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Identification of Phytochemical Compounds Antioxidant Leaf Extract and Clove Flower Extract (*Syzygium aromaticum*)

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Abstract: One plant known as a flavoring spice, but also as a traditional medicinal plant that is thought to contain antioxidant phytochemical compounds, namely cloves (*Syzygium aromaticum*) which are widely found in Maluku, Sulawesi and surrounding areas. This study aims to identify the phytochemical compounds of antioxidants of leaf extract and clove flower extract (*Syzygium aromaticum*) originating from North Sulawesi using leaf methanol extract and clove flowers, where the test was carried out at the Research Center for Spice and Medicine Plant (Ballitro) Bogor included the alkaloid qualitative test , flavonoids, saponins, tannins, and triterpenoids. The results found that there were components of antioxidant phytochemicals in the form of alkaloids, flavonoids, saponins, tannins and triterpenoids in the extraran methanol leaf and clove flowers, with the frequency of existence ranging from there to many.

Keywords: Antioxidants; Cloves; Phytochemicals; *Syzygium aromaticum*

Introduction

Antioxidants are natural or man -made substances that can prevent cell damage due to oxidation processes by oxidants. Oxidants are free radicals in the environment, but are also produced naturally in the body. A better level of safety and broader benefits in the field of food, health and cosmetics, then natural antioxidants that can be found in vegetables, fruits, and other woody plants become feasible to use in treatment. Secondary metabolites in plants derived from alkaloids, flavonoids, saponins, quinons, tannins, and steroids/triterpenoids. According to Razzaghi-Asl et al. (2013), antioxidants play a role in handling oxidative stress -related diseases because it has important structural features for its activity, namely aromatic ring modification, which includes changes in the number and position of the hydroxy group and electron insertion and modification of carboxylic functions.

One plant known as a flavoring spice, but also as a traditional medicinal plant that is thought to contain antioxidant phytochemical compounds, namely cloves (Syzygium aromaticum) which are widely found in Maluku, Sulawesi and surrounding areas. The plant is classified into the Myrtaceae group with the order of Myrtales. Cloves have a very distinctive aroma, produced by Eugenol compounds, namely the main compound of this plant with content ranging from 72-90% (Risitiansyah et al., 2018; Prastya et al., 2019).

Clove (*Syzygium aromaticum* L.) is a tree plant with large hard woody stems that reach 20-30 m high. This plant is able to survive for more than 100 years and grow well in the tropics with an altitude of 600-1000 meters above sea level (DPL). Leaves from clove plants are single leaves that are stiff and thick stem with a length of leaf stems about 2-3 cm. Clove leaf-shaped oval with a pointed tip, flat edge, pinnate leaf bone, leaf length 6-13 cm and a width of 2.5-5 cm. Young clove leaves are light green, while old reddish green clove leaves. Clove leaves are also known to contain eucalyptol compounds, kariofilen, ɑcardinol, and limonene (Mohammed et al., 2015). In addition, clove leaves contain chemical compounds in the form of flavonoids, triterpenoids,

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phenolate, and tannins which are antibacterial compounds (Pratiwi, 2015; Huda et al., 2018; Hu et al., 2018).

Clove plants begin to flower after the age of 4.5-8.5 years, depending on the state of the environment. Clove flowers are a single small flower with a length of 1-2 cm and arranged in one bunch that comes out at the ends of the twigs. Each bunch consists of 2-3 Malai branches that can branch again. The amount of interest per panicle can reach more than 15 buds. Young clove flowers are light green, then turn pale greenish yellow and turn reddish when it is old. Dry clove flowers will be blackish brown and taste spicy because it contains essential oils. Empirical clove flowers are known as oral fragrances, treating toothache and painkillers (Nuraini, 2014).

Previous studies found that leaf and clove powder contain antioxidant compounds such as saponins, tannins, alkaloids, glycosides and flavonoids, while clove flower stalks contain saponins, tannins, glycosides and flavonoids, also contain eugenol (Perwitasari et al., 2013; Haryani et al., 2014; Razafimamonjison et al., 2014; Afrendi et al., 2023), where samples Used originating from outside North Sulawesi, it is necessary to conduct research on the identification of phytochemical compounds antioxidants of leaf extract and clove flower extract (*Syzygium aromaticum*) originating from North Sulawesi as a comparison.

Method

Procurement of Samples

The sample used was clove leaves and clove flowers obtained from Minahasa, North Sulawesi.

Tools and Materials

The tools used in this study were aluminum foil, Petri cup autoclaf, GCMS QP2010S Shimadzu with RSI-5MS Rastek column, Cup Cup, Measuring Cup, Laminar Air Flow (LAF), Oven, Dropper Pipette, Analytical Scales, and Waterbath. While the ingredients used in this research are aquades, alcohol, ampicillin, 1% $BaCl_2$ (barium chloride), $FeCl_3$ (iron chloride), HCl (chloride acid), H_2SO_4 (sulfuric acid), Mg metal (magnesium), 96% methanol, 96% methanol, NaCl (sodium chloride), NaOH (sodium hydroxide), NH_4OH (ammonium hydroxide), starch solvents, dragendorff reagents, Lieberman-Burchard reagents, Mayer reagents and Zn powder (zinc).

