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POLYSACCHARIDES EXTRACTED FROM DEVERRA TORTUOSA WASTES STRUCTURAL, FUNCTIONAL, ANTIOXIDANT, ANTIHYPERTENSIVE AND CYTOTOXIC PROPERTIES

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KEYWORDS : *Deverra tortuosa* polysaccharides ; Waste valorization ; Structural ; Functional ; Antihypertensive ; Antioxidant ; Cytotoxic

ABSTRACT

Water soluble polysaccharides were extracted from waste residues after the industrial production of the essential oil from *Deverra tortuosa* plant as novel approach for valorization of these wastes. The extraction yield was 7.82%. The chemical composition, structural characterization, functional, antioxidant, antihypertensive and cytotoxic properties of the *Deverra tortuosa* water-soluble polysaccharides (DTWSP) were treated. This study was carried out using different techniques such as FT-IR, NMR, XRD, zeta potential and DSC to obtain the structural characterization of DTWSP. The X-ray pattern revealed the semi-crystalline behavior of DTWSP. The analysis of the monosaccharide composition by GC–MS showed the presence of glucose galactose, mannitol, gluconic acid, xylose and ribose. In addition, DTWSP exhibited important techno-functional properties (SWC, WSI, WHC and OHC). Antioxidant activities of DTWSP were determined using different antioxidant assays: DPPH radical-scavenging capacity (IC50 = 1.26 mg/ml), reducing power, ABTS assay and ferrous chelating capacity. The findings indicated that DTWSP displayed excellent antihypertensive (98.92% at 0.8 mg/ml) and antioxidant activities but low cytotoxic effects on HeLa cell lines. Overall, the results suggested that WMRP presents a promising natural source of antioxidants and antihypertensive agents.

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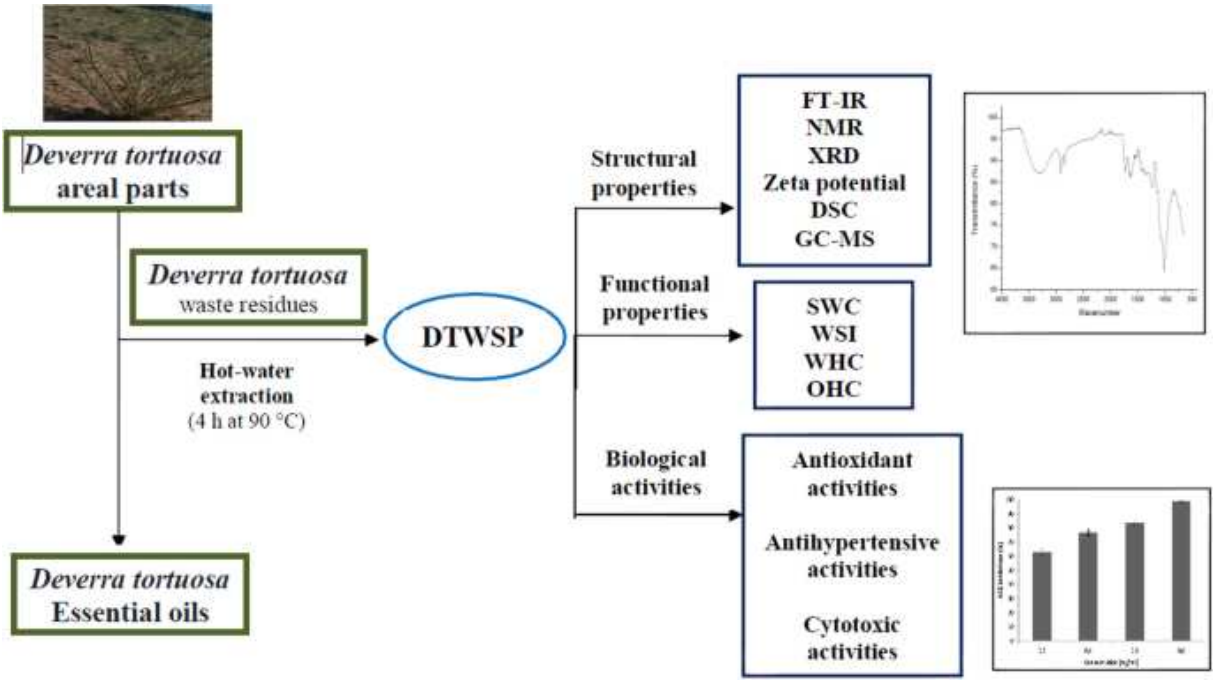
GRAPHICAL ABSTRACT



Statement of Novelty

The novelty of our work consist on enhance of waste residues after the industrial production of the essential oil from *Deverra tortuosa* plant by polysaccharide extraction. The newly produced polysaccharide could be considered as an important source of natural antioxidants and ACE-inhibitory agents that can be incorporated into different food formulations to improve their biological and functional proprieties.

Introduction

Nature is rich with wild plants containing valuable chemical extracts; several herbs have been used since antiquity in food, perfumes and pharmaceutical field.

Deverra tortuosa, a plant species belonging to the *Apiaceae* family widespread in North Africa and the Middle East, is well known by its medicinal, aromatic virtues and essentially as a good source of essential oils [1]. In fact, the essential oils of different parts of *Deverra tortuosa* (stem, flowers, roots and seeds) were investigated as well as their biological activities. [2,3,4,5,6,7].

Industrial processes including essential oil production generated wastes that would be considered the best source for biopolymer production due to their low cost and abundance. In fact, this waste constitutes promising sources of precious biologically active substances such as polysaccharides [8].

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Polysaccharides are natural polymeric carbohydrate molecules found in marine organisms, bacterial, fungal, and vegetal origin [9]. In recent years, these high molecular weight polymers have been received more and more attentions due to their various therapeutic properties, including immune-modulating, antioxidant, anti-inflammatory, antitumor and anti-pathogenic treatments [10]. In addition, several scientific studies had revealed that polysaccharides exhibited ACE inhibitory effect, such as those extracted from watermelon rinds [11], chickpea [10], almond and pistachio [9].

Polysaccharides are generally safe molecules with low cytotoxic side effects and can be potentially used in functional foods or medicines [12]. In fact, this molecule have long been used particularly in food products since they improve emulsions stabilizations, texture and water retention and are also increasingly added into diet foods due to their dietary fibers, prebiotic effect and mimetic fats [13].

However, the exact amount of *Deverra tortuosa* wastes produced by essential oil production industries could not be determined accurately because no official statistics were provided. In addition, the extraction of essential oils in Tunisia is produced mostly at artisanal scale.

Actually, no data is currently available on the extraction, characterization, and biological activities of polysaccharides from *Deverra tortuosa* plant wastes. These polysaccharides are not produced at an industrial scale until now. To the best of our knowledge, our laboratory is the first to valorise these wastes by producing polysaccharides and then to assess their antioxidant, antihypertensive and cytotoxic activities as well as their functional properties.

