

The Extract Standardization Test and Physical Properties Evaluation of *Uncaria gambier* Roxb Extract Catechine Capsules

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Article info	Abstract
<p>History Submission: 03-09-2023 Review: 23-03-2024 Accepted: 12-11-2024</p> <p>*Email: dinamelia11@gmail.com</p> <p>DOI: 10.33096/jffi.v11i3.1048</p> <p>Keywords: catechins; extract standardization test; capsule physical evaluation; assay; antioxidant test</p>	<p><i>Society does not really know that the catechin extract contained in <i>Uncaria gambier</i> Roxb can be used as an anti-inflammatory and antioxidant. The catechins in gambier plant are very complex. They are composed of extracts of catechins (C), epicatechins (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and galocatechin (GC). Thus, the use of this medicinal plants with anti-inflammatory and antioxidant properties needs to be developed as an alternative treatment. In addition, this species of plant has also important effectiveness and relatively smaller side effects. In an effort to increase efficiency and maximize the therapeutic effects of gambier as an anti-inflammatory and antioxidant, the authors need to develop an innovate pharmaceutical product from this plant. One of the selected pharmaceutical preparations is the capsule since it can conceal the taste and the unpleasant smell; besides, it is also easy to consume. The maceration method was carried out in the catechin extraction process. After obtaining pure catechin extracts, the authors tested catechin levels using the HPLC method and antioxidant levels using the DPPH method. Next, the authors performed extract standardization tests and evaluated the physical properties of the capsule preparations. The test results showed catechin levels of 98.697% and IC₅₀ antioxidant levels of 8.54 µg/dl. The results of the standardized extract and capsule evaluation tests indicated that they met the applicable requirements.</i></p>

I. Introduction

Inflammation is a local physiological response caused by injury or tissue damage, which functions to destroy, reduce or eliminate an injuring agent or injured tissue. Symptoms of an inflammatory response include rubor (redness), calor (heat), dolor (pain), turgor (swelling). Treatment that has been carried out so far to slow down or limit the process of tissue damage that occurs in inflammatory areas uses chemical-based drugs, namely non-steroidal anti-inflammatory drugs (NSAIDs) which have side effects on the digestive tract including causing stomach ulcers.

This gambier plant really needs a lot of sunlight. Areas with lots of gambier plants in Indonesia, especially in West Sumatra, Indragiri, West Kalimantan and the Riau Islands (Aditya and Ariyanti, 2016). The Gambier plant contains catechin extracts which function as anti-inflammatories. Apart from being an anti-inflammatory, the gambier plant also has activity as an antioxidant (Damanik, Surbakti and Hasibuan, 2014). Antioxidants are compounds that can prevent and slow down damage caused by free radicals by inhibiting oxidative mechanisms. Catechins exert antioxidant effects through rapid

electron transfer to free radical-induced radical sites on DNA, metal chelate, and reduce lipid peroxidation in vivo (Watanabe, 1998). Catechins are metabolic compounds that are naturally produced by plants and are included in the flavonoid class. Catechins have antioxidant, anti-inflammatory, antibacterial, antiviral and antitumor activities. Catechins in gambier are very complex compounds, composed of extracts of catechins (C), epicatechins (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and galocatechin (GC). The catechin isolates contained in gambier are around 73.3%, while the catechins in tea are around 30% -40% (Miksusanti et al., 2019). Catechin isolates are active compounds derived from the Gambier plant. The catechin isolates were obtained from processed ingredients from the gambier plant. Gambier will then be extracted and its active substance will be taken, namely catechin isolates (Putri, 2010).

Antioxidants can prevent diseases associated with free radicals such as cancer, cardiovascular and premature aging. The production of antioxidants in the human body occurs naturally to balance the production of free radicals. Antioxidants function as a defense system against



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free radicals, but the increased production of free radicals that are formed due to stress, radiation, ultraviolet, air pollution, and the environment causes the defense system to be inadequate, so additional antioxidants are needed from outside. Antioxidants outside the body can be obtained in synthetic and natural forms. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) can effectively inhibit oxidation. However, synthetic antioxidants are limited by government regulations, because if their use exceeds the limit it can actually cause toxins in the body and are carcinogenic, so safe natural antioxidants are needed. One of the plants that have natural antioxidants is gambier. Gambier is the result of extraction from the leaves of the gambier plant (*Uncaria gambier* Roxb) which contains polyphenolic compounds (Aditya and Ariyanti, 2016). The polyphenolic compounds contained in this Gambier extract are catechins which act as anti-inflammatory and antioxidant compounds.

