

Chemical Characterization of Gambir (*Uncaria gambir* Roxb.) Extract by Uv-Vis Spectrophotometry and HPLC, and Its Antibacterial Activity

Desi Widia Kusuma^{1*}, Herri Myzani¹, Suryati², Umar Kalmar Nizar³, Suhendarty⁴

¹Research and Development Agency of West Sumatra Province

²Chemistry Department of Andalas University, Padang

³Chemistry Department of Padang State University

⁴Regional Development Planning, Research, and Innovation Agency of Kaur Regency, Bengkulu

Corresponding Author:
Desi Widia Kusuma
desiranawk@gmail.com

Received: July 2026
Accepted: August 2026
Published: Sept 2026

©Desi Widia Kusuma et al.
This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Gambir (Uncaria gambir Roxb.) is a major commodity of West Sumatra and is rich in polyphenolic compounds, particularly catechins and tannins. This study aimed to extract and chemically characterize the leaves, twigs, pure gambir, and crude gambir using water and ethyl acetate, and to evaluate their antibacterial activity. Pure gambir and crude gambir were extracted by Soxhlet extraction using water and ethyl acetate, whereas leaves and twigs were extracted using water and ethyl acetate. Secondary metabolites were identified through phytochemical screening. Catechin content was determined using UV-Vis spectrophotometry and further confirmed by HPLC, while tannin content was determined by HPLC and expressed as gallic acid equivalents. Total phenolic content was analyzed using the Folin-Ciocalteu method. Antibacterial activity of the crude gambir ethyl acetate extract was evaluated against Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 using the disk diffusion method. The results showed that the ethyl acetate extract of pure gambir produced the highest extraction yield (98.62%) and the highest catechin content by UV-Vis spectrophotometry (78.24%), whereas HPLC showed a catechin concentration of 924.09 µg/mL. The highest tannin content, expressed as gallic acid equivalent response, was observed in the ethyl acetate extract of crude gambir (28.66 µg/mL), with a total phenolic content of 114.86 mg GAE/g. Phytochemical screening indicated that all samples contained phenolic compounds, tannins, and flavonoids. The ethyl acetate extract of crude gambir inhibited both bacterial strains, producing inhibition zones of 6.79–8.37 mm, classified as moderate antibacterial activity overall.

Keywords: Gambir; extraction; catechin; HPLC; antibacterial activity.

Introduction

One of the leading agricultural commodities of West Sumatra, as listed in the Regional Medium-Term Development Plan (RPJMD) of West Sumatra for 2021–2026, is gambir. West Sumatra is the largest gambir-producing region in Indonesia and contributes approximately 80–90% of the country's total gambir production [1],[2]. The major gambir-producing areas in West Sumatra include Limapuluh Kota, Pesisir Selatan, Pasaman, and Agam Regencies.

Limapuluh Kota Regency, the largest gambir-producing region in West Sumatra, had a gambir cultivation area of 17,410 ha in 2024, with a dry gambir resin production of 19,321.33 tons involving approximately 10,600 farming households [3]. Indonesian gambir is exported primarily to India, Pakistan, and Bangladesh, while the remaining exports are distributed to Japan, Malaysia, Singapore, Thailand, Brunei Darussalam, Bahrain, and the United States [4].

Gambir is a dried resin obtained from the extraction of the leaves and twigs of gambir

(*Uncaria gambir* Roxb.). It has high economic value and considerable potential for further development, with promising prospects for both farmers and export markets [5–9],[4]. Gambir is recognized as a rich source of polyphenolic compounds, with catechins and tannins as its major constituents [10–13]. In addition to catechin as the principal component, gambir leaf extract contains several other compounds, including catechu-tannic acid, quercetin, red catechu, gambir fluorescents, fats, and waxes [14]. Phytochemical screening has identified several secondary metabolites in gambir, including flavonoids, alkaloids, tannins, terpenoids, saponins, sterols, carbohydrates, and amino acids [15],[11].

Gambir extract, or the resin obtained from the leaves and twigs of gambir, has a wide range of applications [9], including various pharmacological activities such as antioxidant, antibacterial, anthelmintic, anticancer, antifungal, anti-inflammatory, antihyperglycemic, and antihyperuricemic effects, as well as lipid peroxidation-inhibitory and antihyperlipidemic activities. In addition to its medicinal applications, gambir extract has also been reported to exhibit potential as a corrosion inhibitor and as an adsorbent for dyes and metal ions [15]. The antioxidant and antimicrobial properties of catechin further support its potential application in the food industry [16].

Catechins and tannins exhibit distinct physicochemical properties. Catechins are crystalline compounds that are typically white to yellowish in color and have a sweet taste, whereas tannins are characterized by an astringent taste and a reddish-brown to black color [8],[17]. Catechins can be utilized in the food industry due to their antioxidant and antimicrobial properties [8]. Based on their chemical structures, tannins are broadly classified into hydrolyzed tannins and condensed tannins [18]. Tannins are widely used as raw materials in various industries, including pharmaceuticals, leather tanning, ink and dye production, adsorbent manufacturing, and metal-ion removal [18],[10]. Other reported applications of tannins include their use as

preservatives, adhesives, and raw materials for other chemical industries [19].

Despite its considerable potential, gambir has not yet significantly improved farmers' welfare due to the complex challenges associated with the gambir industry. Product quality and cleanliness remain major production issues, particularly in Limapuluh Kota Regency [20]. More specifically, these challenges are associated with several factors: (1) suboptimal cultivation practices [21]; (2) the continued use of conventional or simple processing technologies, which results in low pressing efficiency and consequently produces low-quality gambir [5],[4]. This condition also contributes to considerable variation in product quality and the limited availability of processed products, which are predominantly marketed as dry crude extracts commonly referred to as crude gambir [22],[16],[23],[24]; (3) inadequate post-harvest handling and processing practices [21]; (4) low selling prices because gambir is predominantly marketed in its raw form [5],[16]; and (5) unstable gambir prices associated with an oligopsony market structure.

The low quality of gambir, as indicated by quality assessments conducted by exporters, is attributed to the high levels of impurities, including moisture, ash, and water-insoluble matter, which exceed the established quality standards [22]. Previous research [25] reported that gambir is sometimes adulterated with foreign materials, such as soil, sand, starch, and fertilizer. Similarly, Kasim, et al [10] reported the addition of other materials, including powder derived from gambir waste, soil, sand, and flour. Another study [26] found that traders encouraged farmers to mix gambir with fertilizer, clay, and flour to increase the weight of the product, resulting in reduced gambir quality and lower market prices.

In the gambir trade, catechin content is one of the key quality parameters used to assess product quality [9],[8],[2],[17],[24]. The quality of gambir can be improved through extraction, which involves transferring soluble compounds from a solid material into a liquid solvent, followed by purification. Gambir purification

can be performed by exploiting differences in the solubility of its constituent compounds [24]. Several factors influence the extraction and purification of phytochemicals and antioxidant compounds from plant materials, including extraction time, temperature, solvent concentration, and solvent polarity [27]. The type of solvent used plays a critical role in determining the efficiency of bioactive compound extraction and the resulting phytochemical composition of plant materials [28],[13]. In particular, the solvent polarity should be compatible with the polarity of the target compounds to facilitate their extraction [29]. The use of different solvents can therefore result in variations in extraction yield and phytochemical composition, both qualitatively and quantitatively [30].

Catechin requires further purification from crude gambir to enhance its quality and commercial value in the international market. According to Yeni et al; Isnawati et al [23],[31], several factors influence the catechin content and quality of gambir, including the characteristics of the raw material (plant material), re-extraction technology, extract purification procedures, and the equipment used. Catechin can be purified using organic solvents and water through an extraction process based on differences in the solubility of catechin and tannins in water. Catechin is readily soluble in hot water, alcohol, and ethyl acetate, but has limited solubility in cold water [8]. In general, nonpolar compounds tend to dissolve more readily in nonpolar solvents, whereas polar compounds exhibit greater solubility in polar solvents [32]. Ethyl acetate is a semipolar solvent with relatively good selectivity for extracting catechin and is therefore suitable for the separation and purification of catechin from gambir extracts [17].

Previous studies have extracted and purified catechin from gambir through three consecutive extraction cycles using ethyl acetate, hexane, and water [7]. Repeated washing has been reported to increase the catechin content while simultaneously reducing the tannin content [33],[31]. Tannins are partially removed during the washing process because of their relatively high

solubility in cold water, whereas catechin is more readily soluble in hot water, alcohol, and ethyl acetate [7],[33]. However, catechin may undergo degradation or transformation during prolonged heating. Heating at 110 °C or treatment in an alkaline carbonate solution has been reported to promote the conversion of catechin into catechu tannic acid or other tannin-related compounds [33],[11].

