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Structural, thermal and rheological characterization of modified *Dalbergia sissoo* gum—A medicinal gum

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ABSTRACT

Dalbergia sissoo gum was purified by ethanol precipitation. The purified gum was modified and hydrolyzed. Gum was modified by performing polyacrylamide grafting and carboxymethylation methods. The hydrolysis was carried out by using mannanase, barium hydroxide and trifluoroacetic acid. The modified and hydrolyzed gums were characterized using thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). The decrease in viscosity was studied by performing the flow test. The modified and hydrolyzed gums were thermally stable as compared to crude gum. There was increase in crystallinity after modification and hydrolysis, determined through XRD. FTIR analysis exhibits no major transformation of functional group, only there was change in the intensity of transmittance. It is concluded that the modified and hydrolyzed gum can be used for pharmaceutical and food industry.

1. Introduction

Dalbergia sissoo, vernacularly known as 'Shisham' in Hindi. It is a deciduous rosewood tree, also known as sisu. *D. sissoo* has many medicinal properties and used as abortifacient, aphrodisiac, anthelmintic, antipyretic and expectorant [1]. Most plant based polysaccharides do not possess any side effects and are nontoxic [2]. Native and unmodified gums solution possess a number of drawbacks to use them for industrial applications, such as lack of clarity, free flowing properties, uncontrolled rates of thickening and hydration, decrease in viscosity on storage, bigger microstructure formation and microbial contamination, which can overcome by changing the physicochemical properties by modification of them [3]. For the use of natural gums as excipients and binders, today the whole world is increasingly interested. Natural gums as binders have advantages over synthetic binders since they are freely available, non toxic and less expensive [4]. Wide varieties of gums have been used as stabilizer and thickener in various food products and considered as harmless for human use [5]. Natural gums or biopolymers are used in drug delivery system because of their well-known biodegradability and biocompatibility [6,7]. Biopolymers are also used as drug carriers [8,9]. Due to their sustainability and biosafety they have been the subject of research [10]. Biopolymers are obtained from natural sources. These polysaccharides are relatively cheap, easily available, non-toxic [6,10]. Currently, much interest is being given to biopolymer's modification [9]. Plant polysaccharides can be modified via chemical and biochemical methods using suitable reagents, [6] resulting in several types of polysaccharide derivatives, because of a variety of derivable groups present on their molecular chains [10–12]. The chemical and physical modifications are used for making the functional properties of gums better as biopolymer [4,10]. Some chemical methods are oxidation [13], graft-copolymerization [14], thiolation [15] and carboxymethylation [3]. For the functionalization of biopolymers carboxymethylation is widely used [4,16], because of low cost of chemicals, ease of processing and product versatility [4].

Gums and the modified derivatives have been extensively used for the food and pharmaceutical industry, as they possess the potential of being safe and non-toxic for animal and human consumption [4,17]. Gums are branched and complex polymeric in structure, and have high cohesive and adhesive properties. Because of this reason, gums are used in pharmaceutical industry for preparations. In pharmaceutical formulation natural gums have been used as binders, thickeners, stabilizer, suspending agent, gelling

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agent, protective colloids, polymer matrices, disintegrant, emulsifying agent and plasma expanders in tablets [18,19]. Literature survey suggested that the research work carried out in present study still have scope for research. Thus the present study was aimed to evaluate the characterization of hydrolyzed and modified gum *D. sissoo*.

2. Material and methods

2.1. Purification of gum

The dried *D. sissoo* was identified and authenticated by Dr. Mansoor Hameed, Associate Professor, Department of Botany, University of Agriculture, Pakistan. The selected crude gum was grinded to powder. The crude gum was collected from tree, dried and used. For the process of purification, the dispersion of gum was formed in water and was left for overnight. The dispersion was filtered and then slowly added to absolute ethanol. White amorphous precipitates were formed when ethanol was added to gum solution. They were collected and washed with ethanol. In hot air oven, at 50 °C the white precipitates were placed to dry and it took 24 h to dry. The dried gum was grinded to powder and stored for further use, as purified gum [9].

2.2. Modification

2.2.1. Polyacrylamide grafting

For chemical modification of *D. sissoo* gum, 0.1 g of purified gum was taken in 25 mL of distilled water. Acrylamide (16×10^{-2} M), silver nitrate (8.0×10^{-5} M) and ascorbic acid (22×10^{-3} M) was added to solution. The solution was thermostated at 35 ± 2 °C in thermostatic water bath (HH-4 China). After 30 min $K_2S_2O_8$ (8.0×10^{-3} M) was added to the solution and reaction was allowed for 1 h. Using pure ethanol the polyacrylamide modified gum was separated from polyacrylamide by precipitating the reaction mixture. The modified gum was washed with ethanol. The final product was oven-dried at 50 °C for further use [20].

