Progress in Engineering Application and Technology Vol. 3 No. 3 (2022) 118–128

PEAT

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/peat
e-ISSN : 2773-5303

Comparison between Eugenol in Essential Oil from Cinnamon Leaves *(Cinnamomum verum)* and Clove *(Eugenia caryophyllata)* Extracted by Soxhlet Extraction

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DOI: https://doi.org/10.30880/peat.2022.03.02.011
Received 27 January 2022; Accepted 20 July 2022; Available online 10 December 2022

Abstract: This study is conducted to produce essential oil from spices that can be inexpensive as spices are cheap and available at all seasons. The essential oils from clove *(Eugenia caryophyllata)* and cinnamon leaves *(Cinnamomum verum)* contain secondary metabolites that are volatile and widely used in various industry. In this study, it was aimed to extract the essential oil from clove and cinnamon leaves, as well as conducting analytical assay such as thin layer chromatography and Fourier transform infrared spectroscopy to determine the presence of the constituent and to characterize eugenol respectively. Extraction of essential oil was extracted using Soxhlet extraction method using ethanol 95.00 % for 3 and 5 hours from clove bud and cinnamon leaves and removal of solvent was done with rotary evaporator at 40 ℃. Extraction of essential oils from clove *(Eugenia caryophyllata)* for 3 hours extraction and 5 hours extraction have the percentage of yield of 0.266 % and 0.363 % respectively. Extraction of essential oil from cinnamon *leaves (Cinnamomum verum)* for 3 hours extraction and for 5 hours extraction has the percentage of yield of 0.0938 % and 0.247 % respectively. The essential oil extracted from clove for 5 hours has the highest yield. The presence of constituent has been identified by thin layer chromatography analysis that was conducted by using a solvent of petroleum ether, toluene, and ethyl acetate with a ratio of 7:2:1. In the analysis of Fourier transform infrared spectroscopy, the functional group of eugenol has been identified in all the samples. By comparing the transmittance percentage, it can be concluded that the clove extract extracted for 5 hours have the higher concentration of functional group of eugenol. In conclusion, the research has helped to establish that using clove oil as essential oil rather than the use of fragrance oil is far more cost-effective and beneficial.

Keywords: Essential Oil, Eugenol, FTIR, TLC

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1. Introduction

Essential oils (EOs) are made up of two type of chemical constituents which are hydrocarbons and their oxygenated derivatives arising from two different isoprenoid pathways [1]. Hydrocarbons is usually made up of terpenes that can be classified into three which are monoterpenes, sesquiterpenes and diterpenes. The oxygenated compounds are esters, aldehydes, ketones, ethers, alcohols, phenols, and oxides. Usually, there are two to three major constituents in essential oil which have high concentration that has the range of 20-95.00 % and other low concentration constituents. The components are 75 to 100 times more concentrated than the oils in dried herbs [2].

Each aromatic plants have unique chemical properties which affect the odor that is produced and is very important to produce essential oil. Other than for food purposes, spices also sometimes used in the production of perfume, cosmetics, religious rituals, and medicine. A majority of the essential oil are stored in the whole form of spices, where it has to be extracted using methods such as Soxhlet extraction, hydrodistillation or cold pressing. Essential oils in spices are widely being used for many applications such as for medicinal purpose, aromatherapy, food preservatives, cosmetics, and perfume industries, and as pesticides. This is due to the presence of a diverse group of phytochemicals in essential oil of spices that act as an antibacterial and antioxidant agent [3].

Essential oils from cinnamon are usually extracted from the bark and leaf. Based on much research done on essential oil from cinnamon leaf, it has been found that there are more than 300 components in the volatile oil, and it has noticeable amount of antioxidant activities [4]. The major constituent in cinnamon leaf, *Cinnamomum verum*, is eugenol which is about 70.00 % to 95.00 % (Rao et al., 2014). Meanwhile, the essential oil that has been produced and extracted from clove bud is being used widely as the availability of the clove bud is high and it also has more properties that benefits humankind compared to the essential oil from the leaf. The percentage of eugenol in the essential oil is around 45-90.00 % which makes eugenol the major constituents of the clove bud oil.

