

The Effect of Different Extraction Solvents on Total Phenolic and Flavonoid Total of Snake Plant (*Sansevieria trifasciata* var. *Laurentii*)

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Abstract

BACKGROUND: The solvent system significantly affects the levels of active substances contained in a plant.

OBJECTIVE: This study was conducted to determine the effect of different extraction solvents on the total phenolic content (TPC) and total flavonoid content (TFC) of *Sansevieria trifasciata* var. *Laurentii*, which is responsible for the antioxidant activity.

DESIGN: The experimental design used in this study was a completely randomized design with various types of solvents as a treatment, using the maceration method.

PARTICIPANTS: The treatment consisted of five types of solvents i.e ethanol (96%, 76%, 56%), methanol, and acetone. All treatments were repeatedly for four times to obtain 20 experimental units. The obtained data were analyzed with a variance analysis. If the treatment had a significant effect, it was followed by the Duncan test.

KEY RESULTS: The results showed that the various types of solvent treatments had a very significant effect ($P < 0.01$) on total phenolic and total flavonoid levels. The results also showed that methanol solvent had the best treatment which resulted in antioxidant activity of 3.96%, total phenol content of 1124 mg GAE/g, and total flavonoid content of 342.15 mg QE/g based on yield.

Keywords: Snake plant, Yield value, Total phenolic; Total flavonoid, Maceration.

INTRODUCTION

Antioxidants are compounds that donate one of their electrons to free radical compounds so that free radical compounds become more stable¹⁷. Polyphenols and flavonoids are the most common phytoconstituents plants which are crucial for their pharmacological activity, especially antioxidant activity¹. These two compounds are natural phenolic group in which its popularity is increasing during the Covid 19 pandemic²⁴. One of the plants containing these two popular compounds is the snake plant (*Sansevieria trifasciata* var. *Laurentii*).

This plant is originally an ornamental plant that has existed in Indonesia since the 16th century, and among international ornamental plant traders, this plant is better known as the snake plant or mother-in-law's tongue^{20,21}.

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Snake plant has many varieties with members of the genus reaching 200 species. Snake plant is also easy to change shapes, such as *Laurentii* which is a deviation from *S. trifasciata*. *Laurentii* has produced various new variants such as *Compacta*, *California*, *gold flame*, *Lilian true*, and *futura*^{26,9}. This plant varieties have been used by people in Indonesia for health purpose. Other varieties of this plant are reportedly contain secondary metabolites in the form of flavonoids and polyphenols.

Polyphenol and flavonoid compounds from snake plants can be extracted with the maceration method. This method does not require complicated equipment, it is also relatively inexpensive, and can avoid the evaporation of thermolabile compounds¹⁵. Various solvents provide different total phenol values for each plant. The water solvent produces a higher total phenol value than the methanol solvent in the extraction of several types of spinach and vegetables. The ethanol solvent is able to produce the highest antioxidant activity in avocado leaves extract²². Methanol extract from *beluntas* (*Pluchea indica*) leaves is able to produce the highest total phenols and flavonoids compared to water and ethanol as solvents¹³.

Extraction of phenolics and flavonoids as antioxidants from snake plant is possible by adding different type of solvents with different types of solvents. It is done based on the consideration that the percentage of extraction is influenced by the type of solvent with various polarities and chemical compositions as well as the physical properties of the sample. Phenols and flavonoids are bioactive compounds that tend to be polar to semi-polar; As the result that in the extraction process, semi-polar to polar solvents are used¹⁹. Ethanol, methanol and acetone solvents are often used to extract phenolic compounds in plants and herbal plants. During the extraction process, the yield will rise as the amount of solvent increases. The increase in yield is due to the higher the amount of solvent used, the more optimal the release of the target compound into the solvent and the avoidance of solvent saturation^{4,22}. This study aims to determine the phenolic and flavonoid content of mother-in-law's tongue from a different variety, namely *laurentii*, using different types of solvents to produce snake plant extracts with the highest phenolic and flavonoid content.

MATERIAL AND METHOD

Raw materials

Fresh leaves of snake plant (*Sansevieria trifasciata* var. *Laurentii*) were procured from Malalak, Agam Regency, West Sumatera, Indonesia and were authenticated by a botanist of the Department of Pharmacognosy, Dwi Farma Academy of Pharmaceutical, Bukittinggi, Indonesia. A voucher specimen (ADF 0721) is kept in our laboratory for future reference

Chemicals

Ethanol, Methanol, Aseton, from Sigma Chemicals, Folin-

Ciocalteu Reagent (Merck) gallic acid, quercetin, sodium carbonate, potassium acetate, and aluminum chloride. All other chemicals used in our study were of analytical grade from local companies.