Previous studies have investigated catechin extraction from gambir using various solvents, extraction techniques, and analytical methods, generally focusing on one or two types of gambir raw materials, such as gambir leaves, leaves and twigs, or dried gambir extract [34],[7],[35],[24],[32],[23],[36]. In the present study, four types of gambir raw materials, gambir leaves and twigs, crude gambir, and purified gambir were investigated using water and ethyl acetate as extraction solvents, with extraction procedures adapted to the characteristics of each raw material. The resulting extracts were subsequently subjected to chemical characterization, including the determination of catechin and tannin contents, phytochemical profiling, and total phenolic content. Finally, the ethyl acetate extract of gambir was further evaluated for its total phenolic content and antibacterial activity.

The use of instrumental analytical techniques can improve the accuracy and reliability of the chemical characterization of gambir. UV-Vis spectrophotometry can be used to estimate catechin content based on a calibration curve prepared from a catechin standard, whereas high-performance liquid chromatography (HPLC) provides more selective separation and quantification of individual components. The Folin-Ciocalteu method is commonly used to determine total phenolic content. Based on these considerations, this study aimed to extract and chemically characterize gambir leaves, twigs, purified gambir, and crude gambir using water and ethyl acetate as extraction solvents, and to evaluate their antibacterial activity.

The chemical characterization included the determination of catechin content using UV-Vis spectrophotometry and HPLC, tannin content using HPLC, identification of secondary

metabolite classes through phytochemical screening, and total phenolic content using the Folin–Ciocalteu method. The antibacterial activity of the extracts was evaluated against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 using the disk diffusion method. This study is expected to provide comprehensive information on the chemical characteristics and antibacterial potential of gambir, thereby supporting the development of high-value-added gambir-based products.

Experimental

Materials

The study was conducted from October to December 2025 and during the fourth week of July 2026 at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, Indonesia. The materials used in this study consisted of gambir leaves, gambir twigs, purified gambir, and crude gambir in cylindrical form. All gambir materials were obtained from Pangkalan Koto Baru District, Limapuluh Kota Regency, West Sumatra, Indonesia.

Methods

Catechin Extraction

Extraction of Gambir Leaves and Twigs

Materials and equipment: Gambir leaves and twigs, distilled water, cold water, ethyl acetate, a thermometer, a 100-mesh sieve, a rotary evaporator, and a UV–Vis spectrophotometer.

Extraction procedure: The extraction of gambir leaves and twigs was performed according to the method reported by Yeni et al ^[21]. The leaves and twigs were ground and extracted with distilled water at approximately 70 °C using a solid-to-liquid ratio of 1:5 (w/v) for 1 h, followed by filtration. The filtrate was allowed to stand for approximately 20–24 h to facilitate precipitation. The resulting precipitate was repeatedly washed with cold water until a yellowish wash solution was obtained. The precipitate was then pressed to remove residual water and air-dried.

The extraction process was subsequently continued by maceration with ethyl acetate at a solid-to-liquid ratio of 1:10 (w/v) for approximately 4 h. The mixture was filtered through a 100-mesh sieve, and the resulting filtrate was concentrated using a rotary evaporator. The dried mass recovered from the ethyl acetate extract relative to the initial mass of the gambir product was expressed as the ethyl acetate extract yield. Catechin content was subsequently determined using UV–Vis spectrophotometry.

Extraction of Purified and Crude Gambir

Materials and equipment: Purified gambir, crude gambir, water, ethyl acetate, a Soxhlet apparatus, a rotary evaporator, and a UV–Vis spectrophotometer.

Extraction procedure: Purified and crude gambir were extracted using two solvents, namely water and ethyl acetate, by Soxhlet extraction for 10 h. The resulting ethyl acetate extracts were analyzed for catechin content using UV–Vis spectrophotometry. The potential for heat-induced changes during the 10-h Soxhlet extraction represents a limitation of the present study. Prolonged exposure to elevated temperatures may affect the stability of catechin and potentially lead to degradation or transformation. Therefore, future studies should compare extraction methods involving different levels of thermal exposure and include dedicated stability or degradation assessments to evaluate the effects of extraction conditions on catechin stability.

Phytochemical Screening

Materials and equipment: The gambir samples analyzed included gambir leaves, gambir twigs, aqueous extracts of purified gambir, aqueous extracts of crude gambir, ethyl acetate extracts of purified gambir, and ethyl acetate extracts of crude gambir. The reagents used were chloroform, methanol, NH₄OH 0.5 N, acetic anhydride, concentrated H₂SO₄, concentrated HCl, magnesium (Mg) powder, FeCl₃, H₂SO₄ 2 N, distilled water, Mayer's reagent, and Dragendorff's reagent. The equipment consisted of test tubes, a water bath, pipettes, and filters.

The sample was placed in a test tube, followed by the addition of methanol. The mixture was heated in a water bath for 15 min, filtered while hot, and allowed to evaporate until the methanol was completely removed.

Chloroform and distilled water were then added in a 1:1 ratio, 5 mL each. The mixture was shaken thoroughly and allowed to stand until two distinct layers, namely the chloroform and aqueous layers, were formed. The lower chloroform layer was used for the detection of triterpenoids and steroids.

Flavonoid test: A portion of the aqueous layer was transferred into a test tube using a pipette, followed by the addition of concentrated HCl and several pieces of magnesium powder.

Phenolic compound test: A portion of the aqueous layer was transferred into a small test tube using a pipette, followed by the addition of FeCl_3 reagent.

Saponin test: A portion of the aqueous layer was vigorously shaken in a test tube. The formation of persistent foam that remained after the addition of several drops of concentrated HCl was taken as a positive indication for saponins.

Triterpenoid and steroid test (Liebermann–Burchard reaction): A small portion of the chloroform layer was transferred into two wells of a spot plate and allowed to evaporate to dryness. One drop of acetic anhydride followed by one drop of concentrated H_2SO_4 was added to one of the wells. The resulting color change was observed as an indication of the presence of triterpenoids or steroids.

Alkaloid test: The sample was dissolved in chloroform and treated with 0.05 M ammoniacal chloroform, followed by the addition of 0.5 mL of H_2SO_4 2 N. The mixture was shaken and allowed to stand until two layers formed. The H_2SO_4 layer was collected and transferred into a test tube, followed by the addition of one drop of Mayer's reagent. The formation of a precipitate was considered a positive indication of alkaloids.

Determination of Catechin Content

Preparation of the Standard Solution

A 10 mg portion of catechin standard was accurately weighed and dissolved in absolute ethanol in a 10 mL volumetric flask to obtain a stock solution with a concentration of 1,000 $\mu\text{g/mL}$. Standard solutions at concentrations of 50, 100, 150, 200, and 250 $\mu\text{g/mL}$ were subsequently prepared by pipetting 0.5, 1.0, 1.5, 2.0, and 2.5 mL, respectively, of the 1,000 $\mu\text{g/mL}$ stock solution into separate 10 mL volumetric flasks. Each solution was then diluted to the mark with absolute ethanol.

Determination of the Maximum Absorption Wavelength

The maximum absorption wavelength (λ_{max}) of catechin was determined by scanning the catechin standard solution using a UV–Vis spectrophotometer over the wavelength range of 200–400 nm.

Determination of Catechin Content

The ethyl acetate extract was dissolved in absolute ethanol. The absorbance of the resulting sample solution was measured using a UV–Vis spectrophotometer at the experimentally determined maximum absorption wavelength (λ_{max}). Catechin content was determined based on the calibration curve constructed from the catechin standard solutions.

Determination of Chemical Components in Samples by HPLC

The chemical components present in the samples were identified using high-performance liquid chromatography (HPLC). The materials analyzed included aqueous extracts of purified gambir, aqueous extracts of crude gambir, ethyl acetate extracts of purified gambir, and ethyl acetate extracts of crude gambir. HPLC-grade methanol, distilled water, catechin standard, and gallic acid standard were used as solvents and analytical standards.

HPLC Analytical Conditions

The HPLC analysis was performed using a Shimadzu LC-20Ai system equipped with an SPD-40 UV-Vis detector. The analytical wavelength was set at 254 nm, with a flow rate of 0.5 mL/min and a column temperature of 40 °C. Separation was performed using a Shim-pack VP-ODS column (250 × 4.6 mm) as the stationary phase. The mobile phase consisted of methanol and water (50:50, v/v).

Catechin standard solutions at concentrations of 50, 100, 150, 200, and 250 µg/mL were prepared in methanol and injected for calibration. Sample solutions were prepared at a concentration of 1,000 µg/mL in methanol. The injection volume was 10 µL.

