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Polysaccharides from the Fruits of *Balanites aegyptiaca* Del.: Preliminary Characterization, Antioxidant, Hypoglycaemic, and Anti-inflammatory Activities

Malika Seddiki[1,2]*, Mohammed D. Ould El Hadj[1], Ahmed Messai-Mohamed[3,4], Zakaria Boual[1], Hakim Belkhalfa[4], Youcef Rahmani[4,5], Philippe Michaud[6]

[1]*Laboratory for the Protection of Ecosystems in Arid and Semi-Arid Zones, Faculty of Science of Nature and Life, University of Ouargla, Ouargla 30000, Algeria*
[2]*Department of Biology, Faculty of Natural Sciences, Life and Earth Sciences, University of Ghardaïa, Ghardaïa 47000, Algeria*
[3]*Laboratory of Valorization and Promotion of Saharan Resources (VPRS), Faculty of Mathematics and Matter Sciences, University of Ouargla, Ouargla 30000, Algeria*
[4]*Scientific and Technical Research Center in Physicochemical Analysis, Tipaza 42000, Algeria*
[5]*Laboratory of Process Engineering, Faculty of Applied Sciences, University of Ouargla, Ouargla 30000, Algeria*
[6]*Institut Pascal, Université Clermont Auvergne, CNRS, Clermont Auvergne INP, 63000 Clermont-Ferrand, France*

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ABSTRACT

Polysaccharides are major cellular macromolecules. They have different pharmaceutical, nutritional, and biological applications depending on their structure, and composition. The current study aim to evaluate the antioxidant, hypoglycaemic and anti-inflammatory activities of polysaccharides from the fruits of *Balanites aegyptiaca* Del. (BA) harvested from the Algerian Sahara. Polysaccharides from BA were extracted by the hot water method. The preliminary characterization of the crude polysaccharides was done by Ultra violet-Visible (UV-Vis) spectrophotometry, Fourier Transform Infrared (FT-IR) spectrophotometry, and Gas Chromatography/Mass Spectrometry-Electron Ionization (GC/MS-EI). The antioxidant activity was determined by the 1,1-Diphenyl-2-picryl hydrazyl (DPPH), 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and Hydroxyl (OH) radical scavenging, ferric reducing power, and total antioxidant capacity assays. Hypoglycaemic activity was evaluated using the *α*-amylase, and *α*-glucosidase inhibitory assays. The anti-inflammatory activity was assessed by the protein denaturation inhibitory assay. The results showed that BA polysaccharides consisted of 34.06% galacturonic acid, 27.82% arabinose, 15.63% galactose, 9.71% glucose, 7.04% xylose, and 5.74% rhamnose. In the antioxidant assays, BA polysaccharides exhibited a significant antiradical activity against DPPH• (IC_{50} = 0.745±0.003 mg/mL), ABTS• (IC_{50} = 1.390±0.018 mg/mL), OH• (IC_{50} = 2.77±0.048 mg/mL), and a strong ferric reducing power and total antioxidant capacity. BA polysaccharides showed moderate hypoglycaemic effect with IC_{50} values of 44.132±0.926 and 27.117±0.737 mg/mL for *α*-amylase and *α*-glucosidase inhibitory activity, respectively. Furthermore, BA polysaccharides displayed a strong anti-inflammatory activity as measured by its inhibition of protein denaturation (67.94% inhibition at 0.5 mg/mL). Therefore, BA polysaccharides could serve as a natural source of antioxidant, hypoglycaemic, and anti-inflammatory agents.

***Keywords*:** *Balanites aegyptiaca*, Polysaccharides, Antioxidant, Hypoglycaemic Activity, Anti-inflammatory Activity

Introduction

Polysaccharides along with proteins and nucleic acids represent the main macromolecules in cells. They are polymers of monosaccharides linked together via glycosidic bonds.[1] They can be classified into diverse categories depending on a number of criteria, such as their chemical composition (homoglycans and heteroglycans), their sources (plant, animal and microbial polysaccharides) or their functions (structural and reserve polysaccharides).[2]

*Corresponding author. E mail: seddiki.malika@univ-ghardaia.dz
Tel: +213671412844

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Polysaccharides are characterized by their structural diversity (monosaccharide composition, glycosidic linkages, molecular weight and branching point),[3] non-toxicity, biodegradability and biocompatibility. They show remarkable rheological properties which are highly dependent on their molecular structure. They act as stabilizers, emulsifiers, gelling agents, thickeners and moisturizers.[4,5] For these properties, polysaccharides are used in many fields including food, cosmetics and pharmacy. However, the focus on these polymers is not exclusively due to their physicochemical properties, but also to their biological activities, such as antioxidant, antidiabetic, immunostimulant, anti-inflammatory and antimicrobial activities.[1,5] A type of polysaccharides that has recently attracted the attention of researchers is the one extracted from land plants.[4] This type of polysaccharides presents an interesting source of biologically active compounds but it is yet to be explored.[6]

Balanites aegyptiaca Del. is a xerophyte native to Africa and the Middle East. It is a spiny shrub or tree that grows in all types of soil (sandy, clay, clay-loam and gravelly). This explains its wide geographical distribution.[7] The plant is used to treat jaundice, asthma, parasitic infections, gastrointestinal disorders, skin diseases, fever and snake bite.[8] Its fruit is edible and rich in carbohydrates, hence its common

name 'desert date'. It also contains proteins, fatty acids, minerals and vitamins.[9] This species has been the subject of several studies designed to identify its secondary metabolites and assess its biological activities, but there is no study on its polysaccharides. Thus, the objective of this study is to characterize the polysaccharides from the fruits of *B. aegyptiaca* Del. and evaluate their antioxidant, hypoglycaemic and anti-inflammatory activities.

