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Chemical Constituent and Antioxidant Activity of Clove (*Syzygium aromaticum*) Bud and Leaf Essential Oils from Bali

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Abstract

Bali is one of clove (*Syzygium aromaticum*) producers in Indonesia. Clove essential oil is mainly produced from the leaves and flowers. Eugenol is the main component in the essential oil of clove. The objective of this research is to determine constituents and antioxidant activity of clove's bud and leaf essential oils from Bali. The essential oils were isolated from clove's bud and leaf samples by steam distillation with the yield of 12.90 and 2.63%. The constituents of the clove essential oils were analyzed by using gas chromatography-mass spectrometry (GC-MS). Thirty-six and twenty-nine constituents were identified based on GC-MS from the clove bud and leaf essential oils, respectively. Major classes of compounds are sesquiterpenes, phenyl propanoids, oxygenated sesquiterpenes, and esters. Different compositions in major constituents were found between both essential oils. Clove bud essential oil (CBEO) contained eugenol (65.29 %), *trans*-caryophyllene (20.06 %), and α-humulene (3.38 %). While, in clove's leaf essential oil (CLEO), the composition was eugenol (64.47 %), *trans*-caryophyllene (27.19 %), and α-humulene (3.62 %). The clove essential oil and its main component show strong antioxidant activity. The antioxidant activity of CBEO, CLEO, and eugenol is 22.58, 29.19, and 17.53 μg/mL, respectively.

Keywords: clove essential oils, chemical constituent, eugenol, antioxidant activity, Bali

INTRODUCTION

Plant essential oils and their other products of secondary metabolism are widely used in traditional medicine, perfume, fragrance, food flavoring, cosmetics, and the pharmaceutical industry. Cloves (*Syzygium aromaticum*), which belongs to Myrtaceae family, are one of aromatic plants that produce essential oil known as clove oil (Hussain et al., 2017). The clove is an indigenous plant species of the Maluku Islands. Indonesia is the largest producer of cloves, with a production volume of 133,604 tons in 2020, which accounted for almost 72.9% of global production (Clove Market, 2021; Danthu et al., 2020). Other large producers of cloves include Madagascar, Tanzania, Comoros, and Sri Lanka. Being the largest producer of cloves, Indonesia is also the largest consumer in the world. Indonesia consumes around 90% of its own clove production (Clove Market, 2021).

Syzygium aromaticum is a source of clove oil which is commonly obtained by hydro distillation, steam distillation, or solvent extraction methods (Haro-González et al., 2021). Various studies have been carried out to find various constituents of *S. aromaticum*. Each origin of clove oils may have its own characteristics and contain different chemical constituent and composition. Previous studies showed essential oil yield and their chemical constituents were influenced by genetic and environmental factors (Tsusaka et al., 2019; Zang et al., 2023). Variation in constituents and composition depends on origin, variety, argo-ecological condition, post-harvest processing, pre-pretreatments, and methods of extractions (Nurdjannah & Bermawie, 2012). Clove buds contain 15-20% essential oil, which is dominated by eugenol (70-85%), eugenyl acetate (15%) and β-caryophyllene (5-12%) (Hussain et al., 2017; Mittal et al., 2014). Other minor essential oil constituents include methyl salicylate, chavicol, α-copaene, α-amorphene and caryophyllene oxide (Amelia et al., 2017). Eugenol and volatile components are responsible for the characteristic pleasant odor and aroma of cloves (Kamatou et al., 2012). They are also contributed to antifungal (Musta & Nurliana, 2019), antibacterial (Nurliana et al., 2020), and antioxidant properties of the oil (Nejad et al., 2017). The antioxidant activities of clove essential oil (Z*yzygium*

aromaticum) and its main constituent eugenol have been tested by DPPH method (Ghadermazi et al. 2017; Alfikri et al., 2020). DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction.

Clove production contributors in Indonesia are mostly coming from Java, Manado and Bali. The chemical constituents of clove bud essential oils from Java and Manado have been reported by Amelia et al. (2017). Thirty-six and thirty-four chemical constituents were identified based on GC-MS from clove oil collected from Java and Manado, respectively. Major classes of compounds are sesquiterpenes, phenylpropanoids, oxygenated sesquiterpenes, and esters. Different compositions in major constituents were found between both origins. Clove oil from Java contained eugenol (55.60 %), eugenyl acetate (20.54 %), caryophyllene (14.84 %), and α-humulene (2.75 %). While, in clove oil from Manado, the compositions were eugenol (74.64 %), caryophyllene (12.79 %), eugenyl acetate (8.70 %), and α-humulene (1.53 %). Moreover, minor constituents β-elemene (0.04 %), α-cadinene (0.05 %) and ledol (0.06 %) were existed only in clove Java, while clove Manado had some unique minor constituents which were not found in clove Java, i.e. β-gurjunene (0.04 %), γ-cadinene (0.03 %), and humulene oxide (0.05 %).

Unfortunately, there are no comprehensive researches on chemical constituents and antioxidant activity of clove essential oil originated from Bali. The aim of this study was to identify the chemical components of clove leave and bud essential oil from Bali obtained by steam distillation, isolate eugenol a main component of the oils, and determine antioxidant activity of the oils and eugenol by using 2, 2-diphenyl-2-picrylhydrazyl (DPPH).

METHODOLOGY

Materials and Instrumentals

The fresh buds and leaves of *S. aromaticum* were harvested and obtained from a local farmer in Buleleng Bali. The buds and leaves were cut and air-dried at room temperature for two weeks. The air-dried buds and leaves were blended to be fine powder. The powder was kept in an air tight container protected from light until used. Chemicals used include sodium hydroxide, hydrochloric acid, sodium sulfate anhydrous, and DPPH (Smart-Lab). Instruments used in this research include Gas Chromatography-Mass Spectrophotometer GCMS QP-2010 Ultra Shimadzu, High Performance Liquid Chromatograph (HPLC-20AD), Infrared Spectrophotometer (FTIR-Afinity) Shimadzu, and UV-2600 Spectrophotometer Shimadzu.

