SCREENING OF SECONDARY METABOLIT COMPOUNDS IN CITRONELLA *(Cymbopogon nardus)* USING REFLUX AND SOCHLETATION METHODS

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Abstract

Introduction: Citronella *(Cymbopogon Nardus)* belongs to the Poaceae family. Citronella *(Cymbopogon Nardus)* has antibacterial, anti-inflammatory, diuretic, antioxidant, anticancer, and antipyretic properties. Therefore, it is necessary to conduct research on the phytochemical screening of n-hexane extract of citronella stems by reflux and soxhletation methods. The purpose of this study was to determine the content of secondary metabolites of Citronella *(Cymbopogon Nardus*) using reflux and soxhletation methods.

Method: This research method is experimental with a variety of methods, namely the reflux method and the soxhlet method, the sample used is Citronella Wangi stems obtained from the Wonosari farm, Kendal. Data analysis was done descriptively.

Results: The results of this study the yield obtained by the reflux method and the soxhletation method did not meet the requirements of the Indonesian herbal pharmacopoeia, namely 7.2% because for reflux the results were 2.4% and the soxhletation method 3.8%.

Conclusion: The conclusion of this study is that the yield obtained does not meet the requirements of Indonesian herbal pharmacopoeias and that Lemongrass contains alkaloids, flavonoids, and tannins.

Key Words: *Fragrant Lemongrass (Cymbopogon Nardus), secondary metabolites,N-Heksana, reflux, Soxhlet, Yield.*

INTRODUCTION

Nurcholis *et al* (2019) states that the diversity of biological natural resources in Indonesia consists of a variety of plant species. Indonesia's geographical location in the tropics is one of the biological centers in the world in the form of various types of plants spread throughout Indonesia. Many plants have been used as traditional medicine for generations. People prefer to use traditional medicines because they are cheap, can be formulated by themselves and have little side effects compared to drugs from pharmaceutical products. The way to develop traditional medicine is by conducting phytochemical screening to determine the active chemical components contained in medicinal plants.

According to Purbowati *et al*. (2021) Phytochemical screening is a method used to determine the components of active compounds contained in samples, namely their chemical structure, biosynthesis, natural distribution and biological function, isolation, comparison of chemical compositions of various types of plants. Susanty and Bachmid (2016) explained that phytochemical compounds contained in plants can be obtained by extraction. Extraction is the process of separating a substance from a mixture using a solvent. Extraction can be carried out by various methods, one of which is reflux and soxhletation, both methods are included in the hot extraction method. Reflux is an extraction method that is carried out at the boiling point of the solvent, for a certain time and a certain amount of solvent using the presence of a cooler(condenser). Soxhletation is extraction using a solvent that is always new, generally carried out using a special tool so that constant extraction occurs using a recooling (condenser). In the extraction process using solvents, the selection of solvents in the extraction process usually uses the principle of like dissolves like, where a non-polar compound will dissolve in a non-polar compound and a polar compound will dissolve in a polar compound.

One of the medicinal plants that have potential as medicinal ingredients is Lemongrass *(Cymbopogon nardus).* In the research of Anwar and Siringoringo (2020) Lemongrass is a plant that is widely cultivated in tropical and subtropical areas. In addition, lemongrass is commonly used by the community. Several studies have reported that lemongrass has antibacterial, anti-inflammatory, diuretic, antioxidant, anticancer, and antipyretic properties. In addition, lemongrass can relieve coughs, treatment of typhus, seizures, and rheumatism. In a previous study conducted by Anwar and Siringoringo (2020) it has been explained that the Citronella plant has several groups of compounds such as D-limonene, citronellal, citronellol, geraniol by means of citronella essential oil identified by Gas–Mass Spectrometry (GC–MS)

chromatherapy)

Previous research conducted by Desmiaty *et al.* (2019) using a different sample, namely *R. fraxinifolius*, it wasfound that the reflux method gave more yield, but the Soxhlet method was able to extract more polyphenoliccompounds and antioxidant compounds. In this study, we want to use the method of reflux and soxhletation.Reflux is used because it is simpler, cheaper, and has a shorter time, while soxhletation is used because it canproduce more extract and use less solvent and can be used repeatedly. Research conducted by Hilmarni *et al.*(2021) the sample used was citronella leaves taken in Bukittinggi, 1 kg in wet sorting, then finely chopped andextracted by percolation with n-hexane as solvent. In the research of Hilmarni *et al*. (2021) it is proven thatlemongrass contains alkaloids, flavonoids, and saponins.

Based on the above background, it is necessary to conduct research on the phytochemical screening of citronella by reflux and soxhletation methods. The results of this study are expected to provide information regarding the content of secondary metabolites in citronella

METHOD

Research Design

The research design used in this study was experimental.

