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Eco-Friendly Catechin's Gambir Extraction Using an Ultrasonic Bath

Eco-Friendly Catechin's Gambir Extraction Using an Ultrasonic Bath

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Abstract. Gambir is an extract from the gambir plant (*Uncaria gambir* Roxb.) which has high economic value and potential for development. In Indonesia, the largest gambier-producing region is West Sumatra where traditional methods are still used for the processes of harvesting, steaming, pressing, clumping, draining, printing, and drying. Traditionally processed gambir products generally have a variety of catechins which need to be removed to improve purity. In this study, a range of treatments was trialed to do this using readily available equipment and no dangerous chemicals so they could be easily used by local farmers. It was expected that the appropriate treatment will result in higher quality gambir that can be sold at a higher price. The highest antioxidant activity (1.8940 mg/ml) and catechin levels (933.45 µg / mg) were obtained using the ultrasonic extraction process for 90 minutes. The highest polyphenol content was found in gambir without ultrasonic treatment and was 5.0776 mg/ml

1. Introduction

Gambir is an extract of the gambir plant (*Uncaria gambier Roxb.) of which Indonesia is the largest exporter worldwide. Much of this is produced* in the South Pesisir and Lima Puluh Kota districts of West Sumatra [1]. Gambir contains polyphenolsincludingtannins, catechin, epicatechin and epigalo catechin many of which are utilized in pharmaceutical, textile and food industries.

In West Sumatra, gambir is generally still processed in the traditional way. Gambir processing includes harvesting, steaming, pressing, agglomerating, draining, shaping and drying [2,3]. Traditional processed crude gambir generally contains 40-60% catechins. The catechin content of gambier is a determiner of quality. This is because the market for catechins is much greater than that for tannins due to this compound having more industrial applications. Pure catechins have a sweet taste, are crystalline and white to yellowish, while tannins taste astringent and are reddish-brown to black [4]. In order to have a high selling value on the international market, further handling is needed to increase the purity of catechins from crude gambier.

Catechin purification can be conducted using an extraction process using organic solvents and water which utilized the differences in the solubility between catechins and tannins in water. In the pure state,catechins are difficult to dissolve in cold water but easily dissolve in hot water, alcohol and ethyl acetate [5]. Crude gambir rinsed with 70oC water has been found to have a catechin content of 65-74% [4]. Catechins obtained by solvent extraction using methanol and ethyl acetate increase the purity of gambir catechins to 95% and 98%, respectively.

Although methanol and ethyl acetate give a much higher yield than water solvents, these chemicals have an impact on the environment, are more expensive and are hard for farmers to use in the field. For this reason, an optimization of water extraction could be both practical and environmentally friendly. Chemical solvents are toxic and not environmentally friendly, as for examples of these solvents

are methanol, dioxane, acetonitrile, acids, formaldehyde, and tetrahydrofuran [6]. While organic solvents can cause environmental problems water is unlikely to cause damage [7].

One way to optimize the process of extracting organic compounds in plants and seeds using organic solvents uses ultrasound and is known as Ultrasonic-assisted extraction (UAE). The cell wall of the material is broken down by ultrasonic vibrations so that the contents in it can come out easily saving time and solvents [8]. Ultrasonic waves are acoustic waves with frequencies greater than 16-20 kHz [9]. Ultrasonic extraction is non-destructive and non-invasive, so it can be easily adapted to various applications. It is faster and safer than thermal extraction or conventional extraction and increases the amount of crude yield. Ultrasonic can also reduce the operating temperature so it is suitable for the extraction of bioactive compounds that are not heat-resistant [10]. Ultrasonic-assisted extraction of supercritical extraction ginger has been found to increase yield 30% and reduce the extraction time [11].

Ultrasonic extraction with water solvent could be expected to produce a product with a high catechin content in a way that local farmers could reproduce. The sale of this improved product would increase their financial return. This study aims to determine the effect of extracting gambir catechin using an ultrasonic bath with water solvents on extraction time, levels of antioxidants, polyphenols, catechins, and epigallocatechingallate.

