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Optimization extraction of polysaccharide from Tunisian *Zizyphus lotus* fruit by response surface methodology: Composition and antioxidant activity

Khaoula Mkadmini Hammi [a,d,*], Majdi Hammami [b], Christophe Rihouey [c], Didier Le Cerf [c], Riadh Ksouri [a], Hatem Majdoub [d,*]

[a] Laboratoire des Plantes Aromatiques et Médicinales (LPAM), Centre de Biotechnologie de Borj- Cédria, BP 901, 2050 Hammam-lif, Tunisia
[b] Laboratoire des substances bioactives (LSBA), Centre de Biotechnologie de Borj- Cédria, BP 901, 2050 Hammam-lif, Tunisia
[c] Normendie Université, Laboratoire de Polymères Biopolymères Surfaces (PBS), UMR 6270 & FR3038CNRS, Université de Rouen, 76821 Mon Saint Aignan, France
[d] Université de Monastir, Laboratoire des Interfaces et des Matériaux Avancés (LIMA), Faculté des Sciences de Monastir, Bd. de l'environnement, 5019 Monastir, Tunisia

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ABSTRACT

Response surface methodology using a Box-Behnken design was employed to optimize extraction temperature, extraction time and ratio of water to material to obtain a maximum polysaccharide yield with high uronic acid content and antioxidant property from edible *Zizyphus lotus* fruit. The optimal conditions were: extraction time of 3 h 15 min, extraction temperature of 91.2 °C and water to solid ratio of 39 mL/g. Under these conditions, the experimental extraction yield, uronic acid content and 2,2-diphenyl-1-picrylhydrazyl scavenging ability (IC_{50}) were 18.88%, 41.89 and 0.518 mg/mL, respectively. Chemical analysis revealed that the extract was composed of 97.92% carbohydrate of which 41.89% is uronic acid. The extracted polysaccharides, with an average molecular weight of 2720 kDa, are composed of arabinose, rhamnose, glucose, fructose, galactose and xylose. Moreover, the polysaccharides exhibited a significant reducing power and anti-lipid peroxidation activities.

1. Introduction

Reactive Oxygen Species (ROS) play a key role related to the degenerative or pathological processes such as inflammation, cataract and cancer (Ksouri et al., 2011). Moreover, in food products, free radicals could cause lipid peroxidation which affects the quality of product (taste, color and flavor). The most effective way to eliminate ROS is to use antioxidants. Notably, synthetic tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) which could be toxic and tumorigenic (Botterweck, Verhagen, Goldbohm, Kleinjans, & van den Brandt, 2000). These antioxidants are being replaced by naturally occurring ones such as polysaccharides which have drawn by far the attention of researchers and consumers due to their antioxidative properties (Xu et al., 2009) and their potential pharmacological and biological activities including antitumour, immunostimulant, anticancer, anticomplementary, anti-inflammatory, anticoagulant hypoglycaemic, antiviral and immunological activities (Li, Liu, Fan, Ai, & Shan, 2011; Majeti & Kumar, 2000).

Published data indicates that natural polysaccharides are abundant in Chinese *Zizyphus jujuba* fruits that exhibited a different antioxidative properties (hepatoprotective, anticancer, and immunobiological) (Chang, Hsu, & Chen, 2010; Wang et al., 2012; Zhao et al., 2006).

In Mediterranean region (Morocco, Algeria and Tunisia), the edible fruit of *Zizyphus* genus is widespreaded (Pottier & Alapetite, 1981). In particular, *Zizyphus lotus* species are abundant in Tunisia and commonly known as "sedra" and the edible fruit is called "nbeg". It is localized in different regions mainly in arid zone like Tozeur. The fruit of this plant is used in folk medicine for the treatment of various diseases such as bronchitis, diabetes, diarrhea and abscess (Mkadmini Hammi, Jdey, Abdelly, Majdoub, & Ksouri, 2015).

As far as we know, there is no report on the antioxidant properties and the chemical characterization of polysaccharides isolated from the *Zizyphus lotus* fruit localized particularly in Tozeur region. In fact, the present study investigates, for the first time, the

* Corresponding authors at: Laboratoire des Plantes Aromatiques et Médicinales (LPAM), Centre de Biotechnologie de Borj- Cédria, BP 901, 2050 Hammam-lif, Tunisia (K. Mkadmini Hammi).
E-mail addresses: khaoulafayrouzahammi@gmail.com (K. Mkadmini Hammi), hatemmajdoub.fsm@gmail.com (H. Majdoub).

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monosaccharide composition, the molecular weight and the conformation of water–soluble polysaccharides from Tunisian *Z. lotus* fruit (pulp and peel) of Tozeur region and their antioxidant effects.

Generally, the extraction yields and antioxidant activity of polysaccharides are mainly affected by various factors such as extraction temperature, extraction time and solvent to solid ratio (Wang, Zhang, Wang, & Wang, 2013; Gan, Abdul Manaf, and Latiff (2010)). Response surface methodology (RSM) is an efficient mathematical and statistical technique used to optimize the effect of independent variables and their interaction on response variables (Myers & Montgomery, 2002).

The objectives of this study were to explore the potential of *Z. lotus* fruit (pulp and peel) in producing polysaccharides and to optimize, using RSM, the conditions for the extraction of water soluble polysaccharides that obtain high extraction yield, uronic acid content and antioxidant property which evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Macromolecular characterization and monosaccharide composition of polysaccharides isolated from Southern Tunisian *Z. lotus* fruit (pulp and peel) were investigated. In order to valorize the extracted polysaccharides as antioxidants in functional food or medicine, two *In vitro* antioxidant assays including reducing power and the anti-lipid peroxidation activity were also evaluated.

