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ANALYSIS OF CLOVE (*Syzygium aromaticum* L.) OIL OBTAINED WITH HYDRODISTILLATION AND ULTRASOUND- ASSISTED EXTRACTION[1]

Arita Sabriu – Haxhijaha, Asst.Mr.Sc.,Suzana Jordanovska, Prof. Dr.Sc., Olga Popovska, Ass.Prof.Dr.Sc.
Faculty of Technological Sciences,
"Mother Teresa" University-Skopje,
*e-mail of the corresponding author:
arita.haxhijaha@unt.edu.mk

ABSTRACT

Essential oils derived from various aromatic plants are frequently used due to their antioxidative, antibacterial and anti-inflammatory activity. Their pleasant odor makes them very useful in pharmaceutical, cosmetic and food industries. Clove oil (CO) can be isolated from dried flower buds of *Syzygium aromaticum* L. and its major component is eugenol presented in 70-90%. The aim of this study was to develop two extraction techniques and analyze the obtained CO with thin-layer chromatography (TLC). Clove buds purchased from the local market were powdered and added distilled water for hydrodistillation and 50% ethanol for ultrasound-assisted extraction. Liquid-liquid extraction was performed with dichloromethane and dried with anhydrous sodium sulfate. Accurate identification of eugenol with TLC was achieved with toluene:ethyl acetate (93:7) mixture as a mobile phase while high-efficiency separation of more active compounds was performed with ultrasound-assisted extraction technique.

KEY WORDS: essential oil, clove oil, ultrasound-assisted extraction, eugenol

[1] original scientific paper

INTRODUCTION

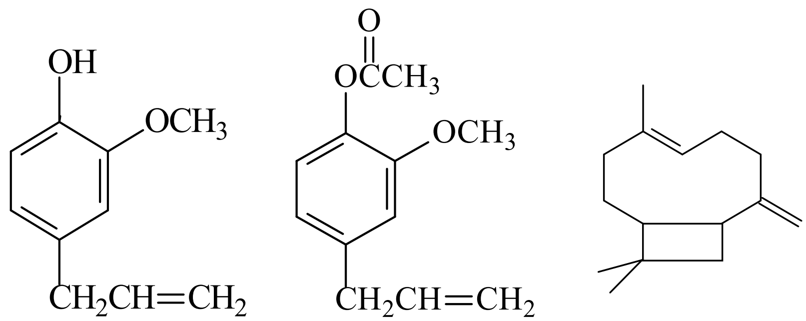
Essential oils contribute to the characteristic aroma of plants, which have been used since antiquity as a source of fragrances and flavorings (Bell *et al*., 2001). These oils occur in all plant's parts; often are concentrated in twigs, flowers, and seeds. Essential oils are generally complex mixtures of hydrocarbons, alcohols, and carbonyl compounds, mostly belonging to thebroad group of plant products known as terpenes (Khalil *et al*., 2017).Some terpenes, such as the hydrocarbon limonene, are found in many different plant species (Bell *et al*., 2001).Certain other compounds, particularly aromatic aldehydes and phenols, occur as a major constituentin only one or in few plants (Yun *et al*., 2010). Thus, the characteristic aromas of clove and cinnamon oils, aremainly due to one compound in each case (Khalil *et al*., 2017). However, the full flavor and bouquetof a spice or flavoring usually depend on a blend of several substances and cannot be reproducedby a single compound (Bell *et al*., 2001; Khalil *et al*., 2017).

Essential oils are isolated from plant tissues by steam distillation-sometimes, in remoteparts of the world, using a very primitive apparatus (Just *et al*., 2016). Individual compounds can be separatedfrom the essential oil by fractional distillation with reduced pressure, crystallization orchromatography (Bell *et al*., 2001; Kapadiya *et al*., 2018).

