



Biochemical, physical, and rheological properties of novel Sudan bean gum synthesized from *Piliostigma thonningii* seeds

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Abstract

Novel Sudan bean gum (SBG) was synthesized from the *Piliostigma thonningii* seeds of the leguminous tree recognized as Camel foot. The SBG is one of edible Polysaccharide is widely spread biopolymers with a variety of structures and properties. It is composed of a long sequence of mannose units to galactose units with a ratio (14/2) M/G, and It may be utilized as a substance that thickens in pharmaceuticals, food, textiles, oil, drilling, and other fields. The main aim of the current work is to investigate the biochemical, physical, and rheological characteristics of novel Sudan bean gum. Chemical analysis outlined the significantly rich carbohydrate content (70.76%), as well as galactomannan, mannose (13.34%), galactose (1.36%), and protein content (12.52%). The rheological properties of SBG have been shown that solubility was higher, 90.80%, and the viscosity of water holding capacity (WHC) of SBG was higher, 23.7%, foam stability (FS), 20.56%, and emulsion stability (ES) reached 1.9 O.D. The samples of SBG have recorded higher Ca, Na, Mg, K, Zinc, and Cu values for all elements. Its molecular structure in aqueous solutions influences its solubility and rheological properties. SBG is an exciting poly saccharide since it works synergistically with various biopolymers like (LBG gum, xanthan gum, etc.). Therefore, the present study provides new findings on the properties of SBG as a future substitute for various industries and Biopharmaceutical Applications.

Keywords Mannose · Galactose · Aqueous solutions · Sudan bean gum

1 Introduction

Polysaccharides are widely distributed natural biopolymers that are characterized by diversity in composition and properties. As it has versatility such as excellent stability, safety, lack of harmfulness, hydrophilicity, and biodegradability in environment [1]. The seeds of *Piliostigma thonningii* (camel foot) have not received much attention for industrial and research purposes. The seeds consist of the outer pericarp layer, which represents about 15–23%, the embryo represents 20–25%, and the endosperm layer 45–50% of the seed weight [2]. This plant is found in the southern regions of central Sudan, including South Kordofan, South Darfur, and Jebel Marra, and extends to Bahr El Ghazal and Upper Nile.

It is widely used and of significant importance because it is a natural source, as it controls the water phase of food [3]. The challenge facing the pharmaceutical and food industry now is to obtain compounds with consistency and stability that enhance health and immunity, which is the motivation behind the interest in finding food sources with properties that contain beneficial components such as gum to enhance the overall value of nutrients [4]. *P. thonningii* seeds are a crop with diverse advantages as product of Mediterranean tree species widespread outside its range, which makes it a promising and important thickening material with functional, nutritional value, and therapeutic potentials [5, 6]. *P. Thonningii* is a crop that has not received attention since ancient times, it can be used as an additive for thickening, stabilizer, emulsifier, and coagulant purposes as in the properties of guar gum [7, 8].

Seed gum is considered a future alternative to petroleum-derived synthetic products because it is widely available, non-toxic, sustainable, and biocompatible. Gum also has a biological effect as it is an effective substance in lowering blood sugar and lowering bad cholesterol, high fiber content

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leads to low density lipoprotein (LDL), [9]. It improves the handling and shaping of the dough in a better manner and with less energy and time [10]. It is also used to increase the size of baked goods, as by adding it, the sticking temperature is reduced, and the size of the piece is increased [11]. Addition gum to baked goods increases viscosity, improves the final quality of flour, and increases the ability to absorb water [12]. One of its most significant uses of gum is as a gluten replacement binding substance for gluten-free products based on starch, as it improves gluten-free baked goods and improves the structure of the dough so that it is easier to shape [13, 14]. Also, in Dairy products uses in full-fat yogurt to improve texture because fat has a very important effect in determining the texture of foodstuffs, as the concentrations of proteins and colloids must be improved to provide the best reaction environment without its presence can cause unwanted interactions for milk, the control of these reactions depends strongly on the concentration of the amount of gum [15]. The solubility of the gum operates well in water that is hot, and creating a syrup requires using heat, that makes it very suitable for making syrup. To make a drink with a stable consistency that is resistant to separating, so the gum is used in the production of the drink [16]. The packaging materials able can be made from biological compounds for instance polysaccharide, and balms by adding plastic materials that reduce surface tension [17]. The gum was used to make eatable films since it is derived from a natural, digestible source and has no adverse effects on fresh foods [18]. Hydrophilic materials have suitable properties that control the permeation of vapors such as water vapor, oxygen and carbon dioxide, viscosity and tensile strength make gum suitable for use. Gum can be used as a carrier material that encapsulates biologically active materials for future advancements in stem cell therapy and tissue engineering [19]. That improved properties of films made of k-carrageenan with gum are attributed to hydrogen bonding proven by using Fourier transform infrared spectroscopy or FTIR [20]. The exploitation of gum in a pharmaceutical industry is due to its ability to bond with other sugary substances. It minimizes Low-Density Lipoproteins (LDL), through its high level of fiber [21]. Substance that has a hypo lipidemic effect and LDL appropriate to the high Fiber content therapeutic and pharmacological action [22, 23]. In the pharmaceutical industry, gum serves a variety of purposes, including binders, viscosity, stabilization agents, enhancement factors, emulsifiers, matrix formers, solubilizers, paints, drug release modifications, disintegrating substances, suspension, gel-forming agents, and the bio sticky substances [24].

