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Extraction and preliminary phytochemical identification of mucilage of *abelmoschus esculentus* as a binder

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Introduction

Natural mucilage is generally normal products of metabolism, formed within the cell (intracellular formation) and/or are produced without injury to the plant[1]. Those are polysaccharide hydrocolloids that slightly soluble upon contact with water and form colloidal solutions. Mucilage is widely used for various applications in cosmetic, food and pharmaceutical industries. Advantages of natural mucilage are biodegradable, less expensive, non-toxic, local availability and edible sources.

Abelmoschus esculentus (Okra, Lady's finger) also known as Krajeab Keaw. Krajeab Keaw is a flowering plant in the Malvaceae family. The physical appearance is green seed pods. Immature fresh and green seed pods are consumed as edible vegetable. It offers mucilaginous consistency after cooking[2]. The medicinally use for plasma expander[3], diuretic and medicinal agent in dental disease (Ndjouenkeu, 1996). Mucilage of *Abelmoschus esculentus* have been reported to have binder for tablet formulation such as furosemide, diclofenac sodium, ibuprofen[4-7] and may be used as a pharmaceutical adjuvant and as a suspending agent in paracetamol suspension[8].

Although some reviews are available describing medicinal properties and some phytochemical of *Abelmoschus esculentus*, but no specific review are present describing phytochemical screening which cultivated and harvested in Thailand. Therefore, the present work was designed to extraction and identification of mucilage from *Abelmoschus esculentus* for the safe and effective material for drug delivery system.

Methods

Extraction of the mucilage

Abelmoschus esculentus fruits were collected from Pathumthani province, Thailand. Cultivated and harvested during August to May. *Abelmoschus esculentus* fruits were cleaned several times with water and sliced to small size (thickness 1-2 mm.). Then, homogenized and extracted with 1% w/v sodium metabisulphite in cold water (4±0.5 °C) with Tefal blender BL312. The crude mucilage was centrifuged (centrifuge, universal 320R Hettich zentrifugen) at 4000 rpm for 10 minutes. The mucilage was filtrated using a multilayer muslin cloth for remove marc from solution. The mucilage was precipitated from the supernatant with acetone and washed several times with acetone until presented white color product. Filtered by using vacuum pump and dried under vacuum in a desiccator. The dried powder was screened through the 80 mesh stainless steel sieve. The dried powder of *Abelmoschus esculentus* was stored in a well-closed container until use.

Phytochemical identification

The phytochemical identification tests confirmed the carbohydrates, proteins, amino acids, alkaloids, mucilage, starch, flavonoids, cardiacglycosides) stearoids, lactone ring and deoxy sugar(and tannins. Phytochemical identification was determined using the appropriate techniques.

Test for carbohydrates

Benedict's test: 25 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with a few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath. Observed for reddish brown precipitate indicates the presence of carbohydrate.

Molisch's test**:** 25 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with a few drops of Molisch's reagent and followed by addition of 1 mL of concentrated sulphuric acid**.** The mixture was then allowed to stand for 2 minutes**.** Observed for red or violet ring color indicates the presence of carbohydrate.

Test for proteins and amino acids

Xanthoproteic test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with 1 mL of concentrated nitric acid and boiled in water bath. Observed for yellow to orange color indicates the presence of protein (tyrosine or tryptophan).

Biuret test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with 1 mL of 10% sodium hydroxide solution and few drops of 0.7% copper sulphate solution. Observed for pink to purplish violet color indicates the presence of protein.

Ninhydrin test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with 0.25% Ninhydrin reagent and boiled in water bath. Observed for purple to blue color indicates the presence of protein (free amino acid).

Test for alkaloids

5 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with 5 mL of 10% hydrochloric solution and boiled in water bath for 15 minutes. The solution was filtered and made alkaline by adding 2 drops of concentrated ammonia solution. The filtered was partitioned between chloroform. The chloroform layer was extracted with 10 mL of acetic acid. The extracts were then used for the following test:

Dragendoff's test: The extract solution was mixed with a few drops of dragendoff's reagent. Observed for reddish brown precipitate indicates the presence of alkaloids.

Hager's test: The extract solution was mixed with a few drops of Hager's reagent (saturated picric acid solution). Observed for yellow precipitate indicates the presence of alkaloids.

Mayer's test: The extract solution was mixed with 1 mL of Mayer's reagent. Observed for greenish or cream color precipitate indicates the presence of alkaloids.

Valser's test: The extract solution was mixed with a few drops of Valser's reagent. Observed for white color precipitate indicates the presence of alkaloids.

Wagner's test: The extract solution was mixed with 1 mL of Wagner's reagent. Observed for reddish-brown precipitate indicates the presence of alkaloids.

Marme's test: The extract solution was mixed with a few drops of Marme's reagent. Observed for white color precipitate indicates the presence of alkaloids.

Test for mucilage

Ruthenium red test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with a few drops of Ruthenium red solution. Observed for pink color indicates the presence of gums and mucilage.

Test for starch

Iodine test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with a few drops of dilute iodine solution. Observed for blue color indicates the presence of starch.

Test for flavonoids

Shinoda's test: 25 mg of dried mucilage powder was dissolved in 2 mL of ethanol, added with a few magnesium ribbon and followed by addition of 1 mL of concentrated hydrochloric acid**.** Observed for pink or red color indicates the presence of flavonoids**.**

Alkaline reagent test**:** 25 mg of dried mucilage powder was dissolved in 2 mL of ethanol, mixed with 2 drops of 10% NaOH solution. Observed for yellow solution indicates the presence of flavonoids and followed by addition of 1 mL of concentrated sulphuric acid becomes colorless**.**

Test for cardiac glycosides

Lieberman-Burchard test: 25 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with 2 mL of acetic acid and followed by addition of 1 mL of concentrated sulphuric acid. Observed for brownish-red ring and greenish ring in acetic acid layer indicates the presence of sterols and triterpenes.