Eugenol, 4-allyl-2-methoxyphenol, is a component of phenol and has a class of phenylpropanoids, and major component in both cinnamon leaves, *Cinnamomum verum*, and cloves, *Eugenia caryophyllata*. Eugenol is also can be defined as an allyl chain substituted guaiacol. Eugenol is used for various of applications due to its biological activities such as antimicrobial, anti-inflammatory, antioxidant, cytotoxic, insect repellent and anesthetic properties. The spices that have been chosen for this study are *Cinnamomum verum* and *Eugenia caryophyllata* because these two spices contain high percentage of eugenol content and the availability of the spices. Thus, it will be easier for this study to conduct the comparison of eugenol extracted from both spices, which are cinnamon leaf and clove.

2. Materials and Methods

The materials and methods section. The essential oil was extracted from clove *(Eugenia caryophyllata)* and cinnamon leaves *(Cinnamomum verum)* using Soxhlet extraction method. The percentage of yield was identified. The essential oil extracted from clove and cinnamon leaves was analyzed to determine the presence of constituents and for its characterization. TLC and FTIR was used for the analysis in order to compare the eugenol content in the essential oil.

2.1 Materials

The raw material used in this study is clove *(Eugenia caryophyllata)* and cinnamon leaves *(Cinnamomum verum).* Absolute ethanol, ethanol 955, silica gel aluminium plate, petroleum ether, toluene, ethyl acetate, acetone and eugenol were the chemicals used in the study. The apparatus and equipment that was used is grinder, drying oven (Memmert), Soxhlet extraction, rotary evaporator (Buchi), weighing balance (Mettler Toledo), Refrigeration (Protech), Cary 600 Fourier transform infrared spectroscopy (Agilent Technologies) and thin layer chromatography (Camag).

2.2 Preparation of raw material

Cinnamon leaves *(Cinnamomum verum)* and clove buds *(Eugenia caryophyllata)* was collected. The cinnamon leaves and clove buds were let to dry in the drying oven for 48 hours at 40 ℃ for moisture content removal before griding process. Then, the dried raw material, clove bud and cinnamon leaves were grinded into fine powder to increase the surface are during extraction. After the grinding process, the powdered raw material was kept in a tight container

2.3 Preparation of stock solution

The stock solution was prepared by using absolute ethanol as a solvent and pure eugenol. The ratio of the pure eugenol oil and the absolute ethanol is 1:10. First, 9 ml of absolute ethanol is measured in a measuring cylinder and 1 ml of eugenol was then added in the measuring cylinder. The solution was then mixed up with the dropper before pouring it into 50 ml volumetric flask. Then, the solution was then mixed well by shaking the flask upside down slowly. The stock solution of eugenol was prepared.

2.4 Extraction of essential oil by Soxhlet extraction

To extract the essential oil, 25 g of each spice was prepared in 3 teabags. Then, 250 ml of 95.00 % ethanol was filled in 250 ml round bottom flask and placed on the heating mantle. After the flow of water has started in condenser, the extraction chamber was set up. The extraction was started after the 95.00 % ethanol starts to boil and the Soxhlet extraction was operated for 3 hours and 5 hours for each spice. The sample has been collected in the round bottom flask. After 3 hours and 5 hours of extraction, the samples were let to cool down for 30 minutes before removing it from the heating mantle. After collecting the extracted sample, the evaporation of solvent by using rotary evaporator was done. The temperature of the water bath was set up at 40 ℃. The evaporation of the solvents from the sample was done under vacuum condition and operated for 30 minutes. The solvent was then collected in the 500 ml round bottom flask and the sample in the 250 ml round bottom flask. The sample was transferred into 10 ml amber glass bottles with correct labelling and kept in it the refrigerator at 4 ℃.

2.5 Thin layer chromatography (TLC) analysis

The TLC analysis was performed with a 10×10 cm CAMAG® twin through chamber. The TLC plate was prepared by cutting it in a measurement of 10×10 cm. Then, 10 ml of solvent has been prepared by using petroleum ether, toluene, and ethyl acetate with a ratio of 7:2:1. A line about 1 cm will be drawn at the bottom of the plate with a light pencil and trace another 5 cm line from the 1 cm line drawn before. A dropper was used to load the stock solution of eugenol and clove oil extracted for 3 hours onto the TLC plate. After the spot have been dried, the plate was placed in the developing chamber. Remove the plate and allow it to dry once the solvent reach the top line of the plate. The observation was then done under the CAMAG® UV light with 366 nm. The thin layer chromatography analysis was then continued with other samples.

