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Extracting antioxidant phenolic compounds from compressional-puffing pretreated *Pinus morrisonicola*: Effects of operational parameters, kinetics and characterization

Pai-Shih Chiang[a], Duu-Jong Lee[a,b,*], Chris G. Whiteley[c], Chun-Yung Huang[d]

[a] Department of Chemical Engineering, National Taiwan University, Taipei 106, Taiwan
[b] Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 106, Taiwan
[c] Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, Taipei 106, Taiwan
[d] Department of Seafood Science, National Kaohsiung Marine University, No. 142, Hai-Chuan Rd., Nan-Tzu District, Kaohsiung 811, Taiwan

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ABSTRACT

Pinus morrisonicola has been reported bio-benefit and contains high amount of antioxidant bioactive compounds. This study followed a previous work to pretreat the *Pinus morrisonicola* by compression-puffing process and then extracted the antioxidant phenolic compounds from the pretreated samples for the measurements. The effects of ethanol concentration, extraction temperature and liquid–solid ratio on the extraction yield (total flavonoids content and total phenolic content) and antioxidant activity (DPPH scavenging activity, FRAP and ABTS scavenging activity) are reported for demonstration the pretreatment efficiencies. Semi-qualitative characterization of the extract compounds was conducted.

1. Introduction

Pinus morrisonicola, also called white pine and Taiwan short-leaf pine, belongs to the Pinaceae family, grows at elevations of 300–2300 m in the Taiwanese mountains. Studies showed that extracts of the leaves of *P. morrisonicola*, called pine needles herein, exhibit specific biological activities, including antioxidant activity, antimicrobial activity, anti-inflammatory effects, antimutagenic and antitumor activity, protection of LDL oxidation, and controlling the differentiation of human embryonic stem cells [1,2]. Recent studies have revealed that pine needles contain abundant flavonoids [3,4], and investigations have shown that phenolic compounds are responsible for the excellent antioxidant activity of the plant extracts [5].

Solvent extraction is commonly applied to isolate bioactive compounds from plants [6–9]). Hsu et al. [10] found that the water extract of pine needles showed the highest scavenging ability on superoxide anion among those from different pine parts and inhibited the growth of leukemia cell line U937. Pine needles extracts also significantly inhibited copper-induced low-density lipoprotein (LDL) oxidation and attenuated excessive NO generation in inflammatory lipopolysaccharide-stimulated RAW 264.7 cells [11]. Most research has implicated that atherosclerosis has a close relationship with modified LDL, which is clearly a main risk factor for coronary heart disease. Therefore, the extract of pine needles would be effective in anti-atherosclerotic and anti-inflammatory abilities [12]. Pine needles have nine essential amino acids and are especially rich in glutamic acid. They are still used in folk medicine and tea, and their essential oil is used in the manufacture of perfumes and deodorants in South Korea. Consumption of pine tree parts is believed to promote health, cure gastrointestinal diseases and neuronal problems, and prevent aging-related chronic diseases such as hypertension, atherosclerosis, and diabetes. According to Buddhist scriptures, pine needle extracts were commonly used as a tonic [13]. The effective components of pine needles are chlorophyll, carotene, dietary fiber, terpenoids, phenolic compounds, tannin, and alkaloids. Extracts from pine needles or pine cones are reported to be effective scavengers of reactive oxygen, lowering serum lipids and possibly helping to delay aging [14–16].

Due to the compact structure formed during the evolvement of plants, transfer of the bioactive compounds from cells to solvents encounters considerable resistance which leads to decreasing extraction efficiency and yield [17]. Therefore, ultrasound, supercritical fluid and microwave are employed to assist in the efficiency and yield of the extraction of phenolic compounds [18,19].

* Corresponding author at: Department of Chemical Engineering, National Taiwan University, Taipei 106, Taiwan.
E-mail addresses: djlee@ntu.edu.tw, djleetw@yahoo.com.tw (D.-J. Lee).

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Nomenclature

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ANOVA	analysis of variance
CCD	central composite design
DPPH	2,2′-diphenyl-1-picryhydrazyl
FTIR	Fourier transform infrared spectroscopy
FRAP	ferric reducing antioxidant power
GAE	gallic acid equivalent
HPLC	high-performance liquid chromatography
MIP	mercury intrusion porosimetry
PE	polyethylene
QE	quercetin equivalent
SEM	scanning electron microscopy
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TFC	total flavonoids content
TPC	total phenolic content
TPTZ	2,4,6-tripyridyl-s-triazine

However, complex operations and high costs of these technologies have so far limited the development of industrial processes. Compressional-puffing is widely used in food industry for cutting food pieces into smaller porous pieces in order to enhance their flavor and productize the pieces more conveniently. Huang et al. [20] applied this process as a pretreatment of bioactive compounds extraction due to the decomposition of the cellular structure of plant. Besides the effect on external destruction of plants' cells, there are numerous advantages that have been introduced including relative simple procedure, reactant-saving, environmentally friendly process and feasibility for a continuous production. Chiang et al. [21] applied the compression-puffing pretreatment to pretreat *P. morrisonicola* and proposed the optimal operational conditions for maximum yields of phenolic compounds and the associated antioxidant activities using response surface methodology with a central-composite design. These authors investigated the effects of extraction temperature, ethanol concentration, and liquid–solid ratio on the extraction yields and noted a promising enhancement of the antioxidant activities of the extraction products.

