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Standardization, Antioxidant, and Antimicrobial Activity Test of Ethanol Extract from Andiroba Bark (*Carapa guianensis*)

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Abstract
Most degenerative diseases are linked to excess free radicals generated by the body's metabolism or exposure to external factors. Antioxidants are used to help neutralize free radicals and treat bacterial infections. Andiroba bark (*Carapa guianensis*) is one of the plants used empirically by the people of the Province of Jeneponto to treat various diseases. This study aims to determine whether the ethanol extract of andiroba bark meets the standardization requirements and exhibits antioxidant activity, and to identify its ability to inhibit the growth of *Staphylococcus aureus, Propionibacterium acnes, Staphylococcus epidermidis,* and *Escherichia coli*. The results of the research showed that the results of specific and non-specific standardization of the ethanol extract from andiroba bark met the specified requirements, the ethanol extract had an antioxidant activity with an IC_{50} value of 15.985 µg/mL, which was included in the group of very strong antioxidants and could inhibit the growth of *Staphylococcus aureus, Propionibacterium acnes, Staphylococcus epidermidis,* and *Escherichia coli* bacteria at concentrations of 0.01 g/mL, 0.05 g/mL, and 0.1 g/mL. Overall, the ethanol extract from andiroba bark meets the standardization requirements and exhibits antibacterial activity.

Keywords: antibacterial, antioxidant, *Carapa guianensis*, standardization

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Standardisasi, Aktivitas Antioksidan, dan Antibakteri dari Ekstrak Kulit Batang Andiroba (*Carapa guianensis*)

Abstrak
Sebagian besar penyakit degeneratif berkaitan dengan kelebihan radikal bebas yang dihasilkan dari metabolism tubuh maupun paparan faktor eksternal. Antioksidan digunakan untuk membantu menetralisir radikal bebas dan mengatasi infeksi bakteri. Kulit batang andiroba (*Carapa guianensis*) merupakan salah satu tanaman yang dimanfaatkan secara empiris oleh masyarakat di Provinsi Jeneponto untuk mengobati berbagai penyakit. Penelitian ini bertujuan untuk mengetahui apakah ekstrak etanol kulit batang andiroba memenuhi standarisasi, aktivitas antioksidan, dan mengidentifikasi kemampuan ekstrak etanol dalam menghambat pertumbuhan bakteri *Staphylococcus aureus, Propionibacterium acnes, Staphylococcus Epidermidis,* dan *Escherichia coli.* Hasil penelitian menunjukkan bahwa hasil standarisasi spesifik dan non spesifik ekstrak etanol kulit batang andiroba memenuhi syarat yang ditentukan, ekstrak etanol mempunyai aktivitas antioksidan dengan nilai IC_{50} sebesar 15,985 µg/mL termasuk dalam kelompok antioksidan sangat kuat dan dapat menghambat pertumbuhan bakteri *Staphylococcus aureus, Propionibacterium acnes, Staphylococcus epidermidis,* dan *Escherichia coli* pada konsentrasi 0,01 g/mL, 0,05 g/mL, dan 0,1 g/mL. Secara keseluruhan, kulit batang andiroba memenuhi uji standardisasi serta menunjukkan aktivitas antibakteri.

Kata Kunci: antibakteri, antioksidan, *Carapa guianensis*, standardisasi

1. Introduction

Infectious diseases and chronic diseases are increasingly suffered by modern society. Chronic diseases such as cardiovascular disease, diabetes mellitus, and aging are some examples of diseases caused by oxidative damage to tissues due to the presence of free radicals in the body. Excessive productio n of an oxidant or free radical in the body affects the pathogenesis of various diseases. Natural antioxidants derived from medicinal plants play an important role in preventing and treating chronic diseases caused by oxidative stress.[1] Exogenous antioxidant intake has been shown to prevent inflammation, atherosclerosis, and oxidative stress in patients with chronic kidney disease.[2]

Infections that induce overproduction of highly reactive molecules in the host or suppress host antioxidant defense mechanisms lead to oxidative stress. The relationship between infection and oxidative stress is a complex and multifaceted process. Both influence each other through specific biological mechanisms: infection can trigger an increase in the production of free radicals, while oxidative stress can worsen the course of infection by affecting the body's immune response and has been studied extensively over the past decades. Free radical detection is advancing rapidly, and it is important to monitor how this technique is used in virology. In addition, the role of free radicals in the progression of infections has been extensively reported and supported by numerous earlier studies.[3]

