Separation and Purification Technology 124 (2014) 201–206

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Alkaline extraction and acid precipitation of phenolic compounds from longan (*Dimocarpus longan* L.) seeds

Xiangnan Li [a,b], Jiale Huang [a], Zidan Wang [a], Xinde Jiang [a], Wencheng Yu [a], Yanmei Zheng [a], Qingbiao Li [a,c,*], Ning He [a,*]

[a] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, People's Republic of China
[b] School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, People's Republic of China
[c] Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen 361005, People's Republic of China

ARTICLE INFO

Article history:
Received 1 September 2013
Received in revised form 4 January 2014
Accepted 17 January 2014
Available online 29 January 2014

Keywords:
Longan seeds
Phenols
Alkaline extraction
Acid precipitation
Radical-scavenging capacity

ABSTRACT

The alkaline extraction and acid precipitation of longan seed phenolic compounds were investigated to develop a more practical process than currently exists to isolate these chemicals. By using an alkaline buffer as the extraction solvent, the yield of total phenols reached 46.86±0.43 mg/g after conventional solid–liquid extraction. Because of the benefit achieved from the preconcentration process, an acid precipitation efficiency of 65.29 ± 0.38% was obtained, and the separation yield of phenols reached 22.04 ± 1.95 mg/g. However, the purity of the phenolic extract was influenced by the presence in the precipitate of proteins and carbohydrates, which both moved together in all four precipitation fractions at different pH values. The FRAP assay and the assay of the radical-scavenging activity revealed the strong antioxidant activity possessed by the phenolic compounds in the precipitate extracts. This preparation process for phenolic compounds has obvious advantages, such as easy operation, safe products and low production cost, which indicated its prospective industrial potential.

1. Introduction

Longan (*Dimocarpus longan* Lour.) originated from Southeast Asia and is a subtropical evergreen plant of the family Sapindaceae [1]. Because of its desirable taste and nutritional value, longan fruit has gained wide popularity with consumers and is in great demand in both domestic and international markets. The seeds, which constitute the main by-product in the further processing of longan fruit, are largely wasted and easily recovered, so it could be treated as an exploitable resource [2]. Previous studies have revealed that longan seeds contain high levels of polyphenolic compounds, including gallic acid, corilagin and ellagic acid [3,4], along with ethyl gallate,1-β-O-galloyl-D-glucopyranose, brevifolin, methyl brevifolin carboxylate and 4-O-α-L-rhamnopyranosyl-ellagic acid [5]. Among these polyphenols, ellagic acid exhibits effective inhibitory activity against mushroom tyrosinase and ultraviolet-induced pigmentation [6], thus possessing potential as a skin-whiting agent for use in cosmetics [7]. There are reports regarding the clinical potential of corilagin for inhibiting HIV-1 protease [8] and the growth of ovarian cancer cells [9]. More noteworthy is the growing attention that the free radical-scavenging capacity of plant polyphenolic compounds has received, suggesting their promising applications in the fields of both food and medicine [10]. Previous study has shown the polyphenolic compounds in longan seeds to exhibit effective radical-scavenging capacity [11]. The SC_{50} values of gallic acid, corilagin and ellagic acid were reported to be 0.80 ± 0.02 μg/mL, 2.01 ± 0.03 μg/mL and 2.20 ± 0.10 μg/mL toward DPPH radicals (compared with 2.13 ± 0.02 μg/mL for ascorbic acid) and 1.04 ± 0.11 μg/mL, 2.37 ± 0.27 μg/mL and 2.56 ± 0.35 μg/mL toward superoxide radicals (for ascorbic acid was not determined), respectively [5]. Antioxidant activity, such as the inhibition of xanthine oxidase and 2-deoxyguanosine, were also found for most polyphenols in longan seed extract [12].

Different processes have been used in the extraction and isolation of polyphenols from longan seeds [13]. Sudjaroen et al. [12] isolated polyphenols from methanol extracts of longan seeds by chromatography on Toyopearl HW-40 and Sephadex LH-20 columns. The obtained polyphenolic fraction (80.90 g/kg dry weight) was dominated by ellagic acid (25.84 g/kg) and the known ellagitannins, which included corilagin (13.31 g/kg), chebulagic acid (13.06 g/kg), ellagic acid 4-O-α-L-arabinofuranoside (9.93 g/kg),

* Corresponding author Address: Xiamen University, Siming South Road No. 422, Xiamen 361005, Fujian Province, People's Republic of China. Tel.: +86 592 2183088; fax: +86 592 2184822.
E-mail addresses: kelqb@xmu.edu.cn (Q. Li), hening@xmu.edu.cn (N. He).

http://dx.doi.org/10.1016/j.seppur.2014.01.030
1383-5866/

isomallotinic acid (8.56 g/kg) and geraniin (5.79 g/kg). Zheng et al. [5] partitioned the 95% ethanol extract of 10.5 kg of longan seed powder with different solvents. A variety of column chromatographic techniques was applied to separate the different soluble extracts. In all, 262 mg of ethyl gallate was isolated from the chloroform-soluble extract. The following yields of compounds were obtained from the ethyl acetate-soluble extract: 9 mg of 1-β-O-galloyl-D-glucopyranose, 6.72 g of gallic acid, 22.4 mg of methyl brevifolin carboxylate, 124 mg of brevifolin, 356 mg of corilagin, 37 mg of 4-O-α-L-rhamnopyranosyl-ellagic acid and 242 mg of ellagic acid.