Methods

Isolation of Clove Essential Oils

Steam distillation of essential oil from *S. aromaticum* buds and leaves employed a modified reported procedure (Muderawan et al., 2022). The air-dried clove (*S. aromaticum*) bud or leave powder (100 g) was steam distilled in Clevenger apparatus for 6 hours. Subsequently, the distillate was extracted with dichloromethane (3 × 50 mL) and the dichloromethane layer was dried with anhydrous sodium sulfate, left to stand overnight, filtered, as well as evaporated with a rotary vacuum evaporator. The essential oils obtained were then weighed to determine the yield, i.e.: 12.90 g, 12.90 % for clove bud essential oil (CBEO) and 2.63 g, 2.63% for clove leave essential oil (CLEO), and stored to preserve freshness, until use.

Gas Chromatography-Mass Spectrometry Analysis

The clove essential oils were analyzed according to reported procedure (Muderawan et al., 2022). The clove bud and leaf oils (CBEO and CLEO) analysis was performed by gas chromatography coupled with mass spectrometry (GC/MS) in a GCMS-QP2010 Ultra, Shimadzu, equipped with an auto sampler, AOC-20s, Shimadzu, auto injector, AOC-20i, Shimadzu, and RTX-5MS (30 m x 0.25 mm ID and 0.25 μm film thickness) columns. The injector temperature was set at 200°C and the oven temperature was initially at 70°C for 2 minutes, programmed to reach 180°C at the rate of 20°C/min and held at 180°C for 3 minutes, then increased from 180°C to 250°C at the rate of 20°C/min, and finally kept constant at 250°C for 16 minutes. Helium was used as the carrier gas with a 35.2 ml/min flow and 100 kPa pressure. The sample (0.2 μL) was neatly injected with a 20:1 split ratio and the mass spectrometer was operated in the electron impact (EI) mode at 70eV, while mass scanning range was varied over 35-500 m/z. Also, the ion source and quadrupole temperatures were set at 230°C and 150° C, respectively. While, the oil components were identified on the basis of their mass spectral fragmentation, using the Wiley 9 GC/MS libraries. Subsequently, the identified compound's percentage was computed from a total ion chromatogram.

Isolation of Eugenol

Clove oil (100 g) was added with 2.5 M NaOH solution (200 mL) and stirred with a magnetic stirrer without heating for 5 hours. Next, the mixture was

transferred into a separating funnel and allowed to stand until two layers formed. The bottom layer, which is a water layer containing salts from eugenolate, was separated and acidified with HCl solution (2.5 M) until the pH was neutral. The mixture was stirred with a magnetic stirrer without heating for 30 minutes. Next, the mixture was transferred into a separating funnel and allowed to stand until two layers formed. The top layer, which is the oil layer, was separated. The lower layer was extracted with DCM (3 x 50 mL) and collected. The oil layer was added with Na_2SO_4 and allowed to stand. Next, the oil layer was filtered. The oil was purified using pressure-reduced fractional distillation to produce pure eugenol (45.60 g). The eugenol obtained was stored in a dark glass bottle. The purity eugenol was tested by HPLC and characterized by UV-Vis and Infrared Spectrometer.