The compounds were identified by comparing the chromatograms of the standards and samples based on their retention times. Catechin and tannin contents were determined using calibration curves prepared from catechin and gallic acid standards, respectively. Tannin content was expressed as the gallic acid equivalent response.

Total Phenolic Content

Equipment: 1 mL and 10 mL graduated pipettes, 10 mL volumetric flasks, an analytical balance, a watch glass, and a UV-Vis spectrophotometer. Materials: Ethyl acetate extract of crude gambir, 10% Folin-Ciocalteu reagent, 7.5% Na₂CO₃ solution, methanol, and gallic acid.

Preparation of the gallic acid standard solution:

A 10 mg portion of gallic acid was accurately weighed and dissolved in methanol in a 10 mL volumetric flask to obtain a stock solution with a concentration of 1,000 ppm. The stock solution was subsequently diluted with methanol to prepare standard solutions at concentrations of 0, 10, 20, 30, 40, and 50 ppm.

Preparation of the sample solution:

A 5 mg (0.005 g) portion of the ethyl acetate extract of crude gambir was accurately weighed and dissolved in methanol in a 10 mL volumetric flask to obtain a sample solution with a

concentration of 500 ppm. The solution was subsequently diluted tenfold in a 10 mL volumetric flask.

Reaction procedure for standards and samples:

A 1 mL aliquot of each standard solution and sample solution was transferred into separate test tubes. Then, 5 mL of 10% Folin-Ciocalteu reagent was added, and the mixture was allowed to react for 10 min. Subsequently, 4 mL of 7.5% Na₂CO₃ solution was added. The mixtures were vortex-mixed and incubated for 30 min. Absorbance was measured at 750 nm using a UV-Vis spectrophotometer. Total phenolic content was determined from the calibration curve prepared using gallic acid and expressed as gallic acid equivalents (GAE).

Antibacterial Activity Test

The antibacterial activity of the ethyl acetate extract of crude gambir was evaluated using the disk diffusion method against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

The ethyl acetate extract of crude gambir was prepared at concentrations of 25, 50, 75, and 100 mg/mL using 10% dimethyl sulfoxide (DMSO) as the solvent. Chloramphenicol at a concentration of 1 mg/mL was prepared as the positive control. A 10% DMSO solution was prepared as the negative control. The test bacteria, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, were subcultured on nutrient agar (NA) and incubated at 37 °C for 24 h. The freshly grown bacterial colonies were suspended in 0.9% physiological saline until the turbidity corresponded to a 0.5 McFarland standard. A 100 µL aliquot of the bacterial suspension was inoculated onto the surface of sterile Mueller-Hinton agar (MHA) and evenly spread over the entire agar surface using a sterile cotton swab. Sterile paper disks with a diameter of 6 mm were individually immersed in the extract solutions, negative control, and positive control. The disks were allowed to dry briefly and then placed on the inoculated agar surface using sterile forceps. The Petri dishes were incubated at 37 °C for 24 h. After incubation, the diameters of the inhibition zones formed around the disks were

measured using a Vernier caliper. The inhibition zone diameters were recorded in millimeters (mm). All antibacterial assays were performed in triplicate for each treatment.

Results and Discussion

Extraction of Purified and Crude Gambir

The extraction of purified and crude gambir was performed using the Soxhlet extraction method based on differences in solvent polarity, allowing compounds to dissolve and be extracted according to their respective polarity characteristics [32]. The extraction results showed that the ethyl acetate extract of purified gambir produced the highest yield (98.62%), followed by the ethyl acetate extract of crude gambir (73.27%), the aqueous extract of purified gambir (54.59%), and the aqueous extract of crude gambir (43.25%) (Table 1).

The results demonstrated that both solvent type and the quality of the raw material influenced the extraction yield. This finding is consistent with previous research [37], which reported that extraction yields vary depending on the solvent used.

In the present study, two types of gambir raw materials, namely purified gambir and crude gambir, were extracted using two solvents, water and ethyl acetate. The ethyl acetate extract of purified gambir produced the highest extraction yield (98.62%), whereas the aqueous extract of crude gambir exhibited the lowest yield (43.25%). The higher yields obtained with

ethyl acetate for both purified and crude gambir suggest that ethyl acetate was more effective in extracting the solvent-soluble constituents present in these materials under the applied extraction conditions.

Ethyl acetate is a moderately polar organic solvent that can effectively dissolve a range of moderately polar compounds, including catechin and other phenolic constituents. Consequently, its solvent properties may favor the extraction of certain gambir constituents compared with water, although extraction efficiency depends on the chemical composition of the raw material and the specific extraction conditions. According to Kasim et al [34], solvent polarity is related to molecular structure and other physicochemical properties; therefore, differences in solvent polarity can affect the solubility and partitioning of compounds during extraction. Ethyl acetate has lower polarity than water and may therefore provide greater affinity for certain moderately polar constituents of gambir, including catechin.

The extraction yield was also affected by differences in raw material quality. Purified gambir, which contains a higher proportion of the desired gambir constituents and fewer impurities, produced a higher yield than crude gambir. The lower yield obtained from crude gambir may be associated with the presence of non-target materials and impurities that can reduce the proportion of extractable gambir constituents.

Table 1. Extraction Yield of Pure Gambir and Raw Gambir using Water Solvent and Ethyl Acetate

No.	Extract	Extract Mass (g)	Sample Mass (g)	Yield (%)
1	Pure gambir water extract	21.836	40	54.59
2	Crude gambir water extract	17.3	40	43.25
3	Pure gambir ethyl acetate extract	39.448	40	98.62
4	Ethyl acetate extract of raw gambir	29.308	40	73.27

Phytochemical Screening

Phytochemical screening was conducted to identify the major classes of secondary metabolites present in gambir. The materials analyzed included gambir leaves, twigs, purified gambir, and crude gambir. Seven classes of secondary metabolites were qualitatively screened, namely phenolics, tannins, flavonoids, saponins, triterpenoids, steroids, and alkaloids.

The results showed that all samples tested positive for phenolic compounds, tannins, and flavonoids. These three classes of compounds are commonly reported as major phytochemical constituents of gambir. The positive flavonoid reactions are consistent with the presence of flavonoid compounds, including catechin, which belongs to the flavan-3-ol subclass of flavonoids. However, a positive flavonoid test provides only qualitative evidence for the presence of flavonoid compounds and cannot be used to confirm the presence of catechin specifically. Therefore, catechin identification and quantification were subsequently performed using high-performance liquid chromatography (HPLC) (Table 2).

The methanolic leaf extract exhibited the most diverse phytochemical profile, containing phenolics, tannins, flavonoids, saponins, triterpenoids, steroids, and alkaloids. This diversity may be associated with the high metabolic activity of leaves and their role in the biosynthesis and accumulation of secondary

metabolites. Previous research ^[40] reported differences in alkaloid and flavonoid profiles between chloroform and ethanol extracts of gambir leaves, which were attributed to differences in solvent properties and polarity. The broad phytochemical profile observed in the present study may therefore be related to the ability of methanol to extract various polar and moderately polar compounds.

The methanolic twig extract contained phenolics, tannins, flavonoids, saponins, triterpenoids, and alkaloids, but not steroids, indicating a less diverse profile than that of the leaves. This observation is consistent with previous research ^[41], which reported higher catechin content in methanolic gambir leaf extract (8.64%) than in twig extract (5.12%). The aqueous extracts of purified and crude gambir showed similar profiles, with positive reactions for phenolics, tannins, flavonoids, saponins, and triterpenoids, but negative reactions for alkaloids and steroids.

The ethyl acetate extract of purified gambir contained phenolics, tannins, flavonoids, triterpenoids, and alkaloids, whereas the crude gambir extract additionally tested positive for steroids. These differences may reflect variations in raw-material composition and processing history. Similar findings were reported by ^[35], who detected alkaloids, flavonoids, phenolics, and saponins in ethyl acetate gambir leaf extracts.

Table 2. Results of Phytochemical Testing of Gambier Extract

Phytochemical Test	Test Results					
	Methanol Leaf Extract	Methanol Extract of Twigs	Pure Gambir Water Extract	Crude Gambir Water Extract	Pure Gambir Ethyl Acetate Extract	Crude Gambir Ethyl Acetate Extract
Phenolic	+	+	+	+	+	+
Tanin	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+
Saponin	+	+	+	+	-	-
Triterpenoid	+	+	+	+	+	+
Steroid	+	-	-	-	-	+
Alkaloid	+	+	-	-	+	+

According to [42], solvent polarity strongly influences phytochemical extraction. Water (polarity index = 9.0) preferentially extracts polar constituents, whereas ethyl acetate (4.4) can extract a broader range of moderately polar compounds. In contrast, nonpolar solvents such as hexane (0.0) are more suitable for lipophilic constituents, including steroids and some triterpenoids.

