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Determination of Alkaloid Content in *Sonchus arvensis* L. Leaf Extract Fractions at Different Fractionation Levels

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Abstract

Tempuyung (*Sonchus arvensis* L.) leaves are traditionally used by communities in South Kalimantan to treat gout and promote external wound healing. The leaves contain secondary metabolites, including alkaloids associated with pharmacological activities such as antibacterial effects. This study determines the total alkaloid content of different solvent fractions of *S. arvensis* leaf extract and identifies the fraction with the highest content. A descriptive observational study was conducted from December 2023 to July 2024 using leaves collected from Sungai Batung Village, Juai District, Balangan Regency, South Kalimantan, Indonesia. The plant material was extracted by maceration with 96% ethanol and subsequently fractionated using n-hexane, chloroform, and methanol. Alkaloids were qualitatively identified using Wagner's and Dragendorff's reagents, while total alkaloid content was quantified by UV–Vis spectrophotometry using caffeine as the reference standard at 289 nm. All three fractions tested positive for alkaloids. The total alkaloid contents of the n-hexane, chloroform, and methanol fractions were 4.821%, 7.867%, and 10.11%, respectively. The methanol fraction therefore contained the highest total alkaloid concentration. These findings demonstrate solvent-dependent differences in the distribution of alkaloids across *S. arvensis* leaf extract fractions and identify the methanol fraction as the most promising fraction for further alkaloid isolation. The study provides a scientific basis for subsequent pharmacognostic, phytochemical, and pharmaceutical investigations of this medicinal plant.

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Keywords: Alkaloids; Fractionation; Methanol Fraction; *Sonchus arvensis* L.; UV–Visible Spectrophotometry

INTRODUCTION

Indonesia is a country endowed with abundant natural resources and one of the world's richest levels of biodiversity. Of the approximately 25,000 plant species found in Indonesia, 9,609 species are reported to possess medicinal properties. Approximately 74% of these medicinal plants grow wild in forests, while the remaining 26% are cultivated. Furthermore, more than 940 plant species have been utilized as ingredients in traditional medicines (Yassir & Asnah, 2019). Despite its considerable potential as an alternative source of pharmaceutical raw materials, Indonesia still imports approximately 90% of its pharmaceutical raw materials from abroad. This condition is attributed to the limited research on the potential and isolation processes of bioactive compounds from locally available plants (Putra et al., 2023). One plant that contains bioactive compounds and has potential for use as a raw material in pharmaceutical development is tempuyung (*Sonchus arvensis* L.).

Tempuyung leaves (*Sonchus arvensis* L.) have been empirically used for generations by the community of Tambak Sirang Baru Village, Gambut District, Banjar Regency, to treat gout and accelerate the healing of external wounds (Wahyuni et al., 2023). The leaves are traditionally prepared by boiling them for oral consumption or by pounding them and applying the resulting material directly to the wounded area (Imelda et al., 2017). This traditional use is supported by the study conducted by Sukmayadi et al., (2023) entitled "Antibacterial Activity Test of Tempuyung (*Sonchus arvensis* L.) Leaf Infusion Against *Escherichia coli* and *Staphylococcus aureus*," which reported that tempuyung leaves were capable of inhibiting bacterial growth due to the presence of secondary metabolites in the leaf extract, including flavonoids, terpenoids, and alkaloids (Fajriyah & LA, 2025).

Tempuyung (*Sonchus arvensis* L.) can be utilized as a traditional medicinal plant because it contains various phytochemical compounds, including flavonoids, tannins, anthraquinones, glycosides, steroids/terpenoids, saponins, and alkaloids (Harahap, 2020). In herbal plants, alkaloids have potential antibacterial activity and may inhibit bacterial growth. The antibacterial mechanism of alkaloids involves the inhibition of enzymes that play a role in DNA replication, thereby preventing bacterial cell division and consequently inhibiting

bacterial growth (Huang et al., 2022). Alkaloids present in plant extracts may also interfere with the components involved in the formation of bacterial peptidoglycan, resulting in an incomplete cell wall structure and ultimately leading to bacterial cell death (Purwanto & Irianto, 2022).

The presence of alkaloids in tempuyung leaves warrants further investigation because these compounds exhibit various pharmacological activities, including antibacterial activity. Therefore, it is necessary not only to identify the presence of alkaloids but also to determine their content in tempuyung leaf extract according to the polarity of the fractions. This approach is expected to provide more specific information regarding the distribution of alkaloid compounds within the extract.

