

Comparison of the Effectiveness of Maceration and Soxhlet Extraction Methods on the Phytochemical Profile and Total Flavonoid Content of *Bandotan* Leaf Fractions (*Ageratum conyzoides* L.)

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Abstract

This study aimed to compare the efficiency of cold (maceration) and hot (Soxhlet) extraction methods in obtaining total flavonoids. Extraction was performed using 96% ethanol solvent, followed by fractionation using n-hexane and ethyl acetate. The content was determined using validated UV-Vis spectrophotometry. The validation results showed that the method met the requirements for linearity ($r=0.9988$), accuracy (99.51%), LOD (2.19 ppm), and LOQ (7.30 ppm). The results of the content determination showed that the ethyl acetate fraction of the Soxhlet extraction method provided the highest flavonoid content of 251.04 ± 0.24 mgQE/g, which was significantly different from the maceration method (194.14 ± 0.49 mgQE/g). In conclusion, the Soxhlet extraction method is more effective in extracting flavonoid compounds from bandotan leaves than maceration due to continuous solvent circulation and heat assistance.

Keywords: *Ageratum conyzoides* L.; Total Flavonoids; Soxhlet Extraction; Method Validation; UV-Vis Spectrophotometry.

INTRODUCTION

Indonesia is a country with extraordinary diversity in habitats, flora, and fauna. Due to this diversity, Indonesia has many types of biodiversity, one of which is flora, which includes all types of plants and vegetation on earth that can be used as herbal medicine and health foods (Harefa *et al.*, 2022a). Herbal medicine is medicine derived from plants that are processed or extracted into powder, pill, or liquid form without the use of chemicals. Herbal medicines derived from natural and non-synthetic ingredients are known to cure diseases with few side effects (Pane *et al.*, 2021). One medicinal plant that is well known to the public is *Bandotan* (*Ageratum conyzoides* L.), which is known as a traditional medicine for healing and preventing diseases (Harefa *et al.*, 2022a).

The *bandotan* plant (*Ageratum conyzoides* L.) is an aggressive weed that causes yield loss in several crops (Erida *et al.*, 2023). The *bandotan* plant has long been widely used for traditional medicine, such as for wounds, digestive problems, and diarrhea (Hasyim & Muh Farid, 2020). *Bandotan* leaves can also prevent and cure diseases such as fever, malaria, cough, and stomach ache (Harefa *et al.*, 2022a). Other benefits include treating itching, flu, intestinal inflammation, and rheumatism.

Applying these leaves to wounds can stop bleeding and accelerate the healing process (Sugara *et al.*, 2016).

Bandotan leaves have antifungal, antibacterial, anti-inflammatory, antioxidant, antiprotozoal, anticancer, analgesic, antidiarrheal, antimicrobial, and antidiabetic properties (Erida *et al.*, 2023). *Bandotan* leaves (*Ageratum conyzoides* L.) contain various secondary metabolites, including steroids, flavonoids, coumarins, tannins, and alkaloids (Kumar *et al.*, 2021). According to (Pay *et al.*, 2022b, Purwanti, 2022), the high content of alkaloids, flavonoids, and saponins plays a role in antidiabetic and antibacterial properties. Alkaloids play a role in the healing process of wounds caused by bacterial infections. Flavonoids function as homeostasis agents in increasing the number of platelets that play a role in stopping bleeding caused by ruptured blood vessels, and work by vasoconstriction to stop bleeding (Astuti *et al.*, 2015). Meanwhile, tannins in *bandotan* leaves can accelerate the release of cell proteins and precipitate blood proteins, thereby accelerating the formation of temporary platelet plugs in injured blood vessels (Safani *et al.*, 2019).

Flavonoids are one of the largest groups of phenolic compounds found in all green plants and can be found in plant extracts (Saputra & Nur, 2016). Flavonoid content analysis can be performed using a UV-Vis

spectrophotometer. Ultraviolet and visible absorption spectra are the most useful way to identify flavonoid structures, because flavonoids contain conjugated aromatic systems and can exhibit strong absorption bands in the UV-Vis region (Herawan, 2020).