However, most research on longan seeds is limited to intricate procedures conducted on a laboratory scale. A simple way to isolate phenolic compounds from longan seeds with alkaline extraction and acid precipitation was studied in this paper. This type of process is a classical method for extracting the flavonoid rutin from *Sophora japonica* and has been employed by various comparison and improvement studies [14,15]. Such processes have also been applied to the production of a variety of cereal proteins concentrates [16], such as soybean protein [17], sunflower [18] and canola meal protein [19], and have the advantages of easy operation, product safety and low production cost.

2. Materials and methods

2.1. Chemicals

2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Aladdin Chemistry Co., Ltd. Gallic acid, the Folin–Ciocalteu reagent, bovine serum albumin, brilliant blue G250 and other common reagents were purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Plant materials

Mature and fresh longan fruit (cultivar Fenglisui) were purchased from local markets in Xiamen, Fujian, China. The fruit was preserved frozen at −20 °C.

2.3. Preparation of seed samples

The longan seeds were manually separated from the frozen fruit and then cleaned with distilled water. After drying for 30 h in a blast oven at 50 °C, the seeds were ground into a homogeneous powder using a stainless-steel grinder. The seed powder was precisely weighed, dispensed into plastic tubes by the weight needed for a single experiment (500 mg or 4 g) and then stored in sealed polyethylene pouches at −20 °C.

2.4. Extraction of phenolic compounds

Aliquots of 500 mg of seed powder were separately extracted with 15 mL of different solvents, included distilled water, sodium phosphate buffer (PB, 10 mM at pH 7), hydrochloric acid (5 mM) and ethanol (50%, V/V). All the extraction processes were maintained at 70 °C in an agitated oil bath for 1–3 h. The extracts were then vacuum filtered through medium-speed filter paper, and the extracts were adjusted with distilled water to the initial volumes and the retentates were discarded. The content of total phenols was determined using a slight modification of the Folin–Ciocalteu phenol method [2]. A 1-mL aliquot of 50-fold diluted sample was mixed with 0.5 mL of the Folin–Ciocalteu reagent (1 N) followed by 3 mL of 13.5% sodium carbonate solution. The mixture was then adjusted to 10 mL and allowed to stand at room temperature for 2 h. The extraction yield of total phenols was expressed as milligrams of gallic acid equivalents per gram of longan seed powder on a dry weight basis (mg/g dry weight, DW).

2.5. Separation of phenols using acid precipitation

Aliquots of 4 g seed powder were extracted with 160 mL of PB (10 mM at pH 11) for 25 min at 70 °C followed by vacuum filtering through medium-speed filter paper, the extract was adjusted with distilled water to its original volume, then concentrated under vacuum below 50 °C for different concentration multiples (2- to 5-fold multiples with an increment of 1-fold). After separately adding 100 μL of acetic acid, phosphoric acid and hydrochloric acid with series of concentrations, 500-μL aliquots of extract in different concentration multiples were thoroughly vortexed and allowed to stand for approximately 5 min until a precipitate was completely generated, then each mixture was centrifuged at 7000 r/min for 5 min. The total phenolic content of the separated supernatant and precipitate, as well as the total carbohydrate and protein content were separately determined using the phenol-sulfuric acid method [20], and the Coomassie brilliant blue method [21]. The assays were performed using a spectrophotometer (UV-1800, Shimadzu, Japan).

Fivefold concentrated extract in 5-mL aliquots was adjusted to different pH values (2–6 in increments of 0.5) by the dropwise addition of hydrochloric acid (2 M). After centrifugation at 9000 r/min for 5 min, the supernatant and precipitate were separated and assayed.

25-mL aliquots of 5-fold concentrated extract were adjusted to pH 6 by using hydrochloric acid (2 M). After centrifuging at 9000 r min^{-1} for 10 min, the supernatant was separated and the precipitate was collected. Then the supernatant was sequentially adjusted to pH 5, 4 and 3 by repeating the process described above.

The precipitates formed at different pHs were separately collected for assay.

Before any content or capacity analysis, all the supernatant and precipitate samples were freeze dried and weighed and then redissolved and diluted with distilled water.

2.6. Determination of antioxidant capacity

The ferric-reducing antioxidant power (FRAP) [22] assay was used to determinate the antioxidant capacity of the supernatant, precipitate and precipitate fractions. Mix acetate buffer (pH 3.6, 100 mM), TPTZ (10 mM, dissolved in 40 mM HCl) and $FeCl_3$ (20 mM) solutions in a proportion of 10:1:1 were used to prepare the FRAP reagent. A sample aliquot of 100 μL was mixed with 3.9 mL of fresh FRAP reagent. After incubation at 37 °C for 10 min, the absorbance of the mixture at 593 nm was measured. The antioxidant capacity was expressed as ascorbic acid equivalents.

2.7. ABTS cation radical-scavenging activity

$ABTS^+$ solution was prepared by mixing 7 mM ABTS with 140 mM potassium persulfate at a ratio of 10:3.5. The mixture was placed in the dark for 15 h at room temperature. Before use, the $ABTS^+$ solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Aliquots of 100 μL of sample were mixed with 3.9 mL of the $ABTS^+$ solution. The absorbance of the mixture was read at 743 nm after incubation for 5 min [23]. Distilled water was used as the blank sample, and ascorbic acid served as the reference standard. The $ABTS^+$ radical-scavenging rate (%) was calculated as $[(A_{743(0)} - A_{743(1)})/A_{743(0)}] \times 100$, where $A_{743(0)}$ is the absorbance with the blank sample and $A_{743(1)}$ is the absorbance with the test sample or reference standard.