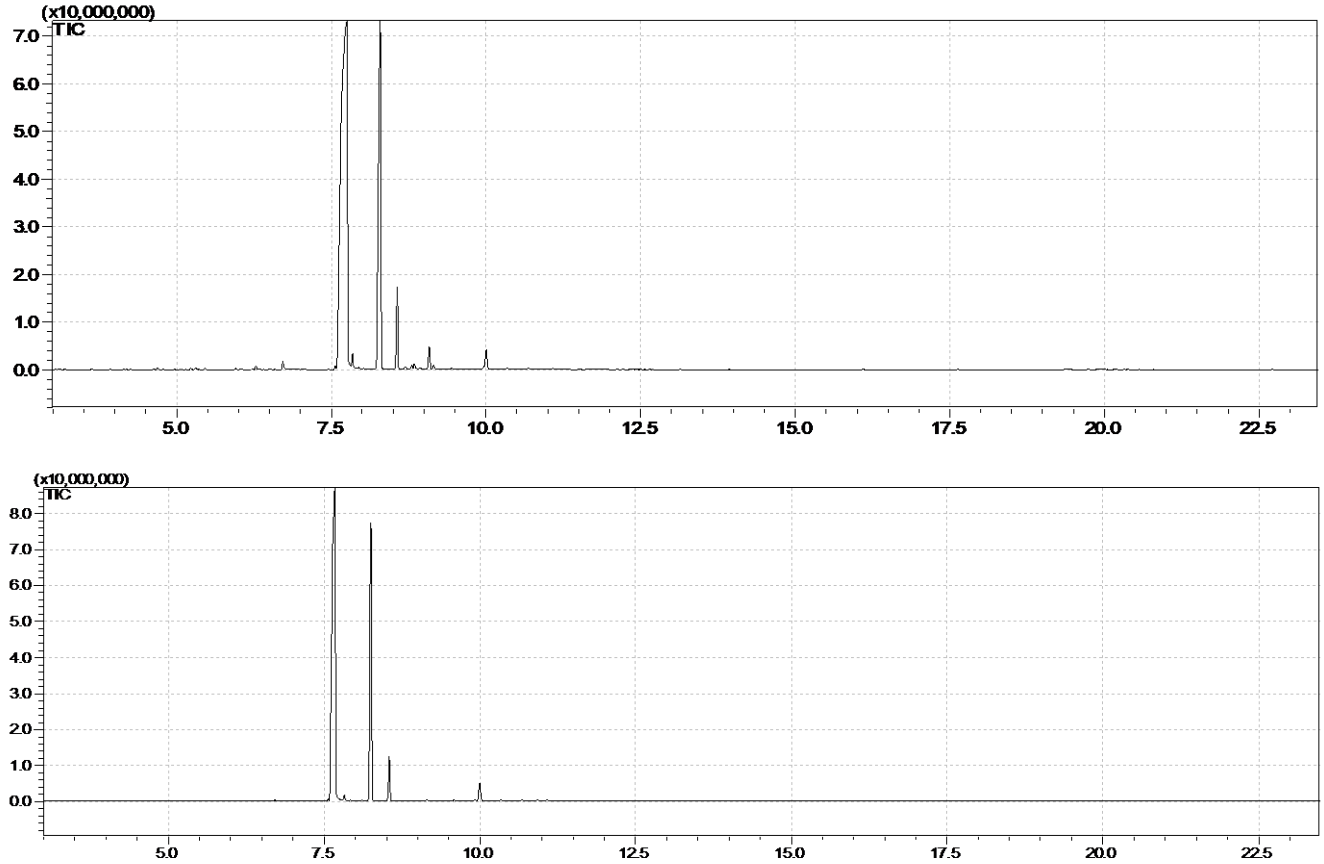


Figure 1. Chromatogram of clove bud (above) and leave (below) essential oils.

Antioxidant Activity Assay

The free radical scavenging activities of the clove oils (CBEO and CLEO) and eugenol were determined spectrophotometrically. The hydrogen atom or electron donation abilities of the clove oils and eugenol were measured from the bleaching of purple-colored methanol solution of stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) according to reported procedure (Shekhar & Anju, 2014; Ali et al., 2018). For this process, 0.5 ml of CBEA, CLEO or eugenol solution in 99.9% methanol, in the final concentration range of 0.025-0.250 mg/ml, or 0.5 ml of methanol (control), were mixed with 3.5 ml of 100 μM DPPH solution (0.0039 g in 100 ml 99,9% methanol prepared daily). The mixture was vortexed thoroughly for a minute and left at room temperature for 30 minutes, then the absorbance was read against control at 517 nm, using UV-2600 spectrophotometer (Shimadzu), and the control probe contained all components except for the radicals. Subsequently, the CBEO, CLEO and eugenol abilities to scavenge DPPH radicals, (DPPH• scavenging activity ($SA_{DPPH•}$)), were calculated using the equation,

$$SA_{DPPH•}\ (\%) = 100 \times (A_{Control} - A_{Sample})/A_{Control}$$

where, $A_{Control}$ is the control reaction's absorbance (containing all reagents except the CBEO, CLEO or eugenol) and A_{Sample} is the absorbance in the CBEO, CLEO or eugenol presence. The CBEO, CLEO and eugenol's radical-scavenging ability were then calculated as IC_{50} (g/ml), from the graph of radical scavenging activity percentage against the oil concentration.

Table 1. Chemical constituents of clove bud and leave essential oils from Bali.

No	RT	Compound	Molecular Formula	M_r (g/mol)	% Area	
					CBEO	CLEO
1	3.11	2-Heptanone	$C_7H_{14}O$	114.18	0.02	
2	3.19	2-Heptanol	$C_7H_{16}O$	116.20	0.02	
3	3.66	3-Hydroxy hexane	$C_6H_{14}O_2$	118.17		0.04
4	3.77	2-Hydroxy hexane	$C_6H_{14}O_2$	118.17		0.05
5	3.93	5-Methyl- 2-furancarboxaldehyde	$C_6H_6O_2$	110.11	0.02	
6	4.15	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	126.20	0.01	0.02
7	4.63	*l*-Limonene	$C_{10}H_{16}$	136.24	0.02	
8	4.69	*sec*-Octyl acetate	$C_{10}H_{20}O_2$	172.26	0.06	
9	4.79	(*E*)-3,7-dimethyl-1,3,6-Octatriene	$C_{10}H_{16}$	136.24	0.01	
10	5.23	2-Nonanone	$C_9H_{18}O$	142.24	0.05	
11	5.31	Linalool	$C_{10}H_{18}O$	154.25	0.06	
12	5.46	Geranyl nitrile	$C_{10}H_{15}N$	149.24	0.04	
13	5.95	Benzyl acetate	$C_9H_{10}O_2$	150.18	0.05	
14	6.03	Ethyl benzoate	$C_9H_{10}O_2$	150.18	0.01	
15	6.28	Methyl 2-hydroxybenzoate	$C_8H_8O_2$	152.15	0.12	0.03
16	6.72	Chavicol	$C_9H_{10}O$	134.18	0.36	0.13
17	7.56	α-Cubebene	$C_{15}H_{24}$	204.35	0.14	0.19
18	7.75	Eugenol	$C_{10}H_{12}O_2$	164.20	65.29	64.47
19	7.84	α-Copaene	$C_{15}H_{24}$	204.35	0.72	0.41
20	7.94	(-)-β-Elemene	$C_{15}H_{24}$	204.35	0.07	0.04
21	8.11	*cis*-Caryophyllene	$C_{15}H_{24}$	204.35		0.07
22	8.29	*trans*-Caryophyllene	$C_{15}H_{24}$	204.35	26.06	27.19
23	8.30	[1S-(1R*,9S*)]-10,10-Dimethyl-2,6- bis(methylene)-bicyclo[7.2.0]undecane	$C_{15}H_{24}$	204.35		0.03
24	8.50	α-Amorphene	$C_{15}H_{24}$	204.35	0.02	
25	8.57	α-Humulene	$C_{15}H_{24}$	204.35	3.38	3.62
26	8.63	γ-Muurolene	$C_{15}H_{24}$	204.35	0.02	
27	8.70	Torreyol	$C_{15}H_{26}O$	222.20	0.14	0.07
28	8.80	Germacrene-D	$C_{15}H_{24}$	204.35	0.19	
29	8.84	Farnesene	$C_{15}H_{24}$	204.35	0.27	0.07
30	8.93	Valencene	$C_{15}H_{24}$	204.35	0.11	
31	8.94	α-Selinene	$C_{15}H_{24}$	204.35		0.06
32	9.07	3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	164.20		0.08
33	9.08	Eugenyl acetate	$C_{12}H_{14}O_3$	206.24	0.98	
34	9.15	Δ-Cadinene	$C_{15}H_{24}$	204.35	0.25	0.21
35	9.29	*trans*-Cadina-1,4-diene	$C_{15}H_{24}$	204.35	0.03	
36	9.44	(+)-Nerolidol	$C_{15}H_{26}O$	222.20		0.04