In biological systems, there are two main types of reactive molecules classified based on their elemental origin: reactive oxygen species (ROS), which are oxygen-centered, and reactive nitrogen species (RNS), which are nitrogen-centered. Both groups can further be classified according to their electron configuration into radicals and non-radicals. Radicals are defined as molecular species that contain at least one unpaired electron, making them highly reactive, whereas non-radical species do not possess unpaired electrons but are still capable of participating in redox reactions and contributing to oxidative stress in living systems.[4] Most articles studying oxidative stress in viral infections focus on reactive molecules derived from oxygen and nitrogen (reactivity can vary widely). Free radicals can affect infections in many ways. Generally, During a viral infection, free radicals and other reactive molecules are produced by invading phagocytic cells, while xanthine oxidase (XO) becomes active in inflamed tissues.[5] In inflamed tissues, inflammatory activation leads to increased production of reactive oxygen species such as superoxide (O_2^-) as well as elevated nitric oxide (NO) levels, and these reactive species not only contribute to oxidative tissue damage but also act as important modulators of immune responses; notably, nitric oxide has been shown to influence the balance of helper T-cell responses by reducing Th1 activity and favoring Th2-associated signaling pathways, thereby shifting immune host responses toward a Th2-biased phenotype.[6] Thus, NO*-induced immunosuppression may contribute to the pathogenesis of infection.

The detrimental effects of *OH radicals produced during infection were also described in other studies. O_2 radiation, during its passage through oxygenated aqueous media and superoxide dismutase, added to the suspension medium, reduces the lethal increase in oxygen caused by irradiation of *Escherichia coli* bacteria. O_2 production by clinically significant activated neutrophils is an important component of their bactericidal activity and inflammatory processes.[3] Since ancient times, plants have contained many secondary metabolites used worldwide in traditional medicine and are considered potent natural medicines. Chemical analysis and purification of plant extracts with therapeutic properties have been shown to yield numerous processed substances that are indispensable in modern medicine. However, only a small number of plant species have been reported to have biological or pharmacological effects, and their potential as sources of new treatments remains unexplored.

Diseases caused by bacterial infections are still a serious problem in Indonesia. In Indonesia, the prevalence of diseases caused by bacterial infections is still quite high. Scientific research has proven various therapeutic effects of plants, so many people use plants to treat infectious diseases of the respiratory, digestive, and urinary systems.[7] Secondary metabolites found in plants, animals, and microorganisms have been proven to be a very promising source of new drug candidates.[7] Treatment of infections and health problems with herbal medicines usually involve active natural compounds that are mostly low-molecular-weight and span a wide range of molecular weights.[8] The use of plant metabolites is currently of interest because they are safer than synthetic ones.

Secondary metabolites usually show a wide range of biological and pharmacological effects. As a result, certain plants or products derived from them have been exploited as sources of antioxidants and also for treating diseases, infections, and other health problems. Indonesia's biodiversity has great potential in the discovery of new compounds.[8] Several plants have been shown to help protect the human body from the dangers of free radicals and microbial contamination. Andiroba bark (*Carapa guianensis*) has traditionally been used in Jeneponto Regency as an alternative therapy to treat skin and digestive problems.

Exploration of the benefits of andiroba plants has not been widely conducted, so this study is an initial step to identify the chemical content, the safety of the extract, and its antioxidant and antibacterial properties.

2. Materials and Method

2.1. Tools

The tools used were an autoclave, stirring rod (Iwaki pyrex), beaker glass (pyrex), blender (Miyako), porcelain cup (Iwaki pyrex), petri dish (pyrex), magnetic stirrer (Iwaki pyrex), funnel (Iwaki pyrex), Erlenmeyer (pyrex), measuring flask (Iwaki pyrex), heating mantle, hot plate, incubator, loop needle, caliper, micropipette (Bio-Rad), oven, pycnometer, rotary evaporator, dropper pipette (Iwaki pyrex), volume pipette (Iwaki pyrex), tube rack, maceration tool set, Spectrophotometer UV-Vis (Shimadzu), test tube (Iwaki pyrex), furnace, analytical balance (Kern), and vacuum rotary evaporator (Heidolph) and vials.

2.2. Materials

The materials used were aluminum foil, $AlCl_3$, distilled water, anhydrous acetic acid, concentrated nitric acid (HNO_3) (supelco), perchloric acid ($HClO_4$), pure bacterial cultures (*Staphylococcus aureus* ATCC-6538P PK/5, *Propionibacterium acnes* ATCC-6919, *Escherichia coli* ATCC-25922) and *Staphylococcus epidermidis* ATCC-12228, DMSO, ethanol extract from andiroba bark (Carapa guianensis), 96% ethanol, $FeCl_3$, HCl, HCL 2N, H_2SO_4 (supelco), filter paper (whatman), ash-free filter paper, chloroform, cursetin (nitra kimia), nutrient agar (NA), NaCl, NaOH, dragendorff reagent (nitra kimia), mayer reagent (nitra kimia), Mg powder (Dwilab mandiri), DPPH powder (smart lab), spirits (kapinis), syringe (onemed), and vitamin C.

