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The Potential of Lime Juice in Reducing Fe Levels and Improving the Quality of Clove Leaf Oil Using Complexometry and Testing Its Antioxidant Activity Using DPPH (*1,1-Diphenyl-2-picrylhydrazil*)

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Abstract

Clove leaf oil from Samigaluh, Kulon Progo has high Fe content and low eugenol content due to an ineffective distillation process. This study aims to analyze the ability of lime juice as a purifying agent in reducing iron content through complexometry, varying the concentration of lime juice (2%, 3%, and 4%) and stirring time (30, 60, and 90 minutes) on 50 mL of clove leaf oil mixed with 50 mL of lime juice. Eugenol isolation was performed using KOH-H_2SO_4 extraction. Testing was also conducted on eugenol content, acid number, refractive index, specific gravity, and antioxidant activity using DPPH. Sample testing was performed using AAS, GC, and UV-Vis spectrophotometry. Optimal conditions were obtained at a lime juice concentration of 4% with 60 minutes of stirring, which successfully reduced Fe content from 17.6 to 1.7 mg/kg, increased eugenol content from 75.908% to 78.730% at a lime juice concentration of 3% and 90 minutes of stirring, reducing the acid number from 1.82 to 0.94 mg NaOH/g, increasing the refractive index from 1.331 to 1.529, and increasing the specific gravity from 1.020 to 1.036. Further redistillation with KOH and H_2SO_4 increased the purity of eugeno1 to 96.476% with strong antioxidant activity (IC_{50} 57.45 ppm).

Keywords: clove leaf oil; complexometry; quality improvement; lime juice; eugenol; antioxidant; AAS; GC; UV-Vis.

Abbreviations: SNI: Standar Nasional Indonesia (Indonesian National Standard), DPPH: 1,1-Diphenyl-2-picrylhydrazyl, Fe: Iron, PA: Patchouli Alcohol, UV-Vis: Ultraviolet-Visible, IC50: Inhibitory Concentration 50%.

INTRODUCTION

Clove (*Syzygium aromaticum*), an indigenous plant of the archipelago from the Myrtaceae family, has an often overlooked component: its leaves. Although clove flowers and stems have been widely utilized in the cigarette and culinary industries, clove leaves are often regarded as waste. However, clove leaves contain significant amounts of essential oil, ranging from 1-4% in both fresh and dry conditions, with eugenol content reaching 80-85% (Tuganyita et al., 2019). This makes clove leaf oil a product with high economic value (Putri et al., 2014).

Nevertheless, the essential oil processing industry in Indonesia, particularly in rural areas, still faces product quality challenges. The distillation methods used by farmers tend to be simple and suboptimal, often producing low-quality oil with dark blackish or greenish colors, caused by metal contamination from leaves and distillation equipment (Nengsi, 2018). According to research by Affifah et al. (2016), the quality of clove leaf oil depends on the quality of its distillation, where distillation equipment such as boilers should be made of stainless steel materials and oil containers must be clean.

According to SNI 06-2387-2006, the eugenol concentration in clove oil should be at least 78%. However, the simple distillation process commonly used only produces clove oil with eugenol content of around 70%. Another parameter affecting essential oil quality is Fe (iron) content. Although SNI 06-2387-2006 does not establish specific limits for Fe levels in clove leaf oil, this parameter remains an important indicator showing metal contamination and degradation of essential components in the oil.

Clove leaf oil contaminated with Fe will produce high acid numbers due to oxidation, alter specific gravity, and potentially affect the oil's refractive index. Increased acid numbers significantly impact the decline in clove leaf oil quality, particularly affecting aroma characteristics and shortening product shelf life. Therefore, to obtain clove leaf oil that meets standards, purification processes are necessary to extend material durability during storage.

Several studies have shown that purification processes can increase eugenol content and marketability

of clove leaf oil in international markets. Complexometry using citric acid purifying has become one method that can be used to reduce metal concentrations while improving oil quality and eugenol content (Fatimah et al., 2014). Research by Saputri et al. (2014)demonstrated that chemical purification with complexometry using citric acid on clove leaf oil can change oil color from dark brown to light yellow and reduce Fe content.