Materials and Methods

Chemicals and reagents

Chemicals used in this study include: standard monosaccharides (D-galacturonic acid, D-glucuronic acid, D-galactose, L-arabinose, L-rhamnose, D-mannose, D-xylose, D-glucose), trifluoroacetic acid (TFA), pyridine, BSTFA (Bistrifluoroacetamide):TMCS (Trimethylchlorosilane) (99:1), meta-hydroxydiphenyl (m-HDP), DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), BHT (butylated hydroxytoluene), Vitamin C, *α*-glucosidase, acarbose, *p*-nitrophenyl-*α*-D-glucopyranoside (*p*-NPG), porcine pancreatic *α*-amylase, 3,5-dinitrosalicylic acid (DNS), Folin-Ciocalteu, starch and dichloromethane which were products of Sigma-Aldrich Chemicals. Salts of Na_2HPO_4, NaH_2PO_4, Na_3HPO_4, Tris, Tris-HCl, NaCl, $FeSO_4$, $FeCl_3$ and Na_2CO_3 were also bought from Sigma-Aldrich. Potassium ferricyanide, potassium persulfate, Bovine Serum Albumin (BSA), salicylic acid, hydrogen peroxide and gallic acid were products of Biochem Chemopharma. Sulfuric acid, acetone and ethanol were products of Honeywell Inc. All chemicals were of analytical grade.

Equipment

The equipment used were: cutting mills (Retsch, SM 100, Germany), centrifuge (Sigma 6-15, 121048, Germany), oven (J.P. Selecta, Spain), centrifuge (Sigma 4-15, 10730, Germany), water bath (Memmert, WB7, Germany), pH meter (Adwa, AD1030, Hungary), vortex mixer (Stuart, SA8, UK), incubator shaker (KS 3000 - i control, Germany), UV-Vis spectrophotometer (Agilent Technologies, Cary 100, USA), FT-IR spectrophotometer (Agilent Technologies Cary 600) and Gas chromatograph (Agilent GC-6890 System) coupled to mass spectrophotometer (Agilent 5973 Network Mass Selective Detector).

Collection and preparation of plant material

The fruits of *B. aegyptiaca* were harvested in January 2021 from Tamanrasset city, Algerian Sahara (geographical coordinates: 22° 53′ 51″ N, 5° 24′ 14″ E). The collected parts were dried, then ground into fine powder.

Extraction of polysaccharides

The powdered fruits of *B. aegyptiaca* (25 g) were macerated in 500 mL of distilled water at 60°C for 4 h with constant stirring. The mixture was filtered via a fine mesh strainer, then centrifuged at 4000 rpm for 15 min. The supernatant was successively filtered under vacuum via a sintered glass filter of porosities 1, 2 and 3. Proteins were precipitated with 4% trichloroacetic acid with continuous stirring, followed by centrifugation at 12000 rpm for 30 min, and neutralization with 1 M NaOH. After centrifugation, the supernatant was precipitated with three volumes of ethanol (96%) at 0°C for 16 h. The precipitate was washed subsequently with ethanol and acetone, and then dried at 50°C for 24 h. The crude extract obtained represented the water-soluble *B. aegyptiaca* polysaccharides (BA).

Determination of biochemical composition

The global composition of the crude extract was determined colorimetrically. The phenol-sulfuric acid assay[10] was used to quantify the total amount of carbohydrates, and the concentration was estimated from a calibration curve of glucose (0.02 to 0.14 g/L). The meta-hydroxydiphenyl (m-HDP) assay[11] was employed to determine the uronic acids content, using standard concentrations of galacturonic acid (0.02 to 0.12 g/L) to prepare the calibration curve. The protein content was estimated from a calibration curve of bovine serum albumin (0.02 to 0.1 g/L) according to the method described by Bradford (1976).[12] Phenolic content was quantified using Folin-Ciocalteu method as described by Singleton *et al*. (1999),[13] gallic acid (0.05 to 0.35 g/L) was used as reference standard to prepare a calibration curve.

Fourier-transform infrared spectroscopy (FT-IR)

The polysaccharide (5 mg) and potassium bromide (100 mg) were mixed, ground and pressed into a disk. FT-IR spectra were recorded on an FT-IR spectrophotometer at room temperature (referenced against air, 32 scans) in the range of 400-4000 cm^{-1}.

Determination of monosaccharide composition

Gas chromatography coupled to mass spectrophotometer (GC/MS-EI) was applied to determine the osidic composition. The polysaccharides hydrolysis was performed using 2 M trifluoroacetic acid (120°C/90 min). The derivatization was performed according to the method described by Pierre *et al*. (2012).[14] The hydrolysates were dissolved in 50 µL of sylon (BSTFA: TMCS. 99:1) and 50 µL of pyridine, then incubated at room temperature for 2 h. After evaporation, the samples were dissolved in 500 µL of dichloromethane, then injected into the column (OPTIMA-1 MS, 30 m x 0.32 mm, 0.25 µm) (Macherey-Nagel). The flow rate was 2.3 mL/min. The temperature was set up as follows: the initial temperature was kept at 100°C for 3 min and increased to 200°C at 8°C/min, then to 215°C at 5°C/min. The ionisation was performed via Electron Impact (EI, 70 eV).

