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Antioxidant activity of water-soluble polysaccharide extracted from *Eucalyptus* cultivated in 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 (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra

In order to identify the structure of fucoidan, NMR (^{1}H NMR and ^{13}C 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:

DPPH scavenging ability (%) = [(Absorbance of control − Absorbance of sample)/Absorbance of control] × 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 ^{1}H and ^{13}C 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 ^{1}H 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 H_4 of 4-sulfated fucose.

The ^{13}C NMR spectrum of fucoidan showed major signals of sulfated L-fucan between 93.8 and 107.0 ppm (C_1) and between 15.0 and 16.7 ppm (C_6). The signal at 57.4 ppm in the ^{13}C NMR spectrum is attributed to C_4 (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-SO_4. 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

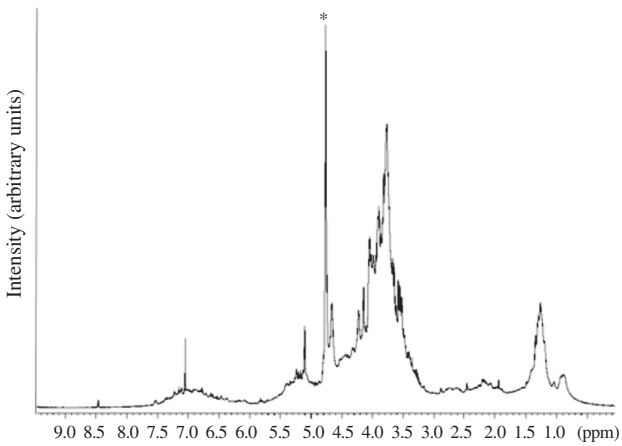

Figure 1. ^{1}H NMR spectrum of fucoidan isolated from the *Eucalyptus* leaves.
*: The residual signal of water.

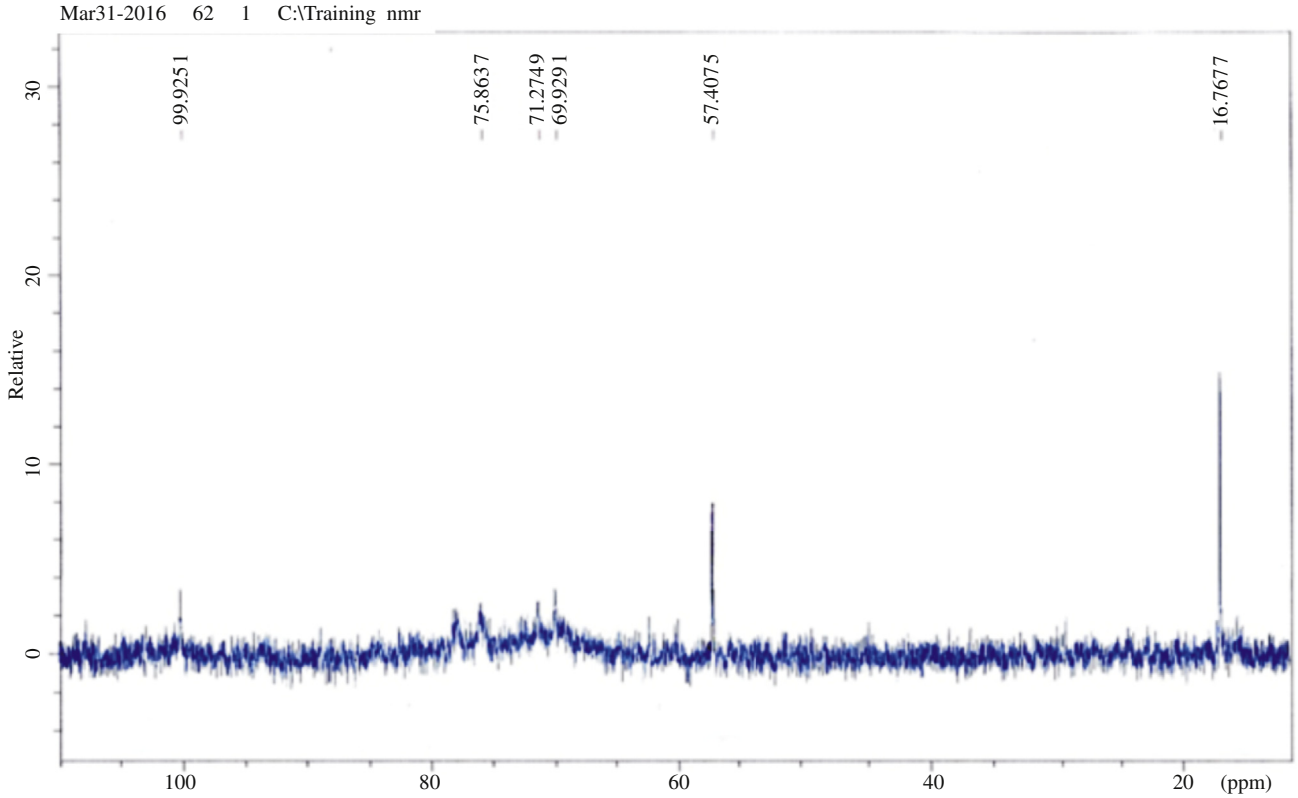

Figure 2. ^{13}C NMR spectrum of fucoidan isolated from *Eucalyptus* leaves.