The use of catechin extracts in the gambier plant (*Uncaria gambier* Roxb) in the community as an anti-inflammatory and antioxidant has not been widely known and applied by the community. Therefore the use of medicinal plants with anti-inflammatory and antioxidant properties needs to be done to find alternative treatments that have great effectiveness and relatively smaller side effects. So far, people still consume gambier plants traditionally. It is feared that the consumption of catechins in the traditional way will cause limitations, namely an unpleasant taste and a distinctive odor from natural ingredients, boiled water for a long time can be damaged by microorganisms, and preparation takes a long time, so as to increase efficiency and maximize therapeutic effects. catechins as anti-inflammatory and antioxidants, it is necessary to innovate in the development of pharmaceutical products.

One of the selected pharmaceutical preparations is the capsule because it can cover unpleasant tastes and odors, is easy to consume, easy to prepare and the medicinal ingredients are protected from external influences (light, humidity). The catechin extract that has been extracted from the gambier plant is then formulated into capsule preparations. The manufacture of pharmaceutical preparations in capsule form aims to make it easier for people to consume catechins. The extracts and catechin capsules were then tested including standardized extract tests, ka extract content tests.

II. Research Method

II.1 Tools and Materials

The tools used are, porcelain cup, desiccator, water bath, analytical balance, beaker glass, measuring cup, disintegration tester, test tube, measuring cup, stirring rod, oven and

refrigerator, moisture balance, furnace. The materials used in this study were catechin extracts from the gambier plant and gambier plant catechin extract capsules.

II.2 Research Procedure

II.2.1 The Preparation of Raw Material

The main source of catechins used in this study was gambier (*Uncaria gambier* Roxb) in cylindrical or cubic form obtained from the Pakpak Bharat area, North Sumatra. The material is ground and then mashed with a machine powder. This aims to increase the surface area so that it is easier to extract. After that, the flour is stored in an airtight container to keep it dry.

II.2.2 Preparation of Gambier Plant Catechin Extract and Standardization Test of The Extract

The solid extract of Gambier was powdered and then the catechin extract contained in it was taken by maceration using ethyl acetate and water as a solvent. Maceration was carried out by soaking 25 kilograms of gambier powder with a ratio of gambier: ethyl acetate, namely 1:10. This maceration process usually takes place for 24 hours, where the ethyl acetate solvent will be heated to a temperature of 50°C and rotated/stirred for about 2 hours so that it is extracted properly. After maceration, a concentrated yellow extract solution was obtained, then evaporated/concentrated. In the evaporation process, approximately 80% of the ethyl acetate solvent is recovered. After that, separation was carried out using a water solvent to separate the catechins and tannins in the gambier extract. Water solvents are used because tannins dissolve in water. After the formation of two phases (catechin phase and tannin phase), the tannin phase is then removed and separated by pressing with a mechanical press, this process can be done repeatedly. The addition of water in the washing process is adjusted to the raw material, the addition is carried out until it is felt to be quite separate. Then, the catechin phase will be given water and stirred before the catechin phase hardens to make it easier to remove the catechin extract from the mechanical press. If you don't have a mechanical press, you can also use a vacuum filter. The catechin extract obtained was then dried in an oven at 50°C for 2 days to remove the water content. After drying, the catechin extract will be in the form of plates and then crushed using a blender.

Furthermore, the catechin extract was tested for standardization of the extract which included organoleptic test, microbiological test, heavy metal content, flow property test, test for catechin compound levels and antioxidant levels, compressibility index, flow time test, flow rate, test for non-specific parameters and specific parameters.