Clove is mainly used as flavoring and food conservation (Yun *et al*., 2010). Besides that, clove is one of the best known medical plants used for diarrhea, digestive disorders, even as an antiseptic (Kumar *et al*., 2010; Overly, 2019). It is also reported that clove can be used in the treatment of certain diseases and conditions such as diabetes, bacterial, fungal and viral infections, as well as toothache reliever (Khalil *et al*., 2017; Rodríguez *et al*., 2014). The wide spectrum of biological activities of clove oil leads its use to be extended in insect repellant, dental freshness, mouthwash and toothpaste (Kapadiya *et al*., 2018).

The plant is aromatic flower bud of a tree *Syzygium aromaticum* L. belonging to the family *Myrtaceae* which is commonly used as a spice (Rodríguez *et al*., 2014). As evergreen tree, the clove grows up to 12 m tall, while the cultivated one is 5 m tall. The flower buds are with pale colour turning to the red when they are ready for harvest (Khalil *et al*., 2017). Availability of clove is during the whole year with different harvest seasons through the world. The substance has a pleasant, specific and strong aroma (Kapadiya *et al*., 2018). The presence of the essential oil in the clove is less than 22% and the oil in flower buds is between 49 and 98% (Rodríguez *et al*., 2014).

The main components in clove oil are eugenol, eugenol acetate, and β-caryophyllene, presented in Figure 1.



a b c

Figure 1. Components in clove oil: eugenol (a), eugenol acetate (b), and β-caryophyllene (c)

Eugenol (4-allyl-2-methoxyphenol) is the main active phenolic compound in clove oil having many health-promoting functions that make it a versatile natural integrant (Khalil *et al*., 2017; Yun *et al*., 2010). Eugenol is considered to be present between 70 and 90% in the plant and is a quality control marker compound (Yun *et al*., 2010). Although the eugenol in general is considered as non-toxic and non-mutagenic substance (Khalil *et al*., 2017), but it is listed as potentially toxic (Bell *et al*., 2001; Kapadiya *et al*., 2018; Saran *et al*., 2013). The concentration of eugenol can be differed not only in specific parts of the plant but also varies between seasons (Khalil *et al*., 2017).

In the literature, the process of extraction preparation steps, hydrodistillation and steam distillation was used for the extraction of the essential oil from clove leaf or bud (Just *et al*., 2016; Kapadiya *et al*., 2018). Microwave assisted extraction can be used for eugenol extraction from clove (Kapadiya *et al*., 2018; Overly, 2019). Spectrophotometric (Sanghai *et al*., 2011) and chromatographic methods have been applied to determine eugenol in clove extracts (Ibrahim *et al*., 2015; Móricz *et al*., 2016; Tekin *et al*., 2015; Yun *et al*., 2010). An isocratic mobile phase consisted of methanol:water (60:40 *v*/*v*)

with a C_{18} column was used to determine eugenol in clove (Saran *et al*., 2013; Yun *et al*., 2010).On the other hand, gas chromatography method usesphenyl methyl siloxane capillary column coupled either with mass spectrometry (Móricz *et al*., 2016; Tekin *et al*., 2015) or with flame ionization detector (Móricz *et al*., 2016). The standardization in the quality control process of the herbal medicine is one of the most important roles to establish the modernization of the alternative medicine (Yun *et al*., 2010).
The aim of this work is to make a comparison of the obtained essential clove extracts using two different extraction procedures, as well as the analysisof the obtained oil. The isolation of the essential oil of cloves was performed by hydrodistillation or ultrasound-assisted extraction and the oil was analyzed with thin-layer chromatography (TLC).

MATERIALS AND METHODS

Dried clove buds (Figure 2a) were bought in the local marketand finely grinded with a coffee mill (Figure 2b). The commercially clove oilused as toothache reliever(Figure 2c) was bought at local pharmacy. Toluene, methylene chloride, ethyl acetate, glacial acetic acid, sodium sulfate were supplied from Merck (Germany). All reagents were with analytical grade purity.

