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Isolation, purification and characterization of galactomannans as an excipient from *Senna tora* seeds

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ABSTRACT

Seed galactomannans are neutral, heterogeneous polysaccharides widely distributed in nature. The Mannose/Galactose ratios differ from gum to gum, resulting in a change in structure, which in turn, determines the various industrial applications of seed galactomannans. *Senna tora* (Family: Fabaceae) is a fast growing and spreading under shrub of which seeds, pods and leaves are extensively used for medicinal applications. The seeds have been found to be an alternative source of commercial gums. The present investigation deals with isolation, purification and characterization of galactomannans from the seeds of *Senna tora* (*S. tora*). The galactomannan extraction was based on mechanical separation of the endosperm, water dissolution, centrifugation and precipitation with acetone. The polysaccharide obtained from *S. tora* seeds was characterized by using physicochemical and chromatographic procedures, as well as FTIR, Mass, ^{13}C NMR and ^{1}H NMR spectroscopy. The results indicated that the gum has the basic structure of galactomannans with a main chain of (1→4)-linked β-D-mannopyranosyl units to which single α-(1→6)-D-linked galactopyranosyl units are attached through block pattern. The rheological studies indicated that the *S. tora* gum (1%, w/w) solution possesses pseudoplastic flow. The viscosity and other rheological properties confirmed its suitability as an excipient in the development of sustained release delivery systems.

1. Introduction

Galactomannans are neutral polysaccharides obtained from the endosperm of seeds of some Leguminosae plant and they have several functions, including reserve of carbohydrates [1]. Galactomannans are polysaccharides built up of a β-(1–4)-D-mannan backbone with single D-galactose branches linked α-(1–6). Their mannose/galactose (M/G) ratios differ according to the species [2]. They are water soluble hydrocolloids which form highly viscous, stable aqueous solutions [3]. The main difference between galactomannans from different plant sources lies in the galactose content as well in its distribution along the mannopyranosyl backbone [1,4]. The degree of substitution of galactose differs in the galactomannans extracted from various plants. The differences of the degree in substitution greatly affect solution properties, including water solubility, thickening ability and synergistic interactions. Galactomannans can often be used in different forms for human consumption. Featuring different physicochemical properties, galactomannans are a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics and food industries [5]. Most galactomannans used in pharmaceutical technology and cosmetics are usually unpurified gums [6]. When associated with other polysaccharides such as xanthan gum and kappa-carrageenan, galactomannans can form gels with new properties [7–10].

The four major galactomannans of commercial importance in food and non-food industries are guar gum (GG, *Cyamopsis tetragonolobo*, M/G ratio: 2:1), tara gum (TG, *Caesalpinia spinosa*, M/G ratio: 3:1), locust bean gum (LBG, *Ceratonia siliqua*, M/G ratio: 3.5:1) and Fenugreek (*Trigonella foenum-graecum* L., M/G ratio: 1:1) [11]. Currently the international trends demand the introduction of alternative sources of seed gums [12] and it is therefore important to search for alternative renewable sources for e.g. the production of edible and biodegradable films and coating materials. In particular, Latin American sources of galactomannans are not well known, in spite of the rich biodiversity of the local flora and of the favorable climate for their production [13].

Senna tora (L.) Roxb. belonging to the family Fabaceae is an annual under shrub which grows all over the tropical countries

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(throughout India, Pakistan, Bangladesh and west China). It grows well in wasteland as a rainy season weed. It is also known as 'Chakramard' in Ayurveda [14,15]. Several studies have been conducted throughout the last decade to investigate chemical and biological properties of *S. tora*. Antihepatotoxic naphtha-pyrine glycosides were reported to be isolated from the seeds of *S. tora* [16]. Antioxidant properties and inhibitory effect of the extract of *S. tora* have already been reported [17,18]. A recent study was conducted by the scientists of the Department of Food Science and Nutrition, Catholic University of Daegu, Korea who concluded that *S. tora* supplements can help to improve serum lipid status in type-II diabetic subjects without significant adverse effect [19]. In view of the easy availability of the plant, the medicinal value of the seeds and the high demand of seed gums throughout the world, the properties of the seed polysaccharide (gum) obtained from *S. tora* were investigated. The present investigation was aimed at isolation and characterization of purified polysaccharide from the seeds of *S. tora* in order to survey its potential applications as an additive/excipient to neutraceuticals and pharmaceuticals.