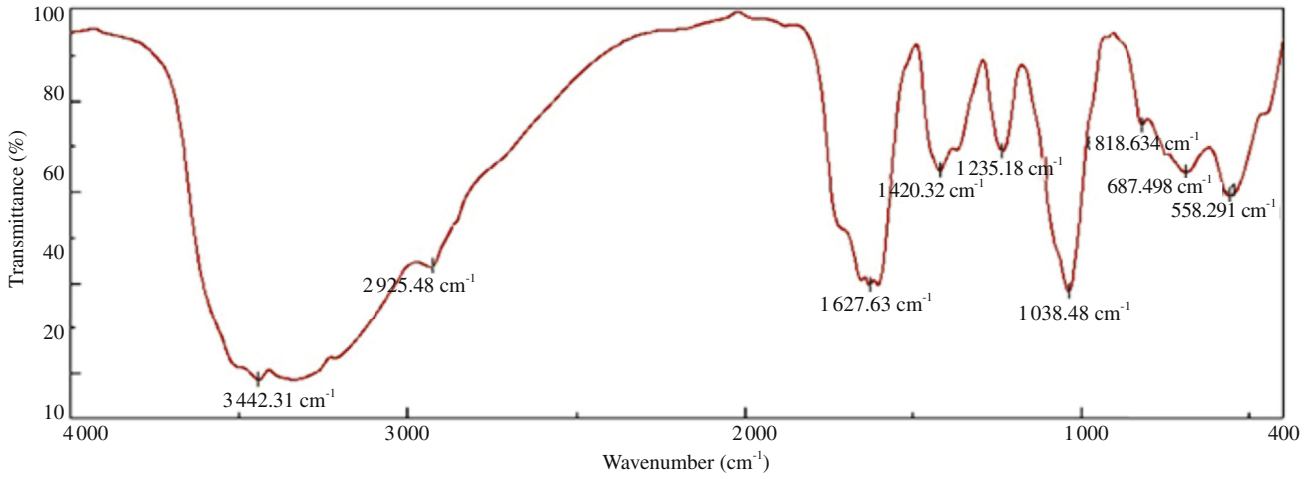

Figure 3. FTIR spectrum of fucoidan isolated from *Eucalyptus* leaves.

Table 1
DPPH radical scavenging activity of fucoidan extracted from *Eucalyptus* (%).

Compounds	Concentrations (μg/mL)												
	0.0	1.0	2.5	5.0	10.0	25.0	50.0	100.0	125.0	200.0	250.0	400.0	500.0
Fucoidan	0.0	25.0	38.0	48.0	59.0	75.0	85.0	95.2	95.3	95.6	95.5	95.6	95.6
Ascorbic acid	0.0	42.0	70.0	85.0	90.0	94.6	95.6	96.0	96.1	96.2	96.2	96.2	96.2

assigned to the $-CH=CH_2$ vinyl compound, a residue of pollution of the environment (Figure 3).

3.3. DPPH radical scavenging activity

Table 1 represents the DPPH radical scavenging activity (in percentage) of fucoidan compared to ascorbic acid. Results showed that the scavenging activity of fucoidan up to 50 μg/mL is lower than that of ascorbic acid but it became almost the same for both compounds at 100 μg/mL and above. These results indicate that fucoidan is an effective antioxidant compared to ascorbic acid.

4. Discussion

Reactive oxygen species and reactive nitrogen species are normally produced in the body as a result of normal cell metabolism. However, the imbalance between the production and the

cells' ability to detoxify free radicals may lead to oxidative stress and contribute to many health problems such as cancer and heart disease. Therefore, great attention has been paid to discovery of natural antioxidants to replace synthetic ones and avoid their undesirable side effects [17].

In this study, the fucoidan extracted from *Eucalyptus* showed a similar antioxidant capacity to that of ascorbic acid depending on the concentration used. This natural antioxidant can detoxify the free radicals through various mechanisms: either by hydrogen donation or chelation of metals and preventing their reactions with reactive oxygen species [18,19]. The hydroxyl hydrogen donor group revealed by analysis of FTIR spectroscopy of the fucoidan can contribute to the antioxidant potential of this compound. The presence of alkenes and carboxylate groups in polysaccharide and the acetyl in fucoidan revealed by ^{13}C NMR can contribute to the stability of the relocation system that improves the antioxidant activity by relocating the resulting free radicals. Thus, the fucoidan isolated from the *Eucalyptus* leaves showed a good antioxidant activity. However, further studies are still needed to evaluate its vital role as therapeutic agent. Finally, these results show the important role of *Eucalyptus* as an important natural source of antioxidant compounds with potential benefits in medicine and health care.