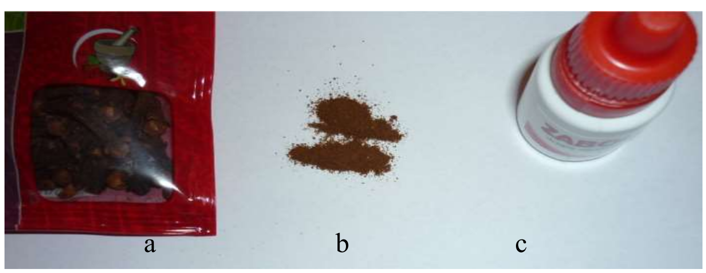


Figure 2. Clove in forms: clove buds (a), grinded clove (b) and clove oil (c)

For the extraction procedure, 5g of clove powder were weighed and quantitatively transferred to avolumetric flask filled with 100 mLof distilled water. One type of extraction was carried out using hydrodistillation with the use of the standard glassware. The apparatus for distillation was set at 90 °C. The distillate was poured into a separatory funnel and methylene chloride (3 x 5 mL) was used in the extraction step. The lower organic layer is drained carefully in Erlenmeyer flask previously weighted and a small quantity of sodium sulfate was added as a desiccant.

Another extraction method utilized in the process of extraction was ultrasound-assisted extraction in an ultrasonic water bath (PRC) with 40 Hz frequency at temperature at 60 °C for 60 minutes. The amount of crushed clove buds was quantitatively transferred into Erlenamayer flask with 100 mL 50% ethanol in it.The obtained filtrate was double filtrated through Whatman No. 1filter paper.The extraction was performed at the same way as with the hydrodistillation method with methylene chloride (3 x 5 mL). The yield of obtained clove oil was calculated using this formula;

$$\text{Yield (\%)} = \frac{theweightofobtainedextract\ (g)}{theweightofstartingmaterial\ (g)} * 100\%$$

The identification of the main compounds was performed with thin-layer chromatography (TLC) using an 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 F_{254}, 250 µm).The first mobile phase (1) was consisted of toluene: ethyl acetate (9.3:0.7 *v*/*v*), the second mobile phase (2) was consisted of ethyl acetate:glacial acetic acid (9.5:0.5 *v*/*v*), the third mobile phase (3) was a mixture of dichloromethane:acetone:glacial acetic acid (7.6:1.5:0.5 *v*/*v*/*v*), while the fourth one (4) was only toluene as an organic solvent used as a mobile phase.

RESULTS AND DISCUSSION

The use of hydrodistillation can provide distillation of a slightly volatile compound at a temperature far below its atmospheric boiling point. Using hydrodistillation is useful when the water-insoluble organic compound is present in a large amount of non-volatile material (Bell *et al*., 2001). The obtained essential oil had strong characteristic odor. The clove oil was a clear colourless to pale yellow liquid and with standing at sun light the colour can be changed to brown. Clove essential oil extracted with hydrodistillation was colourless.

The determined values for yield were 5.67% and 1.07% using hydrodistillation and ultrasound extraction method, respectively. The extraction yield is increased by reducing the particle size of clove buds powder (Khalil *et al*., 2017).

Comparing to literature, yield of the isolated eugenol was 6% using hydrodistillation method (Just *et al*., 2016), while the % of the obtained clove oil varied from 4% to 8% depending on the sample amount, water volume and extraction time (Kapadiya *et al*., 2018). The use of ultrasound-assisted extraction procedure showed more bioactive compounds to be separated effectively (Tekin *et al*., 2015).

The TLC results using different types of mobile phases are shown in Figure 3.