II.2.3 Determination of Catechin Levels by HPLC Method

The mobile phase used was acetonitrile:methanol: H₃PO₄ 0.05% (A) and a buffer solution of 20mM NaH₂PO₄ pH 2.5 (B). Standard catechins were prepared with a stock solution of 6500 ppm, after which a mixture of the three standard solutions was made to 200 ppm. After that, serial dilutions were made with concentrations of 2, 4, 8, 16, 32 and 64 ppm. Determination of sample catechin levels was carried out by dissolving 1 mg of gambir sample with 1000 µL of aquabides as the mobile phase (Andreas et al., 2019).

II.2.4 Capsul Dosage Formulation

After obtaining the catechin extract, the dry catechin powder/granule is then crushed into smaller particles. 500 mg of the extract was weighed and then put into the capsule and evaluated for the physical properties of the capsule which included organoleptic tests, capsule weight uniformity, hygroscopicity, disintegration time tests, and accelerated stability tests.

II.2.5 Catechin Antioxidant Activity Testing

A total of 4 mg of sample extract was weighed in a sample bottle, then dissolved with methanol (4 mL), concentration of 1000 µg/mL. The solution has been diluted into 200 µg/mL, 100 µg/mL, 50 µg/mL, 10 µg/mL. was then put into a test tube and shaken with a vortex and left for 30 minutes. Next, the absorption was measured at a wavelength of 515 nm. If each concentration tested has antioxidant activity, the DPPH radical which is dark purple will be reduced to a non-radical form which is yellow in color. Free radical scavenging activity is expressed as a percentage of inhibition. The percentage of DPPH radical scavenging during incubation was calculated using the equation: % DPPH radical scavenging = (Control Abs – Sample Abs)/Control Abs] x 100%.

III. Results and Discussion

III.1 Results of Catechin Isolation from Gambir

Isolation of catechins from 25 kg of gambir plants obtained from Pakpak Bharat, North Sumatra was extracted using the maceration method. Maceration was carried out using ethyl acetate solvent with a ratio of 1:10. The evaporation process was carried out at a

temperature of 50°C and continued with the filtration and washing process and drying using an oven at a temperature of 50°C. The catechins obtained in this isolation were 12.5 kg with a yield value of 50%.

III.2 Results of Determination of Antioxidant Levels and Tests of Gambier Plant Catechins

The results of the extraction process of the gambier plant are dry extracts of catechins. Furthermore, testing for catechin levels is carried out using the HPLC method and antioxidant levels with the DPPH method. After testing, the catechin level was 98.697% and the IC₅₀ antioxidant level was 8.54 µg/dl. The antioxidant activity of plants is strongly influenced by the extraction solvent, this is because various types of antioxidant compounds have varying chemical characteristics and polarity (Sultana, Anwar and Ashraf, 2009). Pérez-Jiménez and Saura Calixto (2006) reported that the type and polarity of the solvent can affect the mechanism of hydrogen atom transfer and single electron transfer which determine the properties of antioxidants. Testing of antioxidant activity was carried out using the DPPH radical (2,2 diphenyl-1-picrylhydrazyl). The parameter of the DPPH method is the value of 50% inhibition concentration (IC₅₀) or a concentration that can reduce free radical activity by 50% (Widyasanti, Rohdiana and Ekatama, 2016). Lower IC₅₀ value indicates higher antioxidant activity (Pérez-Jiménez and Saura-Calixto, 2006).

III.3 Standardization Test Results for Non-Specific Parameters of Gambier Plant Catechins

Evaluation of non-specific parameters of gambier plant catechin extract was carried out by organoleptic tests and phytochemical tests. Organoleptic tests were carried out by observing the shape, color, and smell of the resulting viscous extract. In the organoleptic test of the gambier plant catechin extract, the results obtained were in the form of a powder extract, bright yellow in color, slightly sour taste, and had a characteristic gambier odor. The results obtained are in accordance with those stated in the Indonesian Herbal Pharmacopoeia (Kemenkes RI, 2017).