2.3. Methods

2.3.1. Sample Management

Preparation of sample Andiroba plant (*Carapa guianensis*) was obtained from Jeneponto Regency, South Sulawesi, in August 2024. Andiroba bark was rinsed with running water until completely clean. Then, Andiroba bark was cut into small pieces and dried by drying in indirect sunlight. After drying, the simplicia were dried-sorted, then weighed and ground in a blender for further use in the extraction process.

2.3.2. Extraction of Sample

Extraction was carried out using the maceration method, which involved soaking 1300 grams of andiroba bark with 15 liters 96% ethanol. This process was carried out by soaking the andiroba bark (*Carapa guianensis*) powder for 1×24 hours in a maceration vessel, stirring occasionally, and performing 2 re-macerations every 1×24 hours. Each filtered result was combined and concentrated using a rotary evaporator at 50°C to produce a thick extract.[9]

2.3.3. Extract Standardization

Specific

Identification of Extract[10]

Description of nomenclature, other names of plants, parts of plants used, and Indonesian names of plants.

Determination of Organoleptic[10]

Organoleptic determination of extracts, including shape, color, odor, and taste.

Determination of Dissolved Compound Levels in Certain Solvents[10]

• Level of Water-Soluble Compounds

A total of 1 g of extract was macerated for 24 hours with 20 mL of water and chloroform in a stoppered flask, with the mixture shaken repeatedly for the first 6 hours. Next, left for 18 hours and then filtered. Evaporated 20 mL of filtrate to dryness in an evaporator cup, and the residue was heated at 105ºC until the weight remained constant. Calculated the percentage of water-soluble compounds in the initial extract weight.

• Level of Ethanol-Soluble Compounds

A total of 1 g of extract was macerated for 24 hours in a stoppered flask with 20 mL of 96% ethanol, with shaking repeated for the first 6 hours. Then, left for 18 hours. The macerate was filtered quickly to prevent ethanol evaporation, and 20 mL of filtrate was then evaporated to dryness in an evaporator cup. The residue was heated at 105ºC until the weight remained constant. The percentage of compounds dissolved in ethanol is calculated against the initial extract weight.

Identification of Chemical Content of Extract[10]

• Identification of Alkaloids

A total of 20 mg ethanol extract was dissolved with a few drops of 2 N sulfuric acid and then tested with 2 reagents, namely Dragendorff's reagent and Mayer's reagent. Positive test results were obtained if an orange-red precipitate was formed on the addition

of Dragendorff's reagent, and a yellowish-white precipitate was formed on the addition of Mayer's reagent.

• Identification of Flavonoid

A total of 20 mg ethanol extract was added with magnesium (Mg) powder, and concentrated hydrochloric acid was added. If an orange, red, or yellow color was formed, it means that it was positive for flavonoids.[10]

• Identification of Saponin

Briefly, 20 mg of the extract were put into a test tube, 10 mL of hot water was added, cooled, and then shaken vigorously for 10 seconds, and 1 drop of HCL 2 N was added. A positive test was indicated by the formation of stable foam as high as 1-10 cm for no less than 10 minutes.[11]

• Identification of Steroids and Triterpenoids

A total of 20 mg of the extract was dissolved in 2 mL of chloroform in a dry test tube then 10 drops of acetic anhydride, and 3 drops of concentrated sulfuric acid were added. The presence of steroids was indicated by a blue or green color, while triterpenoids gave a red color or formed a brownish/violet ring.[11]

• Identification of Tannin

A total of 20 mg of sample extract was put into a test tube, 2 mL of water was added and heat to a temperature of 60°C, then $FeCl_3$ was added. The formation of a blackish-blue or blackish-green color indicates the presence of tannin.[11]

• Identification of Quinone

A total of 20 mg of ethanol extract was added with NaOH 1 N, and the color change was observed. A positive reaction was indicated by the formation of a yellow color.[11]

Non-Specific

Ash Content

• Determination of Total Ash Content

A total of 1 gram of extract was weighed (B) carefully into a crucible and weighed first (A0), slowly ignited. Next, the temperature was gradually increased to 500-600ºC for 10-15 minutes until carbon-free, then cooled in a desiccator, and the ash weight (A1). The ash content was calculated as a percentage of the initial sample weight.[10]

• Acid-Insoluble Ash Content

The ash obtained from the ash content determination was boiled with 25 mL of dilute HCl for 5 minutes, the acid-insoluble part was collected by filtering through previously weighed ash-free filter paper (C), the residue was washed with hot water, filtered, and weighed (A1), and then ignited until the weight remains constant. The acid-insoluble ash content was determined as a percentage of the initial sample weight.[10]

