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Research Article

In Vitro Evaluation of α-Amylase, α-Glucosidase Inhibition Activity and Protein Denaturation Inhibition of Polysaccharides from Leaves and Seeds of *Zygophyllum album* L.

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ABSTRACT

The present study investigates anti-hyperglycemic activity and protein denaturation inhibition of polysaccharide extracts from leaves (ZF) and seeds (ZG) of *Zygophyllum album* L (Zygophyllaceae), a wild plant harvested in the south-east of the Algerian Sahara. The global composition of the extracts was determined by spectrophotometric assays, and the monosaccharide composition was determined by TLC and HPLC-RID. The extraction yields were 0.53 ± 0.04% and 2.33 ± 0.52% for ZF and ZG, respectively. The leaf extract is rich in uronic acids (32.91 ± 0.23%) compared to the seed extract (5.98 ± 1.31%). The analysis of both extracts indicates that their composition of constituent monosaccharides is similar. ZF consists of 20.90% arabinose, 24.16% galactose, 22.88% xylose, 14.71% rhamnose, and 17.34% glucose, and ZG of 21.64% arabinose, 31.76% galactose, 16.95% xylose, 17.25% rhamnose, and 12.39% glucose. Their FT-IR spectra display the characteristic bands of polysaccharides. Seed extract shows a significant inhibitory effect against protein denaturation (IC_{50} of 0.93 ± 0.02 mg/mL & 22.75 ± 1.41 mg/mL for ZG & ZF, respectively). However, the leaf extract, with a low α-amylase inhibitory activity (IC_{50} of 90.16 ± 5.38 mg/mL) and a high α-glucosidase inhibitory power (IC_{50} of 5.44 ± 0.50 mg/mL), seems to be effective in reducing postprandial hyperglycemia.

Key words: α-amylase, α-glucosidase, polysaccharides, protein denaturation, *Zygophyllum*

INTRODUCTION

For centuries, humans have used medicinal plants as natural remedies due to their therapeutic properties (El Youbi *et al*., 2012). They contain several compounds like terpenes, polyphenols, alkaloids, coumarins, and polysaccharides, which exhibit structural complexity, chemical diversity, and a range of biological activities not comparable to synthetic products (Lopez, 2011).

Polysaccharides are high molecular weight polymers; together with oligosaccharides, they are among the most abundant organic compounds in nature. A polysaccharide is made up of a single type of monosaccharide (homoglycan) or of several types of monosaccharide (heteroglycan). Its basic structure can be linear or branched. The sequence of osidic residues can be periodic (a single motif repeats regularly) or aperiodic (Bruneton, 1999; Bauer *et al*., 2010). They play various structural and metabolic roles, and contribute to biological processes, as cell division, immune response, growth, cell communication, and cell adhesion (He *et al*., 2012; Liun *et al*., 2015). The polysaccharides' structural variability, including monosaccharide composition, sugar units' sequence, glycosidic linkages, branching point, and polymerization degree, confers diverse biological activities and physicochemical properties (Colliec-Jouault *et al*., 2004; Zong *et al*., 2012).

Zygophyllum album L. is a halophyte species belonging to the Zygophyllaceae family. It exhibits a wide geographical distribution (Souddi & Bouallala, 2023). It is found throughout tropical East Africa, Arabia, and the Sahara in North Africa (Mohammedi, 2020). *Z. album* is a perennial subshrub with fleshy, compound leaves with two leaflets (Ozenda, 2004). It is found in saline or gypsum soils and sebkha (Ksouri *et al*., 2013). This plant plays an important role in the rehabilitation of degraded soils (Souddi & Bouallala, 2023). In North African folk medicine, this succulent plant is utilized in decoctions, powders, or ointments for the treatment of diabetes, indigestion, dermatosis (Chehma & Djebar, 2008; Lakhdari *et al*., 2016), rheumatism, gout, and asthma (Tigrine-Kordjani *et al*., 2006).

According to published research, *Z. album* is characterized by its richness in secondary metabolites, including flavonoids, saponins, terpenes, alkaloids, tannins, and sterols, which exhibit several biological activities such as the anti-hyperglycemic

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activity, the anti-hyperlipidemic activity, the anti-inflammatory activity, and the antioxidant activity (Ghoul *et al*., 2012; Mnafgui *et al*., 2012; Ksouri *et al*., 2013; Feriani *et al*., 2019; Abdelhameed *et al*., 2022). Kchaou *et al*. (2016) reported that the essential oil extracted from the leaves of *Z. album* is a potent inhibitor of pancreatic α-amylase. Ghoul *et al*. (2012) showed that administration of the ethanolic extract of the plant to streptozotocin-induced diabetic mice for 14 days resulted in a significant reduction in plasma glucose, cholesterol, triglycerides, LDL, and VLDL lipoproteins. Mnafgui *et al*. (2014) reported that the ethanolic extract of leaves and flowers of this plant shows remarkable anti-inflammatory activity, resulting in a decrease in serum levels of C-reactive protein (CRP) and tumour necrosis factor (TNF-α) in diabetic rats.