2. Materials and methods

2.1. Collection of plant material

The pods of *S. tora* were collected from Kalyan taluka (District – Thane, Maharashtra) in the month of September–October 2010. The seeds were manually separated; shade dried and kept in a cool, dry place until further use. Plant was authenticated by Dr. Rajendra D. Shinde, Associate Professor, Blatter Herbarium; St. Xavier's College, Mumbai and was identified as *S. tora* (*L.*) Roxb (Herbarium Specimen no. 8361). The herbarium specimen of *S. tora* was stored in Ultra College of Pharmacy, Madurai for future reference.

2.2. Isolation of gum

The dried seeds were dehusked and de-germed by mechanical treatment followed by milling and screening of the endosperm. The powder was soaked in benzene–ethanol solution (1:1) overnight to remove lipids and then it was dried in vacuum oven. The endosperm powder of *S. tora* seeds (10 g) was soaked in 200 mL of distilled water and stirred under overhead stirrer for 3–4 h. The viscous solution obtained was passed through the muslin. The marc obtained was pressed to remove the mucilage and boiled with 200 mL of water for 1 h. Viscous solution obtained was filtered through muslin cloth. The marc obtained was not discarded but it was sent for multiple extractions with decreasing quantity of extracting solvent, i.e., water with the increase of number of extractions. The isolation was continued until the material becomes free from mucilage. All the viscous solutions obtained were mixed together. An equal quantity of 10% trichloroacetic acid was added to the mixture to precipitate protein. The solution was centrifuged and the supernatant was precipitated out by addition of acetone in the ratio 1:0.5 with continuous stirring. The coagulated mucilage, which formed as a white mass was transferred to an evaporating dish and dried in vacuum oven at 40 °C, powdered and stored in airtight containers.

2.3. Purification of gum

The above obtained crude gum was dissolved in warm water, re-precipitated using ethanol (1:1), dried at 40 °C, powdered and stored in airtight container at room temperature. The process of dissolution in water and precipitation with alcohol was repeated until an almost white precipitate was obtained. The dried polysaccharide was milled and sifted with a 60 mesh for further use.

2.4. Characterization of gum

2.4.1. Organoleptic evaluation

The Organoleptic evaluation of the purified gum sample was carried out. The Organoleptic evaluation refers to the evaluation of color, odor, shape, taste and special features which include touch and texture. The majority of information on the identity, purity and quality of the material can be drawn from these observations.

2.4.2. Identification test for gum

The identification of the isolated polysaccharide was carried out by using the following tests [20,21]:

a. The powder was mounted on a slide with ruthenium red solution and covered with a cover slip. After a few seconds, it was irrigated with lead acetate and the excess stain was sucked off with a blotting paper (lead acetate solution was added to prevent undue swelling of the test solution). The color of the particles was noted.
b. The powder sample was mounted on a slide with freshly prepared corallin soda solution and covered with a cover slip. After a few seconds it was irrigated with 25% sodium carbonate solution. The color of the particles was noted.
c. Polysaccharide was heated with distilled water for some time and then cooled. Formation of gelatinous mass was noted.
d. To 2 mL of polysaccharide solution, 2–3 drops of N/50 iodine solution was added and the color of the particle was noted.
e. Gum solution (3–5 mL) was treated with 4% borax solution.

2.4.3. Physicochemical evaluation

2.4.3.1. Solubility. The solubility is expressed in terms of 'parts' representing the number of mililiters (mL) of the solvent in which 1 g of the solid is soluble. Solubility of powder was determined in different solvents at room temperature as per Indian Pharmacopoeia 1996 [22].

2.4.3.2. pH. The pH of the mucilage was determined using a digital pH meter. This was done by shaking a 1% (w/v) dispersion of the gum sample in water for 5 min and the pH determined using a pH meter. The pH meter was set to neutral (7.4) at a room temperature and the electrode was immersed into the dispersion. The reading on the meter was recorded. Triplicate measurements were made [23].

2.4.3.3. LOD. The inherent moisture in additives may influence the stability of dosage form containing moisture sensitive drugs, so the moisture content of the isolated gum sample was detected by loss on drying method. The gum sample (1 g) was heated at 105 °C until constant weight in a hot air oven and percentage loss of moisture on drying was calculated using the formula:

$$\text{LOD}\,(\%) = \frac{\text{weight of water in sample}}{\text{weight of dry sample}} \times 100$$

2.4.3.4. Ash content. Ash values such as total ash, acid insoluble ash and water-soluble ash were determined according to Indian Pharmacopoeia [22,24]. The following procedures were used for determination of ash values.

2.4.3.4.1. Total ash. About 3 g of sample was accurately weighed and taken in a silica crucible, which was previously ignited and weighed. The powder was spread as a fine, even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to air dried sample.