Materials and Methods

MATERIALS AND EXTRACTION PROCESS OF DTWSP

The waste *Deverra tortuosa* were obtained from a processing factory (Sfax, Tunisia) in December 2017. The waste materials were dried at 50 °C and then reduced to a fine powder.

DTWSP were recovered respecting the method of Liu et al. [14]. The powder of *Deverra tortuosa* (50 g) was pre-extracted at room temperature with 95% ethanol to remove small particules. The obtained residue was extracted twice with 20 volumes of deionized water for 4 h at 90 °C. The filtrates were precipitated using an adequate amount of ethanol 95% (v/v) at 4 °C for 24 h and then centrifuged (4500 × g) for 15 min using a refrigerated centrifuge (Hettich Zentrifugen, ROTINA 380R, Germany).

The final precipitate, obtained after filtrate centrifugation (4500 × g; 15 min; 4 °C), was re-dissolved in distilled water. The water phase was dialyzed against running tap water for 3 days at 4 °C. Finally, the obtained dialysate was lyophilized to obtain DTWSP.

The percentage extraction yield (% w/w)) was calculated as follows:

$$Y(\%) = (W1/W0) \times 100$$

W1: the extracted polysaccharide weight (g).

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W0: the dried sample weight (g).

PHYSICOCHEMICAL CHARACTERISTICS

The moisture and ash content were determined according to the AOAC standard methods [15]. Protein content (N × 6.25) was determined using Kjeldahl method [15]. Crude fat was determined gravimetrically after Soxhlet extraction with hexane. Total carbohydrates were determined by the phenol–sulphuric acid method [16]. All measurements were performed in triplicate.

The sample CieLab parameters (L*, a*, b*) were evaluated with a spectrophotocolorimeter Mini Scan XETM (Hunter lab, In., Reston, VA, USA). According to CieLab system, the L*, a* and b* values is a measure of lightness (from 0 to 100), red–green color (from − 100 to + 100) and yellow–blue color (from − 100 to + 100), respectively.

Structural Analysis of DTWSP

INFRA-RED SPECTROSCOPIC ANALYSIS

The absorption spectra of DTWSP were obtained by FT-IR spectroscopy (Analect Instruments fx-6 160). The FTIR spectra were recorded in a NICOET spectrometer between 400 and 4000 cm−1. The transmission DTWSP sample spectra were recorded using a KBr pallet comprising 0.1% of sample.

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

Spectroscopic analysis of DTWSP was carried out by NMR with CP/MAS technique (cross-polarization, magic-angle-spinning) using a BRUKER-ASX300 instrument. ^{13}C NMR spectra were performed at a frequency of 75.5 MHz (field of 7.04 T). CP/MAS sequence was used with a spin lattice relaxation time was 5 s. DTWSP samples were placed in an alumina rotor used for the double air-bearing-type MAS system and spun as fast as 8 kHz. The contact time was 8 ms. Chemical shifts are expressed in parts per million downfield.

X-RAY DIFFRACTION

The X-ray diffraction (XRD) pattern of DTWSP was obtained using an X-ray diffractometer (Siemens D 5000, Bruker, Germany). The data were collected in the 2θ range 5–100° with a step size of 0.02° and a counting time of 0.78 s/ step.

SURFACE CHARGE (ZETA POTENTIAL) MEASUREMENTS

The surface charges for DTWSP materials were measured at a concentration of 1 g/l at different pH values at 25 °C using the zetasizer Nano series ZS 90 equipment (Malvern instruments, Worcestershire, UK).

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DIFFERENTIAL SCANNING CALORIMETRY (DSC) ANALYSIS

Thermal properties of DTWSP were determined using a differential scanning calorimetry (DSC) experiment according to the method described by Wani et al. [17]. The experiment was conducted using Differential Scanning Calorimetry-DSC METTLER TOLEDO in the temperature range of − 60 to 250 °C at a rate of 5 °C/min. The sample mass was about 5.00 ± 0.20 mg using an empty aluminum pan as reference. All measurements were made at least in triplicate.

Hydrolysis of DTWSP Polysaccharide

Hydrolysis of the DTWSP sample was made by a partially modified version of the method of Boual et al. [18]. A quantity of 50 mg of polysaccharide sample was dissolved in 2 M trifluoroacetic acid (TFA) (2 ml) and incubated for 5 h at 100 °C. The hydrolysis tube was cooled to room temperature. After neutralization to pH 7 with NaOH (1 M), the hydrolisate was mixed and filtered.

Monosaccharide Composition

Determination of the neutral monosaccharide composition was carried out by GC–MS (gas chromatography-mass spectrometry) as previously reported by Kolsi et al. et al. [19]. The obtained hydrolysate, obtained after the hydrolysis of the DTWSP with TFA, was lyophilized and then silylated by a mixture of (pyridine-hexamethyldisilazane-trimethylchlosilane, 9:3:1, v/v/v). Analysis of the obtained trimethylsilyl sugars' derivatives was carried out on a Varian 3800 chromatograph, equipped with a fused silica capillary column (30 m × 0.25 mm) coated with DB-225MS (Durabond) and a Varian Saturn 2000 ITD spectrometer.

Varian Saturn 2000 ITD spectrometer was used with the following parameters: the injected volume was 1 µl, temperature of the injector and the detector was 320 °C, the column temperature was 1 min at 100 °C ramped from 100 to 260 °C. Helium was used as carrier gas at 1 ml min^{-1}.

Functional Proprieties

WATER-HOLDING CAPACITY

Water-Holding Capacity (WHC) absorption of DTWSP was measured by a slightly modified version of the method described by Sosulski [20]. One gram (1 g) of DTWSP sample was dispersed in 25 ml of distilled water and placed in centrifuge tubes. After that, the dispersions were stirred and were left at room temperature for 1 h. After a centrifugation (25 min, 3000 g), the supernatant was collected and centrifuge tube was drained for 25 min at 50 °C, and the sample was reweighed. The WHC of DTWSP was expressed as g of absorbed water per g of sample on dry basis.

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OIL-HOLDING CAPACITY

Oil Holding Capacity (OHC) was determined according to the slightly modified method of Lin et al. [21]. A quantity of 0.5 g of DTWSP was added with 10 ml of soybean oil. The dispersions were stirred and left for 1 h at room temperature. After a centrifugation (20 min; 5000 g), the oil supernatant was removed and the centrifuge tube was drained for 30 min and then reweighed. The OHC of DTWSP was expressed as g of absorbed oil per g of sample on dry basis.

DETERMINATION OF FOAMING PROPERTIES

Foam capacity (FC) and foam stability (FS) were determined according to Lin method [21]. In brief, 50 ml of dispersions of sample in distilled water (0.5, 1, 2 and 3% (w/v)) were homogenized at rapid speed using an ULTRA-TURRAX T 25 (IKA WERKE) for 3 min.

Foam capacity (FC) was expressed as the volume increased due to whipping at 0 min and calculated as follows:

$$\%FC = [(V2 - V1)/V1] \times 100$$

Foam stability (FS) was evaluated as the volume of foam remaining after 30 and 60 min.

$$\%FS = [(V3 - V1)/V1] \times 100$$

V1 = initial volume of solution.

