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Synthesis of Methyl Eugenol from Crude Cloves Leaf Oil Using Acid and Based Chemicals Reactions

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Abstract: *Isolation of methyl eugenol from cloves leaf oil using acid and based chemicals reactions has been done. Cloves leaf oil was isolation from cloves leaf using steam distillation (Step 1). Eugenol was synthesis from cloves leaf oil using sodium hydroxide (NaOH 5%) and sulphuric acid (H_2SO_4) 5% at pH 3 (Step 2). Methyl eugenol was synthesis from eugenol from step 2 using dimethyl sulphate (Step 3). The result is dried with anhydrous Na_2SO_4 and n-hexane is separated by the rotary evaporator. The result of the synthesis was analysis using GC-MS and Fourier Transform Infrared (FTIR). The results of the study showed that the methyl eugenol yield is 99.14%. Methyl eugenol produced from this research according to market needs with a minimum purity of 98.0%. As a conclusion, this method is extremely simple and inexpensive for isolation of methyl eugenol from cloves leaf oil.*

Keywords: *eugenol, methyl eugenol, acid, based*

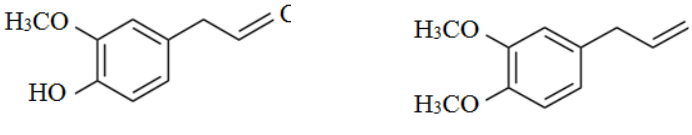
I. Introduction

Clove leaf oil has been use for propose of medicinal uses are related to its antiseptic and analgesic properties. Clove leaf oil has been widely used as a method for preventing tooth decay and toothaches, as well as a cure for nausea, hernia, intestinal gas, diarrhea and fungal infections. Some viral infections respond well to clove oil's antiseptic and bactericidal properties. In tropical Asia, clove is used to combat malaria, cholera, tuberculosis and parasitic illnesses. As a topical ointment, cloves can also be used to alleviate muscle spasms, acne, skin ulcers and styes in the eyes. Clove oil is also a potent insecticide, repelling disease-causing mosquitoes and other insects. Clove oil is an essential oil extracted from clove plants (eugenia caryophyllata thunb), especially from its flowers, stems and leaves. The quality of clove oil is normally indicated by its eugenol and carryophyllene contents. The content of eugenol in oil is mainly affected by quality of raw materials and oil refining methods [1].

Methyl eugenol (ME), as a constituent in leaves, fruits, stems, and/or roots, may be released when that corresponding part of a plant is damaged as a result of feeding by an herbivore. The annual production of methyl eugenol in the United States of America in 1990 was estimated at 11.4 tonnes [2]. Methyl eugenol is a molecule which shows insect pheromone activity for mango fruit flies, although it is believed not to be the natural pheromone itself. If present in sufficiently high concentration, it will immediately deter the herbivore from further feeding on the affected part. ME acts as a deterrent or repellant [3]. ME a constituent of plants used in the human diet, is hepatocarcinogenic in rodents [4]**.** ME is used as a flavouring agent in jellies, baked goods, non-alcoholic beverages, chewing gum, candy, puddings, relishes and ice cream. It is also widely used as a fragrance ingredient in perfumes, toiletries and detergents. Methyl eugenol has been used as an anaesthetic in rodents. It also is used as an insect attractant in combination with insecticides [2,5-6]. Methyl eugenol is a component of several essential oils that are sold for use in aromatherapy, massage oils and alternative medicines [7]. Methyl eugenol is used as a fragrance in perfumes (0.3–0.8%), creams and lotions (0.01–0.05%), and soaps and detergents (0.02–0.2%) [2]**.**

Methyl eugenol is produced by the methylation of eugenol [8]**.** Methyl eugenol was prepared from eugenol in the presence of potassium carbonate (K_2CO_3), using dimethyl carbonate (DMC) as methylating agent. Methyl eugenol (allylveratrol) is a phenylpropene, a type of phenylpropanoid compound, the methyl ether of eugenol. It is obtained by methylation of eugenol, which is produced by fractional distillation (under vacuum) from clove leaf oil, which is extracted from leaves of the clove tree (eugeniacaryophillata) [9].

