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journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2016.11.024>Antioxidant activity of water-soluble polysaccharide extracted from *Eucalyptus* cultivated in LebanonMarianne Haddad¹, Salam Zein¹, Hawraa Shahrouf², Kamar Hamadeh¹, Nadine Karaki¹, Hussein Kanaan^{1*}¹Laboratory of Chemical Synthesis and Extraction of Polysaccharides from Seaweed, Faculty of Pharmacy, Lebanese University, Hadath Campus, Beirut, Lebanon²Laboratory of Microbiology, Department of Life and Earth Sciences, Faculty of Sciences I, Lebanese University, Hadath Campus, Beirut, Lebanon

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ABSTRACT

Objective: To extract and identify the chemical composition of the polysaccharide isolated from the *Eucalyptus* cultivated in Lebanon and to evaluate its antioxidant activity.**Materials:** The water-soluble polysaccharide was isolated from *Eucalyptus* leaves, and its structure was identified by Fourier transform infrared spectroscopy, proton nuclear magnetic resonance, and carbon-13 nuclear magnetic resonance. The antioxidant activity of the active ingredient was screened for its radical scavenging ability using 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) test.**Results:** The results of the DPPH test have shown that fucoidan, the polysaccharide isolated from *Eucalyptus*, exhibited almost the same antioxidant activity against DPPH as the ascorbic acid did at 100 µg/mL.**Conclusions:** This natural molecule extracted from a medicinal plant has a promising antioxidant activity and could be used in pharmaceutical and medical applications.

1. Introduction

Medicinal plants have been widely developed in recent decades and they are considered as the richest natural resources of biologically active compounds [1]. Given the multiple side effects of chemically synthesized drugs and their potential toxic effects, the search for new natural resources having therapeutic effects currently remains of great interest [2]. *Eucalyptus*, also known as “koala tree” or “blue gum”, is a flowering tree of the myrtle family, Myrtaceae. Originally from Australia, *Eucalyptus* spp. are planted in subtropical and temperate regions of the world including the Mediterranean Basin. Today, more than 700 *Eucalyptus* species are recorded worldwide [3]. The medicinal properties of *Eucalyptus* are mainly due to the presence of

eucalyptol (also known as 1,8-cineole), one of the ingredients of *Eucalyptus* oil contained within the leaves.

Several studies have investigated the therapeutic effects of *Eucalyptus* which was found effective in the treatment of respiratory tract diseases such as bronchitis, rhinosinusitis and severe asthma due to its mucolytic properties that make the mucus of the respiratory tract thinner [4–7]. In addition, *in vitro* and *in vivo* studies have indicated that *Eucalyptus* exhibited various properties such as herbicidal, anti-inflammatory, antioxidant, anticancer, antibacterial, antiviral, and antifungal activities [8–14].

One of the most disease-causing factors is the oxidative stress resulting in cell or tissue damage. Nowadays, phytotherapy is being explored to develop viable and safe alternatives to synthetic antioxidants known for their ability to prevent oxidation. Numerous studies have reported the antioxidant activity of the methanolic extracts and the essential oils extracted from *Eucalyptus* leaves [10]. However, to our knowledge, no study has been conducted to examine the *in vitro* antioxidant efficiency of polysaccharides isolated from Lebanese *Eucalyptus*. The aim of this study was to evaluate the antioxidant activity of water-soluble polysaccharide extracted from *Eucalyptus* cultivated in Lebanon.

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2. Materials and methods

2.1. Sample collection

Eucalyptus leaves were collected from the Hadath region in Lebanon in 2015. The samples were air dried at room temperature in the dark for a few weeks to achieve a final moisture content of 10%. Before used, the dried samples were ground using a blender with the particle size between 0.8 and 0.9 mm.

2.2. Extraction of polysaccharides from *Eucalyptus*

Thirty grams of dried *Eucalyptus* leaves were extracted twice with ethanol (96%) for 3 h at 40 °C (ethanol: *Eucalyptus*, 1:0.8, w/w) to remove low molecular weight compounds and for depigmentation. The samples were centrifuged at 4 000 r/min for 20 min. The supernatant was discarded while the residual was dried for 3 h and extracted twice with 150 mL of HCl (pH 2.0–2.3) at 60 °C. Again, the supernatant 1 containing anionic and cationic polysaccharides, called fucoidan and laminarin, respectively was centrifuged (4 000 r/min for 20 min) and chromatographed on a column (polytetrafluoroethylene, 15 cm × 6.5 cm). Fractions were eluted with water to obtain the fucoidan and elution was continued until the test of the phenol-sulfuric acid showed the absence of carbohydrates in the eluate [15].