Previous studies have shown that extraction methods can affect the content of secondary metabolites. Research (Purwanti, 2022) found that extraction methods (maceration vs. sokletation) affect flavonoid concentration and antibacterial activity. However, specific comparisons of the effectiveness of these two methods on the complete phytochemical profile and total flavonoid content in fractionated fractions (n-hexane, ethyl acetate, and ethanol residue) of *bandotan* leaves have not been widely reported. Therefore, this study aims to compare the effectiveness of maceration and Soxhlet extraction methods on the phytochemical profile and total flavonoid content of *bandotan* leaf fractions, validated statistically.

MATERIALS AND METHODS

Equipment

The equipment used included a set of soklet apparatus (Iwaki), a set of fractionation apparatus (Iwaki), a UV-Vis spectrophotometer (Hitachi), a *rotary evaporator* (Rotavapor R-300), a *water bath*, KLT plates (Silica Gel GF254), a maceration vessel, and an analytical balance (Fujitsu).

Materials

The materials used in this study included *bandotan* leaves, 96% ethanol (technical grade), ethyl acetate (technical grade), n-hexane (technical grade), distilled water, concentrated HCl, powdered Mg, Dragendorff's reagent, FeCl₃ 1%, acetic acid, glacial acetic acid, concentrated sulfuric acid, n-butanol, aluminum chloride, potassium acetate, and quercetin standard (Sigma-Aldrich).

Method

Plant Identification

Plant identification of *bandotan* leaves (*Ageratum conyzoides* L.) was conducted at the ANDA Herbarium, Faculty of Biology, Andalas University, Padang, West Sumatra.

Sample Preparation

A total of 20 kg of fresh *bandotan* leaves were harvested from the Kinali area, West Pasaman. The leaves were wet sorted, washed thoroughly, drained, and dried by airing them without direct sunlight for 5–7 days. The dried *simplicia* was then dry-sorted, ground into powder, and sieved to obtain a fine *simplicia* powder ready for extraction.

Extraction and Fractionation

Extraction is carried out using two methods:

1. Maceration: 200 grams of crude drug powder were macerated in 96% ethanol solvent in a closed vessel for 5 days with occasional stirring. The macerate was filtered, and the residue was remacerated until the solvent was clear. The filtrates were combined and concentrated with a *rotary evaporator* (Sidrotullah, 2021).
2. Sokletation: 100 grams of crude drug powder is wrapped in filter paper and placed in a soklet apparatus. Extraction is carried out using 96% ethanol solvent continuously until the solvent droplets are clear. The liquid extract is concentrated with a *rotary evaporator* (Purwanti, 2022).

The ethanol extracts from both methods were fractionated using the liquid-liquid extraction method. Ten grams of concentrated extract were suspended in a small amount of warm water, then partitioned sequentially with n-hexane and ethyl acetate. The fractions obtained (n-hexane fraction, ethyl acetate fraction, and ethanol-water residue fraction) were evaporated to obtain concentrated fractions for further analysis (Yanty *et al.*, 2019).

Phytochemical Screening

Phytochemical screening was performed qualitatively on each fraction to identify groups of secondary metabolite compounds.

Alkaloid Identification

A number of fraction samples were dissolved in test tubes, then 2 mL of HCl and 3 drops of Dragendorff's reagent were added. A positive result was indicated by the formation of an orange precipitate at the bottom of the tube (Purwanti, 2022).

Flavonoid Identification

Identification was carried out by adding 0.1 grams of magnesium (Mg) powder and 3 drops of concentrated HCl to an evaporating dish containing the fraction sample. A change in the color of the solution to red or orange indicated the presence of flavonoids (Purwanti, 2022).

Saponin Identification

The foam formation test was conducted by adding distilled water to the sample in a test tube, then shaking it vertically for 15 minutes. The presence of saponin was indicated by the formation of stable foam 1 cm high that did not disappear after being left to stand (Purwanti, 2022).

Tannin Identification

The sample is dissolved in 10 mL of distilled water, heated for 3 minutes, cooled, and filtered. The filtrate is

then diluted until colorless and reacted with 1-2 drops of 1% FeCl₃ solution. The formation of a blue-black or green-black color indicates the presence of tannin compounds (Purwanti, 2022).