Determination of antioxidant activity

DPPH radical scavenging capacity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of BA was evaluated according to the method described by Ji *et al*. (2020).[15] A mixture of 1 mL of DPPH• solution (0.1 mM in methanol) and 1 mL of the extract (0.1-1 mg/mL) was prepared and incubated at 25°C for 30 min in the dark. The absorbance was read at 517 nm using a UV-Vis spectrophotometer. Vitamin C and BHT were used as standards at concentrations ranging from 0.001 to 0.01 mg/mL and from 0.004 to 0.04 mg/mL, respectively.

ABTS radical scavenging capacity

The assessment of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity of BA was performed according to the method described by Li *et al.* (2015).[16] The ABTS•+ solution was prepared by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1:1, v/v). The mixture was kept at room temperature for 16 h in the dark. The resulting solution was diluted with distilled water to achieve an absorbance of 0.7 at 734 nm. Three hundred microliters of the extract (0.4-3.6 mg/mL) and 2.7 mL of ABTS•+ solution were mixed and incubated at 25°C for 30 min in the dark. The absorbance was read at 734 nm. Vitamin C and BHT were used as standards at concentrations between 0.01 to 0.1 mg/mL.

Hydroxyl (OH) radical scavenging capacity

The hydroxyl radical scavenging capacity of BA was determined according to the method described by Chen *et al*.(2019).[17] A mixture of 1 mL of salicylic acid solution (9 mM in ethanol), 1 mL of the extract (1-10 mg/mL) and 1 mL of 9 mM $FeSO_4$ solution was prepared. After homogenization, 1 mL of 9 mM H_2O_2 solution was added. The mixture was kept at 37°C for 30 min in the dark, then the absorbance was measured at 510 nm. Vitamin C and BHT were used as reference standards at concentrations ranging from 0.03 to 0.3 mg/mL and from 0.2 to 2 mg/mL, respectively.

Reducing power assay

The reducing power of BA was evaluated according to the method described by Zhu *et al*. (2020).[18] A mixture of 2.5 mL of 0.2 M phosphate buffer (pH 6.6), 1 mL of the extract (1-10 mg/mL), and 2.5 mL of 1% potassium ferricyanide was prepared and incubated at 50°C for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added to the mixture. After centrifugation, 2.5 mL of distilled water and 0.5 mL of

0.1% ferric chloride was mixed with the supernatant. After incubation of the mixture at room temperature for 10 min, the absorbance was determined at 700 nm. Vitamin C and BHT were used as standards at concentrations ranging from 0.01 to 0.1 mg/mL and from 0.02 to 0.2 mg/mL, respectively.

Total antioxidant capacity

The total antioxidant capacity of BA was evaluated according to the method described by Ijoma *et al.* (2023).[19] A mixture of 1 mL of reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate) and 0.1 mL of the extract (1-10 mg/mL) was prepared, and incubated in a boiling water bath for 90 min. After cooling the mixture, the absorbance was measured at 695 nm. Vitamin C and BHT were used as reference standards at concentrations ranging from 0.02 to 0.2 mg/mL.

Determination of hypoglycaemic activity

α-Amylase inhibition assay

The assessment of *α*-amylase inhibitory activity of BA was performed according to the method described by Apostolidis *et al*. (2007),[20] with minor modifications. Acarbose (standard) and the extract were prepared at concentrations ranging from 0.1 to 0.8 mg/mL and from 1 to 10 mg/mL, respectively. Then, a mixture of 250 µL of each sample concentration and 250 µL of phosphate buffered saline (0.02 M, pH 6.9, 6 mM NaCl) containing *α*-amylase (0.5 mg/mL) was prepared and kept at 25°C for 10 min. Then, 250 µL of 1% starch solution was added, and the mixture was once again incubated at 25°C for 10 min. After adding 500 µL of dinitrosalicylic acid solution and heating the mixture for 5 min at 95°C in a water bath, the reaction was stopped. After cooling and dilution of the mixture with 5 mL of distilled water, the absorbance was read at 540 nm.

α-Glucosidase inhibition assay

The evaluation of *α*-glucosidase inhibitory activity of BA was performed according to the method described by Deng *et al*.(2020).[21] Acarbose and the extract (BA) solutions were prepared at concentrations ranging from 0.02 to 0.2 mg/mL and from 1 to 10 mg/mL, respectively. Then, the mixture of 120 µL of 0.1 mM phosphate buffer (pH 6.8), 20 µL of each sample and 20 µL of *α*-glucosidase (0.5 U/mL) was prepared and incubated at 37°C for 20 min. After adding 20 µL of p-NPG substrate (2.5 mM), the mixture was re-incubated at 37°C for 10 min. The reaction was terminated by the addition of 80 µL of 0.2 M sodium carbonate, and the absorbance was read at 400 nm.

Determination of anti-inflammatory activity

The anti-inflammatory activity of BA polysaccharides was assessed using the protein denaturation inhibitory activity assay. The protein denaturation inhibitory activity of BA polysaccharides was evaluated according to the method described by Dragan *et al*. (2016).[22] Diclofenac (standard) and the extract were prepared at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. Five hundred microliters (500 µL) of each sample was mixed with 500 µL of 0.2% bovine serum albumin (prepared in Tris-HCI buffer, pH 6.6). The mixture was incubated at 37°C for 20 min in a water bath, then kept 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 calculated as follows:

$$\text{Inhibition (\%)} = \frac{A1 - A2}{A1} \times 100 \qquad \text{Equation 1}$$

Where A1 is the absorbance of control (mixture without sample), and A2 is the absorbance of sample.