2.6 Fourier transform infrared spectroscopy (FTIR) analysis

For this analytical assay, Cary 630 FTIR Spectrometer with a brand of Agilent Technologies was used for the data analysis of the functional group in the extraction from *Cinnamomum verum* and *Eugenia caryophyllata* which was extracted for 3 hours and 5 hours. The solvent used for this analysis is acetone. 2 ml of acetone is mixed with 1 ml of sample extracted and 1 drop of the mixture is placed at the detector until the graph is formed.

2.7 Equation

Using the Beer-Lambert Law equation as a reference, the concentration of chemical species present in the samples has been determined, as indicated in Eq. 1,

$$A = \varepsilon bC \qquad Eq.\ 1$$

Where A is the absorbance, ε id the molar absorptivity, which depends on the nature of the chemical and the wavelength, b is the length of light path traveled in the solution and C is the concentration of given solution.

3. Results and Discussion

Analysis for the extraction of both the raw materials was done. The extracted sample was analyzed by thin layer chromatography to ensure the presence of constituents with a solvent using petroleum ether, toluene, and ethyl acetate with the ratio of 7:2:1. Next, the extracted samples were being analyzed by using Fourier transform infrared spectroscopy in order to identify certain functional group of the constituents in the extracted samples by using acetone as a solvent.

3.1 Extraction of essential oils

The sample of clove buds and cinnamon leaves were extracted for 3 hours and 5 hours using ethanol 95.00 % as solvent yielding the essential oil for each plant. Soxhlet extraction method of the aromatic spices yielded certain amount of oil as shown in Table 1.

Table 1: Yield percentage of essential oil

Essential Oil (EO)	Colour	Yield (%)
Clove bud (3hrs) (*Eugenia caryophyllata*)	Brown	0.266
Clove bud (5hrs) (*Eugenia caryophyllata*)	Dark brown	0.363
Cinnamon leaves (3hrs) *(Cinnamomum verum)*	Green	0.0938
Cinnamon leaves (5hrs) (*Cinnamomum verum*)	Dark green	0.247

3.2 Thin layer chromatography analysis

The sample extracted from clove and cinnamon leaves were analyzed using TLC to detect the presence of chemical constituents in the oil. Petroleum ether, toluene, and ethyl acetate (7:2:1) was used as the mobile phase. The thin layer chromatography plate the was then observed under the long wavelength UV light of 366 nm. Figure 1 and Figure 2 shows the TLC fingerprints for samples extracted for 3 and 5 hours from cloves while Figure 3 and figure 4 shows for the samples extracted for 3 and 5 hours from cinnamon leaves. There were no specific spots identified on the TLC plate under the UV light to calculate the retention factor. This might be occurred because the sample might contain many components where it creates many spots which run together and appear as a streak. However, the presence of the natural compound can be seen in the Figure 1,2, 3 and 4 because there is a red and blue, fluorescent line that is visible on the TLC plate. Eugenol is one of the constituents as it absorbs UV light at wavelengths greater than 290 nm. The fingerprint of the eugenol in Figure 1 and 2 is more compared to eugenol fingerprint in Figure 3 and 4 which is just at the end of the plate. This can be caused by experimental error where the plate is not dried properly after the sample was put on the plate

and after thin layer chromatography analysis before viewing it under the UV light. When the sample is not dried, the activity of the silica can vary tremendously based on water content and can deteriorate the quality of the separation.

