The Influences of Extraction on the Quantity of Oxyresveratrol from *Artocarpus lakoocha* Roxb.

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Abstract: The aim of this study was to develop a process of extraction oxyresveratrol from *Artocarpus lakoocha* heartwood using a maceration method. The influences of types of solvent, a concentration of solvent, and time to extract on the yield of oxyresveratrol were studied. In addition, the antioxidant capacity of the extract was investigated. The quantitation of oxyresveratrol was measured by high-performance liquid chromatography (HPLC) analysis, and antioxidant activity was analyzed by 1,1'-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The results indicated that 70-percent ethanol solvent by volume for 6 hours could extract the optimum oxyresveratrol at 13.09 percent of the dry weight of *Artocarpus lakoocha*, and antioxidant efficiency had an IC_{50} of 47.70 μg/ml.

Key words: Antioxidants, *Artocarpus lakoocha* roxb., extraction, oxyresveratrol.

1. Introduction

Artocarpus lakoocha Roxb. (Moraceae), also known as the Ma-Haad, is a tropical tree widely distributed in the regions of South East, Southwest and South of Thailand. The heartwood of *Artocarpus lakoocha* has a brown color as shown in Figure 1. It is hard and termite resistant with a weight of about 640 kg/cu.m [1]. *Artocarpus lakoocha* is rich in flavonoids, triterpenoids, steroids, and stilbenoids. Also, the extract has a high content of oxyresveratrol (trans-2,4,3',5'-tetrahydroxystilbene) which is a derivative of resveratrol, with the molecular formula of $C_{14}H_{12}O_4$ and a molecular weight of 244.24. Therefore, this is used in medical and cosmetics [2]. Oxyresveratrol has also become known for its supported strong tyrosinase-inhibitory activity, and it is potentially used as a skin-whitening agent [3], [4]. It is also found to possess strong antioxidant [5]. These medicinal properties of the *Artocarpus lakoocha* indicate that plant material may become an important natural source.

Solvent extraction is a soluble-element process of the plant cell with a diffusion method using a solvent to separate from the plant [6]. The extraction of bioactive compounds from the plant is designed to remove the solute from the solid phase with solid-liquid extraction or leaching step. The leaching process can be explained by the theory of mass transfer in a system of substances that are present in different phases. Molecules of bioactive compounds are transferred from high to low concentrations in order to balance the

substance in the system.

Fig. 1. Heartwood of *Artocarpus lakoocha* Roxb. (Moraceae)

Extraction of bioactive compounds from plant matrices by three steps includes mass transfer, solubility, and desorption as shown in Fig. 2. The first steps, the mass transfer, includes external and internal mass transfer. For the external mass transfer, the solvent transfer from bulk solution through the layer of the liquid to the surface of the solid. This stage is called the convective diffusion, which is the transfer of mass with the motion of the medium while the driving force is derived from the concentration gradient between the solution in the bulk solution layer and the solid surface. For the internal mass transfer, the solvent diffusion transfers through the interior of the solid phase, this phenomenon is called the internal diffusion. The solvent diffuses into the cavity or pore of the solid phase, while the driving force is derived from the difference in concentrations.

Next steps, the extraction uses the property of solubility to transfer a solute from a solid phase to liquid phase. The solvent dissolves in the cavity of the solid phase to dissolve the solute whose solubility depends on the chemical properties of solvents and solutes.

And the last steps, the solute is transferred by diffusion from the internal particles to the surface of the solid phase, this phenomenon is called desorption.

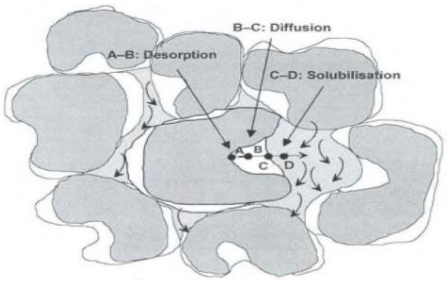

Fig. 2. Three-step solvent extraction of bioactive species from plant matrices.