Overall, the positive reactions for phenolic compounds, tannins, and flavonoids across the samples indicate the presence of these major phytochemical classes. However, phytochemical screening is qualitative and cannot specifically confirm the presence of individual compounds such as catechin. Therefore, the occurrence and concentration of catechin and other target compounds should be established using more selective analytical techniques, such as HPLC.

Determination of Catechin Content by UV–Vis Spectrophotometry

Catechin content in the samples was estimated using UV–Vis spectrophotometry with catechin as the reference standard. Five catechin standard solutions at different concentrations were prepared, and their absorbance values were measured at a wavelength of 280 nm. The resulting absorbance values were used to construct a calibration curve, with catechin concentration plotted on the x-axis and absorbance on the y-axis. The linear regression equation obtained from the calibration curve was subsequently used to estimate the catechin content in the samples. The catechin content was

expressed as a catechin-equivalent value based on the UV–Vis spectrophotometric response relative to the catechin standard (Figure 1). Because UV–Vis absorbance at 280 nm is not entirely specific to catechin and may also arise from other phenolic compounds, absolute catechin content requires further confirmation and quantification using a more selective analytical technique, such as high-performance liquid chromatography (HPLC).

Six sample types, namely methanolic leaf extract, methanolic twig extract, aqueous extract of purified gambir, aqueous extract of crude gambir, ethyl acetate extract of purified gambir, and ethyl acetate extract of crude gambir, were prepared at a concentration of 200 µg/mL, and their absorbance values were subsequently measured. The absorbance value of each sample was substituted into the linear regression equation obtained from the catechin calibration curve to estimate the corresponding catechin concentration. The relative catechin content was then calculated as a percentage of the initial sample concentration of 200 µg/mL (Table 3).

The ethyl acetate extract of purified gambir exhibited the highest estimated catechin content (78.24%), followed by the methanolic leaf extract (69.49%). The relatively high catechin content in leaves may be associated with their role as metabolically active organs involved in the biosynthesis and accumulation of secondary metabolites, including phenolic and flavonoid compounds.

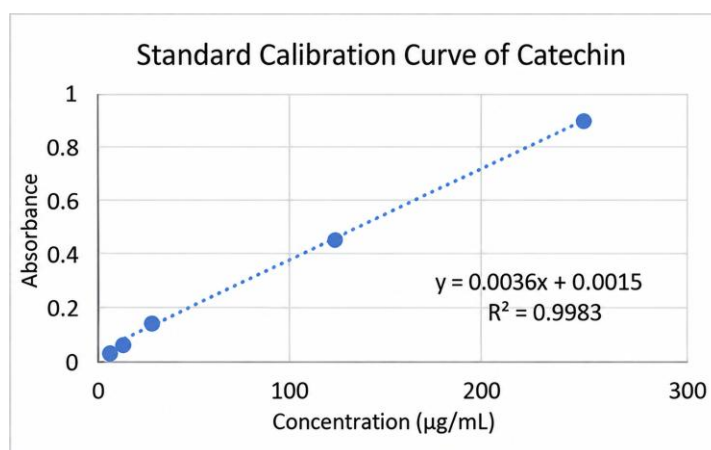


Figure 1. Standard Calibration Curve of Catechin

Table 3. Data on Catechin Content Measurement Results

No.	Ekstrak	Absorbance	Concentration ($\mu\text{g/mL}$)	Catechin content (%)
1	Leaves extract	0.502	138.98	69.49
2	Twigs extract	0.367	101.48	50.74
3	Pure gambir water extract	0.452	125.08	62.54
4	Crude gambir water extract	0.453	125.36	62.68
5	Pure gambir ethyl acetate extract	0.565	156.48	78.24
6	Crude gambir ethyl acetate extract	0.476	131.76	65.88

The ethyl acetate and aqueous extracts of crude gambir showed estimated catechin contents of 65.88% and 62.68%, respectively, while the aqueous extract of purified gambir contained 62.54%. These differences may reflect variations in raw-material composition and processing conditions. The methanolic twig extract showed the lowest estimated catechin content (50.74%), possibly due to the predominantly structural function of twigs and their higher proportion of lignin and cellulose. This finding is consistent with previous research [23], which reported that organic solvents, particularly ethyl acetate, can enhance catechin enrichment compared with ethanol extraction.

Overall, the observed differences in estimated catechin content may reflect the combined effects of raw-material characteristics, extraction procedures, and solvent properties. However, because these factors were not independently controlled or systematically evaluated in the present study, the specific contribution of each factor to the observed catechin content cannot be determined.

HPLC Analysis

Analysis of Catechin and Gallic Acid Standards

HPLC analysis of catechin and gallic acid standards was performed to determine their retention times (Rt) as references for the

tentative identification of catechin and tannin-related responses in gambir extracts. Gallic acid was used as the reference standard for the tannin-related response because it is a simple phenolic compound and a structural component of many hydrolyzable tannins [43]. Accordingly, the tannin-related response was expressed as HPLC-detectable gallic acid equivalents. The catechin and gallic acid standards showed retention times of 2.825 and 2.972 min, respectively, which were used as reference values for the tentative identification of corresponding responses in the gambir extracts.

Peak identification based solely on retention time should be considered tentative because retention time alone does not provide definitive structural confirmation. More robust identification can be achieved using additional analytical approaches, such as standard addition or co-injection, HPLC-DAD with spectral comparison, or liquid chromatography–mass spectrometry (LC–MS). Accordingly, the observed peaks in the gambir extracts were tentatively assigned to catechin and the gallic acid-equivalent response based on their agreement in retention times with the corresponding catechin and gallic acid standards.

Analysis of Gambir Extract Samples

The chromatogram of the aqueous extract of purified gambir showed three major peaks at retention times (Rt) of 2.812, 2.973, and 3.108 min. The peak at 2.812 min was closely matched to the retention time of the catechin standard and was therefore tentatively assigned to catechin. The peak at 2.973 min corresponded closely to the retention time of the gallic acid standard and was therefore assigned to a gallic acid-equivalent response. This response was used to express the tannin-related content in the sample. The third peak, observed at 3.108 min, may indicate the presence of another minor phenolic constituent that was not detected in the analyzed standards (Figure 2).

The ethyl acetate extract of purified gambir showed three peaks at retention times (Rt) of 2.824, 2.976, and 3.220 min. The first two peaks were tentatively assigned to catechin and gallic acid, respectively, based on their agreement with the corresponding standards. The peak at

3.220 min may represent another constituent with relatively lower polarity (Figure 3).

The aqueous extract of crude gambir showed only two major peaks at retention times (Rt) of 2.820 and 2.975 min, corresponding to catechin and gallic acid, respectively. The absence of additional major peaks suggests a relatively simpler phenolic profile in the crude gambir extract (Figure 4).

The ethyl acetate extract of crude gambir showed two peaks at 2.836 and 2.986 min, corresponding to catechin and gallic acid, respectively. The absence of additional major peaks indicates a relatively limited phenolic profile compared with purified gambir (Figure 5).

The methanolic gambir leaf extract showed two peaks at retention times (Rt) of 2.821 and 3.101 min, corresponding to catechin and gallic acid, respectively (Figure 6).