Statistical analysis

The analysis was done in triplicate, and the results were expressed as mean ± standard deviation (SD). The IC_{50} values of the test samples were calculated via linear regression. The data were analyzed using Origin 2024b software and Microsoft Excel 2007.

Results and Discussion

Extraction yield and biochemical composition

Maceration in distilled water at 60°C was the method used to extract water-soluble polysaccharides from *B.aegyptiaca* fruits (BA). This method is commonly used for polysaccharide extraction and offers the advantage of preserving the structural integrity and bioactivity of the polysaccharides.[23] Figure 1 illustrates the aspect of the crude extract that was obtained after alcoholic precipitation and after purification with ethanol and acetone. The extraction yield obtained was low, equal to 1.17±0.01%. Several factors such as temperature, time, water/raw material ratio and number of extractions can influence the extraction yield.[24] Huang *et al.* (2014),[25] reported a yield equal to 1.15% for LPF fraction of *Litchi chinensis* fruit polysaccharides extracted by maceration in distilled water at 85°C which is close to that obtained for BA. Chen *et al.* (2020),[26] reported a yield of 5.88% for CPPh fraction of *Crataegus pinnatifida* fruit polysaccharides extracted by maceration in distilled water at 90°C. Tian *et al.* (2019),[27] reported extraction yields between 7.46 and 7.63% for *Lycium barbarum* fruit polysaccharides obtained by maceration in distilled water at 65°C. These yields are higher than that obtained from *B. aegyptiaca* fruit polysaccharides.

The colorimetric assay results (Table 1) indicated that the crude extract had a high level of total sugars (85.77±0.21%) and a low level of uronic acids (6.16±1.61%). Huang *et al.* (2023),[28] reported a total sugar content of 81.28% in MFP-VI fraction of *Morus alba* fruit polysaccharides extracted by maceration in distilled water at 80°C. These results are close to that obtained from the polysaccharide extract of *B.aegyptiaca* fruits. Xu *et al.* (2018),[29] reported a lower total sugar content of 59.27% in BCP fraction of polysaccharides from *Ribes nigrum* fruits extracted by maceration in distilled water at 80°C.

Table 1: Chemical composition of polysaccharides from *B. aegyptiaca* fruits

Extract	Biochemical composition (%)				Monosaccharide composition (%)					
	Total sugars	Uronic acids	Proteins	Phenolic compounds	Gal A	Ara	Gal	Glc	Rha	Xyl
BA	85.77 ± 0.21	6.16 ± 1.61	7.39 ± 0.06	1.34 ± 0.02	34.06	27.82	15.63	9.71	5.74	7.04

Gal A: Galacturonic acid, Ara: Arabinose, Gal: Galactose, Glc: Glucose, Rha: Rhamnose, Xyl: Xylose.

Figure 1: Extraction of polysaccharides from *B. aegyptiaca* Del. fruits (a) *B. aegyptiaca* Del. fruits, (b) alcoholic precipitation, (c) aspect of the crude extract before drying.

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A deproteination step by trichloroacetic acid method was carried out, and the protein content in the crude extract (BA) was 7.39±0.06%. Dou *et al.* (2019),[30] noted a lower protein content (1.77%) in BBP fraction of water-soluble polysaccharides from Rubus fruits obtained after deproteination by the Sevag method. However, Yang *et al.* (2015),[31] reported a high protein content (13-27%) in the water-soluble polysaccharides of *Lycium barbarum* fruits obtained without deproteination.

The determination of phenolic content revealed a low phenolic content of 1.34±0.02% for *B. aegyptiaca* fruit polysaccharides extract, which was similar to the polyphenols content (1.10 %) in the polysaccharides of *Crataegus azarolus* fruit extracted by maceration in distilled water at 90°C as reported by Rjeibi *et al.*(2020).[32]

FT-IR spectrum of B. aegyptiaca fruit polysaccharide extract

The FT-IR spectrum presented the infrared fingerprint of the polysaccharide extract *of B. aegyptiaca* fruit (Figure 2). A broad and intense absorption band at 3421.71 cm^{-1} and a signal at 2365.99 cm^{-1} were ascribed to the O-H stretch vibrations that characterize polysaccharides.[33] A signal at 2930.83 cm^{-1} was assigned to symmetric and asymmetric C-H stretch vibrations.[33] The broad and intense band around 1600 cm^{-1} characterizes COOH groups of uronic acids.[34] An intense absorption band at 1421.23 cm^{-1} was ascribed to C-H bending vibrations.[35] In the region of 1000 to 1300 cm^{-1}, a strong absorption was observed and was assigned to C=O stretch vibrations.[36] Hong *et al.* (2021) reported that bands at 1740 and 1600 cm^{-1} were attributed to pectin. The signal at 890.01 cm^{-1} was ascribed to C-C-O, C-O-C and C-C-H stretch and bending vibrations.[37] The band at 765.61 cm^{-1} was assigned to symmetrical stretching vibration of pyranose rings.[35] The FT-IR spectrum of BA presented a strong absorption in the region of 800 to 1800 cm^{-1}, with peaks characteristic of uronic acids.

Monosaccharide composition

Gas chromatography is a high resolution and highly sensitive analytical technique. It can be easily coupled with various detectors, including mass spectrometer that provides comprehensive structural information.[38] Due to the limited volatility of sugars, the identification of monosaccharide by gas chromatography is based on an appropriate derivatization of the monosaccharide residues. The determination of osidic composition of BA was accomplished after acid hydrolysis with TFA. The glycosidic residues obtained were then trimethylsilylated and analyzed via GC/MS-EI.

