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Enrichment, purification and *in vitro* antioxidant activities of polysaccharides from *Umbilicaria esculenta* macrolichen

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ABSTRACT

Umbilicaria esculenta as an edible macrolichen possesses nutritional properties, such as antioxidant, immune-boosting, and lipid peroxidation inhibitory properties. A new enrichment and separation technology for the polysaccharides from *Umbilicaria esculenta* (UEP) with AB-8 resin was established, and its adsorption data were well fitted to the pseudo-second-order kinetic model and the Langmuir isotherm, with a process of monolayer coverage of UEP onto it. After purification by DEAE (diethylaminoethyl) Cellulose-52 chromatography, two polysaccharide fractions (UEP-1, UEP-2) were obtained and characterized. UEP-1 was composed of mannose, rhamnose, glucose and galactose in a molar ratio of 0.59:3.12:93.09:3.20 with a molecular weight of 124.7 kD, while UEP-2 comprised mannose and glucose in a molar ratio of 20.66:79.34 with a molecular weight of 249.4 kD. With DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate), superoxide anion, and hydroxyl scavenging capacities as indices, their antioxidative assays *in vitro* indicated that the polysaccharides exhibited the very strong scavenging activities in a concentration-dependent manner. It was worth expecting that UEP could be developed as a novel potential functional component in food industries.

1. Introduction

Lichen symbiosis is a strategy that has resulted in the incorporated of various fungi. About 13,500 distinct species grow worldwide. Most live in extreme places and, thus contain secondary metabolites, such as polysaccharides and antimicrobial peptides. These bioactive substances may protect against physical stresses and suppress the growth of harmful bacteria [1–4]. Some lichen species or their components are used in foods. However, only about 100 lichens have been investigated to determine their polysaccharide components. The best know classes of their polysaccharides are glucans, galactomannans, galactoglucomannans and complex heteroglycans [5–7].

Umbilicaria esculenta, a foliose-type macrolichen, is a popular food known for its culinary properties in Southern China, but relatively unknown for its health benefits. However, the high nutritional values of this macrolichen and its potential health benefits have been reconsidered recently. Consumption of *Umbilicaria esculenta* is associated with reduced cholesterol synthesis and inhibition of tumor growth, and improved glycosidase activity [3,4,7,8]. Studies have analyzed the chemical structure of major polysaccarides from this macrolichen including the β-(1→3)-linked glucan laminarin and the β-(1→6)-linked glucan pustulan [3,4,9]. UEP is also associated with improved immunity and reduced lipid peroxidation *in vitro* [3,4,6,7].

The properties of UEP have been investigated, and its limited extraction and separation methods have been developed, such as solvent (acid or alkaline) and enzyme method [3,4,7,8,10,11]. However, these traditional methods have some disadvantages, such as high solvent dosage, residue toxicology, high cost, and low efficiency. Furthermore, unwanted reactions, such as isomerization and decomposition by acid or alkaline, easily occur and limit industrial applications of these methods for extracting and separating valuable bioactive components. Alternatively, various sorbents are employed to separate bioactive components. Among them, in particular, macroporous resins (MARs) actually get used to enrich and separate substances, such as flavonoids, alkaloids, amino acids, proteins, and polyphenols. MARs possess distinct features, including a range of types, functional groups, pore structure, surface area, mechanical strength, and environmental

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impact. The main interactions between absorbents and absorbates are hydrophobic, electrostatic, or hydrogen bond [12,13]. Given their versatility, low cost, high efficiency and easy regeneration, MARs have been investigated and used to separate target compounds including polysaccharides from tea, rapeseed, *Polyporus*, *Epimedium*, *Gynostemma*, *Ginkgo biloba* endocarp, and *Cyclocarya paliurus* [5–7,14–17]. However, the use of MARs to enrich and separate UEP has yet to be reported, and the adsorption mechanisms between UEP and MARs have not been elucidated. As for its purification, by now, there are no relative reports of any researches. Purification methods of common polysaccharides are solvent precipitation, ion exchange chromatography, gel chromatography and ultra-filtration [10,11], which can be probably used to purify UEP.

In this paper, the macroporous resin adsorption technology was firstly applied to enrich and separate the polysaccharides *from Umbilicaria esculenta*, furthermore DEAE Cellulose-52 was used to purify them. Their monosaccharide composition, molecular weight, infrared spectrum and molecular morphology were also investigated. Finally, their *in vitro* antioxidant activities were deeply evaluated by different free radical scavenging experiments.

2. Materials and methods

2.1. Materials

Unless stated otherwise, all chemicals and reagents were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water was used throughout the experiments and purified by a Mill-Q water purification system from Millipore (Bedford, MA, USA). Fresh thalli of *Umbilicaria esculenta* were collected in July 2010 from Huangshan Mountain in Anhui Province, China. The collected samples were identified on the basis of their morphological characteristics [4,7,8].

