

# Analysis of tannin and catechin levels in ethyl acetate extract of gambir twigs (*Uncaria gambir* Roxb.) using UV-Vis spectrophotometry

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## ABSTRACT

The utilization of gambir twigs (*Uncaria gambir* Roxb.) as a source of bioactive compounds is still relatively minimal, even though gambir has the potential to contain tannins and catechins, which enable it to possess antioxidant, anti-inflammatory, and antimicrobial activities. This study aims to analyze the tannin and catechin content of gambir twig extracts tested using the UV-Vis spectrophotometry method. Extraction was performed using the maceration method with ethyl acetate solvent, followed by qualitative analysis using FeCl<sub>3</sub> reagent. Quantitative analysis was conducted using the Folin-Ciocalteu method to determine the tannin content at a wavelength of 742 nm and catechin analysis at a wavelength of 280 nm. The research results show that the extraction yielded a yield of 17.83% with a DER-native value of 5.60. Qualitative analysis with the FeCl<sub>3</sub> reagent gave a positive result, indicated by the formation of dark greenish-black spots, showing the presence of phenolic compounds. The tannin content was measured at 0.290% w/w, with the regression equation  $y = 0.0072x + 0.2172$  ( $R^2 = 0.9523$ ), while the catechin analysis with the regression equation  $y = 0.0019x + 0.049$  was 0.006%. The analytical method shows good sensitivity with a detection limit of 10.5563 µg/mL and a quantification limit of 35.1877 µg/mL. The results of this study provide important information about the potential of gambir branches as a source of natural bioactive compounds.

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## INTRODUCTION

Gambir (*Uncaria gambir* Roxb.) is a tropical plant commonly found in Southeast Asia, especially in Indonesia and Malaysia (Ibrahim, Mohd Yusoff, & Ahmad, 2016). This plant has the ability to grow in various soil and climate conditions, making it an important natural resource for the local community. For centuries, gambir has been used in various fields of life, including industry, health, and traditional medicine (Mega Silvianti, 2024). Traditionally, gambir has been used for various health purposes. Various scientific studies have demonstrated the pharmacological

potential of gambir, particularly the catechin content found in gambir leaf extracts (Nandika, Syamsu, Arinana, Kusumawardhani, & Fitriana, 2019).

The leaves and twigs of the gambir plant are parts of the plant that are often processed, especially to extract their essence (Rahman, 2016). Gambir extract, particularly from the twigs, contains bioactive compounds such as tannins and catechins known for their beneficial antioxidant and antimicrobial properties (Hidayati & Rahmatulloh, 2022; Aprely et al., 2021). Tannins and catechins are compounds that are widely utilized in health and cosmetic products in the modern industry due to their antimicrobial and antioxidant activities (Munggari et al., 2022; Rahmaddiansyah et al., 2023).

While most previous research has focused on gambir leaves as the primary source of bioactive compounds, emerging studies suggest that gambir twigs represent an underutilized source with significant phytochemical potential. Research by Ibrahim, Yusoff et al. 2016 demonstrated that catechin content in gambir stems reaches 5.12% in methanol extracts, which, although lower than leaves (8.64%), still represents a substantial concentration of bioactive compounds (Ibrahim, Yusoff, & Ahmad, 2016). Importantly, the traditional gambir extraction process has historically utilized both leaves and stems, suggesting that indigenous knowledge recognized the value of these plant parts in combination (Oshima et al., 2019). The different anatomical structure of twigs, characterized by their woody tissue composition and distinct vascular arrangement, may contribute to unique patterns of secondary metabolite accumulation and potentially enhanced stability of phenolic compounds compared to the more delicate leaf tissues (Munggari et al., 2022). Despite their historical use in traditional gambir processing, twigs have been largely overlooked in modern phytochemical research, with most contemporary studies concentrating exclusively on leaf extracts due to established extraction protocols. This research gap represents a significant opportunity for sustainable resource utilization, as twigs are often considered agricultural waste in current gambir processing operations, yet they may harbor substantial bioactive potential (Bancin, 2021). The woody nature of gambir twigs provides advantages in terms of seasonal availability, storage stability, and higher biomass yield compared to leaves, while their different anatomical structure may contribute to unique patterns of secondary metabolite accumulation and enhanced stability of phenolic compounds (Munggari et al., 2022) (Zilullah et al., 2022).

