Research Article

Ultrasound-assisted extraction of phenolic compounds from coconut endocarp and its radical scavenging activity

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Naresuan Phayao J. 2020;13(3): 22-28.

Received; 15 July 2020; Revised: 7 October 2020; Accepted: 15 October 2020

Abstract

Phenolic compounds were extracted from the coconut endocarp waste, a by-product of the processing of coconut fruits. The optimum conditions for extraction of phenolic compounds from coconut endocarp using ultrasound-assisted extraction (UAE) were determined using three main parameters: solvent type, extraction temperature and extraction time. It was found that the extraction of sample with 50% (aq) of ethanol at 50 ºC for 120 minutes provided the highest crude extract yield (29.46% w/w on dry basis) and the highest total phenolic content (962.31 µg GAE/g). The antioxidant activity of crude ethanolic extract under the optimum condition was further investigated and the results showed that the crude extract exhibited an antioxidant activity with IC_{50} values of 288.17 µg/ml by DPPH assay and 10.55 µg/ml by ABTS assay, respectively.

Keywords: Coconut endocarp, phenolic compounds, radical scavenging activity, ultrasound-assisted extraction (UAE)

Introduction

Currently, the global consumption of coconut (*Cocos nucifera* Linn., Family Arecaceae) continues to rapidly increase due to the current health trends recognizing that coconut fruit contains many nutrients and benefits. Coconut is one of the most important economic crops in Thailand, which was ranked sixth among the world's largest coconut producing countries in 2018. During the coconut processing, a large amount of lignocellulosic biomass, coconut endocarp (shell), is generated. In general, this residue is traditionally used in agriculture, made into charcoal, or burnt as a fuel. The chemical composition of coconut endocarp is similar to that of hard wood, which is composed mainly of lignin and cellulose, and is considered as a potent source of phenolics [1]. Nowadays, the uses of natural phenolic extracts in various industries such as food, beverages, medicine, cosmetics, etc. have risen dramatically. Phenolic compounds are reported to exhibit various beneficial activities, such as anticarcinogenic, antiallergenic, antimicrobial, anti-inflammatory, antithrombotic properties and antioxidants [2,3]. Previous studies reported that phenolics play an important role in antioxidant activity, and higher phenolic content leads to stronger antioxidant

activity [4-6]. Today, antioxidants from plant extracts have become a major area of interest due to their possible uses as natural additives to replace those made from synthetic ones [7,8]. Therefore, the isolation of chemical compounds such as phenolics and antioxidants from coconut endocarp is interesting and may be a promising method to utilize this abundant waste.

Extraction is the first important process for separating the bioactive compounds from the raw material. Ultrasound-assisted extraction (UAE) has become an effective alternative extraction method compared to conventional solvent extraction methods such as maceration and Soxhlet, due to its high efficiency, low energy consumption, significantly reduced extraction time, and low temperature, which helps to avoid thermal damage in the extraction of heat-labile products [9]. UAE is based on the principle of acoustic cavitation, which is capable of disrupting the cell walls of the plant samples and thereby favoring the release of bioactive compounds [10]. This extraction method has been successfully used to extract bioactive substances, such as phenolic compounds and antioxidants from various agricultural by-products [11-14].

This research was to determine the optimum extraction condition of phenolics from coconut endocarp waste using the UAE method. In addition, the *in vitro* antioxidant activity of the crude phenolic extract obtained was investigated for its radical scavenging activity.

Materials and method

Raw material

Coconut endocarp (shell) was collected from a coconut processing plant in Samut Songkhram province, Thailand. The sample was cleaned and chopped into smaller pieces and air-dried for several days. Then, the sample was oven-dried at 50 °C to a constant weight. The dried sample was further ground and separated into different particle sizes ranging from 240 to 420 μm using sieves and stored in an airtight container for further analysis.

