EFFECT OF SOLVENT AND ULTRASONIC EXTRACTION ON HYDROXYCITRIC ACID (HCA) IN *GARCINIA CAMBOGIA*

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ABSTRACT

This study evaluates the effect of solvent and ultrasonic application in the extraction process on Hydroxycitric Acid (HCA) content of *Garcinia cambogia*. In this study, the fruit was extracted with methanol, ethanol, water and acetone by using two types of ultrasonic assisted extraction which are Ultrasonic probe and water-bath treatment. The HCA content was analysed by using HPLC-PDA. Ultrasonic probe treatment showed significantly higher yield of HCA for all solvent compared to water bath extraction.The HCA content extracted in methanol, ethanol, water and acetone by using probe was 12.59, 18.58, 26.69 and 13.78 %w/w, respectively meanwhile by using Ultrasonic Water Bath treatment, the HCA content was 11.36, 15.89, 22.11 and 12.43 %w/, respectively. The results obtained showed that the suitable solvent for extraction of HCA was water for both extraction methods that give the highest HCA content. Thus, extraction method by using Ultrasonic probe with water is more efficient in producing higher yield of HCA.

Keywords: Hydroxycitric acid, *Garcinia cambogia*, ultrasonic extraction

1. INTRODUCTION

Garcinia cambogia (Malabar tamarind) had been valued for variety of benefits traditionally to treat constipation, rheumatism, oedema, and intestinal parasites in many Asian countries [1]. Previous reported that Garcinia cambogia contains many different phytochemical including organic acids [2], benzophenones and xanthones [3]. However, HCA, an α-,β-dihydroxy tricarboxylic acid, is the key component present in the fruit rind which exhibited anti-obesity activity including reduced food intake and body fat gain by regulating the serotonin levels related to satiety, increased fat oxidation and decreased *de novo* lipogenesis. The crude extract from the plant also exerted antimicrobial and antioxidant properties [4].

There are many techniques to recover phytochemicals from plants, such as Soxhlet extraction, maceration, supercritical fluid extraction and subcritical water extraction. However, extraction yield not only depend on the extraction method but also on the solvent used for extraction. The presence of various compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent [5]. Polar solvents are frequently used for recovering polyphenols from plant matrices. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols [6]. In research involving plants, the efficiency of ultrasound-assisted extraction methods have been developed for the extraction of phytochemicals in order to increase the extraction yield, shorten the extraction time as well as enhance the quality of extracts. It is simple, inexpensive and efficient alternative to conventional extraction techniques [7]. There are two types of ultrasonic treatment which are ultrasonic probe treatment and ultrasonic bath treatment. A study from Cheok *et al*., (2013), the optimum direct ultrasonic pre-treatment conditions using the probe for maximum total monomeric

anthocyanin (TMA) and total phenolic content (TPC) extractions from mangosteen were compared with an in-direct pre-treatment using a bath and the conventional magnetic stirring extraction as a control [8]. The efficacy of ultrasonic-assisted extraction is not only shown through the improved bioactive compound yield recovery but also helps in reducing the extraction time. This study is carried out to evaluate the usage of ultrasound in overcoming the limitation of conventional extraction of *Garcinia cambogia* as well as its effect on the Hydrocitric acid content of the fruit using different solvent extraction.

2. MATERIALS AND METHODS

2.1. Plant material

The raw material was used for extraction process is the fruits of *Garcinia cambogia* collected from orchard in Perak. All fruits were cleaned and inspected to remove damage, disease or pest infected fruits. The fruits were cut into small pieces and undergo drying in a hot oven at 45°C. Then, the tray dried samples were ground into powder and stored at 4°C until use.

2.2. Chemical and Reagents

(-)-Hydroxycitric Acid Calcium Salt Standard was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Ethanol (99%), acetone, methanol AR grade were obtained from Merck (Massachusetts, USA). Dowex 50WX8, mesh size 100-200 was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phosphoric Acid (85%) and acetonitrile HPLC grade from Sigma Chemical Co. (St. Louis, MO, USA) were used for HPLC analysis. Deionized water was prepared using a Millipore water purification system (Barnstead, California, USA).

