

CHEMICAL COMPOUNDS AND ANTIOXIDANT CONTENTS OF CLOVES LEAVES ESSENTIAL OIL

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ABSTRACT

Essential oils from the distillation of clove leaves could act as anti-bacterial and anti-fungal agents for either food or feed. The purpose of this study was to identify chemical compounds and antioxidant content of clove leaves essential oils. The material used was clove leaves essential oil obtained by soxlet extraction. Clove leaves were taken from trees of over 10 years old in Palu, Central Sulawesi. Chemical compounds of the sample were analyzed by GC-MS method. Each peak that appeared on the chromatogram was identified by comparing to the standard. While the antioxidant analysis was carried out by using the DPPH (IC₅₀) of spectrophotometry method. The results showed that the total essential oils content of clove leaves was 3,957% and there were 6 chemical compounds identified, i.e. caryophyllen (0,96%); phenol, 2-methoxy (guaiacol) (4,16%); phenol, 2-methoxy-4-methyl (9.79%); phenol, 4-ethyl-2-methoxy (1.5%); phenol 2-methoxy-4-propyl (3.87%); phenol, 2-methoxy-4-(2-propenyl) and Eugenol (79.72%). While the results of antioxidant identification based on DPPH and IC₅₀ values before purification were 0.597 and 74.358, and the respective values after purification were 0.597 and 42.255. In conclusion, the antioxidant content of clove leaves essential oils after purification is higher than before purification and it contains 3.957% essential oil.

Keywords: antioxidants, chemical compound, clove leaves, essential oils, eugenol

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INTRODUCTION

Clove (*Syzygium aromaticum*) is one of the spice plants belonging to the Myrtaceae plant in the order Myrtales (Razafimamonjison, et al., 2015). This type of plant is also an herbal plant that has long been used in Middle East and Asian

countries as traditional medicine in curing various diseases and food seasonings (Dehghani et al, 2012). The distinctive clove smell is produced by the compound of eugenol (72-90%) which has antiseptic and anesthetic properties (Razafimamonjison, et al., 2015). Analysis of clove leaves compounds from Bangladesh using the

GC-MS method found eugenol compounds 74.28%, eucalyptol 5.78%, karyofilen 3.85%, α -cardinol 2.43%, limonone 2.08% (Bhuiyan et al., 2010).

Essential oils from cloves have chemical properties and pharmacological effects that function as anesthetics, antimicrobials and antiseptics (Nurhidayati and Sulistiowati, 2013), antioxidants, and immunomodulators (Dehgani et al, 2012). Clove leaves phenolic compounds also contain antioxidants and flavonoid compounds that function as antidotes to free radicals (Dibazar et al., 2015).

Antioxidants are chemical compounds that can contribute one or more electrons to free radicals so that free radicals can be suppressed. Antioxidants could also function to delay, inhibit, preventing oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidized chain reactions (Javanmardia et al, 2003). Based on the source of its acquisition there are two kinds of antioxidants, namely natural antioxidants and synthetic antioxidants (Gitawati 1995).

Naturally, the body can produce antioxidant compounds consisting of enzymatic and non-enzymatic antioxidants. However, these antioxidant compounds are not able to inhibit the oxidants that are formed due to oxidative stress, so antioxidants has to be supplied from outside the body (exogenous) (Halliwell et al, 1995). The body usually does not have excess antioxidant reserves, so if there is excessive radical exposure, the body needs exogenous antioxidants from feed. Concerns about the possible unknown side effects of synthetic antioxidants cause the natural antioxidants becomes indispensable alternatives (Saija et al. 1995).

The use of synthetic antioxidants such as benzoic acid, BHA (Butylated Hydroxy Anisol), BHT (Butylated Hydroxy Toluene) or TBHQ (Tertier Butylated Hydroxy Quinone) can cause side effects on body health. BHA and BHT have been studied can cause tumors in experimental animals if used for a long

time, and can cause liver damage if consumed in excess (Andarwulan et al. 1996). The existence of side effects caused by the use of synthetic antioxidants spurred the development of research on natural antioxidants that are safer and better to reduce free radicals in the body. Natural antioxidants are generally derived from herbs, fruits, vegetables, and seeds.