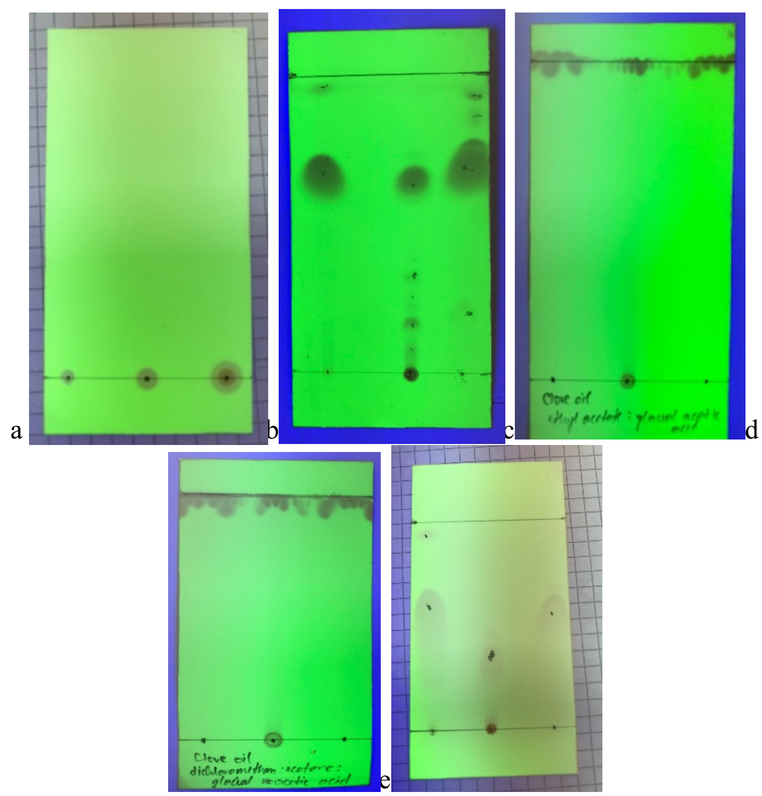


Figure 3. TLC plates: application of the samples (a), results from mobile phase 1 (b), mobile phase 2 (c), mobile phase 3 (d), mobile phase 4 (e)

The first spot was the oil obtained from the hydrodistillation process, the second spot was the one from the ultrasound extraction, while the third spot was from commercially available clove oil in all analyzed samples.

It was found that $R_f$for eugenol in three types of oils was 0.66 with the mixture of toluene:ethyl acetate, 93:7 *v*/*v*as a mobile phase. The determined R_f value for eugenol when we used toluene was 0.81. The results from the developed TLC plates show that the use of the mixture of toluene:ethyl acetate, 93:7 *v*/*v*yielded more active compounds which could be identified comparing to literature. According to Rodríguez *et al*(2014) the mobile phase they used was a mixture of hexane:acetone (9:1 *v*/*v*) and the value of retention factor was found to be 0.65. Comparing our results with literature data, it was found that the value of the retention factor, $R_f$foreugenol was 0.68 using toluene:ethyl acetate, 93:7 *v*/*v*(Sanghai *et al*., 2011).
Using four types of mobile phase for developing the TLC plates it was found that the most satisfactory results in terms of the separation process and the identification of spots was the one using toluene:ethyl acetate (93:7 *v*/*v*).The least satisfactory resultsin terms of separation and identification of eugenol as the main compound in the clove oils were with the use of the mixture of ethyl acetate:glacial acetic acid (9.5:0.5 *v*/*v*) and the mixture of dichloromethane:acetone:glacial acetic acid (7.6:1.5:0.5 *v*/*v*/*v*) for mobile phase where the separation was not complete due to the polarity of the system. The choice of the mobile phase resulted in the impact for the separation process which is based on the behavior of the compounds in the sample.

CONCLUSION

In this study, we extracted essential clove oil with two different methods, hydrodistillation and ultrasound-assisted method. The oil was colourless to pale yellow with characteristic strong odor. The yield of the oil was found to be 5.67% and 1.07% using hydrodistillation and ultrasound extraction method, respectively. The TLC method is a rapid method and easy to be handled. The TLC analysis upon the determined R_f values of the present compounds in the samples provided qualitative control of the obtained oil in comparison with the commercial available clove oil.

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