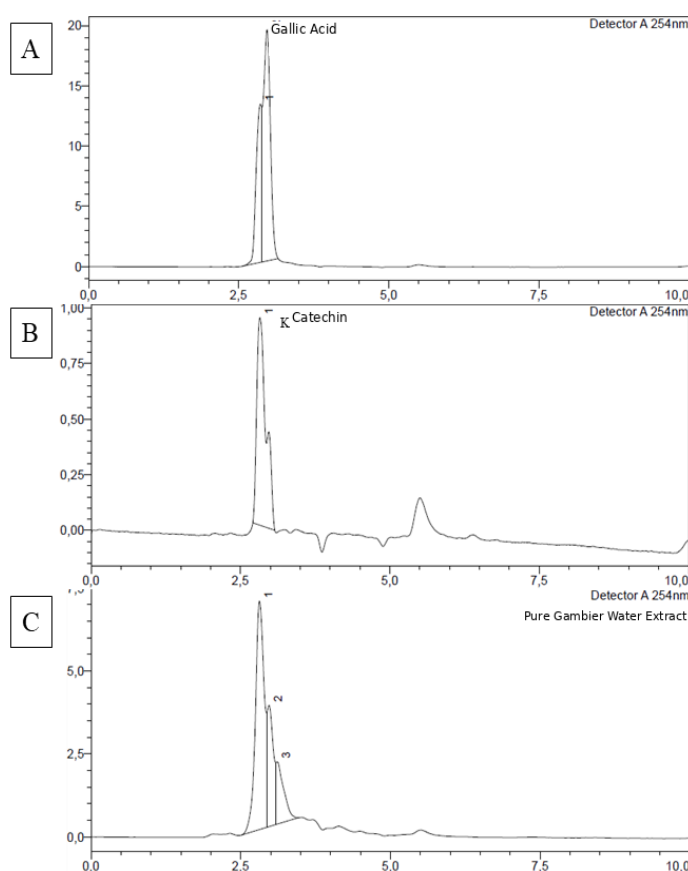


Figure 2. Chromatogram A) Gallic acid standard, B) Catechin standard, C) Pure gambir water extract

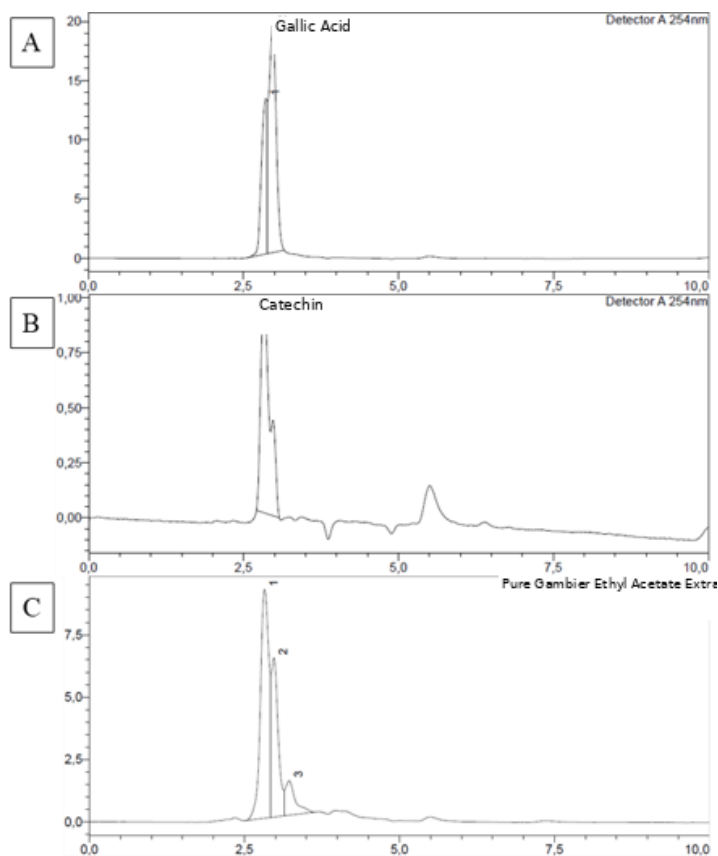


Figure 3. Chromatogram A) Gallic acid standard, B) Catechin standard, C) Pure gambir ethyl acetate extract

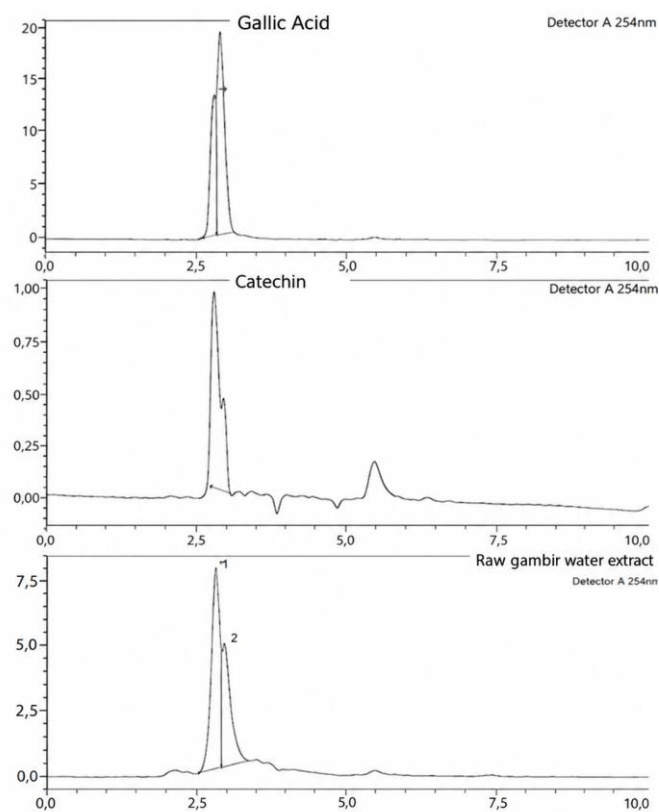


Figure 4. Chromatogram A) Gallic acid standard, B) Catechin standard, C) Crude gambir water extractt

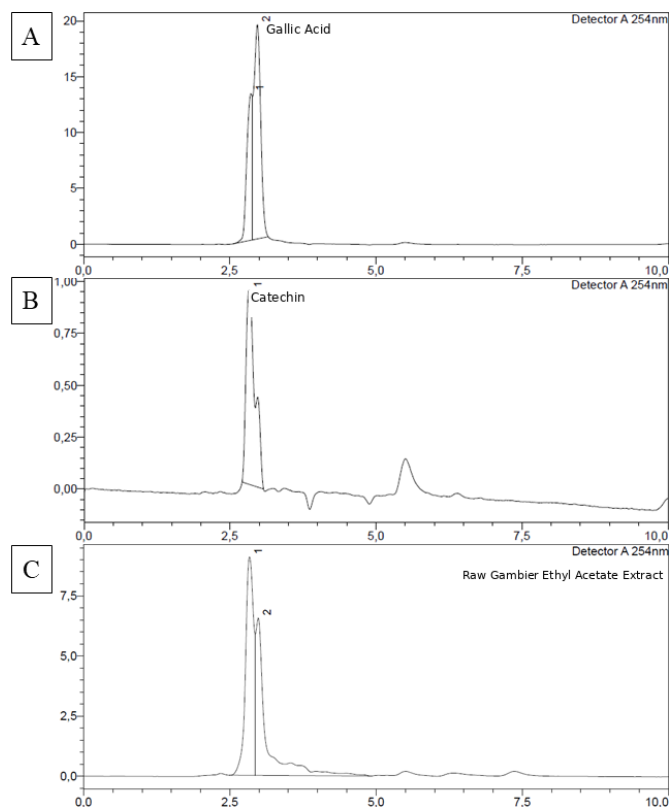


Figure 5. Chromatogram A) Gallic acid standard, B) Catechin standard, C) Crude gambir ethyl acetate extract

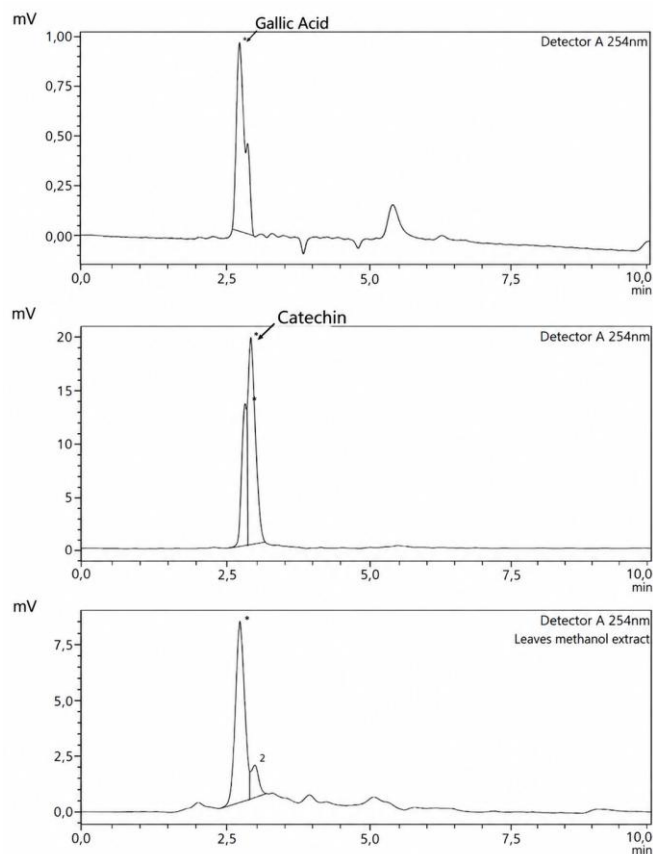


Figure 6. HPLC chromatogram: A) Gallic acid standard, B) Catechin standard, C) Leaves methanol extract

Quantitative Analysis of Catechin and Tannin Content

Quantitative analysis of catechin and tannin-related responses in five extract samples was performed using calibration curves prepared from catechin and gallic acid standards, respectively. The standard concentration was plotted on the x-axis, while the corresponding peak area was plotted on the y-axis (Figure 7).