Lime (*Citrus aurantifolia*) contains citric acid that meets the criteria as a fastener compound and is easily accessible to traditional clove farmers. Lime contains 7% citric acid, higher than lemon which only contains 5% citric acid. A natural material can be categorized as a qualifying fastener if it contains at least 0.5% to 4% citric acid (Tjandrawinata & Julianto, 2018). The use of lime as a purifying agent is expected to be an effective and economical solution for traditional clove farmers to improve oil quality.

The eugenol content in clove oil has antioxidant capabilities (Latifah et al., 2016). Eugenol works as an antioxidant by capturing free radicals through phenolic groups, as its molecular structure allows phenolic hydrogen donation and stabilization of the resulting phenoxyl radicals. The DPPH (1,1-Diphenyl-2-picrylhydrazyl) method is one of the most widely used test methods for antioxidants because of its ease of use, high sensitivity, and ability to analyze large numbers of samples in a short time (Handayani et al., 2018)

This study aims to determine the appropriate treatment combination and the effect of varying stirring times and lime juice concentrations to produce the best physicochemical quality of clove leaf essential oil, as well as to evaluate its antioxidant activity using the DPPH method as an additional quality parameter.

MATERIALS AND METHODS

Study area

This research was conducted from January 10, 2025 to January 30, 2025 at the BSIP Laboratory, Yogyakarta, and from January 31 to July 25, 2025 at the Integrated Laboratory of UIN Sunan Kalijaga, Yogyakarta. The clove leaf essential oil samples were obtained from the Samigaluh area, Kulon Progo Regency, Central Java, Indonesia, which is known as one of the traditional clove cultivation centers in Indonesia.

Procedures

Materials and Equipment

The equipment used in this study included a complete set of glassware, distillation apparatus, analytical balance, measuring pipettes, volumetric pipettes, glass stirrer, magnetic stirrer, burette, separatory funnel, funnel support, glass funnel, reagent bottles, 100 mL volumetric flask, hot plate, 125 mL Erlenmeyer flask, 100 mL measuring cylinder, aquadest bottle, rubber bulb, pycnometer, refractometer, Gas Chromatography Shimadzu 2010, Agilent 240FS AA Atomic Absorption System, and UV-Vis spectrophotometer Thermo Scientific Genesys 20.Sub-procedures-2

The materials used included clove leaf essential oil obtained from the Samigaluh region, Kulon Progo, lime fruit, aquades, pH indicator, 96% ethanol, ascorbic acid p.a., diethyl ether, 1,1-diphenyl-2-picrylhydrazyl (DPPH), filter paper, KOH, and H_2SO_4.

Redistillation of Clove Leaf Oil

The redistillation process began with sample filtration using filter paper to remove coarse particles and visible impurities. After filtration, the oil was weighed using an analytical balance to determine its initial weight. A clean and dry distillation flask was then filled with the filtered oil, and a thermometer was positioned correctly to monitor the temperature during the distillation process. The distillation apparatus was carefully assembled, ensuring all connections were tightly fitted to prevent vapor leakage. The condenser was connected to a cold water flow to ensure effective cooling. The heating mantle was turned on and the temperature was gradually adjusted, slowly raised until reaching the boiling point of clove oil, which ranges between 250-255°C. The main fraction of pure clove oil was collected while maintaining a stable temperature within the clove oil boiling point range. Distillation was stopped when almost all the oil had been distilled. After the distillation process was complete, the redistilled oil was analyzed using GC.

Determination of Citric Acid Concentration in Lime Juice

Fresh limes were washed clean, cut into two parts, and squeezed. The juice was filtered through a cloth filter to remove pulp and seeds. 10 mL of lime juice was placed in a 250 mL Erlenmeyer flask, then 2-3 drops of phenolphthalein indicator were added. Standardized 0.1 N NaOH solution was prepared in a 50 mL burette and the initial volume was recorded. Titration was performed by adding 0.1 N NaOH drop by drop while stirring until the solution changed color to pink which lasted for at least 30 seconds. The final volume of NaOH was recorded for the determination of citric acid concentration in lime juice.