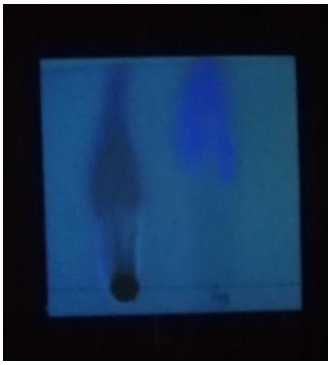

Figure 1: TLC analysis for 3 hours clove extract and eugenol

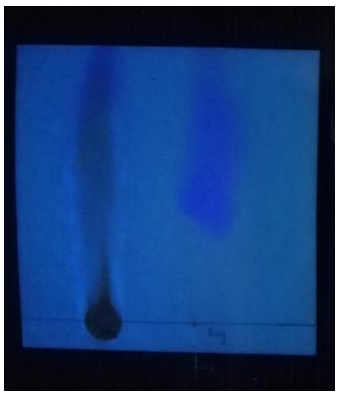

Figure 2: TLC analysis for 5 hours clove extract and eugenol

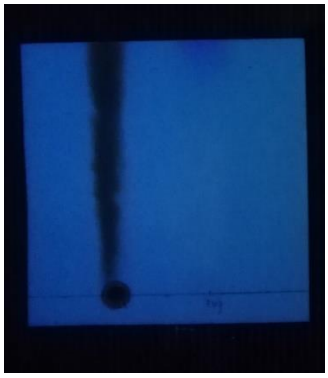

Figure 3: TLC analysis for 3 hours cinnamon leaves extract and eugenol

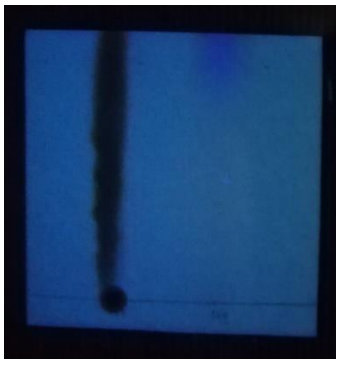

Figure 4: TLC analysis for 5 hours cinnamon leaves extract and eugenol

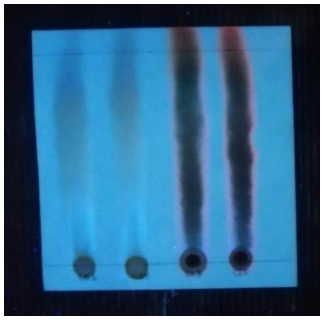

Figure 5: TLC analysis for 3 hours and 5 hours of clove and cinnamon leaves extract

3.3 Fourier transform infrared spectroscopy (FTIR) analysis

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical method used to determine the organic and inorganic materials. FTIR evaluate the absorption of the infrared radiation by the sample substantial versus wavelength. Molecular constituents and configurations are identified by infrared absorption bands. The FTIR was used to identify the functional groups of organic compounds that appear in clove buds and cinnamon leaves samples. The FTIR was tested on two different time of extraction of *Eugenia caryophyllata* and the obtained results from spectroscopy were indicated as Figure 6.

