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Solvent Effects on Phytochemical Screening Test of Red Lemongrass *(Cymbopogon nardus (L.) Rendl.)* Extract and Its Potential as Antidiabetic Agent

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Abstract: Phytochemical screening is a preliminary stage in phytochemical research that aims to provide an overview of class compounds contained in the plants. In this study, distillated water, methanol, and n-hexane were used as solvents. The purpose of this study was to determine the secondary metabolites in red lemongrass (Cymbopogon nardus (L.) Rendl.) extract using different three solvents. Red Lemongrass extract was prepared by maceration method using distillated water, methanol, and n-hexane (10 gram: 100 mL) as solvents and steam distillation using distillated water. The results of phytochemical screening tests, showed that alkaloids, flavonoids, triterpenoids, and saponins were attracted to the distillated water solvent. Meanwhile flavonoids and triterpenoids were drawn to methanol solvents, while citral was appeared in essential oil of red lemongrass. In addition, triterpenoids were selected in n-hexane solvents. The best solvent these results will be used as a reference for testing the ability of the extract as an antidiabetic agent..

Keywords: Red Lemongrass, Phytochemical Screening, The use of solvent, Test tube

1. INTRODUCTION

The red lemongrass plant *(Cymbopogon nardus (L.) Rendl.)* is one of the plants that thrives in Indonesia from ordo Graminales. Red lemongrass is *perennial* (growing throughout the year). Red lemongrass is not only very well known as a spice in Asian cuisine (especially in Thailand and Indonesian cuisine), but it can also be brewed into a herbal tea with a distinctive lemon aroma. Red lemongrass has beneficial properties such as anti-mosquito, anti-fungal, antibacterial, larvicidal, anti-inflammatory, aromatic, antipyretic (can relieve fever and headaches), antispasmodic (acts as a muscle relaxer).), and can be used for cleaning agents [1].

Red lemongrass plants *(Cymbopogon nardus (L.) Rendl.)* contain essential oils. The essential oil from lemongrass leaves averages 0.7% (about 0.5% in the rainy season and can reach 1.2% in the dry season). Red lemongrass oil is pale yellow in colour. The main active ingredients produced are aldehyde compounds (citronellol-$C_{10}H_6O$) of 30-45%, alcoholic compounds (citronellol-$C_{10}H_{20}O$ and

geraniol-$C_{10}H_{18}O$) of 55-65% and other compounds such as geraniol, citral, nerol, metal, heptonone and dipentene [2]. In the previous study was showed that citral as an effective hypolipidemic agent in diabetes, with moderate hypoglycemic property [3]. The root of the lemongrass plant contains approximately 0.52% alkaloids from 300 grams of plant material. Lemongrass leaves and roots contain flavonoids namely luteolin, luteolin 7-O-glucoside (cynaroside), isoscoparin and 2''-O-rhamnosyl isoorientin. The other flavonoid compounds which are isolated from the *aerial* parts of the lemongrass plant are quercetin, kaempferol and apigenin [4]. The compound contents then tested by phytochemical screening test.

Phytochemical screening is an initial stage in a phytochemical research which aims to provide an overview of the class of compounds contained in the plant that being studied. The phytochemical screening method is carried out by looking out the colour testing reaction using a colour reagent. The things that play an important role in phytochemical screening test are the selection of solvents and extraction methods [5]. In phytochemical screening, the several different solvents are used according to the type of polar, non-polar, and semi-polar solvents.

The solvents are liquids that are capable to dissolve the other substances. According to previous research on red lemongrass plant samples, the results obtained that the phytochemical screening test consisted of flavonoids, alkaloids, saponins, tannins, and steroids. Moreover, citral was carried out in essential oil of red lemongrass. In this study, there were used different solvents but producing the same secondary metabolites based on the maceration method. The term maceration comes from the Latin macerare, which means "to soak". Maceration is the most appropriate process because the powder that has been soaked in the solvent until it seeps will soften the cell arrangement so that substances that are easily soluble will dissolve in the solvent [6]. The principle is that the solvent will penetrate the cell wall and enter the cell cavity, because the more concentrated concentration of the solution will force it out so that there is an equilibrium concentration of the solution inside and outside the cell [7]. Based on the information that the authors obtained from several journals and other reading sources, the maceration method has advantages such as the method of processing and the unit of equipment used is simple and the operational costs are relatively low [8]. In this case maceration is used with the appropriate solvent, which meets the specified criteria. In the extraction process, the effectiveness of the active compound depends on the solvent that were used. Some things to consider in choosing a solvent include toxicity, ease of evaporation, selectivity, polarity, and price of the solvent [9]. In this study, several solvents were used in the maceration process, namely n-hexane (non-polar), methanol (semi-polar) and water (polar) solvents. This solvent is used in research because n-hexane is a non-polar solvent that can evaporate easily, so extracts are easily obtained [10]. Methanol is a semi-polar solvent, methanol can attract analytes in the form of alkaloids, steroids , saponins and flavonoids derived from plants [11]. Meanwhile, after this study, it will continue to explore the potential antidiabetic agent of red lemongrass.