Preparation of Lime Juice Solutions

Based on the results of citric acid concentration determination in the previous stage, the volume of lime juice required to make 2%, 3%, and 4% solutions was calculated. The appropriate volume of lime juice for each concentration was placed in separate beakers (16.7; 25.0; 33.3 mL), a small amount of aquades was added, stirred until homogeneous, then transferred to a 50 mL volumetric flask. Aquades was added to the mark, the volumetric flask was closed, and the solution was homogenized. Each volumetric flask was labeled according to concentration (2%, 3%, and 4%).

Atomic Absorption Spectroscopy Analysis for Fe Content

AAS analysis for Fe content was performed in two stages: preparation of a standard solution and calibration curve construction. Standard solution preparation began by dissolving 4.8303 grams of $FeCl_3.6H_2O$ in a beaker glass and adding aqua regia (HNO_3(p): HCl(p)), then heated until dissolved and transferring to a 1000 mL volumetric flask, then diluted with aquabidest to the mark, obtaining a 1000 mg/kg Fe standard solution. The second step began by pipetting 10 mL of 1000 mg/kg Fe standard solution and placing it in a 100 mL volumetric flask to obtain 100 mg/kg. Subsequently, 0, 2, 4, 6, 8, 10 mL were taken from this 100 mg/kg solution and placed in 100 mL volumetric flasks, diluted to the mark and used as standard solutions.

Determination of Acid Number

Acid number determination was performed by taking 4 grams of the sample and dissolving it in 5 mL of neutral ethanol in a saponification flask, then adding 5 drops of phenolphthalein indicator. The solution was then titrated with sodium hydroxide until the color changed to pink.

Determination of Specific Gravity

Specific gravity determination was performed using a pycnometer. The pycnometer was cleaned, rinsed with ethanol and diethyl ether, dried with dry air flow, and weighed (m). The pycnometer was filled with distilled water that had been boiled and cooled to 20°C. After immersion in a water bath at 20°C ± 0.2°C for 30 minutes, the pycnometer was closed, dried, and weighed (m_1). The procedure was repeated with clove leaf oil as a substitute for water (m_2).

Determination of Refractive Index

Refractive index determination was performed using an Abbe refractometer. The refractometer prism cover was opened and the prism was cleaned with tissue paper moistened with aquades. Oil was taken using a pipette and placed on the Abbe refractometer prism (1 drop), then closed again. Temperature was maintained with a tolerance of ± 2°C. The number read on the refractometer was recorded.

Determination of Optimum Conditions and GC Analysis

Analysis of clove leaf oil was performed using GC instrument on samples before and after the purification process. The best results from the four purification quality parameters (Fe content, acid number, refractive index, and specific gravity) were selected from each treatment. The best oil sample obtained was then further analyzed using GC to determine changes in eugenol content before and after purification.

Eugenol Isolation from Optimum Clove Leaf Oil

The eugenol isolation process involved several steps: preliminary treatment with 20 mL clove leaf oil and 6 g KOH dissolved in 100 mL aquades; mixing with strong base at 50°C for 30 minutes using magnetic stirrer; mixing with strong acid using 1.5 N H_2SO_4 solution; second separation process using separatory funnel for 4 hours; eugenol washing with aquades in 1:1 ratio; and water removal by heating on hot plate.

Antioxidant Activity Test

DPPH Solution Preparation (40 ppm)

2 mg DPPH powder was dissolved in a volumetric flask using 96% ethanol to a volume of 50 mL.

Maximum Wavelength Determination

4 mL of 40 ppm DPPH solution was placed in a test tube and mixed with 1 mL of 96% ethanol, homogenized, incubated for 30 minutes in dark conditions, and absorbance was measured at 400-800 nm range using a UV-Vis spectrophotometer.

Test Solution Preparation

0.01 gram (10 mg) of clove leaf oil was measured and placed in a 10 mL volumetric flask. 96% ethanol was added to the mark to produce an initial solution with 1000 ppm concentration. Serial dilutions were performed to obtain test solutions with concentrations of 5 ppm, 10 ppm, 15 ppm, and 20 ppm.

Standard Solution Preparation

Standard solution was prepared using ascorbic acid p.a. To make 1000 ppm stock solution, 10 mg ascorbic acid p.a was dissolved in 96% ethanol to a volume of 10 mL. Serial dilutions were performed to obtain lower concentrations of 5 ppm, 10 ppm, 15 ppm, and 20 ppm.

