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*Original Research Article*

# Optimization of Andrografolid Extraction from the Herb Sambiloto (*Andrographis Paniculata* (Burm.F.) Nees) Using a Combined Maceration and Refluction Method

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ABSTRACT

This study was conducted to investigate the optimal method for extracting andrographolide, the active compound in *Andrographis paniculata* (Sambiloto), using a combination of maceration and reflux. The extraction was carried out with 80% ethanol and methanol as solvents, while the raw material, Sambiloto simplicia, was standardized and screened before extraction. Maceration was performed for 16 hours, followed by reflux extraction, where the liquid extract was collected at 1-hour intervals for 6 hours. Thin-layer chromatography (TLC) was used to identify andrographolide content, and TLC densitometry was used for quantitative analysis at a sample concentration of 5000 ppm, with detection at 230 nm using chloroform: ethyl acetate: methanol mobile phase (6:2:2). The results showed that based on extraction yield calculated for each time point, 80% ethanol at the 5-hour produced a yield of 3.12%. Qualitative analysis with TLC, using vanillin sulfate as a chromogenic reagent, confirmed the presence of andrographolide, with RF values of 0.79 for the methanol and 0.91 for the ethanol extract. Quantitative analysis showed higher andrographolide levels in the 80% ethanol extract, with the third hour yielding the highest concentration at 6.68%. In conclusion, a combination of 16-hour maceration followed by 3-hour reflux using 80% ethanol was found to be optimal for andrographolide extraction. Longer reflux times are not recommended due to the ability to decrease andrographolide levels. These results underscore the importance of optimizing extraction time to improve the efficiency and yield of active compound.

*Keywords*: Optimization of levels, Andrographolide, Densitometry.

## Introduction

Sambiloto (*Andrographis paniculata* (Burm.f.) Nees), commonly known as the "King of Bitters" is a plant native to India and China[1]. Meanwhile, andrographolide ($C_{20}H_{30}O_5$) is a diterpenoid lactone contained in Sambiloto as the main active compound found in many parts of the plant. The highest content is found in the leaves, ranging from 2.5 - 4.8%.[2] It is also present in other parts such as roots, stems, flowers, and shoots but in small quantities.[3] Various pharmacological activities of Sambiloto extract have been reported, including the potential to be used as an analgesic, anticancer, antidiabetic, fertility-inhibiting agent, anti-inflammatory, antimalarial, antimicrobial, antipyretic, antiviral, hepatoprotective, and immunomodulator.[4]

This study was conducted to investigate the optimal method for extracting andrographolide, the active compound in Sambiloto, using a combination of maceration and reflux. The extraction was carried out with 80% ethanol and methanol as solvents, while the raw material, Sambiloto simplicia, was standardized and screened before extraction. Maceration was performed for 16 hours, followed by reflux extraction, where the liquid extract was collected at 1-hour intervals for 6 hours. Thin-layer chromatography (TLC) was used to identify andrographolide content, and TLC densitometry was used for quantitative analysis at a sample concentration of 5000 ppm, with detection at 230 nm using chloroform: ethyl acetate: methanol mobile phase (6:2:2). The results showed that based on extraction yield calculated for each time point, 80% ethanol at the 5-hour produced a yield of 3.12%. Qualitative analysis with TLC, using vanillin sulfate as a chromogenic reagent, confirmed the presence of andrographolide, with RF values of 0.79 for the methanol and 0.91 for the ethanol extract. Quantitative analysis showed higher andrographolide levels in the 80% ethanol extract, with the third hour yielding the highest concentration at 6.68%. In conclusion, a combination of 16-hour maceration followed by 3-hour reflux using 80% ethanol was found to be optimal for andrographolide extraction. Longer reflux times are not recommended due to the ability to decrease andrographolide levels. These results underscore the importance of optimizing extraction time to improve the efficiency and yield of active compound.

Secondary metabolites from a simplicia can be obtained through extraction. Various extraction methods that have been developed for andrographolide include maceration, reflux, percolation, soxhletation, and ultrasonication. However, it is necessary to optimize the extraction with the aim of finding variable values in the process that produce the best and optimal values. Optimization is focused on selecting the right method with a cost-effective and efficient process.

Several studies have developed extraction methods in terms of method development, solvent modification, and extraction cycle times to be used as a comparison, and consideration to obtain pure andrographolide. A previous study[5] carried out extraction using maceration for 16 hours and reflux for 1 hour using methanol solvent. Pure andrographolide isolate which was quite satisfactory was obtained

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and the results can be a reference for further explorations aimed at increasing the isolation of andrographolide compound from sambiloto.[5] Another study by [6] reported that extraction by maceration produced the best andrographolide yield percentage of 3.50%, with the condition parameters including extraction time of 360 minutes, 100 mL of solvent, and an extraction process of 360 minutes with 3 repetitions. Based on the results, the maceration method can optimize extraction conditions in extracting andrographolide in sambiloto [6]. The difference between the results may be because the samples used were collected from different locations, which affected the composition of the compound. However, the studies can be used as a reference to optimize conditions for andrographolide extraction.