Making Methanol Extract

After a simplicia leaf and clove flower powder is made following the way of Wael et al. (2018), then the then made leaf extract and clove flowers using 70% distillation methanol solvents. The use of methanol solvents and clove extraction methods refers to the workings put forward by Dua et al. (2014), Lin et al. (2015), and Lin et al. (2016) with several modifications. Clove samples in the form of flowers, flower stalks (dry sun for 5-7 days) and leaves (litter) are mashed to the size of 60-70 mesh hereinafter called simplicia. As many as 2 kg of simplicia distilled to measure essential oil levels, and as many as 1300-1700 g are extracted. The extraction process begins with soaking simplicia into a 70% methanol solvent with a ratio of 1:10, during the soaking process samples are stirred. Furthermore, the filtering of the solution is carried out every three days, then simplicia is soaked again using methanol, and so on until the filtering process is carried out three times. The resulting filtrate is evaporated using rotary-evaporator until all solvents have evaporated and eventually form a paste called methanol extract.

Phytochemical Screening

The antioxidant phytochemical content test was conducted at the Bogor Ballitro and Medicine Research Center (Ballitro) following the ways of Banu & Cathrine (2015), namely:

1. Alkaloid test is done by weighing 0.5 grams of extract and added 1 ml of 2n HCl and 9 ml of distilled water, heated on a water bath for 2 minutes, cooled and filtered. The filtrate is used for testing on Mayer, bouchardat, and dragendroff reagents. In Mayer reagents, three drops of clove leaf extract are put into a test tube, then add 2 drops of Mayer reagents. When a white or yellow precipitate is formed, it shows the presence of alkaloid compounds. In bouchardate reagents, three drops of clove leaf extract are put into a test tube, then added with 2 drops of bouchardate reagent. When brown to black deposits are formed, it shows the presence of alkaloid compounds. Whereas dragendroff reagents are carried out by taking three drops of clove leaf extracts inserted into a test tube and 2 drops of dragendroff reagents are added, if the orange deposits are formed to red brown or brick red, this shows the presence of alkaloid compounds. If at least 2 of the 3 reagents above are positive, the sample contains alkaloids.
2. The flavonoid test is done by taking the thick extract of clove leaves and then put into a test tube, and 10 ml of hot water is added. The solution is a boil and filtered in a hot state. As many as 5 ml of flitrates are taken and added with 0.1 g of mg powder, 1 ml of HCl, and 2 ml of amyl alcohol. The mixture is shaken and let it separate. When formed yellow, orange or red in the amyl alcohol layer gives an indication of flavonoids.

3. The saponin test on the sample is done by taking a thick extract of clove leaves and put into a test tube. Added hot water to taste, shake for 15 minutes. If after a 2 N hydrochloric acid drops, permanent foam formed for approximately 10 minutes, then it gives an indication of the presence of saponins.
4. The tannin test is done by taking the thick extract of clove leaves added with 10 ml of distilled water and filtered. Filtrate is then diluted with distilled water until colorless. As many as 2 ml of filtrate is inserted into the tube and added 1 to 2 drops of iron (III) chloride reagent. If a dark blue or blackish green color is formed, it gives an indication of the presence of tannins.
5. The triterpenoid test is determined by putting as much as 30 mg of extracts in the drops added with 5 drops of glacial acetic acid and concentrated sulfuric acid as much as 2 drops. The extract is said to contain steroids if it is formed blue or green, while the extract is said to contain triterpenoids if it gives a red or purple color.

Result and Discussion

After an analysis of phytochemical content, the phytochemical component of the leaf methanol extract and clove flowers have plant antioxidant compounds namely alkaloids, flavonoids, saponins, tannins and triterpenoids (Table 1).

Table 1. Results of Identification of Phytochemical Compounds Antioxidants of Leaf Methanol Extracts, and Clove Flowers

The content of compounds /components	Leaf	Flower
Alkaloid	+++	++
Flavonoid	+++	+++
Saponin	+	+
Tanin	+++	+++
Triterpenoid	+++	+++

Description: +++ = Many, ++ = Medium, + = Present

Alkaloids are a group of secondary metabolite compounds that are alkaline with one or more nitrogen atoms which are generally in a combination of cyclic systems. This group of compounds usually has pharmacological activity in humans and animals. The characteristics of alkaloids are generally solid (crystal), although in room temperature there are liquid (for example nicotine), rotate the plane of polarization, taste bitter, the shape of salt soluble in water and soluble in organic solvents in free or basic form. Total phytochemical compounds such as clove flavonoids show efficient antioxidant activity (Elslimani et al., 2013; Abozid & El-Sayed, 2013; Ahmed, 2016). Nam et al. (2013), or it can be said to have an antioxidant effect (Dahham et al., 2015), where the same thing also occurs in clove leaves. Alkaloids contain nitrogen atoms that have free electron pairs so that they can be used to form covalent coordinate bonds with metal ions. In the alkaloid test with Mayer reagents, it is estimated that nitrogen in alkaloids will react with K+ metal ions from potassium tetraiodomerkurat (II) to form a potassium-alkaloid complex that settles (Setyowati et al., 2014).