Table 1. Organoleptic test of gambier plant catechin extract

No.	Organoleptic test	Results	
		Day 1	Day 14
1	Form	Powder	Powder
2	Color	Bright yellow	Bright yellow
3	Aroma	Typical smell of gambier	Typical smell of gambier
4	Taste	Taste Slightly sour	Taste Slightly sour

The phytochemical test was carried out by looking at the content of flavonoids, alkaloids, tannins and saponins in the gambier plant catechin extract. Testing for alkaloid compounds was carried out by reacting the diluted extract with Mayer's reagent, Dragendorff's reagent, and Boucardart's reagent. The positive reaction of alkaloid testing with Mayer's reagent was shown in all types of solvent treatment with the formation of a white precipitate. Meanwhile, in the Dragendorff reagent a positive reaction with the formation of a light brown to yellow precipitate was shown in the treatment of distilled water, acetone, ethanol and methanol. The formation of precipitates occurs due

to the formation of complex compounds from the reaction of alkaloid compounds with K^+ metal ions in each reagent used (Mamahit, Fatimawali and Jayanti, 2023).

The positive results of the flavonoid test with the occurrence of red, yellow or orange colors in the sample after the addition of Mg and HCl powder were shown in all types of solvent treatments. Mg and HCl metal powders function to reduce the benzopiron nucleus found in the flavonoid structure and form red or orange flavilium salts (Prayoga, Nocianitri and Puspawati, 2019).

Table 2. Phytochemical test of gambier plant catechin extract

No	Fitokimia	Test results (- / +)	Information
1	Alkaloid		
	Meyer	+	A white precipitate formed
	Boucardart	+	A white precipitate formed
2	Flavonoid	+	A brown ring is formed
		+	A brick red color change occurred
3	Tanin	+	Blackish blue color formed
4	Saponin	+	

The tannin test was carried out by weighing 0.5 grams of extract and adding 10 ml of distilled water, then filtering it. Furthermore, the filtrate obtained was taken as much as 2 ml and then added 2 drops of 1% $FeCl_3$ reagent. Formation of black or blue color indicates the presence of tannins. This is in accordance with the results of research that has been done, where the results of the tannin test with 1% $FeCl_3$ reagent showed positive results which were indicated by the formation of a black-green color. The tannins contained in the extract will react with Fe^{3+} ions to form complex compounds (Hasibuan, Hedrianto and Purba, 2021).

The saponin test was carried out by weighing 0.5 grams of extract and adding 10 ml of distilled water which was heated then cooled and

then shaken vigorously for 10 seconds until foam formed. Then 1 drop of HCl 2N was added to observe the foam resistance. The presence of saponins was indicated by the formation of foam as high as 1-10 cm for no less than 10 minutes, and the addition of HCl 2N the foam did not disappear. The results of the saponin test that has been carried out show that the catechin leaf extract positively contains saponins which is characterized by the formation of a stable froth. The resulting froth is due to the saponin compound having a hydrophilic (polar) group which bonds with water while the hydrophobic (nonpolar) group will bind with air. The addition of HCl 2N aims to increase polarity so that the hydrophilic groups will bind more stably and the foam that is formed becomes stable (Tandi et al., 2020).

III.4 Standardization Test Results for Specific Parameters of Gambier Leaf Extract

Table 3. Standardization test results for specific parameters of gambier leaf extract

No	Evaluation Test	Results	Requirements
1	Moisture content	5.45%	<14 %
2	Ash Content	0.26%	<0.5 %
3	Heavy metals		
	Pb (Lead)	Below 0.0134	
	As (Arsenic)	Below 0.001	

Determination of water content aims to determine the percentage of water content in the material after drying or thickening through appropriate methods such as titration, distillation or gravimetry (Depkes RI, 2000). Test the water content in this study using a moisture balance tool that functions to determine the moisture content of the extract. The results of the Evaluation Test stated that the water content in the catechin extract of the Gambier plant met the requirements. The result of the moisture test was 5.45% so that it complied with the requirements of the Herbal Pharmacopoeia which required that the extract contain a water content of <14%. The greater the percentage of water content in a material, the easier it is for an extract to suffer damage and decay caused by microbial growth. High water content can also cause decomposition of active compounds in the extract due to the activity of enzymatic reactions. Therefore, the water content determines the quality and stability of an extract as well as the formation of an extract preparation (Aziz Saifudin, Rahayu and Teruna, 2011).

