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Research Article

Antidiabetic Potential of Polysaccharide Extract from *Ferula assa-foetida* Gum Resin Harvested in Algerian Sahara

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ABSTRACT

Ferula assa-foetida (Apiaceae), a naturally occurring medicinal plant found in the Ghardaia region of the Algerian Sahara, has garnered interest for its therapeutic potential. This study aims to partially characterize and assess the antidiabetic activity of water-soluble Polysaccharides isolated from *F. assa-foetida* gum-resin (PGFA). PGFA were obtained through extraction with distilled water at 80°C for 2 hours, followed by polysaccharide precipitation using three volumes of ethanol 96% and subsequent freeze-drying. The extraction yield thus obtained was 24.3%. The total carbohydrate content was 77% ± 0.22%, comprising 46.35% ± 0.015% neutral carbohydrates and 18.7 ± 0.045% uronic acids. Thin-Layer Chromatography (TLC) analysis of PGFA revealed the presence of galacturonic acid, glucuronic acid, arabinose, galactose, rhamnose and xylose. Evaluation of the antidiabetic activity, specifically Alpha (α)-D-glucosidase inhibitors demonstrated a significant inhibitory effect of PGFA (77.66%) at a concentration of 100 mg/ml-1. This inhibition was compared to acarbose, a positive control, which exhibited strong inhibitory activity (100%) at the same concentration. These findings support for the use of polysaccharides extract from *Ferula assa-foetida* in preventing diabetic complications.

Keywords: Polysaccharides, *Ferula assa-foetida*, Gum-resin, Thin-layer chromatography, α-D-glucosidase inhibitors

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INTRODUCTION

Diabetes Mellitus (DM) caused by hyperglycaemia, has already become a global problem which is threatening thousands of people's health. Moreover, the long-term presence of hyperglycaemia can cause chronic damage to various tissues and bring serious complications to the human body (Yang JP, *et al.*, 2012; Zhang L, *et al.*, 2016). Particularly, inhibiting the activity of enzymes related to starch digestion (α-glucosidase and α-amylase) is one of the best ways to control blood glucose. Inhibition of glucosidase is one of therapeutic approach in lowering glucose level in the blood, thereby leading towards management of diabetes mellitus (Ha TJ, *et al.*, 2012; Zhang Z, *et al.*, 2016).

Polysaccharides are the macromolecules which are composed of sugars. They constitute of an important class of bioactive compounds with a wide range of pharmacological activities. The utilization of plant polysaccharides, especially exudate gums (gums), is attributed to their diverse structural and functional properties in the realms of food, pharmaceuticals, cosmetics, textiles and biomedical products (Mirhosseini H and Amid BT, 2012).

Numerous species within the *Ferula* genus have been known since ancient times as sources of gum-resins. *F. assa-foetida*, a spontaneous medicinal plant belonging to this genus, has been the subject of pharmacological and biological studies, which have exposed its anti-oxidative, antiviral, antifungal, anticancer, antidiabetic, antispasmodic and hypotensive properties (Mahendra P and Bisht S, 2012). *F. assa-foetida* is employed as a sedative, analgesic, antispasmodic, and diuretic (Bagheri SM, *et al.*, 2014).

The study aims to extract and partially characterize water-soluble polysaccharides from *F. assa-foetida* gum-resin collected in the Algerian Sahara. Additionally, the study evaluates the anti-diabetic activity of these polysaccharides through *in vitro* testing.

MATERIAL AND METHODS

Plant materials and chemicals

Ferula assa-foetida was collected from Ghardaia (Algeria) in November 2016. The gum-resin was meticulously separated and subsequently stored at room temperature in a dark and dry environment. Standard monosaccharides such as arabinose, rhamnose, galactose, glucose, mannose, glucuronic acid, galacturonic acid, metahydroxydiphenyl, Trifluoroacetic Acid (TFA), α-glucosidase, acarbose and p-Nitrophenyl α-D-Glucopyranoside (p-NPG) from Sigma-Aldrich in Germany were obtained. Additionally, Bovine Serum Albumin (BSA) was used in our experiments.