Table 1 Biochemical characteristics of Novel SBG compared with the locust bean gum (LBG)

Parameters (%)	SBG (%)	LBG (%)
CHO	70.76 ± 0.005	80.05 ± 0.003
Moisture	13.15 ± 0.02	9.9 ± 0.01
Protein	12.52 ± 0.07	7.12 ± 0.08
Magnesium (Mg)	112.400 ± 0.02	160.00 ± 0.006
Sodium (Na)	41.000 ± 0.001	92.800 ± 0.03
Calcium (Ca)	35.200 ± 0.005	85.200 ± 0.005
Copper (Cu)	24.400 ± 0.03	24.600 ± 0.004
Iron (Fe)	7.600 ± 0.001	28.800 ± 0.03
Manganese (Mn)	5.200 ± 0.004	5.400 ± 0.002
Potassium (K)	23.800 ± 0.003	38.400 ± 0.006
Zinc (Zn)	1.400 ± 0.01	2.400 ± 0.041

CHO% Total carbohydrates, SBG Sudan bean gum, LBG: locust bean gum

All values in the table are a means of three replicates. ± S. D

Table 2 Monosaccharide composition of novel Sudan bean gum (SBG) by HPLC analysis

Compound	Retention time	Percentage (w/w)
1 Galactose	2.660	1.36 ± 0.02
2 Arabinose	3.540	0.23 ± 0.08
3 Galacturonic acid	4.310	10.00 ± 0.02
4 Mannose	5.673	13.34 ± 0.05
5 Rhamnose	6.573	23.400 ± 0.02