2.3. Extraction methods of *Garcinia cambogia*

A 700 WATT Sonic Dismembrator, 220V (FB-705, Fisher Scientific, Loughborough, UK) with a 1/2" probe with replaceable tip and 1/8" micro tip with amplitude of 50% was used. *G.cambogia* powder (10 g) was placed in a 250 mL beaker and extracted with 100 ml distilled water, acetone, methanol and ethanol with 10 minutes extraction time. The samples were submerged to a depth of 25 mm in sonicator probe. Meanwhile, the extractions with ultrasonic bath treatment were carried out in a WUC-D06H water-bath sonicator (Daihan Scientific, South Korea). *G.cambogia* powder (10 g) was sonicated in 100 mL of distilled water, acetone, methanol and ethanol in 10 min. The aqueous of extraction was filtered and concentrated by using rotary evaporator to 30 ml and treated with 120 ml ethanol to remove pectinaceous material, then centrifuged at 2000 rpm for 15 min. The supernatant is concentrated under reduced pressure to 25 ml and stored at 4°C until further HPLC analysis.

2.4. HPLC-PDA Analysis of Hydroxycitric Acid

Waters e2695 Alliance Separation Module liquid chromatography system comprising of vacuum degasser, quaternary pump, auto-sampler and Waters 2998 photodiode array detector (Millford, MA, USA) will be used. Empower software is used to control the HPLC system and data processing. Agilent Hi-Plex H, (7.7 × 300 mm, 8 µm) will be used as stationary phase. The isocratic system will be used for the separation is 0.005N Sulphuric Acid with the flow rate of 0.6 ml/min and detection wavelength is 210 nm. Sample injection volume is 20 µl. The total running time for HPLC analysis for (-)-HCA is 10 minutes. The chromatographic peaks of the analytes are confirmed by comparing their retention times and UV spectra with the reference standards.

3. RESULTS AND DISCUSSIONS

Solvents such as methanol, acetone and water have commonly been used for the extraction of phytochemical from Garcinia species [9,10,11]. In this research, distilled water, methanol, acetone and

ethanol were used for solvents extraction. In determining HCA content of G.cambogia rinds, the fine powder of dried material was prepared. The HCA content of the extract were estimated using High Performance Liquid Chromatography (HPLC). The effect of solvent extraction on the release of HCA content extracted by water, acetone, methanol and ethanol as a solvent is shown in Table 1. The yield percentage HCA content extract using probe sonicator by water, ethanol, acetone and methanol was 26.67, 18.59, 13.78 and 12.59 %w/w, respectively. Meanwhile the water-bath sonication gave slightly lower HCA yield than probe sonicator as shown in Table 1. The water extraction gave the highest percentage yield because HCA was dependent on the type of solvent used, its polarity and the solubility of organic acid in the extraction solvents. Jayaprakasha *et al*. (2003) studied the extractability of organic acids from the rinds of *G.pedunculata* with different solvents; water, ethanol and methanol. Among the 3 solvents, water extracted maximum organic acid, which may due to higher polarity of waters than other solvents

Table 1: Composition of HCA in different solvent extraction of dried rinds *Garcinia cambogia*

Extraction solvent	**Probe Sonicator**	**Water-bath Sonicator**
	LC Method (g/100g)	
Water	26.67	22.11
Methanol	12.59	11.36
Ethanol	18.58	15.89
Acetone	13.78	12.43

Thus, it was concluded that HCA was obtained highest with water extraction by using probe sonicator method of extraction. This condition gave the best result and it was taken as the best condition to perform further extraction.

The study for the effect of ultrasonic types was performed by maintaining the time and solid liquid ratio. The optimized direct ultrasonic probe conditions in giving the highest extracted HCA yields with respect to ultrasonic time and amplitude were compared to an indirect ultrasonic bath system. The result shows that HCA extracted with probe ultrasonic was significantly higher than the water-bath ultrasonic for all solvents. A probe sonicator makes direct contact with the sample. This can be an advantage since a more concentrated energy can be added to the sample meanwhile bath sonicators isolate the sample from the energy source in an energized bath of water. They require significantly more energy, are unpredictable, and often over-heat the sample [12]. The improvement of HCA extractions from *Garcinia cambogia* powder with direct ultrasonic pre-treatment over the indirect method of using bath has proven. The advantage of using the probe compared to the bath has also been reported in the extraction of oleuropein from olive fruit [13]. They revealed that the optimized ultrasonic probe conditions of the three extraction step of 20 min, 44 ◦C and pure methanol was more efficient in comparison to the ultrasonic bath and agitation, with up to 33% and 80% enhancement in the extraction of oleuropein from olive fruit.

4. CONCLUSION

The HCA content of *Garcinia cambogia* rind extracted with two types application of ultrasonic were compared in this study. The results clearly indicated that the values of extraction with probe sonicator were significantly higher when water was used as solvent extraction. This study showed that *Garcinia cambogia* extraction can benefit from UAE especially by reducing the extraction time. The use of water as a solvent for extraction can reduced environmental impact, inexpensive and environmentally benign but it is also non-flammable and nontoxic [14] and save for making foods and supplements.

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