To identify the alkaloid compounds targeted in this study, the tempuyung leaves (*Sonchus arvensis* L.) must first undergo an extraction process. Extraction is a separation process employed to transfer and remove soluble components from one liquid phase into another (Poole, 2019). In this study, maceration was selected as the extraction method. Maceration is an extraction technique involving the immersion of plant material in a cold solvent (Saidi et al., 2018). The maceration method was selected because it involves relatively simple procedures and equipment and does not require heating. Consequently, the risk of thermal degradation of natural compounds can be minimized, while allowing a greater number of compounds to be extracted (Susanty & Bachmid, 2016).

The subsequent process involves fractionation using three solvents with different degrees of polarity. The purpose of fractionation in this study is to determine the alkaloid content in each fraction (non-polar, semi-polar, and polar) and to compare the fractions to identify which one contains the highest total alkaloid content in the tempuyung leaf extract (*Sonchus arvensis* L.). The fraction exhibiting the highest alkaloid content indicates the fraction with the greatest concentration of alkaloid compounds, whose chemical characteristics may differ according to the polarity of the solvent used. The analysis is subsequently followed by the determination of total alkaloid content using UV–Vis spectrophotometry.

UV–Vis spectrophotometry is one of the most commonly employed analytical methods for the analysis of chemical compounds and involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution (Palur et al., 2020). UV–Vis spectrophotometry correlates wavelength with the frequency and intensity of radiation absorbed by a substance (absorbance), which is expressed as an absorption spectrum in the

form of lines or bands (Renda et al., 2023). In this study, the compounds of interest are alkaloids; therefore, UV–Vis spectrophotometry is employed for their analysis within a standard wavelength range of 200–800 nm to determine the maximum absorption wavelength of the alkaloid compounds.

Several previous studies have demonstrated the potential of bioactive compounds in tempuyung leaves. Harahap, (2020) reported that phytochemical screening of tempuyung leaves revealed the presence of flavonoids, alkaloids, tannins, anthraquinones, glycosides, steroids/terpenoids, and saponins. Meanwhile, Taris, (2021) demonstrated that 96% ethanol extract of tempuyung leaves exhibited antibacterial activity against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, with various classes of compounds, including alkaloids, identified in the extract. Rahmah et al., (2023), in a study of total alkaloid content in rembusa (*Passiflora foetida* L.) leaf extract at different fractionation levels, reported the highest alkaloid content in the n-hexane fraction (7.161%), followed by the chloroform fraction (3.080%) and methanol fraction (2.340%). These findings indicate that alkaloid distribution may vary depending on plant characteristics and solvent polarity. However, the study used rembusa leaves rather than tempuyung leaves.

Based on these previous studies, a research gap can be identified. Existing studies on tempuyung leaves have predominantly focused on phytochemical screening, simplicia characterization, and antibacterial activity, whereas information regarding the total alkaloid content of tempuyung leaf extract at different fractionation levels remains limited. This study addresses this gap by determining and comparing alkaloid content in the n-hexane, chloroform, and methanol fractions.

Theoretically, fractionation separates compounds according to their polarity. The use of solvents with different polarities allows compounds in an extract to be distributed into fractions according to their physicochemical characteristics. Therefore, differences in alkaloid content among fractions may provide an indication of the polarity characteristics of the alkaloid compounds present in tempuyung leaves. Quantitative determination was subsequently performed using UV–Vis spectrophotometry as an analytical method.

The novelty of this study lies in the determination and comparison of the total alkaloid content of tempuyung (*Sonchus arvensis* L.) leaf extract across three fractionation levels based on solvent polarity, namely n-hexane, chloroform, and methanol. Thus, this

study provides information not only on the presence of alkaloids but also on the fraction exhibiting the highest total alkaloid content.

Based on the foregoing, this study focuses on the determination of total alkaloid content in tempuyung (*Sonchus arvensis* L.) leaf extract at different fractionation levels and comparison of the alkaloid content among the respective fractions. The study aimed to determine the total alkaloid content in the n-hexane, chloroform, and methanol fractions and to identify the fraction with the highest alkaloid content. This focus is consistent with the research questions and objectives of the study.