Antioxidant Activity Determination

The best clove leaf oil sample was tested for antioxidant activity using the DPPH method. 1 mL of test solution from each concentration (5, 10, 15, 20 ppm) was mixed with 4 mL of 40 ppm DPPH, homogenized, and incubated for 30 minutes in a dark room. Absorbance was measured using UV-Vis spectrophotometer at the predetermined maximum wavelength. Control was made by mixing 1 mL of ethanol and 4 mL of 40 ppm DPPH.

Data analysis

The data obtained from quality parameter measurements (Fe content, acid number, specific gravity, refractive index) and antioxidant activity (IC_{50} value) were analyzed using statistical methods. The experimental design used was factorial with two factors: lime juice concentration (2%, 3%, 4%) and stirring time (30, 60, 90 minutes). Each treatment was performed in triplicate to ensure data reliability. Data were analyzed using analysis of variance (ANOVA) to determine significant differences between treatments. The IC_{50} value was

calculated using linear regression analysis between concentration and percentage of DPPH inhibition. The optimum treatment was determined based on the combination of treatments that produced the best quality parameters according to SNI 06-2387-2006 standards.

RESULTS AND DISCUSSION

Result

The results of the Fe levels test

The results of Fe levels testing can be seen in Figure 1.

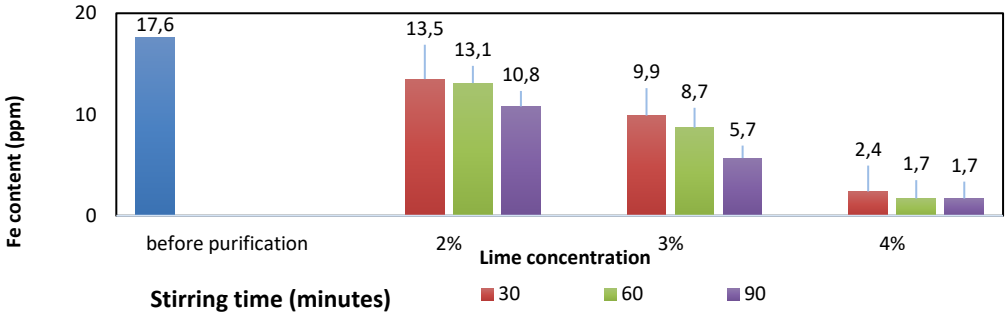


Figure 1. Fe content of clove leaf oil at various concentrations and stirring times.

The result of Acid number of clove leaf oil at various purification conditions

The result of Acid number of clove leaf oil at various purification conditions can be seen in Table 1.

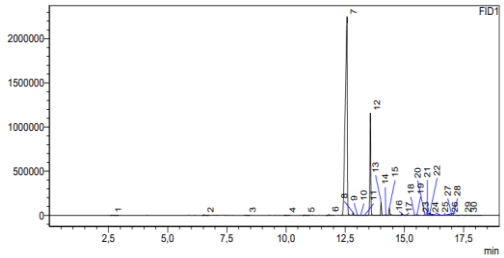
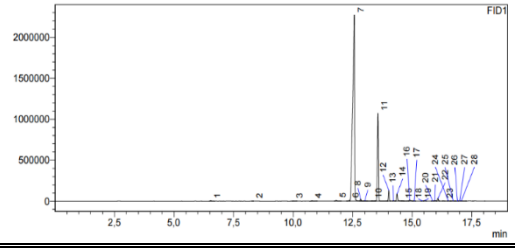
Table 1. Acid number of clove leaf oil at various purification conditions.

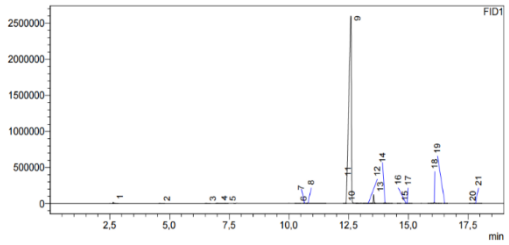
Concentration (%)	30 minutes	60 minutes	90 minutes
Before purification	1.82	1.82	1.82
2%	1.59	1.31	1.26
3%	1.17	1.12	1.03
4%	1.08	0.94	0.94

The result of eugenol content at different purification stages

The result of eugenol content at different purification stages can be seen in Table 2.