37	9.45	2,4,6-Trimethyl-1,3-benzenedimethanol	$C_{11}H_{16}O_2$	180.20	0.08	
38	9.59	1,5-Dimethyl-2,3-divinyl-cyclohexane	$C_{12}H_{20}$	164.29		0.13
39	9.92	(+)-*cis*-Caryophyllene oxide	$C_{15}H_{24}O$	220.35		0.19
40	10.00	(-)-*trans*-Caryophyllene oxide	$C_{15}H_{24}O$	220.35	1.12	1.98
41	10.18	β-Selinene	$C_{15}H_{24}$	204.35		0.06
42	10.34	Humulene oxide	$C_{15}H_{24}O$	220.35	0.09	0.19
43	10.63	*trans*-Longipinocarveol	$C_{15}H_{24}O$	220.35		0.06
44	10.68	(+)-Aromadendrene	$C_{15}H_{24}$	204.35	0.05	0.17
45	10.92	4-Methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane	$C_{15}H_{24}$	204.35	0.03	0.18
46	11.08	Epiglobulol	$C_{15}H_{26}O$	222.20	0.06	0.17
47	11.77	4-(3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.20	0.05	0.05

RESULTS AND DISCUSSION

Chemical Constituents of Clove Essential Oils

The essential oils of *S. aromaticum* buds and leaves gave pale yellowish color with a characteristic clove odor with yield of 12.90 and 2.63%, respectively. The chemical constituents and its composition of the essential oils were determined by GC/MS analyses. Figure 1 shows the chromatogram of clove bud and leaf essential oils and the chemical constituents are given in Table 1. The clove bud and leaf essential oils contain 36 and 29 constituents, respectively, representing 100% of the essential oils, were characterized. The clove bud and leaf oils contain phenylpropanoid compounds (65.46%), sesquiterpenes (31.79%), oxygenated sesquiterpenes (2.06 %), ester (0.14%), alcohol (0.30%), monoterpenes (0.07%), aldehyde and ketones (0.06%), and others (0.14%). Different compositions in major constituents were found between both essential oils.

Clove bud essential oil (CBEO) contained eugenol (65.29%), *trans*-caryophyllene (26.06%), and α-humulene (3.38%) as the major components. While, in the clove leave essential oil (CLEO), the composition was eugenol (64.47 %), *trans*-caryophyllene (27.19 %), α-humulene (3.62 %). Both CBOE and CLOE have eighteen similar components with varying compositions. Moreover, eighteen minor constituents, including *l*-limonene (0.02%), linalool (0.06%), α-amorphene (0.02%), γ-muurolene (0.02%), germacrene-D (0.19%), valencene (0.11%), eugenyl acetate (0.98%), and *trans*-cadina-1,4-diene (0.03%), were existed only in CBEO, while CLEO had eleven unique minor constituents which were not found in clove CBEO, including *cis*-caryophylene (0.07%), α-selinene (0.06%), 3-allyl-6-methoxyphenol (0.08%), and nerolidol (0.04%), β-selinene (0.06%), and *trans*-longipinocarveol (0.06%).

Figure 2 shows the molecular structures of chemical components of clove bud and leaf essential oils. These main components are in agreement with those reported by Jirovetz et al. but differ in their quantities. Jirovetz et al., (2006) found that clove essential oil contained 23 constituents, with eugenol (76.8 %), followed by β-caryophyllene (17.4 %), and α-humulene (2.1 %) as the main components. However, Kalaiselvi et al. (2019) reported eugenol (90.5 %), β-caryophyllene (6.0 %) and *trans*-isoeugenol (3.1 %) while Alma et al. (2017) reported eugenol (87 %), eugenyl acetate (8 %) and caryophyllene (3.56 %). Our result showed that the eugenol content of clove essential oil is lower than that reported value. The variation in components and composition of clove oils depends on variety, agro-ecological condition, pre-treatments, processing and methods of extraction (Tsusaka et al., 2019; Zang et al. 2023; Nurdjannah et al., 2012).

The main component of both clove essential oils was eugenol. Eugenol or 4-allyl-2-methoxyphenol, a volatile bioactive naturally occurring phenolic molecule with a chemical formula $C_{10}H_{12}O_2$, belongs to phenylpropanoids (C_6-C_3) class of natural products. It is weakly acidic molecule and reacts with base such as sodium hydroxide to give sodium eugenolate. The compound is well known for its diverse applications in various fields such as pharmaceutical, food, flavor, cosmetic, agricultural, and numerous other industries. Eugenol is well recognized for its pharmacological properties, viz. antimicrobial, anticancer, antioxidant, anti-inflammatory, and analgesic (Nisar et al., 2021). In aromatherapy, eugenol is used in essential oils for its soothing effects on the body and mind. It is believed to help reduce stress, anxiety, and even pain. It can be used in diffusers or as part of massage oils for its calming and analgesic effects (Aftab & Aftab, 2020).