Experimental design

The design used in this study was a completely randomized design (CRD) which consisted of 5 treatment types of solvents and 3 replications in order to obtain 15 experimental units. 250 g of Snake plant extraction treatment was carried out using different solvents, namely: S1 (Methanol), S2 (Ethanol 96%), S3 (70% Ethanol), S4 (Ethanol 56%), and S5 (Acetone). The obtained data were analyzed using analysis of variance. If the treatment indicate an effect, then Duncan's test was performed^{11,5}.

Determination of Total Phenolic Content (TPC)

The total phenolic content of snake plant extracts was quantified by the Folin-Ciocalteu method from Singleton with some modifications²⁵. Briefly, 0.3 mL of snake plant extracts at 100 µg/mL concentration in distilled water was thoroughly mixed with 1.5 mL of 1:10 diluted Folin-Ciocalteu reagent and incubated for 3 minutes at room temperature. Afterward, 1.2 mL of sodium carbonate (7,5%) was added to each sample mixture and was allowed to stand for a further 30 minutes in the dark at room temperature, and the absorbance was measured at 770 nm. Gallic acid in the range of 0-60 µg/mL was used as a reference standard. Data were expressed as gallic acid equivalent (GAE) mg/g of snake plant extracts (mg of gallic acid/g of the extracted compound).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined using the colorimetric method described by Zhishen et al.¹⁹⁹⁶ with modifications¹⁶. Aluminum chloride complex forming assay was used to determine the total flavonoid content of the extracts. Quercetin was used as standard and flavonoid content was determined as quercetin equivalent. A calibration curve for quercetin was drawn for this purpose. From the standard 2 quercetin solution the dilutions of (10, 15, 20, 25 and 30 µg/mL) concentrations were prepared in methanol. Each dilution of 0.8 mL of quercetin was mixed with 5 mL of distilled water and then 0.3 mL of 5% sodium nitrate and was allowed to stand for 6 minutes. Then 0,3mL of 10% Aluminum chloride solution was added and was allowed to stand for 5 minutes after which 4 mL solution of 4% Sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm on a UV spectrophotometer (Genesys 10s). Total flavonoid content was calculated as quercetin equivalents (mg QE/g).

RESULTS AND DISCUSSION

After extractions were carried out using different solvents, the result indicates that snake plant is concentrated under reduced pressure, and the yield percentage of each extract is calculated against the initial amount of the solid material. As

it is shown in Table 1, as the extraction solvent S1 (Methanol) gave a maximum yield compared to other solvents used in our study.

The results of the yield analysis (Extract Yield (% solid)), total phenol content, and total flavonoid content from the snake plant extract can be seen in Table 1. The results of the variance showed that the type of solvent has a very significant effect ($P < 0.01$) on the yield of snake plant extract. Based on Table 1, it can be seen that the lowest

yield was found in the S4 treatment, i.e. 2.53% and is significantly different from S2 and S3, which are 3.00% and 3.34%, respectively. The highest yield is in the S1 treatment, which is 3.96%. This is not significantly different from the S5 which was 3.92%. This shows that the polarity level of the compounds contained in the snake plant extract is close to the polarity of methanol and acetone which have dielectric constants of 33, 62, and 20,70, respectively.

Table 1: Average yield (Extract Yield (% solid)), total phenol content, and total flavonoid content in snake plant extract.

Solvent	Extract Yield (% solid)	Total Phenol (mg GAE/g)	Total Flavonoid (mg QE/g)
S1 (Methanol)	3.96 ± 0.721 ^a	1124 ± 0.041 ^a	342.15 ± 0.002 ^a
S2 (Ethanol 56%)	3.00 ± 0.358 ^b	271 ± 0.026 ^c	205.00 ± 0.034 ^c
S3 (Ethanol 76%)	3.34 ± 0.384 ^b	173 ± 0.026 ^d	31.05 ± 0.047 ^e
S4 (Ethanol 96%)	2.53 ± 0.143 ^c	82 ± 0.026 ^e	25.35 ± 0.008 ^d
S5 (Acetone)	3.92 ± 0.102 ^a	702 ± 0.041 ^b	283.70 ± 0.001 ^b

Note: The same letter behind the mean value in the same column indicates a non-significant difference ($P < 0.05$).