2. Methods and Materials

2.1. Raw Materials

Dry Gambir(*Uncaria gambir* Roxb.) from Tarusan, South Pesisir, West Sumatra was used.

2.2. Chemicals and Equipment

The chemicals used were Aquabides, Sigma brand catechin, epicatechin, and epigalocatechin gallate standards were used along with DPPH methanol, Na_2CO_3, Folin-Ciocalteu reagent, HPLC methanol, PA methanol, formic acid and water. The equipment used included a blender to break down dry gambier and Universal 320 R centrifuges, HPLC equipment, spectrophotometer, and oven for analysis.

2.3. Gambir Re-extraction with water

A modification by Ref.[4] was used for extraction. Crude gambir was reduced to powder and mixed with distilled water (1: 5), mixed in a vortex mixer, then ultrasonicated (ultrasonic water bath (280 W, 50/60 Hz, S 10H Elmasonic) supplied by Elma (Singem, Germany) for 30, 60 , 90, or 120 minutes at room temperature (approximately 30 °C). The mixture was filtered through a 100 mesh filter. The filtrate was left to stand for 24 hours. The precipitate obtained was repeatedly washed with water until a yellowish suspension was obtained. This was then centrifuged to re-settle out the precipitate which was dried in an oven at 40°C for 15 hours.

2.4. DPPH (Diphenyl Pycryl Hydrazyl) Radical Scavenging Activity

The antioxidant activity test was carried out by determining the levels of the radical compound DPPH (Diphenyl Pycryl Hydrazyl) added to the material. This test was carried out using a modification of the method described by Ref. [12]. 1 gram of sample was added to 10 ml of methanol (105 mg/l) and homogenized. The resulting mixture was diluted with methanol down to 1, 2, 3, 4, and 5 mg/l. Next, 2 ml of each diluted sample was placed with 1 ml of DPPH solution in methanol in a test tube. After incubating in a dark room for 30 minutes, spectrometer readings were carried out at a wavelength of 517 nm. DPPH radical scavenging activity was obtained by the equation below

DPPH radical scavenging activity = [1- A517 (sample) / A517 (blank)] x 100%

2.5. Total Phenol Content

The total phenol content of the sample was determined using the Folin Ciocalteu colorimetric method modified by Ref.[13]. 1 gram of sample was added to 10 ml of methanol and homogenized (105 mg/l). Then the mixture was diluted further with methanol to 5 mg/1. 1 ml of this was homogenized with 2 ml of distilled water and 1 ml Follin-Ciocalteu reagent for 5 minutes. Next 1 ml of Na_2CO_3 was added and the suspension incubated in a dark room for 2 hours at room temperature. Absorbance was measured at

725 nm. Phenol content was calculated using gallic acid as a standard (3,4,5-trihydroxy benzoic acid (C_6H_2 (OH_3) CO_2H)) and expressed as mg of Gallate Acid Equivalent (GAE) / 100 g dry weight.

2.6. HPLC for catechin and epigallocatechingallatecontent

2.6.1. Sample Preparation
The sample was dissolved with methanol (HPLC grade) solvent containing 1% formic acid to a concentration of 200 ppm and homogenized by ultrasonication for 5 minutes

2.6.2. Standard preparation
The standard was prepared by dissolving the standard in methanol HPLC: aquabidest (1: 1) and then adding 0.1% formic acid to the standard concentrations of catechins and epigallocatechingallateeach at 500 ppm.

2.7. Sample Analysis
Dried extract from mashed raw dried gambier that had been obtained after 30, 60, 90, or 120 minutes ultrasonication which had been dried was tested. The tests included antioxidant levels calculated as IC50 and polyphenols by spectrophotometer. Subsequently, an analysis of catechin and epigallocatechin content was carried out on 5 samples of gambier extract by HPLC. The HPLC method used for the analysis of catechin, and epigallocatechin gallate was as in Shimadzu Technical Note L373A. The catechins were determined by a high-pressure pump HPLC system, an autosampler, a column (Prominence HPLC 20A Shimadzu), and a reverse phase chromatography column (agilent Zorbax SB-C18 column) paired with a visible UV detector. The mobile phase A was aquabidest with a mixture of 0.2% phosphoric acid and 1% tetrahydrofuran. The mobile phase B was acetonitrile with 1% tetrahydrofuran. Separation was developed under a polarity gradient system of 5-25% B for 20 minutes with a flow rate of 0.8 ml / min with a wavelength of 230 nm.