The amount of methyl eugenol in an essential oil extracted from a given type of plant differs according to the variety, plant maturity at the time of harvesting, the method of harvesting, storage conditions and the method of extraction [10]. Fig. 1 showed the structure molecular of the eugenol and methyl eugenol. The ether is an almost colorless liquid with a mild-spicy, slightly herbal odor. It is prepared by methylation of eugenol and is used in perfumery (e.g., in carnation and lilac compositions) and in flavor compositions.



(A) (B)

Figure 1. The structural molecular of the eugenol (A) and methyl eugenol (B)

Various techniques have been done for clove leaf oil improvement. Various researchers have been done for improvement quality of clove leaf oil such as the use of bleaching earth method to reduce colour and eugenol content [11]**.** This research has been done using the clay for reduce the colour of clove oil. Other researchers have also been done to improvement quality of clove leaf oil in which citric acid was used as a chelating agent to reduce colour of oil [12-14]. Since citric acid showed more efficient in reducing dark colour, therefore this method was subjected as our further experiment. To compare the adsorption, zeolite was also used. The treatment of natural zeolite was done through chemical and physical treatments by addition of hydrochloric acid and calcinations. Various techniques have been developed only able to improve the quality of eugenol and clove leaf oil, therefore it is very important to do research. Research has been done to increase the price of clove leaf oil through the conversion into a derivative compound eugenol. Eugenol converted into methyl eugenol so as to have a higher price.

The present paper aims to describe the isolation of methyl eugenol from clove leaf oil using acid and based chemicals reactions. Clove leaf oil produced by farmers is crude oil. Clove leaf oil contain eugenol compounds with low concentrations is 76.86%. So that clove leaf oil has a low price. This research will be increase the price of clove leaf oil from farmers to methyl eugenol is a compound with high economic value. Acid and based chemicals reactions method is extremely simple and inexpensive for isolation of methyl eugenol from clove leaf oil. This method also not generate new waste and environmentally friendly.

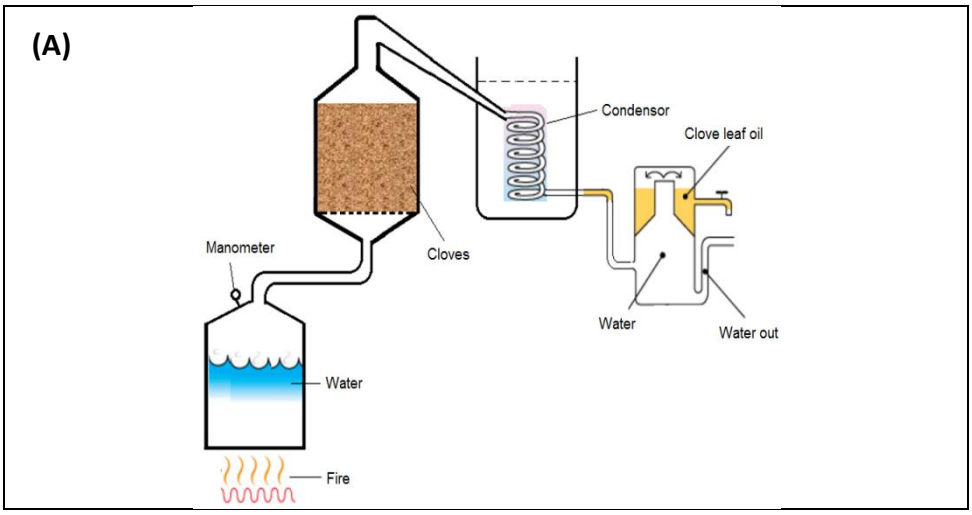
II. Experiments

2.1 Solvents and Chemicals

All solvents used were of analytical grade. Methyl eugenol standard, sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), sodium sulfate (Na_2SO_4), dimethyl sulfate and n-hexana were from Merck. All solutions were prepared by dissolving their analytical grade reagent in deionised distilled water.