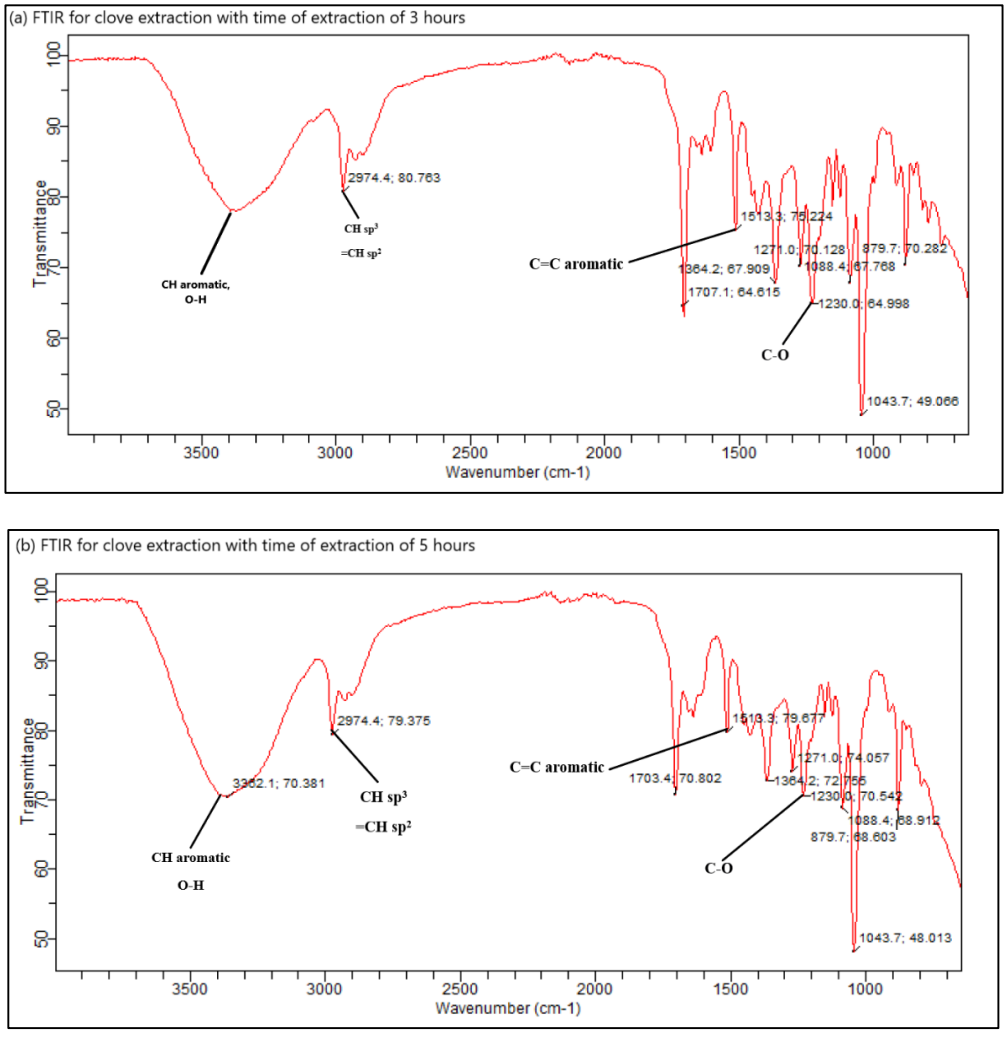

Figure 6: FTIR screening for Clove buds *(Eugenia caryophyllata)* extraction with time of extraction of (a) 3 hours (b) 5 hours

The results of FTIR screening for different time of extraction of clove buds is shown in Figure 6. The absorption bands in Figure 6 (a) and (b) is the same which were detected at the peak of 1230.0 cm^{-1}, 1271.0 cm^{-1}, 1513 cm^{-1}, 2974.4 cm^{-1}, and 3362.1 cm^{-1}. The FTIR band can divide into four sections which is single bond, triple bond, double bond, and fingerprint. The single bond region is from 2500 cm^{-1} and 4000 cm^{-1}, the triple bond region is from 2000 cm^{-1} to 2500 cm^{-1}, the double bond region is from 1500 cm^{-1} to 2000 cm^{-1} and the fingerprint region is from 500 cm^{-1} to 1500 cm^{-1} [6].

The FTIR analysis obtained for the clove bud extracted for both time of extraction, which is for 3 hours and 5 hours, showed a strong, broad absorption band at 3362.1 cm^{-1} indicating the presence of hydroxyl group and C-H aromatic group as it the range of the peak for hydroxyl group and C-H aromatic group is 3100 cm^{-1} to 3690 cm^{-1} and 3000 cm^{-1} to 3200 cm^{-1} respectively. In that range, there is only one peak is visible and this might be because of the intrinsic physical nature of the sample or due to the instrumental resolution that has been used which is 8 cm^{-1} and lead to noisier spectra [7]. Absorption band of 2974.4 cm^{-1} and the peaks that is visible in the range of 2800 cm^{-1} to 3300 cm^{-1} represents the presence of C-H alkene and C-H alkane. Peak at absorption band of 1513.3 cm^{-1} indicated the presence of C=C aromatic group. Absorption band observed at 1230 cm^{-1} and 1271 cm^{-1} detected the presence of C-O proving the presence of the carbon attached to the hydroxyl group. It can be concluded that with all the type of bonds present based on the analysis and previous research, eugenol is present in both the extracted sample from clove bud for 3 hours and 5 hours.