All values in the table are a means of three replicates. D

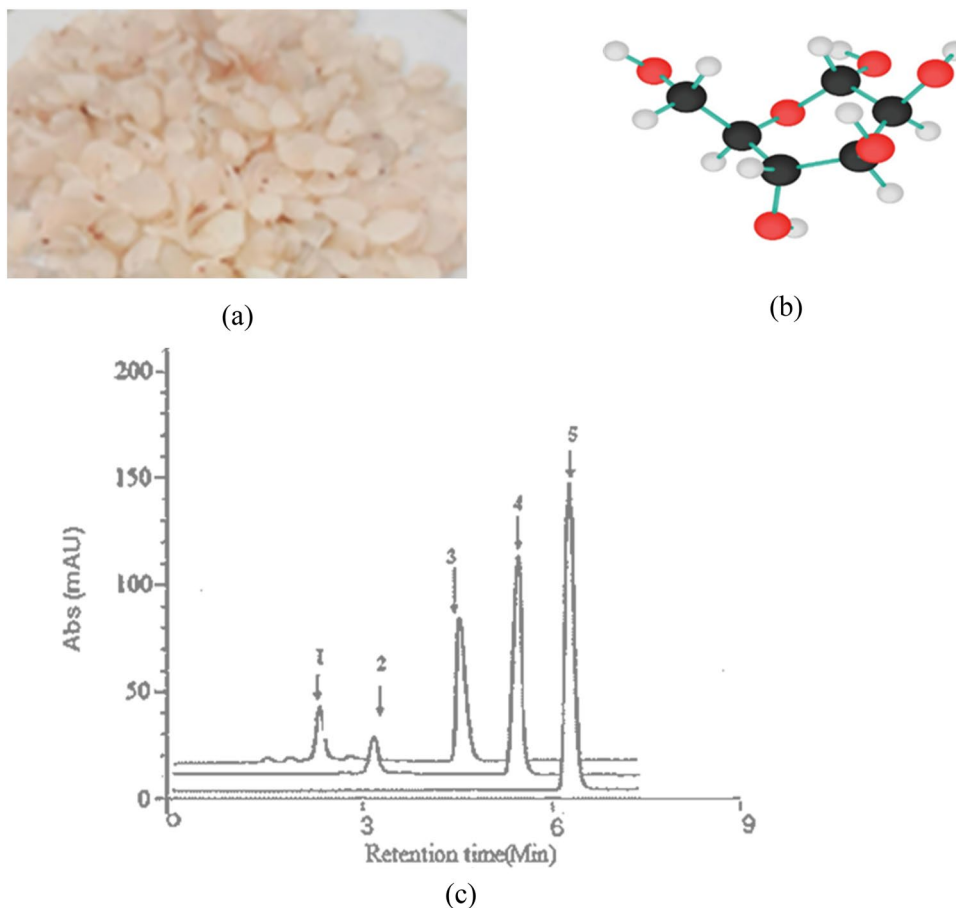
2 Results and discussion

2.1 Biochemical properties of novel SBG

2.1.1 HPLC analysis of monosaccharide

The results of chemical analysis of novel Sudan bean gum (SBG) and Locust Bean Gum (LBG) are displayed in (Table 1), explained that it contains rich carbohydrate content (70.76%), as well as galactomannan, and protein content (12.52%). of SBG (Sudan bean gum recovered of *P. thonningii* seeds), was much higher than the protein content of LBG (*Ceratonia siliqua*). The monosaccharides content of SBG as determined by HPLC, as well as their chromatographic separation shown, (Table 2 and Fig. 1c). The SBG was constituted of distinct monosaccharides with significant differences ($p \leq 0.05$) in terms of amount and retention period. Among the main carbohydrates in extracted SBG, rhamnose appeared to be much higher (23.400 w/w), followed by mannose (13.34 w/w),

Fig. 1 **a** novel gum of SBG synthesized from Seeds of *P. Thonningii*. for the first time. **b** Mannose unit **c** HPLC spectrum analysis of SBG gum hydrolyzed by H_2SO_4



galactose (1.36 w/w), and Arabinose (0.23 w/w). While mannose (133.4 mg/g) and galactose (13.6 mg/g) units were found as minor sugars. The Galactose, Arabinose, Galacturonic acid, Mannose, and Rhamnose standards showed characteristic peaks at 2.660, 3.540, 4.310, 5.673, and 6.573 min of retention time, respectively (Table 2). The role that biochemical composition of SBG plays a significant spot in its physical properties as it shows the mannose or galactose ratio. The aqueous solubility of galactomannan varies with temperature and M/G ratios therefore, a polysaccharides solubility at low temperatures increases with the amount of branching galactose units it contains [45]. The solubility between the swelling solid phase and the pure solvent phases was determined by a thermodynamic equilibrium [46]. Because LBG lacks ionizable functionalities, its solubility is determined according to the solvent composition and the number of hydrogen bonds [47]. This study demonstrated that Sudan bean gum extracted from *P. thonningii* seed (SBG) had high content of carbohydrate, protein, and ash compared to (LBG). Pure mannose backbones exhibit strong hydrogen bonding, allowing for aggregation and precipitation [48]. Galactomannan with higher M/G ratio and higher intrinsic viscosity is frequently utilized in the food industry as an

ingredient in food because of its capacity to generate significant viscosity at low levels. Two functions performed by the branched unit position of galactosyl along the backbone affect the solubility depending on temperature. It is indicated for large, smooth spots and compact galactosyl branching units characterized by high solubility at low temperature [49]. The results of Minerals Content of novel Sudan bean gum (SBG) and (LBG) are presented in the (Table 1). The samples of novel Sudan bean gum (SBG) have recorded higher values conferring to (Fig. 3), for all elements compare with the LBG in, Ca, Na, Zn, Mg, Cu, K, and Fe. These elements and factors affect the gum properties of the aqueous phase and inform desirable properties to the product such as stability and texture for several foods and medical application and cosmetic products.