According to the results shown in Figure 3, the crude extract of polysaccharides from the fruits of *B. aegyptiaca* consisted mainly of galacturonic acid (34.06%), arabinose (27.82%), galactose (15.63%), glucose (9.71%), xylose (7.04%), rhamnose (5.74%), and traces of glucuronic acid. Galacturonic acid and arabinose are the main sugars in the extract. The significant amount of galacturonic acid may indicate the presence of pectin. Feriani *et al.* (2020)[39] mentioned that galacturonic acid (31.21-41.15%) and arabinose (40.55-42.03%) were the main sugars in the water-soluble polysaccharide from *Schinus terebinthifolius* and *Schinus molle* fruits. Yang *et al.* (2020)[40] reported the predominance of arabinose (51.43%) in PMBP fraction of polysaccharides from *Ribes nigrum* fruits, while glucose (41.4%) and galacturonic acid (30.5%) were reported as the predominant monosaccharide in the water-soluble polysaccharides from *Nitraria retusa* fruits.[41] The osidic composition of polysaccharides varies with the raw material, extraction protocol, purification method, hydrolysis technique and monosaccharide analysis.[38]

Determination of the monosaccharide composition is important to understanding the relationship between structure and biological activity of polysaccharides.[24]

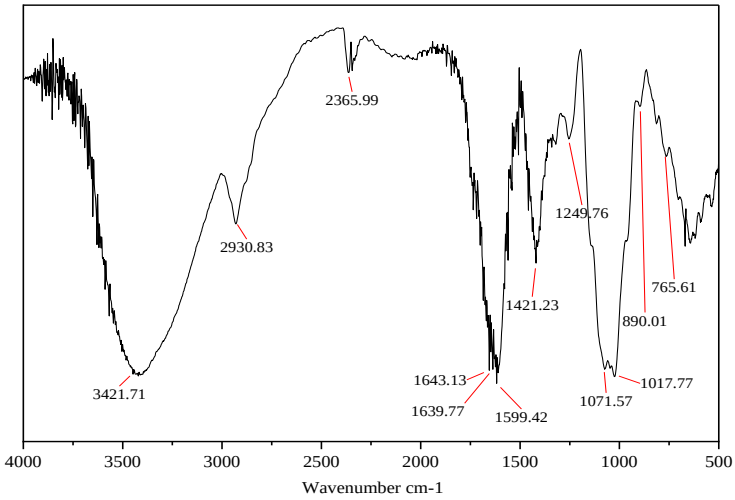

Figure 2: FT-IR spectrum of polysaccharides from *B. aegyptiaca* fruits.

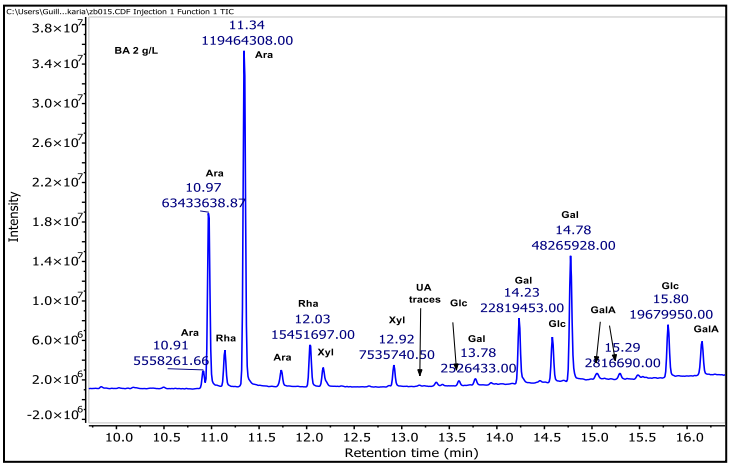

Figure 3: Monosaccharide composition of polysaccharides from *B. aegyptiaca* fruits determined from GC/SM-EI.

DPPH radical scavenging activity

DPPH• is the free radical most commonly employed in the evaluation of the antioxidant activity of polysaccharides. Polysaccharides neutralize DPPH• free radicals by giving up some of their own hydrogens or electrons to form stable molecules (DPPH-H).[42]

BA showed a significant scavenging activity against DPPH radical (69.32%) at a 1 mg/mL concentration (IC_{50} = 0.745±0.003 mg/mL) (Figure 4-A). Nevertheless, BA DPPH radical scavenging activity was lower than that of vitamin C and BHT, whose IC_{50} values were 0.009±6.85E-05 mg/mL and 0.033±2.47E-04 mg/mL, respectively. It has been reported that polysaccharides from *Crataegus azarolus* fruits exhibited a scavenging activity against DPPH radical of 83.2% at a 4 mg/mL concentration (IC_{50} = 1.47 mg/mL).[32] The extract of PZMP1 and PZMP2 of polysaccharides from *Ziziphus jujuba* fruits showed DPPH radical scavenging activity of 52.35% and 53.45% at a 3 mg/mL concentration with an IC_{50} value of 2.19 and 2.01 mg/mL, respectively.[15] Their activity was lower than that of the polysaccharides from the fruits of *B. aegyptiaca* as reported in this study.

A previous study has suggested that galacturonic acid content strongly increases DPPH radical scavenging capacity of polysaccharides.[3] Thus, the antiradical activity of BA could be due to its galacturonic acid content (34.06%).