Based on table 1 above, the highest average value for the total response of phenolic compounds is 1124 mg for methanol solvent, while the lowest average for total phenolic compounds is 82 mg for ethanol 96% solvent. Based on the results of the Tukey test, each type of solvent used had a significantly different effect on the phenolic content, or in other words, there is a significant difference in the average response of phenolic levels due to the type of solvent being tested.

Likewise, with testing of total flavonoids; the highest value of flavonoids is obtained using methanol as a solvent, while the lowest value is obtained using ethanol 96% as a solvent. The results of the Tukey test stated that all the solvents used had an effect on the total flavonoid content.

Extract Yield (% solid)

The results of the analysis of variance showed that the type of solvent has an exceptionally significant effect ($P < 0.01$) on the Extract Yield (% solid) of snake plant extract. The relationship between the type of solvent and the Extract Yield (% solid) of snake palm extract can be seen in Figure 1.

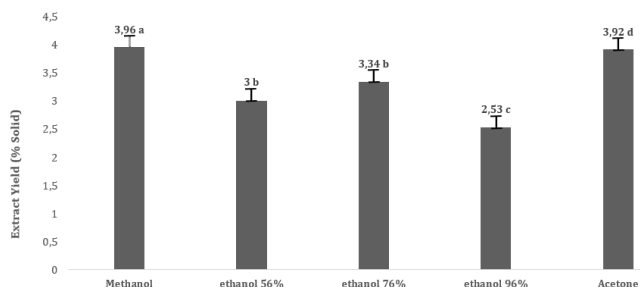


Figure 1: The relationship between the type of solvent and the Extract Yield (% solid) of snake palm extract

Based on Figure 1, it shows that the highest average Extract Yield value is found in methanol solvent with an Extract Yield value of 3.96%, while the lowest Extract Yield value is produced by ethanol 96% solvent. Dunnet's test results showed that methanol and ethanol solvents with various concentrations have the same effect on the Extract Yield, while acetone has a significantly different effect on the Extract Yield value. It indicates that the polarity level of the compounds contained in the snake plant extract is close to the polarity of methanol which has a dielectric constant of 33.62.

The use of solvents with different polarities can affect the result of Extract Yield value. Organic solvents based on their dielectric constant can be divided into two, namely polar and non-polar solvents. The dielectric constant is expressed as the repulsive force between two electrically charged particles in a molecule. The higher the dielectric constant, the more polar the solvent. The dielectric constants in water, methanol, ethanol, and acetone have values of 80, 33, 24, and 21, respectively²³. The high Extract Yield value of the methanol extract of snake plant extract using methanol as a solvent indicates that the methanol in the snake plant is able to extract the compound better because the yield of the compound is based on the similarity of polarity to the solvent. Methanol is a universal solvent so it can attract most of the polar and non-polar compounds in the material¹⁸. Several other studies also showed that extraction using methanol as a solvent resulted in high yields of mangosteen rind extract. Likewise with barley seed extract, the highest yield is obtained using methanol rather than ethanol, and acetone as solvents^{12,2}.

Total Phenolic

The results of the analysis of variance shows that the interaction between the types of solvents has a very significant effect on the total phenolic content of the snake plant extract. The average total phenolic extract of snake plant is described in Figure 2.

