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CHARACTERIZATION OF CLOVE OIL WITH A FT-IR ATR SPECTROSCOPIC METHOD

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ABSTRACT

Clove oil possesses excellent antioxidant and antimicrobial properties, while the main components of clove oil are eugenol, acetyleugenol and caryophyllene. Two types (ground and buds) of clove were used in the analysis. The clove essential oil was obtained using a steam distillation and an ultrasonic assisted extraction method. The separation of eugenol from other components was achieved with benzoyl chloride reaction. The analysis of clove oil was with a FT-IR ATR spectroscopic method along with the use of a TLC method using mobile phases, toluene:ethyl acetate (9.3:0.7 *v*/*v*), phase A and chloroform:hexane (1:2 *v*/*v*), phase B. The UV-Vis spectrum of eugenol was with maximum at 280 nm. The obtained yield was found to be 8.79% and 12.41% for ground clove and 1.36% and 6.75% for clove buds using ultrasonic assisted extraction method, respectively. The characteristic band for the ester group C-O of eugenol acetate was observed at 1765 cm^{-1}. The R_f value for eugenol was 0.66 with phase A, and 0.46 using phase B. The methods were easy to be handled, rapid and can be used in the routine analysis.

Key words: Eugenol, TLC method, Steam distillation, Ultrasonic assisted extraction.

INTRODUCTION

The volatile and essential oils contribute to the characteristic aroma of plants (Khalil et al., 2017). Terpenes as a broad group of plant products influence to the aroma along with hydrocarbons, alcohols, and carbonyl compounds. The oil can be concentrated in any part of the plant such as twigs, flowers, and seeds (Tekin et al., 2015, Cortés-Rojas et al., 2014). The essential oils were known since ancient time where the application was in perfumes, spices and antiseptic substances. A blend of several substances play role in the full flavor and bouquet of a spice or flavoring (Kapadiya et al., 2018). Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants (Bell et al., 2001, Jirovetz et al., 2006).

The isolation of essential oil from clove either from leaf or bud is performed using a steam distillation or hydrodistillation (Cassel et al., 2009; Vanin et al., 2014). The most of the components which are in the essential oil are volatile. Assisted extraction can be done using microwave (Rojas et al., 2021), spectrophotometric (Sanghai et al., 2011, Saran et al., 2013) and chromatographic methods (Ibrahim et al., 2015; Móricza et al., 2016; Yun et al., 2010) have been applied to determine eugenol in clove extracts (Rodríguez et al., 2014, Yugatama et al., 2017).

The clove belongs to the *Myrtacea* family where the essential oil is made from light and liquid, warm spicy aroma. As evergreen tree, native from Indonesia, the clove grows up to 12

m tall, while the cultivated one is 5 m tall. The essential oil is found in flowers (15–18%), stem (4–6%), and leaves (2–3%) (Gülçin et al., 2012).

The beneficial usage of clove oil is because of the advantages in antimicrobial activity against gram positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Lactobacillus* sp., *Actinomyces* sp., *Streptococcus* sp.), gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Aeromonas* sp., *Pseudomonas* sp., *Candida albicans*, *Aspergillus* sp. in *Penicillium* sp.) (Kumar et al., 2010; Park et al, 2011, Xie et al., 2015). Oil of clove is aromas and safety, as well as an alternative for food preservatives. It shows also antioxidant, anticarcinogenic and antiviral properties. The broad usage of the clove oil is in various fields and industries such as medicine, dentistry, cosmetology, perfumery, and tobacco industry (Sanghai et al., 2011, Vanin et al., 2014).

The clove oil is reach in 4-allyl-2-methohyphenol (eugenol), while the amount of other compounds such as sesquiterpene caryophyllene (Figure 1) and eugenol acetate is low (Yugatama et al., 2017). The main components in the flower clove are eugenol (70–95%), acetyleugenol (2–27%), caryophyllene (5–12%), methyl salicylate, α-humulene, methyl eugenol, benzaldehyde, and furfural (Bell et al., 2001, Cortés-Rojas et al., 2014). The amount of eugenol in the stem is present from 90% to 95%, while in the leaves between 82% and 88%. Eugenol benzoate can be converted from eugenol presented in Figure 1. Eugenol, the main bioactive component of clove oil has been accepted as a food preservative by many countries (Ibrahim et al., 2015). On the other hand, many oral care products contain eugenol showing antimicrobial and antiallergic properties (Ibrahim et al., 2015, Jirovetz et al., 2006, Kumar et al., 2010, Park et al., 2011). Although eugenol as a substance is promising agent in many fields, it can be considered also as potential toxic substance (Bell et al., 2001, Kapadiya et al., 2018, Saran et al., 2013).