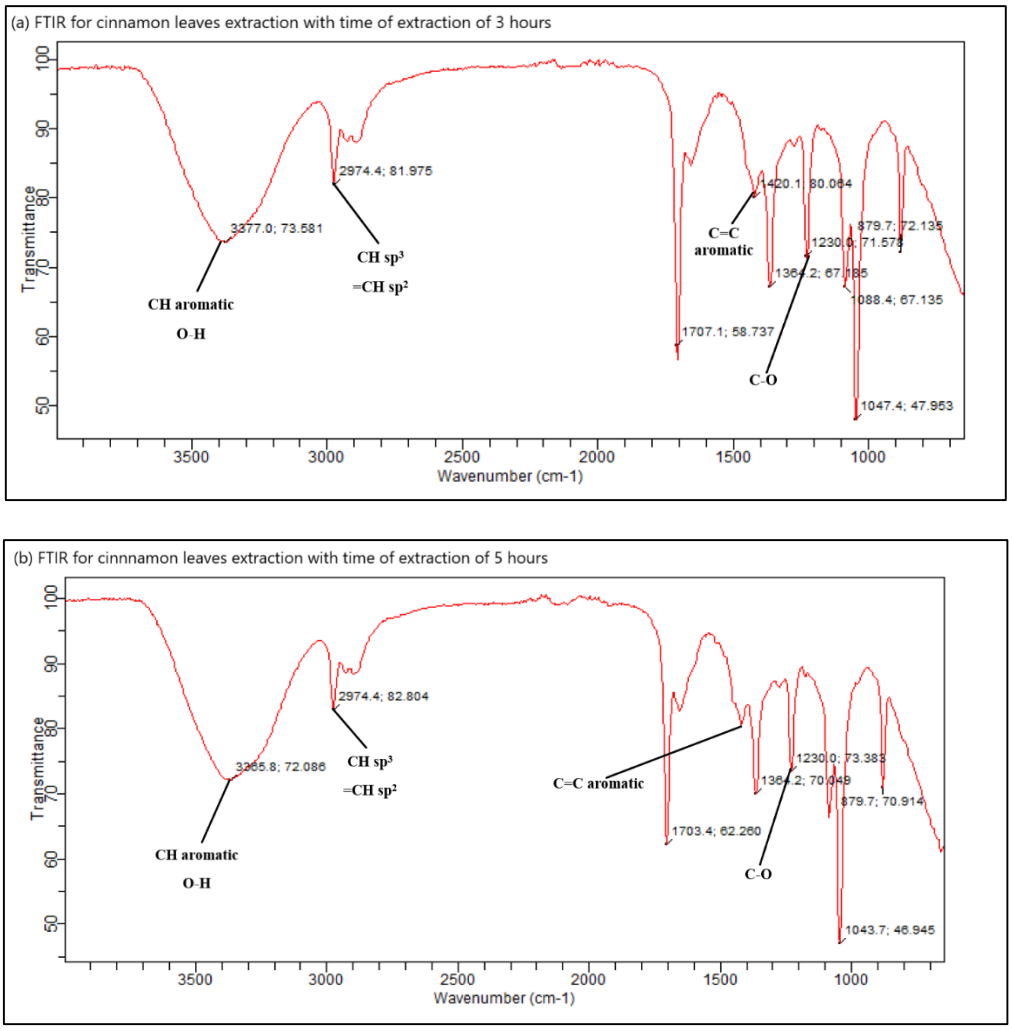

Figure 7: FTIR screening for Cinnamon leaves *(Cinnamomum verum)* extraction time of extraction of (a) 3 hours (b) 5 hours

The results of FTIR screening for different time of extraction of cinnamon leaves is shown in Figure 7. The absorption bands in Figure 7 (a) were detected at the peak of 1230 cm^{-1}, 1420.1 cm^{-1}, 2974.4 cm^{-1}, and 3377 cm^{-1} meanwhile in Figure 7 (b) the peaks were detected at 1230 cm^{-1}, 1420.1 cm^{-1}, 2974.4 cm^{-1}, and 3365.8 cm^{-1}. The FTIR analysis obtained for the cinnamon leaves extracted for both time of extraction, which is for 3 hours and 5 hours, showed a strong, broad absorption band at 3377 cm^{-1} and 3365.8 cm^{-1} indicating the presence of hydroxyl group and C-H aromatic group as it the range of the peak for hydroxyl group and C-H aromatic group is 3100 cm^{-1} to 3690 cm^{-1} and 3000 cm^{-1} to 3200 cm^{-1} respectively. In that range, there is only one peak is visible and this might be because of the intrinsic physical nature of the sample or due to the instrumental resolution that has been used which is 8 cm^{-1} and lead to noisier spectra [7]. Absorption band of 2974.4 cm^{-1} and the peaks that is visible in the range of 2800 cm^{-1} to 3300 cm^{-1} represents the presence of C-H alkene and C-H alkane. Peak at absorption band of 1420.1 cm^{-1} indicated the presence of C=C aromatic group. Absorption band observed at 1230 cm^{-1} detected the presence of C-O proving the presence of the carbon attached to the hydroxyl group. It can be concluded that with all the type of bonds present based on the analysis and previous research, eugenol is present in both the extracted sample from cinnamon leaves for 3 hours and 5 hours.