V2 = volume of solution after whipping.

V3 = total volume after leaving at room temperature for different times (30 and 60 min).

DETERMINATION OF EMULSION PROPERTIES

The emulsifying proprieties of DTWSP were determined according to the method described by Pearce and Kinsella [22]. Emulsions were prepared by homogenizing 50 ml of DTWSP solution with 2 ml of soybean oil using an ULTRA-TURAX T 25 basic (IKA WERKE) at speed 3 for 1 min. After homogenization, 100 µl of emulsion sample was taken at 0 and 10 min and diluted with 7.5 ml of 10 mM sodium phosphate buffer (pH 7.0) containing 0.1% sodium dodecyl sulphate (SDS).from the bottom of the tube and diluted in 7.5 ml of 10 mM sodium phosphate buffer (pH 7.0) containing 0.1% sodium dodecyl sulphate (SDS). The absorbance of the emulsions was measured at 500 nm.

Emulsifying Activity Index (EAI) and the Emulsion Stability Index (ESI) were calculated according the following equations:

$$EAI(m2/g) = (2 \times 2.303 \times A0 \times N)/(C \times \phi \times 10000)$$

$$ESI(min) = [A0/(A0 - A10)] \times t$$

where.

A0: Absorbance of the emulsion immediately after homogenization.

A10: Absorbance of the emulsion after 10 min.

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: N: Dilution factor(N = 150).

C: Concentration (g/mL).

ϕ: Oil volume fraction of the emulsion.

In Vitro Antioxidant Activity

REDUCING POWER

The ability of DTWSP to reduce iron (III) was determined as described by Yildirim et al. [23]. The absorbance DTWSP solutions at different concentrations (1–20 mg/ml) compared to BHT (used as the reference antioxidant) was measured at 700 nm. The highest absorbance recorded reflected the best reducing power.

DPPH RADICAL-SCAVENGING CAPACITY

The DPPH radical-scavenging capacity of DTWSP was determined according to the method previously described by Bersuder et al. [24]. Scavenging capacity of DTWSP solutions (1–5 mg/ml) was measured spectrophotometrically at 517 nm. The IC50 value (defined as the concentration of test compound required to produce 50% maximal inhibition) of DTWSP was calculated. BHA was used as positive control. DPPH radical-scavenging capacity was calculated as follows:

$$DPPHradical - scavengingcapacity(\%) = \frac{Ablank - Asample}{Asample} \times 100$$

where *A blank:* Absorbance of the control reaction.

A sample is the absorbance of Bersuder (with the DPPH solution).

ABTS ASSAY

The free radical-scavenging activity was determined by ABTS radical cation decolorization assay as described by Re et al. [25]. Scavenging capacity of DTWSP solutions (0.5–2 mg/ml) was measured spectrophotometrically at 734 nm. BHT was used as positive control The $ABTS^{+}$ scavenging activity was given by:

$$ABTSradicalscavengingactivity(\%) = [(1 - (A/A0) \times 100]$$

where A and A0 are the absorbance values of $ABTS^{+}$ solution with and without samples, respectively. All determinations were performed in triplicate.

FERROUS CHELATING CAPACITY

Chelating capacities of DTWSP for Fe^{2+}were measured according to the method described by Dinis et al. [26]. A volume of 0.5 ml of DTWSP at different concentrations (0.2 to 1 mg ml^{-1}) were added to

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1.6 ml distilled water, 0.05 ml of $FeCl_2$ (2 mM) and 0.1 ml of ferrozine (5 mM). After 10 min at room temperature, the absorbance of the Fe^{2+}–ferrozine complex was measured at 562 nm. EDTA was used as a standard. The chelating antioxidant activity was given by the following formula:

$$Chelatingrate(\%) = \frac{Acontrol - Asample}{Asample} \times 100$$

where A control: Absorbance of the control reaction.

A sample is the absorbance of the sample.

Determination of ACE Inhibition Activity

The ACE inhibition activity was measured in triplicate according to Nakamura method [27]. A sample solution (80 µl) containing different concentrations (0.2 to 0.8 mg/ml) of DTWSP was mixed with 200 µl of HHL (5 mM) and pre-incubated for 3 min at 37 °C. DTWSP and HHL were prepared in 100 mM borate buffer (pH 8.3) containing NaCl (300 mM). The reactions were initiated by adding 20 µl of 0.1 U/ml ACE from rabbit lung prepared in the same buffer. After incubation for 30 min at 37 °C, the enzyme reactions were stopped by the addition of 250 µl of HCl (0.05 M). The liberated hippuric acid (HA) was extracted with ethyl acetate (1.7 ml) and subsequently evaporated for 10 min at 90 °C. The residue was dissolved in distilled water (1 ml). The absorbance was measured at 228 nm. The ACE inhibition rate was calculated as follows:

$$ACEinhibition(\%) = [(B - A)/(B - C)] * 100$$

where,

A: Absorbance of HA generated in the presence of ACE inhibitor.

B: Absorbance of HA generated without ACE inhibitors (100 mM borate buffer pH 8.3 was used instead of DTWSP).

C: Absorbance of HA generated without ACE (corresponding to HHL autolysis in the course of enzymatic assay).

Cytotoxic Properties

HELA CELL CULTURE

The continuous human cell lines HeLa (epithelial cervical cancer cell line) was investigated for cytotoxicity effect of extracts. Adherent cell line was grown in RPMI 1640 medium (Gibco) containing foetal calf serum 10% (v/v) and L-glutamin (2 mM) in tissue culture flasks (Nunc). It was passed twice a week and kept at 37 °C in a humidified atmosphere (5% CO_2 and 95% air).

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MTT CELL PROLIFERATION ASSAY

The MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) cell proliferation assay were performed as previously described by Makhlouf et al. [28].

In brief, HeLa Cells were grown on microtiter plates in 96 well microplates with dilutions of extracts for 72 h before addition of 20 µl of a MTT solution (5 mg/ml in PBS). After incubation at 37 °C for 4 h in a CO_2 incubator, 180 µl of medium were removed from each well and 180 µl of DMSO/methanol (50:50) were added to each well. The solutions were mixed with the cells containing crystals of formazan. The absorbance was measured at 570 nm after dissolution of all the crystals with a microplate reader (Elx800).

STATISTICAL ANALYSES

All experiments were carried out in triplicate. Statistical Package for the Social Sciences software (SPSS for windows version 16.0) was used to analyze Data. Duncan's test was performed at the level of $p \leq 0.05$ to determine significant differences between mean values [29].

Results and Discussion

CHEMICAL COMPOSITION OF DTWSP

Physico-chemical characterization of DTWSP is shown on Table 1. The extraction yield of the obtained crude water-soluble polysaccharides was 7.82%. Although the low yield, polysaccharides from *Deverra tortuosa* wastes (DTWSP) were isolated by the traditional water extraction method because of its simplicity and low cost [30]. In addition, this methods is known by the preservation of the bioactivity and the reduce of damage which can affect the structure of crude polysaccharides [31].