Steroid Identification

Identification was performed using Liebermann-Burchard reagent. The sample was dissolved with 20 drops of glacial acetic acid, shaken gently, and then 1 drop of concentrated sulfuric acid was added. A positive result was indicated by a change in the color of the solution to blue or green (Purwanti, 2022).

Thin Layer Chromatography (TLC) Analysis

The fraction chromatography profile was analyzed using a 10 cm long and 3 cm wide silica gel 60 F₂₅₄ stationary phase plate. The mobile phase (eluent) used was a mixture of n-butanol:acetic acid:water in a ratio of 4:1:5 (v/v). The sample was spotted using a capillary tube at a distance of 1 cm from the bottom edge of the plate, then eluted in a saturated chromatography chamber. The elution spots were observed under UV light at wavelengths of 254 nm and 366 nm. The analysis was carried out until at least three separate spots were observed, then the *retention factor* (R_f) value was calculated for each spot (Koirewoa, 2020).

Determination of Total Flavonoid Content

Preparation of Quercetin Stock Solution

A total of 10 mg of standard quercetin was carefully weighed and dissolved with 96% ethanol in a 100 mL measuring flask up to the mark to obtain a stock solution with a concentration of 100 ppm.

Determination of Maximum Wavelength

One milliliter (1 mL) of the 100 ppm quercetin stock solution was diluted in a 10 mL volumetric flask to obtain a concentration of 10 ppm. A total of 0.3 mL of this solution was mixed with 1.5 mL of 120 mM potassium acetate and 1.5 mL of 2% aluminum chloride (AlCl₃). The mixture was incubated for 30 minutes at room temperature (operating time). Absorption was measured in the wavelength range of 400–550 nm to determine the maximum wavelength (Aminah, 2017).

Preparation of Calibration Curve

Working standard solutions were prepared by pipetting 1, 2, 3, 4, and 5 mL of the quercetin stock solution (100 ppm), then diluting each to 10 mL to obtain a series of concentrations of 10, 20, 30, 40, and 50 ppm. Each concentration series was reacted with 1.5 mL of 2% AlCl₃ and 1.5 mL of 120 mM potassium acetate, then incubated for 30 minutes. Absorbance was measured at the maximum wavelength obtained (Aminah, 2017).

Determination of Sample Concentration

A total of 50 mg (0.05 g) of concentrated extract was dissolved with 96% ethanol in a 50 mL measuring flask.

A certain volume of sample was pipetted, 1 mL of 10% AlCl₃ and 1 mL of 1 M potassium acetate were added, and the volume was adjusted with the appropriate solvent. The mixture was homogenized and incubated before measuring its absorbance at the maximum wavelength. The test was performed in triplicate (Herawan, 2020). The total flavonoid content was calculated based on the linear regression equation of the calibration curve using the following formula:

$$KTF = \frac{V(ml) \times X \left(\frac{mg}{ml} \right) \times fp}{g \text{ ekstrak}}$$

KTF : Total Flavonoid Content

V : Volume during extraction

X : Flavonoid content

fp : Dilution Factor

g : Sample Weight (g)

Data analysis

Absorbance data were analyzed using the Lambert-Beer equation ($y = ax + b$), where y is absorbance and x is concentration ($\mu\text{g/mL}$). The results of flavonoid content calculations are presented descriptively in tables and graphs (Herawan, 2020).

RESULTS AND DISCUSSION

Botanical Identification and Extraction Efficiency

The plant samples were identified as *Ageratum conyzoides* L. (family Asteraceae) at the ANDA Herbarium, Department of Biology, FMIPA, Universitas Andalas. The extraction process revealed a significant performance disparity between the two methods: the Soxhlet extraction method produced an extract yield of 18.14% (18.14 g), whereas the maceration method yielded 9.17% (8.49 g).