2.2 Isolation of Cloves Leaf Oil from Clove

The clove leaf used in this research was obtained collection from farmers in Kulon Progo District, Yogyakarta, Indonesia. Isolation of cloves leaf oil from clove using water steam distillation method. Fig. 2 shown the water steam distillation equipment for this research. Fig. 2A shown the scheme of water steam distillation for isolation of clove leaf oil. Fig. 2B shown the equipment of water steam distillation for isolation of clove leaf oil with industrial scale. One hundred kilograms of clove leaf was used for isolation eugenol. Distillation has been done using a pressure of 1 bar and then opened the faucet and the hot steam of the sample for about 6 h. Essential oils are mixed with hot steam, and then separated in the separator. The essential oils were separated from the aqueous solution, dried over anhydrous Na_2SO_4, then transferred into an amber glass bottle and stored at 4°C until use.



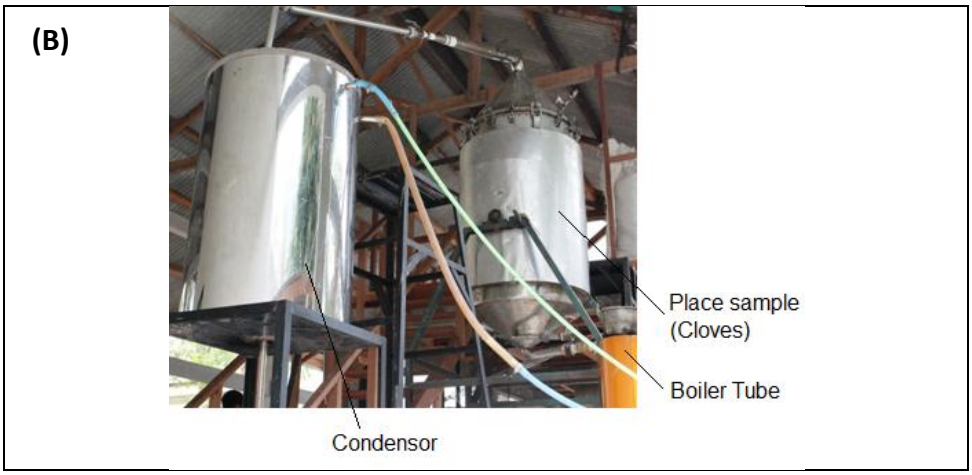


Figure 2. Schematic diagram of steam distillation (A) and (B) steam distillation with industrial scale (100 kg materials)

2.3 Isolation of Eugenol from Clove Leaf Oil (step 1)

A total of 200 grams of clove leaf oil in a 500 mL glass beaker and 5% NaOH solution prepared from 41.21 g of NaOH is dissolved in 824.2 mL of distilled water. The mixture was stirred with a magnetic stirrer for 30 minutes and put in a separating funnel and then allowed to stand overnight, there will be two layers, the top layer is the organic layer and the bottom layer is a layer that contains sodium eugenolate. Bottom layer was taken of 0.5 cm of the top layer, plus 5% H_2SO_4 to pH 3 with stirring will be two layers. This result washed with distilled water until neutral and anhydrous Na_2SO_4 to the remaining water is then filtered. The results were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) [15-16].

2.4 Synthesis of Methyl Eugenol (step 2)

Eugenol 10 g (0.06098 mol) was added to a 100 mL three-neck flask which was equipped with air balls, magnetic stirrer, a thermometer and a funnel dropper then added 3.5 g (0.0875 mol) of NaOH in 20 mL of distilled water and stirred with a magnetic stirrer. Amount of 8.06 mL (0.5 mol) of dimethyl sulfate included in the distillation flask drop wise for 1 hour while stirring and refluxing the mixture at a temperature of 103°C for 1 hour. The mixture was diluted with 25 mL of distilled water. The organic layer was separated and the water layer was extracted 3 times with 5 mL of n-hexane to take the remainder of methyl eugenol. Top layer or organic layer was extracted 2 times with 5 mL of 10% NaOH solution and the upper layer was washed with distilled water until neutral. The result is dried with anhydrous Na_2SO_4 and n-hexane is separated by the evaporator. The result was analysis using GC-MS and Fourier Transform Infrared (FTIR).