The gambir twigs specifically have interesting potential because they contain phenolic compounds that play important roles in both industry and pharmacy. There are two main compounds in gambir twigs, namely tannins and catechins, which have important therapeutic benefits and bioactivity. The chemical composition of gambir twigs is very important for developing their potential use in various applications (Yeni, Syamsu, Mardliyati, & Muchtar, 2017). Research shows that tannins and catechins in gambir twigs exhibit significant biological activities such as antioxidant and antimicrobial properties (Nandika et al., 2019); (Saad et al., 2020). Tannins in gambir extract have a concentration of up to 15% of dry weight, indicating its potential for development in health and cosmetic products (Hasanah, 2019); (Rosaini, Makmur, Lestari, Sidoretno, & Yetti, 2021). Tannin and catechin compounds also play an important role in oxidative prevention, providing therapeutic benefits in combating degenerative diseases, such as cancer and cardiovascular diseases (Auliana, Ifora, & Fauziah, 2022).

Additionally, research by Rahmaddiansyah et al., (2023) shows that catechins found in gambir twigs have potential in medicinal applications due to their anti-inflammatory properties and ability to inhibit the growth of pathogenic bacteria. This makes gambir twigs a rich source of bioactive compounds that can be further utilized in the development of safe and effective health products (Rahmaddiansyah et al., 2023).

This research aims to qualitatively and quantitatively explore the tannin and catechin compounds found in gambir twigs, which have significant potential in the health and cosmetics industries. The extraction process was carried out using ethyl acetate solvent on the dried powder

of gambir twigs to extract the tannin and catechin compounds present in the gambir twigs. The determination of the bioactive compound content was carried out using the UV-Vis spectrophotometry method. This finding opens up opportunities for further research to explore the applications of gambir twig compounds in the health and cosmetics industries, while also supporting the added value and sustainability of the gambir plant as a natural raw material

## RESEARCH METHOD

### Materials

The materials used in this study are gambir twigs (*Uncaria gambir* Roxb) obtained from Nagari Sari Lamak, Harau District, Lima Puluh Kota Regency, West Sumatra Province. Tannin and catechin standards from Henan Senyuan Biological Technology, co.ltd., sulfuric acid, Folin-Ciocalteu reagent, sodium carbonate, ferric chloride, distilled water, ethanol p.a., ethyl acetate p.a.,

### Equipment

A set of UV-Vis spectrophotometer (1240 mini shimadzu), analytical balance (Ohaus), rotary evaporator (Heidolph), filter paper, volumetric flask, volumetric pipette, micropipette, dropper pipette, test tube, spatula, dark bottle, funnel, beaker, timer, evaporating dish.

### Sample Processing

The gambir branches are cleaned of impurities and foreign organic materials, air-dried, dried away from sunlight, and the dried branches are ground using a blender until gambir branch powder is obtained.

### Preparation of Ethyl Acetate Extract

The method used for extract preparation is maceration with ethyl acetate as the solvent, at a ratio of 1:10. Extraction is carried out for 3 days, then repeated using fresh ethyl acetate twice. The extract was concentrated using a rotary evaporator and an oven at 40°C, resulting in a thick extract.

### Qualitative Tannin Test

100 mg of gambir twig extract was dissolved in 96% ethanol and FeCl<sub>3</sub> was added. If a color change to dark blue-green occurs, it indicates that the sample is positive for tannin compounds (Irianty & Sembiring, 2012).

### Qualitative test for catechin

Weigh 2 grams of the extract, then add 100 mL of water and boil for 15 minutes. After that, cool and filter to obtain the filtrate. The filtrate is pipetted 2 mL and placed into a test tube, then added with 1% FeCl<sub>3</sub> solution. If a greenish-purple or black color forms, this result indicates that the sample contains catechins (Fajrina, Jubahar, & Sabirin, 2017)

### Determination of Tannin Content

The determination of tannin content was carried out using the general phenolic analysis method, namely the Folin-Ciocalteu method. (Chaovanalikit & Wrolstad, 2004): a) Determination of maximum wavelength, 0.3 mL of a 120 µg/mL tannin standard solution was added to 1.5 mL of 10% Folin-Ciocalteu Reagent, allowed to stand for 3 minutes. Then, 1.2 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added, allowed to stand for 5 minutes in the dark. Absorbance is measured at a wavelength range of 600-800 nm; b) Preparation of the tannin standard curve, 0.3 mL of tannin standard solution with concentrations of 20, 30, 40, 50, 60 µg/mL was added to 1.5 mL of 10% Folin Ciocalteu reagent, allowed to stand for 3 minutes. Then, 1.2 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added, allowed to stand for 5 minutes in the dark. All solutions were measured for their absorbance at a wavelength of 742 nm, with three repetitions conducted. A calibration curve was created to show the relationship between tannin concentration and absorbance; c) Determination of tannin content, 10 mg of extract is dissolved in ethyl acetate to a volume of 10 mL, then 5 mL is