2.1.2 Structure, processing and properties

Novel SBG is a galactomannan. It is composed of a straight sequence of mannose units linked together by the β -D (1 $\rightarrow$ 4) links; this linear chain has α -D-galactose units connected by (1 $\rightarrow$ 6) linkages. The percentage of mannose% units to galactose % units (M/G) ranges from 13.34: 1.36 to 14:2, it suggests that the galactose side groups should be

more consistently distributed along the mannose backbone, (Fig. 2). The repeating unit of Sudan bean gum and partially hydrolyzed gum, and manufacturing gum from seed beans of *P. Thonningii*. Locust bean gum (LBG) is a polymer obtained from plants that refers to the galactomannan family [38]. Also, Locust bean gum is made up of β -(1 $\rightarrow$ 4) mannose unit backbones that are casually branched with α -(1 $\rightarrow$ 6) galactose, [39]. The method by Blakeney [40], determines mannose galactose ratios, with varied by galactomannan type and source [41]. As follows, galactomannans containing ordinary galactose, sufficient and randomly distributed can be illustrious [42]. Thus, the ratios found for different gums range between 1 to 1 and 10 to 1 42: Fenugreek gums 1:1; Al Guar gum 2:1; The Tara gum 3:1; The LBG gum 4:1; Cassia gum 5:1, etc., [43]. The initial presented explanation of the clear structure of Louct bean gum in 1975 using an alkaline degradation approach [44].

2.2 Figures, and tables

See Figs. 2 and 3, Tables 1 and 2.

2.3 Physical characteristics of novel SBG

The results of physical characteristics of Sudan bean gum (SBG) are demonstrated in (Table 2), it showed that the lower 1.46 g/mL Refraction of SBG than Locust bean gum (LBG) had 1.53 g/mL, and the greatest solubility (92.13%) of LBG, Sudan bean gum had the lower solubility (90.80%), Table 2. Some studies have been conducted on the dynamic

viscosity of galactomannans, positively LBG. The pH-values of gum solution and gel ranged from 6.5 to 7.0., of SBG and LBG, respectively. This hydrocolloid exhibits pseudoplastic activities in solutions, like several other polysaccharides [50]. The dynamic viscosity of solutions is influenced by galactomannan concentration and microstructure, which are linked to intramolecular interactions and the smooth locust bean gum region [57]. Viscosity is influenced only slightly by ionic strength, temperature, and pH [41]. The pH and Temperature can break polysaccharides, altering the ultimate viscosity due to variations within the molecular weight of polymer sequences.

The solution affects its viscosity, as a higher temperature allows for more effective solubilization and promotes more solubility [51]. The viscosity of solutions will increase as it cools compared to when it was dissolved at a lower temperature. Among galactomannans, LBG needs heat for complete hydration, when combined with other hydrocolloids, it forms gels even if it does not gel by itself [54]. That may be explained through a higher possibility of tangling once the solubility of hydrocolloid has improved [56]. The considerable influence of wiping on the monosaccharose composition of the gum side chains and backbone might account for the variance [37], demonstrated that polymeric chains with branched components are more soluble than those with linear components. Some high molecular weight, low galactose molecules containing galactomannan are insoluble at low temperatures and rapidly soluble at elevated emperatures.

Viscosity is the term used to describe the liquid's resistance to flow via the shear motor capillary tube. The specific