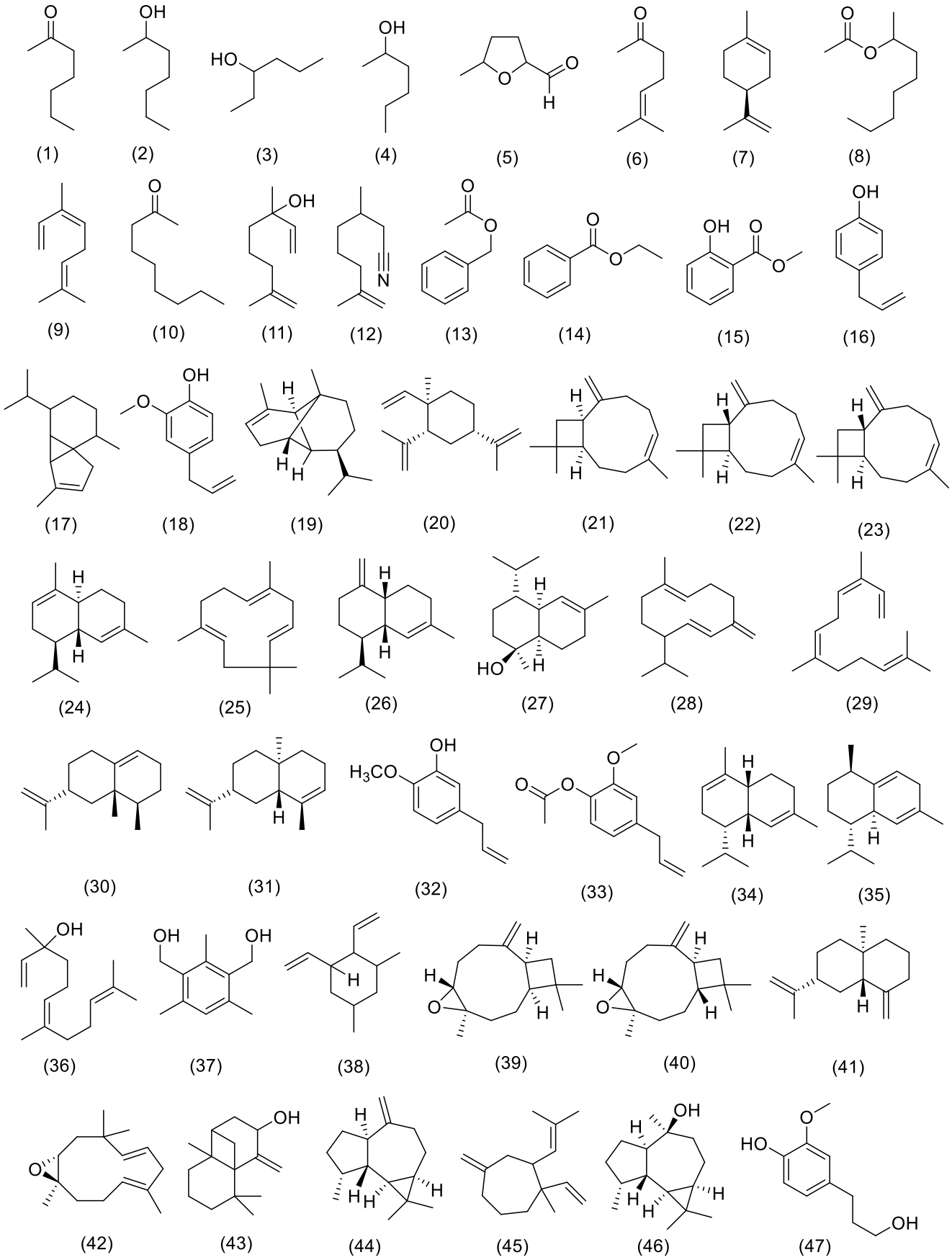
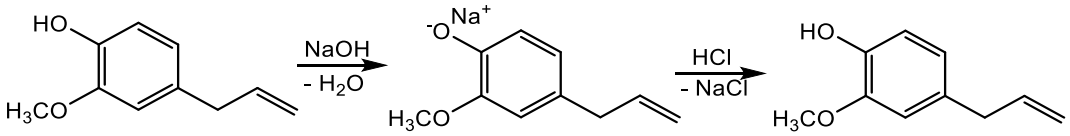


Figure 2. Molecular structure of chemical components of clove essential oil.

Isolation of Eugenol

Isolation of eugenol from clove oil was carried out using the base-acid extraction method, Scheme 4.1. The crude eugenol obtained was purified using distillation under reduced pressure to give pure eugenol (45.60%) with purity of 99.54%. This yield is slightly different from that reported (50.8% yield) (Sharma, 2019) and is very dependent on the eugenol content in clove oil used.



Scheme 1. Reaction during isolation of eugenol from clove oil.

The characterization of the eugenol obtained was carried out using a UV-Vis and Infrared spectrophotometer and Gas Chromatography Mass Spectrometer (GCMS). The maximum wavelength of eugenol measured in ethanol is 283 nm, whereas according to the literature it is 280 nm (Daryono, 2015). The mass spectrum shows base peak of m/z =164, related to M_r = 164 gmol^{-1}, Figure 3. The IR spectrum gives a characteristic absorption at 3227.05-3377.50 cm^{-1} is the stretching vibration region of the O-H bond, 2848.98-2978.22 cm^{-1} for the C-H bond, 1647.28-1674.26 cm^{-1} for the C bond =C aliphatic, 1516.10-1614.47 cm^{-1} for C=C aromatic ring systems, 1047.39-1294.29 cm^{-1} for C-O and 696.33-979.88 cm^{-1} for =C-H bonds.