So far, a few methods for extracting oxyresveratrol from *Artocarpus lakoocha* are mainly based on solvents. These methods take a long time to extract and use organic solvents in large quantities, which are often expensive and potentially harmful. Therefore, the optimal condition for extraction of *Artocarpus lakoocha* is highly desirable to be able to extract effectively to reduce the cost and the difficulty of extraction.

Thus, the aim of this study was to develop a process of extraction oxyresveratrol from *Artocarpus lakoocha* by low energy, and to assay the activity on antioxidation of extract.

2. Materials and Methods

2.1. Materials

Dried heartwood samples of *Artocarpus lakoocha* were obtained from the Varangporn (Chonburi, Thailand). Oxyresveratrol and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ethanol and ethyl acetate (HPLC Grade) were obtained from LabScan (Thailand).

2.2. Sample Preparation

The heartwood was washed with deionized water and then dried at 50°C for 48 hours to reduce the size of the heartwood. The powder was separated into the particle sizes of 850 and 150 micrometers by a sieve shaker as shown in Fig. 3.

Fig. 3. The heartwood powder of *Artocarpus lakoocha*: a) 850 and b) 150 micrometers

2.3. Solvent Extraction

The effects of extracting solvent were investigated for three solvents, i.e., deionized water, 99% (v/v) ethanol and 99.97 (v/v) ethyl acetate. One gram of *Artocarpus lakoocha* dried powder was extracted with 50 ml of each solvent at room temperature for 72 hours.

The effects of solvent concentration were investigated in the range of 60–80% by using selected solvent. One gram of dried powder was extracted with 10 ml of each solvent at 40°C for 6 hours.

The effects of extraction time were investigated for different duration time (4-8 hours) by using the optimal solvent and solvent concentration. One gram of dried powder was extracted with 10 ml of solvent at 40°C for each extraction time.

Each extraction was performed in triplicate. All samples were filtered with No.1 Whatman filter paper. The clear supernatant was collected and evaporated by a rotary evaporator under vacuum at 45°C. The concentration of oxyresveratrol was determined by using HPLC.

2.4. High Performance Liquid Chromatography Analysis

The concentration of oxyresveratrol in the solvent extract from *Artocarpus lakoocha* was measured by high-performance liquid chromatography (HPLC) as similar to the previous study [7]. The analysis was performed with Luna-Pak® C-18 (5 µm, 4.6 mm i.d. × 250 mm). The mobile phase was acetonitrile (CH_3CN) and 10-mM phosphoric acid (H_3PO_4) (35:65, v/v). The flow rate was 1.0 ml/min, and the effluent was monitored at 325 nm with a photodiode array detector. The purified oxyresveratrol described above was used as the standard.

2.5. DPPH Radical Scavenging Assay

The free-radical scavenging capacity of *Artocarpus lakoocha* heartwood extracts was analyzed by using a

method adapted from that described by Singhatong [2]. A volume of 100 µl of the extract was added to 0.3 mM of DPPH solution and mixed in 96-well plate. These samples were left to incubate in the dark for 30 minutes at room temperature, after which their absorbance at 517 nm was measured. Ethanol was used as the blank solution and ascorbic acid as a positive control. The scavenging activity of the DPPH radicals was calculated using the following formula:

$$DPPH\ scavenging\ activity(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where A_{sample} is the absorbance of the test sample, and A_{control} is the absorbance of the control.

After that, the inhibition curves were prepared, and IC_{50} values were obtained.

3. Results and Discussion

3.1. The Effects of Types of Solvent

Artocarpus lakoocha heartwood powder of 850 micrometers was extracted with three different solvents by the maceration method at the heartwood-to-solvent ratio (solid/liquid ratio) of 1:50 for 72 hours, the results were shown in Table 1. From the results, the ethanol was the suitable solvent to obtain the highest extraction yield and the highest concentration of oxyresveratrol due to its high polarity and the solubility of bio-active components. In addition, the crude extracts from ethanol and ethyl acetate are solid, clear, and yellowish brown, whereas the crude extracts from water is a solid yellow transparent as shown in Figure 4.