Fig. 2 Chemical structure of novel SBG gum

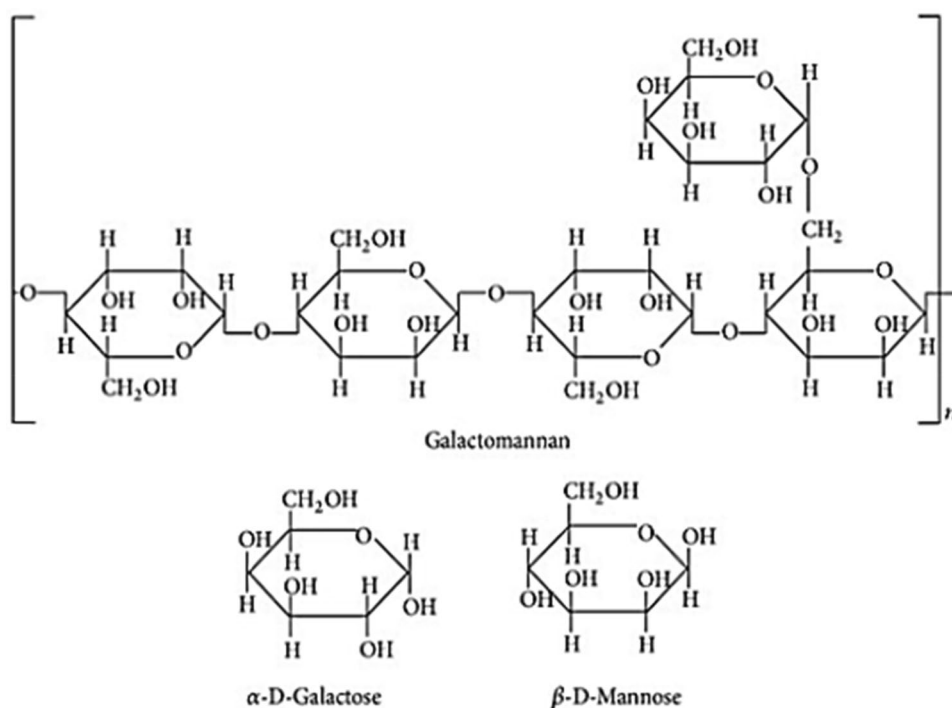
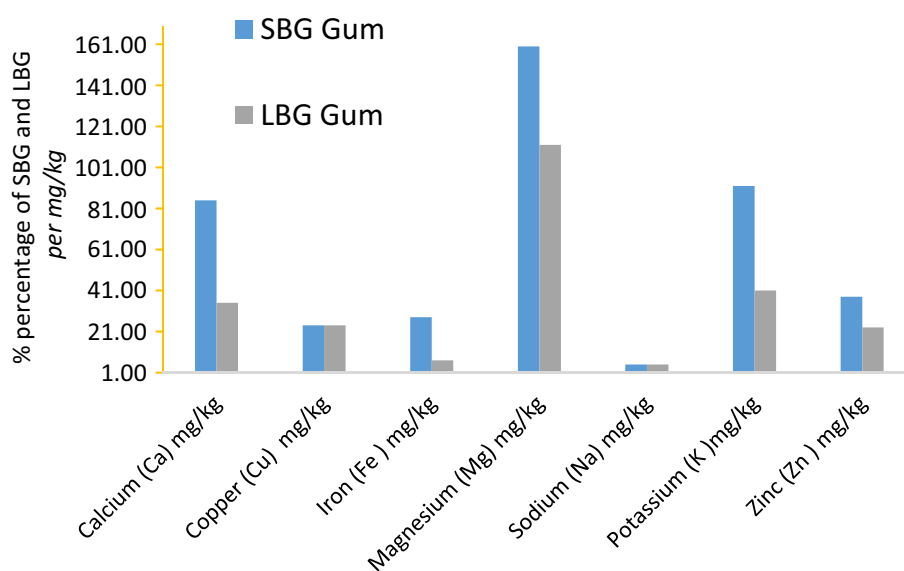


Fig. 3 Minerals content of SBG, compared to LBG gum

Minerals content % of novel SBG and LBG

Table 3 Physical characteristics of novel SBG compared with the locust gum (LBG)

Parameters (%)	SBG (%)	LBG (%)
Solubility	90.80 ± 0.003	92.13 ± 0.001
Viscosity (CPS)	3600 ± 13.710 CPS	4400 ± 57.284
Specific rotation	2.26 Deg-dm1gm	2.13 Deg-dm1gm
Ref. Index R.V	1.46 g/mL	1.53 g/mL
PH	6.65 ± 0.002	6.96 ± 0.004
Yellow color	7.1 ± 0.051	8.3 ± 0.003
Blue color	0.2 ± 0.0001	0.28 ± 0.0001
Red color	0.1 ± 0.0003	0.3 ± 0.00004
Electrical conductivity (S/m)	0.264 ± 0.003	0.218 ± 0.003

All values in the table are a means of three replicates ± S. D

SBG Sudan bean gum, LBG locust bean gum, R.V (cps) Relative viscosity in centipoises

rotation of a pure substance at a given wavelength and temperature must also be associated to the heat at which the sample was taken and the solvent in that the substance was dissolved. Fix Sudan gum *P. thonningii* seed gum 2.26 Deg-dm1g-1. (Table 3). The colours of gums vary from water-white (colour less) through shades of yellow to blue.