2.5 Product Analysis

Product oil sample analysis was performed using Gas Chromatography-Spectrometry Mass (GC-MS) Shimadzu QP-2010 SE, connected to an ion trap detector operating in electron impact mode at 70 eV; EI (Eletro Impact), employing the following conditions: coloum Rastek Rxi-5MS (30 m), temperatur coulom 60^{o}C/5 minute, temperature coloum 290^{o}C, gas helium as the carrier gas, detector temperature 250^{o}C, injector temperature 300^{o}C and total flow 80 mL/second. The oven temperature was programmed from 60°C (isothermal for 3 min), with an increase of 1°C min^{-1}, to 80°C and then an increase of 3°C min^{-1} to 120°C, ending with 4°C min^{-1} to 220°C. The compounds were identified by matching their mass spectral fragmentation patterns with those stored in the spectrometer database, using the National Institute of Standards and Technology Mass Spectral database. The Fourier Transform Infrared (FTIR) spectra were measured on a Thermo Nicolet Avatar 320 FTIR spectrometer (Thermo Nicolet Corporation, Madison, WI, USA), equipped by a Smart Diffuse Reflectance accessory (Nicolet, Madison, USA).

III. Result and Discussions

3.1 Chemicals composition analysis of clove leaf oil using GC-MS

Fig. 3 shows the analysis of clove leaf oil using Gas Chromatography Mass Spectrometry (GC-MS). The GC-MS result shows 5 peak of the chromatogram peak with the retention time of 11.832 to 14.081 min. The impurity components in clove oil are dominated by beta-caryophyllene and alpha humulene. Clove oil contains two major components, namely eugenol (76.86%) and beta-caryophyllene (18.90%) as well as some of small quantities components such as α-copaene, alpha humulene and delta cadinene.

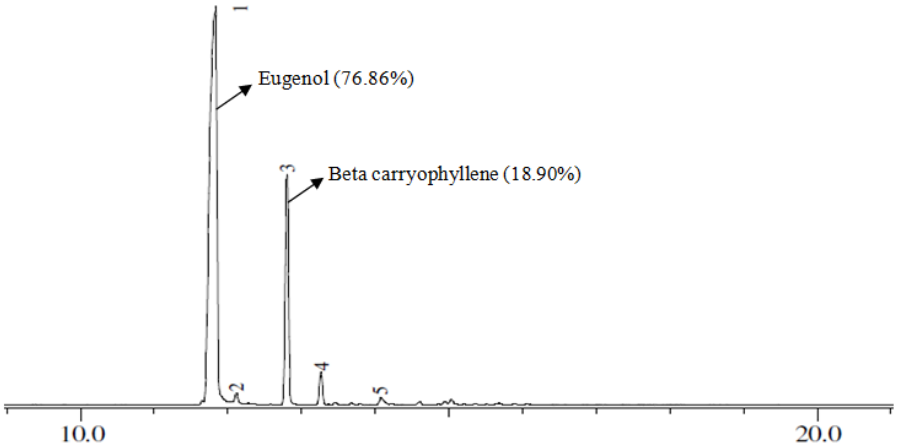


Figure 3. Chromatogram from analysis of initial cloves leaf oil

The clove leaf oil chromatograms are 5 peaks. The first peak with a retention time (tR) = 11.832 minutes and yield 76.86% is the peak of eugenol which is the main component of cloves leaf oil. The second peak with a retention time (tR) = 12.112 minutes, and yield of 1.03% is the peak of the alpha copaene. The third peak with a retention time (tR) = 12.805 minutes, and yield of 18.9% is the peak of the beta carryophyllene. The fourth peak with a retention time (tR) = 13.262 minutes, and yield of 2.53% is the peak of alpha humulene. The fifth peak with a retention time (tR) = 14.081 minutes, and yield of 0.67% is the peak of the delta cadinene. Standard quality for clove leaf oil requirements minimum content of compound eugenol was 78%. Eugenol contain in clove leaf oil from steam distillation is 76.86%, so it is below standard trade. The content of eugenol in clove leaf oil is influenced by various factors such as soil type, distillation time, type of plant, distillation equipment. Because the content of eugenol below the standards it needs to be improved with purification and then change to derivative compound eugenol which methyl eugenol.