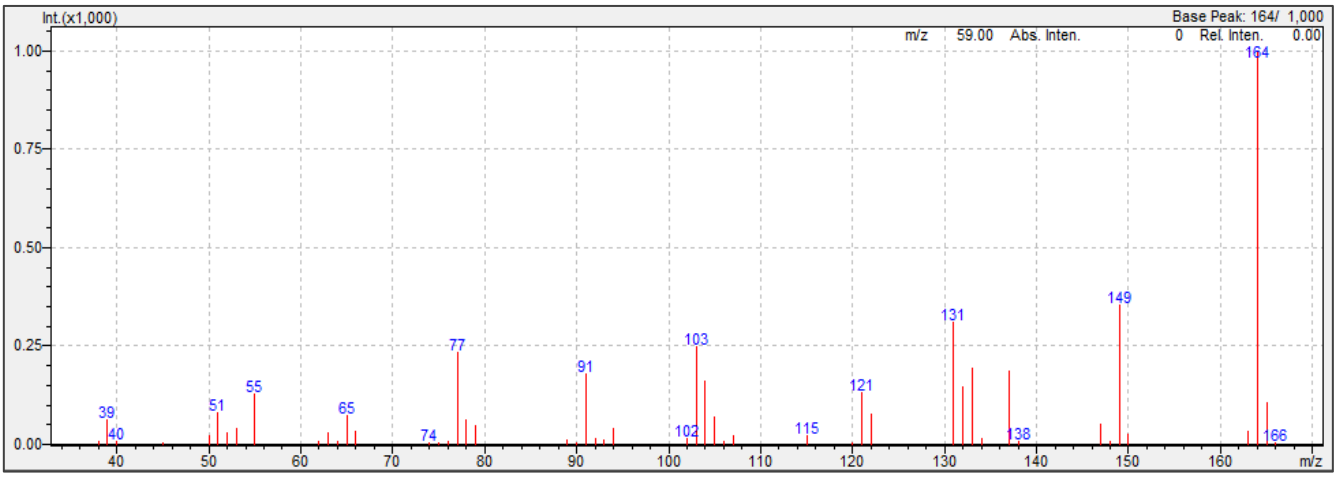


Figure 3. Mass spectrum of eugenol.

Antioxidant Activity

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Shekhar et al., 2014). In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. With this method it was possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm resulting in a color change from purple to yellow, the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule (Ali et al., 2018). In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. In this study, the antioxidant activity of the clove bud essential oil, clove leave essential oil, and eugenol has been evaluated by DPPH free radical scavenging and was compared to ascorbic acid, Figure 4.

DPPH free radical scavenging activity of eugenol, clove bud oil, and clove leave oil increased with an increasing concentration (r^2 = 0.9985-0.9995). The scavenging effect of eugenol and clove oils on the DPPH radical decreased in the order of eugenol > clove bud essential oil > clove leave essential oil. As shown

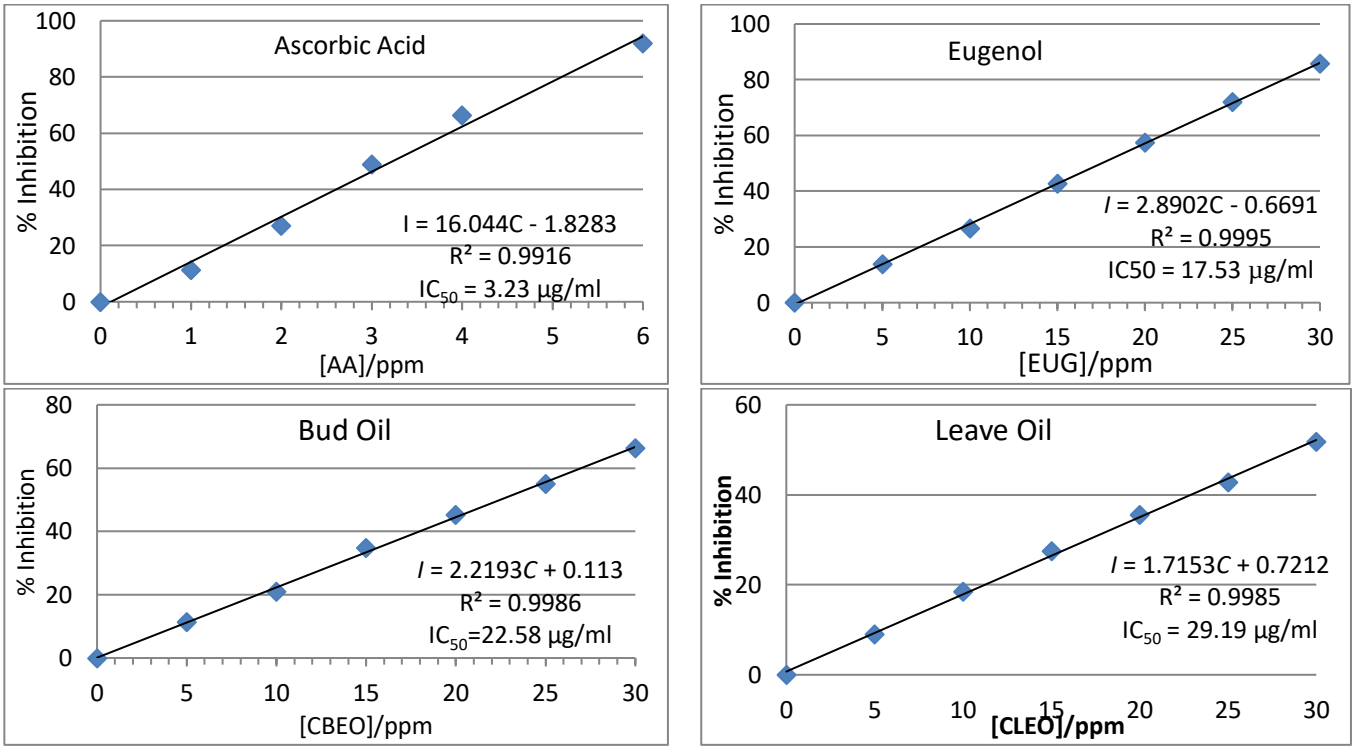


Figure 4. Antioxidant activity of Ascorbic acid, Eugenol, CBEO, and CLEO.