Based on the background described, this study aimed to identify optimal conditions for extracting andrographolide from Sambiloto using a combination method over a period of 1 - 6 hours. The andrographolide levels were also measured qualitatively and quantitatively using TLC and TLC-Densitometry methods. The results will offer insights for selecting an efficient extraction method to obtain pure andrographolide from sambiloto and provide useful information on the potential use of the plant for the development of pharmaceutical products and health supplements.

## Material And Methods

Materials

The materials used include Sambiloto herb, Methanol (Emsure®), Methanol Pro Analysis (Merck®), Aquadest, Chloroform (Emsure®), Ethyl Acetate (Emsure®), anisaldehyde (Emsure®), sulfuric acid, Ethanol (Kimia Market), and Andrographolide Standard ( TCI®). Other materials include Hydrochloric Acid, Mayer, Bourchardat, and Dragendorff reagents, as well as Magnesium powder, Amyl Alcohol, Iron (III) Chloride 5 %, n-hexane (Emsure®), Acetic Acid Anhydrite, and Vanillin Sulfate.

*Plant materials and preparation of Sambiloto Herb Simplicia*

Sambiloto (*Andrographis paniculata* (Burm. F) Nees) herb was obtained from Lembang Manoko Experimental Garden located in Kampung Sukalaksana RT 01 RW 02, Cikahuripan Village, Kec. Lembang, Kab. West Bandung, West Java. Plant determination was carried out at the Jatinangor Herbarium Center, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The simplicia was then subjected to dry sorting of the leaves and soft stems. The parts collected were ground using a grinder until smooth and sifted using a 60-mesh sieve.

*Standardization of simplicia and phytochemical screening*

The quality of simplicia was determined using a standardization method according to the provisions.[7] The determination methods used include drying loss, total ash content, water-soluble content, ethanol-soluble, and water content.

All samples were analyzed using amyl alcohol reagent to detect flavonoid compound[8], $FeCl_3$ 10% for detecting phenolic compound[9], gelatin reagent for tannin detection[10], as well as Dragendorf and Mayer reagent for alkaloid detection.[11-12] The quinone group was identified using a 5% KOH reagent.[13] Steroid and triterpenoid secondary metabolites were identified using Lieberman-Buchard reagent, while volatile compound in the monoterpene and sesquiterpenoid subgroups were identified using vanillin sulfate reagent (10% vanillin in $H_2SO_4$).[14,15,16] Additionally, saponins, another type of secondary metabolite, were determined by observing continuous foaming after shaking the aqueous extracts for 10 minutes.[17]

*Optimization of Bioactive Compound Extraction from Sambiloto (Andrographis paniculata)*

The extraction process was carried out in 2 stages to maximize the yield of andrographolide from Sambiloto. The first stage was the maceration method, where dried and ground plant material (simplicia) was soaked in a solvent. The process was conducted for 16 hours at room temperature to ensure the solvent could penetrate the plant cells and extract the bioactive compound efficiently. During this phase, the solvent slowly diffused through the plant matrix, dissolving and extracting secondary metabolites, including andrographolide. Maceration is widely considered an effective method for the extraction of thermolabile compound, as it operates at ambient temperatures and avoids degradation of bioactive constituents[18].

After the maceration, the liquid macerate was collected and subjected to the second stage of extraction, namely reflux extraction. This method entailed heating the macerate with additional solvent in a reflux apparatus for 6 hours. Refluxing was selected to further enhance the extraction efficiency by increasing the temperature, which in turn raised the solubility and diffusion rate of the bioactive compound from the plant material into the solvent. Throughout the reflux process, 300 ml of the liquid extract was collected at 1-hour intervals, resulting in a total of 6 separate liquid extract samples[19].

The solvents used for both maceration and reflux were methanol and 80% ethanol, selected due to the ability to dissolve both polar and non-polar compound. Ethanol is commonly used in herbal extractions due to the safety profile and effectiveness in extracting both hydrophilic and hydrophobic phytoconstituents.[20] The liquid extracts were collected and then concentrated using a rotary evaporator to remove the solvents under reduced pressure, leaving behind the crude extract. The percentage yield of each extract was calculated by comparing the weight of the dried extract to the weight of the starting plant material.

The yield percentages were analyzed to determine the optimal solvent and extraction time for maximizing andrographolide content. This process ensured that the extract produced contained a high concentration of the desired compound while minimizing degradation or loss during the extraction process.