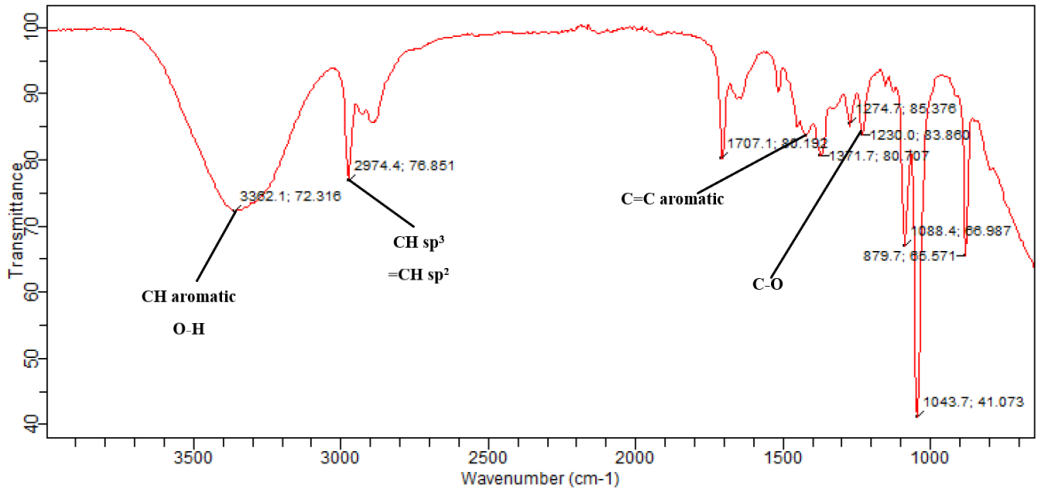

Figure 8: FTIR screening for Eugenol stock solution

The results of FTIR screening for eugenol stock solution is shown in Figure 8. The absorption bands in Figure 8 were detected at the peak of 1230 cm^{-1}, 1450.4 cm^{-1}, 2974.4 cm^{-1}, and 3362.1 cm^{-1}. The FTIR analysis obtained for the eugenol, showed a strong, broad absorption band at 3362.1 cm^{-1} indicating the presence of hydroxyl group and C-H aromatic group as it the range of the peak for hydroxyl group and C-H aromatic group is 3100 cm^{-1} to 3690 cm^{-1} and 3000 cm^{-1} to 3200 cm^{-1} respectively. In that range, there is only one peak is visible and this might be because of the intrinsic physical nature of the sample or due to the instrumental resolution that has been used which is 8 cm^{-1} and lead to noisier spectra [7]. Absorption band of 2974.4 cm^{-1} and the peaks that is visible in the range of 2800 cm^{-1} to 3300 cm^{-1} represents the presence of C-H alkene and C-H alkane. Absorption band of 1703.4 cm^{-1} indicated the presence of C=C aromatic group. Absorption band observed at 1230 cm^{-1} and 1274.7 cm^{-1} detected the presence of C-O proving the presence of the carbon attached to the hydroxyl group. Based on the analysis, it can be concluded that with all the type of bonds present that eugenol is present.