Extraction of PGFA

The water polysaccharides extraction process followed the methodology described by Wang M, *et al.*, 2013, Chidouh A, *et al.*, 2014 and Chen G, *et al.*, 2016. Twenty grams of powdered gum-resin from *Ferula assa-foetida* underwent a pretreatment process using petroleum ether for 24 hours at room temperature. Subsequently, the insoluble residue was macerated in distilled water (v/v) for 2 hours at 80°C with continuous agitation. After this maceration, the mixture was filtered. The resulting filtrate was then subjected to centrifugation at 4000 × g for 15 minutes. Following centrifugation, three volumes of 96% ethanol were added to the supernatant and the mixture was stored at -4°C for 24 hours. The precipitate that formed was collected by centrifugation at 10000 × g for 15 minutes and subjected to three washes with acetone. Finally, the precipitate was freeze-dried to obtain the water-soluble polysaccharides fraction, which is named PGFA.

Biochemical composition

The chemical composition of PGFA was comprehensively ana-

lyzed. The total sugar content was determined using the phenol-sulfuric method (Dubois M, *et al.*, 1956), while neutral sugar content was assessed through the resorcinol-sulfuric acid assay (Monsigny M, *et al.* 1988). In both cases, glucose was served as the standard for calibration. Total uronic acid content was quantified colorimetrically using the m-hydroxydiphenyl assay, with glucuronic acid as the standard reference (Blumenkrantz Z and Asboe-Hansen, 1973). To determine the protein content, the micro-Bradford method was employed, as outlined by Bradford in 1976, with BSA by using as the standard for calibration.

TLC of PGFA

25 grams of PGFA was hydrolyzed with TFA for 4 hours at 100°C, followed by evaporation and recovery with 1 ml of distilled water. The hydrolysate and standard sugars were separated using TLC on silica-gel plates as the stationary phase, employing two distinct mobile phase systems. Regarding the mobile phases utilized, they were as follows, system 1 consisting of chloroform, n-butanol, methanol, acetic acid and water (Yang C, *et al.*, 2010) and system 2 comprised of acetonitrile, ethyl acetate, propanol and water (Han NS and Robyt JF, 1998). Further, spot visualization was carried out and the Retention Factor (R_F) values for the separated spots, including the PGFA hydrolysate and various standards, were calculated.

Antidiabetic activity of PGFA

The antihyperglycemic activity of PGFA was investigated by assessing its inhibition of α-glucosidase activity. The α-glucosidase inhibition assay was conducted following the methodology described by Bisht S, *et al.*, 2013 with slight modifications. In dry tubes, 500 µl of α-glucosidase solution (2 IU/l^{-1}) was mixed with 100 µl of each PGFA dilution (2.5 to 100 mg/ml^{-1}), acarbose (positive control), or ultrapure water (negative control). The mixture was pre-incubated at 37°C for 15 minutes. Subsequently, 100 µl of a p-NPG (p-nitrophenyl-α-D-glucopyranoside) solution (4 mM) was added to each tube. The tubes were shaken and then incubated at 37°C for 20 minutes. To stop the enzymatic reaction, 1 ml of Na_2CO_3 (0.2 M) was added and the absorbance were measured at 405 nm. All experiments were conducted in triplicate. The results of the inhibition activity were expressed using the following equation:

$$\text{Inhibition (\%)} = (A_{control} - A_{sample})/(A_{control}) \times 100$$

Statistical analysis

The data was analyzed using Origin Pro8 software and Microsoft Excel 2007.

RESULTS AND DISCUSSION

Biochemical composition

The extraction yield of PGFA was 24.3% (w/w), which exceeded the yields of polysaccharides found in *Ferula gumosa* Boiss gum, where it was reported as 5%, but was slightly lower than the yield of polysaccharides extracted from *Ferula assa-foetida* gum harvested in Iran, which was reported as 27.1% (Saeidy S, *et al.*, 2018), and also lower than the polysaccharides extracted from *Ferula communis* gum, which had a yield of 33.5% (Youmbai A, *et al.*, 2021).

The biochemical analysis of PGFA is presented in *Table 1*. PGFA consisted of approximately 77 ± 0.22% of total sugars, with neutral sugars accounting for 46.35±0.015% and uronic acid at 18.7 ± 0.045%. Additionally, the protein content was found to be relatively low at 2.06 ± 1.02%. These findings are comparable to the water-soluble polysaccharides extracted from *Ferula communis* gum resin, which contained 5.29% (m/m) protein, 71.45% (m/m) uronic acid, and 27.90% neutral sugars (Youmbai A, *et al.*, 2021). Saeidy S, *et al.*, 2018, reported that polysaccharides extracted from *Ferula assa-foetida* gum, which contained 67.39% total sugars and 5.2% uronic acid.