This disparity is fundamentally driven by extraction thermodynamics. In Soxhlet extraction, continuous heat application reduces solvent viscosity and surface tension, thereby increasing the diffusion rate and the solvent's ability to penetrate the pores of the leaf cell matrix to optimize the release of secondary metabolites (Zhang & Ye, 2018). Furthermore, the mechanism of repeated circulation of fresh solvent in the Soxhlet method ensures that the plant material remains in contact with unsaturated solvent, maintaining a maximum concentration gradient between the solute inside the cells and the solvent outside. This serves as the primary driving force for mass transfer, a factor absent in static maceration where diffusion equilibrium is often reached prematurely before complete extraction is achieved (De Castro & Priego-Capote, 2010).

Phytochemical Profile and Chromatographic Characterization

Phytochemical screening of the n-hexane and ethyl acetate fractions confirmed the presence of alkaloids, flavonoids, saponins, tannins, and steroids. The detection of alkaloids in the organic solvent fractions (n-hexane and ethyl acetate), but their absence in the residual ethanol-water fraction, suggests that the alkaloids in *A. conyzoides* leaves exist predominantly as free bases rather than water-soluble salts.

TLC analysis using an n-butanol:acetic acid:water (4:1:5) mobile phase visualized two distinct regions: a green spot (R_f 0.13–0.27) and an orange spot (R_f 0.52–0.80). This chromatographic pattern confirms a complex mixture of flavonoids with varying polarities, likely spanning from less-polar flavonoid aglycones (found in the ethyl acetate fraction) to more polar flavonoid glycosides.

Table 1. Results of Phytochemical Screening of Bandotan Leaf Fractions Using the Maceration Method.

Compound	Positive Results Based on Theory	n-Hexane Fraction	Ethyl Acetate Fraction	Ethanol-Water Fraction
Flavonoids	Color change to orange/red	+	+	+
Alkaloid	Formation of orange-colored precipitate	-	+	+
Saponin	The formation of foam	+	+	+
Tannin	Blue-black/green-black color change	-	+	+
Steroid	Color change to blue/green	-	+	+

Description: (+) detected, (-) not detected.

Table 2. Results of Phytochemical Screening of Bandotan Leaf Fractions Using the Soxhlet Method.

Compound	Positive Results Based on Theory	n-Hexane Fraction	Ethyl Acetate Fraction	Ethanol-Water Fraction
Flavonoids	Color change to orange/red	+	+	+
Alkaloid	Formation of orange-colored precipitate	-	+	+
Saponin	The formation of foam	+	+	+
Tannin	Blue-black/green-black color change	+	+	+
Steroid	Color change to blue/green	-	+	+

Note: (+) detected, (-) not detected.

Quantitative Analysis of Total Flavonoid Content

Validation of the UV-Vis spectrophotometry method yielded parameters within acceptable criteria: linearity ($r=0.9988$), accuracy (99.51%), LOD (2.19 ppm), and LOQ (7.30 ppm). These metrics ensure the reliability of the quantitative data.

The ethyl acetate fraction obtained via Soxhlet extraction achieved the highest flavonoid concentration (251.04 mgQE/g), significantly outperforming the maceration method (194.14 mgQE/g). This confirms ethyl acetate as the optimal selective solvent for isolating flavonoids, effectively separating target compounds from non-polar impurities (e.g., fats and chlorophyll in the n-hexane fraction) and polar impurities (e.g., sugars and proteins in the water fraction).

The efficacy of Soxhlet extraction for most flavonoids can be attributed to the thermal energy at the boiling point of ethanol (78°C), which facilitates the cleavage of complex bonds between flavonoids and the

cell wall or polysaccharide matrix, releasing more free compounds for extraction (Endarini, 2016). Interestingly, the maceration method yielded a slightly higher flavonoid content in the n-hexane fraction (139.17 mgQE/g) compared to the Soxhlet method (123.16 mgQE/g). This anomaly suggests that certain non-polar flavonoids, such as methylated flavonoids or isoflavones, may be thermolabile and undergo partial degradation or structural transformation under the continuous heat of the Soxhlet cycle.