Ash content is a parameter that provides an overview of the internal and external mineral content from the initial process to the formation of the extract. At this stage, the extract is heated until the organic compounds and their derivatives are destroyed and evaporate until only mineral and inorganic elements are left (Maryam, Taebe and Toding, 2020). The results of testing the ash content of the gambier plant catechin extract were 3.03%. The ash content produced in the gambier plant catechin extract complies with the quality requirements, which is less than 5% (Depkes RI, 2000). The ash content should have a small value because this parameter indicates the presence of heavy metal contamination that is resistant to high temperatures (Maryam, Taebe and Toding, 2020).

Arsenic is a form of heavy metal which is divided into two forms, namely the reduced form which occurs under anaerobic conditions called arsenite and the oxidized form which occurs under aerobic conditions which is called arsenate (Hazimah and Triwuri, 2018). A person

experiencing arsenic poisoning shows signs of severe inflammation of the stomach and intestines, starting with a burning feeling in the throat, difficulty swallowing and severe stomach pain, these symptoms are followed by nausea, vomiting, to acute diarrhea which causes the stool to mix with water and mucus (WHO, 2022). Lead is a xenobiotic substance that is foreign to the body which can cause various health problems (Wallach, 2007). The heavy metal lead can affect the function of the hematopoietic, neurological, endocrine, kidney, gastrointestinal, hematological, and reproductive systems. In children, lead reduces the level of intelligence, growth and hearing, causes anemia and can cause attention deficit disorder and behavior disorders. In high exposure cases it can cause severe brain damage or death. Young children are particularly vulnerable to lead poisoning. Their central nervous system is still at a developing stage causing them to absorb lead from their environment compared to adults. In the metal contamination test in the catechin extract of the gambier plant, it showed that Pb (Lead) metal was below 0.0134 and As (Arsenic) metal. The maximum limits for Pb and As metal contamination are respectively ≤ 10 mg/kg extract and ≤ 0.3 mg/kg (BPOM, 2022). Based on these provisions, the contamination of Cd and Pb metals in the extract does not exceed the maximum limit value. The presence of heavy metal content in extracts can come from various sources such as soil conditions where the material grows, the process of washing the material with water, the extraction equipment used, fertilizers and pesticides, smoke from burning garbage and motorized vehicles, and industrial waste whose process is not perfect (Aziz Saifudin, Rahayu and Teruna, 2011).

Table 4. Microbiological evaluation test results for gambier catechin extract

No	Preparation Evaluation Test	Unit Result	Unit Result
1	Total Plate Number (ALT)	7.0x10 ¹ Colonies/g	7.0x10 ¹ Colonies/g
2	<i>E. Coli</i>	Below 3.0** MPN/g	Below 3.0** MPN/g
3	Yeast Mold Number (AKK)	3.3x10 ² Colonies/g	3.3x10 ² Colonies/g
4	<i>Salmonella aeruginosa</i>	Negative Colony/25g	Negative Colony/25g
5	<i>Staphylococcus aureus</i>	Under 10* Colonies/g	Under 10* Colonies/g
6	<i>Pseudomonas aeruginosa</i>	Under 10* Colonies/g	Under 10* Colonies/g

This test is one of the mandatory tests from BPOM. Microbiological testing includes testing for Total Plate Count (ALT), Yeast Mold Number (AKK) and testing for several bacteria. The aim is to provide assurance that the extract may not contain pathogenic and non-pathogenic microbes and fungi that exceed the specified limit because it affects the stability of the extract and its toxicity to health. The total plate number is a quantitative test to determine the number of microbes present in a sample. In this study, the ALT test was used, which is a test based on the growth of aerobic mesophyll bacteria colonies after the extract was inoculated on agar plate media by pouring and incubating at the appropriate temperature (Depkes RI, 2000). ALT can be used as an indicator of product hygiene, environmental microbial analysis of finished products, process monitoring indicators and can be used as a basis for suspicion of whether or not a product is accepted based on its microbiological quality. While testing the Yeast Number (AKK) in principle, calculates fungal contamination so that it does not exceed the set limits. Yeasts are a group of microscopic unicellular fungi that can cause pathogens in humans, while molds are multicellular fungi that have filaments that can also be toxic (Segal-