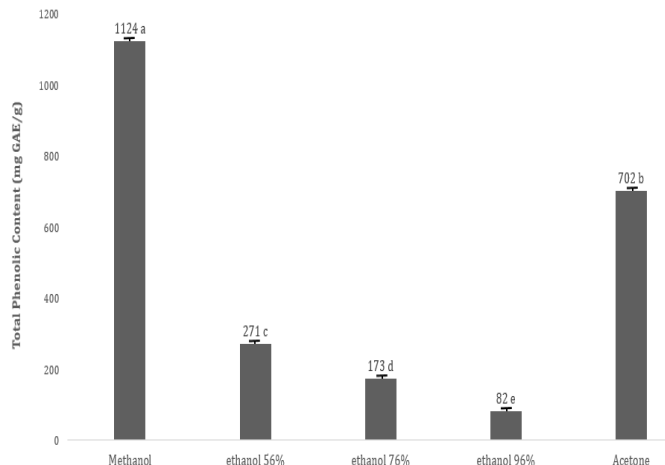


Figure 2: The average total phenolic extract of snake plant

The results indicates that methanol and acetone are effective when they are used to extract the snake plant because they produced the highest total phenol content. This can be understood that the polarity of the phenolic compounds of snake plant extract corresponds to methanol and acetone which have dielectric constants of 33.62 and 20.70. The use of methanol and acetone as solvents makes compounds such as phenolic in plants extracted, because polar compounds will dissolve compounds that are also polar, and most of the phenolic components of plants are generally polar. The total phenol content in the extract with ethanol 96% is lower than other extracts. The components contained in the snake plant extract tend to have a solubility close to methanol and acetone. In plants, phenolic compounds can be in the form of simple phenols, benzoquinone, phenolic acid, acetophenone, naphthoquinones, xanthenes, coumarin bioflavonoids, stilbenes, tyrosine derivatives, hydroxycinnamic acid, flavonoids, lignans, and tannins^{8,14}. One of the natural antioxidants is gallic acid (3, 4, 5-trihydroxy benzoic acid). Gallic acid is a phenolic compound and has strong antioxidant activity, and the determination of the total phenolic content can be done using the Folin-Ciocalteu reagent. This method is based on the reducing power of the phenolic hydroxy groups. All phenolic compounds including simple phenols can react with Folin Ciocalteu reagent. The presence of an aromatic nucleus in phenolic compounds (phenolic hydroxy groups) can reduce phosphomolybdate phosphotungstate to molybdenum which is blue in color. The total phenolic content in plants is expressed in GAE (gallic acid equivalent) which is the equivalent number of milligrams of gallic acid in 1 gram of sample^{10, 3}.

The measurement results of gallic acid calibration against

Folin Ciocalteu reagent can be seen in Figure 3 below:

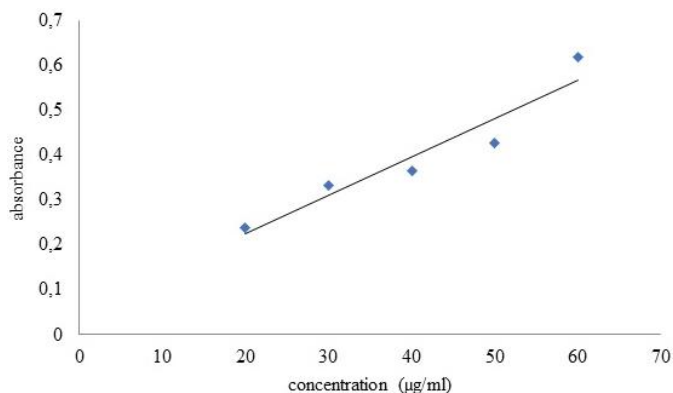


Figure 3: Gallic Acid Calibration Curve in Reagan Folin-Ciocalteu

Total phenolic content (TPC) is calculated based on equivalence to gallic acid (mg GA/g extract) using the regression equation = $0.0085x + 0.055$ with $R^2 = 0.9122$, meaning that 91.2% of the absorbance is affected by concentration, while the rest are influenced by other factors such as temperature, light, storage, chemical substances, and so on.

The results shows that methanol extract and acetone extract of snake plant had a fairly high total phenolic content, i.e. 1124 mg/g EAG, using the standard gallic acid curve ($R^2=0.9122$). This illustrates that the polar compounds contained in the snake plant extract are well dissolved in methanol^[14]. The total phenol content in the sample is determined by the Folin-Ciocalteu method which is based on the ability of the phenolic compounds in the extract to react with the fosmolybdcic acid-phosphotungstic acid contained in the Folin-Ciocalteu reagent. This reaction produced a blue molybdenum-tungstat compound. The bluer the color intensity of the solution, the greater the total phenol content in the sample.

Total Flavonoids

The results of the analysis of variance describes that the type of solvent used in the treatment had a very significant effect ($P<0.01$) on the total flavonoids of the snake plant extract. The relationship between the type of solvent and the total flavonoids of the snake plant extract can be seen in Figure 4.