Moreover, no research has been reported on the study of polysaccharides of *Z. album*. Thus, the objective of this research is to preliminarily characterize polysaccharides from the leaves and seeds of this species, and evaluate the anti-hyperglycemic and anti-inflammatory activities.

MATERIALS AND METHODS

Plant material

Z. album's leaves of comparable morphological appearance were harvested in March 2019 in El Bour, Ouargla city (Septentrional Algerian Sahara, 32° 5′ 32.87″ North latitude & 5° 17′ 51.43″ East longitude). The Seeds were collected in November 2019 in the same locality. The collected parts were dried at room temperature for three weeks and then ground into a fine powder (<500 µm) using cutting mills (Retsch, SM 100, Germany).

Chemicals

Standards monosaccharide (D-galacturonic acid (48280), D-glucuronic acid (G5269), D-galactose (G0750), L-arabinose (13256), L-rhamnose (W373011), D-mannose (M6020), D-xylose (X1500), D-glucose (G8270)), meta-hydroxydiphenyl (m-HDP) (262250), trifluoroacetic acid (TFA) (T6508), α-glucosidase (G5003-100UN), acarbose (A8980-1G), p-nitrophenyl-α-D-glucopyranoside (p-NPG) (N1377-1G), porcine pancreatic α-amylase (A3176), 3,5-dinitrosalicylic acid (DNS) (128848) and starch (S9765) were bought from Sigma-Aldrich. The rest of the used chemicals were of an analytical grade.

Extraction of polysaccharides

Fifty grams of dried and ground leaf powder of *Z. album* were pretreated with 500 mL of ethanol under reflux using a Soxhlet; then, the depigmented extract was dried at 50°C for 24 hr. The depigmented leaf powder was macerated in distilled water (1:20, w/w) at 60°C for 4 hr under constant stirring. The obtained mixture was filtered via a fine mesh strainer to remove insoluble debris, then filtered successively under vacuum on a sintered glass filter (DURAN, Germany), with decreasing porosity. Proteins were precipitated later by adding 4% trichloroacetic acid while stirring. After centrifugation (12000 rpm / 30 min), alcoholic precipitation of the supernatant was performed by three volumes of ethanol (96%) at 0°C for 16 hr. After centrifugation, the precipitate was washed subsequently with ethanol and acetone and then dried at 50°C for 24 hr. The crude extract was dissolved in distilled water, then precipitated with ethanol (0°C, 16 hr), and subsequently dried at 50°C for 24 hr. Finally, the crude extract was ground into a fine powder and coded ZF.

The extraction of polysaccharides from seeds of *Z. album* followed the same procedure as described for the leaves, excluding the initial depigmentation and the final desalting steps. Briefly, the seed powder was directly extracted with hot distilled water, followed by filtration, ethanol precipitation, washing with ethanol and acetone, and drying to obtain the crude extract (ZG).

Biochemical composition

The phenol-sulfuric acid method was applied to quantify the total amount of carbohydrates (Dubois *et al*., 1956), and the concentration is determined by reference to a glucose calibration curve from 0.02 to 0.14 g/L. The meta-hydroxydiphenyl assay was used to estimate the uronic acid content (Blumenkrantz & Asboe-Hansen, 1973), and standard concentrations of galacturonic acid range from 0.02 to 0.1 g/L. The Bradford method (1976) was utilized to determine protein content in the crude extract, and standard concentrations of Bovine Serum Albumin (BSA) range from 0.02 to 0.1 g/L. The Folin-Ciocalteu assay was employed to quantify the polyphenols (Singleton *et al*., 1999), and the concentration is calculated by reference to a gallic acid calibration curve from 0.05 to 0.35 g/L. Each concentration of the standard or sample was analyzed in triplicate.

Fourier-transform infrared spectroscopy (FT-IR) analysis

The mixture of 5 mg of polysaccharide and 100 mg of KBr was ground and pressed into a disk. Infrared spectra (400 - 4000 cm^{-1}) were recorded at room temperature (referenced against air, 32 scans) using a FT-IR spectrophotometer (Agilent Technologies Cary 600).

Monosaccharide composition analysis

The acid hydrolysis of the polysaccharide for 4 hr at 110°C was carried out using 2 M TFA (Morrison, 1988). The monosaccharide composition was determined by Thin-Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) with Refractive Index Detector (RID).