kischinevzky *et al.*, 2022). The total plate number of the extract was 7.0×10^1 colonies/g while the total fungal and mold contamination was 3.3×10^2 . The quality of a traditional medicinal ingredient does not exceed the maximum limit for microbial and mold/yeast contamination, respectively, namely $\leq 10^4$ colonies/g and $\leq 10^3$ colonies/g (BPOM, 2019). From these limits, it shows that the results of the determination of ALT and AKK in catechin extracts are below the specified requirements. Bacterial and mold contamination parameters determine the microbiological presence of pathogenic microbes and fungi. Possible bacteria that can damage the extract and cause health problems include *Salmonella typhi*, *Escherichia coli*, *Bacillus substilis*, *Aspergillus flavus*, and *Staphylococcus aureus*. Therefore it is necessary to extract raw materials, store raw materials and product packaging to minimize the presence of these bacteria (Saifudin et al, 2011). In this study the results of the *Salmonella aeruginosa* bacteria contamination test showed negative results, and the results of the *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria contamination tests showed results below 10^* colonies/gram respectively. Contamination by *E. Coli* bacteria below 3.0^{**} colonies/gram.

III.5 Evaluation Test Result for the Physical Properties of Gambier Plant Catechin Powder/Granule Extract

Table 5. Results of evaluation of the physical properties of gambier plant catechin powder/granule extract

No	Preparation Evaluation Test	Condition Results	Condition Results
1	Flow Time	1.06 sec < 10 sec	1.06 sec < 10 sec
2	Flow rate	23.5 g/sec > 10 g/sec	23.5 g/sec > 10 g/sec
3	Rest Corner	$6.8^\circ < 25^\circ$	$6.8^\circ < 25^\circ$
4	Compressibility Index	12.06 % < 20%	12.06 % < 20%

Another parameter to assess the quality of the granules produced is the flow properties of the granules. Flow time is the time required to flow a number of granules in a device. Flow rate is affected by shape, size, surface condition, granule moisture and addition of lubricant. Flow time is influenced by shape, size, surface condition, granule moisture and addition of lubricant if the granule has good flow properties. The granule mixture is said to have good flow properties if the flow rate is not less than 10 g/sec or 100 g granules has a flow time of not more than 10 seconds. The granule used in this test is 25 g so that a good granule fulfills the requirements if the flow time is less than 2.5 seconds or the flow rate is not less than 10 g/sec. Flow properties can be measured by the direct method (flow time test) and the indirect method (default angle test and setting index).

The angle of repose is the fixed angle that occurs between the heap of cone-shaped particles and the horizontal plane. The size of the angle of

repose is influenced by the shape, size and humidity of the granules. If the angle of repose is less than 25° it usually indicates that the material can flow freely, if the angle is greater than or equal to 40° it usually means that the flow is not good.

Tapping is a decrease in the volume of a number of granules or powders due to tapping and vibration. Granules with a compressibility index of less than 20% show good flow properties. The index is determined by observing the change in volume before and after the measurement. The index determination is carried out by placing 25 grams of granules in a measuring cup, then recording the initial volume of the measuring cup and then tapping it 100 times until the granule volume is constant. %.

The results of the evaluation of the flow properties of the granules stated that all tests met the specified requirements. The flow properties are affected by the size and shape of the particles; larger and rounder sizes indicate better flow. The

maximum flow velocity is reached after the flow decreases when the particle size approaches the hole size. Sometimes, poor flow can be caused by the presence of damp conditions.