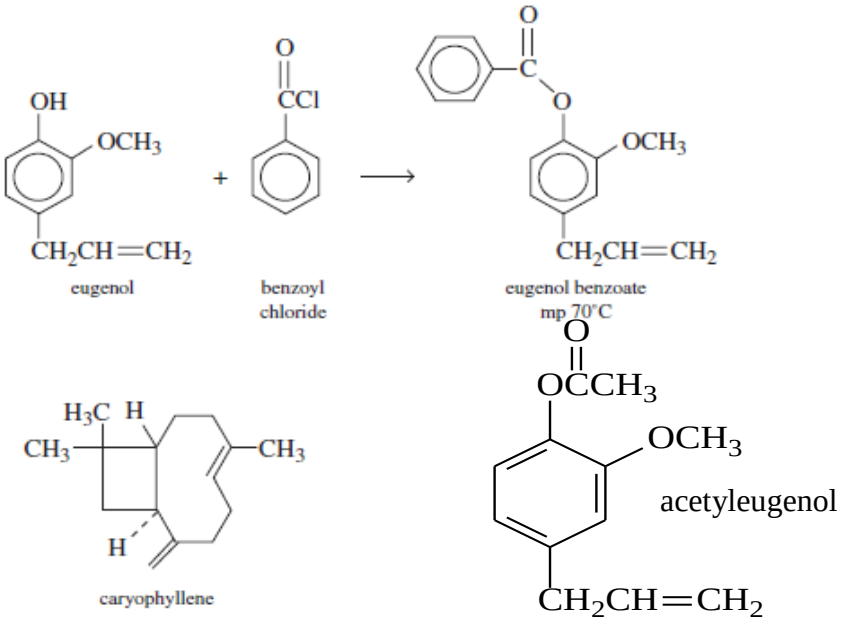


Figure 1. Derivatization of eugenol and main components of clove oil

The main goal of this work is to make a comparison of the obtained essential clove oil from buds and ground clove using steam distillation and ultrasound extraction method. The FT-IR spectroscopic method, UV-Vis spectrophotometric method and Thin-Layer Chromatography (TLC) method were used to analyze clove oil. In order to isolate the main component, eugenol, benzoyl chloride was used in the reaction.

MATERIALS AND METHODS

Two types of clove commercially available were used in the analysis (in form of buds and ground clove). All reagents: hydrochloric acid, chloroform, sodium sulfate, sodium hydroxide, diethyl ether, benzoyl chloride, toluene, potassium permanganate, iron(III) chloride, heksane, and ethyl acetate were with analytical purity (Merck, Germany). Analytical balance (VWR, Austria) with 0.1 mg accuracy was used in the analysis.

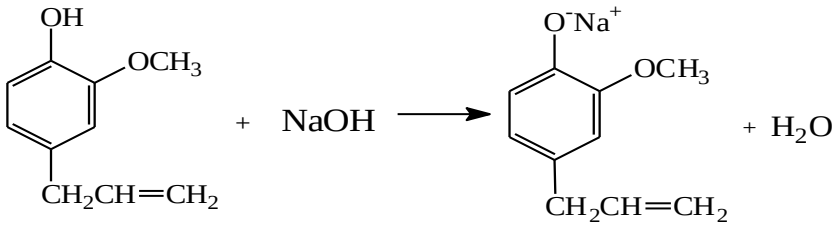
Steam distillation of essential oil from clove. Freshly ground spice of clove and in a form of twigs (10 g) was placed in a 500 mL round-bottom flask and 150 mL water was added. Occasionally water was added to maintain the original level. The distillate was collected until no further drops of oil can be seen (100 mL of distillate). The distillate was poured into a separatory funnel, and chloroform was used for the extraction process. The aqueous layer was extracted three times with 5 mL of chloroform followed by draining the organic layer into a vessel. A small amount of sodium sulfate was added into the organic layer and transferred the solution in a funnel into another tared flask. The evaporation of chloroform was done on the steam bath until the solution has been concentrated to an oily residue. The percent yield based on the weight of plant material was calculated after determination of the oil mass.