Thin-layer chromatography

Hydrolysates and standards monosaccharide were analyzed on silica gel plate (Silica gel 60 F_{254}, Merck, Germany), using two elution systems: The 1st system consists of acetic acid, n-butanol, water, pyridine, ethyl acetate in proportions of 2:10:4:4:5 respectively (Hoton-Dorge, 1976); and the 2nd system consists of water, acetic acid, methanol, n-butanol, chloroform, in proportions of 1.5: 1.5: 5: 12.5: 4.5 respectively (Cheng *et al*., 2010). After elution, the plates were taken out, dried, sprayed with

Diphenylamine–aniline–phosphoric acid (DPA) reagent, and dried again at 100°C for 10 min to visualize the colored spots. The monosaccharide was identified by the retention factor (R_f) of the sample and by comparing it with the standards. TLC analysis was performed once per sample for preliminary qualitative identification of monosaccharides, without replicates.

High-performance liquid chromatography

Chromatographic separation was carried out using HPLC (SHIMADZU, Japan) equipped with Shim-pack GIST NH2 column (5 µm, 250 x 4.6 mm), LC-20ADXR pump, SIL-20ACXR automatic sampler, and RID-20A detector. Ultrapure water (20%) and acetonitrile (80%) were used as the mobile phase, at a flow rate of 1 mL/min. The analysis was performed in isocratic mode, and the injection volume was 10 µL. The column and RID temperature were set at 35°C (Xu *et al*., 2014).

Anti-hyperglycemic activity

The enzymatic inhibitory activity of α-amylase and α-glucosidase was applied to assess the anti-hyperglycemic activity of the extracted polysaccharides, using acarbose as a standard.

α-Amylase inhibition assay

By inhibiting the enzyme's ability to hydrolyze starch, the activity was performed. The 3,5-dinitrosalicylic acid (DNS) is used to quantify the hydrolysis of starch. The enzymatic hydrolysis of starch into monosaccharides is inversely proportional to the coloration's intensity (Thangaraj, 2016). The α-amylase inhibitory activity was carried out using the protocol of Apostolidis *et al*. (2007). The mixture of 500 µL of each extract and 500 µL of phosphate saline buffer (0.02 M, pH 6.9, 0.5 mg/mL of α-amylase, 6 mM NaCl) was prepared and incubated for 10 min at 25°C. After adding 500 µL of 1% starch solution, the mixture was re-incubated for 10 min at 25°C. The reaction was stopped by adding dinitrosalicylic acid solution (1 mL), and heating the mixture in a boiling water bath for 5 min. After cooling, the mixture was diluted by 10 mL of distilled water, and the absorbance was read by a UV-Vis spectrophotometer (Agilent Technologies, Cary 100) at 540 nm.

α-Glucosidase inhibition assay

In this assay, the enzyme hydrolyzes *p*-nitrophenyl-α-D-glucopyranoside into α-D-glucopyranoside and *p*-nitrophenol (p-NP). The latter is a colored product, and the enzymatic activity is correlated to its concentration (Thangaraj, 2016).

The inhibitory activity was performed by the protocol of Deng *et al*. (2020). A mixture of 20 µL of the sample, 20 µL of α-glucosidase (0.5 U/mL), and 120 µL of phosphate buffer (pH 6.8, 0.1 mM) was prepared and incubated at 37°C for 20 min. After the addition of 2.5 mM of *p*-NPG substrate (20 µL), the mixture was re-incubated at 37°C for 10 min. The reaction was terminated by the addition of 0.2 M sodium carbonate (80 µL), and the absorbance was determined at 400 nm.

Protein denaturation inhibition

The anti-inflammatory test was performed by the protocol of Drăgan *et al*. (2016) with slight changes, and diclofenac (CLOFENAL, 25 mg/mL, SAIDAL Group**)** was used as a standard. In this assay, the denaturation of protein was induced by heat, keeping the mixture at a temperature exceeding 50°C (Thangaraj, 2016). Bovine serum albumin was used as a protein source. A reaction mixture of 500 µL of Tris-HCl buffer (0.2 % BSA, pH 6.6) and 500 µL of the sample was prepared and incubated for 20 min at 37°C, then heated in a water bath at 70°C for 5 min. After cooling the mixture, the absorbance was read at 660 nm.

The percentage inhibition of the different tests was determined as follows:

$$Inhibition\left(\%\right) = \frac{Abs\ \ of\ \ control\ -\ Abs\ \ of\ \ sample}{Abs\ \ of\ \ control} \times 100$$

The control represents the mixture without the sample.
Each assay was conducted three times to ensure reproducibility.

Statistical analysis

The analysis is determined in triplicate, and the findings are reported as mean ± standard deviation (SD). The significance of differences in biochemical composition between extracts at a level of $p < 0.05$ was established by the Student's t-test for independent samples. The data analysis was performed using SPSS Statistics 29. IC_{50} values were determined using linear regression, with R^2 values ranging from 0.97 to 0.99, indicating a good fit of the regression models.

RESULTS AND DISCUSSION

Biochemical composition

The extraction yields of the polysaccharides from *Z. album* leaves (ZF) and seeds (ZG) are 0.53 ± 0.04% and 2.33 ± 0.52% respectively. The total sugar content of ZG (59.33 ± 1.48%) is higher than that of ZF (49.02 ± 3.86%) (p=0.049). A highly significant difference in the uronic acid content was noted between the two extracts (p=0.001). The uronic acids are major in ZF (32.91 ± 0.23%), and minor in ZG (5.98 ± 1.31%) (Table 1).