Water Content

Weighed the porcelain cup, dried it using an oven at a temperature of 105ºC for 30 minutes, and then put it in a desiccator for 30 minutes. Next, the porcelain cup was reweighed. After that, 2 grams of extract were added, heated using an oven at a temperature of 105ºC for 30 minutes, finally placed in a desiccator for 30 minutes, and reweighed.[10]

Microbial Contamination

Weighed 1g of extract and dissolved it in 10 mL of diluent, namely NaCl solution, shaken until homogeneous to obtain a dilution of 10-1. Three tubes were prepared, then 9 mL of diluent was put into each tube. The 10-1 dilution was pipetted into the first tube, shaken until homogeneous to obtain a 10-2 dilution, then into the 10-3 and 10-4 tubes. Each dilution was pipetted into each petri dish using a sterile pipette up to 1 mL, and 15 mL of melted Nutrient Agar (NA) at 45°C was then poured into each dish, and the suspension was shaken to ensure even distribution. After the media solidified, the petri dishes were incubated at 37°C for 24 hours.[12]

Mold and Yeast

1 g of extract was dissolved in 10 mL of diluent (NaCl solution) and shaken until homogeneous to obtain a 10-1 dilution. Three tubes were prepared, and 9 mL of diluent was added to each. The 10-1 dilution was pipetted into the first tube, shaken until homogeneous to obtain a 10-2 dilution, then into the 10-3 and 10-4 tubes. Each dilution was pipetted as much as 1 mL with a sterile pipette into each petri dish containing 15 mL of Potato Dextrose Agar (PDA) medium, which was still liquid at a temperature of 45°C, then shaken so that the suspension was evenly distributed, and finally incubated at a temperature of 25°C for 3 days.[10]

Contamination of Heavy Metal

Determination of As (arsenic), Pb (lead), and Cd (cadmium) levels using the Atomic Absorption

Spectroscopy (AAS) method. Briefly, 1 g of extract was prepared and added to 10 mL of concentrated HNO_3, then heated on a heating mantle until it reached half its volume or more. The thick, cold extract was added to 10 mL of distilled water and 5 mL of perchloric acid, then heated until the white smoke disappeared and allowed to cool. Next, rinsed with distilled water and filtered into a 50 mL measuring flask. Added distilled water up to 50 mL. The sample was measured using AAS. The As metal content was measured at a wavelength of 193.7 nm, Pb metal at a wavelength of 283 nm, and Cd metal at a wavelength of 288.8 nm.[10]

2.3.4. Determination of Total Flavonoid Levels

A standard solution then making several concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm.13 The determination of total flavonoid content was measured using the AlCl3 colorimetric method. 20 mg of andiroba (*Carapa guianensis*) bark extract was taken and diluted with 100 mL of ethanol. 1 mL of the diluted sample was taken and added to 1 mL of 10% $AlCl_3$ and 8 mL of 5% acetic acid. The sample was left for 30 minutes. The solution's light absorption was measured at the maximum wavelength using a UV-Vis Spectrophotometer. The linear regression equation was determined from the absorbance measurement results and the total flavonoid content was calculated.[14]

2.3.5. Antioxidant Activity Testing

Around 10 mg of sample and comparison solution were prepared, the dissolved with ethanol until all dissolved, diluted to the mark to obtain a 100 ppm stock solution. The comparison solution was made in 3 concentrations of 5 ppm, 10 ppm, and 15 ppm with 3 replications each.

Ethanol extract of andiroba bark was pipetted 1 mL each and the stock solution, put into a test tube, then added 0.5 DPPH solution and 3.5 ml of ethanol, followed by 25 minutes incubation before being measured the absorbance at a wavelength of 517nm.[15] The percentage of inhibition and The extract concentrations were represented on the x- and y-axes, respectively, and the corresponding line equation determined was used to calculate the inhibition concentration of 50% (IC_{50}).[15]

2.3.6. Antimicrobial Activity Testing

Medium and tools used must be in sterile condition. In testing antibacterial activity using the agar diffusion method with the well method, 15 mL of NA media that had been sterilized in an autoclave was taken using a sterile syringe and then inserted into a test tube. The 1 mL suspension of *Staphylococcus aureus, Propionibacterium acnes, Staphylococcus epidermidis,* and *Escherichia coli* bacteria was taken, placed in a test tube containing NA media, then homogenized and the media was poured into a petri dish and waited until it solidified. A hole was made on the agar plate containing the test bacteria, which was then filled with test substances such as andiroba bark extract with a concentration of 0.01, 0.05, and 0.1 g/mL positive control (clindamycin), and negative control (DMSO) with 3 replications each. After that, it was incubated at room temperature and time for 1×24 hours with the test microbes, then observations were made to see whether or not there was an inhibition zone around the hole.[16]

3. Result

3.1. Results of Ethanol Extraction of Andiroba Bark (*Carapa guianensis*)

The extraction results of samples of andiroba (*Carapa guianensis*) bark and are expressed in yield values (%), with a total of 12.08% of yieled was obtained following the extraction with ethanol (Table 1).