Kedde test: 25 mg of dried mucilage powder was dissolved in 2 mL of methanol, mixed with 1 mL of 2% solution 3, 5-dinitrobenzoic acid in methanol and 1 mL of a 5.7% aqueous sodium hydroxide. Observed for violet color indicates the presence of carbenolide. And reddish-brown to brownish-yellow precipitate indicates the presence of lactone ring.

Keller-Killiani test: 25 mg of dried mucilage powder was dissolved in 2 mL of methanol, mixed with a few drops of glacial acetic acid and ferric chloride solution and followed by addition of 1 mL of concentrated sulphuric acid. Observed for reddish brown and bluish green layer indicates the presence of glycosides.

Test for tannins

Ferric chloride test: 100 mg of dried mucilage powder was dissolved in 5 mL of distilled water, boiled and filtered through filter paper. A few drops of 0.1% ferric chloride were added. Observed for blue or greenish-black color indicates the presence of tannins.

Formulation and evaluation of tablets

Table 1 Formula of tablet

Ingredients	Weight per tablet (mg)		Categories
	F1	F2	
Microcrystalline cellulose	567	564	Diluent
Talcum	30.0	30.0	Diluent/Glidant
Magnesium sterate	3.0	3.0	Lubricant
Abelmoschus esculentus mucilage powder	-	3.0	Binder
Total	600	600	-

All ingredients as shown in Table 1 will be mixed and compressed by direct compression using a single punch tablet machine to make a tablet. Humidity in the room will be controlled to be lower than 30%RH. The evaluation data composed of weight variation, thickness, hardness, friability, and disintegration time.

Results

Extraction of the mucilage

The physical appearance of *Abelmoschus esculentus* mucilage was fine, white in color and hygroscopic. The yield of crude *Abelmoschus esculentus* mucilage was 2.25 g /kg immature fruits.

Table 2 Phytochemical identification

Identification tests	Name of tests	Observations
Test for carbohydrates	Benedic test	+
	Molisch's test	+
Test for proteins and amino acids	Xanthproteic test	+
	Biuret test	+
	Ninhydrin test	+
Test for alkaloids	Dragendorff's test	-
	Hager's test	-
	Mayer's test	-
	Valser's test	-
	Wagner's test	-
	Marme's test	-
Test for mucilage	Ruthenium red test	+
Test for starch	Iodine test	-
Test for flavonoids	Shinoda test	+
	Alkaline reagent test	+
Test for cardiacglycosides	Liebermann-Burchard test	-
	Kedde test	-
	Keller-Killaini test	-
Test for tannins	Ferric chloride test	-

Table 3 Data of physical properties

Physical properties	F1	F2
Weight variation (mg)	608.20±0.007	594.81±0.007
Thickness (mm)	6.01±0.07	5.98±0.08
Hardness (kP)	2.18±0.67	2.05±0.34
Friability (%)	0.58	0.19
Disintegration time (mins)	7.03±0.02	8.52±0.03

Discussion

Phytochemical tests carried out on *Abelmoschus esculentus* mucilage confirmed the carbohydrates, proteins, amino acids, alkaloids, mucilage, starch, flavonoids, cardiacglycosides (stearoids, lactone ring and deoxy sugar) and tannins. The results of phytochemical identification of mucilage are summarized in Table2. The phytochemical tests comfimed the absence of alkaloids, starch, cardiacglycosides (stearoids, lactone ring and Deoxy sugar) and tannins. Identification test showed the presence of carbohydrates, proteins and amino acids, mucilage and flavonoids. In this study, ninhydrin test showed the presence of proteins and amino acids that different from the previous study[8-9]. Therefore, identification test of proteins and amino acids were repeated by xanthproteic test and biuret test. The results confirmed the presence of proteins and amino acids. Test for flavonoids, shinoda test were selected for these chemical. The test showed the presence of flavonoids that different from the study of Ravi, 2009[8]. Repeated with alkaline reagent test, the results confirmed the presence of flavonoids.

Tablets were prepared by direct compression method. The physical appearance of tablets were round shape, white (F1), off white (F2) in colour and smooth surface. The physical properties of F1 and F2 were compared and shown in Table 3. All of formulations presented the weight variation, thickness, hardness, friability and disintegration time were within the pharmacopoeial limits. In preparation process, F1 was found capping problem but not found in F2. F2 was composed of 0.5% w/w *abelmoschus esculentus* mucilage powder. The effect of mucilage on tablets could be decrease friability from 0.58% (F1) to 0.19% (F2). Moreover, it had given increase in disintegration time from 7.03±0.02 (F1) to 8.52±0.03 (F2). Therefore, *Abelmoschus esculentus* mucilage was exhibited binder properties.

Conclusion

Abelmoschus esculentus fruits were local availability. Tablet of *abelmoschus esculentus* mucilage as a binder was successfully prepared to achieve friability decrease. The mucilage obtained from the fruits or seed pods is a promising natural binder, suspending agent and sustained release agent. The extraction method was simple and found to remain stable in desiccators. The phytochemical properties presented that non-toxic. The mucilage of *Abelmoschus esculentus* as a natural alternative polymers for binder, suspending agent, sustained release agent in pharmaceutical industries.

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