Ultrasonic extraction. The ultrasonic method was used as an assisted extraction method using an ultrasonic bath with 40 Hz at 60 °C for 1 h. The amount of clove in both forms which were used in the analysis was quantitatively transferred into Erlenmayer flask with 100 mL 50% ethanol. The procedure was followed by steps as were described in steam distillation process.

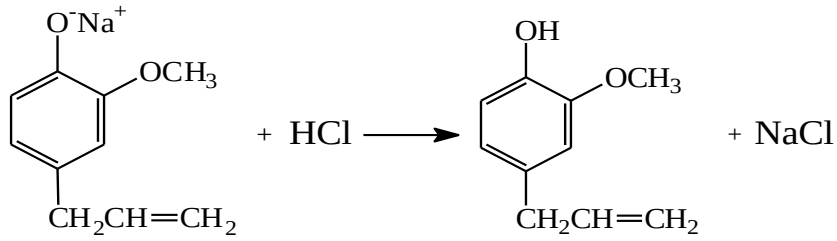
Determination of physic-chemical properties. The tested parameters such as colour, refraction index, reaction with 1% $KMnO_4$ and with 1% $FeCl_3$ were compared to the literature (Bell et al., 2001).

Thin-Layer Chromatography (TLC). A TLC analysis was performed plates (254 nm) visualized under UV lamp (VWR, Austria). For chromatographic determination, 2.5 µL of sample solutions were spotted on 20 x 20 cm Merck (Germany) pre-coated TLC plates (60 F254, 250 µm). The mobile phase was consisted of toluene:ethyl acetate (9.3:0.7 *v*/*v*), phase A, and chloroform:hexane (1:2 *v*/*v*), phase B.

Preparation of eugenol benzoate. To the obtained oil, 1 mL of water was added, followed by several drops of 1M NaOH solution until the oily layer dissolves. The clear solution will not result, but there should be no oily droplets (phenolic eugenol, ArOH is converted into the water-soluble ArO^-). The small amounts of other substances such as acetyl eugenol and neutral components will tend to interfere with crystallization of the derivative. In order to remove these components, 3 mL of diethyl ether was added in the vessel and after shaking, the organic layer was removed. The procedure was repeated three times. To the aqueous eugenol solution, 4–5 drops of benzoyl chloride were added. The mixture was gently warmed on a steam bath for approximately 5 minutes. After cooling, 3 mL of diethyl ether was added and the vessel was shaken. The lower aqueous layer from the tube was removed with a transfer pipette and bulb. A small amount of anhydrous sodium sulfate was added to dry the ether solution. After the transfer to another test tube, 1 mL methanol was added followed by evaporationg the solution where crystals of ester were obtained. The chemical reactions are given in Figure 2.



Eugenol (soluble in diethyl ether) Sodium eugenolate (soluble in water)



Sodium eugenolate Eugenol

Figure 2. Isolation of eugenol from clove oil

FT-IR Spectroscopy. FT-IR Spectroscopic specific parameters were within running on Shimadzu FT-IR Spirit-L type (Japan) with ZnSe, 20 scans, Range 400-4000 cm^{-1}, Apodization Happ-Genzel, Measurement mode %Transmitance, Attenuated Total Reflection (ATR) mode Single Reflection ATR Accessory (QATR-S).

UV-Vis spectrophotometry. The UV-Vis spectrophotometer *Varian Cary Scan 50* (Switzerland) with 10 mm quartz cuvette was used in the analysis at 25 °C. A portion of 3 μL clove oil was transferred to a 100 mL volumetric flask and water was added to the mark. A few drops (2–3) of 1M NaOH were added to the cuvette and spectrum was recorded. In new portion of clove solution 2–3 drops of "HCl" were added and the spectrum was recorded. The blank sample was distilled water.

