Original Article

คุณสมบัติทางกายภาพและชีวภาพของสารสกัดเมือกจากลำต้นผักปลัง และการเตรียมเป็นผลิตภัณฑ์เจล

Physical and Biological Properties of Mucilage from *Basella alba* L. Stem and Its Gel Formulation

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บทคัดย่อ

ผักปลัง *(Basella alba* Linn*)* เป็นพืชวงศ์ Basellaceae ประกอบด้วยสารเมือกปริมาณมากในส่วนต่างๆ ของต้น ซึ่งเป็นสารกลุ่มโพลีแซคาไรด์ที่ละลายน้ำ สารเมือกสกัดด้วยน้ำเมื่อทำให้แห้งมีคุณสมบัติในการดูดความชื้นสูง พองตัวในน้ำได้ มีค่า Swelling capacity เท่ากับ 4.33 $\pm$ 0.29 mL/g dry weight เมื่อละลายน้ำได้สารละลายที่มีความหนืด มี pH ในช่วง 5.3-5.4 และสามารถจับตัวเป็นแผ่นฟิล์มเมื่อแห้ง สารโพลีแซคาไรด์ที่ได้จากการแยกสารเมือกด้วย Anion exchange chromatography เมื่อไปวิเคราะห์ ด้วยวิธี Thin layer chromatography พบว่ามีองค์ประกอบหลักเป็นน้ำตาลกาแลคโตส สารสกัดเมือกจากผักปลังมีฤทธิ์ต้านอนุมูลอิสระเมื่อทดสอบด้วยวิธี DPPH scavenging assay (IC_{50} = 514.41 µg/mL) แต่ไม่แสดงฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนส การศึกษาการมีชีวิตรอดของเซลล์เพาะเลี้ยงตับ (Chang liver cell) เมื่อได้รับสารสกัดเมือกของผักปลัง พบว่าที่ความเข้มข้นของสารสกัด 2 mg/mL ร้อยละการรอดชีวิตของเซลล์มีค่า 84.4% ซึ่งแสดงความเป็นพิษในระดับต่ำ ผลการเตรียมเจลเบื้องต้นจากสารสกัดเมือกจากผักปลัง สำหรับการวิจัยขั้นต่อไปเพื่อพัฒนาเป็นเครื่องสำอางและยาใช้ภายนอก ได้เจลที่มีคุณสมบัติทางกายภาพที่ดีและมีความคงตัว

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Abstract

Basella alba Linn is a succulent mucilage plant in Basellaceae family. Its mucilage is the water soluble polysaccharide. The aqueous extracts from stem of *Basella alba* Linn were investigated for the general physical properties and pharmacological activities. Lyophilized mucilage extract was hygroscopic and could swell in water with a swelling capacity of 4.33 $\pm$ 0.29 mL/g dry weight. Its aqueous solution was viscous with pH 5.3-5.4 and film forming upon drying. The TLC analysis of hydrolyzed polysaccharide which was purified by using anion exchange chromatography yielded D-galactose as the major component. *Basella alba* extract presented antioxidant activity by DPPH scavenging assay (IC_{50} = 514.41 μg/mL) but not antityrosinase. The mucilage extract 2 mg/mL exhibited relatively mild toxicity to Chang liver cell with 84.4% cell viability. Preliminary gel formulations from *Basella* mucilage for further development as cosmetic and topical medicine showed good physical properties and stability.

Keywords : *Basella alba* Linn, Mucilage, Polysaccharide, Antioxidant activity, Cell viability
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Introduction