Table 2. The result of eugenol content at different purification stages.

Purification Stage		Retention Time (minutes)	Eugenol Content (%)	Number of Peaks
Before purification		12.574	75.908	30
After lime juice (3%, 90 min)		12.570	78.730	28

Purification Stage		Retention Time (minutes)	Eugenol Content (%)	Number of Peaks
After KOH-H_2SO_4 extraction		12.603	96.476	21

The result of refractive Index

The result of refractive index testing can be seen in Table 3.

Table 3. The result of refractive index.

Concentration (%)	30 minutes	60 minutes	90 minutes
Before purification	1.5287	1.5287	1.5287
2%	1.5294	1.5298	1.5301
3%	1.5305	1.5308	1.5310
4%	1.5309	1.5312	1.5312

The result of spesific gravity of clove leaf oil at various purification conditions

The result of spesific gravity of clove leaf oil at various purification conditions can be seen in Table 4.

Table 4. The result of spesific gravity of clove leaf oil at various purification conditions.

Concentration (%)	30 minutes	60 minutes	90 minutes
Before purification	1.020	1.020	1.020
2%	1.025	1.027	1.029
3%	1.031	1.033	1.035
4%	1.034	1.037	1.037

One way anova test results for all tested parameters results

One way anova test results for all tested parameters results can be seen in Table 5

ANOVA

Table 5. One way anova test results for all tested parameters. Description: * : significant, ns : not significant.

Parameter	Factor	F-value	p-value	significance
Fe content	Concentration	4.306	0.030	*
	Stirring time	0.263	0.772	ns
	Interaction	0.049	0.995	ns
Acid number	Concentration	15.428	0.000	*
	Stirring time	12.847	0.000	*
	Interaction	3.952	0.026	*
Refractive index	Concentration	8.742	0.002	*
	Stirring time	6.158	0.008	*
	Interaction	2.847	0.045	*
Specific Gravity	Concentration	2.134	0.148	ns
	Stirring time	1.867	0.186	ns
	Interaction	0.398	0.836	ns

(Significant at $\alpha = 0.05$)

The result of antioxidant activity

The result of antioxidant activity can be seen in Table 6.

Table 6. Antioxidant activity results.

Sample	Concentration (ppm)	Average Absorbance	% Inhibition	Linear Regression Equation	R^2	IC_{50} (ppm)	Category
Clove Leaf Oil	10	0.024	20.00	y = 0.4486x + 24.228	0.8682	**57.45**	**Strong**
	25	0.017	43.33				
	50	0.015	50.00				
	75	0.012	58.89				
	100	0.010	65.56				
Vitamin C	10	0.019	36.67	y = 0.2834x + 37.483	0.9279	**44.16**	**Very Strong**
	25	0.016	46.67				
	50	0.014	54.44				
	75	0.012	60.00				
	100	0.011	63.33				
Control	0	0.030	0.00	-	-	-	-

Discussion

Physical Parameter Analysis of Purification Process

Iron (Fe) Content

The purification process of clove leaf oil aims to remove impurities or unwanted compounds, which consequently reduces the oil volume during processing. Analysis of Fe content in clove leaf oil after purification was conducted to determine the Fe components removed, enabling more efficient processes for industrial-scale applications. Fe content in a material indicates the amount of iron contained within it. Iron in clove leaf oil is considered an unwanted component that must be reduced, as excessive Fe content can trigger oxidation reactions causing the oil to appear dark and blackish, affecting the physicochemical properties of the resulting clove leaf essential oil. Based on the analysis results, the Fe content in unpurified clove leaf oil was 17.6 mg/kg. After purification with lime juice addition at concentrations of 2-4% and stirring time variations of 30, 60, and 90 minutes, significant Fe content reduction occurred with results ranging from 13.5-1.7 mg/kg (Figure 1). The ability of lime juice to reduce Fe content in clove leaf oil can be explained through complex formation mechanisms. The main component of clove leaf oil is eugenol containing phenolic groups, where Fe^{3+} ions initially bind to these phenolic groups forming -OFe^{3+} structures.