III.6 Catechin Capsule Making Process from Gambir Leaves

Gambir leaves used come from the Pakpak Bharat area, North Sumatra, the leaf yield can reach 50-60% to get more than 90% catechins. The process of extracting gambir is still done traditionally. Gambir leaves are pressed while heated, then the sap or extract of gambir leaves is obtained which is then molded into various shapes, some are round or tube-shaped and many other shapes. After that, it is dried in the yard with the help of the sun like the usual drying process. However, because it is still done traditionally, sometimes in the dry extract of gambir there are impurities which are indicated by the color of the dry extract which is rather dark. This process is carried out in the area producing gambir leaves and sent or sold in the form of a solidified extract.

The solid extract of gambir is then powdered and then the catechin extract contained in it is taken by maceration using ethyl acetate and water solvents. Maceration is carried out in a tube with a capacity of 25 kilograms of gambir powder with a ratio of gambir: ethyl acetate of 1:10. This maceration process usually occurs for 24 hours, where the ethyl acetate solvent will be heated to a temperature of 50°C and rotated/stirred for approximately 2 hours to be properly extracted. After maceration, a thick yellow extract solution is obtained, then evaporated/concentrated. In the

evaporation process, approximately 80% of the ethyl acetate solvent is recovered. After that, separation is carried out using a water solvent to separate the catechine and tannin in the gambir extract. Water solvent is used because tannin is soluble in water. After two phases are formed (catechin phase and tannin phase), the tannin phase is then removed and separated by pressing with a mechanical press, this process can be repeated. In this process, washing or separating catechin from tannin, the addition of water is adjusted to the raw materials, the addition is done until it is considered separate enough. Then, the catechin phase will be given water and stirred before the catechin phase hardens to facilitate the removal of the catechin extract from the mechanical press. If you do not have a mechanical press tool, you can also use a vacuum filter. The catechin extract obtained is then dried in an oven at a temperature of 50 °C for 2 days to remove water content. After drying, the catechin extract will be in the form of plates and then powdered using a blender. Then the extract is analyzed for catechin content using HPLC and IC50 as well. Each capsule production has a different catechin content, but the difference is not too great, the catechin content depends on the raw materials obtained.

III.7 Evaluation of Gambier Leaf Extract Capsules

The capsules that are ready are then subjected to evaluation tests to ascertain how effective the capsules are. The evaluation tests carried out were organoleptic tests, weight uniformity tests, and disintegration time tests.

Tabel 6. Evaluation test results for gambier plant catechin extract capsules

No	Preparation Evaluation Test	Results	Condition
1	Organoleptic	As per requirements	Clean, Dry, no particles attached to the shell capsule
2	Weight Uniformity	14.43%	< 10 %
3	Ruined Time	5.85 minutes	< 15 minutes

The capsules that are ready are then subjected to evaluation tests to ascertain how effective the capsules are. Evaluation tests carried out were organoleptic tests, weight uniformity tests, and disintegration time tests. Organoleptically, the results meet the requirements, namely the capsule is clean, dry and there are no particles attached to the shell. Based on the requirements of the Indonesian Pharmacopoeia edition III that capsules with an average weight of more than 120 mg may not have a difference in the percentage of the content weight of each capsule to the average weight of the capsule contents of more than 7.5% and 15%. Based on the weighing of the capsules for the weight uniformity test, it showed that nothing deviated more than the requirements. This shows that the formula meets the criteria for weight uniformity. Capsules can provide a therapeutic

effect if they are first crushed into smaller particles, so that the contents of the capsule can be absorbed in the digestive tract.

According to the Indonesian Pharmacopoeia Edition III, the disintegration time test requirement is under 15 minutes. The results of the evaluation test show that the capsule disintegration time is 5.85 minutes and meets the requirements of the capsule disintegration time test, which is less than 15 minutes.

IV. Conclusions

The results of extract standardization tests have met the specified requirements. The results of HPLC measurements showed catechin levels of 98.697%. Antioxidant activity testing using the DPPH method (2,2 Diphenyl -1-Pikrylhidrazyl) resulted in an IC50 of 8.54 µg/dl, categorized as a

strong antioxidant. The results of the capsule evaluation showed that organoleptically, weight uniformity and disintegration time met the requirements.

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