ABTS radical scavenging activity

Among all the free radicals, ABTS• is the most suitable radical to assess the antiradical activity of hydrophilic compounds.[43] In the presence of

H• donor, the transition from ABTS•+ radical to non-radical ABTS+ form is accompanied by the discoloration of the solution.[44]
The scavenging capacity of BA against ABTS radical increased with increasing concentration (0.4-3.6 mg/mL). The maximum ABTS radical scavenging activity (89.91%) was attained at a concentration of 3.6 mg/mL (Figure 4-B). Therefore, BA displayed a strong scavenging activity with an IC_{50} of 1.390±0.018 mg/mL. However, this activity was lower than that of vitamin C (IC_{50} = 0.054±0.001 mg/mL) and BHT (IC_{50} = 0.068±0.001 mg/mL). Polysaccharides from the fruits of *Schinus terebinthifolius* and *Schinus molle* showed higher antiradical activity against ABTS• than BA, with IC_{50} values of 0.19 mg/mL and 0.28 mg/mL, respectively.[39]on the other hand, Mohammed *et al.* (2020)[45] reported a lower scavenging activity of water-soluble polysaccharides from *Medemia argun* fruits with IC_{50} value of 13.61 mg/mL.
The abundance of uronic acids and the presence of aldehyde or keto groups contribute to the high ABTS• scavenging capacity of polysaccharides.[43]

Hydroxyl radical scavenging activity
Hydroxyl (OH) radical is a very powerful oxidant that promotes the generation of other reactive oxygen species, and can react with macromolecules, causing serious cellular damage.[16,15] The hydroxyl group of polysaccharide can give hydrogen to hydroxyl radical in order to obtain stable compounds and block chain reaction.[16,43]
BA exhibited a strong radical scavenging activity (96.33%) at a 10 mg/mL concentration (Figure 4C). Nevertheless, its activity (IC_{50} = 2.77±0.048 mg/mL) was lower than that of vitamin C (IC_{50} = 0.18±8.77E-05 mg/mL) and BHT (IC_{50} = 0.94±0.006 mg/mL). The polysaccharides from *Ziziphus jujuba* fruits have been shown to have scavenging activity against OH radical with 60.23% inhibition at 3 mg/mL concentration, and an IC_{50} of 1.919 mg/mL.[15] ZHAO *et al.* (2018)[46] reported that the polysaccharides from *Nitraria tangutorum* fruits had a strong antiradical activity, with IC_{50} of 0.82 mg/mL.
The transition metal ions catalyze the production of OH free radicals from superoxide and hydrogen peroxide.[3]The hydroxyl radical scavenging capacity of polysaccharides is strongly associated with their ability to chelate iron.[47] Thus, they can prevent free radical generation by chelating metal ions.[3] Wu *et al.* (2014)[48] suggested that the presence of arabinose in the polysaccharide structure reduces the production of hydroxyl radicals through Fe^{2+} chelation. Thus, the OH radical scavenging activity of BA may be attributed to its arabinose content (27.82%).

Ferric reducing power
Ferric reducing power assay is used to evaluate the ability of a sample to reduce iron (III) present in potassium ferrocyanide complex to iron (II). The reaction produces a coloured complex which absorbs maximally at 700 nm.[49]
As illustrated in Figure 4D, a rapid increase in absorbance was noticed between 0.02 and 0.2 mg/mL concentrations of vitamin C and BHT, while the absorbance gradually increased with increasing concentration of BA. Consequently, the extract is said to have iron reducing power, but its activity remains moderate compared to vitamin C and BHT.
The reducing power of polysaccharides is an important indicator of their antioxidant activity. Their ability to reduce ferric ions is probably attributed to their hydroxyl and carboxyl groups, which can act as electron and hydrogen donors.[43]

Total antioxidant capacity
The total antioxidant capacity assay is a is spectrophotometric method based on the reduction of molybdenum (VI) to molybdenum (V) by a sample.[49] The crude extract of *B.aegyptiaca* fruit polysaccharides had an interesting reducing activity. The total antioxidant capacity of BA extract was found to be proportional to the concentration of the extract. A rapid increase in absorbance from 0.340 to 2.086 was noticed from 1 to 10 mg/mL concentrations of BA (Figure 4E).
The antioxidant activity of polysaccharides depends on their structure, including uronic acid content, functional groups present, molecular weight, glycosidic bond and degree of branching.[3,42]

Hypoglycaemic activity
Pancreatic α-amylases and intestinal α-glucosidases are the main enzymes responsible for the digestion of carbohydrates in the gastrointestinal tract. α-Amylase catalyses the hydrolysis of α-(1,4)-glycosidic bonds producing maltose and glucose from starch, while α-glucosidase catalyses the final stage of the carbohydrate digestion, acting on α-(1,4)-glycosidic bonds, to produce glucose.[50] The digestive enzymes inhibition assay is one of the principal methods adapted for the evaluation of hypoglycaemic activity *in vitro*.
The results of the present study showed that α-glucosidase and α-amylase inhibitory activity of BA increased slowly with increasing concentration of the extract (Figure 5). The percentage α-glucosidase inhibitory activity of BA was found to be 23.75% at 10 mg/mL concentration, and IC_{50} value was 27.117±0.737 mg/mL. Its activity was lower compared to acarbose (IC_{50} = 1.108±0.006 mg/mL).
In addition, the α-glucosidase inhibitory activity of *B.aegyptiaca* fruit polysaccharides was low compared to some types of fruit polysaccharides reported in the literature. For example, the MFP-VI fraction from *Morus alba* fruit exhibited an α-glucosidase inhibitory activity higher than BA, with an IC_{50} of 5.38 mg/mL.[28] Strong α-glucosidase inhibitory activity has been reported in the polysaccharide extract (MBP) of *Ribes nigrum* fruit with an IC_{50} of 0.82 mg/mL,[51] and in water-soluble polysaccharide extract of *Schinus terebinthifolius* and *Schinus molle* fruit, with IC_{50} values of 0.22 mg/mL and 0.17 mg/mL, respectively.[39]
For the α-amylase inhibitory activity, BA exhibited an activity (IC_{50} = 44.132±0.926 mg/mL) lower than than that of acarbose (IC_{50} = 0.051±0.001 mg/mL). Previous studies have shown strong α-amylase inhibitory activity by water-soluble polysaccharides from *Crataegus azarolus* fruits (IC_{50} = 1.81 mg/mL),[32] and alkali-soluble polysaccharides from *Annona squamosa* fruits (IC_{50} = 1.36 mg/mL).[52]
One of the mechanisms implicated in the inhibition of digestive enzymes is the modification of their molecular conformation caused by their association with polysaccharides. Hydroxyl groups of polysaccharides may play an essential role in the binding process.[53]