Basella alba Linn. (Indian Spinach, Phak Plang Khaw, Phak Plang Daeng) belongs to family Basellaceae with mucilage contained in most parts of the whole plant. In Thailand, there are 2 distinctive types of *B. alba* as the colors of the stem appeared in red and green. However, they are classified to be the same species of *B. alba* which *B. rubra* is its synonym (Smitinand, 1992). This plant serves as a Thai traditional vegetable. The fruit provides dark violet color for food colorant. *Basella* mucilage has been used in Thai traditional medicine as topical application for irritant, bruise, ringworm, and laboring. Stem and leaves are used as mild laxative, diuretic, and antipyretic (Chote-Anan et al, 2550). In India, it has been used for antipruritis, and burn (Saikia et al, 2006), and has been used in Bangkladesh for acne and freckle treatment (Akhter, 2008). The Ayurvedic treatment in India has been used *B. alba* leaves and stem for anticancer such as melanoma, leukaemia, and oral cancer (Premalatha and Rajgopal, 2005). *B. alba* contains basellasaponins (Toshiyuki, 2001), amino acid such as arginine, leucine, isoleucine, lysine, threonine and tryptophan (Khare, 2007), peptide, phenolic compounds in 95% extract (15.5 mg GAE/g DW) (Maisuthisakul et al 2008). *Basella* fruit contains gomphrenin derivative which is betalain pigment (Glassgen et al., 1993). The mucilage of *B. alba* consists of mixture of polysaccharides 2.6-5.35% (Palanuvej et al 2009; Lin et al 2009); and starch-type glucan which can be separated by starch iodine complex (Haq et al., 1969). Plant mucilage composes of water soluble polysaccharide. It functions as water retention, germination, food reservoir and secondary metabolite storage. The sticky properties of mucilage can be used as medicine and cosmetic proposes, for example marshmallow mucilage is used for cough relief; Psyllium mucilage is used for bulk laxative; the mucilage from okra possesses stomach cytoprotective activity and gastric reduction (Jadhav, 2008); Aloe vera mucilage is used as antiinflammation, UV protective, wound healing, and

moisturizer (Dracelos, 2001); mucilage from yam *(Dioscorea batatas)* is used as skin lubricant, and it also contains allantoin and allantoic acid that promoted wound healing and anti skin cancer (Fu et al, 2006). For the pharmaceutical aid, the mucilage can be used as thickener, water-retention agent, gelling agent, suspending agent, and film former (Jani et al, 2007). To various properties and usages of plant mucilage, *Basella* mucilage was also proposed for those applications of medicine and cosmetics. This study was to detect for some physical properties as well as the sugar composition of the *Basella* mucilage. The antityrosinase which was accorded to traditional used for anti freckle was tested. Antioxidant was related to the inflammation mechanisms which caused by free radical was assay. Then formulation of gel from *Basella* mucilage was prepared. This study can be used for further developing as topical use for cosmetic and anti-inflammatory purposes.

Material and methods

1. Plant material

Basella alba L. was purchased from local market, Khon Kaen province, Thailand. The plant was identified by Assistant Prof. Dr. Srisomporn Preprame. The voucher specimen was kept at the Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.

2. Chemical and instruments

D-glucose anhydrous (Univar, Australia), D(+)raffinose pentahydrate (Himedia, India), D(+)mannose (Himedia, India), D(+)xylose (Himedia, India), L(+)rhamnose (Himedia, India), D(-)fructose (Fisher Chemicals, UK), D(+)galactose (Univar, Australia), L(+)arabinose (Calbiochem, Germany), D-sorbital (Fluka, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Fluka, Germany), L-DOPA (Sigma, USA), mushroom tyrosinase (Sigma, USA), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromine (Molecular Probes, USA), DEAE 650 M (Toyopearl Japan), TLC SiO_2 GF254 precoated plate (Merck, Germany), freeze dryer (Flexi-Dry, UK), UV-VISIBLE spectrophotometer (Shimadzu UV-1700, Japan), dialysis bag MWCO 12000-14000 (Cellu Sep, France), micro plate reader (Bio-Rad, USA), rheometer model DV-111 programmable (Brookfield, UK).

3. Extraction of mucilage

500 g Fresh stems were cleaned, extracted twice by homogenization in water at the ratio of 1:1, boiled, and filtered through the cheesecloth. The combined aqueous extract was lyophilized to dryness and kept in the desiccator until used.