This paper is a complementary document for ref. [21], particularly emphasizing on the characterization of the pretreated pine needles, the effects of operational parameters on the antioxidant activities of the extract, the apparent kinetics, and the semi-quantitative characterization of the extract compounds.

2. Materials and methods

2.1. Materials

Pine needles, *Pinus morrisonicola*, were collected from Nantou County, Taiwan and were washed with tap water, drained and then dried at room temperature to 6% w/w moisture content. After drying, the sample was packed in polyethylene (PE) bags and stored at 4 °C until used.

Ethyl alcohol (99.5%) was purchased from Shimakyu's Pure Chemicals (Osaka, Japan). Aluminum chloride (anhydrous), quercetin, sodium hydroxide and sodium nitrite were purchased from Acros Organics (New Jersey, USA). 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), 2,2′-diphenyl-1-picryhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, formic acid (≥95%), gallic acid and Iron(II) sulfate heptahydrate were purchased from Sigma-Aldrich (St. Louis, USA). Folin–Ciocalteau reagent, hydrochloric acid, potassium persulfate and sodium carbonate were purchased from Merck (Darmstadt, Germany). Acetonitrile was purchased from Burdick & Jackson (New Jersey, USA).

2.2. Pretreatment and extraction protocols

The pretreatment and extraction protocols followed those used in [21]. In brief, the pine needle samples were cut into <5 mm pieces. The pretreatment process was conducted using a continuous and patented compressional-puffing machine (MIBO R2, Yuan Chuang Food Machinery Co. Ltd., Taiwan) equipped with a separable cylindrical chamber (65 mm diameter, 6 mm deep) [20] at 180, 200 or 220 °C. The mechanical compression force of approximately 5 kg/cm^2 was applied to the pine needle sample with subsequent rapid release of pressure to induce puffing.

The treated pine needles were extracted in different extraction environments involving four variables (extraction time, extraction temperature, ethanol concentration and liquid–solid ratio). The solution was filtered through 0.3 μm membrane filter and the filtered samples were store at -4 °C until used.

All tests were repeated at least in triplicate.

2.3. Analyses

2.3.1. Total flavonoid contents

The determination of the total flavonoid content was conducted using the method of [21,22] with modifications. In brief, 0.5 mL of the extract solution was added to a test tube containing 5 mL of ethanol. 0.25 mL of 0.5 M sodium nitrite solution and 0.25 mL of 0.3 M aluminum chloride was added and allowed to stand for 5 min. Then, 1.5 mL of 1 M sodium hydroxide was added. The absorbance of the mixture was measure at 510 nm immediately after the mixture with an UV–Vis spectrophotometer (DR2700^{TM} portable spectrophotometer, HACH, Colorado, USA), reported in quercetin equivalents (QE) mg/g dried wright (DW) of the pine needles. All determinations were estimated in triplicate.

2.4.2. Total phenolic content (TPC)

The determination of the total phenol content was conducted using the method of [21,23] with modifications. In brief, 0.1 mL of the extract sample and 0.5 mL of Folin–Ciocalteau's reagent was added into a test tube containing 5.9 mL deionized water and allowed to stand for 2 min. Then, 1.5 mL of 20% (w/w) sodium carbonate was added, then the mixture was incubated at 40 °C for 30 min and the absorbance of the mixture was measure at 765 nm immediately after the mixture with an UV–Vis spectrophotometer with gallic acid as model compounds. The final values were expressed as gallic acid equivalents (GAE) mg/g DW of the pine needles.

2.4.3. DPPH radical scavenging assay

The DPPH method employed to determine the radical scavenging activity of each sample was based on [21,24]. In brief, the stock solution was prepared by dissolving 39 mg 2,2′-diphenyl-1-picrylhydrazyl (DPPH) in 1000 mL ethanol and storing at 20 °C until required. 0.1 mL of example was added to 3.9 mL of the DPPH solution. The mixture was vortexed for 1 min and left in the dark for 30 min at room temperature. The absorbance of all samples was measured at 517 nm using an UV–Vis spectrophotometer using Trolox as the model compounds. The scavenging activity of DPPH radical was calculated by considering the variation of the absorbance obtained as follows:

$$\text{Scavenging } activity(\%) = \left(1 - A_{\text{sample}}/A_{\text{control}}\right) \times 100 \quad (9)$$

where A_{control} represents absorbance of the ethanol solution of DPPH without the sample, and A_{sample} is absorbance of the ethanol

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