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Ceylon spinach: A promising crop for skin hydrating products

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ABSTRACT

Ceylon spinach, or *Basella alba*, is a mucilaginous vegetable crop having high economic, nutritional and medicinal value. It has long been used in traditional skin remedies, although to date few reports have focused on the scientific basis of its skin hydrating properties, or protocols for extract quality control. Extracts of this crop were therefore prepared, and evaluated for their efficacy towards skin hydration. An extraction time of 3 h provided the highest yield, and maximum total polysaccharide content, with the following extract specifications: yield (2.93 ± 0.83%), moisture content (12.51 ± 0.52%), total polysaccharide and total tannin contents (0.37 ± 0.00 mg glucose/g and 4.18 ± 0.09 μg tannic acid/g, respectively), astringency (245.86 ± 0.05 mg tannic acid/g), solubility and swelling capacity in water (0.02 ± 0.00% and 4.17 ± 0.58%, respectively), viscosity (1541.33 ± 22.12 cps) and water and oil absorption capacities (1.13 ± 0.03 g/g and 2.82 ± 0.12 g/g, respectively). Evaluation in 22 Thai volunteers indicated that the extract caused no skin irritation, and that skin hydrating efficacy (0.05–0.10%) was 7–28% better than the control in the short term (0–210 min; Corneometer® CM 825). These results highlight the potential for *B. alba* usage in healthcare products including pharmaceutics and cosmetics, and provide useful guidelines for extract preparation, quality control, standardization and specifications for industrial processing.

1. Introduction

Mucilage is a rich source of polysaccharides, actives that are widely used in the pharmaceutical, food, and cosmetic industries (Archana et al., 2013). Polysaccharides, naturally derived molecules constructed from simple sugars, are hydrated under aqueous conditions forming a gel structure (hydrogel or hydrocolloid), which is highly prevalent in mucilage vegetables. Extracts of plants enriched with polysaccharides impart a skin moisturizing effect allowing them to be used as active biopolymers in cosmetic formulations. Their excellent compatibility with biological tissues, including edibility in some cases, and biodegradable nature, make them key ingredients for bio-based healthcare products (García-González et al., 2011). This is underpinned by their low cost and ready availability from natural sources (Kanlayavattanakul and Lourith, 2015a); these, and the previously mentioned factors fit with consumer preferences, and their perceptions of safety (Kanlayavattanakul and Lourith, 2015b).

Ceylon or Malabar spinach (*Basella alba* and *B. rubra*) is important vegetable food crop, with *B. alba* (green leaves and stems) being more widely cultivated than *B. rubra* (red leaves and stems) due to its faster growth and greater active content. Extract of *B. alba* have long been used as an Ayurvedic remedy for skin diseases (Kuhnlein, 2000; Reddy et al., 2014), and in Thai traditional medicine for treatment of inflammation. The anticancer, antioxidant (Siriwatanametanon et al., 2010) and antidiabetic properties of *B. alba* extract as a consequence of the presence of bioactive polysaccharides (Palanuvej et al., 2009), has also been reported. However, to this point the use of *B. alba* as a source of active skin hydration polysaccharides has not garnered significant attention. In this context, the present study aims to widen the applications of this mucilaginous vegetable to the cosmetic industry, through preparation and standardization of *B. alba* extract, and clinical investigations into its safety and skin hydration efficacy for its substantiating evidence claimed (de Boer et al., 2016).

2. Materials and methods

2.1. Preparation of polysaccharides

Fresh aerial part of *B. alba* cultivated in Chiang Rai were cleaned and extracted with boiling distilled water (50 °C) as it is consumed (Kuhnlein, 2000; Palanuvej et al., 2009) for 1, 2, 3, 4, and 5 h (Samuelsen et al., 1998) with shaking at 150 rpm. The slurry was filtered and the polysaccharide was obtained in the dryness form by means of freeze dry. The extractive yield was calculated and each of the

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extraction condition was repeated for more 2 times.

2.2. Quality control and standardization of polysaccharides

2.2.1. Total polysaccharide content

The polysaccharide was quality controlled in terms of total polysaccharide content using phenol-sulfuric acid assay of which glucose was the standard (Fournier, 2001).

2.2.2. Solubility, viscosity and swelling capacity

The solubility of *B. alba* extract (10 mg) was determined in deionized water or propylene glycol (10 ml). Briefly, the mucilaginous *B. alba* extract was saturated in each solvent overnight, and the clear supernatants were transferred into evaporating dishes, evaporated to completely dried. The weight of the dried residue with the reference were determined and expressed as the solubility (%). Viscosity of the polysaccharide (0.3 g) saturated in water (7.5 ml) for 24 h, adjusted to 10 ml and mixed thoroughly at 100 rpm under room temperature for 2 h was recorded with the viscometer (Brookfield DVII+ Pro, USA) at the ambient condition (spindle no. 21, 20 rpm). The sample (0.5 g) in a measuring cylinder was hydrated with distilled water (10 ml) for 24 h, recorded the occupied volume (ml), and calculated as swelling capacity (%) (Archana et al. 2013).

2.2.3. FT-IR characterization of polysaccharide

FT-IR (Perkin Elmer Spectrum-GX FT-IR spectrometer) of the extract in KBr (2 mg/200 mg) was recorded with the transmittance mode from 4000 to 400 cm^{-1}.