*Qualitative Analysis of Andrographolide Compound*

The qualitative analysis process for andrographolide compound in the extract used the TLC method, the stationary phase used was silica Gel $GF_{254}$, and the mobile phase was Chloroform: ethyl acetate: methanol (6:2:2). Analysis was carried out using UV light at wavelengths of 254 and 366 nm. Sample preparation was conducted by dissolving 50 mg of sambiloto extract in 10 ml of methanol and 10 mg of andrographolide standard in 10 ml of methanol. The andrographolide samples and standards were spotted on each plate with a spot volume of 10 µl at a distance of 1 cm from the bottom edge of the plate with a distance of 1 cm between spots, then eluted in the chamber. The spots that appeared were RF counted and the TLC plate was sprayed with a chromogenic reagent, namely vanillin-sulfuric acid.[21]

*Quantitative analysis of Andrographolide compound using Densitometry TLC*

The standard solution preparation used andrographolide with a concentration of 3000 ppm, then dilution was carried out with concentrations of 3000, 2800, 2600, 2400, 2200, 2000, 1800, 1600, 1400, 1200, and 1000 ppm. Andrographolide standards of various concentrations were spotted in 10 µL each on a $GF_{254}$ silica gel plate, eluted with the mobile phase Chloroform: ethyl acetate: methanol (6:2:2), and inserted into a Densitometer (TLC Scanner) (Camag®). Scanning was carried out at a wavelength of 230 nm and the peak area under the curve (AUC) was read.[22]

To prepare the sample, 50 mg of sambiloto extract was weighed, dissolved in methanol, and diluted to 10 mL in a measuring flask to obtain a concentration of 5000 ppm. The test was carried out 6 times with sample replications. About 10 µL of each sample was placed on a GF254 silica gel plate, then observations were made by detecting with a Densitometer (TLC Scanner) at a wavelength of 230 nm. The peak area under the curve or area under curve (AUC) was read and andrographolide content in the extract was calculated using the linear regression equation from the comparison standard solution (concentration on the x line and AUC value on the y line).[22]

## Results and Discussion

*Plant materials and preparation of Simplicia Herba Sambiloto*

The major material used in this study was sambiloto obtained from the Manoko Lembang plantation. The identification process showed that the sambiloto herb belongs to the species *Andrographis paniculata* (Burm.f.) Nees, commonly known locally as herba Sambiloto. This identification was confirmed through a plant determination process conducted at the Plant Taxonomy Laboratory, Department of Biology, FMIPA UNPAD, on December 5, 2022, under plant identification sheet number 20/HB/01/2023. The collected sample was in a dry condition and subjected to a dry sorting process to separate it from impurities. This sorting step was essential to ensure that only the leaves and soft-stemmed parts of the plant, which are non-woody or contain minimal woody tissue, were retained for further processing. The removal of unwanted parts helped to maintain the quality and consistency of the raw material used in subsequent analyses.

Following the sorting, the herbs were ground using a grinder to reduce the particle size and were sieved using a 60-mesh sieve. Grinding the simplicia (dried plant material) into smaller particles increased the surface area, which is crucial during the extraction process as it allows for more efficient contact between the sample and the solvent. This increased surface area enhanced the dissolution rate of the secondary metabolites, facilitating optimal extraction conditions. Smaller particle sizes lead to faster solvent penetration, improving the overall yield and efficiency of the extraction. The use of a 60-mesh sieve served multiple purposes including separating finer particles from larger ones, ensuring a uniform particle size distribution. This homogeneity is particularly important when dealing with herbal samples, as it contributes to a consistent extraction process. By achieving uniform powder size, the efficiency of the extraction process was improved, and the rate at which active substances are released from the material was accelerated[20]. This step is crucial in obtaining a high-quality extract rich in bioactive compound, which can then be used for further pharmacological studies. Additionally, achieving uniform particle size through grinding and sieving also aids in the reproducibility of results, especially in experimental setups where precise control over extraction parameters is necessary. This standardization helps in comparing results across different batches and ensures the reliability of the data obtained during subsequent testing phases.

*Standardization of simplicia and phytochemical screening*

Simplicia characterization aims to analyze the quality specifically and non-specifically to obtain raw materials with quality that meets the standard requirements.[7] Characterization tests carried out on the sambiloto herb simplicia included drying loss, total ash, water-soluble, ethanol-soluble, and water content. The simple characterization test was carried out repeatedly 3 times to minimize inaccurate results. The results of repeated measurements were closer to the actual value and the uncertainty of repeated measurements was smaller than a single measurement which could result in errors during testing. Data from the characterization results are presented in Table 1.