Clove essential oil (*Syzygium aromaticum L*) obtained from the extraction or refining process, namely flowers (10-20%), flower stalks (5-10% and leaves (2 - 3.5%). Biologically it can function as an anti-bacterial, antifungal, insecticidal and antioxidant and traditionally used as flavoring and anti-microbial ingredients in food (Lee and Shibamoto, 2001; Huang et al, 2002; Velluti et al, 2003). This study aims to identify the types of chemical compounds contained in clove leaves essential oil and determine the antioxidant contents.

RESEARCH METHODS

Research on the identification of chemical compounds and the antioxidant content of clove leaves essential oils was carried out in two stages. The first step was identification of chemical compounds of clove leaves essential oil, and the second step was determining the antioxidant content before and after purification. The identification of clove leaves essential oil chemical compounds was carried out at the Organic Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta, and the analysis of the antioxidant content of clove leaves essential oils was carried out at the Chemical Laboratory, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu. Procedure for conducting the analysis as follows:

Determination of Essential Oil

Determination of the content of essential oils of clove leaves is carried out as follows:

Sample Preparation

Clove leaves were taken from Palu, Central Sulawesi with a clove tree age of \pm

10 years old. The leaves were cleaned and then dried until the water content reached \pm 14% and then it is ground.

Distillation Process

The distillation process was carried out by taking 50 g of clove leaves flour into a 1000 ml distillation flask that has been filled with 500 ml of distilled water. The next stage distillation flask is placed on an electric water bath and heated at a temperature of 100 ° C until it boiled for 4 hours. The resulting distillate was collected by using 200 ml erlenmeyer flask. Distillation results were repeated to get the volume of essential oil needed. It was then subsequently concentrated by using rotary evaporator at a temperature of 40°C to obtain a distillation of pure clove leaves essential oil (free of water).

Determination of Chemical Compounds

Determination of essential oil chemical compounds was carried out by the GC-MS method. The essential oil distillate was injected into the GC-MS injector (Shimadzu-QP-2010S) with the AGILENT column (dimension 0.25mm 0.25um) and helium carrier gas at a flow rate of 20 ml / min. The injector temperature was 310°C, while the programmed column temperature began with 70°C being held for 2 minutes, then slowly changed with a raised rate of temperature of 5°C/min to reach 300°C and held for 8 minutes. The condition of the mass spectrometer was 70 eV ionization energy, electron collision ionization mode, split ratio: 25.0, and the detection area was 40-500 m/z. Every peak that appeared on the chromatogram was then identified by comparing m/z standard data contained in the MS index library.

Determination of Antioxidant

The method for determining the antioxidant content of clove leaves essential oil samples was by using the DPPH method (1,1-diphenyl-2-picrylhydrazil). DPPH method was used to test the ability of a component as a free radical scavenger in an ingredient or extract. The advantage of the DPPH method was quick and simple (Pamarti, 2005), sensitive and only

required small sample. The parameter used for the DPPH radical capture test was IC₅₀, which was the concentration of the extract or test fraction needed to capture the DPPH radical by 50% (Zouand Wei, 2004).

The antioxidant analysis procedure of the DPPH (IC₅₀) method was as follows: extract of the sample (distillate) was weighed as much as 25 mg then put in a 25 ml volumetric flask, then added with ethanol solvent up to a concentration of 1000 ppm. Then a dilution series was carried out to obtain a solution of 10, 30, 50, 70 and 90 ppm. The solution was then taken as much as 0.2 ml and added with 3.8 ml of DPPH 50µM solution. The mixture was homogenized and left for 30 minutes in a dark place. Then the absorption was measured at a wavelength of 517 nm with spectrophotometer. Tests were also carried out on DPPH solutions. The absorbance value obtained was used to determine the % inhibition.