Location and Research Time

This research was conducted at the Chemical Labrotarium of Stikes Mitra Keluarga Bekasi Timur, the research was carried out for approximately 3 months from March to May 2022.

Population and Research Sample

The population as well as the sample in this study were 15 kg of fragrant lemongrass *(Cymbopogon nardus)* stems obtained from Wonosari, Kec. Kendal, Kendal Regency, Central Java.

Research Variabel

In this study, the independent variable is a single variable (Sugiyono, 2017). The independent variables in this study were the percentage yield of the thick extract and the phytochemical screening of the lemongrassstem extract using reflux and soxhletation methods.

Research Tools and Materials

Tools

The tools used in this study were knife, cutting board, dropper, analytical balance, test tube (Iwaki Pyrex®),measuring cup (Iwaki Pyrex®), blender (Philips), beaker glass (Iwaki Pyrex®), reflux, soxletation, rotary evaporator, filter paper, mattress thread, steam dish (Iwaki Pyrex®) stir bar, thermometer, horn spoon, spatula.

Research Materials

The materials used in this study were Citronella, magnesium powder, concentrated HCL, 2 N HCL (Hydrochloric acid, distilled water), N-Hexane, mayer (chloride, aquades, potassium iodide), ethyl acetate, concentrated sulfuric acid, dragendorff (Nitrate), concentrated nitric acid, potassium iodide, distilled water), Wagner (Aquades, iodine, potassium iodide), 1% $FeCl_3$ ($FeCl_3$, aquades).

Method Reflux

In the reflux method, the mashed Lemongrass sample was then weighed as much as 50 g, then put in a round bottom flask and 500 ml of N-Hexane solvent was added. After that, a reflux device was assembled, until it was extracted at 50℃ for 2 hours. The solution that has been extracted is filtered through filter paper and then put in an Erlenmeyer.

Method Soxletation

In the soxhletation method, the first method is to weigh the Simplicia Citronella Wangi *(Cymbopogonnardus)* as much as 50 g then wrapped in filter paper and then tied with mattress thread. Then assemble the

soxhlet apparatus. Next, filter paper containing Citronella *(Cymbopogon Nardus)* was added, then put it into the soxlet apparatus plus 500 ml of N-Hexane solvent, then heated at 50°C with a hot plate until the cycle drops were colorless.

Extract Evaporation

After obtaining the results of the reflux and soxhletation methods, the next step is to separate the solvent from the Citronella extract using a rotary evaporator and then evaporate it at a temperature of 50°C with a speed of 100 rpm. After obtaining the results, the yield is calculated using the following formula:

$$\text{Thick extract yield \%} = \frac{\text{Extract weight}}{\text{Citronella weight}} \text{x } 100\%$$

(Susanty and Bachmid, 2016)

RESULTS

The results of the yield on the thick extract of Citronella stem stems using N-hexane as solvent can be seen in table 5.3 below.

Table 5.3 Yield Results of Concentrated Lemongrass Stem Extract

Extraction Method	Extracted Powder Weight (grams)	Weight of Extracted Extract (grams)	Yield Value (%)
Reflux	50	1,2	2,4
Soxletation	50	1,9	3,8

From table 5.3 it can be seen that the yield of the thick extract of the Citronella stem extract showed the higher yield value was the soxhletation method of 3.8%.

Phytochemical Screening

When a thick extract is obtained, phytochemical screening is carried out including alkaloids, flavonoids,saponins, tannins, steroids/terpenoids. The results of phytochemical screening can be seen in table 5.4. **Table 5.1 Phytochemical Screening**

Phytochemical Screening	Reagent	Method Reflux	Method Soxletation	Description
Alkaloid	Mayer	+	+	Sediment white
	Dragendroff	+	+	Sediment orange
	Wagner	+	+	Sediment brown
Flavonoid	Mg + Hcl pekat	+	+	yellow
Saponin	Aquadest	-	-	No foam
Tanin	$Fecl_3$ 1%	+	+	Brownish green
Steroid/terpenoid	Etil + asam asetat anhidrat + asam sulfat pekat	-	-	Unstable green color

Description: + = Positive result
- = Negative result

The results of phytochemical screening of citronella stem extract using the reflux method and the soxhletmethod showed the presence of alkaloids, flavonoids and tannins

a b c d

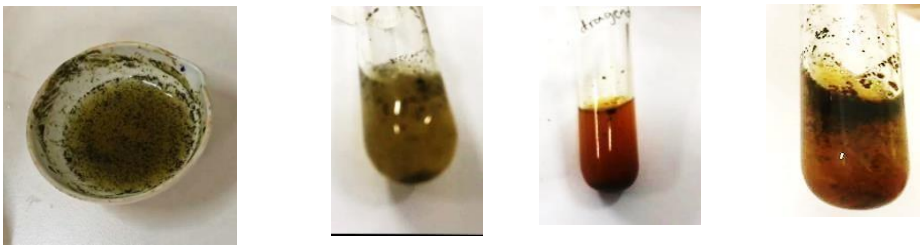

Figure 5.5. The results of the alkaloid test using the reflux method and the soxhlet method before and after the reagent was given

Description:
a = Before
b, c, d = After added reagents (b. mayer, c. dragendrof, d. wagner)

Figure 5.5 shows the results of the alkaloid test with three reagents, namely Mayer, Wagner and Dragendroff reagents. In Mayer's reagent positive for alkaloids was shown a white precipitate, Wagner's reagent was positive for alkaloids indicated the presence of brown deposits, and positive Dragendroff's reagent indicated the presence of red precipitates.

