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Introducing Organic Chemistry Students to the Extraction of Natural Products Found in Vegetal Species

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Abstract This work constitutes a new strategy for teaching-learning the chemistry of natural products, being the students active participants in the process. Results showed in this activity contributed not only to the understanding of the subject but also turned out to be an important pillar of motivation. An essential oil is any of a class of volatile oils that give plants their characteristic odor. Essential oils are also known as ethereal oil, volatile oil, aetherolea, or simply as the oil of the plant from which they are extracted, such as oil of clove. The oil is "essential" in the sense that it contains the "essence of" vegetal specie fragrance, the characteristic fragrance of the plant from which it is derived. These can be extracted from different parts of plants such as stems, leaves and flowers. In this work, two conventional methods (steam distillation and Soxhlet extraction) and one non-conventional method (microwave-assisted extraction), was developed under different conditions from clove. The yield of clove oil and yield of eugenol were obtained; and the concentration of eugenol present was assessed by gas chromatography. The main objective was that the experimental activity with the theoretical foundation will provide to the students of organic chemistry the ability to link concepts and develop skills in the laboratory.

Keywords: *organic chemistry, essential oils, natural products, methods of extraction, eugenol*

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1. Introduction

An experimental work have the potential to be an important medium for introducing students to developing practical skills and conceptual understanding in science [1]. In April 2013, the Next Generation Science Standards (NGSS) were released, which are composed of three strands: disciplinary core ideas, crosscutting concepts, science and engineering practices [2]. Science and engineering educators, and employers, agree that students should graduate from college with expertise in their major subject area as well as the skills and competencies necessary for productive participation in diverse work environments [3]. In this sense, this work proposes an introducing to organic chemistry students to the extraction process of natural products found in plants. The students used three methods of extraction of clove oil: steam distillation (SD), Soxhlet extraction and microwave-assisted extraction (MAE). Thus, the extraction and isolation of organic molecules from vegetal material can be used to reinforce in the students fundamental principles. It provides an overview of aromatic plants, essential oils (EOs), fundaments of extraction methods, different aspects of solubility, polar and non-polar characteristic of diverse compounds, fundaments of gas chromatography (GC). We expect to students develop critical thinking skills, team working, mastery of laboratory techniques, management and distribution of time.

The vegetal species produce an amazing diversity of secondary metabolites, which have multiple functions throughout the plant's life cycle [4,5]. In that direction have been investigated different methods for the extraction of EOs from aromatic plants. EOs are represent complex mixtures of up to hundred components. Between them these can be founded low molecular weight aliphatic compounds (alkanes, alcohols, aldehydes, ketones, esters, acids, monoterpenes, sesquiterpenes, and phenylpropanes) [6,7]. EOs are used by aromatize perfumes or fragrances [8].

Clove oil has biological activities, such us antibacterial, antifungal, insecticidal and antioxidant properties, and traditionally is used as flavoring agent and antimicrobial material in food [9,10,11]. Eugenol is the major component in clove oil which also contains smaller amounts of eugenyl acetate and the terpenoid β- caryophyllene [12] (Figure 1). Eugenol belongs to the class of volatile phenylpropenes that are widely distributed across the plant kingdom [13]. Various therapeutic effects of this compound have been demonstrated, including antivirus, antibacterial, antipyresis, analgesia, anti-inflammatory, anticoagulation, anti-oxidation, antihypoxia and anti-ulcer [14].

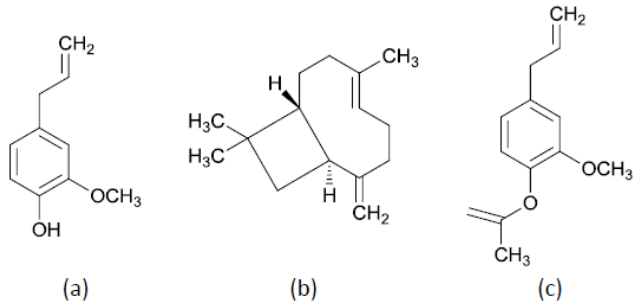

Figure 1. Main components of clove oil (a) Eugenol, (b) β-Caryophyllene, (c) Eugenol acetate