pipetted and the volume is adjusted to 10 mL with ethyl acetate. 0.3 mL is pipetted and 1.5 mL of 10% Folin Ciocalteu reagent is added, left for 3 minutes. 1.2 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution is added and left in the dark for the operating time. The absorbance was measured at a wavelength of 742 nm, with three repetitions performed.

#### Determination of Catechin Content

Preparation of Catechin Standard Solution 10 mg of pure catechin was weighed and dissolved using 10 mL of ethyl acetate solvent in a 10 mL flask to obtain a pure catechin solution concentration of 1000 µg/mL. Then, 1 mL, 1.5 mL, 2 mL, 2.5 mL, and 3 mL of the 1000 µg/mL pure catechin solution were pipetted and diluted with ethyl acetate solvent in a 10 mL flask to the mark, resulting in concentrations of 100 µg/mL, 150 µg/mL, 200 µg/mL, 250 µg/mL, and 300 µg/mL (Nur, 2020): a) Determination of Maximum Wavelength, the determination of the maximum wavelength of catechin is conducted by measuring the standard catechin solution (300 µg/mL) with a spectrophotometer at a wavelength of 200-400 nm; b) Determination of Catechin Content, the determination of catechin content was carried out using the method from Syamsu Nur et al. (2020), which involved weighing 10 mg of extract dissolved in ethyl acetate up to 10 mL. Pipetting 5 mL into a 10 mL volumetric flask and then adjusting the volume to the mark. The sample solution's absorbance is measured using a UV-Visible spectrophotometer at a maximum wavelength of 280 nm. (Fajrina et al., 2017).

#### Data Analysis

The research data were analyzed based on the linear regression equation  $y = bx \pm a$  obtained from the absorbance and concentration data of the standard solution (Sugiyono & Alfabet, 2003).

## RESULTS AND DISCUSSIONS

In this study, the sample used was gambir twigs (*Uncaria gambir* Roxb.). Before testing, the gambir twigs were cleaned to remove dirt, then underwent an air-drying process to eliminate any residual water from the washing process. After that, the twigs were further dried and ground to reduce moisture content, which serves to prevent microbial growth and extend shelf life (Martiningsih, Kusumawati, Pgri, Puri, & Kartini, 2023). This grinding process is important for preparing the material in the extraction process, as finer particles will increase the surface area of the sample, thereby accelerating and enhancing the efficiency of extracting active compounds such as catechins and tannins. The smaller the particle size, the larger the surface area, which allows for optimal extraction of active compounds (Munggari et al., 2022).



Figure 1. (a) Gambir bransh (b) gambir branch powder

Ethyl acetate is used as a semi-polar solvent in the extraction of catechins from gambir twigs because it has a polarity that matches the moderate lipophilic nature of catechins (Anova & Yeni, 2020). This solvent is more selective towards simple phenolic compounds with small molecular weights, allowing it to extract catechins in higher quantities compared to tannins, which

are more polar and have larger molecular weights. (Suputri, Ananto, & Andayani, 2021)(Afriwardi, Wahyuni, Husni, Alianta, & Aldi, 2022). Extraction with ethyl acetate yields significant concentrations of catechins and high antioxidant activity (Malrianti, Kasim, Asben, & Yeni, 2020). In addition, ethyl acetate also allows for extraction at low temperatures due to its low boiling point, preserving the stability of heat-sensitive bioactive compounds (Masela, 2021). To obtain a more complete extract profile, ethyl acetate is often combined with more polar solvents, such as ethanol or water (Jhon Rouberd Yomilena, Muhammad Yusuf, & Andi Meinardwi Rantisari, 2023). The maceration method is used in this process to maintain the stability of heat-sensitive secondary metabolites, ensuring they can still provide the desired pharmacological activity (Candra, Andayani, & Wirasisya, 2021).