A good percentage soaking value is if the initial weight of the simplisia is 500 grams and should not be less than 7.2%. If the initial weight of the simplisia is 1000 grams, then the percentage soaking value should not be less than 10%. If the percentage soaking is more than 10%, then the secondary metabolite compounds obtained are more.17,18 Based on the specific and non-specific standardization analysis, the bioactive compounds in the ethanolic extract that dissolved in water and ethanol were unable to pass the requirements (Table 2).

Furthermore, the results of flavonoid identification were positive with concentration of 1.168% (Table 3). Flavonoid compounds are secondary metabolites that

Table 1. Results of Ethanol Extraction of Andiroba Bark (*Carapa guianensis*)

Types of Solvents	Simplisia (g)	Extract (g)	Yield (%)	Organoleptic
Ethanol 96%	1300	157	12.08	Color : Dark Brown, Smell: Aromatic andiroba, Teste: Bitter, Shape: Thick

Table 2. Results of specific and Non-Specific Extract ethanol Standardization Analysis

No	Parameters	Evaluation	Test Results
1	Spesific	Water soluble essence	0.30%
2		Ethanol soluble essence	0.92%
3		Alkaloid test	+
4		Tannin test	+
5		Flavonoid test	+
6		Saponin test	+
7		Quinone test	-
8		Steroid and triterpenoid test	+
9		Water Content	0.49 %
10	Non-Specific	Total Ash Content	0.19%
11		Acid insoluble ash content	0.18%
12		Microbial contamination	3750/cfu/gr
13		Mold/yeast contamination	100/cfu/g
14		Metal Contamination: a. Cadmium b. Lead c. Arsen	 0.0012 mg/kg 0.0011 mg/kg 0.0006 mg/kg

have the ability as antioxidants that can be used to ward off oxidative stress in the body.

The reaction between flavonoids and free radicals can occur through two main mechanisms, namely quenching and proton transfer. Quenching is a mechanism in which flavonoids can bind free radicals by forming stable compounds, this causes free radicals to be reduced and inactive. Therefore, the researchers determine the total flavonoid content to determine how much flavonoid content is contained in the ethanol extract of andiroba bark. This test was carried out using the $AlCl_3$ colorimetric method using quercetin as a comparator. In addition, DPPH test was conducted to assess the antioxidant capacity of the extract, resulted in the IC_{50} score of 15.985 µg/mL (Table 4), which belonged in the very strong category.

3.2. Results of Antimicrobial Test

Antimicrobial activity testing using four types of bacteria. The antimicrobial test results showed that inhibition after incubation was highest for *Staphylococcus aureus,* followed by *Propionibacterium acnes, Staphylococcus epidermidis,* and *Escherichia coli* (Figure 1).

4. Discussion

To obtain the extract, maceration is used. Maceration is a cold extraction method in which procedures and equipment are simple and do not require heating, so natural ingredients do not decompose, and many compounds can be extracted.[19] Andiroba bark (*Carapa guianensis*), which is chopped and blended before extraction, it is employed to enable the solvent to pass through the cell wall and reach the cell cavity that holds the active compound. The active compound then dissolves because of the concentration gradient between the inside and outside of the cell, causing the most concentrated portion of the solution to be expelled from the cell. This will continue until the concentration of the solute is balanced between the outside and inside of the cell. Another reason for using this method is that some active substances in the simplicia are not yet known to be stable under heating,[20] for example, bioactive compounds such as flavonoids, phenolics, alkaloids, and tannins.[21]

The extraction process uses 96% ethanol as a solvent. Ethanol is a polar, versatile solvent and is very good for

Table 3. Total Flavonoid Content of Andiroba Bark (*Carapa guianensis*)

Sample Weight (g)	Absorbance	Average Absorbance	Quercetin Equivalent Level (mg/L)	% Total Flavonoid Content
0.02	0.325 0.324 0.323	0.324	23.353	1.168

Table 4. Results of Antioxidant Test of Ethanol Extract from Andiroba Bark (*Carapa guianensis*)