METHODS

This study employed an observational research design with a descriptive observational approach aimed at determining the total alkaloid content of tempuyung (*Sonchus arvensis* L.) leaf extract in different fractions based on their degree of polarity. The study was conducted from December 2023 to July 2024 at the Pharmaceutical Chemistry Laboratory, Universitas Sari Mulia, Banjarmasin. Plant identification was performed at the Basic Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Banjarbaru. The samples consisted of fresh, green tempuyung leaves collected from Sungai Batung Village, Juai District, Balangan Regency, South Kalimantan, Indonesia.

A total of 1 kg of tempuyung leaves was washed under running water and dried under sunlight while covered with a black cloth. The dried leaves were then subjected to dry sorting and pulverized into simplicia powder. A total of 300 g of simplicia powder was extracted using the maceration method with 96% ethanol for 1 × 24 h, followed by three successive remaceration cycles. The resulting filtrate was concentrated using a *rotary vacuum evaporator* at 50°C until a concentrated extract was obtained.

Fractionation was performed using 10 g of the concentrated extract, which was dissolved in 50 mL of distilled water and subsequently partitioned using solvents with different degrees of polarity, namely n-hexane, chloroform, and methanol, each at a volume of 50 mL. The n-hexane, chloroform, and methanol fractions represented the non-polar, semi-polar, and polar fractions, respectively. Each fraction was collected separately and concentrated using a *water bath* at 50°C.

Qualitative identification of alkaloids was performed using Wagner's and Dragendorff's reagents. Each fraction was treated with 2 N HCl, dissolved in distilled water,

and subsequently filtered. The resulting filtrates were then reacted separately with Wagner's and Dragendorff's reagents. A positive result for alkaloids was indicated by the formation of a brown precipitate with Wagner's reagent and a red coloration with Dragendorff's reagent.

The alkaloid content was determined using UV–Vis spectrophotometry with caffeine as the standard solution. The maximum absorption wavelength was determined within the range of 200–400 nm, yielding a maximum wavelength of 289 nm. The *operating time* was determined over 60 min at 2-min intervals, and the absorbance was found to be stable at 34 min. A calibration curve was prepared using caffeine standard solutions at concentrations of 2, 4, 6, 8, 10, and 12 ppm. The absorbance of each standard solution was measured at 289 nm in triplicate.

Solutions of each fraction were prepared at a concentration of 100 ppm. A total of 2 mL of each sample solution was added to 5 mL of phosphate buffer and 5 mL of BCG solution, followed by extraction with 5 mL of chloroform. The chloroform phase was transferred into a 10-mL volumetric flask, and chloroform was added to the mark. The absorbance of each sample was measured at 289 nm using a UV–Vis spectrophotometer, with three replicates performed for each fraction.

The data obtained consisted of qualitative and quantitative data. Qualitative data were derived from the results of alkaloid identification, whereas quantitative data consisted of absorbance values obtained from UV–Vis spectrophotometric measurements. Data analysis was performed using univariate analysis based on the linear regression equation of the caffeine calibration curve ($y = bx + a$). The absorbance values of the respective samples were substituted into the regression equation to determine the alkaloid concentration, followed by calculation of the total alkaloid content in each fraction.

RESULTS

1. Extraction and Fractionation Results

The processing of tempuyung (*Sonchus arvensis* L.) leaves yielded 300 g of simplicia powder. Extraction was performed using the maceration method with 96% ethanol, after which the filtrate was concentrated using a *rotary evaporator* at 50°C. The extraction process yielded 19.63 g of concentrated extract, corresponding to an extraction yield of 6.54%.

Table 1. Extraction and Fractionation Results of Tempuyung (*Sonchus arvensis* L.) Leaves

Sample	Weight	Yield
96% ethanol extract	19.63 g	6.54%
n-Hexane fraction	5.46 g	54.60%
Chloroform fraction	3.39 g	33.90%
Methanol fraction	0.72 g	7.20%

Subsequently based on table 1, 10 g of the concentrated extract was fractionated according to solvent polarity using *n*-hexane, chloroform, and methanol. The fractionation results showed that the *n*-hexane fraction produced the highest fraction weight, amounting to 5.46 g (54.6%), followed by the chloroform fraction at 3.39 g (33.9%), while the methanol fraction yielded 0.72 g (7.2%).