**Table 1:** Results of Characterization of Simplicia Herb Sambiloto

| Characterization Test | Results | Literature [23] |
|---|---|---|
| Water content | 1.24 % | Not more than 10% |
| Total ash content | 22.35 % | 3.9% to 43.92% |
| Drying Shrinkage | 0.49 % | Not more than 10% |
| Water soluble materials | 19.3 % | Not less than 12.7 % |
| Etanol soluble extract | 11.7 % | Not less than 5.5 % |

The analysis results showed that most of the simplicia characterization tests were consistent with the standards[24], particularly for the herba Sambiloto monograph. However, the ash content often exceeds the recommended levels, which suggests an excess of mineral content, probably from organic and inorganic sources, including potential silicate contamination from soil or sand. This high ash content may also result from contamination during production, processing, or storage, where foreign particles or environmental pollutants contribute to elevated mineral levels, impacting the overall quality of the herbal material.[20]

Phytochemical screening is critical in identifying the compound classes present in the plant. The results showed that simplicia and extracts in 80% methanol and ethanol contain various secondary metabolites, including flavonoids, tannins, phenols, saponins, steroids/triterpenoids, monoterpenes, and sesquiterpenes. Andrographolide, a key active compound found within the phenol group, is particularly known for therapeutic properties, such as anti-inflammatory and antioxidant effects. This compound validates the traditional uses of Sambiloto and supports the documented pharmacological activities, including anticancer, antihypertensive, and anti-inflammatory effects.[25] These results underscore the importance of strict quality control measures to ensure that the simplicia meets established standards, thereby maintaining the safety and efficacy of Sambiloto in both traditional and modern herbal medicine formulations.

*Optimization of Bioactive Compound Extraction from Sambiloto (Andrographis paniculata)*

The extraction process of sambiloto simplicia was carried out using a combination of maceration and reflux methods to produce a high yield of andrographolide. Extraction by maceration is a simple and cheap method, but this process often requires quite a long time to achieve maximum yield because it only entails soaking without any heating process. The purpose of maceration for 16 hours first is to wet the cells in the plant to ensure solvent can attract all the contents in the cells of the plant part. Therefore, during the reflux extraction process, the temperature of the solvent is raised to near or above the boiling point. This process allows extraction to be carried out at higher temperatures and helps speed up the release of secondary metabolite compound from the sample into the solvent. By combining the 2 methods, there is an increase in extract yield, as reflux helps increase the extraction rate and reduces the contact time needed to achieve maximum yield. In this study, the solvents used were methanol and 80% ethanol. Both solvents can dissolve polar and non-polar compound indicating great potential for extracting secondary metabolite compound contained in the samples used.[26] Methanol and 80% ethanol have good solubility properties for the active compound contained and sambiloto has andrographolide as the main compound.

Based on the results, the yield value of the methanol extract refluxed from 1 to 6 hours had different results, similar to the yield of the 80% ethanol extract. The methanol extract yield data was very small, while the yield for 80% ethanol extract was higher. However, the yield for both extracts was less than the requirements specified in the monograph for sambiloto extract, which should not be less than 9.6%. Low extract yields occur when the reflux extraction process is less controlled in terms of temperature usage which is unstable over time due to bumping and no addition of solvent at any time. This potentially causes a reduction in the yield, resulting in shrinkage of the solvent which may lead to a decrease in the solubility of the active compound.

Regarding the methanol extract, the yield value increased with a reflux time of 1 hour to 6 hours. This is in accordance with the statement that the longer the extraction and the higher the temperature used, the more secondary metabolites will be attracted. The yield value will also be higher, but attention must be given to temperature control.[27] The yield value increased periodically but not significantly as shown in Table 2.

For the ethanol extract, there was a decrease in the yield value in the 3-hour reflux time which was caused by the high temperature and prolonged extract thickening time. Increasing the temperature and time when thickening the extract causes the loss of water content, leading to a reduction in weight. In a previous study by,[28] a combination of maceration and reflux extraction increased the yield value of the extract, producing higher levels of andrographolide. According to another study,[29] separate extractions only produced a yield percentage of 0.10% for maceration and 0.72% for the reflux method, with andrographolide content of 0.69 grams and 0.80 respectively. This comparison can further confirm that extraction using a combination will produce a fairly high yield.

*Qualitative Analysis of Andrographolide Compound*

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Qualitative analysis to determine the presence of andrographolide compound in the extract was carried out using the TLC method. Analysis using TLC is the separation of chemical components based on the principle of adsorption and partition determined by the stationary (adsorbent) and the mobile phase (eluent).[30]

Extract samples and andrographolide standards were dissolved in methanol because andrographolide can dissolve well in methanol. About 10 µl samples of the extract and standard andrographolide were added to ensure visibility under the UV lamp. This is important because an extremely small amount causes the stain to be thin, making it unreadable by the instrument, while an excessive amount leads to the stain being too thick. Consequently, separation on TLC will be difficult and may produce tailed spots. Factors that influence spotting on silica gel plates include the concentration of the sample being spotted, which must be adjusted to the right level.[31] The sample concentration was prepared at 5000 ppm and the standard for andrographolide was 1000 ppm to obtain good and clearly visible stains. The distance between the first and the next spot was 1 cm. To create good separation, the distance between the spots can be increased to ensure optimal separation.