Table 1. Proximate composition and monosaccharide composition of *Deverra tortuosa* water-soluble polysaccharides (DTWSP)

Parameters	**DTWSP**
Yield (%)	7.82 ± 0.5
Dry matter (%)	92.4 ± 1.01
Polysaccharides (%)	85.74 ± 1.56
Proteins (%)	4.93 ± 0.2
Fat (%)	3.7 ± 0.12
Ash (%)	1.49 ± 0.1
Cie color	

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L*	47.03 ± 0.4
a*	5.12 ± 0.06
b*	9.86 ± 0.24
Monosaccharide composition (% m/m)	
Glucose	50.06%
Galactose	31.11%
Mannitol	6.53%
Gluconic acid	5.9%
Fructose	3.8%
Xylose	1.43%
Ribose	1.17%

Physico-chemical composition was calculated based on the dry matter. Values are given as mean ± SD from triplicate determinations (n = 3)

% m/m: % mass per mass

The extraction yield of DTWSP was higher than that obtained for polysaccharides extracted from adzuki beans (6.31%), black cumin seeds (5.18%) [32] and Chickpea (5.56%) [10]. It is though lower than that obtained for polysaccharides extracted from *Salicornia arabica* (11.90%) [33] and *Sorghum bicolor* (L.) seeds (15.20%) [13]. These differences can be assigned to extraction parameters (time, temperature, and ratio of water to raw material) as well as the initial raw material which affected significantly the polysaccharide extraction yield [11].

The sample was characterized by a relatively low moisture (about 7%) and protein content (4.93%). This protein amount was higher than that reported for *Salicornia arabica* (1.24%) [33], almond by-product (2.02%) and pistachio by-product (1.16%) [9] but lower than that recorded for Chickpea (11.22%) [10]. In fact, protein content is significantly affected by the extraction method as well as deproteination processes [34]. The ash and fat contents were 1.49% and 3.7%, respectively.

The colors of the DTWSP expressed in terms of L^*, a^* and b^* are presented in Table 1. The high value of L* (47.03) linked with the low values of a* (5.12) and b* (9.86) explicates the light yellow color of DTWSP which is well appreciated by consumers. In fact, color constitutes one of the aesthetic properties that determine the suitability of any components to its application [10]. Accordingly, the light yellow color of DTWSP promotes their relevance in food and non-food formulations.

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Structural Analysis of DTWSP

FT-IR SPECTROSCOPY

FT-IR spectroscopy is often used to study the carbohydrates due to its ability to identify and characterize main functional groups of plant polysaccharides. The infrared spectrum of DTWSP was obtained in the region of 4000–500 cm^{-1}. As presented in Fig. 1A, DTWSP displayed typical peaks of polysaccharides at 3280, 2916, 1733, 1636, 1142, 1069 and 890 cm^{-1}. The bands at 3280.05 cm^{-1} represented the stretching of the hydroxyl [9]. The small band at around 2916.64 $cm^{-1}$was attributed to C-H stretching vibration including CH, CH2 and CH3 [35]. The peak obtained at 1733.21 cm^{-1} was characteristic of C = O stretching vibration. The bands at around 1636.30 cm^{-1} represented the stretching vibrations of C-O bonds in the acylamino group. The bands at 1069.74 cm^{-1} and 1142.42 cm^{-1} represented the C–C and C–O–C glycosidic bond vibrations which are related to the presence of pyranose ring in polysaccharides [9]. The bands at 890.83 cm^{-1} were assigned to the corresponding β-D-pyranosidic bond C1-H deformation mode [14].

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

The chemical structure of DTWSP was also studied using NMR spectroscopy. The ^{13}C NMR spectra of DTWSP are plotted in Fig. 1B.

In ^{13}C-NMR spectrum, the signals at 33.7 ppm correspond to CH_2C group [35]. The signal at 71.1 ppm was due to C5 of α–D-Galactopyranose residues. The signals at 81.37 ppm was assigned carbon atoms with secondary hydroxyl groups (C2, 3, 4 in pyranoses and C2, 3 in furanoses) [36]. The signal at 103.15 ppm was attributed to anomeric carbon of β –galactopyranose [13].

X-RAY DIFFRACTION (XRD)

The X-ray diffraction (XRD) constitutes a strong method widely used to examine the crystallography of polysaccharides. The XRD pattern of DTWSP recorded between 5 and 80° was performed (Fig. 1C) and suggests that DTWSP was a semi-crystalline polymer: crystal domains coexist with amorphous ones. This structural arrangement is known to directly affect various physical properties, including tensile strength, flexibility, solubility, swelling or opaqueness of the bulk polymer and therefore the final application of these polysaccharides. In fact, semi-crystalline polysaccharides have been utilized to reinforce the polymer composites for various applications ranging from packaging uses to biomedical applications [37]. In the other hand, physical properties are dependent on the degree of order within the material. Indeed, Seidi et al. [38] reported that mechanical strength and stiffness of cellulose increase with the crystallinity.

Similar structure have been previously reported for chickpea polysaccharides [10], the potatoes peel polysaccharides [39] and *Sorghum bicolor* (L.) seeds [13].

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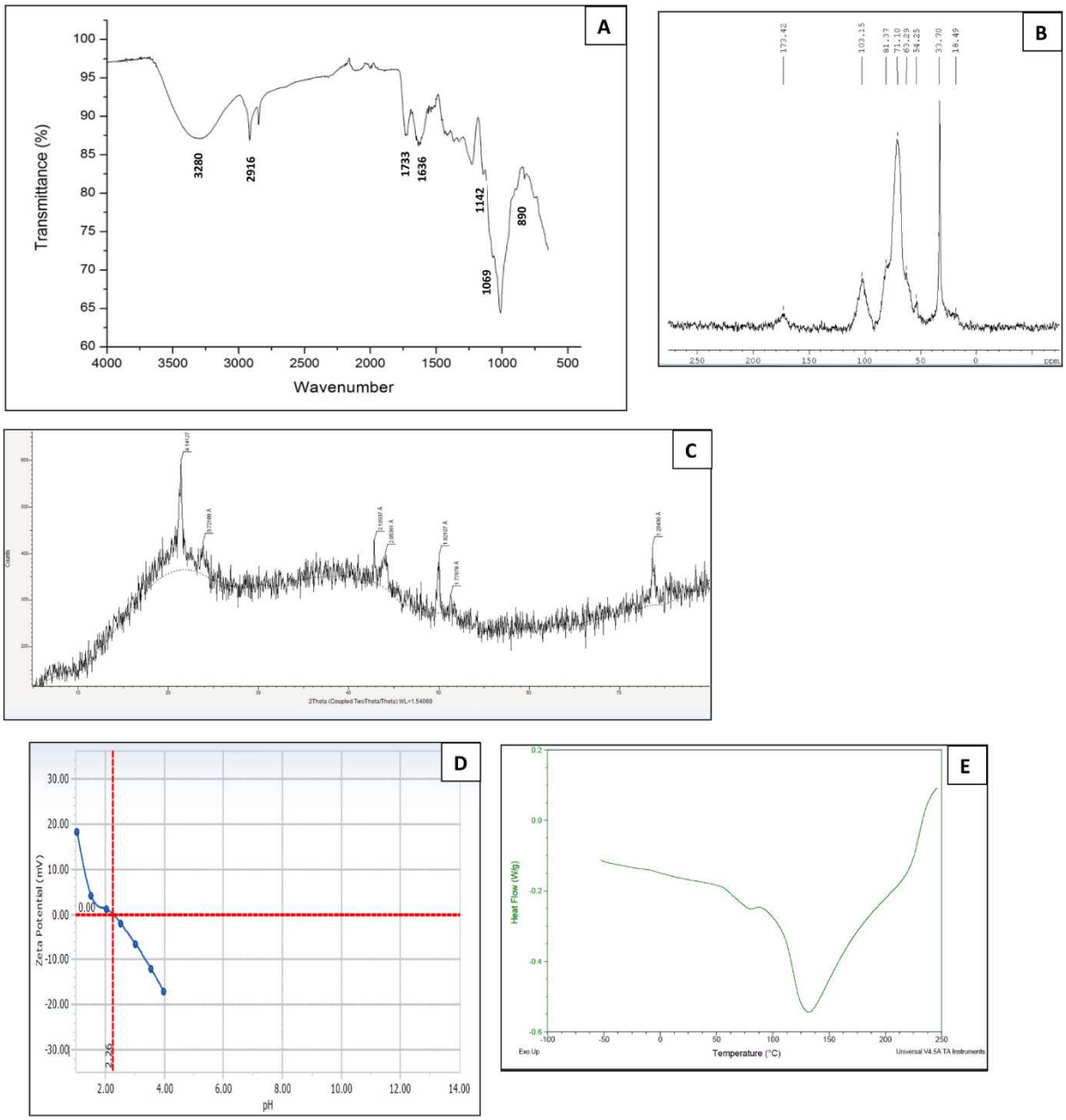