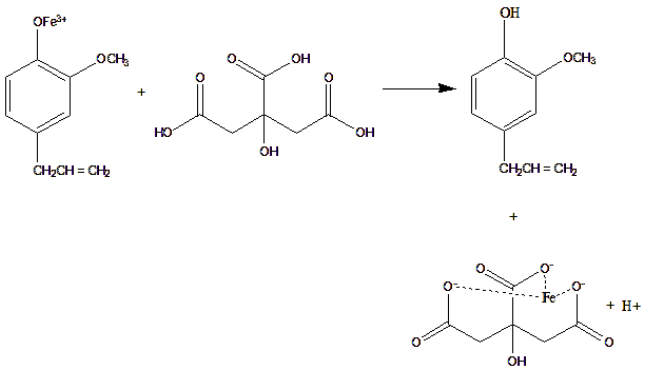


Figure 2. Mechanism of Fe^{3+} binding from clove leaf oil by citric acid

The active component in lime juice serving as the primary binding agent is citric acid ($C_6H_8O_7$), with additional contributions from ascorbic acid (vitamin C) and other organic acids. Citric acid possesses high binding capacity due to its structure containing four potential binding sites: three carboxyl groups (-COOH) and one hydroxyl group (-OH). When lime juice containing citric acid interacts with the Fe^{3+}-eugenol complex, deprotonation of carboxyl groups occurs, producing citrate ions ($C_6H_5O_7^{3-}$). These negatively charged groups provide free electron pairs that can strongly bind Fe^{3+} ions while simultaneously releasing Fe^{3+} from eugenol phenolic groups, forming coordination bonds with oxygen atoms from citric acid carboxyl groups.

Before purification After purification

Figure 3. Clove leaf oil before and after purification.

The Fe content reduction process can be visually observed through color changes during purification. Before purification, clove leaf oil appeared darker and brownish due to Fe^{3+} ions that can trigger oxidation reactions. After purification with lime juice, the oil became more yellow-clear and bright, indicating successful Fe^{3+} binding and extraction from the oil phase.

Concentration of 4% showed the highest Fe reduction effectiveness with results of 2.4 mg/kg (30 minutes), 1.7 mg/kg (60 minutes), and 1.7 mg/kg (90 minutes). At this concentration, Fe reduction reached a stable point at stirring times of 60 and 90 minutes with identical values of 1.7 mg/kg, indicating that at 4% concentration, the Fe-citrate complex formation process reached equilibrium conditions where additional stirring time no longer provided significant Fe reduction. Based on research data, the optimum conditions for Fe reduction in clove leaf oil were at 4% lime juice concentration with 60 minutes stirring time, producing Fe content of 1.7 mg/kg. Statistical analysis showed that lime juice concentration had significant effect on Fe content ($F = 4.306$, $p = 0.030$), while stirring time showed no significant effect ($F = 0.263$, $p = 0.772$).

Eugenol Content

Eugenol is the main component in clove leaf essential oil, comprising 70% to 90% of the total content. GC chromatography analysis of three clove leaf oil samples showed eugenol presence with relatively similar retention times but different percentage contents (Table 2). Before purification, eugenol was detected at retention time 12.574 minutes with 75.908% content, showing 30 peaks indicating various impurity components. This percentage did not meet Indonesian National Standard (SNI 06-2387-2006) requirements of minimum 78% eugenol content.

Table 7. Quality Standards for Clove Leaf Essential Oil SNI 06-2387-2006.

No.	Test Type	Unit	Requirements
1.	Color	-	Yellow-brown to brownish clove oil
2.	Specific Gravity 20°C / 20°C	-	1.025-1.049
3.	Refractive Index	-	1.528-1.535
4.	Solubility in 70% Ethanol	-	1:2 clear
5.	Total Eugenol	%, w/v	Min. 78
6.	Beta Caryophyllene	%	Max. 17

After purification using 3% lime juice with 90 minutes stirring time, significant improvement occurred in clove leaf oil quality. Eugenol was detected at nearly identical retention time (12.570 minutes) but with increased percentage to 78.730%, representing a 3.73% increase from initial content. This purified oil successfully met the minimum SNI standard for eugenol content. The purification process also reduced impurity components, indicated by peak reduction from 30 to 28, with elimination of peaks at retention times 17.413 and 17.656 minutes, likely corresponding to α-copaene and β-elemene compounds (Amelia et al., 2017; Sulistyoningrum et al., 2017).