3.2 Isolation product of eugenol from clove leaf oil

Fig. 4 show the chromatogram essential oil result from synthesis step 1. The GC-MS result at Fig. 4 shows 5 peak of the chromatogram peak with the retention time of 11.832 to 14.081 min. Synthesis product contains one mayor component is eugenol with the retention time and concentration are 11.832 min and 98.43%, respectively. The impurity components in synthesis product are dominated by beta-caryophyllene (1.13%), delta cadinene (0.20%) and alpha copaene (0.11%).

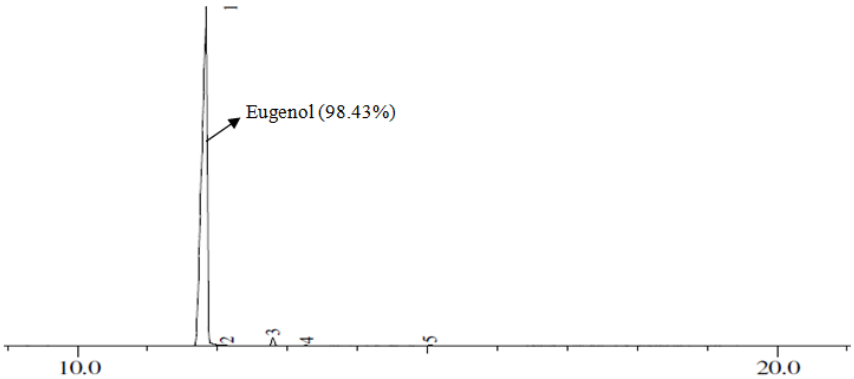


Figure 4. Chromatogram the essential oil analysis result from synthesis step 1

The eugenol and derivatives compounds with high purity that have a use in various industries, such as pharmaceuticals, cosmetics, food and beverages, cigarettes, pesticides, fishery, mining, active packaging and other chemical industries. In the clove oil quality requirements minimum content of compound eugenol was 78% [17]**.** Eugenol contain in clove leaf oil from steam distillation (step 1) is 76.86%. The results of the study showed clove leaf oil can improved eugenol contain form 76.86% to 98.43% (Fig. 4). Clove oil prices in the world market is US $ 4.75/kg, eugenol price of US $ 7.80/kg, the price isoeugenol US $ 10.80/kg. Eugenol is one of these key phenyl propenes. The natural product is a major aromatic constituent (up to approximately 80% by weight) of the essential oil of clove. Eugenol is commercially available in large quantities with a market price around US$ 5 per kg.

3.3 Isolation product analysis of methyl eugenol from eugenol

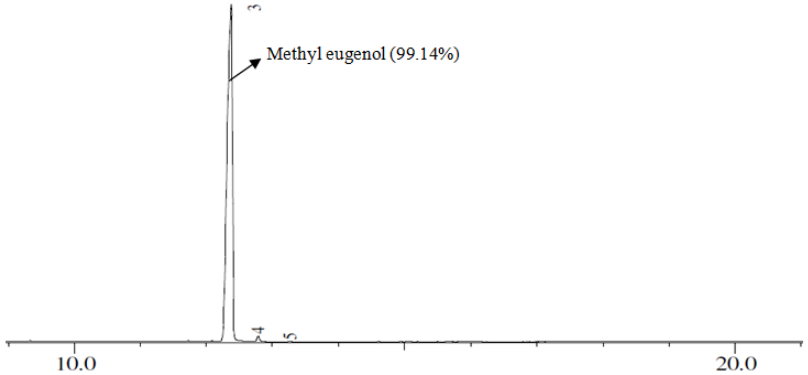


Figure 5. Chromatogram of the methyl eugenol result from synthesis step 2

Fig. 5 shows the chromatogram of the essential oil result from synthesis step 2. The first peak with a retention time (tR) = 12.374 minutes and yield 99.14% is the peak of methyl eugenol (1-allyl-3,4-dimethoxybenzene). The second peak with a retention time (tR) = 12.805 minutes, and yield of 0.68% is the peak of beta carryophyllene. The third peak with a retention time (tR) = 13.262 minutes, and yield of 0.08% is the peak of alpha humulene. Methyl eugenol or benzene, 1,2-dimethoxy-4-(2-propenyl) is commercially available with the following specifications purity of 98.0% min. Methyl eugenol from this research have purity 99.14%. Methyl eugenol with high purity used as a flavorings agent in jellies, baked goods, non-alcoholic beverages, chewing gum, candy, puddings, relishes and ice cream. Process improvement clove leaf oil to eugenol and final product to methyl eugenol have been done with successfully.