The highest catechin concentration was found in the ethyl acetate extract of purified gambir (924.09 µg/mL), followed by crude gambir (922.22 µg/mL) and methanolic leaf extract (853.65 µg/mL). Aqueous extracts showed lower concentrations, at 798.95 µg/mL for crude gambir and 715.25 µg/mL for purified gambir. The highest gallic acid-equivalent response was

observed in the ethyl acetate extract of crude gambir (28.6632 µg/mL), followed by methanolic leaf extract (24.3528 µg/mL), ethyl acetate extract of purified gambir (17.10 µg/mL), aqueous crude gambir (16.53 µg/mL), and aqueous purified gambir (11.74 µg/mL). Overall, ethyl acetate produced higher catechin concentrations and gallic acid-equivalent responses than water for both gambir types (Table 4).

A previous study ^[36] demonstrated that the use of ethyl acetate during the percolation stage significantly increased catechin purity from 92.45% to 99.80%. This effectiveness may be attributed to the semipolar nature of ethyl acetate, which facilitates the selective extraction and separation of catechin from other constituents.

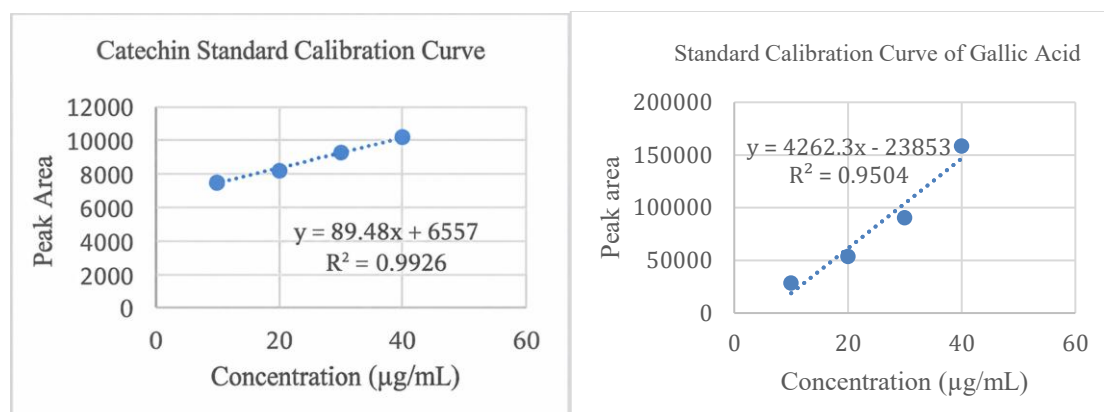


Figure 7. Calibration Curve of Catechin and Gallic Acid Standards

Table 4. Results of Catechin and Tannin Content Analysis

No.	Extract	Catechin Content	Tannin as a response to Gallic
		(µg/mL)	Acid Equivalents (µg/mL)
1	Methanol extract of leaves	853.6544	24.3528
2	Pure gambir water extract	715.2548	11.7422
3	Crude gambir water extract	798.9495	16.5352
4	Pure gambir ethyl acetate extract	924.0948	17.1027
5	Ethyl acetate extract of crude gambir	922.2173	28.6632

In contrast, tannins are predominantly polar compounds and can therefore be more effectively extracted using polar solvents [39]. The present results indicate an inverse relationship between catechin concentration and the gallic acid-equivalent response attributed to tannins, whereby higher catechin concentrations were generally associated with lower tannin-related responses. This finding suggests that extraction conditions favoring catechin enrichment may simultaneously reduce the relative extraction of tannin-related constituent.

Determination of Total Phenolic Content

The total phenolic content of the sample was determined using UV-Vis spectrophotometry with gallic acid as the reference standard. The analysis was performed in duplicate using the ethyl acetate extract of crude gambir, based on a calibration curve prepared from the gallic acid standard (Figure 8).

The total phenolic content of the ethyl acetate extract of crude gambir was determined using

the Folin–Ciocalteu method, with gallic acid as the standard, and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The first and second replicates yielded total phenolic contents of 114.63 and 115.09 mg GAE/g, respectively, with a mean value of 114.86 mg GAE/g (Table 5).

The mean total phenolic content of 114.86 mg GAE/g indicates that approximately 11.49% of the extract, expressed as gallic acid equivalents, produced a response equivalent to gallic acid under the applied analytical conditions. This value indicates that the ethyl acetate fraction of crude gambir is relatively rich in phenolic constituents. This finding is consistent with the chemical characteristics of gambir, which contains various polyphenolic compounds, with catechin as one of its major constituents. Analysis of gambir products has shown that catechin is the predominant flavan constituent, whereas epicatechin and several dimers, including procyanidin B1, procyanidin B3, and gambirin A1, occur at lower levels [44].

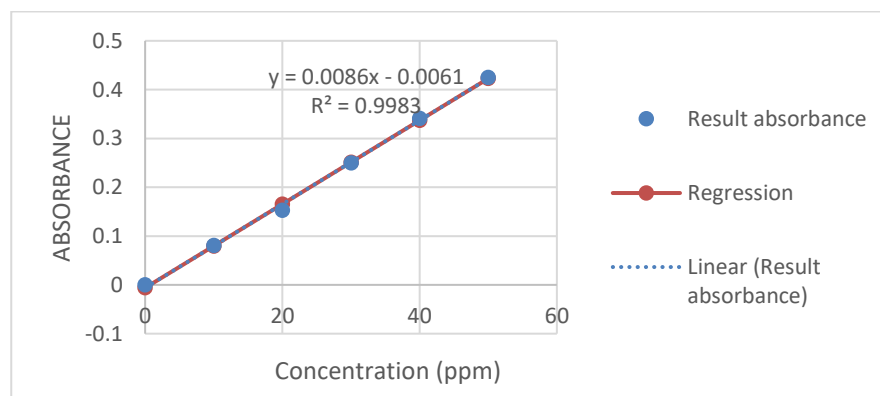


Figure 8. Standard Calibration Curve for Gallic Acid

Table 5. Results of the Analysis of Total Phenolic Content

No.	Extract	Weight of extract (g)	Concentration (ppm)	Total phenolic content (mg GAE/g)
1.	Ethyl acetate extract of gambir 1 (repeat 1)	0.0050	114627.9	114.63
2.	Ethyl acetate extract of gambir 2 (repeat 2)	0.0050	115093.0	115.09
Average				114.86

The present finding is also consistent with Kassim et al ^[34], who compared the total phenolic contents of gambir extracts obtained using different solvents and reported values of 113.43 mg GAE/g for ethyl acetate, 99.25 mg GAE/g for methanol, and 76.75 mg GAE/g for hot-water extracts. The relatively high phenolic content of the ethyl acetate extract may also be relevant to its antioxidant potential. Phenolic compounds, particularly flavonoids such as catechin, contain hydroxyl groups that can participate in hydrogen- or electron-donation mechanisms to neutralize radical species. The antioxidant properties of gambir have been associated with its catechin and polyphenolic constituents. Munggari et al ^[14] reported that catechin is among the most abundant phenolic constituents of gambir and that its content is associated with the antioxidant activity of gambir extracts.

Antibacterial Activity Test

The antibacterial activity of the ethyl acetate extract of crude gambir against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 was evaluated using the disk diffusion method. The results showed that the extract inhibited the growth of both tested bacterial strains, exhibiting moderate antibacterial activity (Table 6).

For *Escherichia coli* ATCC 25922, the ethyl acetate extract of crude gambir produced inhibition zones of 7.05 ± 0.05 , 7.79 ± 0.46 , 7.60 ± 0.83 , and 6.79 ± 0.27 mm at concentrations of 25, 50, 75, and 100 mg/mL, respectively. The largest inhibition zone was observed at 50 mg/mL, whereas the smallest was observed at 100 mg/mL. In contrast, against *Staphylococcus aureus* ATCC 25923, the extract produced inhibition zones of 7.16 ± 0.25 , 7.41 ± 0.27 , 8.25 ± 0.39 , and 8.37 ± 0.47 mm at 25, 50, 75, and 100 mg/mL, respectively.