2.4 Rheological properties of novel gum

The Table 4 demonstrated that the rheological Properties of novel Sudan bean gum (SBG) from *P. thonningii* seeds has a high-Water holding capacity (W.H.C) compared to the locust bean gum (LBG), refers to how well materials can retain water even when gravity is pulling it down. The

Table 4 Rheological properties of Novel SBG compared to the locust bean gum (LBG)

Parameters (%)	SBG (%)	LBG (%)
Water holding capacity (WHC)	23.7 ± 0.001	18.730 ± 0.003
Emulsion stability (ES)	1.9 ± 0.002	1.89 ± 0.001
Foam stability (FS)	20.5 ± 0.01	22.34 ± 0.0007

SBG Sudan bean gum, LBG locust bean gum, All values in the table are a means of three replicates ± S. D

gum sample was found to retain an average of 23.7% water, surpassing the 18.73% retention of *C. siliqua* LBG.

The Emulsifiers properties, including agents with surfactant activity, help stabilize the mixture of two liquids incompatible, essential for creating and maintaining an emulsion. *P. thonningii* seeds gum enhances the emulsification and dispersion of Sudanese SBG, although this emulsifying activity is not found in sugars. The emulsion stability when protein impurities are present in Sudan bean gum extracted from *P. thonningii* seeds (Camel foot), the emulsion stability of SBG gum ranged from 1.89 to 1.9 O.D (Table 4).

The Foam stability is important in whipped materials such as ice cream, whipped desserts, and frozen desserts in this study showed that the foam reached 20.5 cm of gum SBG. The ability of protein to form good Foam stability depends on its ability to change nature and form cohesive layers, create biomechanical force to prevent ripping and merging of droplets, and that material possesses sufficient viscosity when near the air-liquid interface.

Sudan bean gum reacts strongly with polysaccharides, such as Locust gum, Xanthan, and alginate gum. The rheological synergy may be desirable for medicinal applications due to its non-toxicity and possibility for many entanglement levels, according to Ref. [52, 53].

Gel Foams Stabilized by Xanthan Gum had a maximum foam thickness of 14.6–16.2 cm after 5 min. After a brief period of stability near the apex, the bubble started towards imploding. But, the foam viscosity curve of Xanthan Gum has been decreasing since the beginning, which contradicts the occurrence of foam expansion. The findings indicate that they are increasing concentration of Xanthan Gum improved the foam layer stability under heat [55].

3 Material and methods

3.1 Synthesis of novel bean gum

The novel gum is synthesized from the *P. thonningii* seeds, Sudan bean gum (SBG), extracted for first time by treating seeds with amount of H_2SO_4 , %, the *P. thonningii* seeds is softened in H_2SO_4 : H_2O (60:40 v/v) at 60 °C for 60 min, to break the hardness of the peel. Then they were washed well with distilled water to get rid of the traces of the acid [11]. They were soaked in water for 8 h, after which they were peeled manually to separate the peel from the inner seed components that represent layer (Endosperm) gum shown in (Fig. 1a, and the yellow protein layer (germ).

3.2 Biochemical characteristics of novel SBG

3.2.1 HPLC analysis of monosaccharide

The sugar compositions of SBG gum in the upper phase were determined following hydrolysis by using a Shimadzu HPLC, high-performance liquid chromatography [25, 26]. After precisely weighing the gum samples into Pyrex test tubes that had been stopped (15 cm, 0.03 g), 10 ml of H_2SO_4 acid 4.0% w/w was added to each tube. For four hours, the tubes were immersed in a water bath heated at 100 °C. Barium carbonate (2.0 g $BaCO_3$) was added to the hydrolysate to neutralize it, and it was then allowed to mix overnight at room temperature and shaken for 12 h. Whatman filter paper (0.45 μm) was used to filter the hydrolysates, and an HPLC system was used for analysis. Auto sample controller, linked to LC column, and refractive index detector (RID-10A) at 30 °C were used. A mobile phase consisting of 75:25 v/v acetonitrile was used to elute the sample (10 ml hydrolysate), and the water was filtered and dehydrated before using, samples were eluted at 30 °C with a flow rate of 0.1 mL/min. Using a differential refractometer (RID-10A, water), the monosaccharide retention times (RT) were recorded. Each

sugar retention time was determined from chromatograms, and standard curves for each sugar standard were obtained. The RT observed were compared to those calculated using D (+) Mannose and D (+) Galactose as standards. The spot ratio of every peak was evaluated by the Miltinum database, which is related to the RI-IQA detector.