2. Qualitative Identification of Alkaloids

Table 2. Results of Qualitative Alkaloid Identification

Sample	Wagner's Reagent	Dragendorff's Reagent	Result
96% ethanol extract	+ (brown precipitate)	+ (red coloration)	Positive
n-Hexane fraction	+ (brown precipitate)	+ (red coloration)	Positive
Chloroform fraction	+ (brown precipitate)	+ (red coloration)	Positive
Methanol fraction	+ (brown precipitate)	+ (red coloration)	Positive

The qualitative identification results based on table 2 indicated that the 96% ethanol extract and all fractions tested positive for alkaloids. Testing with Wagner's reagent resulted in the formation of a brown precipitate, whereas Dragendorff's reagent produced a red coloration. Positive results were observed in the 96% ethanol extract, *n*-hexane fraction, chloroform fraction, and methanol fraction.

3. Results of UV–Vis Spectrophotometric Analysis

The determination of alkaloid content was performed using UV–Vis spectrophotometry with caffeine as the standard. The maximum absorption wavelength of caffeine was determined to be 289 nm. The *operating time* test demonstrated stable absorbance values within the range of 34–40 min.

Table 3. Mean Absorbance of Caffeine Standard Solutions

Concentration (ppm)	Mean Absorbance
2	0.104
4	0.153
6	0.196
8	0.276
10	0.347
12	0.446

Calibration curve equation: $y = 0.0348x + 0.0088$; $R^2 = 0.9867$

Based on table 3, a calibration curve was constructed using caffeine standard solutions at concentrations of 2, 4, 6, 8, 10, and 12 ppm. The mean absorbance values at each concentration were 0.104, 0.153, 0.196, 0.276, 0.347, and 0.446, respectively. The relationship between caffeine concentration and absorbance exhibited a linear trend. The resulting linear regression equation was $y = 0.0348x + 0.0088$, with a coefficient of determination of $R^2 = 0.9867$.

4. Results of Total Alkaloid Content Determination

Absorbance measurements were performed at a wavelength of 289 nm with three replicates. The mean absorbance values of the 96% ethanol extract, *n*-hexane fraction, chloroform fraction, and methanol fraction were 0.382, 0.159, 0.265, and 0.343, respectively.

Based on the linear regression equation obtained from the caffeine calibration curve, the calculated concentrations were 11.22 ppm for the 96% ethanol extract, 4.821 ppm for the *n*-hexane fraction, 7.867 ppm for the chloroform fraction, and 10.11 ppm for the methanol fraction.

Table 4. Total Alkaloid Content of Tempuyung Leaf Extract and Fractions

Sample	Mean Absorbance	Concentration (ppm)	Total Alkaloid Content (%)
96% ethanol extract	0.382	11.22	11.12
n-Hexane fraction	0.159	4.821	4.821
Chloroform fraction	0.265	7.867	7.867
Methanol fraction	0.343	10.11	10.11

The results demonstrated based on table 4 that the methanol fraction had the highest total alkaloid content among the three fractions, at 10.11%, followed by the chloroform fraction at 7.867% and the *n*-hexane fraction at 4.821%. Thus, based on the findings of this study, fractions with a higher degree of polarity exhibited higher alkaloid contents than the *n*-hexane and chloroform fractions.

5. Data Visualization

To fulfill the "data visualization" criterion in the journal rubric, two main figures are recommended for inclusion:

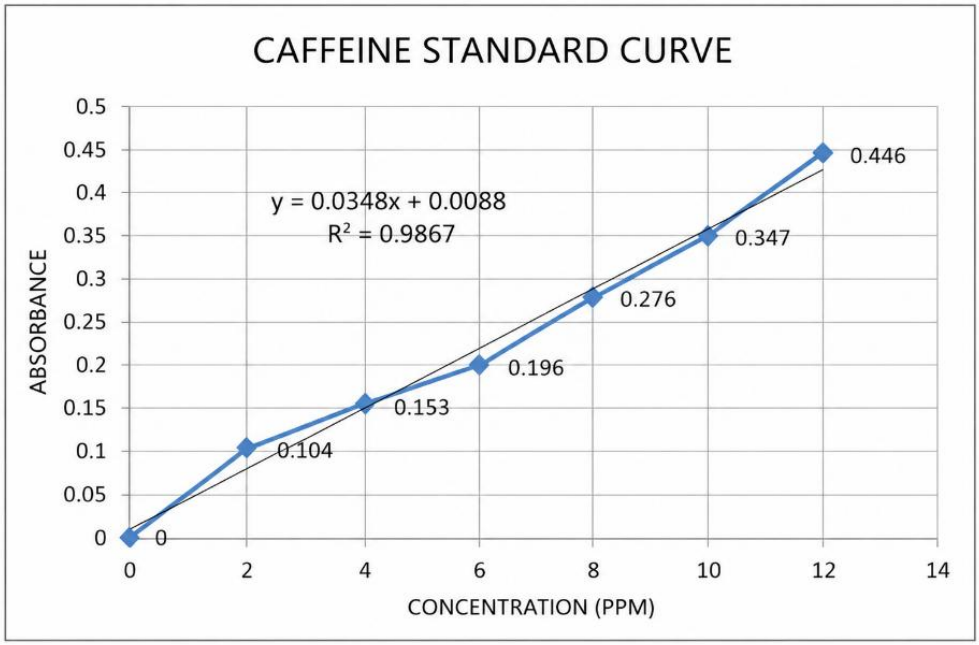


Figure 1. Caffeine Calibration Curve at a Wavelength of 289 nm

Figure 1 illustrates the concentrated tempuyung (*Sonchus arvensis* L.) leaf extract obtained through maceration using 96% ethanol as the extraction solvent. A total of 300 g of tempuyung leaf simplicia powder was extracted for 3 days, with the solvent replaced daily and periodic stirring performed throughout the extraction process. The resulting liquid extract was subsequently filtered and concentrated using a rotary evaporator to obtain a concentrated extract. The extraction process yielded 19.63 g of concentrated extract, corresponding to an extraction yield of 6.54%.

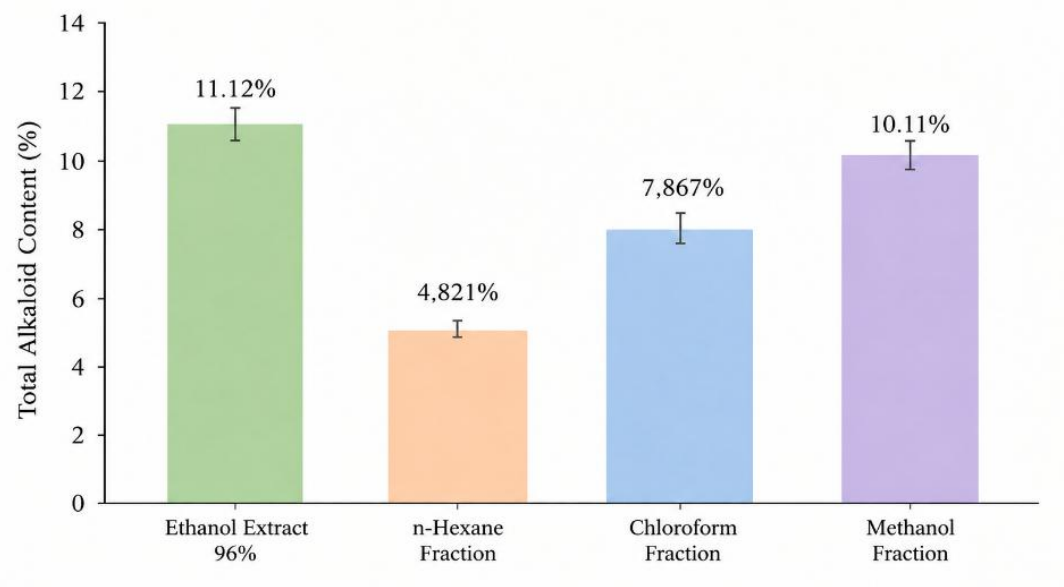


Figure 2. Total alkaloid content in the extract and various fractions of the leafflower plant

Figure 2 illustrates the total alkaloid content of the 96% ethanol extract of tempuyung (*Sonchus arvensis* L.) leaves and each fraction obtained according to solvent polarity. The results showed that the 96% ethanol extract exhibited the highest total alkaloid content, at 11.12%. Following the fractionation process, the total alkaloid content was 4.821% in the *n*-hexane fraction, 7.867% in the chloroform fraction, and 10.11% in the methanol fraction. Thus, among the three fractions, the methanol fraction exhibited the highest total alkaloid content, whereas the *n*-hexane fraction exhibited the lowest. These findings indicate differences in alkaloid content among the fractions, which may be associated with the differences in solvent polarity used during the fractionation process.

Overall, the results demonstrated that tempuyung leaves (*Sonchus arvensis* L.) tested positive for alkaloids in both the 96% ethanol extract and all fractions examined. Among the fractions, the methanol fraction exhibited the highest total alkaloid content (10.11%), followed by the chloroform fraction (7.867%) and the *n*-hexane fraction (4.821%). Thus, the methanol fraction showed the highest total alkaloid content, whereas the *n*-hexane fraction showed the lowest.