Anti-inflammatory activity by inhibition of protein denaturation
Protein denaturation implies the destruction of native three-dimensional structure of proteins through the alteration of electrostatic, hydrogen, hydrophobic, and disulfide bonds.[54] It is among one of the causes of chronic inflammatory diseases, mainly rheumatoid arthritis.[55] Diverse kind of non-steroidal anti-inflammatory drugs (NSAIDs) are usually used for the treatment of inflammatory diseases. These drugs (NSAIDs) binds to plasma albumin and prevent its denaturation.[54,56] However, their long-term use can damage the functions of the gastrointestinal tract, liver, heart and kidneys. These secondary and toxic effects of NSAIDs lead to a continuous search for alternative sources of anti-inflammatory agents of natural origin.[56]
In the present study, BA showed a 67.94% inhibitory activity against protein denaturation at 0.5 mg/mL concentration, which was comparable to that of diclofenac (73.98%) at the same concentration (Figure 6). Its activity was higher than that of the polysaccharides of *Olea vera*, which exhibited an inhibitory capacity of 39.35% at 1 mg/mL concentration.[55] However, BA protein denaturation inhibitory activity was lower than that of the polysaccharides of *Opuntia ficus* fruit peel which displayed an inhibitory activity of 77.77% at 0.15 mg/mL concentration.[57]
Based on the results obtained from this study, the crude polysaccharides of *B. aegyptiaca* fruit have an excellent capacity to inhibit protein denaturation, and can serve as a source of anti-inflammatory drug. Their activity may be attributed to pectin which is recognized for its significant anti-inflammatory activity compared to other types of polysaccharides. The anti-inflammatory activity of pectin depends on its molecular structure, particularly its molecular weight and monosaccharide composition.[58]

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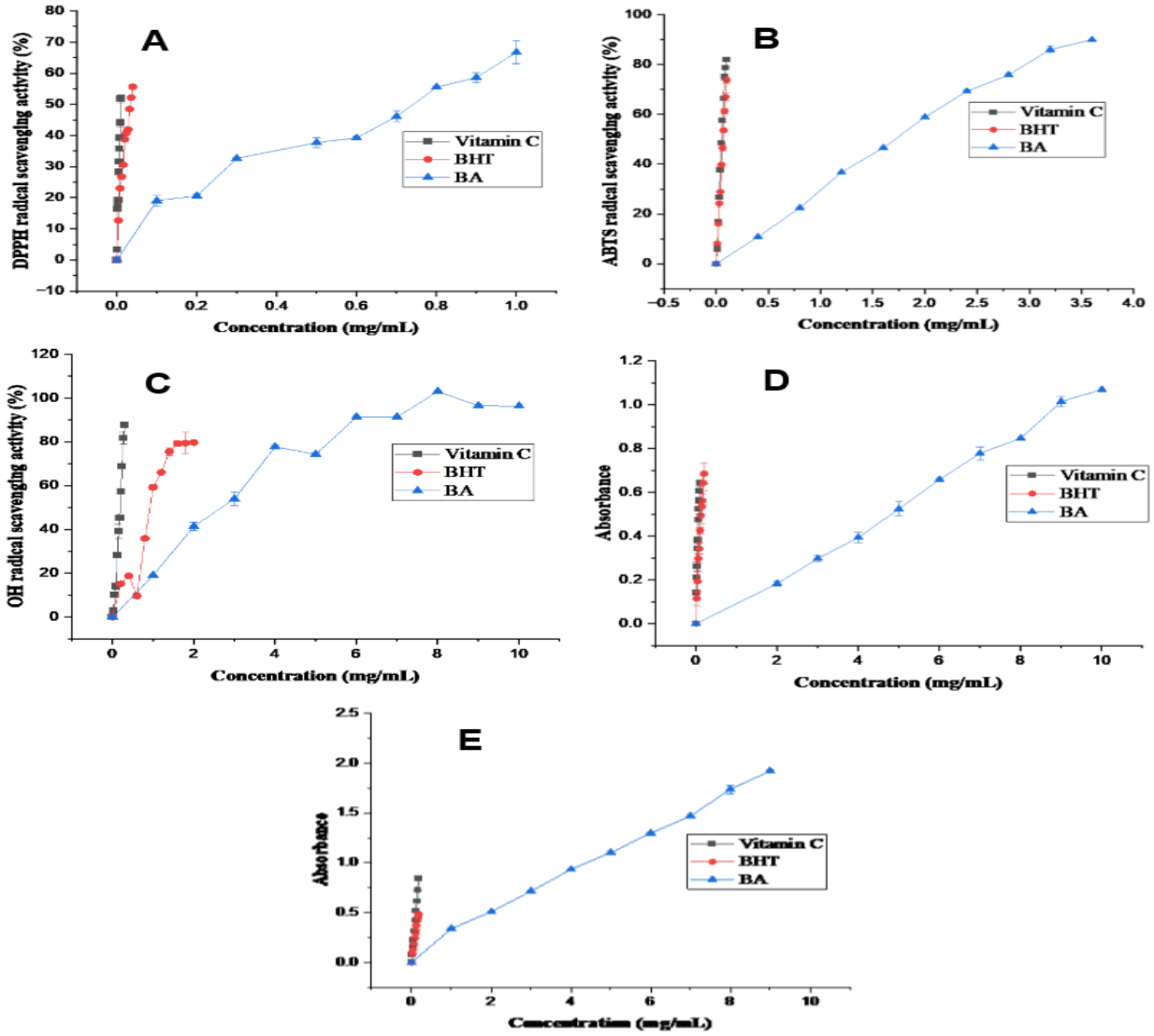