Fig. 1. Structural properties of DTWSP: FT-IR spectrum (**A**), 13C NMR spectrum (**B**), X-ray diffraction pattern (**C**), Effect of pH on the zeta potential (**D**), Thermal comportment by differential scanning calorimetry (**E**)

SURFACE CHARGE (ZETA POTENTIAL) MEASUREMENTS

Zeta potential provides a measure of the net surface charge and potential charge distribution at the interface. The ζ-potential of DTWSP solutions recorded at different pH values (1 to 4) was illustrated in Fig. 1D. Results revealed that pH affected significantly ($p < 0.05$) the surface charge. In fact, the ζ-potential decreased from 18.2 to − 17.26 mV when the pH increased from 1 to 4. The isoelectric point of DTWSP was 2.26. The DTWSP solutions were negatively charged at a pH value superior at 2.5 which suggest that DTWSP were potential compounds that are able to provide electron and assure the

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solution's stability. Similar results are in accordance with those previously reported on soy and black cumin seeds [32].

THERMAL ANALYSIS

The differential scanning calorimetry (DSC) curve of DTWSP is shown in Fig. 1E. A major endothermic peak was observed for the dried DTWSP sample around 130 °C which corresponds to the glass transition temperature. A similar curve was observed in the case of polysaccharide isolated from *Cymodocea nodosa* which revealed a glass transition temperature at 150 °C [19]. Furthermore, Jallabert reported a similar thermal profile in the case of cellulose with a majority endothermic peak from 120 °C to 160 °C [40]. The different thermal states are awarded to chemical nature of polymers and to temperature. The glass transition temperature of the polysaccharide depends on the monosaccharide composition and increases with the polymerization degree [19].

IDENTIFICATION OF MONOSACCHARIDE COMPOSITION

The monosaccharide composition of DTWSP is shown on (Table 1). Results revealed that The DTWSP was mainly composed of glucose (50,06%), followed by galactose, mannitol, gluconic acid and with low contents of xylose and ribose. The ribose was also detected in herbal plants with medicinal purposes such as *Polygonatum cyrtonema* [41] and *Panax notoginseng* [42]. The mannitol was detected in many organisms such as bacteria, fungi and seaweeds and higher plant. Taking into account that this photosynthetic product is responsible for regulating osmotic pressure in cells under abiotic stresses [43] and the arid growth environment of *Deverra tortuosa*, we can explain the presence of mannitol in polysaccharides extracted from this desert aromatic plant.

Functional Proprieties

WATER-HOLDING AND OIL- HOLDING CAPACITIES

Water-holding capacity (WHC) and oil-holding capacity (OHC) constitute the most significant functional properties in food processing that are related to texture enhancement by the interaction between components, including water and oil.

The WHC of DTWSP was in the order of 4.004 ± 0.01 g H_2O/g sample. This capacity was higher than those obtained by water soluble polysaccharides isolated from *Sorghum bicolor* (L.) seeds (3.01 g H_2O/g sample) [13] watermelon rinds (2 g H_2O/g sample) [11], Chickpea (5.14 g H_2O/g sample) [10], almond by-product (1.95 g H_2O/g sample), and pistachio by-product (1.46 g H_2O/g sample) [9]. Nevertheless it was lower than the value reported on galactomannans (15.20 g H_2O/g sample) and *Cymodocea nodosa* (10.73 g H_2O/g sample) [19].

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As stated by Ben Slima et al. [13], WHC depends on pore size, capillarity of the molecules, conformational structure, experimental conditions (temperature, pH, time, and centrifugation), and polysaccharide source.

Oil holding capacity (OHC) constitutes an important characteristic of polysaccharides and a technological property depending on the chemical structure of polysaccharides. The OHC of DTWSP was in the order of 3.184 ± 0.01 g oil/g sample.

This capacity was significantly higher than that obtained for *Sorghum bicolor* (L.) seeds polysaccharide (1.02 g/g) [13], *Cymodocea nodosa* polysaccharide (1.56 g/g) [19], pea (0.13 g/g) and okara (0.27 g/g) polysaccharides [44]. According to Elleuch et al. [45], OHC depends on hydrophilic character and overall charge density of constituents.

FOAMING PROPERTIES

Foams are an important category of food colloids [46]. The foam capacity (FC) and foam stability (FS) of DTWSP at different concentrations (0.5%, 1%, 2% and 3%; w/v) are presented in Table 2. The results revealed that FC and FS increased in a dose dependent manner at a concentration range of (0.5–3%). These interesting FC and FS values of DTWSP could be attributed to the ability of these polysaccharides to enhance the aqueous phase viscosity and then to create a system that stabilizes the interfacial film of gas–liquid. In fact, polysaccharides commonly remain in the aqueous subphase and control its rheology because of their predominantly hydrophilic nature [10]. Accordingly, DTWSP could be potentially used for improving functional proprieties in various food formulations [47].