**Table 1.** Results of gambir branch extraction using ethyl acetate solvent

No	Type	Calculation Result
1	Gambir twig powder	60 g
2	Gambir twig extract	10,7 g
3	DER-native	5,60
4	Yield	17,83%

The use of ethyl acetate as a solvent resulted in a yield of 17.83% with a native DER of 5.60, indicating that approximately 5.6 grams of raw material are needed to obtain 1 gram of extract. This value indicates that the extraction efficiency is not yet optimal. As a semi-polar solvent, ethyl acetate is effective in extracting phenolic compounds and flavonoids such as catechins and tannins from gambir, but tends to yield lower yields compared to polar solvents like methanol or water, which are more efficient in extracting hydrophilic compounds (Shoviyyah, 2019). Therefore, a combination of solvents or a multi-solvent approach can be considered to obtain a broader spectrum of bioactive compounds.

This moderate yield of 17.83% has the potential to be improved with modern extraction techniques, such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), which have been proven to enhance the efficiency and speed of extraction by reducing the diffusion resistance between the solvent and the raw material (Husna, Noor, Fahma, & Sunarti, 2022). The use of a combination of polar and non-polar solvents can also increase the extraction yield of phenolic-rich materials such as gambir, while the use of a single solvent like ethyl acetate can limit the spectrum of bioactive compounds obtained. To optimize the content of active compounds, a stepwise extraction using more polar solvents, such as ethanol or water, can also be considered. (Ihsan, Anggraini, & Azzahro, 2023).

Phytochemical screening was conducted using  $\text{FeCl}_3$  reagent to detect phenolic content, indicated by a color change to dark green, suggesting the presence of tannins in the sample (Noviyanty, 2020). The tannin content is measured using the Folin-Ciocalteu method, which is a spectrophotometric method based on oxidation-reduction reactions that produce a blue-colored complex and can be measured at a wavelength of 742 nm, which is the optimal value for detecting phenolic compounds (Dominguez-López, Pérez, & Lamuela-Raventós, 2023). The combination of the  $\text{FeCl}_3$  test and the Folin-Ciocalteu method provides a comprehensive overview of the catechin and tannin content in gambir twigs, thereby enabling the assessment of the extract's quality and pharmacological potential. Measurements at a wavelength of 742 nm ensure the accuracy of phenolic content detection, supporting the utilization of gambir in pharmaceutical and food applications due to its antioxidant activity. This method can serve as a basis for further research to explore the bioactivity potential of gambir in antioxidant products and food additives. Further studies are recommended to verify the influence of environmental factors on tannin levels and the potential bioactivity of gambir extract (Kim et al., 2018).

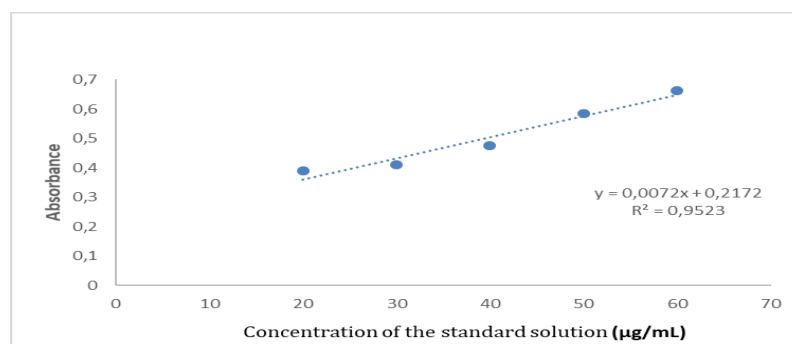


Figure 2. Calibration curve of standard tannin solution (n=3, RSD = 0.0253)

Based on the calibration curve obtained at a wavelength of 742 nm, the tannin content analysis in the sample shows a concentration of 0.290% weight per weight (w/w). This value is obtained by utilizing the regression equation  $y = 0.0072x + 0.2172$  from the calibration curve, where the absorbance measured at this wavelength can be used to determine the tannin concentration in the test sample. The spectrophotometric method with Folin-Ciocalteu reagent is very suitable for quantifying tannins in plant-based samples such as gambir, where tannins are one of the main active compounds with pharmacological and industrial potential.