Further enhancement was achieved using reactive extraction method with KOH and H_2SO_4 solutions. The eugenol purification process through reactive extraction involves two main stages utilizing the acidic properties of eugenol's phenolic hydroxyl group. In the first stage, KOH reacts with eugenol's phenol group, where the hydroxyl group acts as a weak acid due to electron resonance in the aromatic ring. KOH removes the proton from the hydroxyl group, creating water-soluble potassium eugenolate (K-Eugenolate) salt that separates from other non-polar clove oil components, while the allyl side chain remains stable under mild reaction conditions.

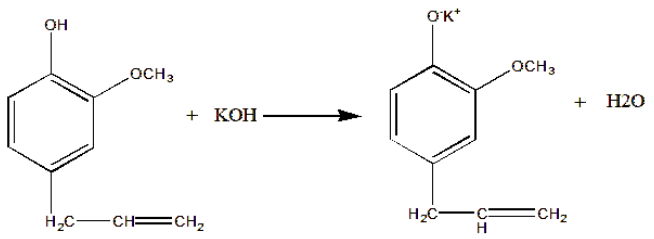


Figure 4. Image of the reaction forming K-Eugenolate salt

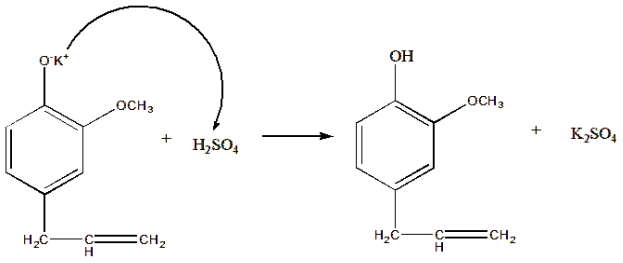


Figure 5. Image of the reaction between K-eugenolate and H_2SO_4 forming eugenol

In the second stage, H_2SO_4 neutralizes the K-Eugenolate salt by providing H^+ ions that combine with eugenolate ions, reforming the hydroxyl group and returning eugenol to its original hydrophobic form that naturally separates from the aqueous solution. This method successfully increased eugenol purity from 78.73% to 96.476%, representing a 17.746% improvement and demonstrating the effectiveness of reactive extraction in achieving high-purity eugenol.

Acid Number

Acid number represents an important parameter for determining oil age, purity, and hydrolysis level. Although SNI 06-2387-2006 for clove leaf oil does not mention acid value test parameters, high acid content in clove leaf oil can cause unpleasant odor and taste, reducing product quality and commercial value. Acid value indicates the amount of free fatty acids present in oil (Hendra Wijaya et al., 2024)Research results showed acid number differences between unpurified and purified clove leaf oil ranging from 8.4-4.9 mg NaOH/g (Table 1). Unpurified clove leaf oil had higher acid number of 1.82 mg NaOH/gram, while purified oil showed acid number reduction. At 4% concentration with 60 and 90 minutes contact time, acid numbers were identical at 0.94 mg KOH/g, indicating that the lime juice purifying agent reached the saturation point. This saturation condition occurs because the chelating agent adsorption capacity is maximized, so contact time extension does not provide significant effectiveness improvement. Results align with research by Rosdiana (2014) regarding citric acid concentration effects on acid content and used cooking oil opacity reduction, and Purbaningtias et al. (2014) on patchouli oil acid value reduction with natural adsorbent.

Two-way ANOVA analysis showed significant effects from both lime juice concentration ($p<0.05$) and stirring time ($p<0.05$) on clove leaf oil acid number, with significant interaction between both factors ($p<0.05$), indicating combined effects in improving oil purification effectiveness.

Refractive Index

Refractive index represents the ratio of light speed in air to light speed in the tested substance at a specific temperature, indicating clove leaf oil quality or purity (Erliyanti et al., 2020). Research results showed that refractive index values at various treatment variations met SNI standards (1.331-1.535), though average values remained at the lower SNI boundary (Table 3). Refractive index tends to increase with purifying agent concentration addition, though not significantly.