2.2.4. In vitro hydration and oil absorption capacity

In vitro hydration retaining capacity or water adsorption was determined by the mixing of the polysaccharide (100 mg) and water (6 ml) in a centrifuge tube and hydrated for 18 h. A centrifugation at 3000*g* was undertaken for 20 min. The supernatant was decanted and the residue weight was recorded. The capacity was calculated as the amount of water retained by the pellet (g water per g sample dry weight). In addition, oil absorption capacity was examined with the same practice but mixed with olive oil instead of water (Mateos-Apricio et al., 2010).

2.2.5. Total tannin content and astringent activity

Total tannin content of the polysaccharide in DMSO and water was determined by incubation with 5% Na_2CO_3 and 1 N Folin-Ciocalteu for 60 min prior to an absorbance recorded at 725 nm (Biochrom Libra S22, UK). Of which, tannic acid was used as the standard. Astringent activity was measured by mixing the sample in ethanol with hemoglobin in phosphate buffer saline (pH 6.8) at 1:1 ratio. Following centrifugation at 3000 rpm for 10 min, the supernatant was analyzed at 407 nm in a comparison with tannic acid, the positive control (Son et al., 2013).

2.2.6. Moisture content

The polysaccharide extract was additionally quality control in term of moisture content using the moisture analyzer (Ohaus MB45, USA) (AOAC, 2000).

2.3. Skin hydrating efficacy

Thai healthy volunteers aged between 20 and 30 years old were enrolled in the study. All recruited subjects were informed about the study both in writing and verbally and signed a written consent form, which was approved by the ethical committee of the Mae Fah Luang University prior to enrollment (REH-59031). All of the study in human volunteers was delineated by the Declaration of Helsinki.

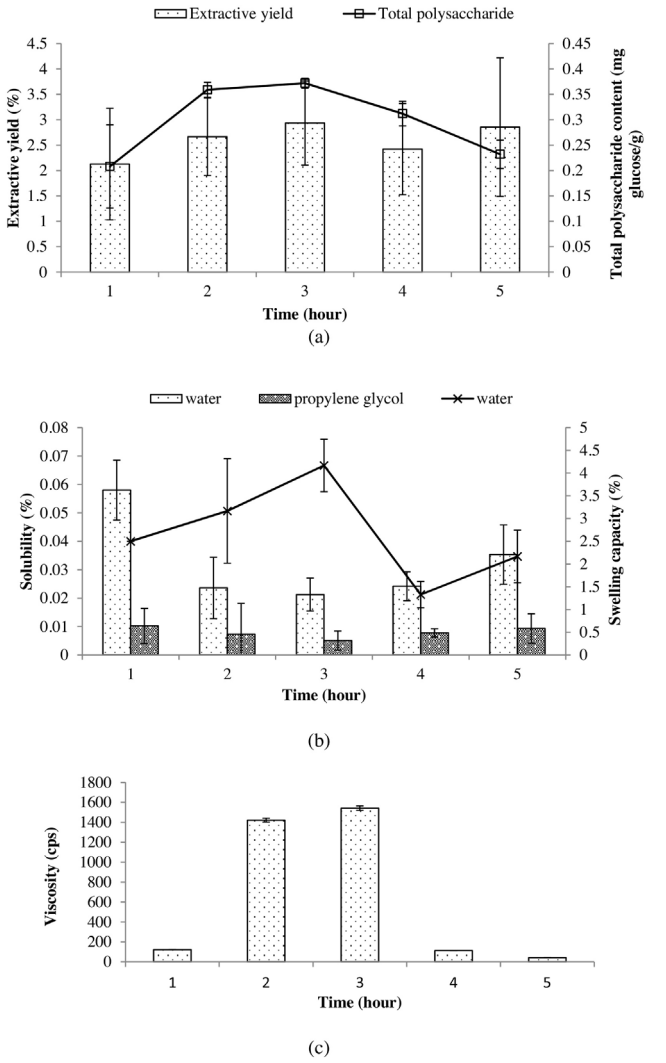

Fig. 1. Extractive yields and total polysaccharides (a), solubility and swelling capacity (b) and viscosity (c) of Ceylon spinach polysaccharides extracted at different times.

2.3.1. Skin irritation test

Safety assessment of *B. alba* extract with the greatest polysaccharide content was examined at 0.05 and 0.10 (w/v) in water by means of a single application closed patch test. Water was used as a negative control. Skin irritation severity was graded 0–4. Observation was undertook immediately, 24, 48 and 72 h following Finn chamber® (8 mm, SmartPractice, USA) removal. Mean Irritation Index (MII) was calculated. The MII < 0.2 was interpreted as non-irritation (Kanlayavattanakul et al., 2012).

2.3.2. In vivo skin hydrating efficacy

Skin hydrating efficacy of *B. alba* polysaccharide was evaluated using a previously described method (Kanlayavattanakul et al., 2012). Volunteers were instructed not to use moisturizers, body lotions, soap, or occlusive cosmetic preparations on the area tested for 12 h prior to the *in vivo* study. All subjects rested in a room maintained at 25 ± 1 °C and 40–60% relative humidity for 15 min prior to skin hydration monitoring using Corneometer® CM 825 (Courage + Khazaka, Germany) at the center of the inner forearm. Baseline skin hydration levels of 22 volunteers were recorded, followed by application of 0.05 and 0.10% *B. alba* polysaccharides onto the skin in a randomized single-blind procedure. The volunteers were directed to rest in the environmentally controlled room. Skin hydrating efficacy was recorded 15, 30, 45, 60, 90, 120, 150, 180 and 210 min after application. All of the