2. Materials and methods

2.1. Plant material

A ripe fruits of *Zizyphus lotus* (L.) were harvested from Tozeur, South of Tunisia, in August 2013, then washed in distilled water and stored at 20 °C until use. A portion of the ripe fruit was taken and seeds were carefully separated from pulp and peel. Afterwards, the pulp and peel fraction were air–dried, cut into small pieces, grounded in a blender and sieved to obtain a fine powder (Particle diameter size: 400–500 μm).

2.2. Chemicals and reagents

Several chemical reagents and solvents were used in this study including DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium phosphate, linoleic acid, iron(II) sulfate heptahydrate, trichloroacetic scid (TCA), iron (III) chloride, potassium ferricyanide, lithium nitrate, trifluoroacetic acid, N,O-bis(trimethylsilyl)-trifluoroaceta mide (BSTFA), Acetic acid, butylated hyroxytoluene (Reference standard), ascorbic acid (Reference standard), pyridine, petroleum ether, methanol and ethanol (HPLC grade). Moreover, D-myo-inositol, D-(+)-glucose, L-rhamnose, D-(+)-galactose, D-(−)-fructose, D-(−)-arabinose, D-(+)-xylose were used as standards for GC–MS analysis. Ultrapure water was obtained from the Millipore system and the dialysis membrane with molecular weight cut-off of 14 kDa was also employed.

2.3. Extraction of polysaccharides

Dried *Zizyphus lotus* fruit (pulp and peel) were defatted with petroleum ether for 48 h to remove lipids and depigmented with pure ethanol for 48 h in a soxhlet apparatus. 5 g of dried pretreated samples were extracted three times with deionized hot water in a 500-mL batch reactor with mechanical stirrer. The reactor (jacketed beaker) was kept at the desired temperature by a thermostatic water bath (±0.1 °C). The extraction temperature was varied from 80 to 95 °C, the extraction time selected from 1 to 4 h and the ratios of water to raw material were chosen from 20 to 40 mL/g. The water extraction solutions were separated from insoluble residue by centrifugation (2800*g* for 15 min) and then precipitated by the addition of dehydrated alcohol to a final concentration of 80% (v/v) at 4 °C for 24 h. The resulting precipitate from the ethanol dispersion was collected by vacuum filtration on a glass G2 funnel, redissolved in deionized water (Milli Q process) and then dialyzed against deionized water using dialysis membrane tubing with molecular weight cut-off of 14 kDa in order to eliminate compounds of low molecular weight and salts. The dialysis was carried out for 5 days at 4 °C until the conductivity becomes similar to deionized water (Chaouch, Hafsa, Rihouey, Le Cerf, & Majdoub, 2015). The extensively dialyzed extract was lyophilized to provide the fraction termed as ZLP. The obtained fractions according to Box–Behnken design were weighed with a balance and the extract yield percentage (%) was calculated by Eq. (1).

$$\text{Extraction yield}\,(\%, \text{W/W}) = \left(\frac{\text{Weight of dried crude extraction (g)}}{\text{Weight of Zizyphus lotus pulp powder (g)}}\right) \times 100 \quad (1)$$

2.4. Analytical methodology

2.4.1. Determination of carbohydrate, protein and uronic acid contents

Total carbohydrate content of ZLP was measured by Phenol-sulfuric acid method using galactose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The uronic acid was estimated by a carbazole method using the glucuronic acid as standard for calibration (Bitter & Muir, 1962). Protein content was determined by the method of Bradford (Bradford, 1976).

2.4.2. Antioxidant activity of polysaccharides

2.4.2.1. DPPH free radical Scavenging activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of ZLP samples were performed using a method described elsewhere (Yamaguchi, Takamura, Matoba, & Terao, 1998) with slight modifications. 1 mL of each ZLP extract with various concentrations was mixed with 2 mL of freshly prepared DPPH solution (0.2 mM in methanol). The mixture was incubated at 25 °C for 30 min, and the absorbance of the mixture was measured at 517 nm using a UV–vis spectrophotometer (Perkin Elmer Lambda 40 UV/Vis Spectrophotometer). The radical scavenging activity was expressed as percentage of DPPH radical elimination calculated according to Eq. (2).

$$\text{Percent Inhibition}\,(\%) = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right] \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of the control (DPPH solution with no sample), and A_{sample} is the absorbance of the samples (DPPH solution with sample). All tests were run in triplicate and the average value was calculated. Antiradical DPPH activity is expressed as IC_{50} in (mg/mL) which denoted the concentration of sample required to scavenge 50% of DPPH free radicals.

2.4.2.2. Ferric- reducing antioxidant power (FRAP) activity. The reducing power was determined according to the method described elsewhere by Wang, Yang, and Wei (2012). Briefly, 250 μL of each ZLP sample in deionized water solution with various concentrations (0.5–4 mg/mL), was mixed with 625 μL of sodium phosphate buffer (0.2 M, pH 6.6) and 625 μL of $K_3Fe(CN)_6$ (1%, w/v). The mixtures were incubated at 50 °C for 20 min. After cooling, 625 μL of trichloroacetic acid (TCA, 10%, w/v) was added to mixtures to stop the reaction, then samples were centrifuged at 2000*g* for 10 min. Afterwards, 625 μL of aliquot was mixed with 625 μL of distilled water and 125 μL of 1% (w/v) $FeCl_3$, and set for 10 min. The absorbance of the reaction mixture was measured at 700 nm against a blank. A standard curve was plotted using different concentrations (25–2000 μmol/L) of $FeSO_4{\cdot}7H_2O$ and the