Determination of the optimum extraction condition for phenolics from coconut endocarp powder by UAE

Determination of the optimum extraction solvents

Extraction of phenolic compounds from dried coconut endocarp powder was conducted using an ultrasonic cleaning bath (Bandelin sonorex digitec, DT 510H, 35 kHz, 16 W). The samples were extracted with three different solvents [70% (aq) of acetone, 50% (aq) of ethanol, and distilled water] with a solvent-to-sample ratio of 100 (v/w). UAE was conducted at 50 °C for 60 minutes. The mixture was then filtered through filter paper, the filtrate was evaporated to achieve dryness, and the yield of each extract was determined and was further examined the total phenolic content by the Folin-Ciocalteu method [15] to evaluate the suitable solvent for phenolic extraction. The total phenolic content was reported as μg gallic acid equivalents (GAE)/g DW.

Determination of the optimum extraction conditions by UAE

The optimum operating conditions (temperature and time) for extraction of phenolics from coconut endocarp powder by UAE were determined. Samples were extracted with the optimum solvent (50% (aq) of ethanol) with a solvent-to-sample ratio of 100 (v/w). UAE was carried out in an ultrasonic bath at different temperatures (30 and 50 °C) and durations of time (30, 60, and 120 minutes). The extraction yield and phenolic contents were determined using the same procedures described above.

Determination of antioxidant activity

DPPH assay

The *in vitro* DPPH radical scavenging activity of 50% (aq) ethanolic extract, under the optimum extraction condition for use with UAE (at 50 °C for 120 minutes) was carried out according to the method used by Siramon et al. (2007) [16]. The crude ethanolic extract was prepared in various concentrations. The extract solution (1 mL) was mixed with 0.25 mM DPPH in ethanol (5 mL). The samples were kept in the dark for 30 minutes at room temperature, and then the absorbance of the mixture was measured at 515 nm on an ultraviolet-visible (UV-VIS) spectrophotometer. The DPPH scavenging ability was expressed as IC_{50} (the extract concentration required to inhibit 50% of the DPPH activity). Standard antioxidants: butylated hydroxytoluene (BHT) and α-tocopherol were used for comparison.

ABTS assay

The ABTS scavenging activity of the extract under the optimum extraction condition was measured by the ABTS cation decolorization assay [17]. The ABTS•+ solution was prepared by the reaction of 7 mM ABTS diammonium salt solution with 2.45 mM potassium persulfate and left in a dark condition for 12 hours, and then diluted with ethanol to obtain an absorbance of 0.70 measured at 734 nm on an UV-VIS spectrophotometer. The crude ethanolic extract was prepared in various concentrations. The extract solution (1 mL) was reacted with the ABTS•+ solution (2 mL) at the same condition and the absorbance was measured at 734 nm. The IC_{50} values were then calculated

Statistical analysis

All data were expressed as means ± standard deviations (SD) of three replications. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) were used to determine significant differences between the parameters. P-values (< 0.05) were regarded as statistically significant.

Results and discussion

Effects of solvents on the total phenolic content of crude extract

The effects of three different solvents 70% (aq) of acetone, 50% (aq) of ethanol, and distilled water, are shown in **Table 1**. The extraction solvents significantly affected the phenolic contents of the crude extract. Ethanol (50%) aqueous solution was demonstrated to be the most effective solvent as it provided the highest percentage yield of extract (25.96% w/w on dry basis) and the highest total phenolic content (790.93 µg GAE/g dry weight of the sample).

Table 1. Effects of solvents on the extraction yield and the total phenolic content of coconut endocarp extract using UAE.

Solvent	Yield (%, w/w)[1,2]	Total phenolic content (µg GAE /g)[1,2]
70% (aq) acetone	25.31 ± 0.29[b]	797.89 ± 3.07[a]
50% (aq) ethanol	**25.96 ± 0.43[a]**	**790.93 ± 7.16[a]**
distilled water	23.08 ± 0.02[c]	748.48 ± 8.69[b]

[1] Values are means of three replication ± SD. Numbers followed by different alphabetical among each column are significantly different ($P < 0.05$).

[2] Data were based on dry weight basis.

The results of this study were consistent with the previous study that used 50% (aq) of ethanol for optimizing the extraction condition for phenolics from coconut shell powder by response surface methodology [1,3]. Polar solvents such as aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate were recommended to be used in extraction of phenolic compounds from plants. Ethanol is frequently used due to its harmlessness for human consumption [18].