Figure 5.6. The results of the flavanoid test using the reflux method and the soxhlet method before and after the reagent was given

Description:
a = Before
b = After

In Figure 5.6, the positive flavonoid test results with mg and HCL powder reagents show a yellow color. The data obtained in table 5.4 is complemented by Figure 5.7 which can be seen in the stem samples of Citronella *(Cymbopogon nardus)* during phytochemical screening.

a b

Figure 5.7. The results of the steroid/terpenoid test using the reflux method and the soxhlet method before and after the reagent was given

Description:
a = Before
b = After

Figure 5.7 shows the negative steroid/terpenoid test results with ethyl reagent, anhydrous acetic acid, and concentrated sulfuric acid. Because it does not form a green color if positive for steroids and red if positive for terpenoids. The data obtained in table 5.4 is complemented by Figure 5.8 which can be seen in the stem samples of Citronella *(Cymbopogon nardus)* during phytochemical screening.

a b

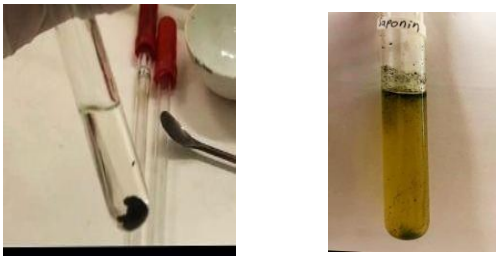

Figure 5.8. The results of the saponin test using the reflux method and the soxhlet method before and after the reagent was given

Description:
a = Before
b = After

In Figure 5.8, the results of the saponin test are negative because it does not produce foam after being shaken for ± 1 minute. The data obtained in table 5.4 is complemented by Figure 5.9 which can be seen in the stem samples of Citronella Wangi *(Cymbopogon nardus)* during phytochemical screening.

a b

Figure 5.8. The results of the tannin test using the reflux method and the soxhlet method before and after the reagent was given

Description:
a = Before
b = After

In Figure 5.9 the results of the positive tannin test with FeCl3 reagent produce a brownish green color.

DISCUSSION

Based on the results of the viscous extract research, the yield calculation was carried out, the results obtained were 2.4% for the reflux method and 3.8% for the soxhlet method. The yield percentage results in this study did not meet the requirements of the Indonesian Herbal Pharmacopoeia because it was less than 7.2% (DEPKES, 2017).

According to Sari *et al*. (2021) the longer the simplicia is extracted, the lower the yield will be. This is because the temperature and pressure increase so that the yield of the viscous extract decreases. In addition to the extracting factors, the moisture content of the sample also affects the yield of the thick extract, so that prior to extraction, drying is carried out to reduce the moisture content of the material. The yield of thick extract was influenced by the way of handling before and after extraction.

In this study, positive results were obtained on phytochemical screening including alkaloids, flavonoids andtannins. The alkaloids were tested using extracts and reacted with Mayer, Dragendroff, and Wagnerreagents. The Mayer reagent produces a white precipitate because nitrogen in the alkaloids will react with metal ions K+ from K2[HgI4] (potassium tetraiodomercurate (II) to form a precipitated potassium-alkaloid complex. Dragendroff's test obtained positive results indicated by orange color, because nitrogen is used to form coordinate covalent bonds with K+ which is a metal ion. The Wagner test got a positive result to get a brown precipitate, because the metal ion K+ will form a coordinate covalent bond with nitrogen in the alkaloid. The flavonoid test got positive results with yellow results, due to the addition of magnesium powder and the addition of HCL, to reduce the benzopyron core bonds contained in the flavonoid structure that form flavylium salts. The tannin test got a positive result because it showed a brownish green color and it can be concluded that the citronella has a phenol group (Harahap, 2021).

CONCLUSION

Based on the research that has been done, it can be concluded that the results of the yield of the thickextract of Lemongrass Wangi showed that none of the results met the requirements contained in the Indonesian herbal pharmacopoeia as much as 7.2%. Citronella thick extract using two methods, reflux and soxhletation, was proven to contain secondary metabolites in the form of alkaloids, flavonoids, and tannins.

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