RESULTS AND DISCUSSION

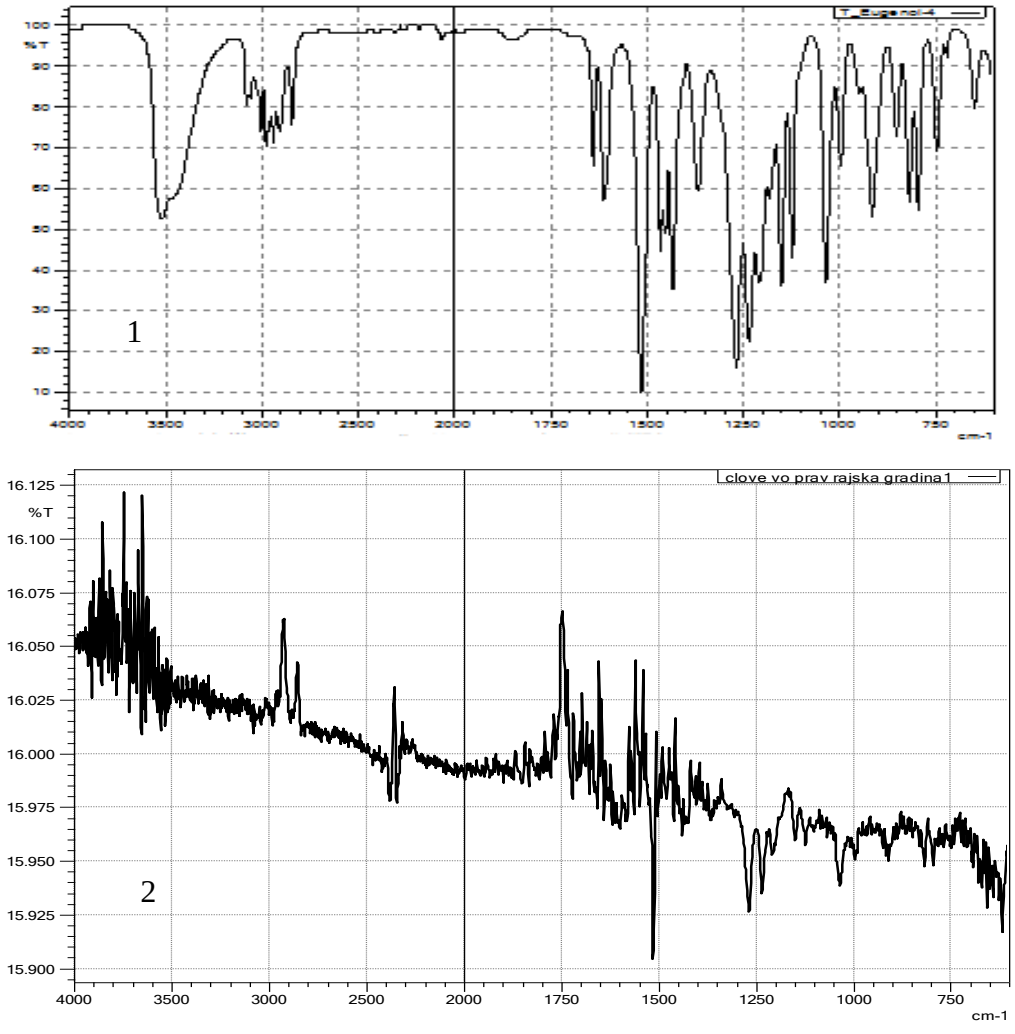
The determined values for obtained yield from clove ground form were 8.79% and 12.41% using ultrasonic and steam distillation method, respectively. The determined values of 1.36% and 6.75% for clove buds were using ultrasonic assisted extraction method, respectively. The extraction yield is increased by reducing the particle size of clove buds powder (Khalil et al., 2017). In the comparison to the literature, the yield of the isolated eugenol was 6% with hydrodistillation method, while water volume and extraction time contribute to the % of the obtained clove oil in the range from 4% to 8% (Bell et al., 2001). More bioactive compounds can be separated effectively using ultrasound-assisted method (Tekin et al., 2015).