Data analysis

Data were analyzed descriptively based on observations and measurements, then compared with research results from the literature being reviewed.

RESULTS AND DISCUSSION

Essential Oils Content and Chemical Compound

The results of identification of clove leaves essential oil based on the analysis of using GC-MS were 3,957%. Chemical composition identification based on chromatogram resulted before purification and after purification as shown in Table 1.

The clove leaves essential oil from the results of this study contained 79.72% eugenol. This value is higher than SNI 06-2387 (1998), which is 78% with specific gravity (1.03-1.06) and refractive index (1.52-1.54). This finding was closely in range with that reported by Ross and Ross (1999) who found that the main component of clove leaves essential oil was eugenol, ranging from 70 to 90%. Hutajulu, et al. (2007) stated that the respective contents of eugenol and β -caryophyllene of clove

leavesoil using Gas Chromatography (GC) were 68.46% (eugenol) with retention time of 26 minutes and 23.57% (β -caryophyllene) with retention time of 11 minutes.

Other researchers such as Nurdjannah (2004) reported that the respective essential oil contents in clove flowers, flower stalks and leaves were 10-20%, 5-10% and 1-4%, of which the eugenol content of each component ranged from 70-80%. In addition, Jayanudin (2011) stated that the chemical composition of cloves leaves essential oil through steam distillation process obtained the greatest

yield of 1.84% with eugenol levels of 65.03% and trans-caryophyllene 20.94%. Alma et al. (2007) found that the chemical composition of clove flower essential oil from Turkey through steam distillation process obtained the largest components of eugenol 87%, eugenyl acetate 8.01% and β -caryophyllene 3.56%. While Jirovetz et al. (2006) analyzed the chemical composition in clove leaves oil using GC and GCMS obtained 23 chemicals and their largest components were eugenol(76.8%), β -caryophyllene (17.4%), R-humulene (2.1%), and eugenylacetate (1.2%).

Table 1. Chemical Compounds Essential Oil of Clove Leaves Before and After Purification.

Name of Compound	Before Purification (%)	After Purification (%)
Eugenol (C ₁₀ H ₁₂ O ₂)	64.81	79.72
Caryophylen (C ₁₅ H ₂₄)	-	0.96
Guaiacol (C ₇ H ₈ O ₂)	-	4.16
Methylguaiacol (C ₈ H ₁₀ O ₂)	-	9.79
phenol, 4-ethyl-2-methoxy (C ₉ H ₁₂ O ₂)	-	1.5
phenol, 2-methoxy-4-propyl (C ₁₀ H ₁₄ O ₂)	-	3,87
Beta-caryophyle (C ₁₅ H ₂₄)	1.43	-
Isocaryophylen (C ₁₅ H ₂₄)	2.77	-
Trans-caryophylen (C ₁₅ H ₂₄)	26.58	-
Alpha-humulent (C ₁₅ H ₂₄)	4.42	-

Source: Results of GCM analysis at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Gadjah Madah University, Yogyakarta

Some other research results show the existence of clove leaf essential oil chemical compounds with different compositions. Eugenol compounds are the main components contained in clove oil (*Syzygium aromaticum*), with a content of up to 70-96%, although clove oil contains several other components such as eugenol acetate and β -caryophyllene (Amlaet *al.*, 2007), Bhuiyanet *al* (2010) reported that

clove leaf essential oil contains eugenol (74.28%), eucalyptol (5.78%), karyofilen (3.85%), α -cadinol (2.43%), limonene (2.08 and caryophyllene (1.52%). These differences may be due to the different locations of clove leaf extraction. The concentration of essential oil varies and depends on plant species, tissue used, soil and environmental conditions, and time of harvest (Lee *et al.*, 2004)

Table 2. DPPH, IC₅₀ and Eugenol Value of Clove Leaves Essential Oils.