The refractive index is influenced by the number of double bonds and carbon chain length, where higher indices indicate better quality due to higher eugenol content (Idris et al., 2014; Pratiwi et al., 2016). After purification, refractive index increased from 1.5287 to values ranging 1.5294-1.5312, indicating improved oil quality. ANOVA analysis showed that both lime juice concentration and stirring time significantly affected refractive index ($p<0.05$) with significant interaction between factors.

Specific Gravity

Specific gravity represents the ratio between oil mass and distilled water mass at equal volume and temperature (Kristian et al., 2016). Research results showed specific gravity increased from 1.020 before treatment to 1.037 after purification, meeting SNI 06-2387-2006 standards (1.025-1.049) (Table 4). Specific gravity increase indicates better oil quality as it shows more mass within the same volume unit, caused by light-fraction components evaporating during purification (Dewi et al., 2019). ANOVA analysis indicated that neither lime juice concentration nor stirring time significantly affected specific gravity ($p>0.05$), with no interaction between factors ($p>0.05$).

Antioxidant Activity

Previous analysis showed that eugenol separation from clove leaf oil using KOH and H_2SO_4 yielded 96.476% eugenol content. To prove separation success, antioxidant activity analysis was conducted on purified eugenol. Antioxidant activity analysis on clove leaf oil eugenol used DPPH (1,1-diphenyl-2-picrylhydrazyl) method with UV-VIS spectrophotometer assistance.

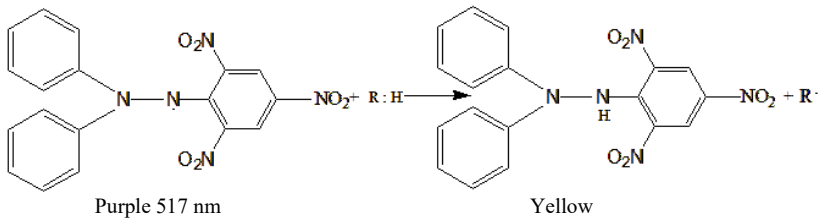


Figure 6. Reaction mechanism of DPPH radicals with antioxidant compounds (RH = radical scavenging antioxidant: R = antioxidant radical)

DPPH radical scavenging mechanism showing color change from purple to yellow. The method was chosen for its simplicity, high sensitivity, and ability to evaluate antioxidant activity in relatively short time. The main principle lies in DPPH reaction as free radical representation (Kolompoy et al., 2024). Antioxidant compounds donate radical electrons while DPPH becomes radical electron acceptor. This reaction is marked by color change from purple to yellow, measurable through absorbance decrease at 517 nm maximum wavelength.

Based on results, clove leaf oil obtained an IC_{50} value of 57.45 ppm, indicating strong antioxidant category (50-100 ppm), while vitamin C extract obtained IC_{50} value of 44.16 ppm, classified as very strong antioxidant (<50 ppm). This aligns with research by Lusiana et al. (2024) reporting a vitamin C IC_{50} value of 4.245 ppm and Irnawati et al., (2017) showing vitamin C IC_{50} value of 24.63 mg/l, both categorized as very strong antioxidants. This difference occurred because vitamin C is a pure compound with very high antioxidant activity, while clove leaf oil eugenol, despite 96.467% purification, still contains other components in small amounts.

Clove leaf oil antioxidant activity originates from eugenol structure which has hydroxyl groups that can donate hydrogen atoms to neutralize free radicals. Other phenol derivative compounds such as eugenol acetate and β-caryophyllene also contribute to antioxidant effects. The concentration and percentage inhibition comparison graph showed strong positive correlation, indicating that eugenol antioxidant activity depends on the concentration used.

CONCLUSIONS

Based on the research results of clove leaf oil purification using the complexometric method with lime juice, it can be concluded that the purification successfully improved oil quality with optimum conditions achieved at 4% lime juice concentration and 60 minutes stirring time. The Fe content decreased from 17.6 mg/kg to 1.7 mg/kg, and all parameters met SNI 06-2387-2006 standards. Further redistillation increased eugenol purity to 96.476%. The purified oil demonstrated strong antioxidant activity with an IC_{50} value of 57.45 ppm, showing potential as a high-quality natural antioxidant.

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