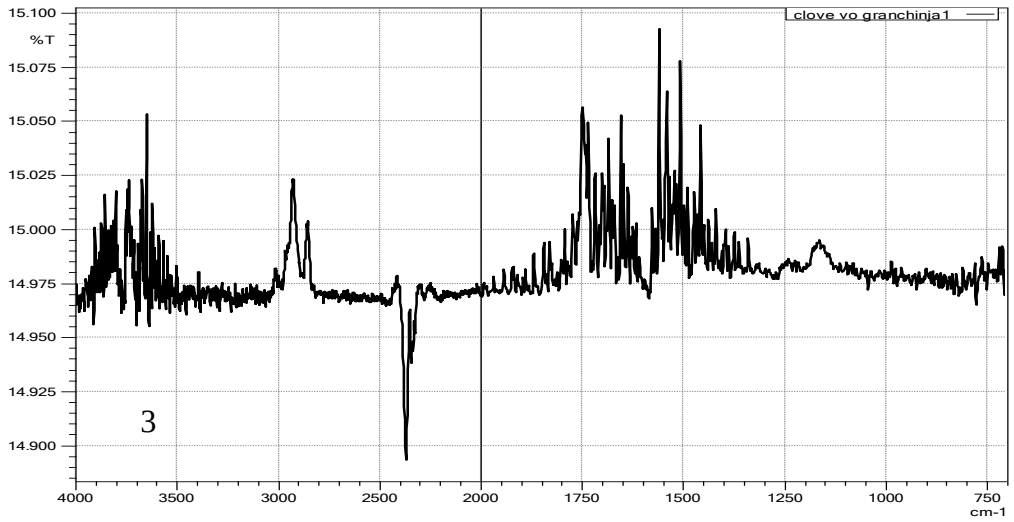
Determination of physic-chemical properties. The colour of the clove essential oil was yellow. The reaction with 1% $FeCl_3$ was positive with blue colour solution, while the sediment colour of the positive reaction with $KMnO_4$ was brown. The refraction index was 1.54 at 23 °C.

Thin-Layer Chromatography (TLC). It was found that the Retention factor (R_f) for eugenol was 0.66 with using mobile phase toluene:ethyl acetate (9.3:0.7 *v*/*v*) and 0.46 using mobile phase chloroform:heksane (1:2 *v*/*v*).

FT-IR Spectroscopy. The observed main peaks of clove essential oil observed in Figure 3 are due to the main component in the clove oil, eugenol. Bands at 3432, 3071, and 2920 cm^{-}

[1] ascribed to O-H, =C-H, and C-H stretching, respectively. The peaks at 1637 and 1606 cm^{-1} originated from C=C stretching vibration of the aromatic part, whereas the peak at 1269 cm^{-1} specified to C-O stretching. The CH_2 deforming vibration, CH out-of-plane, and ring deformation are observed at 1431, 990, and 804 cm^{-1}, respectively. The stretching vibration absorption bands of the allyl group are located at 1637 and 995 cm^{-1}. The ester group C-O of eugenol acetate is observed at 1765 cm^{-1}. The peak at 1027–1032 cm^{-1} is ascribed to CH-in-phase wag. The asymmetric C-O-C stretching band of eugenol is observed between 1100 and 1210 cm^{-1}. The peaks at 1637 and 1606 cm^{-1} originated from C=C stretching vibration of the aromatic part of the molecules, whereas the peak at 1269 cm^{-1} specified to C-O stretching. The spectral signal obtained at the frequencies of 1431, 990, and 804 cm^{-1} can be attributed to the presence of CH_2 deformation vibration, CH out-of-plane, and ring deformation, respectively. The stretching vibration absorption bands of the allyl group are located at 1637 and 995 cm^{-1}. The ester group C-O of eugenol acetate is assigned with band at 1765 cm^{-1}. The peak at 1027–1032 cm^{-1} is ascribed to CH-in-phase wag. The band between 1100 and 1210 cm^{-1} is assigned to the asymmetric C-O-C stretching of eugenol.