2.2.2. Carboxymethylation

The purified *D. sissoo* gum (2 g) was dissolved in 100 mL of distilled water. 10 mL of (5% NaOH) was added to gum dispersion at the rate of 1 mL within 15 min at room temperature with continuous stirring. One gram of choloroacetic acid was dissolved in 15 mL of water (6.6% solution) and it was added at a period of 10 min to the reaction mixture. Using ethanol the reaction product was extracted. The precipitated gum was repeatedly washed by ethanol. Modified gum was oven-dried at 50 °C [21].

2.3. Hydrolysis

2.3.1. Acidic hydrolysis

Purified gum samples ranging from 0.2 mg were taken up in 2 M trifluoroacetic acid (100 μL/0.2 mg sample) and hydrolyzed in capped glass vials at 110 °C for 2 h. After acidic hydrolysis the hydrolyzed sample was collected through ethanol precipitation method and at 50 °C it was dried in oven [22].

2.3.2. Basic hydrolysis

Basic hydrolysis of purified *D. sissoo* gum was performed by using the method given by Beltran [23]. The 5 g of purified gum was hydrolyzed with saturated solution of barium hydroxide (200 mL) for 8 h at 100 °C. The hydrolyzed sample was neutralized with 1 M H_2SO_4. The resultant product was precipitated with ethanol, filtered and at 50 °C oven-dried.

2.3.3. Enzymatic hydrolysis

For enzymatic hydrolysis of gum sample a method described by Tester and Sommerville [24] was used with some modifications. In 10 mL capped tubes, 10 mg of gum samples was taken and 0.5 mL of distilled water was added and mixed thoroughly. Sample solution was placed in water bath for 30 min at 40–60 °C and 1.5 mL of acetate buffer having pH 4.7 was added to the gum solution. The mixture was mixed thoroughly. Then 5 mL (0.02/100 mL) of mannanase (enzyme) was added. The mixture was incubated for 15 min at 30 °C. Using ethanol the mixture was precipitated; the hydrolyzed gum was collected and dried in oven for further analysis.

2.4. Characterization

2.4.1. Scanning electron microscope

Scanning electron microscope (S-2380N, Hitachi, Japan) was used for analyzing the surface morphology of crude, purified, hydrolyzed and modified gum. Powder form of samples was used for the analysis [25].

2.4.2. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of crude, purified and processed gums for the study of functional groups were studied using FTIR spectroscopy (Bruker Tensor 27 FTIR Spectrometer). The samples were analyzed directly, in the form of powder, KBr was not used. The range of spectrophotometer was from 500 cm^{-1} to 4000 cm^{-1} [25].

2.4.3. Thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC)

For the thermal behavior of gum samples they were analyzed using TGA analyzer (Hi-Res. 2950 Thermogravimetric analyzer). The temperature range for DSC and TGA was from 0 to 400 °C under N atmosphere at a heating rate of 10 °C min^{-1} [33]. Differential scanning calorimeter (DSC Q-2000) was used to study the difference in amount of heat required for the increase of temperature of gum samples and the reference was measured as a function of temperature [19].

2.4.4. X-ray diffraction (XRD)

X-ray diffraction (X'Pert PRO, PANalytical, Almelo, Holland) was performed for the phase analysis of polycrystalline of samples, which reveals that whether the sample is amorphous or crystalline in nature [26].

2.4.5. Flow test

Rheological analyses of crude and modified *D. sissoo* gum at 1% concentration (w/w) were performed on controlled stress rheometer. The rheometer, TA instruments, AR 1000 N (Leatherhead, Surrey, UK) equipped with rheology advantage software was used. The cone diameter was 60 mm. A simple flow curve may be generated by applying a series of shears and recording resulting viscosity. The shape of the curve may be used to predict flow behavior in polymers. Flow test was performed to determine the flow behavior of gum solution using the crude, purified, hydrolyzed and modified gum. Steady state flow test is destructive test; therefore sample after this test was discarded.

3. Results and discussion

The crude *D. sissoo* gum was purified for further use. After performing the purification the gum was hydrolyzed and modified. The hydrolyzed and modified gum was subjected to different characterization techniques.