in Figure 3, eugenol, clove bud oil, and clove leave oil were effective DPPH radical scavenger in a concentration-dependent manner (5-30 µg/mL) with EC_{50} values of 17.53, 22.58, and 29.19 µg/mL, respectively compared with standard ascorbic acid (3.23 µg/mL). Lower EC_{50} indicates a higher DPPH free radical scavenging activity.

It has been reported that clove oil has high antioxidant activity with EC_{50} value of 21.50 µg/mL (Ghadermazi et al., 2017) and 22.20 µg/mL (Alfikri et al., 2020). Moreover, eugenol was an effective DPPH radical scavenger in a concentration-dependent manner (10-30 µg/mL; r^2 = 0.9823) with EC_{50} value of 16.06 µg/mL (Gülçin, 2011). Eugenol can undergo

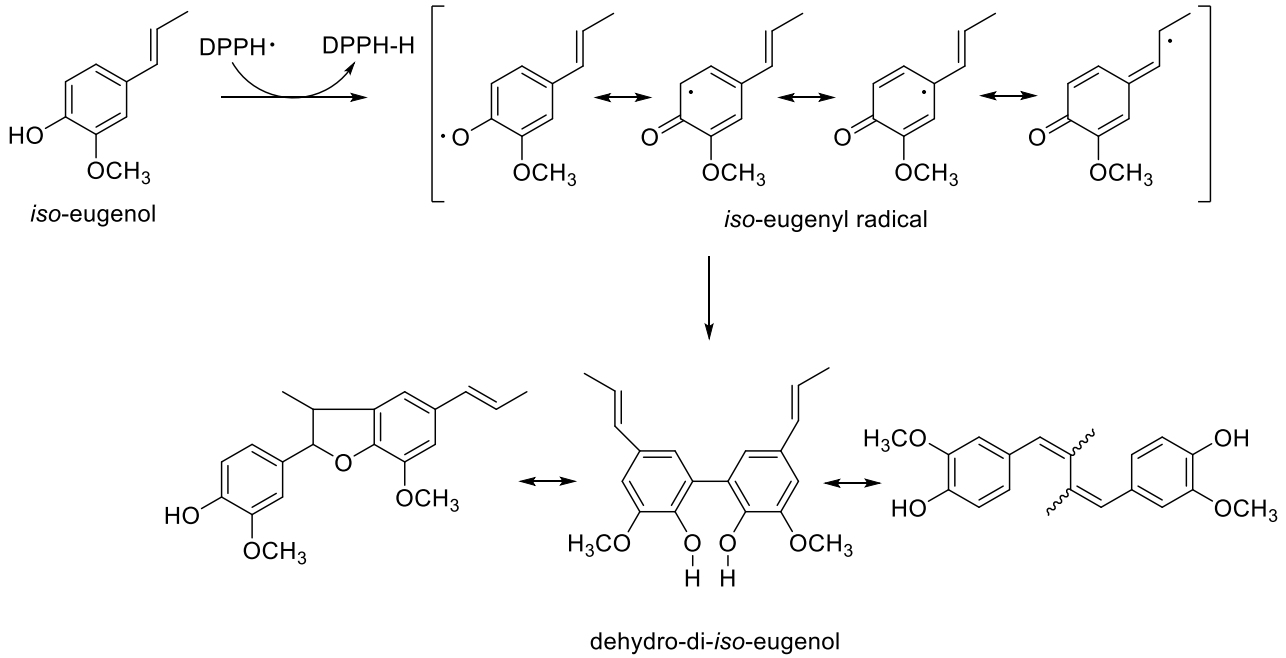


Figure 5. The proposed mechanism between eugenol and DPPH radicals and formation of dehydro-dieugenol.

isomerization to *iso*-eugenol. Eugenol has an aromatic ring stabilized a radical formed on an-oxygen with conjugation in the eugenol molecule. As can be seen in Figure 5, eugenol scavenged the radical on this aromatic ring. It is well known that phenolic groups stabilize a radical formed on phenolic carbon with their resonance structure (Gülçin, 2011). The eugenyl radical intermediates can form a dimer of eugenol called as dehydro-dieugenol.

CONCLUSION

In conclusion, both clove bud essential oil (CBEO) and clove leave essential oil (CLEO) from Bali contained similar major chemical constituents, eugenol, *trans*-caryophyllene, and α-humulene, but different in their composition. Eugenol with molecular formula $C_{10}H_{12}O_2$ is the main component in the both essential oils of clove. In addition, some minor constituents existed only in clove bud essential oil (CBEO). The clove bud essential oil, clove leave essential oil and eugenol as main component of the oils have high antioxidant activities with EC_{50} values of 22.58, 29.19, and 17.53 µg/mL, respectively.

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REFERENCES

Amelia, B., Saepudin, E., Cahyana, A.H., Rahayu, D.U., Sulistyoningrum, A.S., & Haib, J. (2017). GC-MS analysis of clove (*Syzygium aromaticum*) bud essential oil from Java and Manado. *AIP Conference Proceedings*, 1862, 030082.