In all samples of methanol and 80% ethanol extracts andrographolide compound was detected. This was indicated by the Rf value of each extracted sample being the same and close to the standard Rf of andrographolide. Based on the results, the Rf value was 0.79 and 0.91 for the methanol and 80% ethanol extract respectively. These values meet the requirements for a good Rf, namely between 0.2 and 0.8. The 254 nm UV TLC visualization showed stains of the andrographolide compound due to the fluorescent properties resulting from the interaction of the chromophore group bound by auxochrome.[33] At a wavelength of 254 nm UV lamp, the compound absorbs energy from UV light and then re-emits in the form of light which appears as a greenish-blue color.

In this study, spotting was used to identify compound present in the form of color spots on the plate. Andrographolide is a compound belonging to the terpenoid group which causes a purple color after being sprayed with vanillin sulfuric acid.[34] Vanillin sulfuric acid is used as a reagent to identify compound containing hydroxyl and carbonyl groups. In this case, andrographolide contains a hydroxyl and a carbonyl group, hence, it reacts with vanillin sulfuric acid, producing a purple color in the appearance of the spots. This reaction can help in the identification of andrographolide in layer chromatography[32] as shown in Figure 1.

**Table 2:** Extract Yield

| Extract | Methanol | | Ethanol 80% | |
|---|---|---|---|---|
| | Extract Weight (g) | Yield (%) | Extract Weight (g) | Yield (%) |
| E1 | 2.62 | 1.04 | 6.93 | 2.77 |
| E2 | 2.82 | 1.12 | 7.67 | 3.07 |
| E3 | 3.61 | 1.44 | 6.83 | 2.73 |
| E4 | 3.74 | 1.49 | 7.01 | 2.8 |
| E5 | 4.11 | 1.64 | 7.77 | 3.12 |
| E6 | 4.36 | 1.74 | 7.61 | 3.04 |

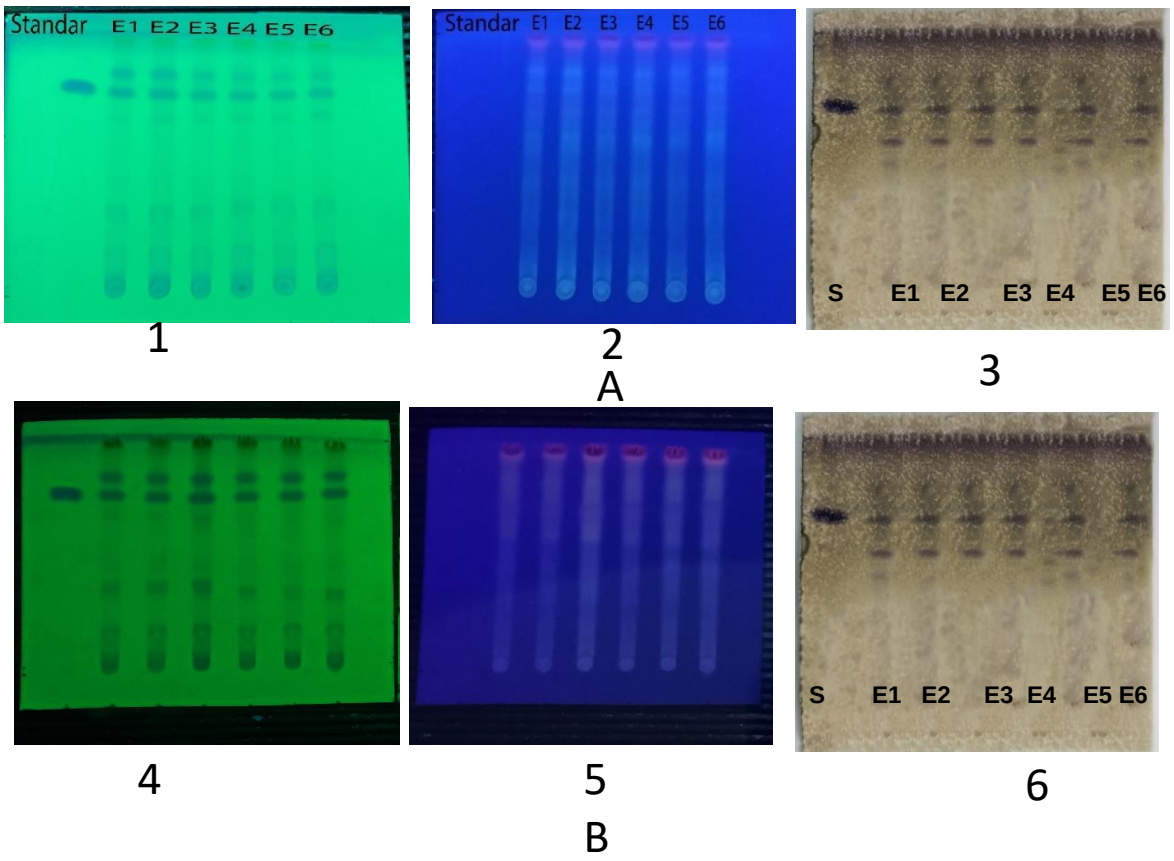

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**Figure 1:** Visualization of Thin Layer Chromatography (TLC) Results (A) 80% Ethanol Extract a) Visualization of UV 254 nm; (b) 366 nm UV visualization; (c) Visualization with Vanillin Spots - Sulfuric Acid
(B) Methanol Extract a) Visualization UV 254 nm; (b) 366 nm UVvisualization; (c) Visualization with Vanillin Spots - Sulfuric Acid