2. MATERIALS AND METHODS

2.1. Collection and processing of red lemongrass (Cymbopogon nardus (L.) Rendl.)

Red lemongrass is collected from Sorogenen, Ambarketawang, Gamping, Sleman Regency, Indonesia. The stems of red lemongrass are washed to remove dirt and microbes. The red lemongrass stems are dried in an oven at 60^{o}C, until it was completely clean from other impurities [12].

Parameters to determine which red lemongrass have been dried was easy to break as well as constant weight [13].

2.2. Preparation of red lemongrass extract

Red lemongrass was prepared in 1-2 cm, then extracted by maceration method using n-hexane, methanol and distillated water. As much as 500 grams of red lemongrass was added to each solvent until the sample was submerged (solvent was 1 cm above the sample). Red lemongrass was macerated repeatedly until the solvent was clear (three times of maceration). The extract of red lemongrass was obtained and stored at room temperature.

2.3. Extraction of red lemongrass essential oil

Red lemongrass is obtained through steam distillation. Red lemongrass (7 kg) was dissolved by distillated water and put it into a distillation apparatus, then distillate for 4 hours at 100^{o}C. The liquid that coming out of the condenser is allowed to stand for 24 hours. After that, the water was removed and the oil was taken and then it was purified by anhydrous Na_2SO_4. Pure essential oil was put into the bottle. Red lemongrass essential oil has a strong odor.

2.4. Phytochemical Screening

2.4.1. Flavonoids

The flavonoid test was carried out by dissolving the maceration results, then add 3 mL of 96% ethanol, then as much as 2 drops of the maceration results were dripped on the filter paper, then the filter paper was placed on the mouth of a beaker containing ammonia above the heater. Positive results are indicated by a change in the color of the filter paper to yellow or orange [14].

2.4.2. Alkaloids

Alkaloids are identified with Mayer's reagent. Maceration results of 1 ml were added with 0.5 ml of 2% HCl [13]. Then added with Mayer's reagent by dripping 3 drops of Mayer's reagent, the maceration results of red lemongrass will give a white precipitate if the result is positive for alkaloids.

2.4.3. Tannins

The tannin test was carried out by taking 1 ml of maceration results and then add 2-3 drops of 1-10% $FeCl_3$. A positive result if the color changes to blackish blue [15].

2.4.4. Saponins

The saponin test was carried out by taking 1 ml of maceration results, adding water (1:1) and stirring in a test tube or glass container for 15 minutes. If foam formation occurs, it indicates the presence of saponins (positive) [16]. 1 N HCl is added if it is still formed with a thickness of 1-3 cm then it is positive for saponins.

2.4.5. Terpenoids

The terpenoid test was carried out by taking 5 mL of red lemongrass maceration results, adding 0.5 ml of chloroform, adding 0.5 ml of acetic anhydrous acid, adding concentrated sulfuric acid through the tube wall. Samples containing terpenoid group compounds will change color to form brown or violet rings [14].

2.4.6. Citral

The citral was identified by thin layer chromatography (TLC). Citral standard (Citral 95%, mixture of cis-trans from Sigma) was carried out as a standard. Essential oil of red lemongrass was spotted on a GF_{254} TLC plate. A mobile phase of n-hexane:ethyl acetate (8:1) was used in this experiment [14]. As much as 10 mL of the mobile phase was put into the chromatography chamber and saturated for 20 minutes. After that, the TLC plate which is containing the sample was put into the chromatography chamber. Then the TLC plate was removed, dried, and detected. Detection was carried out to see the bands that appeared on the TLC plate using UV254.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

Phytochemical screening is an initial stage in a phytochemical study which aims to provide an overview of the class of compounds contained in the plant being studied [5]. These compounds can be identified by reagents that are able to provide characteristics of each class of secondary metabolites. Phytochemical screening in this study aims to determine the content of secondary metabolites contained in n-hexane, methanol and distillated water solvent extracts in red lemongrass. The phytochemical screening test is shown in Table 1.