Aftab, S., & Aftab, S. (2020). The role of aromatherapy in reducing stress and anxiety. *Journal of Complementary Therapies in Clinical Practice*, 39, 101183.

Alfikri, F.N., Pujiarti, R., Wibisono, M.G., & Hardiyanto, E.B. (2020). Yield, quality, and antioxidant activity of clove (*Syzygium aromaticum* L.) bud oil at the different phenological stages in young and mature trees. *Scientifica (Cairo)*, 9701701.

Ali, H., Yesmin, R., Satter, M.A., Habib, R., & Yeasmin, T. (2018). Antioxidant and antineoplastic activities of methanolic extract of *Kaempferia galanga* Linn. rhizome against Ehrlich ascites carcinoma cells. *Journal of King Saudi Univiversity Sciences*, 30(3), 386-392.

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Danthu, P., Simanjuntak, R., Fawbush, F., Leong Pock Tsy, J.M., Razafimamonjison, G., Abdillahi, M.M., Jahiel, M., & Penot, E. (2020). The clove tree and its products (clove bud, clove oil, eugenol): prosperous today but what of tomorrow's restrictions? *FRUITS The International Journal of Tropical & Subtropical Horticulture*, *75*(5), 224–242.

Daryono, E.D. (2015). Reactive Extraction process in isolation of eugenol of clove essential oil (*Syzigium aromaticum*) based on temperature and time process. *International Journal of ChemTech Research*, 8(11): 564-569.

Ghadermazi, R., Keramat, J., & Goli, S.A.H. (2017). Antioxidant activity of clove (*Eugenia caryophyllata* Thunb), oregano (*Oringanum vulgare* L) and sage (*Salvia officinalis* L) essential oils in various model systems. *International Food Research Journal*, 24(4): 1628-1635.

Gülçin, İ. (2011). Antioxidant activity of eugenol: a structure-activity relationship study. *Journal of Medicinal Food*, 14(9): 975-985.

Haro-González, J. N., Castillo-Herrera, G. A.,Martínez-Velázquez, M., & Espinosa-Andrews, H. (2021). Clove Essential Oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. *Molecules*, 26(21): 6387.

Hussain, S., Rahman, R., Mushtaq, A., & Zerey-Belaskri, A. E. (2017). Clove: A review of a precious species with multiple uses. *International Journal of Chemical and Biological Sciences*, 11:129-133.

Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A., & Schmidt, E. (2006). Chemical composition and antioxidant properties of clove leaf essential oil. *Journal of Agricultural and Food Chemistry*, 54(17): 6303–6307.

Kalaiselvi, A., Aswin Jeno, J. G., Roopan, S. M., Madhumitha, G., & Nakkeeran, E. (2019). Chemical composition of clove bud oil and development of clove bud oil loaded niosomes against three larvae species. *International Biodeterioration & Biodegradation*, 137, 102-108.

Kamatou, G.P., Vermaak, I., Viljoen, & A.M. (2012). Eugenol - from the remote Maluku Islands to the international market place: A Review of a remarkable and versatile molecule. *Molecules*, 17(6), 6953-6981.

Mittal, M., Gupta, N., Parashar, P., Mehra, V., & Khatri, M. (2014). Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: a comprehensive review. *International Journal of Pharmcy & Pharmaceutical Sciences*, 6(8): 67-72.

Mohammadi Nejad, S., Özgüneş, H., & Başaran, N. (2017). Pharmacological and toxicological properties of eugenol, *Turkish Journal of Pharmaceutical Sciences*, 14(2): 201-206.

Muderawan. IW., Mudianta, IW., & Martiningsih, NW. (2022). Physicochemical properties, chemical compositions, and antioxidant activities of rhizome essential oils of two varieties of *Kaempferia galanga*, Linn. *Indonesian Journal of Chemistry*, 22(1): 72-85. Q3.

Musta, R., & Nurliana, L. (2019), Studi Kinetika Efektifitas Minyak Daun Cengkeh (*Syzigium aromaticum*) Sebagai Antifungi *Candida albicans*. *Indonesian Journal of Chemical Research*, 6(2): 107-114.

Nisar, M. F., Khadim, M., Rafiq, M., Chen, J., Yang, Y., & Wan, C. C. (2021). Pharmacological Properties and Health Benefits of Eugenol: A Comprehensive Review. Oxidative Medicine and Cellular Longevity Volume, ID 2497354.

Nurdjannah, N., & Bermawie, N. (2012). Handbook of Herbs and Spices. 2nd Edition, edited by K. V. Peter, Woodhead Publishing, Cambridge, pp. 197-215.

Nurliana, L., Kurniawati, D., Musta, R., Kadir, L. A., & Dewi, F. (2020). Tinjauan Kinetika Kimia Daya Hambat Minyak Daun Cengkeh (*Syzygium aromaticum*) dan Hasil Mikroenkapsulasinya Terhadap *Eschericia coli*. *Indonesian Journal of Chemical Research*, 7(2): 151-158.

Sharma, S., Bhagat, A., Vishwakarma, G. S., & Mittal, S. (2019). Physicochemical characterization of the inclusion compounds of eugenol and β-caryophyllene in β-Cyclodextrin. *Nanotecnology Perceptions*, 15, 143-149.

Shekhar, T.C., & Anju, G. (2014). Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn leaves. *American Journal of Ethnomedicine*, 1(4), 244-249.

Tsusaka, T., Makino, B., Ohsawa, R., & Ezura, H. (2019). Genetic and environmental factors influencing the contents of essential oil compounds in Atractylodes lancea. *PLoS ONE*, 14(5): e0217522.

Zhang, T., Zheng, Y., Fu, C., Yang, H., Liu, X., Qiu, F., Wang, X., & Wang, Z. (2023). Chemical variation and environmental influence on essential oil of *Cinnamomum camphora*. *Molecules*, 28, 97.