Partial structural characterization of PGFA by TLC

TLC chromatography analysis was employed to elucidate the composition of the polysaccharide extract, focusing on its constituent sugars. This was accomplished by comparing the R_F values of hydrolyzed spots with those of reference standards (*Table 2*). In system 1 (*Figure 1*), we observed four distinct R_F values for the sugars are 0.166, 0.416, 0.527 and 0.638. These R_F values were found to correspond to the following sugars: 0.166 (galacturonic acid), 0.416 (galactose), 0.527 (arabinose) and 0.638 (rhamnose). In contrast, system 2 (*Figure 1*) exhibited 5 different R_F values for the sugars are 0.136, 0.191, 0.520, 0.438 and 0.643. These values were identified as 0.136 (galacturonic acid), 0.191 (glucuronic acid), 0.520 (galactose), 0.438 (arabinose) and 0.643 (rhamnose). These findings collectively suggest that the crude extract of water-soluble polysaccharides from *Ferula assa-foetida* gum comprises a heteropolysaccharide consisting of galacturonic acid, glucuronic acid, galactose, arabinose, rhamnose, and xylose.

In *Figure 2*, PGFA exhibited inhibitory effects on α-D-glucosidase activity, albeit with a lower potency compared to acarbose. As the concentration of PGFA increased from 2.5 to 100 mg/ml^{-1}, a dose-response relationship was observed, with the inhibition rate of PGFA increasing. The inhibition rate reached a maximum of 77.66% at a concentration of 100 mg/ml^{-1}.

Comparatively, Youmbai A, *et al.*, 2021, reported that the inhibitory efficacy of acarbose reaches its maximum of 100% at a concentration of 6.45 mg/ml^{-1}, while polysaccharides extracted from *Ferula communis* gum achieves similar inhibition at 100 mg/ml. Bisht S, *et al.*, 2013, reported that polysaccharides isolated from Acacia tortilis gum had an Half-maximal Inhibitory Concentration (IC_{50}) of 0.5 mg/ml^{-1} for α-D-glucosidase inhibition. Wang XT, *et al.*, 2016, found even stronger inhibition of α-D-glucosidase at 0.8 mg/ml^{-1} using heteroglucan extracted from *Fagopyrum tartaricum*. Jia X, *et al.*, 2017, demonstrated that among three polysaccharide fractions isolated from *Rhynchosia minima* root, the fraction richest in arabinogalactan displayed the strongest inhibition of α-D-glucosidase activity, with an IC_{50} value of 8.85 mg/ml^{-1}.

Table 1: Biochemical characterization of polysaccharides from *F. assa-foetida* gum

Extraction yield (% w/w)	Carbohydrate (w/w %)			Proteins (% w/w)
	Total	Neutral	Uronic acid	
24.3	77 ± 0.22	46.35 ± 0.015	18.7 ± 0.045	2.06 ± 1.02

Table 2: The R_F values of the separated spots of PGFA and the standards using system 1 and 2

Sugar names	L-galactose	Glucuronic acid	Arabinose	Galactose	Glucose	Mannose	Rhamnose	Xylose
Standards (system 1)	0.166	0.208	0.527	0.416	0.458	0.472	0.638	0.569
PGFA	0.166	/	0.527	0.416	/	/	0.638	/
Standards (system 2)	0.136	0.191	0.52	0.438	0.493	0.465	0.643	0,561
PGFA	0.136	0.191	0.52	0.438	/	/	0.643	/

