile-water 8.5:1.5 (v/v). Pre-saturated chamber (30 minutes at room temperature) was used for development of the plates with a distance of 95 mm from lower edge of the plate using ascending mode. The developed plates were detected with color reaction using 10% aqueous sulfuric acid, the color reaction occurred when heat at 105°c for 15 minutes.

3.4.5 Fehling's test for monosaccharides

Fehling's reagent was freshly prepared by combining solution A (7 g of copper sulfate in 100 mL water) and B (35 g of potassium tartrate and 12 g of sodium hydroxide in 100 ml water) in the ratio of 1:1. One mL of Fehling's reagent was added to 2 mL of hydrolyzed mucilage and mixed. The mixture was heated in water bath for 5 minutes. The brick red precipitate would confirm the presence of monosaccharides.

3.4.6 Fourier transform infrared spectral analysis

Functional groups presenting in the isolated mucilage were determined by fourier transform infrared (FTIR) spectrometer (PerkinElmer Spectrum 100). The spectrum was recorded between 4000 and 650 cm^{-1}.

3.5 Preparation of raft forming anti-reflux suspension using the isolated mucilage

Five different formulations (F1 – F5) were prepared using the isolated mucilage at the concentration of 6% w/v as shown in table 1. HPMC and Na CMC were used as suspending agents in different concentrations. Calcium carbonate was used as antacid. Sodium bicarbonate was used as carbon dioxide generating agent. Methyl- and propyl-paraben were used as preservatives. Saccharin sodium and peppermint oil were used as sweetening and favoring agents, respectively. Primarily the isolated mucilage and suspending agents each was separately dispersed in DI water before mixing. Calcium carbonate, sodium bicarbonate, and saccharin sodium were dissolved in DI water and added. Then, the remain excipients were added and stirred continuously till uniform dispersion was obtained.

Table 1 Composition of raft forming anti-reflux suspensions (F1 - F5)

Excipients	Amount (%W/V)				
	F1	F2	F3	F4	F5
Isolated mucilage	6.00	6.00	6.00	6.00	6.00
HPMC	-	0.75	1.00	-	-
Na CMC	-	-	-	0.50	0.75
Calcium carbonate	1.60	1.60	1.60	1.60	1.60
Sodium bicarbonate	2.67	2.67	2.67	2.67	2.67
Saccharin sodium	1.00	1.00	1.00	1.00	1.00
Peppermint oil	0.20	0.20	0.20	0.20	020
Methylparaben	0.40	0.40	0.40	0.40	0.40
Propylparaben	0.06	0.06	0.06	0.06	0.06
DI water q.s.	100.00	100.00	100.00	100.00	100.00

3.6 Evaluation of physical properties of suspension

3.6.1 Physical appearance

Physical appearance of suspension was evaluated by observing its color, odor, and consistency.

3.6.2 pH measurement

Suspension was sent to measure pH value using pH meter at room temperature.

3.6.3 Sedimentation volume

Each formulation (25 mL) was transferred to a 25 mL graduated cylinder and allowed to stand at room temperature without agitation. The volume occupied by the solute was recorded at 1, 4, 8, and 12 weeks. The percentage sedimentation volume was calculated by the equation as shown below.

$$\% \text{ Sedimentation volume} = 100 (V_u)/V_o$$

Where: V_u = ultimate sediment volume, mL

V_o = original sediment volume, mL

3.6.4 Redispersibility rate

Each formulation (25 mL) was transferred to a 25 mL graduated cylinder and allowed to stand at room temperature. On 1, 5, 10, 15, 20, 30, and 45 days the cylinders were moved upside down until the sediment was uniform redispersed and the number of times used to invert the cylinders was recorded.

3.6.5 Raft forming

Each formulation (2 mL) was added in 0.1 M hydrochloric acid (10 mL) and maintained at 37°c in 30 mL glass beaker for 10 minutes. The character of raft formation was observed and recorded.