4. Determination of the physical properties of *Basella* mucilage

The physical properties of mucilage were determined, such as appearance, color, flavor, and pH. The swelling test was performed by bed volume technique (Kuniak and Marchessault, 1972). Water was gently added into 0.2 g dried *Basella* mucilage until reached the final volume of 5 mL and kept overnight at room temperature. The swelling capacity could be obtained from the swelling volume (ml) divided by *Basella* mucilage (g). Film forming was observed from spreading 6.7% aqueous mucilage (1 g dried *Basella* mucilage in 15 ml water) onto the petri dish 10 cm diameter and incubated at 50 °C for one day. The viscosity was measured by using rheometer, spindle number 31, RPM 60.0, 30 sec per cycle at room temperature.

5. Partial purification of polysaccharide from *Basella* mucilage

Dried *Basella* mucilage extract 450 mg was redissolved in 30 mL water. The crude polysac charide was precipitated by adding 70 mL ethanol to obtained 70% v/v ethanol final concentration, and placed overnight at room temperature. The pellet was collected by centrifugation at 4,000 rpm for 10 min at room temperature, and washed with ethanol. Then it was redissolved with 120 mL water, and applied

onto DEAE 650M which was pre-equilibrated with water. The column was eluted by 50 mL of 0, 0.5, 1.0, 1.5, and 2 M NaCl in water, respectively. The fractions of 15 ml were collected. Each fraction was detected for protein by UV at 280 nm, and assayed for the total sugar content by phenol-sulfuric acid method. The sugar containing fractions were pooled, dialyzed against water, and lyophilized to dryness.

6. Phenol-sulfuric acid method

0.2-mL Sample was mixed with 0.8 mL conc. H_2SO_4, then 0.6 mL of 5% aqueous phenol was added. The mixture was detected by UV at 490 nm.

7. Determination of monosaccharide composition in polysaccharides

One mg of the partial purified polysaccharide from *B. alba* was hydrolyzed with 1 mL 10% HCl, and boiled for 2 hr. The reaction mixture was then applied onto SiO_2 TLC using 3 mobile phase systems of CH_3CN:H_2O (8.5:1.5), CH_3CN:EtOH (8:2), and CH_3CN:EtOH (13:2), and detected by spraying with 10 % aqueous H_2SO_4, and heated. The R_f value of authentic monosaccharides was compared with that of acid-hydrolyzed sample.

8. DPPH radical-scavenging assay

DPPH radical-scavenging assay was performed in 96-well plate according to Chan et al, 2008. Ascorbic acid was used as positive control. The *Basella* mucilage was diluted with water into concentrations of 0.125, 0.25, 0.5, 1, 2.5 mg/ml before reacting with 0.25 mM DPPH in MeOH in a ratio 1:1 for 30 min. Mixture was protected from light at room temperature, and measured for UV absorbance at 550 nm. The percentage of radical scavenging was calculated:

$$\% \text{ radical scavenging} = \frac{(A_{DPPH} - (A_{sample} - A_{blank})) \times 100}{A_{DPPH}}$$

When A_{DPPH} is absorbance of DPPH in MeOH; A_{sample} is absorbance of mixture of *Basella* mucilage, and DPPH; A_{blank} is absorbance of mixture of *Basella* mucilage and MeOH. IC_{50} was calculated as the final concentration of *Basella* mucilage to reduce the absorbance of DPPH by 50%.

9. Tyrosinase inhibitor assay

By using method of Chan et al., 2008, *Basella* mucilage was dissolved in 0.1 M sodium phosphate buffer pH 6.8 into concentrations of 0.125, 0.25, 0.5, 1, 2 mg/ml, respectively. 80-µL of sample was added to 40 µL of 100 units/mL tyrosinase in 96-well plate. After 10 min, 80 µl of 12 mM L-DOPA was added, and incubated at 37 °C for 10 min, protected from light. The reaction mixtures were measured for UV absorbance at 490 nm, and calculated for % inhibition of each sample. Ascorbic acid was used as positive control.

$$\% \text{ inhibition} = \frac{(A_{control} - (A_{sample} - A_{blank})) \times 100}{A_{control}}$$

When $A_{comtrol}$ is absorbance of mixture of tyrosinase and L-DOPA; A_{sample} is absorbance of mixture of *Basella* mucilage, tyrosinase, and L-DOPA; A blank is absorbance of mixture of Basella mucilage, and tyrosinase. IC_{50} was calculated as the final concentration of the tested sample that reduces the absorbance of the reaction mixture by 50%.