Table 2. Foaming and emulsifying properties of DTWSP at different concentrations

Concentration (g/100 ml)	FC (%)	FS (%)		EAI (m^2/g)	ESI (min)
		30 min	60 min		
0.5	62.5 ± 0.82^a	8.3 ± 0.02^a	4.16 ± 0.01^a	39.85 ± 1.15 ^{a}	30.14 ± 0.4 ^{a}
1	86.32 ± 0.27^b	9.8 ± 0.01^b	6.45 ± 0.1^b	30.43 ± 0.52 ^{b}	20.53 ± 0. 97 ^{b}
2	101.25 ± 0.94^c	10.2 ± 0.12^c	8.31 ± 0.13^c	27.85 ± 0.98 ^{c}	15.69 ± 0.62 ^{c}
3	120.45 ± 0.36^d	12.76 ± 0.09^d	10.63 ± 0.8^d	22.45 ± 0.6 ^{d}	11.45 ± 0.41 ^{d}

Means with the different superscript letters within the same column are significantly different ($p < 0.05$)

FC foam capacity, *FS* foam stability, *EAI* emulsion activity index, *ESI* emulsion stability index

EMULSION PROPERTIES

Emulsion is defined as a mixture of two or more liquids that are generally immiscible. EAI and the ESI of DTWSP at different concentrations (0.5%, 1%, 2% and 3%; w/v) are shown in Table 2. Statistical analysis indicated that emulsifying proprieties were affected by DTWSP concentrations ($p < 0.05$). The EAI and ESI values of DTWSP were noted to decrease when polysaccharides concentrations increases. Indeed, the small fractions of proteins in DTWSP may affect emulsifying properties of the

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polysaccharide. The low polysaccharide concentrations improve the repulsion between proteins and polysaccharides. Therefore, protein hydrophobic regions are more exposed and thus the diffusion rate to the interface is enhanced. However, at high polysaccharide concentrations, the formation of the complexes protein–polysaccharide could sterically hinder the surface hydrophobic areas on the protein and consequently decreases their diffusion to the interface [32].

In Vitro Antioxidant Activity

DPPH RADICAL-SCAVENGING CAPACITY

The DPPH radical-scavenging activity of DTWSP at varying concentrations (0.5–5 mg/ml) was determined and compared to BHA as presented in Fig. 2A. As illustrated in this figure, both DTWSP and BHA showed a concentration dependent DPPH radical scavenging activity. Although DPPH radical scavenging activity of DTWSP was lower than that of BHA at all tested concentrations, DTWSP exhibited a good antioxidant capacity and the highest DPPH radical scavenging activity was 77.3% at 5 mg/ml. The IC50 value of DTWSP was calculated and estimated to be 1.26 mg/ml. This value was lower and hence better than that of the water-soluble polysaccharides extracted from *Sorghum bicolor* (L.) seeds (8.5 mg/ml) [13] and potatoes peels (11.578 mg/ml) [39]. Thus, DTWSP can act as hydrogen donator to scavenge DPPH free radicals and convert them into stable products proving its usefulness into food and pharmaceutical formulations. The mechanism of DPPH scavenging activity of polysaccharides may be due hydroxyl and carboxyl groups of uronic acids in polysaccharides [33].

REDUCING POWER

The reducing power assay is a useful method to evaluate the aptitude of an antioxidant to donate an electron or hydrogen to reactive radicals and convert them into more stable compounds [28]. As shown in Fig. 2B, the reducing power of DTWSP increased with increasing of the polysaccharides concentrations. However, it stayed significantly ($p < 0.05$) lower than that of BHA used as a standard. According to Trigui et al. [32], the reducing power of DTWSP depended probably on their molecular weight as well as monosaccharide composition. The potential reducing power recorded for DTWSP can be assigned to gluconic acid. In fact, Uronic acids are acknowledged as an important role in antioxidant activity. Based on the reported literature, polysaccharides containing higher content of uronic acids possessed stronger antioxidant activities [48]. Furthemore, it has been reported that polysaccharides with high content of Mannose and Rhamnose displayed higher antioxidant activity [49]. However, the exact mechanism the reducing power of polysaccharides is still under extensive investigation.

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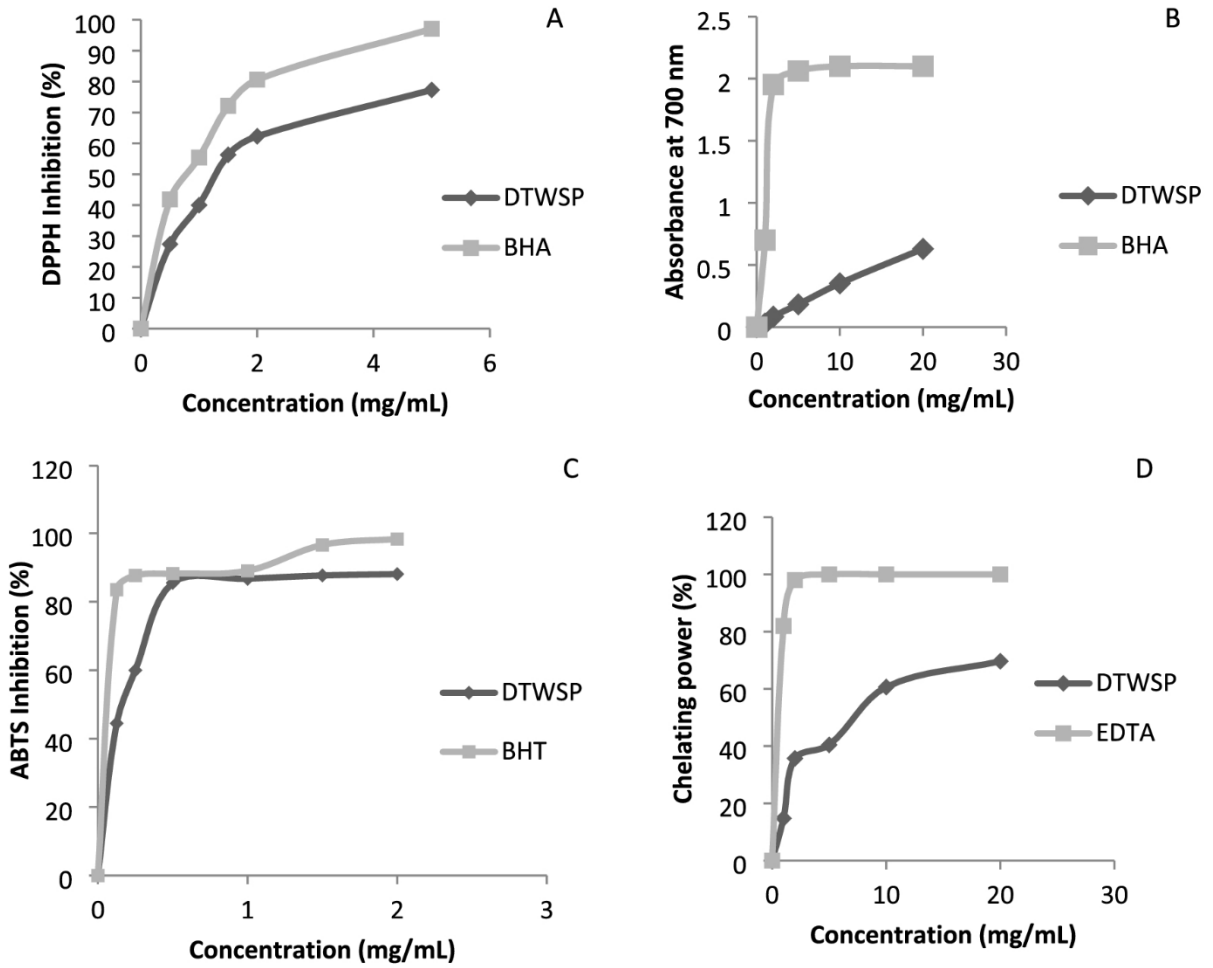