The uronic acid content, around 34.64%, was recorded in the water-soluble polysaccharides from the leaves of *Allium roseum* (Teka *et al*., 2022), close to that obtained by ZF. A higher content (47.5%) is mentioned in the crude extract of polysaccharides from the leaves of *Suaeda fruticosa* (Mzoughi *et al*., 2018).

Table 1. Biochemical composition of water-soluble polysaccharides from leaves (ZF) and seeds (ZG) of *Z. album* L.

Extract	Total sugars	Uronic acids	Proteins	Phenolic compounds
ZF	49.02 ± 3.86 a	32.91 ± 0.23 b	19.15 ± 0.35 a	1.64 ± 0.02 b
ZG	59.33 ± 1.48 b	5.98 ± 1.31 a	18.22 ± 0.28 a	1.31 ± 0.01 a

a and b show statistical groups that are significantly different (P<0.05)

However, the protein contents in both extracts are high despite the deproteinization step with trichloroacetic acid, i.e., 19.15 ± 0.35% and 18.22 ± 0.28% for ZF and ZG extracts, respectively (p=0.067). Rjeibi *et al*. (2019) reported high protein content even after the deproteinization step by the Sevag method, i.e., 18.67%, for water-soluble polysaccharides extract from the fruits of *Nitraria retusa*.

The levels of phenolic compounds in the polysaccharide extracts of *Z. album* remain low, at 1.64 ± 0.02% and 1.31 ± 0.01% in ZF and ZG, respectively, with a highly significant difference between the two extracts (P=0.002). Higher polyphenol levels than those obtained in *Z. album* extracts were recorded in the mucilaginous polysaccharides from the leaves of *Corchorus olitorius* (8%) (Benyakoub *et al*., 2020), and in the polysaccharide extracted by hot water from the leaves of *Ficus carica* (13.55%) (Jiang *et al*., 2023).

It is important to note that, so far, there has been no research on the extraction, structural characterization, and biological activity of polysaccharides from *Z. album* or its genus, unlike other phytochemicals. As a result, comparisons were made with polysaccharides extracted from other medicinal plants with well-characterised compositions, to contextualise and interpret the current results.

FT-IR analysis

Infrared spectra of both extracts display the typical peaks of polysaccharides (Figures 1 & 2). A broad and strong absorption band observed in the 3000 cm^{-1} to 3600 cm^{-1} range is typical of O-H stretching vibrations (Zhang *et al*., 2015; Hong *et al*., 2021). The peak recorded at 2920 cm^{-1} in both spectra is likely due to symmetrical and asymmetrical C-H stretching vibrations (Liu *et al*., 2014; Zhang *et al*., 2019). A broad and strong band with a peak around 1750 cm^{-1} attributed to C=O stretching vibrations (Synytsya *et al*., 2010; Zhang *et al*., 2019).

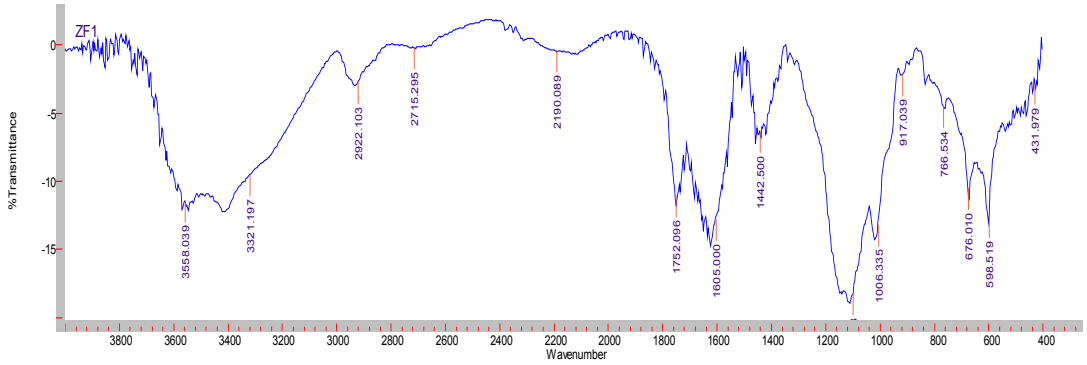

Fig. 1. FT-IR spectrum of ZF.