The complete results of analysis are presented in Table 1 including clove leaf oil, eugenol and methyl eugenol content. Table 1 show the methyl eugenol product has purity 99.14% and very low impurity. Methyl eugenol is a permitted food additive, provided that it is used in the minimum quantity required to produce its intended effect, and otherwise in accordance with all the principles of good manufacturing practice.

Table 1. Major compound result from synthesis

No	Compound	Retention time (Rt)	Initial Clove leaf oil (%)	Step 1 Eugenol isolation (%)	Step2 Methyl eugenol synthesis (%)
1	Eugenol	11.832	76.86	98.43	-
2	Alpha copaene	12.112	1.03	0.11	-
3	Methyl eugenol	12.374	-	-	99.14
4	Beta carryophyllene	12.805	18.90	1.13	0.68
5	Alpha humulene	13.262	2.53	0.13	0.08
6	Delta cadinene	14.081	0.67	0.20	-

3.4 Analysis of isolation product of methyl eugenol using FTIR

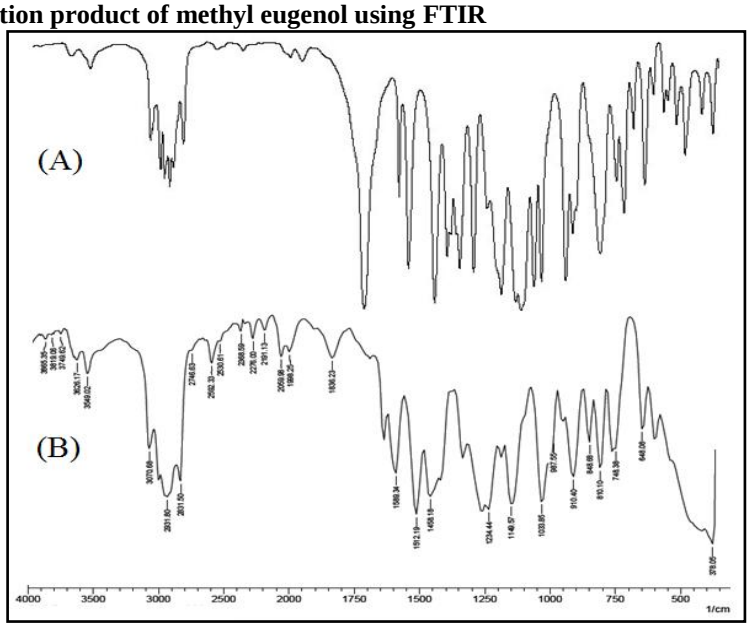


Figure 6. Comparison of FTIR Spectra of (A) cloves leaf oil and (B) synthesis product (methyl eugenol)

Fig. 6 shown the comparison of FTIR spectra of (A) cloves leaf oil and (B) synthesis product (methyl eugenol). Fig. 6 shown the spectra of infrared (IR) absorption above looks at 3070.68 cm^{-1} indicate the presence of an alkene group (sp^2) which is the uptake in the presence of an aromatic group that is characteristic peaks at 1512.19 cm^{-1} and 1589.34 cm^{-1}. Absorption at 810.10 cm^{-1} indicates the presence of the aromatic group substitution. Alkyl CH stretch (sp^3) looks at 2931.80 and 2831.50 cm^{-1}. Absorption at 1458.18 cm^{-1} show presence of methyl groups (CH_3). Based on FTIR spectral data in accordance with the above groups are present in the structure of methyl eugenol (1-allyl-3,4-dimethoxybenzene). The complete results of interpretation analysis of FTIR spectra are presented in Table 2. Fig. 7 show spectra FTIR methyl eugenol from National Institute Standard and Technology (NIST) [17]. Based on spectra FTIR methyl eugenol from National Institute Standard and Technology (NIST), Standard Reference Data Program, methyl eugenol have specific wave number are 3070, 2931, 2831, 1512, 1589, 1458 and 1033 cm^{-1}.