Fig. 2. Scavenging effect on DPPH free radical (**A**), reducing power (**B**), ABTS radical scavenging activity (**C**), and Ferrous chelating capacity (**D**) of DTWSP at different concentrations. BHA, BHT and EDTA were used as positive controls. All analyses were carried in triplicate

ABTS RADICAL SCAVENGING ACTIVITY

The $ABTS^+$ radical is commonly applied to evaluate the total antioxidant potential of compounds. $ABTS^+$ scavenging potentials of the crude polysaccharides DTWSP compared to BHT (used as control) were presented in Fig. 2C. As illustrated, the DTWSP was found to exhibit a strong radical scavenging activity on ABTS which increased in a concentration-dependent manner from 0 to 2 mg/ml and reached 88.07%. at 2 mg/ml. Compared to BHT, the $ABTS^+$ scavenging potentials of the crude polysaccharides DTWSP was relatively lower at the concentration range of (0–2 mg/ml) except at 0.5 and 1 mg/ml where similar scavenging potentials has been noted. Based on these findings, DTWSP can be considered as an important natural antioxidant to scavenge ABTS radical due to the active hydroxyl groups associated to monosaccharides by providing hydrogen or an electron to free radicals [33].

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FERROUS CHELATING CAPACITY

Iron is an essential microelement for life most notably the oxygen transport, respiration, and activity, of many enzymes, but overloaded iron would catalyze the oxidation of many such as lipid and protein. Accordingly, chelating iron may stabilize transition metals and then reduce oxidation damage. Compounds containing two or more of the following functional groups: -OH, -SH, -COOH, C = O and -O- were reported to exhibit metal chelation activity [34]. Figure 2D illustrated the chelating activities of DTWSP and EDTA in relation to Fe^{2+}. Both DTWSP and EDTA showed a concentration dependent ferrous chelating capacity ($p < 0.05$). In fact, this activity increased with increasing concentration at the range of 0–20 mg/ml. Nevertheless, DTWSP had a lower antioxidant activity than the EDTA one. The same observations have been previously reported for polysaccharides extracted from black cumin seeds [32], fenugreek [34] and almond and pistachio byproducts [9]. The IC50 of DTWSP was estimated to be 28.7 mg/ml. The Fe^{2+}–chelating activity of DTWSP appeared to be lower than that of the polysaccharide from almond and pistachio byproducts (0.22 and 0.19 mg/ml, respectively) [9] but in the range of that from floral mushroom (42.68% at 5 mg/ml) [50].

ACE Inhibitory Activities of DTWSP

The inhibition of angiotensin I-converting enzyme (ACE) by dietary anti-hypertensive agents constitutes a promising hypertension management approach. Indeed, ACE inhibition has often been considered as a useful therapeutic approach for the treatment of high blood pressure [10]. Taking into account that synthetic ACE inhibitors may be a source of adverse side effects, plants molecules can present natural and cost-effective alternative ACE inhibitors for the prevention and the treatment of hypertension [9].

As shown on Fig. 3, DTWSP exhibits a very important ACE inhibitory activity which increased with increasing concentrations increased in a concentration-dependent manner at the range of 0.2 to 0.8 mg/ml).

The ACE inhibitory effect of DTWSP (98.92 ± 1% at 0.8 mg/ml) was significantly ($p < 0.05$) higher than that of water-soluble polysaccharides extracted from watermelon rinds (93.93 ± 0.68% at 1 mg/ml) [11], chickpea (87.83 ± 0.8% at 1 mg/ml) [10], almond by-product (79.5 ± 2.8% at 5 mg/ml) and pistachio byproduct (81.78 ± 1.1% at 5 mg/ml) [9].

The IC50 value of DTWSP was also determined (IC50 = 0.18 mg/ml) and it was lower than that of watermelon rinds (IC50 = 0.21 mg/ml) [11], almond by-product polysaccharides (IC50 = 2.81 mg/ml) and pistachio by-product polysaccharides (IC50 = 2.59 mg/ml) [9].

Although the polysaccharides ACE inhibitory mechanism remains unidentified, the inhibitory capacity of WMRP was supposed to be caused by to the gluconic acid present in the extract. In fact, after the dissolution of DTWSP in water, gluconic acid is ionized and subsequently liberated

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hydrogen ions. Consequently, the envionment becomes acid and causes denaturation of ACE enzyme whose optimum pH was 8.3 [51].

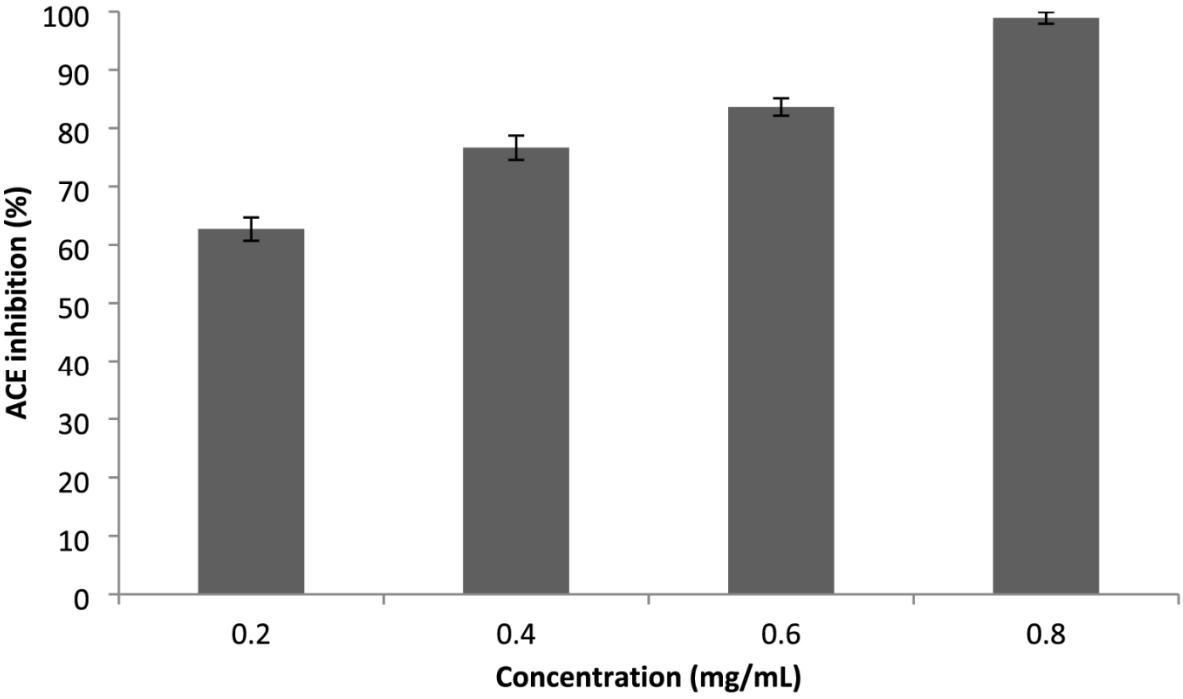

Fig. 3. ACE-inhibitory activities of DTWSP at different concentrations. Values are given as mean ± SD from triplicate determinations