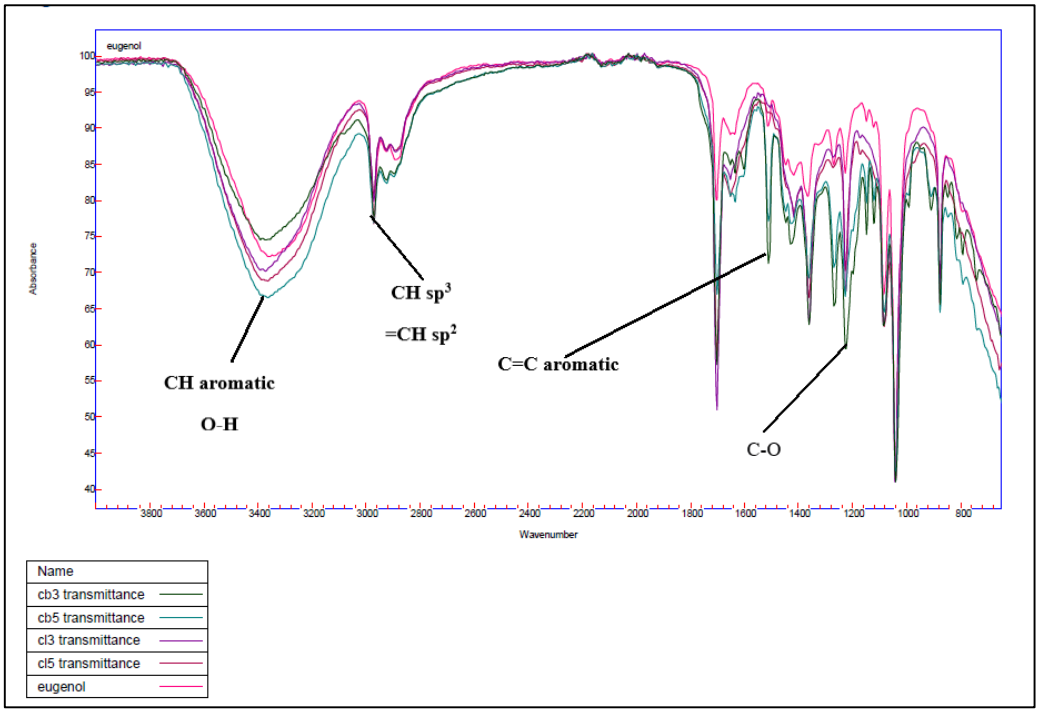

Figure 9: Overlaying screening graph of FTIR analysis of all samples

Table 2: The comparison of transmittance for different type of bonds present in clove bud extract, cinnamon leaves extract and eugenol

Type of bonds	Transmittance				
	Clove bud extract		Cinnamon leaves extract		Eugenol
	3 hours	5 hours	3 hours	5 hours	
C-O	64.998	70.542	71.578	73.383	82.740
C=C aromatic	75.224	79. 677	80.064	80.737	80.192
CH sp^3 =CH sp^2	80.763	79.375	81.975	82.804	76.851
CH aromatic O-H	77.282	70.381	73.581	72.086	72.316

Figure 9 shows the overlaying screening of FTIR analysis for all the samples which are clove extraction that is extracted for 3 and 5 hours, cinnamon leaves extraction that is extracted for 3 and 5 hours as well and eugenol stock solution to observe the difference of the peak of each functional group in each sample. The Table 2 shows the comparison of transmittance for different type of bonds present in clove bud extract, cinnamon leaves extract where the extraction was for 3 and 5 hours each, and eugenol stock solution. The transmittance was being compared in order to find the concentration approximately of each compound in the sample extract. This is to determine the concentration of chemical species present in the samples and with the reference of Beer-Lambert Law equation (Eq. 1), it is known that the transmittance of the compound in each sample is inversely proportional to the absorbance and absorbance is linearly proportional towards the concentration. Thus, when the percentage of the transmittance is lower, then the absorbance of the infrared light will be high. The high absorbance indicates the high population of bonds which have vibrational energies corresponding to the infrared light. Based on the table above that shows the comparison between the percentage of transmittance for every sample and the type of bond, clove extract that is extracted for 5 hours is more

concentrated with eugenol compared to other extracts due to its constant and low percentage of transmittance for all the type of bonds. Hence, it has high absorbance of infrared light, proving that it is more concentrated with eugenol.

4. Conclusion

As a conclusion, this study was carried out to compare the eugenol in the essential oil that is extracted for 3 hours and 5 hours from clove bud *(Eugenia caryophyllata)* and cinnamon leaves *(Cinnamomum verum)* by Soxhlet extraction. The first objective has been achieved when the Soxhlet extraction is done for 3 hours and 5 hours. It can be concluded that essential oil that was extracted from clove for 5 hours has the highest percentage of yield which is 0.363 %. Next, the second objective was also achieved by conducting thin layer chromatography analysis to isolate and identify the presence of the components consists in the essential oil of cinnamon leaves *(Cinnamomum verum)* and clove *(Eugenia caryophyllata)*. By the observation that was done, there are constituents present in each extract due to the observation done under the UV light of 366 nm. In addition, the third objective which is to characterize the components was achieved by using Fourier transform infrared spectroscopy (FTIR) analytical assay. The FTIR analysis of clove bud and cinnamon leaves extract indicates that it consists of functional group of the C-O stretch, C=C aromatic stretch, CH sp^3, =CH sp^2, CH aromatic and OH stretch as these functional group is existed in the compound of eugenol. The clove extract that is extracted for 5 hours is more concentrated with eugenol compared to other extracts due to its constant and low percentage of transmittance for all the type of bonds. Hence, it has high absorbance of infrared light, proving that it is more concentrated with eugenol.

However, further studies can be done to the essential oils of clove bud *(Eugenia caryophyllata)* and cinnamon leaves *(Cinnamomum verum)* by using other extraction method such as cold extraction, carbon dioxide extraction or hydrodistillation method for more yield of essential oil. It is recommended to use HPLC in order to separate a mixture of compound in order to identify and quantify. In addition, to determine the concentration of the components in the essential oil precisely, it is recommended to use gas chromatography mass spectrometry (GC-MS). It is also recommended to extract the samples for at least five different time of extraction to improve the findings.

Acknowledgement

The author would like to thank the Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia for the facilities offered. The author is very appreciative for all the assistance and guidance received in order to accomplish this research paper.

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