2.2. Crude UEP preparation

The thalli of *Umbilicaria esculenta* (100.0 g) were cleaned, lyophilized (a Model 2K-XL Lyophilizer, Virtis Corporation, American), and ground with a Model ZN-100 Grinder (Zhongnan Pharmaceutical Machinery Factory, Shanghai, China). Thalli powders (70.8 g) were obtained and successively extracted with deionized water (800 mL) at 100 °C for 3 h (× 3) to isolate the residue. Each aqueous extract was added to excess absolute ethyl alcohol (ratio of 3:1; v/v) to form a precipitate (14.5 g). The precipitate was isolated through centrifugation (Model 5804R, Eppendorf Corporation, Germany) at 4680*g* for 20 min at 25 °C and then lyophilized. Hence, crude UEP (10.2 g) was obtained and stored at 4 °C.

2.3. UEP enrichment and separation

2.3.1. Adsorbents

MARs including H107, H103, DM301, DM130, D101, X-5, S-8, and AB-8 were provided by Anhui Sanxin Resin Technology Co., Ltd. (Bengbu, China). ADS-7, ADS-17, NKA-9, CAD-40, DA201, HPD300, HPD400, HPD500, HPD600, HPD722, HPD750, and HPD800 were obtained from Cangzhou Bon Adsorber Technology Co., Ltd. (Cangzhou, China). Table 1 shows their physical and chemical properties.

Some monomers and porogenic agents were trapped inside the pores of the resins during their synthesis. Thus, the pretreatment of macroporous adsorption resins was deemed necessary. Resins were washed with ethanol (95%) for 120 min, treated with 1 mol/L HCl and NaOH solution, and then vacuum dried at 60 °C. The dried resins were weighed, soaked in ethanol overnight, and finally washed thoroughly with deionized water.

2.3.2. Static adsorption and desorption tests

Twenty MARs were tested with static adsorption/desorption experiments. Static adsorption tests were performed as follows. In brief, 0.1 g of pretreated resin was introduced into a 100 mL Erlenmeyer flask. Subsequently, approximately 20 mL of crude aqueous solution of UEP (2.0 mg/mL, with deionized water as solvent) was adjusted to pH 7 and added to each flask. The flasks were shaken (100 rpm) at 25 °C for 24 h in an incubator shaker (HYL-A, Taicang Experiment Equipment Co., Taicang, China). The solutions were analyzed after reaching the adsorption equilibrium.

Adsorption capacity was evaluated as follows [18]:

$$q_e = \frac{(C_0 - C_e)V}{W} \quad (1)$$

where q_e is the equilibrium adsorption capacity (mg/g); C_0 and *Ce* are the initial and equilibrium concentrations of UEP in the solutions (mg/mL), respectively; V is the volume of crude aqueous solution of UEP (mL); and W is the weight of the resins (g).

Table 1
Physical and chemical properties of MARs.

Resin series	Resin name	Polarity	Particle size	Pore size	Specific surface area
			(mm)	(nm)	(m^2/g)
1	S-8	Strong-polar	0.3–1.25	28.0–30.0	100–120
2	HPD500	Strong-polar	0.3–1.20	8.5–9.0	650–700
3	HPD600	Strong-polar	0.3–1.20	10.0–12.0	500–550
4	ADS-7	Strong-polar	0.3–1.25	25.0–30.0	100–120
5	NKA-9	Strong-polar	0.3–1.25	15.5–16.5	250–290
6	ADS-17	Moderate-polar	0.3–1.25	25.0–30.0	90–120
7	HPD400	Moderate-polar	0.28–0.34	7.5–8.0	500–550
8	DM301	Moderate-polar	0.3–1.25	9.0–10.0	330–500
9	HPD750	Moderate-polar	0.3–1.20	8.5–9.0	650–700
10	HPD800	Moderate-polar	0.3–1.25	9.0–11.0	450–500
11	AB-8	Weak-polar	0.3–1.25	13.0–14.0	480–520
12	DM130	Weak-polar	0.25–0.84	9.0–10.0	500–550
13	HPD722	Weak-polar	0.30–1.25	13.0–14.0	485–530
14	CAD-40	Weak-polar	0.25–0.84	5.0–6.0	485–530
15	DA201	Weak-polar	0.3–1.25	9.0–10.0	550–650
16	X-5	Non-polar	0.3–1.25	29.0–30.0	500–600
17	HPD300	Non-polar	0.3–1.2	8.5–9.0	650–700
18	D101	Non-polar	0.2–0.6	10.0–12.0	400–600
19	H103	Non-polar	0.3–1.25	8.4–9.4	1000–1100
20	H107	Non-polar	0.3–1.25	8.5–10.0	1000–1300