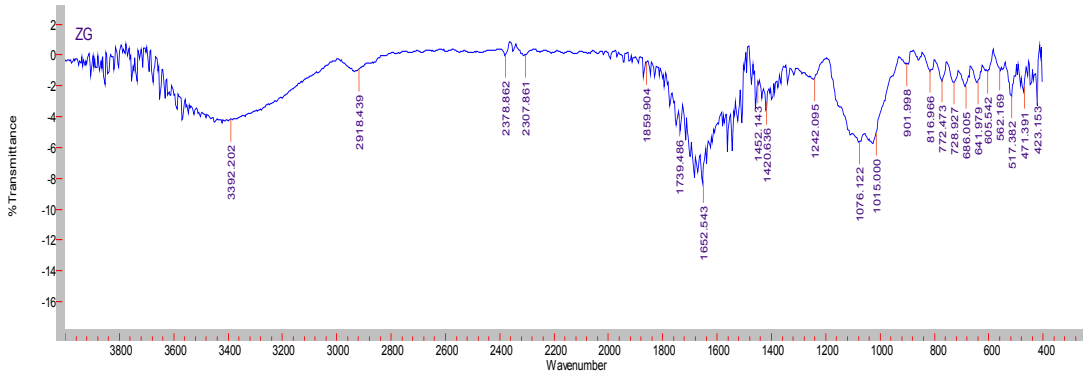

Fig. 2. FT-IR spectrum of ZG.

ZF shows a peak at 1605 cm^{-1} characteristic of the carboxyl groups (Ye & Lai, 2014; Wang *et al*., 2020). ZG displays a peak at 1652.54 cm^{-1} corresponding to water bending vibrations (Synytsya *et al*., 2010; Zhang *et al*., 2015). In both spectra, an intense band detected at 1430 cm^{-1} corresponds to C-H bending vibrations (Zhang *et al*., 2015). There is a band at 770 cm^{-1} assigned to the symmetric stretching vibration of the pyranose ring (Liu *et al*., 2014; Zhang *et al*., 2015).

Monosaccharide composition

Table 2 compiles the results obtained by TLC. The comparison of R_f values of standards with the water-soluble polysaccharides allows for the identification of their monosaccharide.

Table 2. R_f values of the standards, monosaccharide, and water-soluble extracted polysaccharide of Z. *album* L.

	R_f of system 1							
	Gal A	Glc A	Ara	Gal	Glc	Man	Rha	Xyl
Standards	0.126	0.138	0.463	0.337	0.397	0.439	0.692	0.560
ZF	/	/	0.464	0.340	/	/	/	0.560
ZG	/	/	0.460	0.337	/	/	/	0.560
	R_f of system 2							
	Gal A	Glc A	Ara	Gal	Glc	Man	Rha	Xyl
Standards	0.181	0.218	0.547	0.481	0.518	0.531	0.662	0.612
ZF	/	/	0.550	/	0.517	/	/	0.620
ZG	/	/	0.548	/	/	/	0.661	0.614

Gal A : galacturonic acid, Glc A : glucuronic acid, Ara : Arabinose, Gal : Galactose, Glc : Glucose, Man : Mannose, Rha : Rhamnose, Xyl : Xylose

Elution system 1 reveals the presence of arabinose, galactose, and xylose in both ZF and ZG extracts. Meanwhile, elution system 2 identifies the presence of arabinose, glucose, and xylose in ZF, and the presence of arabinose, xylose, and rhamnose in ZG (Figure 3).