The positive results of the alkaloids in the dragendorff test are characterized by the formation of young to yellow brown deposits. The precipitate is potassium-alkaloid. In making dragendorff reactions, bismut nitrate is dissolved in HCl so that the hydrolysis reaction does not occur because bismut salts are easily hydrolyzed to form bismutil ions (bio+). In order for BI^{3+} to remain in the solution, the solution is added to the acid so that the equilibrium will shift to the left. Furthermore, BI^{3+} ions from bismut nitrate react with potassium iodide to form bismut (iii) iodide which then dissolved in excess potassium iodide. Testing alkaloid compounds with dragendorff reagents uses nitrogen atoms to form covalent coordination with K+ ions which are metal ions (Setyowati et al., 2014).

Flavonoids are one of the important secondary metabolites in plants. In general, the classification of flavonoids consists of flavones, flavonols, flavanol, flavanone, Ansotianidin, and calcon. This flavonoid classification depends on differences in the substitution of flavonoid structures and these differences cause diverse pharmacological activity. Differences in pharmacological activity of flavonoids include anti - inflammatory, antioxidants, antidiabetic, and antibacterial. Identification of flavonoids on the leaves and clove flowers using the Wilstater test shows the orange color which means positive the presence of flavonoids. Magnesium and hydrochloric acid in the Wilstater test react to form bubbles which are H2 gas, while concentrated MG and HCl metals in the test function to reduce the nucleus of benzopiron found in the structure of flavonoids, so that the color changes to red or orange. If in a plant extract there is a flavonoid compound a flavilium salt will form when the addition of red or orange MG and HCl (Setyowati et al., 2014).

Saponin is a detergent or natural glycoside that has a surface active properties that are amphyphilic, has a large molecular weight and molecular structure consisting of aglycone steroids or triterpens called sapogenin and glycone which contains one or more sugar chains (Sirohi et al., 2014). Saponins are polar so that they can dissolve in solvents such as water and saponins are also nonpolar because they have hydrophobic groups, namely aglys (sapogenin). The foam produced in the saponin test is caused by glycoside

which can form foam in water and hydrolyzed into glucose and other compounds.

Tannins are organic substances found in water extracts that dissolve in water. In addition, tannins are polyphenol compounds that can form complexes with polysaccharides and can precipitate proteins. Tanin testing is carried out by adding $FeCl_3$ that reacts with one of the hydroxyl groups in the tannin. The function of $FeCl_3$ is to hydrolyze the tannin group so that it will produce a change in blackish blue and tannins that produce blackish green color (Pardede et al., 2013).

Triterpenoids are secondary metabolitic compounds in terpenoid derivatives whose carbon frame derived from six isoprene units (2-methylbuta-1, 3-Diene), which is a carbon frame that is built by six C5 units and is reduced from the C30 Asiklic Hydrocarbons, namely scalene. This compound is cyclic or ashymik and often has alcohol, aldehyde, or carboxylic acid groups (Widiyati, 2006). Triterpenoid group compounds in plants containing triterpenoid compounds there are pharmacological values because these compounds work as antifungus, insecticides, antipemangsi, antibacterial and antivirus, anti -inflammatory and antioxidants (Nassar et al., 2010).

Identification of triterpenoids in leaf methanol extract and clove flowers gives positive results with the formation of a brown ring at the boundary between chloroform and H_2SO_4. In addition, when added 2 ml of sulfuric acid, it looks green color to a jet green color. This color change is caused by oxidation in the group of terpenoid or steroid compounds through the formation of conjugated double bonds. The principle of the reaction in the terpenoid test is the condensation or release of H_2O and the combination of carboxy and causes electrophilic additions followed by the release of hydrogen. The hydrogen group and electrons are released so that they experience a conjugate extension that shows the presence of chocolate rings (Pardede et al., 2013; Windarini et al., 2013). Figueiredo et al. (2008) put forward the results and composition of secondary metabolites in plants, especially in leaves and flowers and roots are influenced by several factors including physiological variations, environmental conditions, geographical variations, genetic factors and evolution, and variations of several soil nutrients such as Ca, Mg, P, P, P K and Cu (Sampaio et al., 2015).

Conclusion

Phytochemical components of leaf methanol extract and clove flowers contain plant antioxidant compounds in the form of alkaloids, flavonoids, saponins, tannins and triterpenoids with frequency of presence ranging from there to many.

Author Contributions
Adeleyda M. W. Lumingkewas conceptualized the research idea, designed of methodology, management and coordination responsibility, analyzed data, conducted a research and investigation process; Adrien Jems Akiles Unitly conducted literature review and provided critical feedback on the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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