Then the sugar (percent) was calculated as follows:

$$\text{Component Sugar (i)} = \frac{\text{Area percent of component (i)}}{\text{Total area}} \times 100 \quad (1)$$

X-ray spectroscopy (XRF) was utilized by Ref. [33], to determine the mineral content of gum and the percentage of protein was determined by Ref. [28], Moisture and CHO in the gum sample, according to Ref. [27].

3.3 Physical characteristics of novel SBG

Refractive index of the extracted SBG gum was determined according to, [27], using Refractometer, Kruss/Alsterdorferstaz-D22297 D E157167706. The refractive index of extracted guar gum (1-gm gum/100 ml distilled water) was read directly.

The reading was repeated three times for each sample

$$\text{specific gravity (at } 30^\circ\text{C)} = \frac{A - B}{C - B} \times 100 \quad (2)$$

Where: A = Weight in gram of specific gravity bottle with liquid. B = Weight in gram of specific gravity bottle (empty). C = Weight in gram of specific gravity bottle with water.

The specific rotation of gum measured by using an Automatic digital Polari meter/Krossr P3001R with dm column tube at 29 °C according to the method described by Ref. [31]. The solubility and viscosity were assessed based on Mantella method by Ref. [29], 1 g of the sample was dissolved in 100 ml of distilled water. The optical density was determined according to [30]. The pH of the gum was measured by the Ref. [27], and the color measured with a Lovibond colorimeter following the method outlined by Ref. [32].

3.4 Rheological properties determination

3.4.1 Water holding capacity (WHC)

The method for determining water holding capacity (WHC) is provided by [34]. The WHC of SBG and LBG was calculated using the following equation:

$$\text{WHC(\%)} = \frac{W_2}{W_1} \times 100 \quad (3)$$

WHC = water Holding Capacity. W1 = weight added water. W2 = weight formulation (added water after time).

3.4.2 Emulsion stability (Es)

The ES of novel gum was measured by using the procedure described according to [35]. A gum solution (concentration 20%) was mixed with sunflower oil in the ratio 80:20 w/w. One minute of use of a mixer. The solution was then diluted 1:1000 and read at 520 nm using a spectrophotometer with serial number SP-1105/25 ~ 264 VAC. After an hour, a second reading was obtained. The emulsion stability (ES) = was calculated.

$$E.S = \frac{\text{first reading}}{\text{reading after 1 hour}} \quad (4)$$

3.4.3 Foaming stability

Foam stability (FS) and foam capacity (FC) were assessed as stated by Kabirullah and Wells method [36]. The process includes combining 30 ml of gum (% 1) at 25 degrees Celsius for two minutes. The mixture was moved into the measuring cylinder (100 ml) to determine the FS and FC. The foam volume after 30 s is recorded as a percentage.

$$FC (\%) = \frac{\text{Volume after whipping} - \text{vol before whipping} \times 100}{\text{volume before whipping}}$$

3.4.4 Statistical analysis

The study results were statistically evaluated as means and standard errors of mean for each group. The values were analyzed using the student t-test and found to be significant based on the statistical analysis of group differences. Correlation coefficients and levels were established at $p \leq 0.05$ and $p < 0.01$. (According to the t-test calculator for 2 independent means [37].

4 Conclusions

In this research involved the extraction and purification of novel Sudan bean gum (SBG) from *P. thonningii* seeds, The biochemical and physical, and rheological properties of purified SBG was evaluated and compared with LBG, revealed that SBG was close to Locust gum in General, in ingredients and properties, the study suggests that *P. thonningii* seeds gum is a promising source to produce Gum. Two interesting features of SBG have been identified, namely in terms of the possibility of deriving their chains and / or constructing very attractive interlocking bridges to investigate various options. On the other hand, its possibility of synergy with

other biopolymers is impressive, which expands its potential uses. Further study is needed to identify bioactive compounds of SBG.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [SOMIA A. AGAB1] and [MOHAMED O. ELSAMANI2*]. All authors conducted data investigation, writing, read and approved the final manuscript.

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Data availability Data will be made available on reasonable request, in the main manuscript file.

Declarations

Competing interests The authors declare no conflict of interest.

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