Sample	Concentration (ppm)	Blank Absorbance	Sample Absorbance	% Inhibition	IC_{50} µg/mL
Ethanol Extract of Andiroba Bark	5		0.497	44.531	
	10	0.896	0.473	47.210	15.985
	15		0.453	49.442	
Vitamin C	5		0.552	38.393	
	10	0.896	0.485	45.871	11.281
	15		0.375	58.147	

initial extraction. In other research, samples extracted with ethanol yielded a higher percentage in both hot and cold extractions.[22] Processing samples using heat can destroy metabolites that are not heat-resistant, such as flavonoids.[23]

Standardization was performed in this study to ensure a consistent parameter value for the extract.[10] To determine the standardization value, a reference is needed to confirm that the extract meets the established requirements. For andiroba bark (*Carapa guianensis*) extract, there is no official standardization reference published by the Ministry of Health or other sources, so researchers use general extract requirements as a reference. The Standardization of the extract aims to provide an objective identity for the name and the compound's specificity, while the organoleptic properties of the extract serve as an initial introduction, using the five senses to describe the shape, smell, color, and taste.[10]

Water and ethanol are permitted solvents and meet pharmaceutical requirements.[24] Determining the levels of compounds dissolved in water and ethanol provides an initial picture of the amount of each compound dissolved in a particular solvent. The determination of extract content is also carried out to assess the results of the extraction, so that a suitable solvent for extracting a particular compound can be identified. Based on Table 2, the test results for the levels of compounds dissolved in water and ethanol do not exceed the requirements. The determination of the levels of water-soluble compounds and ethanol-soluble compounds is not something that has an impact on their pharmacological effects but rather a rough estimate of compounds that are polar (water-soluble) and active compounds that are semi-polar-nonpolar (ethanol-soluble).[25]

Identification of chemical compound groups contained in andiroba bark was carried out using chemical reactions (color and sediment). In Table 4, the results of chemical group identification showed that the extract contains alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. These results are consistent with previous studies, which report that the

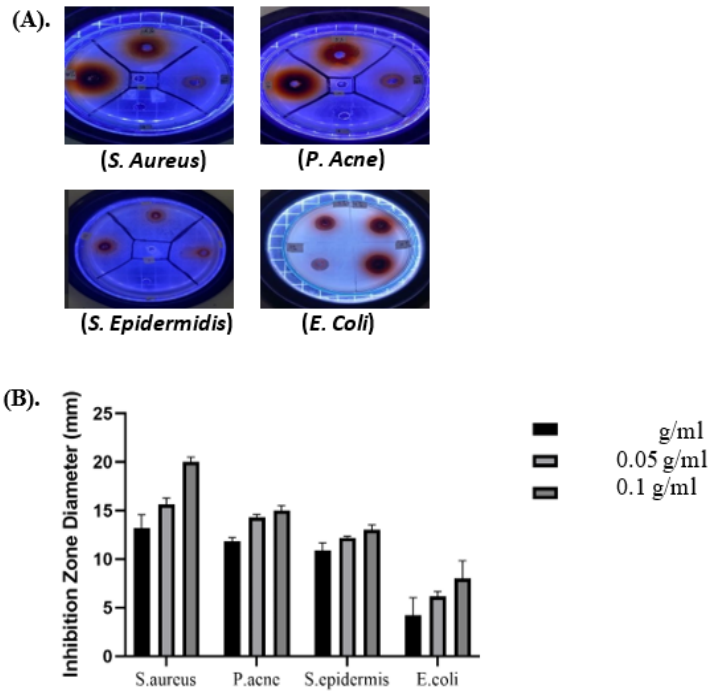

Figure 1. Results of (A) antimicrobial activity inhibition zone against bacteria and (B) the measurement of inhibition zone diameter of each bacterial activity

ethanol extract of andiroba bark contains flavonoids, steroids, tannins, phenolics, and terpenes.[23]

The purpose of determining ash content is to provide an overview of the level of impurities in the form of inorganic compounds, such as alkali metals (sodium, potassium, lithium), and mineral content. Based on Table 2, the ash content still meets the requirement of ≤5%.10 Total ash content in the extract is determined by complete combustion until only inorganic/mineral residues remain, so that the ash value is directly related to the mineral content in the sample.[25] This is because the Andiroba plant (*Carapa guianensis*) is a plant that grows on the coast, where the coastline soil is alluvial soil, which is one type of soil that contains quite high minerals.[23]

The acid-insoluble ash content test is used to determine the level of contamination from sand and soil. The acid-insoluble ash content indicates that the andiroba bark extract still complies with the standard requirement (below the established limit of ≤2%), demonstrating the purity and quality of the extract.[26] The presence of acid-insoluble ash indicates the presence of sand or other impurities.[10] This is caused by external factors, such as sand from the soil and dust that adheres during drying.[25]