2.3. Proton nuclear magnetic resonance (¹H NMR) and carbon-13 nuclear magnetic resonance (¹³C NMR) spectra

In order to identify the structure of fucoidan, NMR (¹H NMR and ¹³C NMR) was performed. Three milligrams of the water-soluble polysaccharide were dissolved in 0.5 mL of 99% deuterium oxide. NMR spectra of the sample were recorded using Ultrashield Broker 300 spectrometer at room temperature with a frequency of 300 MHz, an acquisition time of 5.29 s and a pulse duration of 11 μs. Tetramethylsilane was used as an internal standard.

2.4. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectrum of the fucoidan extracted from *Eucalyptus* was recorded on a JASCO FT/IR-6300 spectrometer. The resolution was 4 cm⁻¹. Data were collected in the range of 4 000–400 cm⁻¹. All samples were prepared for the measurement in the form of KBr pellets.

2.5. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity

The radical scavenging activity of fucoidan isolated from *Eucalyptus* leaves depends on its capacity to scavenge DPPH free radicals. The DPPH free radical scavenging activity was evaluated according to the method described by Karaki *et al.* with minor modifications [16].

A total of 2.62 mg of DPPH was dissolved in 50 mL methanol and 12 concentrations of fucoidan (1–500 μg/mL) were prepared. Then, 1 mL of each concentration of fucoidan was added to 1 mL of DPPH solution, agitated and maintained for 30 min at room temperature in the dark. The absorbance was

measured at 517 nm using a UV–visible spectrometer (Shimadzu UV-1605, Japan). The DPPH scavenging capacity was calculated from the following equation:

$$\text{DPPH scavenging ability (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Ascorbic acid was used as a positive control. Each value represents the average of three independent experiments.

3. Results

3.1. Fucoidan isolation and characterization via ¹H and ¹³C NMR analysis

The amount of the fucoidan extracted from 30 g of *Eucalyptus* leaves was 0.63 g. Fucoidan was subjected to NMR analysis and results have shown that the ¹H NMR spectrum of the fucoidan isolated from Lebanese *Eucalyptus* showed signals spread from 5.0 to 5.4 ppm and the broad methyl peak at 1.2–1.3 ppm. These signals corresponded to α-L-fucopyranosyl units (Figure 1). Moreover, a signal at 4.65 ppm can be attributed to H₄ of 4-sulfated fucose.

The ¹³C NMR spectrum of fucoidan showed major signals of sulfated L-fucan between 93.8 and 107.0 ppm (C₁) and between 15.0 and 16.7 ppm (C₆). The signal at 57.4 ppm in the ¹³C NMR spectrum is attributed to C₄ (Figure 2).

3.2. FTIR spectroscopic analysis of fucoidan

The FTIR spectrum of fucoidan isolated from *Eucalyptus* leaves is shown in Figure 3. A wide band centered at 3 442.31 cm⁻¹ is assigned to the hydrogen bonded O-H stretching vibration; a weak band at 2 925.48 cm⁻¹ is assigned to a C-H stretching vibration.

The band centered at 1 627.63 cm⁻¹ is assigned to the carbonyl group C=O and the one at 1 420.32 cm⁻¹ is assigned to the C-O bond of the carboxylate group. The band at 1 235.18 cm⁻¹ is assigned to an S=O stretching vibration; the peak at 1 038.48 cm⁻¹ is assigned to the sulfate ester group and the small peak at 818.634 cm⁻¹ is attributed to the C-O group of C-O-SO₄. The peak between 510 and 560 cm⁻¹ is assigned to the C-C=O bending. The band between 545 and 555 cm⁻¹ is

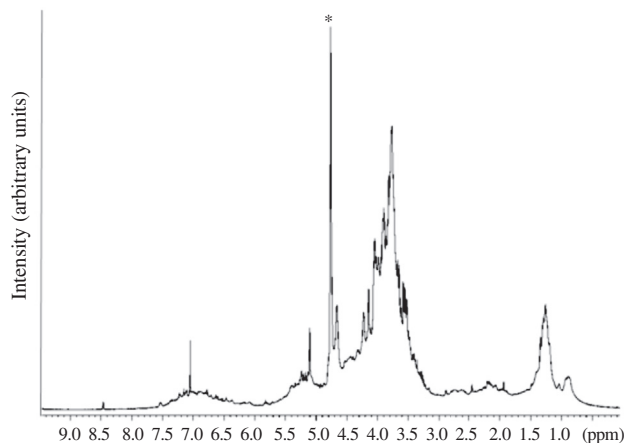


Figure 1. ¹H NMR spectrum of fucoidan isolated from the *Eucalyptus* leaves.

*: The residual signal of water.

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