4. Results and discussion

The plant powder (1.543 kg) was defatted with a non-polar solvent, petroleum ether to remove all non-polar compounds before extracted by boiling in deionized water. The aqueous extract was viscous liquid after left to cool at room temperature. And mucilage was isolated from the aqueous extract by ethanol precipitation at low temperature (2°c). The isolated mucilage (76.88 g) was obtained as brown to grey green fragment shown in figure 1 with the percentage yield of 4.98% w/w of plant powder. The percentage loss on drying of the mucilage was 8.95% ± 0.51. The pH and swelling index of the mucilage dispersion were 6.61 ± 0.11 and 10.07 ± 0.03, respectively. The acid-hydrolysis mucilage was tested with Fehling's reagent and the brick red precipitates occurred that confirmed the presence of monosaccharides. Additionally, thin layer chromatography analysis of the acid-hydrolysis mucilage exhibited spots comparable to glucose and galactose which in agreement with previous reports. Chatchawal (2010) indicated that D-galactose was the major monosaccharide in the mucilage extracted from stems and leaves of *B. alba* by TLC analysis (p.101-112). Gas chromatography analysis of mucilage extracted from aerial parts of *B. alba* revealed the composition of galactose, glucose, arabinose, rhamnose, and galacturonic acid in the ratio of 41:16:24:5:13 (Palanuvej, 2009, p.837-850).

The FTIR spectrum of the isolated mucilage indicated the presence of stretching vibration of hydroxyl groups (O-H) at 3,369 cm^{-1} (broad), stretching vibration of alkyl group (C-H) at 2,928 cm^{-1}, stretching vibration of carboxyl and carbonyl (COO^-) at 1,638 cm^{-1}, bending vibration of alkyl group (C-H) at 1,369 cm^{-1}, and polysaccharide group (C-O-C) at 1,015 cm^{-1}. The band corresponding to carboxylate group indicated the presence of uronic acid which is commonly found in mucilage (Quintero-García, 2021, p.1-18). As well as the wavenumbers between 800-1200 cm^{-1} characterizes the fingerprint region for carbohydrate (Singh, 2014, p.713-725).

Figure 1 The isolated mucilage from *Basella alba* L.

Five different formulations (F1 – F5) were prepared using the isolated mucilage at the concentration of 6% w/v. Among five formulations of raft forming anti-reflux suspensions, F1 did not contain any suspending agents, and consequently the isolated mucilage could not suspend throughout the liquid media. Accordingly, F1 was not subjected to further studies. Physical appearance and pH of F2 - F5 are shown in table 2. The viscosity of all suspensions increased when the amount of suspending agents increased. Formulations prepared with HPMC (F2 and F3) were found to be more viscous than that with Na CMC (F4 and F5). The highest viscosity was shown in F3 whereas the lowest viscosity was shown in F4.

Table 2 Physical appearance and pH of suspensions (F2 - F5)

Physical appearance	F2	F3	F4	F5
Color	Brown	Brown	Brown	Brown
Odor	Peppermint	Peppermint	Peppermint	Peppermint
Taste	Sweet	Sweet	Sweet	Sweet
Viscosity	+++	++++	+	++
pH*	9.12 ± 0.01	9.11 ± 0.01	9.13 ± 0.01	9.12 ± 0.01

(+) = very low; (++) = low; (+++) = moderate; (++++) = high

* Values are presented as mean ± SD; n = 3

The percentage sedimentation volume of suspensions (F2 - F5) over storage period of 2 months are shown in table 3. The results showed that sedimentation volumes of suspensions decreased with an increase time of storage and varied depend on concentrations and types of suspending agents. The sedimentation volumes of F2 and F3 which used HPMC as suspending agent were higher than of F4 and F5 which used Na CMC as suspending agent. At 4 weeks of storage, the sedimentation volumes of F4 and F5 were constant.

Table 3 The percentage sedimentation volume of suspensions (F2 - F5)

Periods	Sedimentation volume (%V/V)			
	F2	F3	F4	F5
1 weeks	60	62	46	42
4 weeks	56	56	44	40
8 weeks	54	54	44	40

Redispersibility rates of each suspension after stand for several days at room temperature were shown in figure 2. The results showed that the redispersibility rates of suspensions increased when the storage periods increased. Furthermore, F4 and F5 which used Na CMC as suspending agent were easily redispersed than F2 and F3 which used HPMC as suspending agent. At 45 days of storage, F4 containing Na CMC at 0.50%. was easily redispersed by 10 times of human shaking.