Samples Measured	DPPH	IC ₅₀	Eugenol (%)
Clove leaves essential oils (before purification)	0.5975	74.3580	64,81
Clove leaves essential oils (after purification)	0.5975	42.2552	79,72
Vitamine C (as standard)	0.6068	32.7088	-

Source: Results of antioxidant analysis at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu

Antioxidant Contents

The antioxidant content is obtained from the calculation of the IC₅₀ value of the compound. DPPH and IC₅₀ value from samples of clove leaves essential oils are listed in Table 2.

Table 2 shows that the IC₅₀ value of clove leaves essential oil before purification was higher than the values after purification and of vitamin C as standard. The smaller the IC₅₀ value of a compound, the higher the antioxidant content. IC₅₀ value of cloves leaf essential oil after refining was 42,255, indicating that the antioxidant was higher than before purification with IC₅₀ value of 74,358. This is in accordance with the standards set by Badarinath (2010), that antioxidant compounds are very high if the IC₅₀ value is less than 50, high (50-100), moderate (100-150), and low (151-200). The lower the IC₅₀ value, the higher the antioxidant content. The higher antioxidant content in clove leaves essential oil after purification is due to the higher levels of its eugenol content.

Antioxidants are divided into two types namely synthetic and natural. Synthetic antioxidants that are widely used are harmful to health because they are toxic if consumed in excessive concentrations. Therefore, natural antioxidants are needed which tend not to have side effects and are beneficial to health (Sarastani et. al. 2002). Trilaksani (2003), stated that natural plant antioxidant compounds are phenolic or polyphenolic compounds which can be in the form of flavonoids, tocopherols and

polyfunctional acids. Phenolic compounds can be found in herbs such as clove leaves. Sastrohamidjojo (2002), reported that clove leaves oil contained phenolic components such as eugenol and eugenol acetate. In addition there are also caryophyllene and sesquiterpenes.

Lumingkewas et al (2014) reported that the phenolic, flavonoid, and tannin content of clove leaves extracted by using three types of solvents (acetone, methanol and ethanol) were different and the use of methanol solvent tended to produce the highest content of eugenol (270 µg / mL) followed by kaempferol, routine, quercetin and gallic acid, respectively 15.19; 11,37; 8.79 and 7.28 µg / mL. Gallic acid and its derivatives (tannin) and some flavonoids (kaempferol, quercetin and routine) have many hydroxyl groups especially ortho-dihydroxyl groups (catechol structures) with potential free radical suppression activities. Therefore, these compounds contribute significantly to the most powerful antioxidant and antipotoxidant activities of clove leaves extract.

Eugenol and its derivatives normally contains one hydroxyl group and have relatively lower free radical scavenging activity than other phenolic compounds. However, the highest levels of essential oil compounds such as eugenol and acetyl eugenol in clove leaves extract also made an important positive contribution to the antioxidant activity of clove leaves extract (Shan et.al., 2005). It was further reported that the component of eugenol is a major

component in clove leaves and has antioxidant activity. Eugenol can also dissolve in organic solvents such as ether, chloroform, alcohol, and glacial acetic acid. In addition, eugenol can also be soluble in oil which is a nonpolar component. Jadhav et al. (1996), stated that antioxidants that are donating hydrogen radicals are chain-breaking antioxidants, otherwise known as primary antioxidants. Primary antioxidants will react with peroxy radicals which are then converted to radicals that are more stable or non-radical.

CONCLUSION

The conclusions from the results of this study are as follows:

1. The essential oil content of clove leaves is 3.957% and comprises of 6 compounds which are identified namely: caryophyllen (0.96%); phenol, 2-methoxy (guaiacol) (4.16%); phenol, 2-methoxy-4-methyl (9.79%); phenol, 4-ethyl-2-methoxy (1.5%); phenol 2-methoxy-4-propyl (3.87%); phenol, 2-methoxy-4-(2-propenyl) or eugenol (79.72%).
2. The antioxidant content of clove leaves essential oil after purification is higher than before purification, but lower than the standard antioxidant content (vitamine C). The respective DPPH and IC₅₀ values resulted from this study, are: before purification (0.597 and 74.358), after purification (0.597 and 42.255), standard using vitamin C (0.606 and 32, 708).

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