3. Results and discussion

3.1. Raw Crude Gambir
IC50 analysis of 1, 2, 3, 4 and 5 ppm concentration samples showed the antioxidant activity of the original gambir was 1.939 mg / mL and the crude gambir polyphenol content in the 5 ppm concentration solution was 6.004 mg / ml solution.

The DPPH method was chosen to test for antioxidant compounds because it is simple, easy, fast, sensitive and does not require a large sample. DPPH is a free radical compound that can react with compounds that can donate hydrogen atoms changing the color of DPPH from purple to yellow [14]. Antioxidant compounds in the crude gambir donate hydrogen atomsinto DPPH reducing the intensity of the color purple. Crude gambir was shown to have an IC50 of 1.939 mg/mL indicating strong antioxidant activity. The raw crude gambir used is shown in Figure 1.

Antioxidant activity can be considered very high because low concentrations of 1, 2, 3, 4 and 5 ppm were used for IC50 testing. According to Ref.[15], if the IC50 value is less than 50 ppm, antioxidants are classified as strong antioxidants. According to Ref. [3], the antioxidant content in gambir is from polyphenols, catechins, epicatechins, caffeic acid and in testing antioxidant activity using DPPH, the optimal incubation time is 30 minutes. In this test, the incubation process was only carried out for 15 minutes.

Fig.1. Raw Crude Gambir

3.2. Re-extraction to produce a catechin-rich product

Before extracting, dry crude gambir was broken up in a blender to increase the surface area. Ultrasonication maximized the solubility of catechin and tannin compounds in water. Five different extraction times were used, 30 minutes, 60 minutes, 90 minutes, and 120 minutes. The separation of gambir from impurities such as sand was achieved, as is traditional, by the use of 100 mesh sieves. Deposition then separated out the catechins that are barely soluble in cold water from tannins that remained dissolved due to their many hydroxide functional groups [5].

The residue was then rinsed with cold water so that any remaining might be dissolved. Then the catechins were precipitated out using centrifugation to speed up the process. The precipitate was dried in an oven at 40^{o}C so that no color changes occurred.

3.3. Antioxidant Activity of the purified Extract

Antioxidants are electron donors for compounds that function as radicals or Reactive Oxygen Species (ROS) [16]. The antioxidant activity of phenol compounds is due to their ability to form phenoxide ions which can donate an electron to free radicals. The antioxidant phenol compounds (PhH) reacts with free radical (ROO •) to form ROOH and a radical phenol compound (Ph •) which is relatively unreactive but can react again with another free radical (ROO •) to form compounds that are not radicals [17].

DPPH is a free radical compound that is able to react with compounds that donate hydrogen atoms such as the antioxidant compounds (polyphenols) in gambir. As DPPH binds to this hydrogen a reduction in the intensity of the color purple occurs which can be measured by spectrophotometer at 517 nm. The results can be seen in Table 1.

Table 1. DDPH Scavenging Activity of the catechin-rich product extracted using Ultrasonication in water

Extraction Time (Minutes)	DDPH Scavenging Activity (%)				
	1 mg/mL	2 mg/mL	3 mg/mL	4 mg/mL	5 mg/mL
30	24.13043	49.30435	73.47826	88.69565	89.13043
60	23.47826	41.95652	67.60870	79.56522	88.69565
90	31.52174	66.95652	83.47826	85.43478	88.47826
120	27.38386	46.21027	70.41565	88.99756	89.24205

IC50 testing was successfully carried out at extract concentrations of 1 to 5 mg / L indicating very strong antioxidant activity. As the extraction time increased, the DPPH Radical Scavenger activity also increased. The longer contact between the sample and the water during the extraction process, the more antioxidants extracted [18]. However, after 120 minutes, antioxidant activity decreases, perhaps due to oxidation or hydrolysis of the antioxidant compounds. According to Re.[15], if the IC50 value is less than 50 ppm, the antioxidants in the ingredients are classified as strong antioxidants. The results of the

analysis of the antioxidant activity of gambir extraction ultrasonic methods with water solvents can be seen in Table 2.