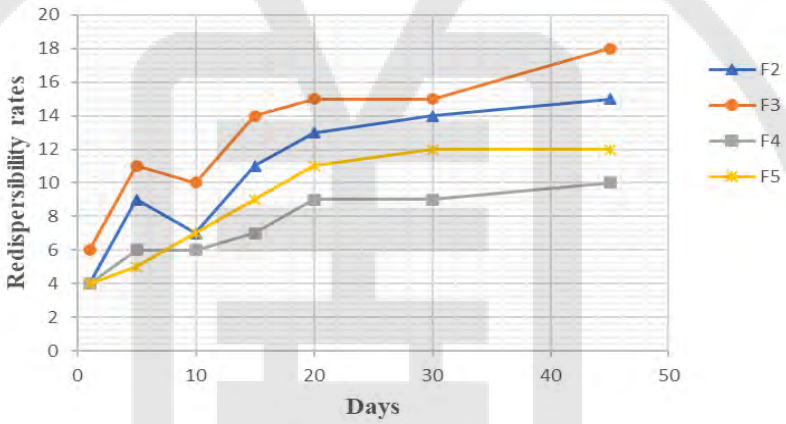

Figure 2 The redispersibility rates of suspension (F2 - F5) after stand for 1, 5, 10, 15, 20, 30, and 45 days at room temperature.

Raft forming systems have been used in the treatment of heartburn and acid reflux which are the symptoms of gastro-oesophageal reflux disease (GERD). Alginates, natural polysaccharide polymers from brown seaweed, have been used to form raft that can act as a physical barrier to reduce the acid reflux. The mechanism involved in the raft formation includes the formation of viscous cohesive gel in contact with gastric fluids, wherein each portion of the liquid swells forming a continuous layer called a raft. The raft floats on the gastric fluids and acts as a barrier between the stomach and oesophagus to prevent the reflux of the gastric acid into the oesophagus (Yaswantrao, 2015, p.178-192). The character of raft formation of each formulation (F2 - F5) was observed under simulated gastric acid conditions, 0.1 M hydrochloric acid at 37°c for 10 minutes. The results showed that no raft was formed in all formulations. This may be due to the amount of calcium carbonate and sodium bicarbonate in each formulation is not enough to provide raft. Raft formation and raft strength depend on several factors. Calcium ion was reported as an extrinsic factor which increase raft strength by its ability to cross-link polysaccharide polymers. Bicarbonate, another extrinsic factor, acts as a carbon dioxide generating agent which produces carbon dioxide bubbles in the presence of gastric acid. The bubbles are entrapped within the gel matrix allowing the gel to float on the surface of gastric fluid (Mandel, 2000, p.669–690). In addition, the concentration of mucilage as well as its physicochemical properties also impact to raft formation. The process used for drying mucilage after isolation from plant material influences its physicochemical properties. Conventional drying using an oven causes the collapse of the porous structure of hydrogel which produces a massive volume shrinkage due to the capillary pressure gradient established during the solvent removal. This phenomenon can reduce the consistency and water holding capacity of the mucilage that has a highly aggregated and densely packed without pore (Auriemma, 2020, p.3156).

5. Conclusion

The isolated mucilage from stems and leaves of *B. alba* was composed of glucose and galactose with a swelling index of 10.07 ± 0.03, pH 6.61 ± 0.11, and loss on drying of 8.95% ± 0.51. Suspension containing 6% of the isolated mucilage and 0.5% of Na CMC exhibited good physico-chemical properties. It was easily redispersed by 10 times of human shaking with the percentage of sedimentation volume of 44%. However, raft-forming performed under simulated gastric acid conditions revealed that no raft was formed in all formulations. Different concentrations of mucilage and carbon dioxide generating agent for formulation of good raft forming anti-reflux preparation should be further study.

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