Table 2. Specific of functional group of the methyl eugenol analysis using FTIR (Fig 6B)

No.	Wave number from this research (cm^{-1})	Wave number form reference (cm^{-1})	Specific of functional group
1	3070.68	>3000	Alkenes (sp^2)
2	2931.80 and 2831.50	<3000	Alkanes (sp^3)
3	1640 – 1680	1650	Aliphatic alkenes
4	1512.19 and 1589.34 810.10 (1 peak)	1500 and/or 1600 800 (1 peak)	Substituted aromatic
5	1350	1370	Methyl (CH_3)
6	1458.18	1450	Methylen (CH_2)

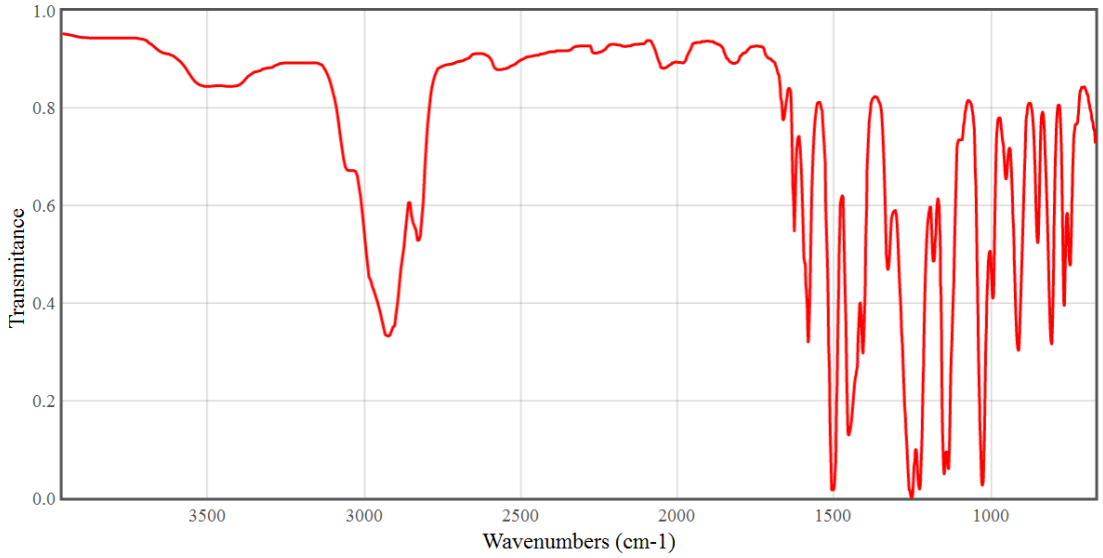


Figure 7. Spectra FTIR methyl eugenol standard from National Institute Standard and Technology (NIST) [17]

3.5 Mechanism reaction of isolation of methyl eugenol from cloves leaf oil

Fig. 8A showed mechanism of isolation of eugenol from cloves leaf oil and Fig. 8B showed mechanism of synthesis of methyl eugenol from eugenol. Synthesis of methyl eugenol from eugenol has been done using basic ingredients isolated eugenol clove oil. Eugenol is reacted with sodium hydroxide to form sodium eugenolate. Methylation reaction has been done using the addition of alkylating agent is dimethyl sulfate (DMS) and then refluxed with stirring frequently. The addition has been done using of DMS with drop wise, because the reaction is exothermic and the reaction is complete.

The result of reflux is formed two layers, the top layer is the organic layer and the bottom layer is a layer of water. The organic layer was separated from solution. The water layer extracted with n-hexane to take the remainder of methyl eugenol. Furthermore, the top layer or the organic layers were combined with 5 mL of 10% NaOH solution to take unreacted eugenol. This solution must be washing with distillate water until neutral. The result is dried with anhydrous Na_2SO_4 residual water with anhydrous Na_2SO_4 and n-hexane are separated by the evaporator. Analysis of the levels of methyl eugenol synthesized by GC-MS was obtained at 99.14%.