Fucoidan, a water-soluble polysaccharide, was isolated for the first time from the leaves of *Eucalyptus* and its antioxidant activity was investigated. The results obtained in this study showed that fucoidan has an antioxidant activity close to that of the ascorbic acid, allowing its potential use in the treatment of several diseases including cancer. In conclusion, this study demonstrated the antioxidant role of active *Eucalyptus* substances that may be available in powder form and can be used, after further analyses, in the fields of medicine, pharmacy and food industry.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *Int Pharm Sci* 2011; **1**(1): 98-106.

[2] Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. *J Med Plants Res* 2010; **4**(2): 104-11.

[3] Maciel MV, Morais SM, Bevilaqua CM, Silva RA, Barros RS, Sousa RN, et al. Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. *Vet Parasitol* 2010; **167**(1): 1-7.

[4] Fischer J, Dethlefsen U. Efficacy of cineole in patients suffering from acute bronchitis: a placebo-controlled double-blind trial. *Cough* 2013; **9**: 25.

[5] Lai Y, Dilidaer D, Chen B, Xu G, Shi J, Lee RJ, et al. *In vitro* studies of a distillate of rectified essential oils on sinonasal components of mucociliary clearance. *Am J Rhinol Allergy* 2014; **28**(3): 244-8.

[6] Worth H, Dethlefsen U. Patients with asthma benefit from concomitant therapy with cineole: a placebo-controlled, double-blind trial. *J Asthma* 2012; **49**: 849-53.

[7] Bhowal M, Gopal M. Eucalyptol: safety and pharmacological profile. *RGUHS J Pharm Sci* 2016; **5**: 125-31.

[8] Barton AF, Dell B, Knight AR. Herbicidal activity of cineole derivatives. *J Agric Food Chem* 2010; **58**(18): 10147-55.

[9] Juergens UR. Anti-inflammatory properties of the monoterpene 1.8-cineole: current evidence for co-medication in inflammatory airway diseases. *Drug Res (Stuttg)* 2014; **64**(12): 638-46.

[10] Mishra AK, Sahu N, Mishra A, Ghosh AK, Jha S, Chattopadhyay P. Phytochemical screening and *Antioxidant* activity of essential oil of *Eucalyptus* leaf. *Pharmacogn J* 2010; **2**(16): 25-8.

[11] Sebei K, Sakouhi F, Herchi W, Khouja ML, Boukhchina S. Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves. *Biol Res* 2015; **48**: 7.

[12] Zhou LJ, Li FR, Huang LJ, Yang ZR, Yuan S, Bai LH. Antifungal activity of *Eucalyptus* oil against rice blast fungi and the possible mechanism of gene expression pattern. *Molecules* 2016; **21**(5): 621.

[13] Elaissi A, Rouis Z, Ban Salem NA, Mabrouk S, ben Salem Y, Salah KBH, et al. Chemical composition of 8 *Eucalyptus* species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. *BMC Complement Altern Med* 2012; **12**: 81.

[14] Karaki N, Haddad M, Hammoud M, Kassem Z, Kanaan H, Makhour Y, et al. Structural characteristics, antitumor, antibacterial properties of polysaccharides and essential oil, isolated from *Eucalyptus* cultivated in Lebanon. *World J Pharm Sci* 2016; **4**(9): 281-6.

[15] Imbs TI, Shevchenko NM, Sukhoverkhov SV, Semenova TL, Skriptsova AV, Zvyagintseva TN. Seasonal variations of the composition and structural characteristics of polysaccharides from the brown alga *Costaria costata*. *Chem Nat Compd* 2009; **45**(6): 786-91.

[16] Karaki N, Aljawish A, Muniglia L, Humeau C, Jasniewski J. Physicochemical characterization of pectin grafted with exogenous phenols. *Food Hydrocoll* 2016; **60**: 486-93.

[17] Emam AM, Mohamed MA, Diab YM, Megally NY. Isolation and structure elucidation of antioxidant compounds from leaves of *Laurus nobilis* and *Emex spinosus*. *Drug Discov Ther* 2010; **4**(3): 202-7.

[18] Floira SJS. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxid Med Cell Longev* 2009; **2**(4): 191-206.

[19] Farhan H, Rammal H, Hijazi A, Daher A, Reda M, Annan H, et al. Chemical composition and antioxidant activity of a Lebanese plant *Euphorbia macroclada schyzoceras*. *Asian Pac J Trop Biomed* 2013; **3**(7): 542-8.