Unlike *E. coli* ATCC 25922, the inhibition zone against *S. aureus* ATCC 25923 generally increased with increasing extract concentration, although the increase was relatively small. Nevertheless, the antibacterial activity remained lower than that of the positive control. Chloramphenicol (1 mg/mL) produced an inhibition zone of 11.42 ± 1.97 mm, indicating greater antibacterial activity than all tested extract concentrations. In contrast, the negative control (10% DMSO) produced no inhibition zone against either bacterial strain, confirming that the solvent itself did not exhibit detectable antibacterial activity under the applied conditions.

Table 6. Inhibition Zones of Gambir Extract Against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923

No.	Ethyl acetate extract of gambir (mg/mL)	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923
1.	25	7.05 ± 0.05	7.16 ± 0.25
2.	50	7.79 ± 0.46	7.41 ± 0.27
3.	75	7.6 ± 0.83	8.25 ± 0.39
4.	100	6.79 ± 0.27	8.37 ± 0.47
5.	negative control (DMSO 10%)	-	-
6.	Positive control (chloramphenicol 1 mg/mL)	11.42 ± 1.97	17.19 ± 0.57

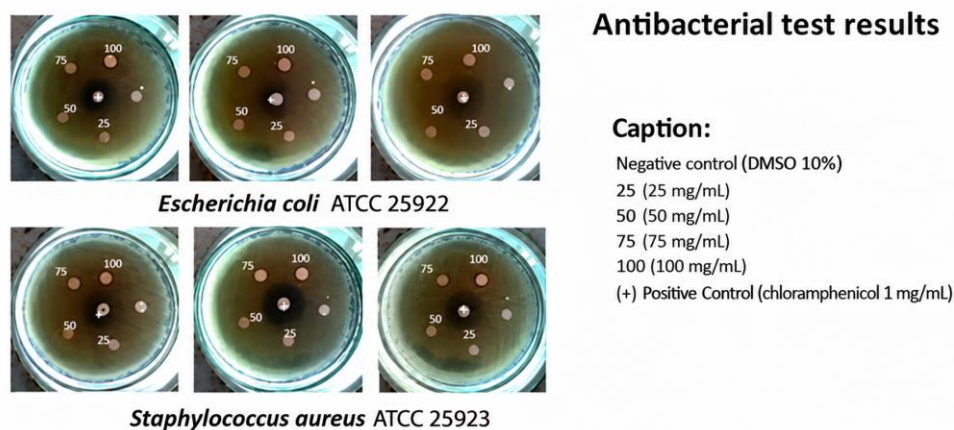


Figure 9. Inhibition Zones in Test Strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923

In the present study, the inhibition zones of the ethyl acetate extract of crude gambir ranged from 6.79 to 8.37 mm, corresponding to moderate antibacterial activity according to the classification of Davis and Stout [39], which defines inhibition zones of <5, 5–10, 10–20, and >20 mm as weak, moderate, strong, and very strong activity, respectively.

Melia et al [42] reported stronger antibacterial activity of gambir ethyl acetate extract against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, with inhibition zones of 15.6 and 34.2 mm, respectively, which was associated with catechin and tannin contents. Similarly, Uyun et al [43] reported inhibition zones of 12.18 and 12.53 mm against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, respectively, for gambir-derived catechin at 10%. These effects may be related to gambir secondary metabolites, particularly catechin, epicatechin, tannins, flavonoids [41], and other phenolic compounds that can disrupt membrane permeability, precipitate proteins, interfere with enzymes, and inhibit nucleic acid synthesis [38].

Differences in antibacterial activity may result from variations in extraction solvent, catechin content, raw-material origin, extraction method, extract concentration, assay method, and bacterial strain. Ethyl acetate generally extracts semipolar fractions rich in phenolic and flavonoid compounds, although their composition depends on the extraction process and plant source [14]. The lack of a proportional

increase in inhibition zones with increasing extract concentration, particularly against *E. coli*, may be attributed to reduced diffusion at higher extract viscosity and interactions among secondary metabolites, including binding, precipitation, or antagonistic effects. Thus, the relationship between extract concentration and inhibition-zone diameter in disk diffusion assays is not necessarily linear [40].

Conclusions

Under The Applied Extraction Conditions, Ethyl Acetate Extraction Of Purified Gambir Produced The Highest Yield (98.62%), Whereas Aqueous Extraction Of Crude Gambir Yielded 43.25%. Phytochemical Screening Confirmed Phenolics, Tannins, And Flavonoids In All Samples, While Saponins, Triterpenoids, Steroids, And Alkaloids Varied With Raw Material And Solvent.

Uv–Vis Analysis Showed The Highest Catechin-Equivalent Response In The Ethyl Acetate Extract Of Purified Gambir (78.24%), Consistent With The Highest Catechin Concentration Obtained By HPLC. The Highest Gallic Acid-Equivalent Response Attributed To Tannin-Related Constituents Was Found In The Ethyl Acetate Extract Of Crude Gambir (28.66 µg/mL), Which Also Showed A Total Phenolic Content Of 114.86 Mg Gae/G. This Extract Exhibited Moderate Antibacterial Activity Against *Escherichia Coli* ATCC 25922 And *Staphylococcus*

Aureus ATCC 25923, With Inhibition Zones Of 6.79–7.79 And 7.16–8.37 Mm, Respectively. Overall, Ethyl Acetate Extraction Yielded Higher Extract Yields Than Aqueous Extraction, While Raw-Material Type And Extraction Conditions Influenced The Chemical Characteristics And Antibacterial Activity Of Gambir Extracts. These Findings Support Further Optimization Of The Extraction Process And Evaluation Of Its Economic Feasibility.

Acknowledgments

The authors would like to express their sincere gratitude to the Research and Development Agency of West Sumatra Province (Balitbang Provinsi Sumatera Barat) for funding this research. The authors also gratefully acknowledge Prof. Dr. Dra. Suryati, M.Si., for facilitating the research, as well as all parties who contributed directly or indirectly to the completion of this study.

Declaration of Generative AI and AI-assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

Conceptualization, D.W.K. U.K.N. and S.; methodology, S.; validation, D.W.K.; formal analysis, S.; investigation, D.W.K.; resources, S. and D.W.K.; data curation, D.W.K.; writing—original draft, D.W.K and S.; writing—review and editing, D.W.K.; visualization, S.; supervision, S.; project administration, H.M.; funding acquisition, H.M.

Funding

This research received no external funding.

Declaration of Conflicts of Interest

The authors declare no conflict of interest.

References

1. Nasution, AH., Asmarantaka, RW dan Baga, L. Efisiensi pemasaran gambir di kabupaten lima puluh kota, sumatera barat. Buletin Ilmiah Litbang Perdagangan. 9(2): 221-240 (2015).
2. Widiyarti, G., Sundowo, A., Filaila, E and Laksmo, JA. The mechanically extraction process from leaves and twigs of gambier (*Uncaria gambier* Roxb) and its antioxidant activity. J. Pure App. Chem.Res. 9(1): 8-15 (2020).
3. Badan Pusat Statistik Sumatera Barat. Provinsi Sumatera Barat dalam Angka (2025).
4. Malik, D., Aflizar, Afner, SOG., Fukuda, A.Masunaga, T. Socio economical evaluation of uncaria gambier cultivation systems in West Sumatra, Indonesia. Tropics. 29(3): 77-87 (2020).
5. Ferdinal, N., Nazir, N. Phytochemical screening and fractionation of gambier (*Uncaria gambier*). The Journal of Biomaterial Chemistry. 1(1): 23-26 (2013).
6. Rauf, A., Rahmawaty, Siregar, AZ The condition of uncaria gambier roxb. as one of important medicinal plants in north sumatra indonesia. Procedia Chemistry. 14: 3 – 10 (2015).
7. Ferdinal, N. A simple purification method of catechin from gambier. Int.J. on Adv Sci Engg Inform Tech. 4(6): 53 – 55 (2014).
8. Anggraini, T., Rini, Aini, L. The extraction of gambier by using ultrasonic assisted at various temperature. IOP Conf. Series: Earth and Env. Sci. 757: 012058 (2021)
9. Monica, S., & Husna, Z. Antibacterial activity test of (+)- catekin and gambir (*uncaria gambier* roxb.) against some types of grammbled bacteria negative bacteria and their mechanism. International Journal on Health and Medical Sciences, 1(1): 29–37 (2022).
10. Kasim, A., Asben, A., Mutiar, S. Kajian kualitas gambir dan hubungannya dengan karakteristik kulit tersamak. Majalah Kulit, Karet dan Plastik. 31(1): 55-64 (2015).
11. Arumsari, A.G. Analysis of catechins in gambir west sumatra. ARRUS J of Engg and Tech, 1(2):