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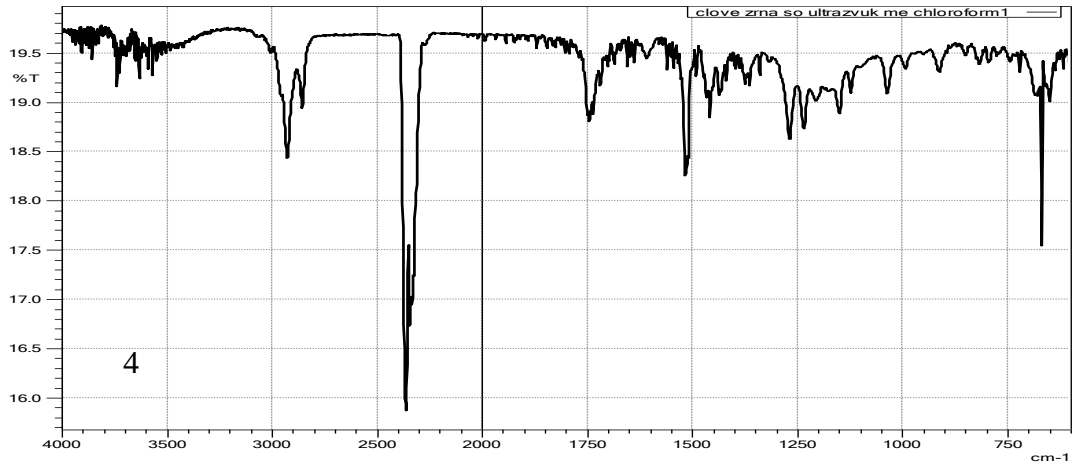
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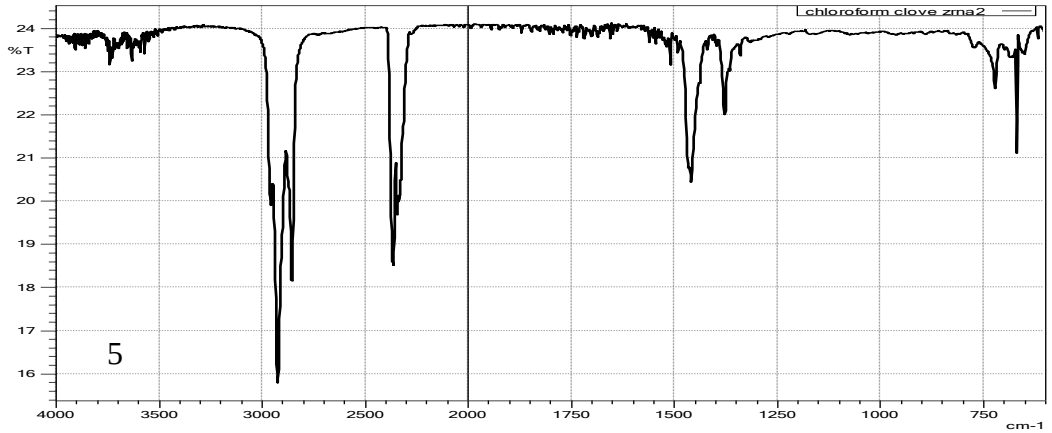
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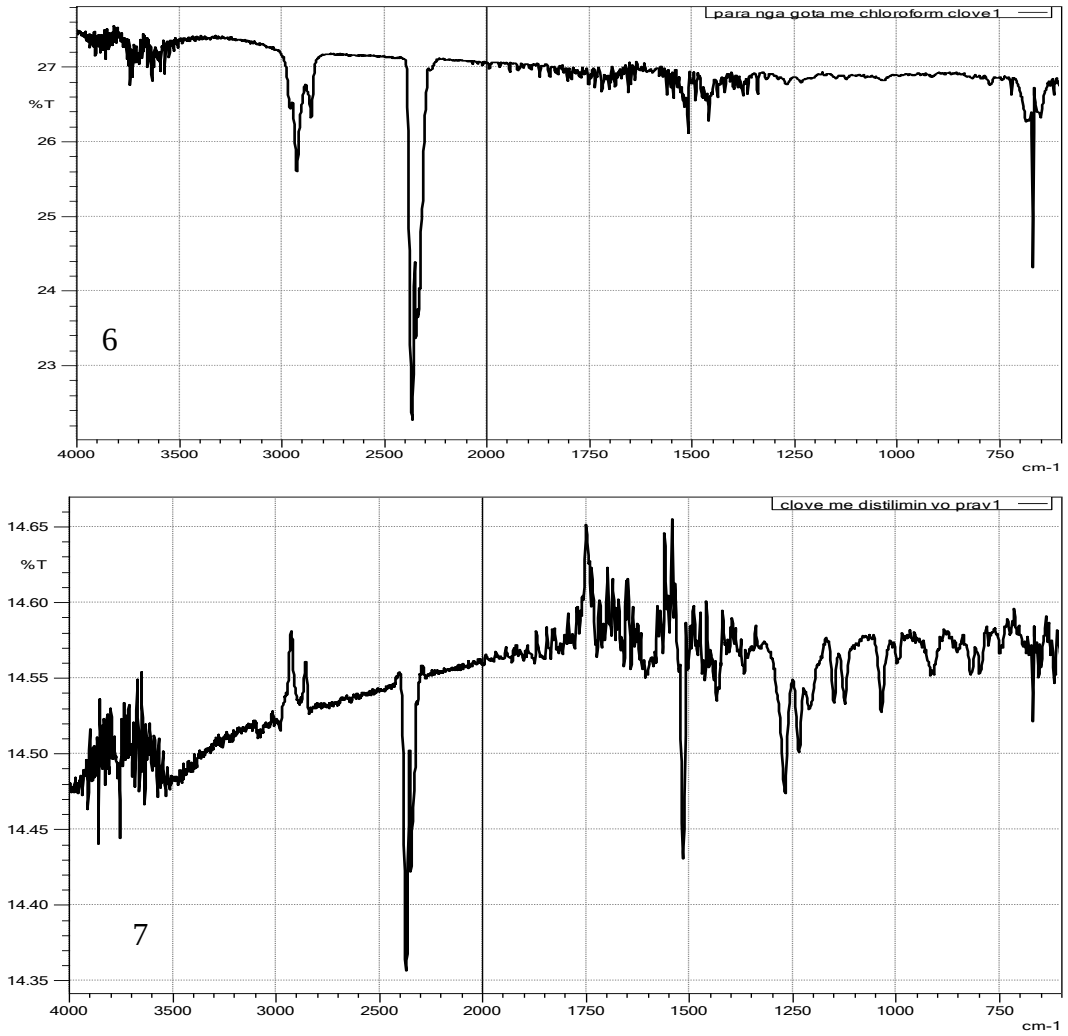