Water content is determined to assess the remaining water in the extract, which helps ensure its quality and storage. Water content can determine the stability of the extract and subsequent dosage forms. High water content can promote the growth of harmful fungi.[25] In the determination of non-specific parameters, there is a test for microbial contamination and mold/yeast. Microbial contamination testing is one of the purity tests for extracts. This test includes the number of microorganisms allowed to indicate the presence or absence of bacteria in the extract. Contamination in the extract can occur during sample processing and storage, and may also be caused by air in the storage area.[12]

The determination of the levels of cadmium, lead, and arsenic metal content using an atomic absorption spectrophotometer. The results obtained show that the levels of cadmium, lead, and arsenic contamination do not exceed the established limits, namely 0.5 mg/kg cadmium, 1.0 mg/kg lead, and 1.0 mg/kg arsenic. Testing for heavy metals in extracts is important because high levels that exceed established limits can have toxic effects on health.

Quercetin is chosen because it is the most widely distributed compound in plants, including one of the flavonoid compounds that has a keto group at C-4, and a hydroxyl group at the C-3 or C-5 atom neighboring flavone and flavonol, which can react with $AlCl_3$ to form a complex.[27] Quercetin is a flavonoid with unique biological properties that can improve physical and mental health and reduce viral infections28. Quercetin has been reported to exert protective effects against atherosclerosis through antioxidant and anti-inflammatory mechanisms involving the modulation of key signaling pathways. A preclinical systematic review has shown that quercetin reduces the expression of NADPH oxidase components such as NOX2 and p47phox and decreases oxLDL formation, thereby attenuating vascular oxidative stress. In addition, quercetin activates the AMPK/SIRT1 pathway, inhibits NF-κB activation, and helps preserve endothelial function through regulation of the AKT/eNOS pathway, collectively contributing to the suppression of atherosclerosis progression and the improvement of vascular function.[29]

Quercetin has been reported to enhance cellular antioxidant capacity by activating intracellular signaling pathways such as p38 MAPK, increasing intracellular glutathione (GSH) levels, and acting as a hydrogen-donating molecule for free radical scavenging. Recent studies indicate that adverse environmental factors elevate reactive oxygen species (ROS) production, largely through increased activity of the mitochondrial electron transport chain, a primary intracellular source of ROS. The body defends itself against oxidative stress using two main systems: non-enzymatic antioxidants, including vitamins and trace elements such as vitamin C, manganese, vitamin E, enzymatic antioxidants, copper, and selenium such as superoxide dismutase (SOD) and catalase. Quercetin contributes to the maintenance of oxidative homeostasis not only by regulating both enzyme-independent and enzyme-mediated antioxidant defense systems but also by modulating ROS-induced signaling pathways, including MAPK, NF-κB, and AMPK, thereby strengthening the overall cellular antioxidant defense network.[30] By influencing signal transduction pathways, quercetin can modulate antioxidant enzymes and other substances, thereby increasing antioxidant capacity and preventing disease development.[31]

Quercetin has been shown to enhance the body's antioxidant capacity by regulating intracellular glutathione (GSH) levels, which plays a critical role in neutralizing free radicals generated during normal metabolic processes; these free radicals can otherwise cause genetic mutations, damage cell membranes, contribute to the development of diseases such as liver disease, cardiovascular disease, accelerate aging, and diabetes. Superoxide dismutase (SOD) rapidly converts superoxide (O_2^-) into hydrogen peroxide (H_2O_2), which is subsequently detoxified into non-toxic water (H_2O) through reactions involving GSH

as a hydrogen donor, with GSH levels influencing the rate of this detoxification. This mechanism, in which quercetin modulates GSH and supports antioxidant defenses including SOD activity, has been discussed in the context of quercetin's biological effects on oxidative stress in the article.[32]

The antioxidant activity test of ethanol extract from andiroba bark uses the DPPH method because it is a fast, simple, and inexpensive method for screening free radical scavenging activity. In addition, it has been shown to be accurate and practical for determining antioxidant capacity.[33] Vitamin C is chosen as a comparator because it is an antioxidant that captures free radicals and prevents chain reactions; it has very high antioxidant activity, is easy to obtain, and is non-toxic. Each concentration is measured for absorbance using UV-Vis spectrophotometry at 517 nm, as DPPH exhibits strong absorption. The percent inhibition (% antioxidant activity) is a parameter that indicates an antioxidant's ability to inhibit free radicals.[34,35]

The results in Table 4 show the antioxidant measurements of the ethanol extract from andiroba bark and vitamin C. The IC_{50} value of the ethanol extract from andiroba bark is 15.985 µg/mL, which is in the very strong category. A compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 ppm, strong activity if the IC_{50} value is between 50-100 ppm, moderate activity if the IC_{50} value is between 100-150 ppm, and weak if the IC_{50} value is between 150-200 ppm.[36]