10. Cell viability test by MTT assay

Liver cells (Chang liver cells) were cultured in 96 well plate with 135 µl DMEM supplemented with 10% fetal bovine serum, penicillin, and streptomycin, at 37 °C in the CO_2 incubator (5% CO_2) prior to add 15 µl *Basella* mucilage in phosphate saline buffer (PBS) to the final concentrations from 0-2 mg/ml. The culture was then incubated for 24 hr. The positive control was 0.01 %v/v H_2O_2, and negative control was PBS. After the culture was washed with PBS, 100 µl 5 mg/mL MTT was added and

incubated at 37 °C for 1 hr. The formazan crystal was redissolved with 150 µl DMSO, and measured for UV absorbance at 550 nm.

11. Gel formulation from *Basella* mucilage and stability test

Gel was formulated into 3 formulae: formula A, B, and C which contained 0.05 , 0.1 and 0.15 % w/w *Basella* mucilage, respectively. The gel base composed of 0.9% w/w Carbopol 934, 4% v/w 1N NaOH, and 15% w/w propylene glycol. The finished products were tested for stability by using heating and cooling cycles method at 4 °C and 50 °C cycle, 12 hr each for 6 cycles. The physical appearances before and after stability test such as texture, color, flavor, pH, and viscosity were recorded.

Results

1. Physical properties of *Basella* mucilage

Lyophilized powder of *Basella* mucilage was in brown-green color with characteristic odor and hygroscopic. It could be swollen in water with the swelling capacity of 4.33 ± 0.29 mL/g dry weight. The redissolved *Basella* mucilage in water at 1-4% w/w yielded the viscous solution with pH 5.3-5.4. The higher mucilage concentration showed the lower pH, but increasing of viscosity (Table 1) and more green brown color intensity. *Basella* mucilage formed brown fragile film after it had been incubated at 50 °C for 24 hr. When exposed to the air, the film became sticky, flexible, and stretchable (Fig. 1).

Table 1 Physical properties of Basella mucilage

Appearance	Concentration (%w/w)		
	4	2	1
pH	5.3	5.3	5.4
Viscosity (centipoises)	195.67	143.33	132.33
Color intensity*	+++	++	+

*+++ = the most intense dark green color, ++ = moderate intense color, + = the less intense color (n = 1)

Figure 1 Basella mucilage film

2. Partial purification and monomeric sugar determination of *Basella* polysaccharide

The white precipitate of polysaccharide from *Basella* mucilage was obtained by EtOH precipitation. After the purification by using DEAE 650M, poly saccharide 11.7 mg (2.6% yield from 450 mg *Basella* mucilage dry weight) was obtained from 0.5 M NaCl elution (Fig. 2). TLC of the acid-hydrolysis poly saccharide clearly indicated that the major sugar was D-galactose (Fig. 3) with other sugars such as L-arabinose (Fig. 3B, 3C).

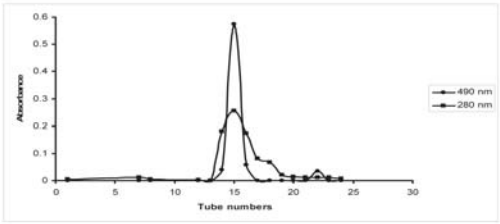

Figure 2 Anion chromatography profile of *Basella* polysaccharide purification.