Table 1. The Effects of Solvent Type on the Yield and the Oxyresveratrol Content

Solvent	Extraction yield (%)	Oxyresveratrol (%)
Ethanol	13.17 ±0.76	5.51 ±0.88
Ethyl acetate	6.17 ±0.58	4.14 ±0.46
Water	1.94 ±0.31	0.42 ±0.22

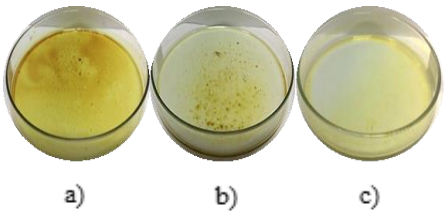

Fig. 4. Crude extracts obtained by the solvent extraction: a) ethanol, b) ethyl acetate, and c) water.

3.2. The Effects of Ethanol Concentration

The extraction of *Artocarpus lakoocha* heartwood powders of 150 micrometers at the heartwood-to-solvent ratio of 1:10 (solid/liquid ratio) were stirred at 40°C for 6 hours. The effects of the concentration of ethanol solvents were studied at 60, 70, and 80 percent by volume. As shown in Table 2, the content of oxyresveratrol was found to increase with increasing ethanol concentrations from 60 to 70 percent, while those of 70 and 80 percent of its were almost the same value. The amount of water in the

solvent of 70 percent increased the strength of the hydrogen bond of the hydroxyl group. It was attributed to that of the strength of the hydrogen bond in which the polarity of the solvent was higher and dissolved the important substances with the same polarity very well [8], [9]. However, the results of the extraction yield were differed owing to the extraction of other compounds as shown in Table 2.

Table 2. The Effects of Ethanol Concentration on the Yield and the Oxyresveratrol Content

Ethanol concentration (%)	Extraction yield (%)	Oxyresveratrol (%)
60	25.60 ±0.53	7.70 ±1.55
70	26.07 ±0.58	11.20 ±1.22
80	27.93 ±1.70	11.47 ±1.49

3.3. The Effects of Extraction Time

Artocarpus lakoocha heartwood powders of 150 micrometers were extracted by a maceration method using 70-percent ethanol as a solvent (solid/liquid ratio is 1:10). The solution which depends on the time of extraction (4, 6, and 8 hours) was stirred at 40°C. The results of the experiment were expressed in Table 3. From the results, the extraction yield at 6 hours was highest, while the amount of oxyresveratrol increased with an extraction time increased. So the extraction time of 6 hours is enough for the extraction of oxyresveratrol. This is because the time from 4 to 6 hours provided the increasing results to 1.55 percent. However, the extraction time from 6 to 8 hours increased only by 0.13 percent, because the concentration of solute in the solid phase was similar to that of solvent. Also, the time of interaction of solid phase and solvent was enough for the concentrations of both phases not significantly different [10].

Table 3. The Effects of Extraction Time on the Yield and the Oxyresveratrol Content

Time (hours)	Extraction yield (%)	Oxyresveratrol (%)
4	25.73 ±1.53	11.54 ±1.93
6	28.33 ±1.62	13.09 ±1.99
8	27.67 ±0.58	13.11 ±1.22

3.4. Antioxidant Activity

Artocarpus lakoocha heartwood contains a potent antioxidant, oxyresveratrol, which exhibits more than 50-percent ability to scavenge free radicals in all conditions. By demonstrating a correlation between free-radical inhibition (percent) and level concentration of the extract, it was found 50-percent inhibition (IC_{50}) to be 47.70 µg/ml.

4. Conclusion

The results revealed that the highest oxyresveratrol was obtained from extracting *Artocarpus lakoocha* heartwood by maceration in 70-percent ethanol at 40°C for 6 hours. The obvious advantages appeared in this step of our study were time and energy saving. In addition, this technique had a potential for industrial application in the medical and cosmetic industries.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

KJ designed and conducted the research, collected the data, analyzed and interpreted the data, including wrote the paper. CS and DC substantial contributed and designed the research, and revised the paper for important content. All authors had approved the final version.

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