The increasing resistance of microorganisms to antibiotics has driven the search for new and more effective antibacterial compounds, including those derived from natural sources such as plants. Various plant secondary metabolites, including alkaloids, flavonoids, glycosides, terpenoids, tannins, and polyphenols, have been demonstrated to exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria, including strains resistant to conventional antibiotics. Furthermore, recent studies have reported synergistic effects between these phytochemical compounds and existing antibiotics, which may enhance therapeutic efficacy, reduce required antibiotic dosages, and help overcome resistance mechanisms such as efflux pump activity and biofilm formation.[37]

In much of the literature, antioxidant and antibacterial activities are often reported simultaneously, especially when bioactivity-guided samples are involved. Therefore, it is also equally important that the methods used for antibacterial testing are standardized and optimized to ensure reporting accuracy. The diffusion method is usually used as an initial screening method to determine whether the sample under test has any antimicrobial activity.[38]

Antibacterial activity testing aims to determine the ability of ethanol extract from Andiroba bark (*Carapa guianensis*) to inhibit bacterial growth. Antibacterial activity is tested using the well method. The well method is chosen because it is easier to measure the area of the inhibition zone formed, since bacteria are active not only on the upper surface of the nutrient agar but also at the bottom. In addition, because the test sample in the well method is inserted into a well that has been prepared to induce osmosis, it is more homogeneous, efficient, and effective at inhibiting bacterial growth.[39]

In antibacterial testing, Nutrient Agar is used to cultivate bacteria. Nutrient Agar is a universal medium that contains agar, meat extract, yeast extract, and peptone. This medium is often prepared in large quantities and used for bacterial culture because its composition provides the nutrients required for microbial growth.[40]

Based on the inhibition zone measurements in Figure 1, the ethanol extract of andiroba bark inhibits bacterial growth, as evidenced by the inhibition zone diameter in each treatment group. This is due to the presence of active substances, or secondary metabolites, in the ethanol extract of andiroba bark, which can inhibit bacterial growth, including alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids.

The mechanism of action of flavonoid compounds can inhibit bacterial growth by damaging cell walls, deactivating enzymes, binding to adhesins, and damaging the cell membrane.[41,42] Alkaloid compounds, as antibacterials, disrupt components of the peptidoglycan in bacterial cells, preventing the cell wall from forming completely and causing cell death.[43] Tannins can inactivate microbial cell adhesion, inhibit enzymes, and affect DNA and topoisomerase, thereby disrupting the formation and function of bacterial cells.[43] Saponins damage erythrocyte membranes by forming complexes with cholesterol, leading to membrane defects in the form of large pores that increase membrane permeability and ultimately cause hemolysis. The mechanism of hemolysis is explained through pore formation and a colloid-osmotic mechanism, which enhances the leakage of ions and hemoglobin out of the cell.[44] Then, steroid and triterpenoid compounds, as antibacterials, are related to lipid membranes and to sensitivity to components that cause leakage in liposomes. Steroids and triterpenoids can interact with cell membranes that are permeable to lipophilic compounds, reducing membrane integrity and altering cell morphology,

leading to fragile cells and lysis.[45]

Overall, there is a significant difference in antibacterial inhibition between the treatment groups. Several factors that influence the formation of inhibition zones include the turbidity of the bacterial suspension, temperature, and thickness of the medium. If the bacterial suspension is more turbid, the resulting inhibition zone will tend to be larger; vice versa. Incubation temperature also affects the formation of inhibition zones. If the incubation temperature is below 35°C, the inhibition zone diameter tends to be larger. In addition, the thickness of the agar media, the effective thickness of the media to form an inhibition zone, is usually around 4 mm.46 The ethanol extract of andiroba bark shows good antioxidant and antibacterial activity. The bioactive compounds in the extract show increasing bioactivity with concentration. so that the Andiroba bark extract has the potential to be developed into a preparation sourced from natural ingredients.

5. Conclusion

Based on the research results, it can be concluded that the specific and non-specific standardization of the ethanol extract from Andiroba bark (*Carapa guianensis*) meets the requirements. The total flavonoid content of ethanol extract from andiroba bark was 11.676 mgQE/g, which is equivalent to 1.168% of the extract's total weight, and ethanol extract of andiroba stem bark has antioxidant activity classified as very strong with an IC_{50} value of 15.985 µg/mL (<50 µg/mL). Furthermore, the ethanol extract of Andiroba bark (*Carapa guianensis*) can inhibit the growth of *Staphylococcus aureus, Propionibacterium acnes, Staphylococcus epidermidis*, and *Escherichia coli* at extract concentrations of 0.01, 0.05, and 0.1g/mL.

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Conflict of Interest

The authors declare no conflicts of interest.

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