Figure 4: Antioxidant activity of BA, vitamin C and BHT. (A) DPPH• scavenging capacity, (B) ABTS• scavenging capacity, (C) OH• scavenging capacity, (D) ferric reducing power, (E) total antioxidant capacity.

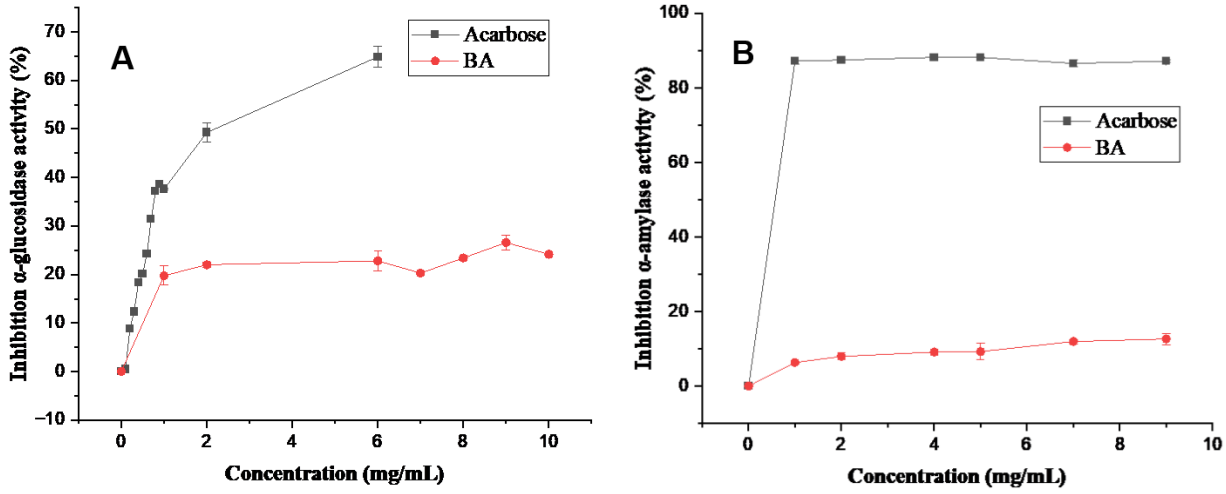

Figure 5: Hypoglycaemic activity of BA and acarbose, (A) α-glucosidase inhibitory activity, (B) α-amylase inhibitory activity.

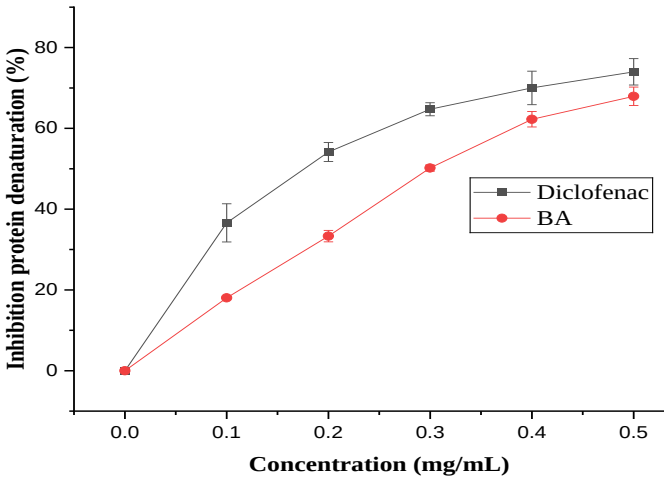

Figure 6: Protein denaturation inhibitory activity of BA and diclofenac

Conclusion

The present study may be considered as a preliminary study involving the extraction, characterization, and biological activity screening of the crude polysaccharides extract from *B. aegyptiaca* fruits. The FT-IR spectrum of BA displayed peaks that are characteristic of polysaccharides and uronic acids. GC/MS-EI analysis indicated that galacturonic acid and arabinose are the main sugars of BA polysaccharides. The extract had a remarkable antioxidant potential, scavenging DPPH•, ABTS• and OH• radicals, and a good reducing power of iron and molybdenum. Its hypoglycaemic activity via inhibition of *α*-glucosidase and *α*-amylase were moderate. In addition, BA polysaccharides had a strong inhibitory effect on protein denaturation, which warrants further investigation of its anti-inflammatory activity *in vivo*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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