Cytotoxicity Effect

To investigate the cytotoxic effect of DTWSP on HeLa human cell line, cells were treated with various concentrations of DTWSP for 72 h, and then submitted to the MTT test (Fig. 4). Results revealed that cell viabilities still remained between 84–99% over DTWSP concentrations ranging from 0.02 to 5 mg/mL which suggest that DTWSP doesn't exhibited significant cytotoxicity. These findings are in accordance with several studies that reported the non-toxicity of polysaccharides from natural resources [44]. Consequently, DTWSP could be consumed in large quantities as potential safe functional food to make them beneficial for human health.

Accordingly, this scientific study revealed that these polysaccharides could be considered as an important source of natural antioxidants and ACE-inhibitory agents that could be potentially used for improving functional proprieties in various formulations in food industries and thus improves biological and functional proprieties of foods.

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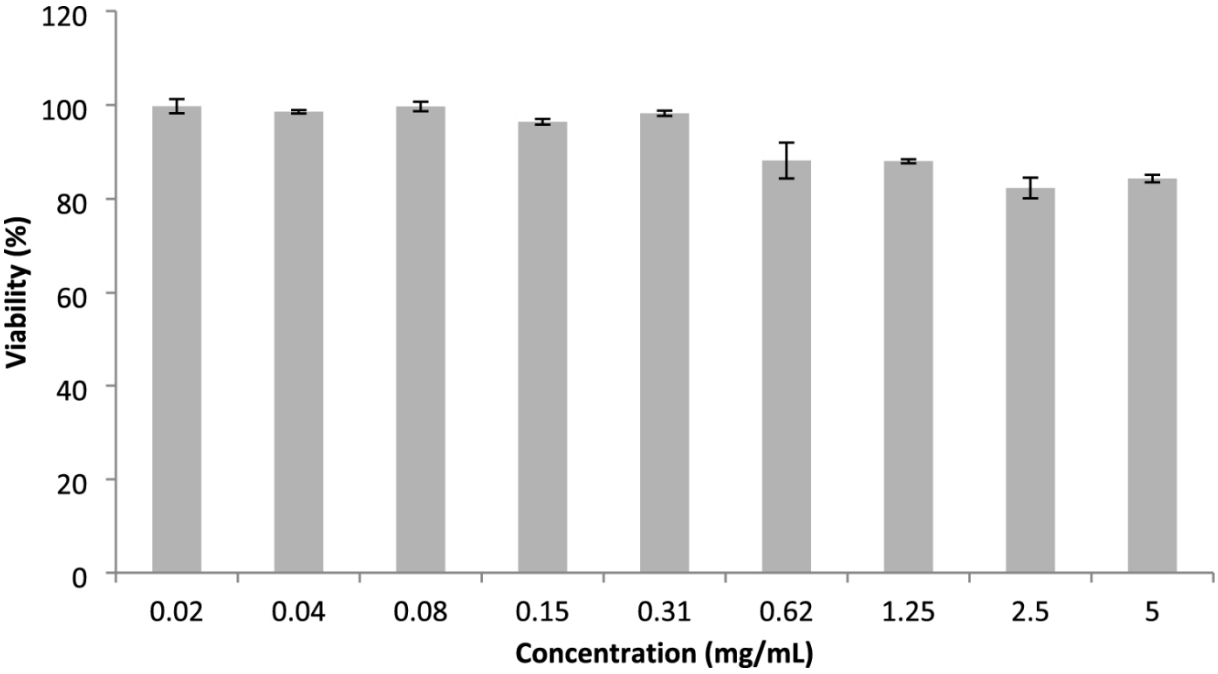

Fig. 4. Cytotoxic activity determined by the MTT assay of DTWSP at different concentration. Values are given as mean ± SD from triplicate determinations

Therefore, this scientific study should be followed by an economic one focusing on the passage from the laboratory scale to the industrial scale similar to the work studying the extraction of polysaccharides from Baobab which are applied in the flour and baking industry in Nigeria [52]. In fact, an assessment of the economic viability of extracting such polysaccharides on a large scale is equally important, along with investigating the potential market for such products. Considering the polysaccharide extraction from *Deverra tortuosa* wastes is a novel process, it is ambiguous to assume an exact price for the final product in order to determine the probable incomes and, thus, evaluate the economic efficiency of the investment.

Impact of DTWSP Extraction on the Industrial Biowaste Management Strategies

The extraction of essential oils from green plants is generally known by the low yield and the large amount of waste generated (more than 90% of the dry weight of the green plant). The valorisation of these waste residues via the production of polysaccharides would be a very interesting issue and could be potentially applied for improving functional proprieties in various formulations in food industries. Thereby, this waste valorisation has obviously two benefits. The first is set as a waste reduction, because it decreases the amount of waste which would be treated by local waste management systems; the second is set as a recycling measure, since an important waste fraction is transformed to consumable products followed by a promising impact on the local economy.

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A suite of the present study is to deal with the optimization of the process taking into account the quality of the product and the availability of the raw material while respecting the sustainability of the material flow management.

Conclusion

Biopolymers constitute nowadays an increasing interest field for researchers because they discovered that this type of molecules have promising beneficial effects on human health.

This current research emphasizes actually this trend, it was undertaken to characterize water-soluble polysaccharides (DTWSP) isolated from the waste of essential oil plant *Deverra tortuosa* and to investigate their functional properties as well as antioxidant, antihypertensive and cytotoxic activities.

The characterization of DTWSP showed a typical NMR and IR spectra characteristic of polysaccharides and a XRD pattern distinctive for semi-crystalline polymers.

Glucose galactose, mannitol, gluconic acid, xylose and ribose were identified in the isolated DTWSP by GC–MS. In addition, extracted polysaccharides exhibited good water-holding capacity, oil-binding ability, foaming properties, and emulsion capacity.

Furthermore, the tests recorded important antioxidant activities in vitro and in a concentration-dependent way also an ACE inhibition and a non-significant cytotoxicity exhibition however.

Accordingly, DTWSP could be considered as an important source of natural antioxidants and ACE-inhibitory agents that can be incorporated into different food formulations to improve their biological and functional proprieties.

Data Availability

All data generated or analysed during this study are included in this published article.

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The authors have not disclosed any funding.

Conflict of interest

The authors declare no competing interests.

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