*Quantitative Analysis of Andrographolide Compound*

Andrographolide content in the extract was determined using TLC-Densitometry based on the relationship between the peak area (AUC) of the chromatogram of the compound and the concentration. The peak area of a compound is directly proportional to the concentration contained in the stain. The higher the concentration of a compound, the greater the chromatogram peak area. Identification of andrographolide was carried out by scanning at a wavelength of 230 nm to obtain the maximum wavelength at which the compound produced the maximum response. The results showed a maximum wavelength of 230 nm, as also reported in a previous study.[7] Analyte measurements were carried out using stain spots to determine the levels in the wavelength range of the andrographolide compound. The success of the analysis will be influenced by the accuracy of the sample and standard volume. When observing spot in the elution results on TLC, detection was carried out at a maximum wavelength of 230 nm to obtain the AUC value for each standard solution and sample. The linear relationship between andrographolide standard concentration and AUC was determined by making a dilution series of standards. In this study, measurements were carried out on 11 standard dilution series solutions with a concentration range of 3000 ppm - 1000 ppm at a wavelength of 230 nm.[28] The results of the TLC visualization and densitogram are shown in Figures 2, 3, and 4.

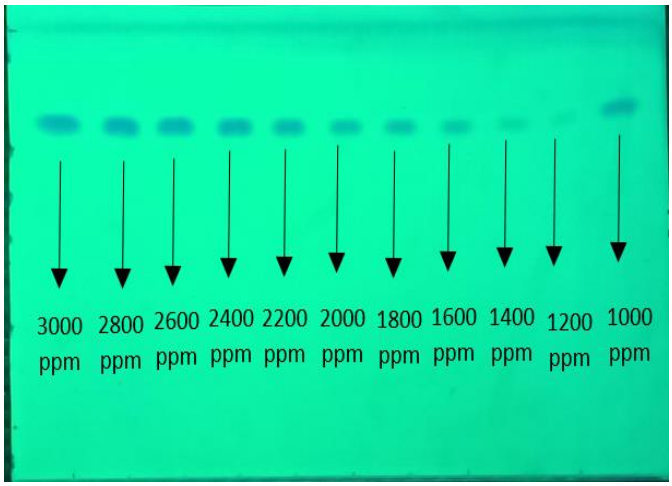


**Figure 2:** Visualization Results of Andrographolide Standard Series TLC (254 nm).

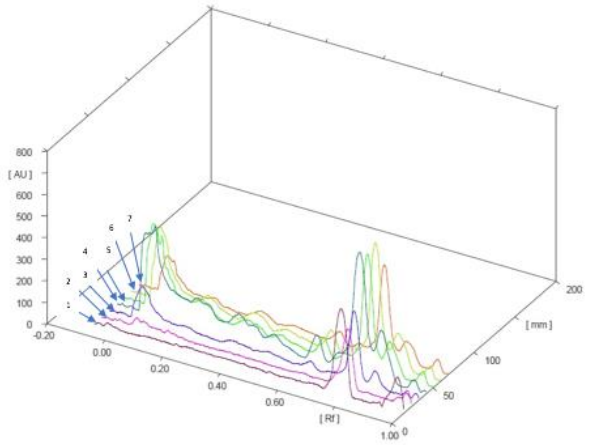

**Figure 3:** Densitogram of standard and methanol extract Description: 1. Standard Andrographolide 2. 1st hour extract 3. Extract 2nd hour 4. Extract 3 hours 5. Extract 4th hour 6. Extract 5th hour 7. Extract 6th hour

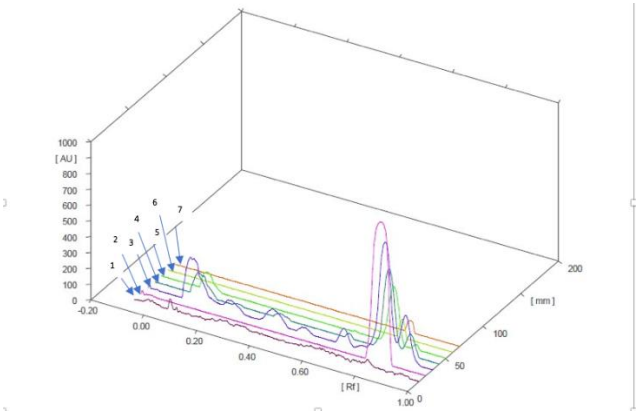

**Figure 4:** Densitogram of standard and ethanol extract Description: 1. Standard Andrographolide 2. 1st hour extract 3. Extract 2nd hour 4. Extract 3 hours 5. Extract 4th hour 6. Extract 5th hour 7. Extract 6th hour

The results showed that only 5 standard series solution points were used including concentrations of 2200, 2000, 1800, 1600, and 1400 ppm. These 5 standard solutions showed linear results indicating that a proportional line was formed with good correlation. The results obtained are concentration vs AUC, then a linear regression line equation is created and the correlation coefficient can be determined.[32] The analyte concentration can be calculated by creating a calibration curve between concentration and AUC. The standard calibration curve for andrographolide is shown in Figure 5.