Nevertheless, the low flavonoid content in the ethanol-water residue (0.19–16.27 mgQE/g) highlights the efficiency of the liquid-liquid partitioning process. The combination of Soxhlet extraction and ethyl acetate fractionation is, therefore, a robust strategy for obtaining standardized extracts. This protocol is recommended for industrial applications, as it improves time efficiency and ensures maximum yield of flavonoid active ingredients for potential pharmaceutical development.

Table 3. Total Flavonoid Content Using The Maceration Method.

Fraction	Measurement	KTF	Average	SD	KTF + SD
n-Hexane	0.215	16.59	16.27	0.28	16.27 ± 0.28
	0.212	16.30			
	0.208	15.91			
Ethyl acetate	2.033	194.83	194.14	0.49	194 ± 0.49
	2.024	193.95			
	2.021	193.65			
Ethanol-Water	1,472	139.83	139.17	0.46	139.17 ± 0.46
	1,461	138.75			
	1,463	138.95			

Table 4. Total Flavonoid Content by Soxhlet Extraction Method.

Fraction	Measurement	KTF	Average	SD	KTF + SD
n-Hexane	0.05	0.42	0.192	0.25	0.192 ± 0.25
	0.049	0.32			
	0.044	-0.16			
Ethyl acetate	2,607	251.10	251.04	0.24	251.04 ± 0.24
	2,609	251.30			
	2,603	250.71			
Ethanol-Water	1,297	122.67	123.16	0.21	123.16 ± 0.21
	1,302	123.16			
	1,301	123.06			

The superior extraction efficiency of the Soxhlet method for the ethyl acetate fraction can be attributed to the principle of continuous solvent circulation. Unlike the static environment in maceration, the Soxhlet apparatus ensures that the plant matrix is continuously exposed to fresh solvent vapor that has been condensed and heated. This process maintains a steep concentration gradient between the plant matrix and the solvent, which is a key driver for mass transfer (Azwanida, 2015). Furthermore, the elevated temperature inherent in the Soxhlet process reduces the solvent's viscosity and surface tension, facilitating deeper penetration into the cellular matrix and enhancing the desorption of flavonoid compounds from the *Ageratum conyzoides* leaf tissues (Zhang *et al.*, 2018).

Conversely, the finding that maceration yielded a higher flavonoid content in the n-hexane fraction is particularly intriguing. This discrepancy suggests that certain flavonoid glycosides within *Ageratum conyzoides* may possess high thermal sensitivity (Kotta *et al.*, 2020). While the Soxhlet extraction is generally more efficient, the prolonged exposure to thermal stress—even at the boiling point of the solvent—may inadvertently catalyze the degradation of heat-sensitive (thermolabile) compounds (Chaaban *et al.*, 2017). Maceration, performed at ambient temperature, mitigates this risk by avoiding thermal stress, thereby preserving the structural integrity of these specific flavonoids. These results highlight that the optimal extraction technique is not only a function of mass transfer efficiency but also a strategic balance between yield and the preservation of thermolabile phytochemical profiles.

Implications for Pharmaceutical Development

The evidence presented suggests that the multi-stage fractionation process—specifically utilizing Soxhlet extraction followed by ethyl acetate partitioning—is a crucial strategy for obtaining standardized extracts. Given the superior recovery of flavonoid markers and the process efficiency observed, this protocol is highly recommended for the industrial-scale production of *A. conyzoides* extracts intended for pharmacological applications, such as anti-ulcer or antibacterial treatments. Future research should focus on further isolating these high-yield fractions to characterize the specific flavonoid compounds responsible for these biological activities.

CONCLUSIONS

The Soxhlet extraction method proved to be more effective and efficient than the maceration method in isolating bioactive compounds from leaves (*Ageratum conyzoides* L.), as indicated by a higher extract yield (18.14%) and maximum total flavonoid content. The ethyl acetate fraction from the Soxhlet extraction method produced the highest total flavonoid content of 251.04 mgQE/g, surpassing the maceration method, which only reached 194.14 mgQE/g. This study recommends the use of the Soxhlet extraction method followed by ethyl acetate fractionation as the optimal procedure for obtaining flavonoid-rich bandotan leaf extracts.

Competing Interests: The authors declare that there are no competing interests.

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