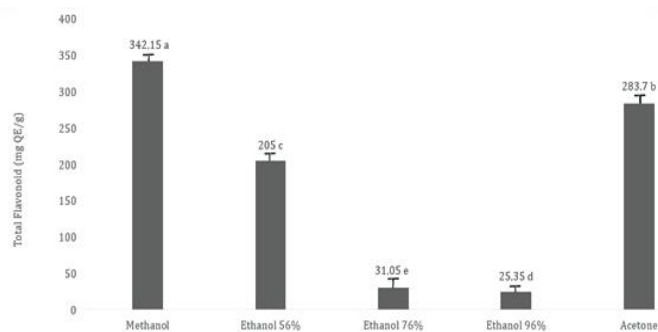


Figure 4: The relationship between the type of solvent and the total flavonoids of the snake plant extract

Based on the figure, the highest total flavonoid is obtained when methanol solvent is used, namely 342.15 mg QE/g extract, and the lowest total flavonoid is obtained when ethanol 96% solvent is used, i.e. 25.35 mg QE/g extract.

A compound will dissolve in a solvent of the same polarity. Flavonoid compounds are divided into several types, and each type of flavonoid has a different polarity depending on the number and position of the hydroxyl groups of each type flavonoids so that it will affect the solubility of flavonoids in solvents⁶. The total flavonoids in the snake plant extract with methanol as a solvent showed that the methanol solvent has a similar level of polarity and way more effective in dissolving flavonoid compounds in lemon peels so that the snake plant extract using methanol as a solvent produced the highest flavonoid compounds.

The basis for determining the flavonoid content in the aluminum chloride method is the formation of a complex between aluminum chloride with a ketone group on the C-4 atom and a hydroxyl group on the C-3 or C-5 atom which is neighboring from the flavone and flavonol groups.

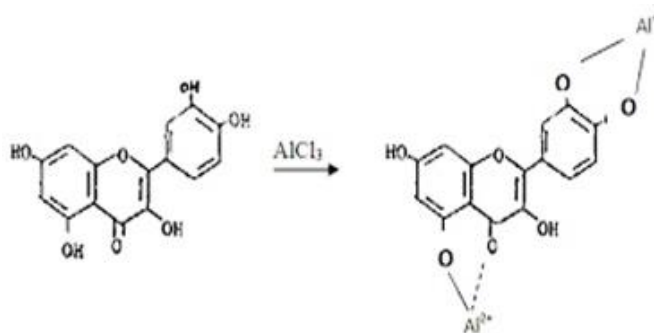


Figure 5: Formation of the quercetin-aluminum chloride complex

The ability of flavonoids to form complexes with AlCl_3 produced an orange color due to an oxidation-reduction reaction between flavonoids and AlCl_3 where flavonoids acted as reducing agents and AlCl_3 as oxidizing agents. Then potassium acetate which is a strong base formed a red color whose absorbance was measured at a maximum wavelength of 430nm with 3 repetitions. The addition of potassium acetate served to detect the presence of a 7-hydroxyl group. The red color was the result of the reaction between aluminum ions and flavonoids (quercetin) in an alkaline environment (potassium acetate). The formation of aluminum complexes is one of the most common procedures for determining the total flavonoid content in food and medicinal plants. The results of the analysis of variance indicates that the type of solvent had a very significant effect ($P < 0.01$) on the total flavonoid content of the snake plant extract. Based on Table 1, it can be seen that the lowest total flavonoid content was found in the S4 treatment, which was 25.35 mg QE/g. The highest total flavonoid level was found in the S1 treatment, which was 342.15 mg QE/g. This shows that methanol solvent is effective when used to extract snake plants. The polarity

level the methanol solvent showed compatibility with the flavonoid compounds in the snake plants so that it could produce extracts with the highest flavonoid content. Methanol has a dielectric constant of 33.62 which indicates that methanol is a polar solvent. Ethanol solvent produced higher total flavonoid content than other solvents in snake plant extract^{7,21}.

The results showed that the total flavonoids are directly proportional to the total phenol and yield value of the snake plant extract. Phenolic components such as flavonoids which are known as primary antioxidants from plants are polar. Flavonoid levels are lower than phenolic levels because flavonoids are part of phenolics.

CONCLUSION

The results of this study indicates that the type of solvent has a very significant effect on antioxidant activity. The highest total phenol content is obtained using methanol solvent, i.e. 1124 mg GAE/g, which is directly proportional to the total flavonoid content and Extract Yield (% solid).

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