DISCUSSION

Tempuyung (*Sonchus arvensis* L.) is a traditional medicinal plant commonly found in local communities and is widely utilized for its therapeutic properties. The leaves are the plant part most frequently used as traditional medicine, particularly for the treatment of gout and to accelerate the healing of external wounds. Traditionally, the leaves are prepared by boiling them for oral consumption or by pounding them and applying the resulting material directly to the wounded area. This traditional use is supported by the findings of Sukmayadi et al., (2023), who reported that tempuyung leaves (*Sonchus arvensis* L.) exhibited antibacterial activity due to the presence of secondary metabolites, including alkaloids. Alkaloids are known to possess antibacterial activity; therefore, tempuyung leaves have potential for traditional medicinal applications, including as a wound dressing. To determine the alkaloid content of tempuyung leaves (*Sonchus arvensis* L.), further investigation is required. Accordingly, this study was conducted to determine the alkaloid content of tempuyung leaf extract (*Sonchus arvensis* L.) at different fractionation levels.

The tempuyung leaf samples used in this study were collected from the Juai District, Balangan Regency, South Kalimantan, Indonesia. The collected leaves were subsequently

processed into simplicia. The preparation of simplicia began with the collection of raw materials and continued through the drying process. Following collection, the raw materials underwent wet sorting to remove impurities and foreign materials adhering to the samples, such as soil, gravel, and twigs. The leaves were then washed under running water to remove remaining dirt and other foreign materials. The washed leaves were subsequently cut into smaller pieces to facilitate the drying process. Drying was performed under direct sunlight for three days, with the tempuyung leaves covered with black cloth to minimize potential degradation of their secondary metabolites. The drying process was intended to prevent enzymatic reactions, reduce the moisture content of the simplicia, and minimize the risk of fungal or microbial growth. Once completely dried, the simplicia were pulverized into powder and weighed. A total of 300 g of simplicia powder was obtained.

The simplicia powder was subsequently extracted using an appropriate solvent. Extraction is a process of separating compounds from plant material using a suitable solvent. In this study, maceration was employed as the extraction method. This method was selected because maceration is a relatively simple cold-extraction technique and is suitable for materials that are sensitive to heat, thereby reducing the risk of degradation of secondary metabolites during extraction. The maceration process was performed by immersing the simplicia powder in a vessel containing an appropriate solvent for 1 × 24 h, with occasional stirring. 96% ethanol was used as the extraction solvent. Ethanol was selected because it is readily available and is considered an effective solvent with relatively low toxicity and good extraction capacity, while also being less susceptible to fungal contamination. In addition, ethanol has broad solvent properties and can extract secondary metabolites with different polarities, including non-polar, semi-polar, and polar compounds. Increasing the concentration of the solvent may facilitate its penetration into plant cells, thereby enhancing the extraction of compounds and producing a more concentrated extract (Mujipradhana et al., 2018). During the maceration process, the solvent was replaced periodically through three remaceration cycles. This procedure was intended to prevent the solvent from becoming excessively saturated, which could reduce further extraction due to the establishment of a concentration equilibrium between the solution outside and inside the plant cells.

The maceration process yielded a liquid extract, which was subsequently concentrated using a *rotary evaporator* at 50°C to obtain a concentrated extract. The use of a *rotary evaporator* was intended to remove the solvent while minimizing degradation of the secondary metabolites, thereby producing a concentrated tempuyung leaf extract. The

resulting concentrated extract was weighed, and the extraction yield was calculated. Extraction yield provides an indication of the amount of compounds extracted from a sample; however, a higher extract yield does not necessarily indicate a higher concentration of a particular bioactive compound (Ramadhani et al., 2020). In this study, 19.63 g of concentrated tempuyung leaf extract was obtained, corresponding to an extraction yield of 6.54%.

The subsequent stage involved fractionation using solvents with different degrees of polarity. Fractionation is a separation process based on the principle of *like dissolves like*, whereby compounds tend to dissolve preferentially in solvents with similar polarity characteristics. In this study, fractionation was performed using a separatory funnel, resulting in the formation of two liquid phases with different densities. The solvents used were *n*-hexane, chloroform, and methanol. Fractionation was intended to separate compounds according to their polarity, allowing them to be distributed preferentially into the corresponding solvent phases. The resulting fractions were the *n*-hexane, chloroform, and methanol fractions, with different fraction weights. The *n*-hexane fraction weighed 5.46 g, the chloroform fraction weighed 3.39 g, and the methanol fraction weighed 0.72 g. The corresponding fraction yields were 54.6%, 33.9%, and 7.2%, respectively.