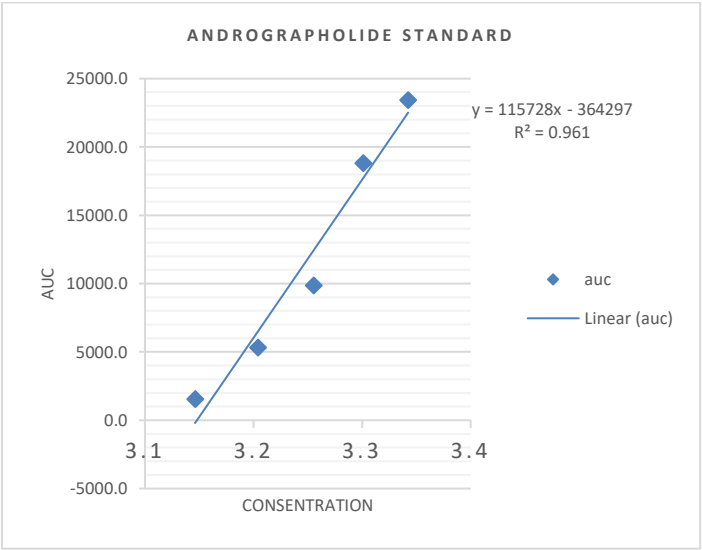


**Figure 5:** Andrographolide Standard Series Calibration Curve

Based on the data analysis results, the linear regression equation obtained was y = 115728x - 132842 with R2 = 0.961. The regression value obtained was close to 1, suggesting that the linear regression model has a good ability to predict compound concentrations based on color intensity on the densitometer chromatogram.

From the results of the standard chromatogram (Figure 2), the Rf value for each of the 5 standard concentrations used was in the same position, at Rf 0.77 and no peak was detected, suggesting the standard contains 1 compound, namely andrographolide. The peak position of

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andrographolide follows the standard peak which is at Rf 0.77. Based on the Rf values of the 6 extracts, the peaks of each chromatogram were in a position close to the standard value, where the results respectively from 1 to 6 were 0.76, 0.76, 0.75, 0.74, and 0.75; 0.75. The results showed that the extracted sample contained andrographolide compound. The results of determining andrographolide levels in methanol and 80% ethanol extract samples of sambiloto herb are shown in Figure 6.

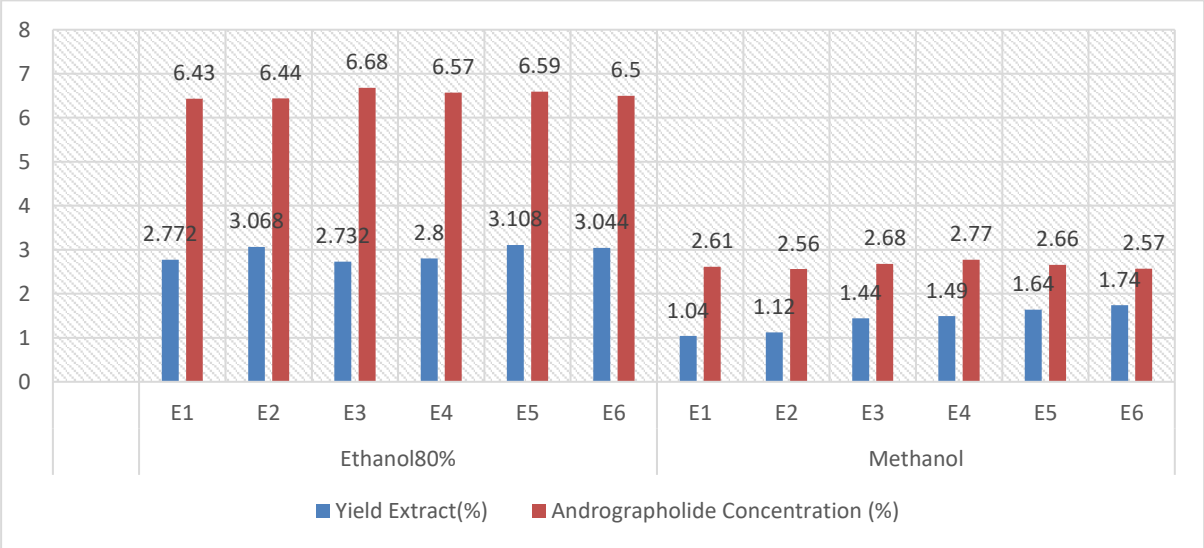


**Figure 6:** Comparison of Yield with Andrografolide levels

The results of the 6 extract samples showed different percentage levels. In the methanol extract, the andrographolide content was relatively contained in the 4-hour extract because there was a significant increase with a concentration percentage of 2.77%.