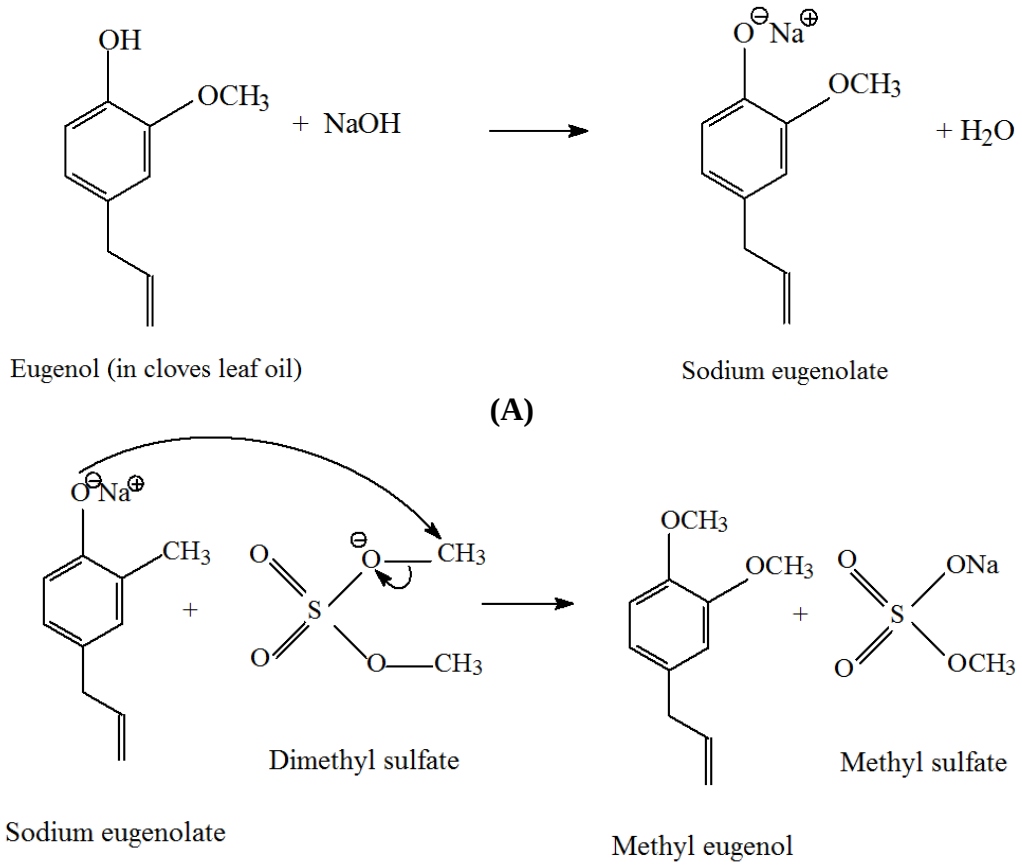


Figure 8. Mechanism of isolation of eugenol from cloves leaf oil, step 1 (A) and synthesis of methyl eugenol from eugenol, step 2 (B)

IV. Conclusions

The isolation of methyl eugenol from cloves leaf oil using acid and based chemicals reactions have been done. Cloves leaf oil was isolation from cloves leaf using water steam distillation (Step 1). Eugenol was synthesis from cloves leaf oil using sodium hydroxide (NaOH 5%) and sulphuric acid (H_2SO_4) 5% at pH 3 (Step 2). Methyl eugenol was synthesis from eugenol from step 2 using dimethyl sulphate (Step 3). The result is dried with anhydrous Na_2SO_4 and n-hexane is separated by the rotary evaporator. The result of the synthesis was analysis using GC-MS and Fourier Transform Infrared (FTIR). The results of the study showed that the methyl eugenol yield is 99.14%. As a conclusion, the method is extremely simple and inexpensive.

Acknowledgment

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