Table 1. This is a table of the result phytochemical screening red lemongrass.

No	Phytochemical Screening Test	Solvent		
		n-Hexane	Methanol	Distillated water
1.	Flavonoid	-	+	+
2.	Alkaloid	-	-	+
3.	Tannin	-	-	-
4.	Saponin	-	-	+
5.	Triterpenoid	+	+	+

Table 1 describes the qualitative results through phytochemical screening produced on red lemongrass. The results of the phytochemical screening showed that alkaloids, flavonoids, triterpenoids, and saponins were attracted to distilled water as a solvent. While the flavonoids and triterpenoids are attracted to the methanol solvent. The test also showed that the triterpenoids were attracted to the n-hexane solvent. The best solvent for maceration results will then be chosen as a reference to test the ability of the extract as an antidiabetic agent. In Table 1 it can be explained, among others:

3.1.1. Flavonoids

The results of the analysis show that the red lemongrass is carried out by taking the maceration results, taking 1 ml added with 3 mL of 96% ethanol, 2 drops of the solution are then dripped onto filter paper, then the filter paper is placed on the mouth of a beaker glass containing ammonia over a heater. Positive results are indicated by a change in the color of the filter paper to yellow or orange [14]. Ammonia is a base and flavonoid compounds are acidic compounds, the reactions that occur cause the formation of salts and form a kinoid structure which makes the double bond longer causing the color intensity on the filter paper to increase [17].

3.1.2. Alkaloids

The results of alkaloid analysis on red lemongrass were identified with Mayer's reagent. Maceration results of 1 ml were added with 0.5 ml of 2% HCl [13]. Then added Mayer's reagent by dropping 3 drops of Mayer's reagent. Alkaloid screening is basically a precipitation reaction that occurs due to a change in ligand on a nitrogen atom that has a lone pair of electrons with iodine ions in Mayer's reagent. The results of the red lemongrass phytochemical screening showed positive results in the presence of a white precipitate in Mayer's reagent [18].

3.1.3. Tannins

The maceration results of red lemongrass were tested for tannins by taking 1 ml of maceration results and then adding 2-3 drops of 1-10% $FeCl_3$. Testing for tannin compounds was carried out by adding the FeCl3 reaction to produce a bluish black or green color. The addition of $FeCl_3$ reagent can react with the hydroxyl groups in tannin compounds and cause a blue-black color change [19]. The tannin test on red lemongrass showed negative results in n-hexane, methanol, and distillated water solvents.

3.1.4. Saponins

Saponins are a form of glycosides and sapogenins. Testing for saponins generally results in the form of foam which lasts for 15 minutes. The foam produced is due to saponins having hydrophilic and hydrophobic groups. When shaking, the hydrophilic groups will bind to water, while the hydrophobic groups will bind to air to form foam [18]. The red lemongrass saponin test showed positive results in distillated water solvent, the saponin test was carried out by adding the macerated product with water and then shaking it and creating foam for 5 minutes. After adding 1 N HCl, a thickness of 1-3 cm was still formed, so the results of maceration with distillated water positively contained saponins.

3.1.5. Triterpenoids

Testing for terpenoids was carried out by taking 5 ml of red lemongrass maceration results, adding 0.5 ml of chloroform, adding 0.5 ml of acetic anhydrous acid, adding concentrated sulfuric acid through the tube wall. The three results of maceration with different solvents (n-hexane, methanol and distillated water) positively contain terpenoid group compounds because the color changes to form brown or violet rings [14].

3.1.6. Citral

The band of essential oil showed the same band with Citral standard (Citral 95%, mixture of cis-trans from Sigma). Both of the band have Rf 0.875. This result indicated that essential oil of red lemongrass contains citral.

4. CONCLUSION

The results of phytochemical screening showed that alkaloids, flavonoids, triterpenoids, and saponins were drawn attracted to the distillated water solvent. Meanwhile flavonoids and triterpenoids were drawn to methanol solvents. In addition, triterpenoids were drawn to n-hexane

solvents. The essential oil of red lemongrass also contains citral which is potential as an antidiabetic agent.

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