The 80% ethanol extract showed that the andrographolide content was relatively contained in the 3-hour extract with a percentage of 6.68%. Based on a previous study [6], the measurement of andrographolide levels carried out using TLC- Densitometry must not be less than 3.80% of the thick extract. The 80% ethanol extract can provide a high value compared to methanol. In other words, the 80% ethanol solvent is able to optimally extract andrographolide in the sambiloto herb which has a polarity close to the polarity of the 80% ethanol solvent. The time between reflux and the levels obtained is presented in Figure 7.

At the beginning of the reflux time from 1 hour to 2 hours in the methanol extract, as shown by the graph, there was a decrease, indicating there was a situation that was not optimal. This could be because the reflux time at the 2-hour extraction had not yet reached an optimum point. There was an increase in levels at the 3-hour of reflux and the highest andrographolide levels were produced at 4 hours of reflux, while at 5 and 6 hours, there was a decrease in the amount of andrographolide produced. At the beginning of the reflux in the 80% ethanol extract, there was an increase, with the most optimal reflux time being 3 hours, followed by an unstable decrease. It is possible that as time increases, the amount of other dissolved compound increases,[35] thereby reducing the amount of andrographolide compound extracted. Extraction times that exceed the optimum limit will cause damage to phytochemical compound, affecting the yield and the levels of active compound contained.

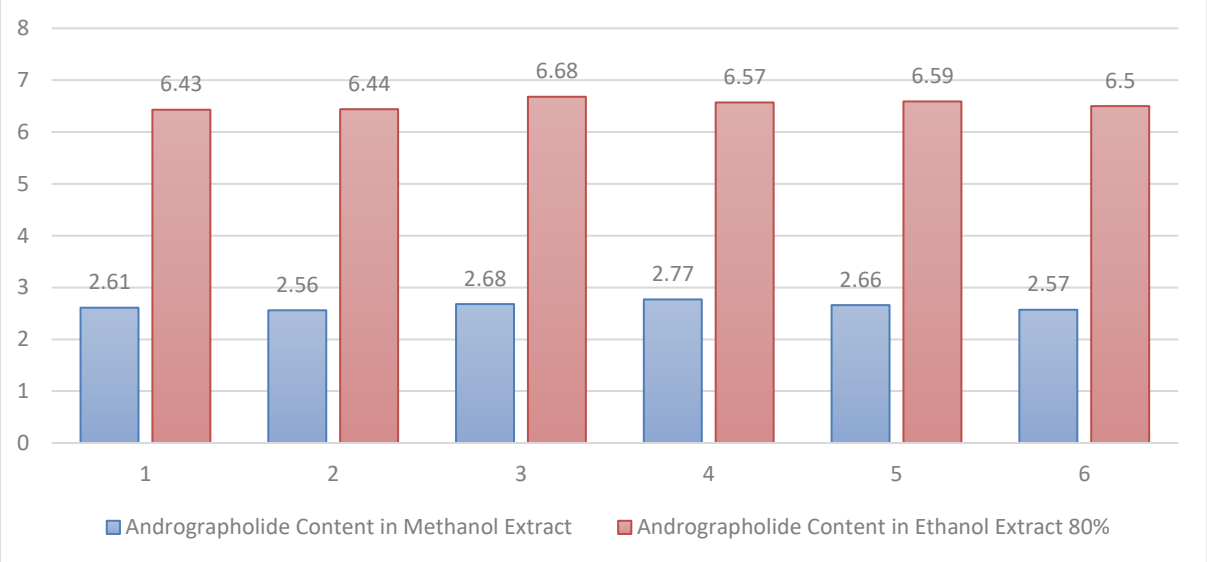


**Figure 7:** Graph of the relationship between extraction time and andrographolide levels

## Conclusion

In conclusion, this study successfully optimized the extraction process of andrographolide, the primary bioactive compound in Sambiloto, using a combination of maceration and reflux methods. A 16-hour maceration followed by a 3-hour reflux with 80% ethanol as the solvent

produced the highest yield (6.68%). TLC and TLC-densitometry analyses confirmed the presence and concentration of andrographolide, underscoring the efficiency of this method. Longer reflux times led to a reduction in andrographolide levels, indicating that 3-hour reflux was optimal for maximizing compound yield. These results provide valuable insights for improving the efficiency of andrographolide extraction, which can be applied to the development of pharmaceutical and health supplement products.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and any liability for claims relating to the content of this article will be borne.

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