The fraction yields indicate that the *n*-hexane fraction produced the highest yield, suggesting that a relatively large amount of non-polar extractable material was distributed into this fraction. In contrast, the chloroform and methanol fractions accounted for lower yields. However, fraction yield alone cannot be used to determine the amount of a specific metabolite, because the total mass of a fraction may comprise various classes of secondary metabolites with different chemical characteristics. Therefore, the distribution of alkaloids should be evaluated specifically through qualitative and quantitative analyses rather than inferred solely from fraction yield.

Each concentrated fraction was subsequently subjected to qualitative alkaloid identification using two different reagents, namely Wagner's reagent and Dragendorff's reagent. For each fraction, 1 mL of 2 N HCl was added to the sample in a test tube, followed by two drops of Wagner's reagent and two drops of Dragendorff's reagent in separate tests. The addition of 2 N HCl was intended to facilitate the dissolution and protonation of alkaloid compounds, thereby enhancing their reaction with the respective reagents. A positive alkaloid reaction with Wagner's reagent was indicated by the formation of a brown

precipitate, whereas a positive reaction with Dragendorff's reagent was indicated by the development of a red coloration. In this study, all tested fractions showed positive reactions for alkaloids. These qualitative findings provided the basis for subsequent quantitative determination of alkaloid content in the tempuyung leaf extract and its respective fractions.

The quantitative determination of alkaloids in the tempuyung leaf extract and its fractions was performed using UV–Vis spectrophotometry. This analytical technique measures the absorbance of standard or sample solutions at a selected wavelength. Caffeine was used as the reference standard in this study. The caffeine standard solution was scanned using UV–Vis spectrophotometry over the wavelength range of 200–400 nm, and the maximum absorption wavelength was determined to be 289 nm. Subsequently, the *operating time* was determined to identify the period at which the reaction system exhibited stable or constant absorbance. A 10 ppm caffeine standard solution was monitored for 60 min at 2-min intervals. The results indicated stable absorbance within the 34–40 min interval.

A series of caffeine standard solutions at concentrations of 2, 4, 6, 8, 10, and 12 ppm were subsequently measured at 289 nm in triplicate. The mean absorbance values obtained were 0.104, 0.153, 0.196, 0.276, 0.347, and 0.446, respectively. The caffeine calibration curve demonstrated a positive relationship between concentration and absorbance, indicating that increasing the concentration of the standard solution resulted in an increase in absorbance (Wahyuni & Marpaung, 2020). The linear regression equation obtained from the caffeine calibration curve was $y = 0.0348x + 0.0088$, where y represents absorbance and x represents concentration. The coefficient of determination was $R^2 = 0.9867$, indicating a strong linear relationship between caffeine concentration and absorbance. A high R^2 value indicates that a substantial proportion of the variation in absorbance can be explained by the concentration of the standard solution (Winahyu et al., 2019).

The total alkaloid content of the extract and individual fractions was determined using UV–Vis spectrophotometry at 289 nm, with three replicates for each sample. The mean absorbance values were 0.382 for the 96% ethanol extract, 0.159 for the *n*-hexane fraction, 0.265 for the chloroform fraction, and 0.343 for the methanol fraction. These absorbance values were substituted into the linear regression equation, $y = 0.0348x + 0.0088$, to estimate the alkaloid concentration in each sample. Based on the reported calculations, the alkaloid contents were 11.22% in the 96% ethanol extract, 4.821% in the *n*-hexane fraction, 7.867% in the chloroform fraction, and 10.11% in the methanol fraction.

Among the fractions, the methanol fraction exhibited the highest reported alkaloid content (10.11%), followed by the chloroform fraction (7.867%) and the *n*-hexane fraction (4.821%). Methanol is a polar solvent; therefore, the higher alkaloid content observed in the methanol fraction may indicate that a substantial proportion of the alkaloid constituents in tempuyung leaves possesses relatively polar characteristics. Alkaloids in their protonated or salt forms generally exhibit greater solubility in polar solvents. These findings suggest that the fractionation process resulted in the preferential distribution of alkaloid compounds into the methanol fraction, despite this fraction exhibiting the lowest fraction yield.