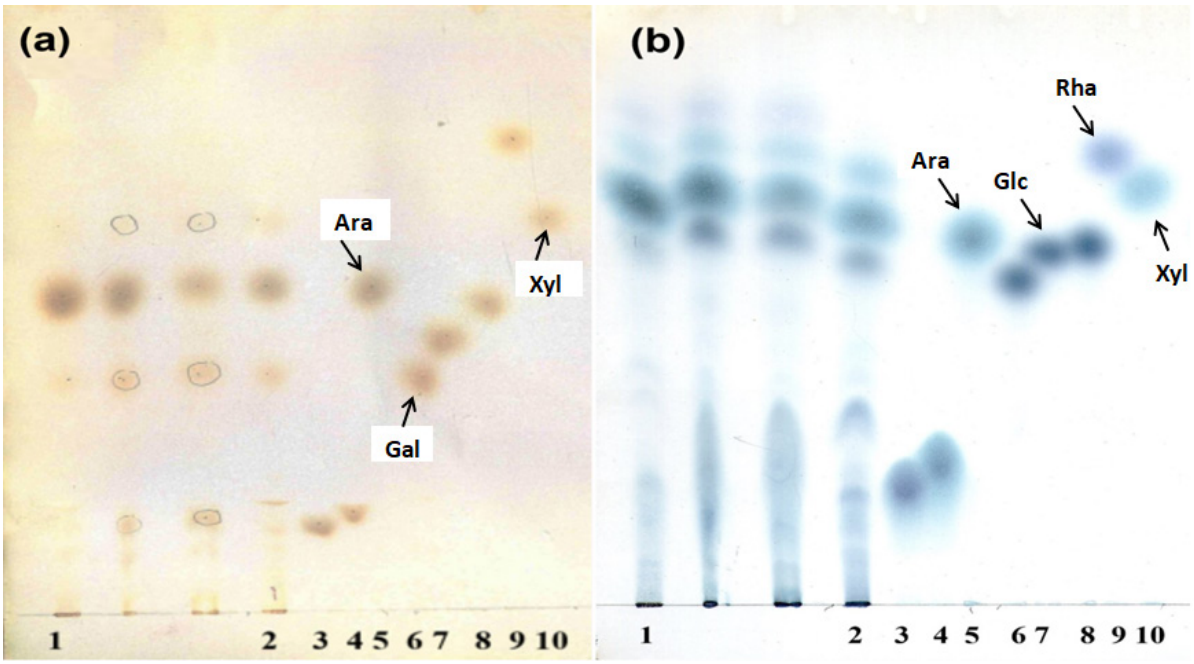

Fig. 3. (a)- TLC of ZF and ZG hydrolysates using elution system 1, **(b)-** TLC of ZF and ZG hydrolysates using elution system 2, (1: ZG, 2: ZF,3: galacturonic acid (Gal A), 4: glucuronic acid (Glc A), 5: Arabinose (Ara), 6: Galactose (Gal), 7: Glucose (Glc), 8: Mannose (Man), 9: Rhamnose (Rha), 10: Xylose (Xyl)).

HPLC-RID analysis of the polysaccharides indicated the existence of five neutral sugars. These are arabinose (20.90%), galactose (24.16%), xylose (22.88%), rhamnose (14.71%) and glucose (17.34%) for the leaf polysaccharide extract (Table 3), and arabinose (21.64%), galactose (31.76%), xylose (16.95%), rhamnose (17.25%) and glucose (12.39%) for the seed polysaccharide extract (Table 4).

Table 3. Monosaccharide composition of water-soluble polysaccharides from leaves of *Z. album* L.

Monosaccharide	Retention time (min)	(%)
Arabinose	6.852	20.90
Galactose	8.739	24.16
Xylose	6.306	22.88
Rhamnose	5.668	14.71
Glucose	8.266	17.34

The analysis of both extracts by HPLC-RID reveals their similar composition in constituent monosaccharides. Galactose and arabinose are the major sugars in ZF and ZG. The water-soluble polysaccharides in the leaves and seeds of *Z. album* are rich in arabinogalactans, which are essential structural polymers in the plant cell wall (Cipriani *et al*., 2006). Arabinogalactans are branched polysaccharides, constituted by β-D-galactopyranose (in the main chain) and α-L-arabinofuranose (in the side chain) units. Residues of rhamnose, glucose, galacturonic acid, and xylose may also be present (Saeidy *et al*., 2021). This type of heteropolysaccharide can be associated with proteins (Cipriani *et al*., 2006).

Table 4. Monosaccharide composition of water-soluble polysaccharides from seeds of *Z. album* L.

Monosaccharide	Retention time (min)	(%)
Arabinose	6.874	21.64
Galactose	8.741	31.76
Xylose	6.290	16.95
Rhamnose	5.660	17.25
Glucose	8.241	12.39

Anti-hyperglycemic activity

One therapeutic strategy of diabetes treatment, particularly type 2, is reducing the intestinal absorption of glucose by inhibiting enzymes that hydrolyze carbohydrates in the digestive system, including α-glucosidases and α-amylases (Kumar *et al*., 2011; Li *et al*., 2018). Since α-glucosidase is an essential enzyme in the hydrolysis of carbohydrates, most studies on natural antidiabetic drugs focus on its inhibition. Though the α-amylase inhibition leads to a reduction in the release of glucose, too, its total inhibition causes digestive disorders. Yet, partial inhibition allows for the modulation of glucose release rate (Kim *et al*., 2014). Many studies discuss the inhibition of α-amylase but do not specify which isoform is being referred to. Our study tested the pancreatic form, which is significant for postprandial glycemic control.

Inhibitory activity of α-amylase

The Inhibitory effect of α-amylase by ZG is considerably higher than that of ZF for all concentrations tested, with a low IC_{50} (22.107 ± 3.955 mg/mL) for ZG compared to ZF (90.163 ± 5.384 mg/mL).