Figure 3. IR spectra: eugenol (1), ground clove (2), clove buds (3), essential oil obtained from clove buds using the ultrasonic assisted extraction method (4), essential oil obtained from clove buds using the steam distillation (5), essential oil obtained from ground clove using the ultrasonic assisted extraction method (6), and essential oil obtained from ground clove using the steam distillation (7)

UV-Vis spectrophotometry. A maximum peak was recorded at 280 nm. After addition of few drops of 1M NaOH in the spectrum one peak was recorded at 244.7 nm and the peak which was at 280 nm was shifted at 295.8 nm. This is a result for the OH^- group from eugenol which in alkaline medium passed in phenolate. In acidic medium, the peak is at 280 nm, but the peak is smaller due to the dilution (Figure 4).

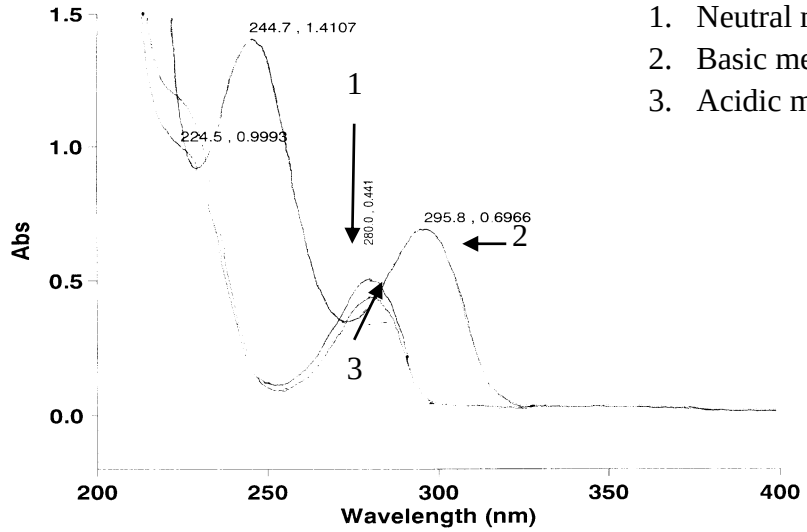

Figure 4. UV-Vis spectrum of clove oil

CONCLUSIONS

The obtained clove essential oil from clove buds and ground clove was obtained using two methods, the steam distillation method and the ultrasonic assisted extraction method. The oil was analyzed in terms of its characteristics, physical properties, as well with chemical reactions obtaining characteristic colour. The spectroscopic methods, FT-IR with ATR and UV-Vis spectrometry were used in order to determine the characteristic compounds which are consisted in the clove essential oil. Moreover, the main component, eugenol was derivatized with benzoyl chloride in order to be isolated from other components in the clove essential oil. The results were satisfactory with easy, rapid and methods easy to be handled.

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