This observation highlights that fraction yield does not necessarily correspond to the concentration of a specific class of secondary metabolites. Although the methanol fraction had the lowest yield (7.2%), it exhibited the highest reported alkaloid content among the fractions. This may indicate that the compounds present in the methanol fraction were relatively enriched in alkaloids compared with other extracted constituents. Therefore, the amount of fraction obtained cannot be used as the sole indicator of alkaloid abundance. The differences in alkaloid content among the fractions may be associated with the polarity of the solvents used during fractionation.

The findings of this study demonstrate that tempuyung leaf extract (*Sonchus arvensis* L.) contains alkaloids, as confirmed by qualitative testing, and that the highest reported alkaloid content among the fractions was observed in the methanol fraction (10.11%). Alkaloid content may vary among plant species and fractions because plants differ in the types and relative abundance of their alkaloid constituents. The distribution of alkaloids may also vary according to their chemical structures and polarity. For comparison, Rahmah et al., (2023) reported an alkaloid content of 0.323% in the *n*-hexane fraction of rembusa leaves. Differences between these findings may reflect variations in plant species, extraction and fractionation procedures, solvent polarity, and the chemical composition of the respective plant materials.

The relatively high alkaloid content reported in the methanol fraction of tempuyung leaves may be related to the polarity of methanol, which can preferentially solubilize relatively polar constituents. However, the presence of alkaloids in a particular fraction should not be interpreted as evidence that all alkaloid compounds are polar, because alkaloids comprise structurally diverse compounds with different physicochemical properties. Therefore, further

studies are required to characterize and identify the specific alkaloid constituents present in the methanol fraction.

Overall, the findings indicate that solvent fractionation can influence the distribution and apparent concentration of alkaloid compounds in tempuyung leaf extract. The methanol fraction showed the highest reported total alkaloid content among the three fractions, suggesting that this fraction may be a promising target for further phytochemical investigation. Nevertheless, additional studies involving compound-specific identification, isolation, structural elucidation, and pharmacological evaluation are required to establish the identity and biological significance of the alkaloid constituents in *S. arvensis* leaves.

The findings indicate that the methanol fraction yielded the highest total alkaloid content in tempuyung (*Sonchus arvensis* L.) leaf extract, at 10.11%. This result may provide preliminary information for identifying a potentially promising fraction for further investigations of alkaloid compounds in *S. arvensis* L.

Scientifically, this study complements existing information on the chemical constituents of tempuyung leaves, which have previously been investigated predominantly through phytochemical screening and antibacterial activity assays. The quantitative data on alkaloid content across fractions with different polarities may serve as a basis for further studies involving the characterization, isolation, and identification of alkaloid compounds present in tempuyung leaves.

This study has several limitations. The analysis focused exclusively on the determination of total alkaloid content across different fractionation levels; therefore, it could not determine the specific types or structures of alkaloid compounds present in each fraction. Furthermore, the study did not evaluate the relationship between alkaloid content and specific pharmacological activities.

Therefore, future studies should focus on the isolation and identification of individual alkaloid compounds, the application of different analytical methods, the use of varied concentration ranges, and the evaluation of biological activities of each fraction. Further investigation is also needed to clarify the influence of solvent polarity on the content and characteristics of alkaloid compounds in tempuyung leaves.

CONCLUSION

Based on the findings, tempuyung (*Sonchus arvensis* L.) leaf extract was confirmed to contain alkaloid compounds across all fractionation levels. The total alkaloid content varied among the fractions, namely 4.821% in the n-hexane fraction, 7.867% in the chloroform fraction, and 10.11% in the methanol fraction. Thus, the highest total alkaloid content was observed in the methanol fraction (10.11%). These findings indicate that solvent polarity during the fractionation process is associated with the distribution of alkaloid compounds in tempuyung leaf extract, with the methanol fraction exhibiting the highest alkaloid content.

This study contributes to phytochemical and pharmacognostic research by providing information on the total alkaloid content of tempuyung leaves at different fractionation levels. The findings may serve as preliminary data to support further investigations into the potential of alkaloid compounds in *S. arvensis* L. and their utilization as natural materials. Further studies are recommended to isolate and identify the specific alkaloid compounds present in tempuyung leaves, determine alkaloid content using different concentration ranges and analytical methods, and further investigate the influence of fractionation levels on the content and characteristics of alkaloid compounds.

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