This practical work was proposed for students of chemical engineering and bachelor's degree in chemistry; and consisted of five stages. In the first place, the theoretical content was developed. In the second stage, the first practical work of SD technique was realized. The third stage involves the extraction of EO of clove through Soxhlet technique. The fourth stage consisted in performing the MAE like a method alternative. In the fifth stage, the concentration of eugenol was determined by GC. At the end of stages the yields of EO and eugenol present in the same were calculated. After these stages was possible arrival to the conclusions of experimental work realized.

Then, the basic theory of different techniques that were carried out in the laboratory will be explained.

1.1. Steam Distillation

Distillation is used for the purification of liquids and to separate components from a liquid mixture of different boiling points. In general, a distillation process involves heating the liquid mixture to the vapor state so as to enable the selective condensation and withdrawal of the components of interest [15,16].

SD is based in the coexistence of two immiscible liquids (water and essential oil). The saturated vapors of immiscible liquids follow Dalton's Law on partial pressures. The boiling point of the mixture will be the temperature which the sum of the vapor pressure is equal to the atmospheric pressure. This temperature will be less than the boiling point of the most volatile component [17]. SD is used to separate essential oil from vegetal species [18].

Figure 2 shows the steam distillation equipment. In this case the generated steam comes in contact with clove by dragging the volatile components. The mixture, saturated steam and essential oil, flows into the condenser, where it is condensed and subsequently separated by a Florentine tube.

1.2. Soxhlet Extraction

Soxhlet is a standard technique and the main reference for evaluating the performance of other solid–liquid extraction methods. In a conventional Soxhlet system (Figure 3), plant material is placed in a thimble-holder, and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. Solute is left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction is achieved. Soxhlet extraction strongly depends on matrix characteristics, particle size and the internal diffusion may be the limiting step during extraction [19]. This method is used by extraction of inorganic and organic pollutants. Besides have been used for extraction of pesticides from soil samples and natural products from medicinal plants [20,21].

In this study, the effects of influential extraction parameters such as extraction time and solvents were studied to set up the optimal extraction conditions to obtain the maximum yield of EO and eugenol.

1.3. Microwave-Assisted Extraction

Microwave is an electromagnetic wave. It consists in a combination of an electric and magnetic fields which oscillates perpendicularly to each other in frequency ranged from 0.3 to 300 GHz [22]. MAE operates through cell bursting, which facilitates the entry of the extraction solvent to solubilize out the target compounds; this leads to faster extraction and prevents the degradation of target compounds [23].

As advantages can be named; time reduction, rapid heating, less volume of solvent. The efficiency of MAE strongly relies on the selection of the operating conditions and the parameters affecting the extraction mechanisms and yield. The factors that may influence the performance of MAE are solvent nature, solvent to feed ratio, extraction time, microwave power, temperature, sample characteristic and effect of stirring [24].