The optimum conditions for extraction of coconut endocarp by UAE

The extraction yield and total phenolic contents of the samples were strongly affected by the extraction parameters: temperature and time as shown in **Table 2**. Samples extracted from coconut endocarp powder with 50% (aq) of ethanol (the optimum solvent) at 50 °C for 120 minutes in an ultrasonic bath gave the best results. These extraction conditions produced the highest percentage yield of extract (29.46% w/w on dry basis) and the highest total phenolic content (962.31 µg GAE/g dry weight of the sample) among all of the condition tests.

Table 2. Extraction yield and total phenolic content of coconut endocarp extract using 50% (v/v) ethanol as the extraction solvent at different temperatures and duration by UAE.

Extraction conditions		Yield (%)[1,2]	Total phenolic content (µg GAE /g)[1,2]
Temperature (^{0}C)	Time (min)		
30	30	23.20 ± 1.67[c]	812.68 ± 7.82[c]
	60	24.49 ± 0.56[bc]	815.29 ± 7.53[c]
	120	25.24 ± 0.12[b]	870.06 ± 5.02[b]
50	30	25.40 ± 0.79[b]	786.46 ± 7.00[d]
	60	25.96 ± 0.43[b]	790.93 ± 7.16[d]
	120	**29.46 ± 1.49[a]**	**962.31 ± 7.93[a]**

[1] Values are means of three replication ± SD. Numbers followed by different alphabetical among each column are significantly different ($P < 0.05$).

[2] Data were based on dry weight basis.

Determination of the antioxidant activity

The antioxidant activity of 50% (aq) ethanolic extract of coconut endocarp under the optimum extraction condition by UAE (at 50 °C for 120 minute) was investigated using DPPH and ABTS radical scavenging assays and its activities were compared with the reference antioxidants: BHT and α-tocopherol. These two assays are widely used spectrophotometric methods for determination of the antioxidant capacity of plant extracts because of the simple, rapid, and reproducible procedures [19]. **Table 3** presents the results of the antioxidant activity expressed in terms of the amount of extract needed to reduce the DPPH and ABTS concentration by 50% (IC_{50}).

Table 3. Antioxidant activity (IC_{50}) by DPPH and ABTS assays of crude ethanolic extract from the optimum extraction conditions.

Sample	IC_{50} (μg/mL) by DPPH assay	IC_{50} (μg/mL) by ABTS assay
Crude ethanolic extract of coconut endocarp[1]	288.17 ± 3.23	10.55 ± 1.28
Butylated hydroxytoluene (BHT)	180.32 ± 1.53	215.45 ± 0.79
α-Tocopherol	383.14 ± 2.01	375.01 ± 1.78

[1] Data were based on crude sample weight.

According to data from Table 3, the crude ethanolic extract was found to be an effective antioxidant in different *in vitro* assay. The scavenging effect of crude extract and standard on the DPPH assay decreased according to the following order: BHT > crude extract > α-tocopherol. The crude extract was shown to be more effective for scavenging free radicals in the ABTS assay compared to both of the standard controls: crude extract> BHT> α-tocopherol. Due to the presence of different active compounds in the crude extract like phenolics and flavonoids which have difference mechanism to react with chemical testing in each method. That's the reason why the crude extract exhibited different antioxidant activity [20].

Conclusions

This work evaluates the use coconut endocarp as a natural source of phenolics. The optimum conditions for extraction of phenolic compounds from the sample by UAE were determined. They were found to be the use of a 50% (aq) of ethanol solution, with a solvent-to-sample ratio of 100, and a temperature of 50 °C for 120 minutes when carrying out the process. Extraction under these conditions provided the highest percentage yield of extract (29.46 % w/w on dry basis) and the highest total phenolic content (962.31 μg GAE/g dry weight of the sample) among all of the condition tests. The antioxidant activity of crude ethanolic extract derived from the optimum extraction conditions exhibited potent antioxidant activity. Thus, coconut endocarp is an abundant agricultural waste product that is suitable as an alternative source of natural phenolic antioxidants for various industrial application uses.

Acknowledgements

The project was financially supported by the Office of the Higher Education Commission (OHEC), Ministry of Education, Thailand and Chiwadi Products Co., Ltd.

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