(DEAE 650M column; ◆ UV absorbance at 490 nm for sugar detection by using phenol-sulfuric method; ■ UV absorbance at 280 nm for protein detection)

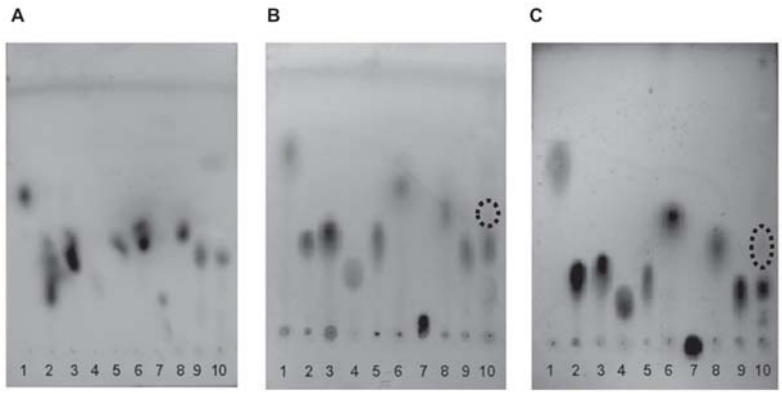

Figure 3 TLC Chromatogram of authentic monosachharides and acid-hydrolyse *Basella* polysaccharide A. mobile phase $CH_3CN:H_2O$ (8.5:1.5); B. Mobile phase CH_3CN:EtOH (8:2), C. Mobile phase CH_3CN:EtOH (13:2). Detection by spraying with 10 % aq. H_2SO_4 and heat. Dotted circles located the faint spots of L-arabinose.
(lane 1: L-rhamnose; lane 2: D-glucose; lane 3: D-fructose; lane 4: D-sorbitol; lane 5: D-mannose; lane 6: D-xylose; lane 7: D-raffinose; lane 8: L-arabinose; lane 9: D-galactose; lane 10. acid-hydrolyse *Basella* polysaccharide fraction from DEAE650M column

3. Antioxidant and tyrosinase inhibitory activities of *Basella* mucilage

DPPH radical-scavenging assay of *Basella* mucilage exhibited IC_{50} at 514.41 μg/mL compared to that of ascorbic acid (2.17 μg/mL). However, *Basella* mucilage showed no inhibitory effect to enzyme tyrosinase when using ascorbic acid as positive control (IC_{50} of ascorbic acid was 120 μg/mL).

4. Cell viability effect of *Basella* mucilage

Basella mucilage showed mild toxicity to Chang liver cell as shown in Fig. 4. The mucilage of concentration 2 mg/ml exhibited 84.4% cell viability. The estimate IC_{50} at 5.16 mg/mL could be obtained from the equation y = -10.904x + 106.23 of the linearity range.

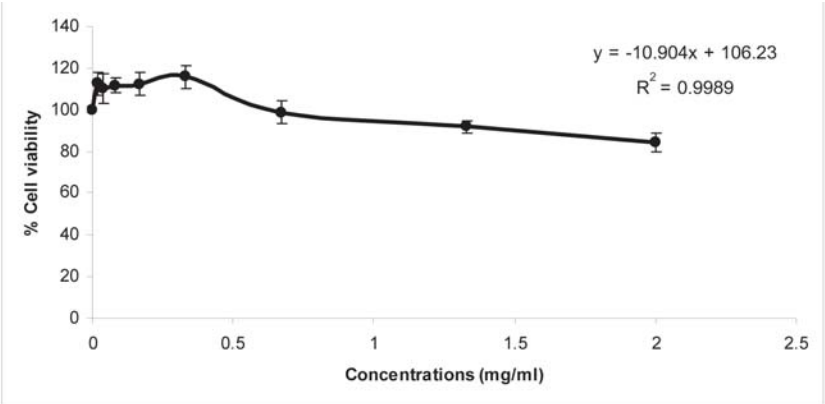

Figure 4 Percent viability of Chang liver cell by MTT assay after exposing to various concentration of *Basella* mucilage. The estimated IC_{50} was 5.16 mg/mL

5. Gel formulation and stability test

As shown in Table 2, gel formulations from *Basella* mucilage were physical stable. No phase separation, no precipitation, and no changing of color, odor, or pH were observed after stability test by heating and cooling cycles method. The viscosities of gels were 2-4% decreased.