A tannin content of 0.290% b/b indicates a significantly substantial tannin content in gambir extract. The significantly higher tannin concentration (0.290%) compared to catechin (0.006%) can be attributed to several chemical factors. First, tannins represent a broader class of polyphenolic compounds including both hydrolyzable and condensed tannins, while catechins are specific monomeric flavonoids. The semi-polar nature of ethyl acetate, despite its theoretical selectivity for smaller molecules, actually facilitates better extraction of polymeric tannins due to their varied polarity ranges and stronger matrix interactions. Additionally, catechins in gambir twigs may exist predominantly as precursors that have undergone polymerization to form condensed tannins during plant development, explaining their lower individual concentrations. Building upon this chemical rationale, comparison with commercial gambir leaf extracts containing 15-25% tannins and 7-33% catechins (Lukas, et al, 2020) reveals that while our twig extracts show significantly lower absolute concentrations, they exhibit a unique tannin-dominant profile with a remarkably high tannin:catechin ratio (48:1). This compositional uniqueness, reflecting the physiological role of twigs as structural tissues where condensed tannins predominate for mechanical protection, provides distinct pharmacological advantages including enhanced antioxidant stability, potent antimicrobial activity, and superior astringent properties (Jensen, 2023). Consequently, twig extracts represent valuable ingredients for specialized therapeutic applications requiring long-term stability and broad-spectrum bioactivity, particularly in formulations where moderate phenolic concentrations are preferred for safety considerations. Tannins are known to have strong antioxidant activity, so this content has the potential to provide significant therapeutic benefits, such as in the application of natural antioxidant products and antimicrobial agents (Sunani & Hendriani, 2023). Additionally, this method supports the analysis of tannin levels at lower concentration ranges, as evidenced by the Limit of detection (LoD) and Limit of quantification (LoQ) of 10.5563 µg/mL and 35.1877 µg/mL, respectively. This means that this calibration method is not only accurate for measuring tannin concentrations in the medium to high range but is also sensitive enough to detect tannins at low concentrations that may be encountered in further phytochemical research (Yaqin, Mahatmanti, Sulistyarningsih, & Nurcahyo, 2019). Additionally, this method supports the analysis of tannin levels at lower concentration ranges, as evidenced by the detection limit (DL) and quantification limit (QL) of 10.5563 µg/mL and 35.1877 µg/mL, respectively. This means that this calibration method is not only accurate for measuring tannin concentrations in the medium to high range but is also sensitive enough to detect

tannins at low concentrations that may be encountered in further phytochemical research. (Yaqin et al., 2019). This concentration of tannin also strengthens the developed calibration curve, which is relevant in the quantitative analysis of phenolic compounds in medicinal plants. This value serves as a reference for the development of tannin-based products from gambir, such as antioxidant tests or tannin stability in certain formulations (Aini, Salam, Syam, & Amir, 2021). This calibration curve provides a valid and reliable method for determining tannin content in various samples, facilitating research on the health benefits of phenolic compounds in tropical plants

Calibration analysis at a wavelength of 742 nm shows a linear relationship between tannin concentration ( $\mu\text{g/mL}$ ) and absorbance, with the regression equation  $y = 0.0072x + 0.2172$  and a determination coefficient  $R^2 = 0.9523$ . The  $R^2$  value approaching 1 indicates that 95.23% of the absorbance variability is explained by changes in tannin concentration, indicating very good linearity and showing that the spectrophotometric method at a wavelength of 742 nm is effective for quantitative analysis of tannins. The standard deviation of  $0.0253 \mu\text{g/mL}$  reflects the precision of absorbance measurements, while the detection limit (BD) and quantification limit (BK) of  $10.5563 \mu\text{g/mL}$  and  $35.1877 \mu\text{g/mL}$ , respectively, indicate the sensitivity of this method at low to medium concentrations, which is important for phytochemical analysis (Anggraini, Tai, Yoshino, & Itani, 2011). This method uses the reaction of tannins with the Folin-Ciocalteu reagent, which produces a blue complex and is measured at the optimal wavelength of 742 nm, sensitive to phenolic compounds. The regression equation of this calibration curve serves as the basis for calculating the tannin content from the sample absorbance. The intercept of 0.2172 indicates the presence of baseline absorbance, possibly from the solvent or other matrix influences, which need to be considered for accurate results.

Catechin is a flavonoid compound commonly found in gambir plants and possesses strong antioxidant properties. In the qualitative identification test, a 1%  $\text{FeCl}_3$  solution is used to detect phenolic compounds such as catechin based on the reaction of  $\text{Fe}^{3+}$  ions with the phenolic groups of catechin, which forms a colored complex as a visual indicator. In the gambir twig samples, the  $\text{FeCl}_3$  test results show a color change to purple-green or black, indicating the presence of catechin. This color is characteristic of the reaction between  $\text{Fe}^{3+}$  and catechin or other phenolic compounds with aromatic hydroxyl groups, indicating the bond between  $\text{Fe}^{3+}$  ions and the phenolic groups in catechin (Sukaesih, 2021).