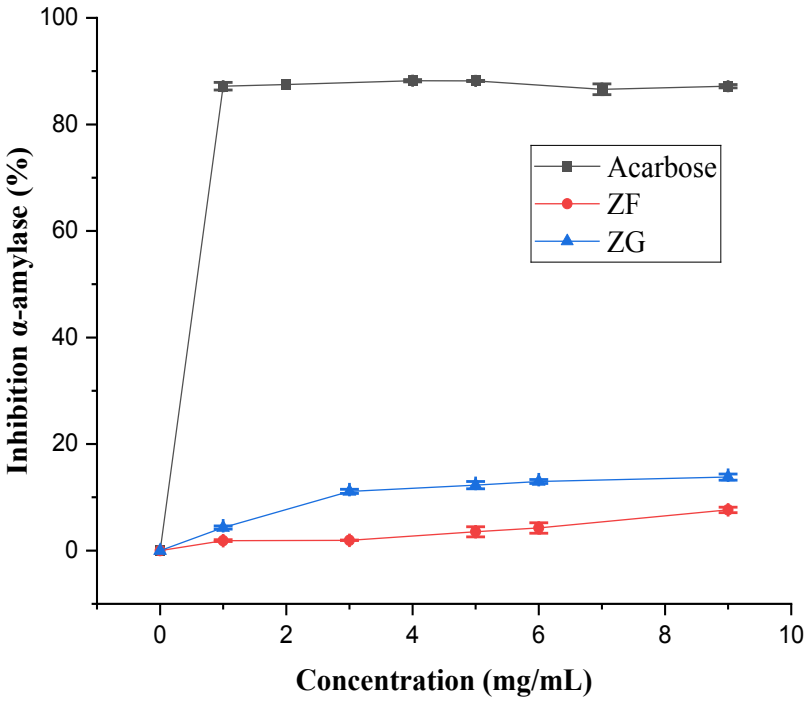

Fig. 4. Inhibition of *α*-amylase activity of water-soluble polysaccharides of *Z. album* L., error bars represent the standard deviation of the mean (*n*=3).

One of the mechanisms implicated in the *α*-amylase inhibition is the formation of a complex between the inhibitor and the enzyme's active site, directly obstructing substrate binding and subsequent catalytic activity. This is the case with acarbose. 0r it does not directly block the active site, but rather modulates interactions between the enzyme and substrate. This can occur through various mechanisms, such as increasing viscosity to hinder substrate diffusion or inducing conformational changes in

the enzyme that impact substrate binding affinity. Consequently, the inhibition leads to a slowing of glucose release by delaying the enzymatic degradation and intestinal absorption of carbohydrates (Kim *et al*., 2014).

Inhibitory activity of α-glucosidase

ZF showed a significant inhibitory effect on α-glucosidase at 7 mg/mL, with an inhibition percentage of 60.83% compared to ZG, that prove a low inhibition percentage (17.65%) for the same concentration (Figure 5). It demonstrated that the leaves' extract revealed a high capacity to inhibit α-glucosidase (IC_{50} = 5.448 ± 0.502 mg/mL), close to that of acarbose (IC_{50} = 1.108±0.006 mg/mL). Its higher uronic acid content (32.91%) compared to the seed's extract (5.98%) could explain its activity.

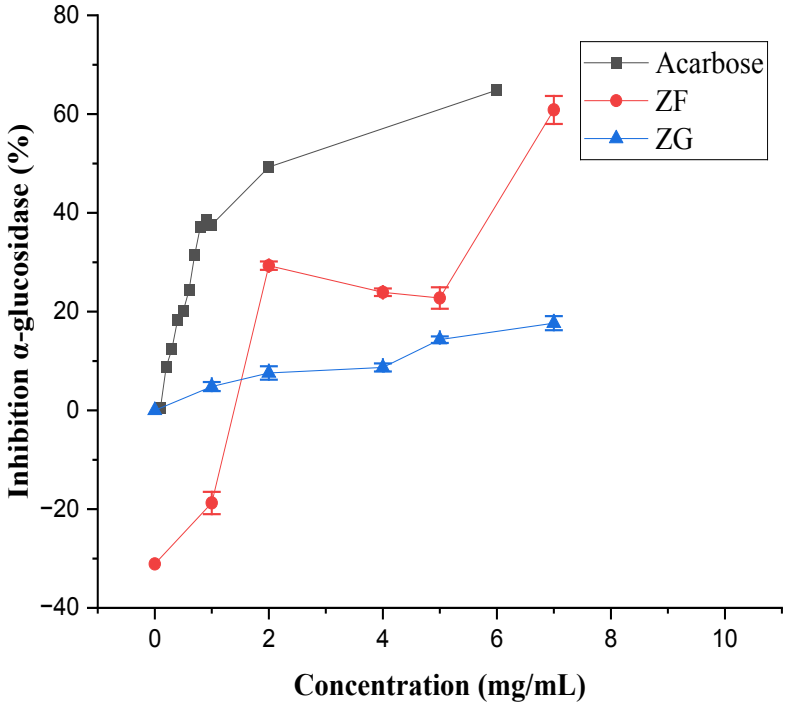

Fig. 5. Inhibition of *α*-glucosidase activity of water-soluble polysaccharides of *Z. album* L., error bars represent the standard deviation of the mean (*n*=3).