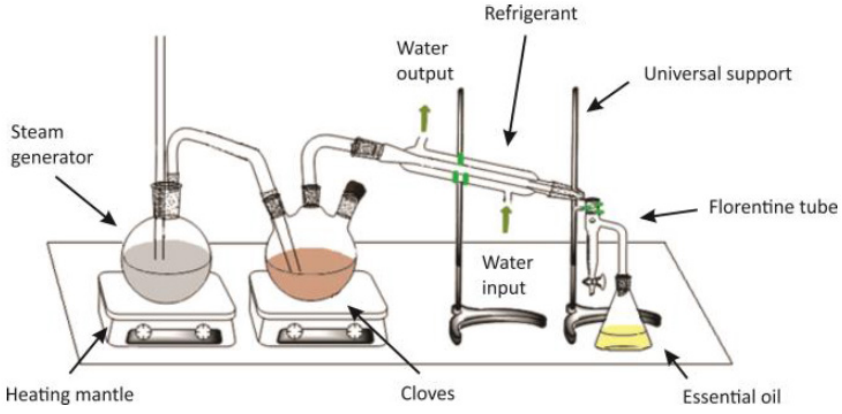

Figure 2. Steam Distillation equipment

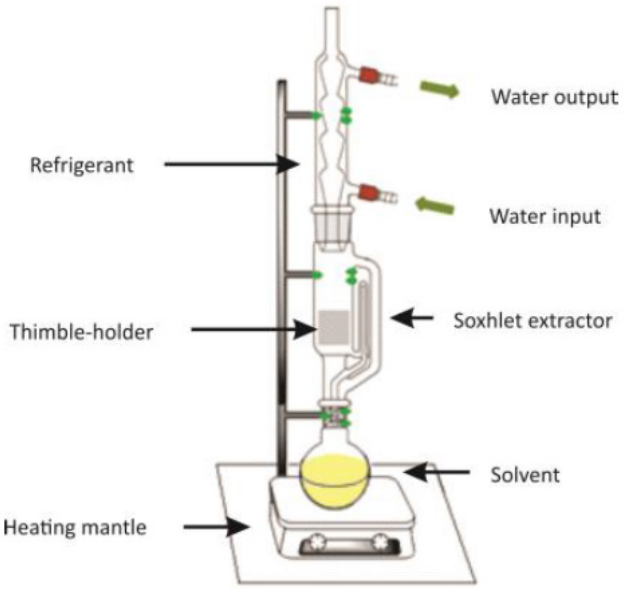

Figure 3. Soxhlet equipment

1.4. Gas Chromatography

The chromatographic has as purpose the separation of mixtures of different capacity of interaction with components of others substances. Consist of the passage of a mobile phase through a stationary phase. The components of the samples overpass the stationary phase at different rates and therefore at different times, so that, each has a characteristic retention time.

The chromatographic process is one in which mixtures of volatile compounds are separated by molecular size, boiling point, and polarity [25]. Basically, a small sample to be analyzed is injected into a flowing stream of a carrier gas and is forced through a column which allows the lighter components to easily flow through the column for detection. The heavier components are also separated from the column to the detectors in their respective weight order [26].

2. Materials and Methods

2.1. Plant Material

Cloves were commercial one. The samples were dried in stove at a temperature of 40°C until constant weight, and then ground in a mortar.

2.2. Steam Distillation

The distillation equipment consisted of a 1000 ml steam generator, a 500 ml round-bottom flask where the sample is placed, a refrigerant and a collecting vessel. Water was placed in a steam generator and heated by a heating blanket. The sample (30 g) was placed in the contiguous round-bottom flask. This method was carried out at different times and the concentration of eugenol was determined for each one by GC. The yield was calculated by equation 1.

$$Yield\ of\ EO\,(\%) = \frac{mass\ of\ EO\,(g)}{mass\ clove\,(g)}.100\%. \qquad (1)$$

2.3. Soxhlet Extraction

This method was carried out using three solvents: water, n-hexane and ethanol. The choice of these was due to their polarity; the least polar is the n-hexane, the more polar the water and the ethanol of intermediate polarity.

The samples (30 g) were extracted with of each solvent during 6 h. After extraction the extract was concentrated on rotary evaporator and the concentration of eugenol was analyzed by GC. The following extractions were performed with the solvent that obtained higher concentration of eugenol at different times (4, 6 and 8 h) and 30 g of sample.

2.4. Microwave-Assisted Extraction

The essential oil was extract from the plant under various conditions according to Taguchi method of 5 factors and 5 levels, which was realized with Minitab 17 [27] (Table 1).