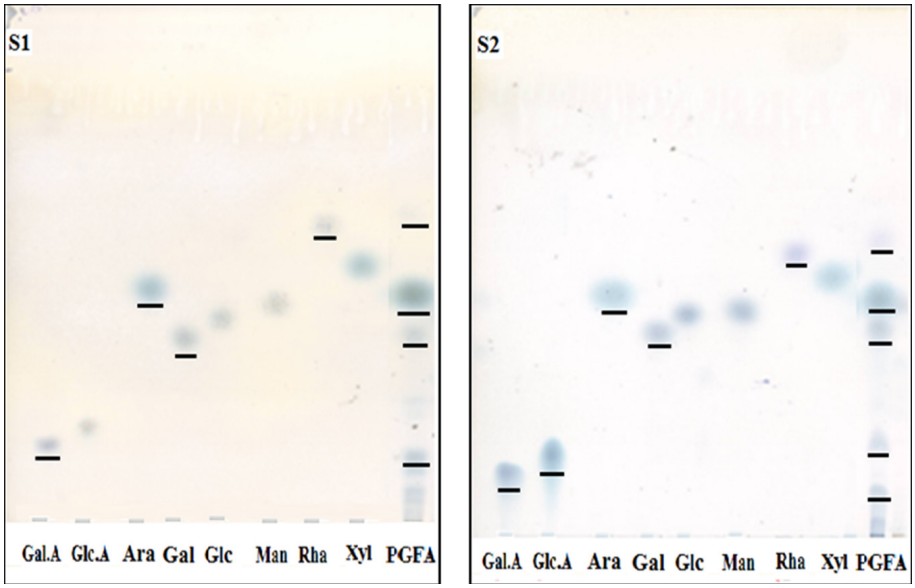

Figure 1: Chromatogram of polysaccharide hydrolysates of PGSA using system 1 and system 2

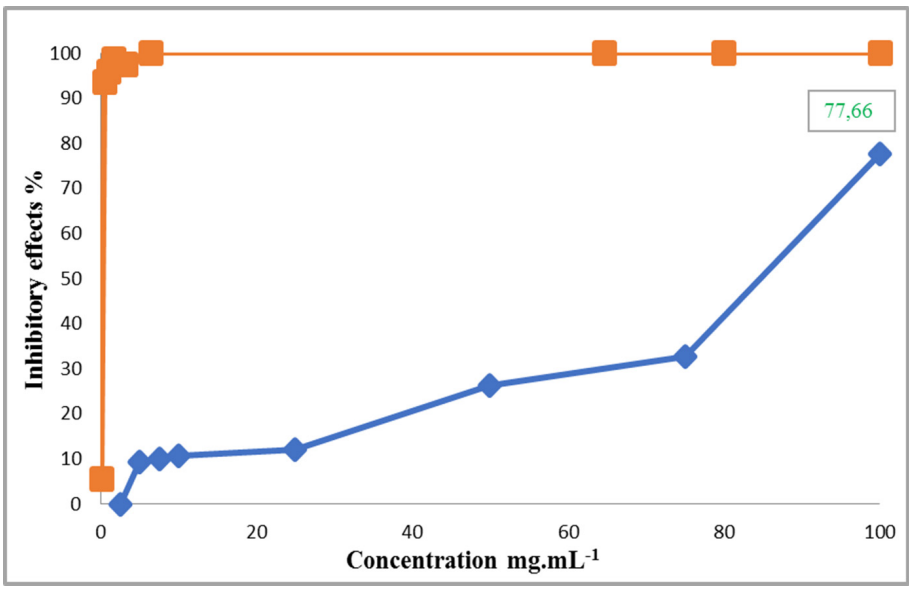

Figure 2: Inhibitory effects of PGFA and acarbose on α-D-glucosidase activity
Note: (—◆—): *Ferula assa-foetida*; (—■—): Acarbose

PGFA has shown a significant inhibitory activity against α-D-glucosidase, suggesting its potential as a promising therapeutic agent for the management of blood glucose. This potential could further be harnessed in the health food industry. It is well-established that the effects of polysaccharides may vary depending on factors such as glycosidic linkage, molecular weight, conformation, and degree of branching. Therefore, it is recommended that further research be conducted to investigate the structural characteristics of *Ferula assa-foetida* gum polysaccharides.

CONCLUSION

The gum-resin of *Ferula assa-foetida* is rich in polysaccharides. Structural analysis of the polysaccharide extract by thin-layer chromatography using two separation systems revealed the presence of galacturonic acid, glucuronic acid, arabinose, galactose, rhamnose, and xylose. The polysaccharides from the gum-resin exhibited a significant inhibitory effect on the α-D-glucosidase enzyme at the tested concentrations. This finding could provide a scientific basis for the traditional use of this plant for its anti-diabetic effect.

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