Several mechanisms are involved in the inhibition of α-glucosidase activity. One of these mechanisms is hydrogen scavenging, as α-glucosidase provides the hydrogen required to catalyse the hydrolysis of the α--(1®4) glucosidic bond. The inhibitor acts by intercepting the hydrogen ions released from the enzyme's catalytic site. Certain compounds, such as acarbose, act as competitive inhibitors by imitating the enzyme substrate (Kim *et al*., 2014). They, therefore, selectively inhibit the enzymatic hydrolysis of carbohydrates and slow down their intestinal absorption (Bellien & Cracowski, 2016). The binding of polysaccharides to α-glucosidase can lead to changes in the enzyme's polarity and molecular conformation, resulting in a partial loss of its activity (Deng *et al*., 2020). Ji *et al*. (2022) suggest that polysaccharide side chain groups can be combined at the active site of the enzyme in order to reduce its catalytic activity, and the smaller the molecular weight, the greater the combination of side chain groups at the active site (Ji *et al*., 2022).

The inhibitory activity of ZF and ZG against *α*-glucosidase and *α*-amylase is different. ZF exhibited a high capacity to inhibit *α*-glucosidase compared to ZG; yet, its inhibitory capacity on *α*-amylase is lower than that of the latter.

Inhibition of protein denaturation

Chronic inflammation contributes to the development of degenerative diseases, including rheumatoid arthritis, atherosclerosis, cardiovascular disease, and Alzheimer's disease (Iwalewa *et al*., 2007). The inflammatory response is characterised at the tissue level by increased vascular permeability, increased protein denaturation, and alteration of cell membranes. Protein denaturation is one of the causes of inflammatory diseases, including rheumatoid arthritis (Dey *et al*., 2011; Iffath & Caroline, 2018). Agents that can prevent protein denaturation are, therefore, of interest in the treatment of inflammatory diseases (Dey *et al*., 2011; Bailey-Shaw *et al*., 2017).

There is proportionality between extract concentration and percentage inhibition of denaturation for all concentrations used. A concentration of 0.5 mg/mL, the inhibition percentage of ZG and ZF are 33.76% and 11.37% respectively (Figure 6). The denaturation inhibitory effect of ZG (IC_{50} = 0.931 ± 0.029 mg/mL) remains better than ZF (IC_{50} = 22.751 ± 1.415 mg/mL). Whereas, its inhibitory power is lower than diclofenac (IC_{50} = 0.197 ± 0.009 mg/mL).

Various types of plant polysaccharides are known for their anti-inflammatory properties, such as starch, β-glucans, arabic gum, inulin, and pectins (Jin *et al*., 2020).

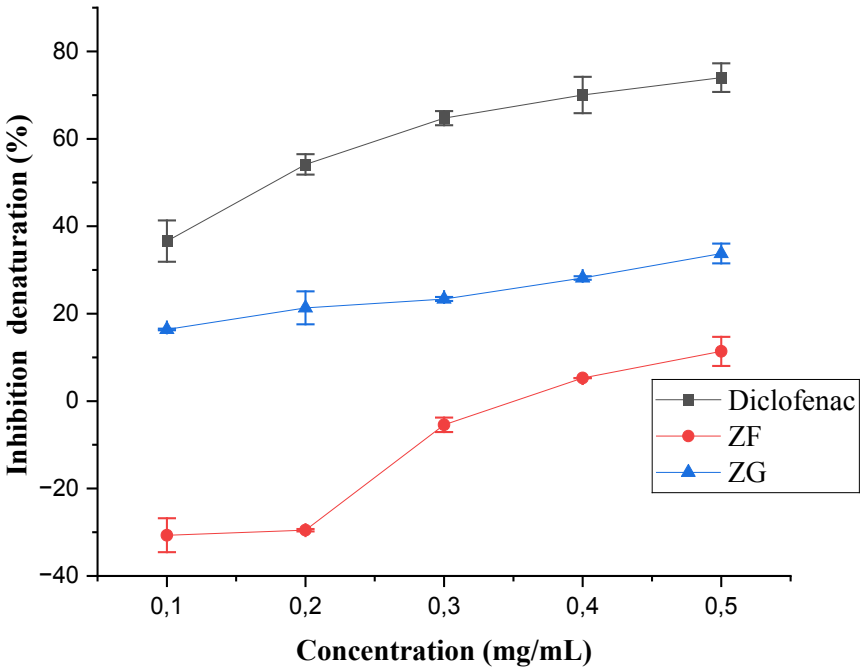

Fig. 6. Inhibition of protein denaturation of water-soluble polysaccharides of *Z. album* L., error bars represent the standard deviation of the mean (*n*=3).

CONCLUSION

The analysis of the chemical composition of water-soluble polysaccharides of *Z. album* shows that uronic acids are predominant in ZF and minor in ZG, with high protein content. FT-IR spectra show the characteristic bands of polysaccharides. They consist mainly of arabinogalactans. The results indicate that ZG exhibits a greater inhibitory effect on protein denaturation compared to ZF. Yet, the polysaccharide extract of *Z. album* leaves shows its hypoglycemic potential with a significant inhibitory effect of α-glucosidase, and a moderate inhibitory effect of α-amylase. However, further studies are needed to determine the fine structure and better understand the relationship between structure and activity.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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