Table 2. Antioxidant Activity of the catechin-rich product extracted with different durations of Ultrasonication

Extraction Time (Minutes)	Antioxidant Activity (mg/ml)± SD
30	2.6094 ± 0.7426[a]
60	2.6867 ± 0.4195[a]
90	1.8940 ± 0.6957[a]
120	2.2703 ± 0.1946[a]

The IC50 values are closely related to the length of ultrasonication when extracting the catechin-rich product using water as a solvent. The highest antioxidant activity was obtained when the ultrasonication was 90 minutes (1.8940 mg/ml). There were no significance difference among the treatment.That is, it takes 1.8940 mg/ml of the sample to inhibit 50% of the oxidation activity. This is stronger than the IC50 antioxidant activity of crude gambir which is 1.939 mg/ml.

3.4. Extract polyphenol levels

The Polyphenol Content of the extract can be seen in Table 3.

Table 3. Polyphenol Content of the catechin-rich productafter using Water as a solvent and Ultrasonication

Extraction Time (Minutes)	Polifenol content (mg/ml) ± SD
30	4.0862 ± 1.2496 [a]
60	3.5043 ± 1.280 [a]
90	5.0776 ± 0.5791 [a]
120	4.6681 ± 0.1829 [a]

SD: Standard Deviation(α<0.05)

In the 5 mg / L sample, the best polyphenol levels were obtained in the gambier extract which was processed for 90 minutes (5.0776 mg/ml). There were no significance difference among the treatment. Polyphenols are secondary metabolites of plants and include catechins and tannins. Tannins are complex phenolic compounds which have a molecular weight of 500-3000 and are colloidal in water and weakly acidic [3, 19]. The high solubility of tannins increases further when dissolved in hot water [19].

With prolonged rinsing with cold water, tannins can dissolve and be lost so that the tannin content in repeated extraction gambier samples is much lower than in crude gambier. Catechins are barely in cold water but may also be lost with prolonged water exposure at room temperature. This would explain why 90 minutes appears to be the optimal length of time for extraction of crude gambier using this method, optimising DPPH Radical Scavenging Activity, IC50 and total polyphenol content.

3.5. Catechin and Epigallocatechin Gallate levels in catechin-rich gambir product

These levels can be seen in Table 4. Epigallocatechin gallate was not detected in any sample. The highest levels of catechins were obtained after 90-minute processing (933.45 μg / mg). However, extraction time did not significantly affect gambir extract catechin levels. The yields of 75.7 - 93.3% were far higher than that measured in West Sumatran raw gambir extract by Ref.[16] who found Cubadak Gambir catechin content was 26%, Gambir Udang 25 %, and Riau Mancik Gambir 27% [3]. Again 90 minutes proved to be the optimal time for ultrasonication. This indicates that ultrasonication can improve

the extraction of the components in gambier. The advantage of ultrasonic-assisted extraction is the direct contact between solvents and solids achieved with ultrasonic waves [20].

Table 4. Catechin and Epigallocatechin Gallate levels in catechin-rich gambir product Purified using Water as a solvent and Ultrasonication

Extraction Time (Minutes)	Catechin Content (μg/mg)
30	847.05±12.4
60	756.88±15.8
90	933.45±10.7
120	872.1±12.9