- 47-54 (2021).
12. Sovira, GDJ., Mariam, M.S., Satari, M.H. Antimicrobial properties of various solvents combinations for phytochemical fraction derived from *Uncaria gambier* extract against *Enterococcus faecalis* ATCC 29212. *Padjadjaran Journal of Dentistry*. 33(1): 32-38 (2021).
 13. Yunarto, N., Elya, B., Konadi, L. Potensi fraksi etil asetat ekstrak daun gambir (*Uncaria gambier* Roxb.) sebagai antihiperlipidemia. *Jurnal Kefarmasian Indonesia*. 5(1): 1-10 (2015).
 14. Munggari, IP., Kurnia, D., Deawati, Y and Julacha, E. Current research of phytochemical, medicinal and non-medicinal uses of *Uncaria gambier* Roxb.: A Review. *Molecules*. 27:6551 (2022).
 15. Yeni, G., Syamsu, K., Suparno, O., Mardiyati, E. and Muchtar, H. Repeated extraction process of raw gambiers (*Uncaria gambier* Robx.) for the catechin production as an antioxidant. *International J. of Applied Engineering Research*. 9(24): 24565-24578 (2014).
 16. Anova, IT., dan Yeni, G. Rasio pelarut etanol dan etil asetat pada proses ekstraksi terhadap karakteristik katekin dari gambir. *Jurnal Litbang Industri*. 10(2): 121 – 127 (2020).
 17. Fakhruzy, Kasim, A., Asben, A., Anwar, A. The effect of microwave time and power on the tannin extraction process from gambier (*uncaria gambier* roxb.) using the microwave assisted extraction (MAE) Method. *J. of Appl Agr. Sci and Tech*. 9(2): 276-285 (2025).
 18. Awaliyan, HMR., Rosamah, E. dan Sukaton, E. Karakteristik tanin dari ekstrak kulit kayu leda (*Eucalyptus deglupta* Blume.). *Jurnal Hutan Tropik*. 1(1): 16-28 (2017).
 19. Evalia, NA., Sa'id, E.G., dan Suryana, RN. Strategi pengembangan agroindustri dan peningkatan nilai tambah gambir di Kabupaten Lima Puluh Kota Sumatera Barat. *Jurnal Manajemen & Agribisnis*, 9(3): 173-182 (2012).
 20. Sa'id, E.G., Syamsu, K., Mardiyati, E., Herryanie, A., Evalia, NA., Rahayu, DL., Puspitarini, Ahyaruddin, Hadiwijono, A. Agroindustri dan Bisnis Gambir di Indonesia. IPB Press (2009).
 21. Yeni, G., Khaswar Syamsu, Mardiyati, E. dan Muchtar, H. Penentuan teknologi proses pembuatan gambir murni dan katekin terstandar dari gambir asalan. *Jurnal Litbang Industri*, 7 (1): 1-10 (2017).
 22. Rahman, ED., Sari, E., Burmawi, Frizka, Endah. Determination of extraction process conditions of gambier catechin (*uncaria gambier* roxb) from Solok Bio-Bio Lima Puluh Kota District – West Sumatera. *IOP Conf. Ser.: Mater. Sci. Eng*. 316 (2018).
 23. Satria, D., Eranisa, Y., Arel, A. Rapid detection and quantification of gambir adulteration using ATR-FTIR spectroscopy coupled with chemometric analysis. *J. Ris. Kim*. 16(2): 108-121 (2025).
 24. Rohima, N., Syahardi, A dan Ibnu sina, F. Analisis faktor penyebab fluktuasi harga gambir pada tingkat petani di Nagari Harau Kabupaten Lima Puluh Kota. *Jurnal Agri Sains*. 9 (1): 61-71 (2025).
 25. Nawaz, H., Shad, MA., Najiha Rehman, N., Andaleeb, H., Ullah, N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Braz. J. Pharm. Sci*. 56:17129: 1-9 (2020).
 26. Muhamad, N., Muhmed, S.A., Yusoffand, M.M., Gimbun, J. Influence of solvent polarity and conditions on extraction of antioxidant, flavonoids and phenolic content from *averrhoa bilimbi*. *J of Food Sci and Eng*. 4: 255-260 (2014).
 27. Dewi, JR., Estiasih, T. dan Murtini, ES. Aktivitas anti oksidan dedak sorgum lokal varietas coklat (*sorghum bicolor*) hasil ekstraksi berbagai pelarut. *Jurnal Teknologi Pertanian*, 8(3): 188-197 (2007).
 28. Rauf, R., Santoso, U., Suparmo. Aktivitas penangkapan radikal DPPH ekstrak gambir (*Uncaria gambier* roxb.). *Agritech*, 30(1). (2010).
 29. Isnawati, A., Raini, M., Sampurno, O.D Mutiatikum, D., Widowati, L., Gitawati, R. Karakterisasi tiga jenis ekstrak gambir ((*uncaria gambier* roxb) dari Sumatera Barat. *Bul. Penelit. Kesehat*, 40(4): 201–208 (2012).

30. Herdiana, I., Aji, N. Fraksinasi ekstrak daun sirih dan ekstrak gambir serta uji antibakteri streptococcus mutans. *Jurnal Kesehatan Ilmiah*. 19(3): 100-106 (2020).
31. Muchtar, H., Yeni, G., Hermianti, W., Diza, Y.H., Pembuatan konsentrat polifenol gambir (*Uncaria gambir Roxb*) sebagai bahan antioksidan pangan. *J. Ris. Ind.* 4(2): 71–82 (2010).
32. Damanik. D., Surbakti, N., & Hasibuan, R. Ekstraksi katekin dari daun gambir (*Uncaria gambir roxb*) dengan metode maserasi. *Jurnal Teknik Kimia USU*, 3 (2) : 10-14 (2014).
33. Kurniatri, A.A., Sulistyaningrum, N., Rustanti, L. Purifikasi katekin dari ekstrak gambir (*uncaria gambir roxb.*). *Media Litbangkes*, 29(2): 153 – 160 (2019).
34. Kassim, M.J., Hussin, M.H., Achmad, N., Dahon, H., Suan, K., Hamdan, S., Determination of total phenol, condensed tannin and flavonoid contents and antioxidant activity of *uncaria gambir* extracts. *Majalah Farmasi Indonesia*. 22(1): 50-59 (2011).
35. Sakti, AS., Saputri, FC., Mun'im, A. Microscopic Characters, Phytochemical screening focus on alkaloid and total phenolic content of *uncaria gambir roxb.* and *uncaria sclerophylla roxb.* leaves. *Pharmacognosy Journal*. 11(1):119-123 (2019).
36. Ibrahim N, Mohd Yusoff NZ, Ahmad R. Quantification of catechin in leaves and stems of malaysian *Uncaria gambir* (hunter) *roxb.* by HPLC-DAD. *Malaysian J Anal Sci.*; 20(3):567–72 (2016).
37. Hersila, N., Chatri, M., Vauzia, & Irdawati. Senyawa metabolit sekunder (tanin) pada tanaman sebagai antifungi. *Jurnal Embrio*, 15, 16–22 (2023).
38. Daglia, M. Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, 23(2) : 174–181 (2012)
39. Davis, W. W., & Stout, T. R. Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. *Applied Microbiology*, 22(4): 659–665 (1971).
40. Eloff, J. N. Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. *BMC Complementary and Alternative Medicine*, 19(1), 106 (2019)
41. Marlina, P. Pemanfaatan Gambir (*Uncaria Gambir Roxb*) sebagai Antioksidan dan Pengaruhnya terhadap Umur Simpan Minyak Goreng. *Jurnal Dinamika Penelitian Industri*, 21(1) (2010)
42. Melia, S., Novia, D., & Juliyarsi, I. Antioxidant and Antimicrobial Activities of Gambir (*Uncaria gambir Roxb*) Extracts and Their Application in Rendang. *Pakistan Journal of Nutrition*, 14(12 SE-Research Article) : 938–941(2015).
43. Uyun, H. S. K., Putra, P. M., Suhardana, G., Bellatasie, R., Wiliantari, S., Fajrina, A., & Ramadhani, P.. Extraction of Gambier using the boiling method and antibacterial activity test. *Riset Informasi Kesehatan*, 14(2): 276–282 (2025).
44. Taniguchi, S., Kuroda, K., Doi, K.-I., Inada, K., Yoshikado, N., Yoneda, Y., Tanabe, M., Shibata, T., Yoshida, T., & Hatano, T. Evaluation of gambir quality based on quantitative analysis of polyphenolic constituents. *Yakugaku Zasshi*, 127(8), 1291–1300 (2007).