Table 2 Physical properties of *Basella* mucilage gel preparation before and after the heating and cooling cycles test

Properties	Physical properties of *Basella* gel preparation					
	Formula A 0.05% w/w		Formula B 0.10% w/w		Formula C 0.15% w/w	
	Before	After	Before	After	Before	After
Gel appearance	Clear, homogenous	Clear, homogenous	Clear, homogenous	Clear, homogenous	Clear, homogenous	Clear, homogenous
Flavor	normal	normal	normal	normal	normal	normal
Color[a]	+	+	++	++	+++	+++
pH	5.76	5.77	5.61	5.59	5.65	5.65
Relative viscosity (%)	100%[b]	98%	100%[c]	96%	100%[d]	99%

[a] +++ = the most intense dark green color, ++ = moderate intense color, + = the less intense color

[b] 20.7 kilopoises

[c] 20.8 kilopoises

[d] 19.0 kilopoises

Discussion

The stem part of *Basella alba* was selected for mucilage extraction as it provided highly viscous material when crushing by hand indicated that it contained more mucilage than leaves. The partial purification of *Basella* mucilage composed of only 2.6% polysaccharide because of high water content of this plant (92%, Maisuthisakul et al, 2007; 2008). Dried *Basella* mucilage swells in water and gel formed because of its viscous characteristic and water-holding capacity. Its mucilage exhibited mild DPPH scavenging activity as from the *Basella* polysac charide reported by Palanuvej 2009. From our phytochemical screening, the aqueous extract of *Basella* gave negative result for phenolic compound assay, therefore it was possible to possess no tyrosinase inhibitory activity. For the purification process, protein, and polysaccharide could be coprecipitated by ethanol precipitation, while chlorophyll was soluble in ethanol, so the white precipitation was obtained and the chromatogram (Fig. 2) showed the mixing of protein and polysaccharide in the same fractions. Three solvent systems of TLC were used to confirm for sugar component identification. We found that D-galactose was the major monosaccharide in the acid-hydrolyzed polysaccharide. The minor amount of L-arabinose was also clearly detected although it could not

be seen in Fig 3A because of the lower amount of sample loaded onto TLC to assure the position of D-galactose. Our TLC results were accordance to the results from GC detection of polysaccharide reported by Palanuvej et al., 2009. They showed that the composition of *Basella* polysaccharide was galactose, arabinose, glucose, galacturonic acid and rhamnose (41:24:16:13:5). Aqueous extract of *B. alba* leave also consisted of the starch-type glucan (0.35%) that contained mainly L-arabinose and D-galactose with minor quantities of uronic acid and L-rhamnose (Haq et al., 1969). In this study, gel preparations of *Basella* mucilage with relatively concentrations 1, 2, 3 fold of IC_{50} of antioxidant were prepared as formulae A, B, and C, respectively. All preparations showed good stability. The pH of *Basella* mucilage at 5.3-5.4 was also suitable for skin. Our preliminary work for gel formulation showed that, up to 80% mucilage could be added into the gel preparation. The anti-inflammatory effect which was related to antioxidant activity will be further investigated from these preparations. From cell viability test in Chang liver cells, it showed tendency of weak toxicity. However, mutagenicity and antimutagenicity of aqueous extract of *B. alba* were tested by Yen et al, 2001 which showed no mutagenicity towards *Samonella typhimurium* TA98 and TA100 (using benzo[a]pyrene, and 4-nitroquinoline-N-oxide as mutagen) and weak to moderate against 2-amino-3-methyl-imidazo [4,5-f] quinoline mutagen. This could be used as the information for toxicological study of *Basella* mucilage in the future.

Conclusion

Basella mucilage was viscous with low swelling capacity. Its pH was good for skin (5.3-5.4). Crude aqueous extract possessed mild antioxidant, and no tyrosinase inhibitory activity. Partial purification of *Basella* mucilage composed of polysaccharide which contained D-galactose as a major compound. The cell toxicity to Chang liver cell showed tendency of mild toxicity. The gel preparation of *Basella* mucilage provided good stability that served for further development as cosmetic and medicine for skin diseases.

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