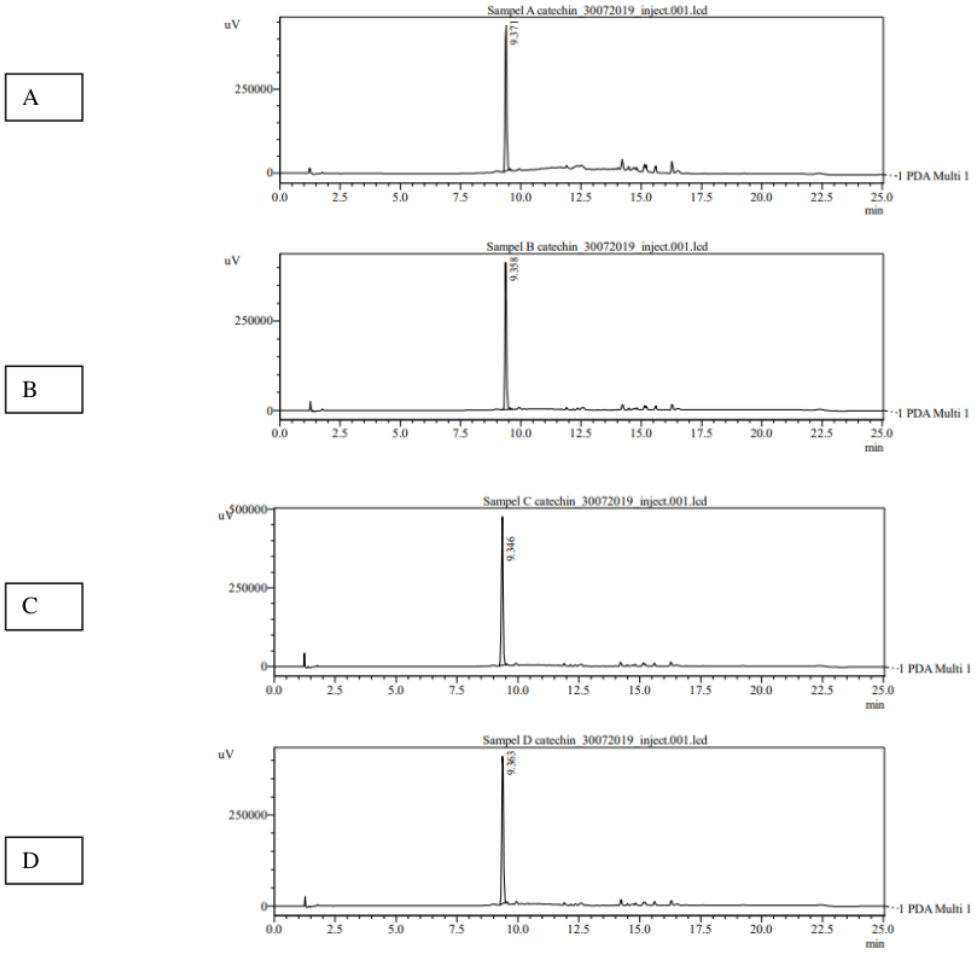

Fig.2. Catechin Levels incatechin-rich gambir productafter ultrasonication in water durations of (A) 30 minutes, (B) 60 minutes, (C) 90 minutes, (D) 120 minutes.

Ref. [21] found up to 87.14% of catechins could be extracted using a 60^{O}C maceration temperature over 6 hours using ethyl acetate 95%, as the solvent. But this present study acheived comparable results using water which is safer and environmentally friendly solvent.HPLC confirmed that the catechin-rich gambier extract contained no detectable epigallocatechin content.

4. Conclusion

Crude gambier can be successfully purified in water with the use of ultrasonication. The optimal sonication time for this was found to be 90 minutes. The resulting diluted extract had strong antioxidant properties LC50 1.8940 mg / ml, catechin and polyphenol content are 933.45µg / mg, and 5.0776 mg / ml respectively.

This method avoids the ongoing use of dangerous and expensive chemicals. It also removes the need to separate chemicals out of the final product so making the process more efficient in time and labor. However, it requires the use of an ultrasonic bath. Mass production of large ultrasonic baths, which could be powered by micropower generation (solar or hydro) for use by farmer cooperatives would allow the farmers to significantly increase the quality and prices they get for their product while avoiding environmental damage.

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