Qualitative testing with  $\text{FeCl}_3$  provides initial indications of the presence of catechins in the sample, but to accurately determine their concentration, quantitative methods such as HPLC or UV-Vis spectrophotometry are required. The HPLC method can measure catechin levels with high precision, which is important for the standards of gambir-based products, especially in the pharmaceutical or health product fields (Li, Zhang, & Sun, 2020). The  $\text{FeCl}_3$  test, which produces a greenish-purple or black color, supports previous research indicating that *Uncaria gambir* has a high catechin content and functions as a natural antioxidant, neutralizing free radicals and protecting cells from oxidative damage (Sari, Rita, & Anas, 2018).

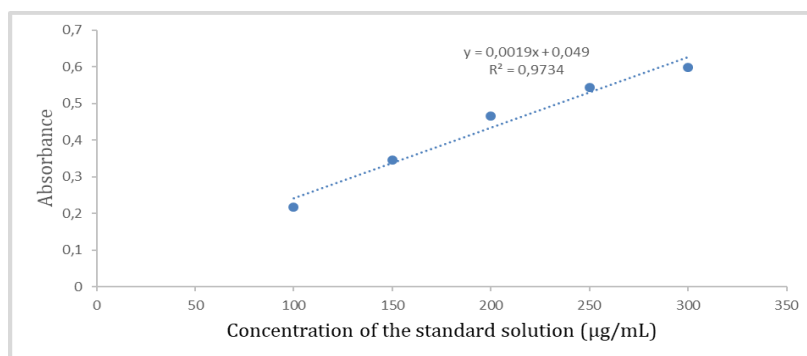


Figure 3. Calibration curve of pure catechin

Figure 3 shows the calibration curve of pure catechin detected at a wavelength of 280 nm. The selection of this wavelength is based on the characteristics of catechin as a flavonoid compound that exhibits optimal absorbance peaks and high selectivity at 280 nm, making it commonly used for the analysis of phenolic and flavonoid compounds in natural materials (Nur, 2020). The calibration curve yields the regression equation  $y=0.0019x+0.049$  with a coefficient of determination ( $R^2$ ) of 0.9734, indicating a very good linear relationship between catechin concentration and absorbance. This  $R^2$  value indicates that 97.34% of the absorbance variation can be explained by changes in catechin concentration, while the slope of the equation shows that each increase in catechin concentration by 1  $\mu\text{g}/\text{mL}$  will increase absorbance by 0.0019 units (Gil-Martín et al., 2022). The high linearity of this calibration curve validates the reliability of the method for the quantitative analysis of catechins within the tested concentration range. With good accuracy and precision, this method can be applied to determine the catechin content in various plant extracts, particularly in *Uncaria gambir*, which is known to be rich in catechin compounds with high antioxidant activity.

## CONCLUSION

From the research conducted, it can be concluded that the extraction of gambir twigs (*Uncaria gambir* Roxb.) using the maceration method with ethyl acetate solvent resulted in a yield of 17.83% (DER-native 5.60). The results of the spectrophotometric analysis showed catechins and tannins with concentrations of 0.006% and 0.290% w/w, respectively. Although the catechin concentration is lower than that of tannins, these results confirm the effectiveness of ethyl acetate in extracting both compounds. The higher tannin concentration actually provides superior commercial advantages for phytopharmaceutical and cosmetic applications, as tannins exhibit greater antioxidant stability, stronger astringent properties, and more potent antimicrobial activity than monomeric catechins. This tannin-dominant profile makes the extract particularly suitable for anti-aging formulations, wound healing products, and long-shelf-life nutraceutical applications, while the 17.83% yield ensures commercial feasibility. This finding reinforces the potential of gambir as a source of natural antioxidants applicable in pharmaceutical and food products with distinct competitive advantages over conventional catechin-based extracts. Future studies should explore mixed solvent optimization (ethyl acetate-methanol combinations), advanced purification techniques (solid-phase extraction, column chromatography), and comprehensive bioactivity testing (antioxidant, antimicrobial, anti-aging assays) to fully validate the commercial potential and develop standardized phytopharmaceutical applications.

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