Table 1. Taguchi method of 5 factors and 5 levels

Run N°	Factor 1 Temp. (°C)	Factor 2 Stirring (rpm)	Factor 3 Time (min)	Factor 4 Ethanol conc. (%)	Factor 5 Liq: sol ratio (ml/mg)
1	30	240	5	20	7
2		480	10	40	10
3		720	15	60	13
4		960	20	80	16
5		1200	25	100	19
6	40	240	10	60	16
7		480	15	80	19
8		720	20	100	7
9		960	25	20	10
10		1200	5	40	13
11	50	240	15	100	10
12		480	20	20	13
13		720	25	40	16
14		960	5	60	19
15		1200	10	80	7
16	60	240	20	40	19
17		480	25	60	7
18		720	5	80	10
19		960	10	100	13
20		1200	15	20	16
21	70	240	25	80	13
22		480	5	100	16
23		720	10	20	19
24		960	15	40	7
25		1200	20	60	10

The experiments were carried out in an Anton Para Monowave 300 which has the characteristic that a stage controlled by power and temperature cannot be combined in an experiment. For this reason, of the two variables, only the temperature was analyzed. The range of variation of the same was from 30°C to 70°C, because the higher temperatures favor the degradation of the extracted compounds. In order to avoid the risk of thermal degradation and oxidation, MAE extraction time usually ranges from a few minutes to half an hour for the extraction of EOs [24]. The reaction time was set between 5 and 25 minutes. Another of the chosen parameters is the stirring. The values established in the levels correspond to 20, 40, 60, 80 and 100 % of the maximum value that can reach (1200 rpm).

The solvent used was ethanol in 20 to 100 % solutions. The choice of it was because it is a good microwave absorber. The solvent volume was selected according to the volumetric capacity of the reaction cells, which varies between 6 and 20 ml. The solvent/solid ratio is important because it assure a homogenous and effective heating [28].

2.5. Gas Chromatography

The concentrate was analyzed by GC to determinate the percent total peak-area of aroma compounds [29]. The concentration of eugenol was calculated from the calibration line obtained (Equation 2).

$$\frac{eugenol\ area\ (mV)}{IP\ area\ (mV)} = 46.191\frac{eugenol\ concentration\left(\frac{g}{l}\right)}{IP\ concentration\left(\frac{g}{l}\right)} + 0.415. \quad (2)$$

From this result, the yield of eugenol was calculated by equation (3).

$$Yield\ of\ Eugenol\,(\%) = \frac{eugenol\ concentration\left(\frac{g}{l}\right)}{mass\ of\ EO\,(g)}.100\%. \quad (3)$$

3. Results and Discussion

3.1. Extraction of Essential Oil by Steam Distillation

Figure 4 shows the yield of clove oil and eugenol obtained by SD. It can be observed that the yield of eugenol maximum was 49.15 % at a time that 6 h.

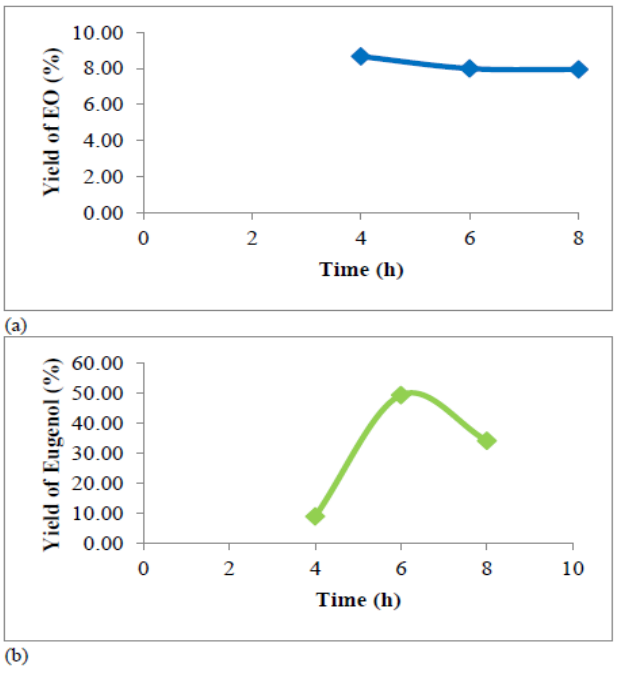

Figure 4. (a) Yield of EO and (b) Yield of Eugenol obtained by SD at different times

3.2. Extraction of Essential Oil by Soxhlet

Figure 5 shows the result obtained for different conditions. Although the highest yield of EO was obtained with n-hexane, it presented a low yield of eugenol (equal to water) compared to ethanol, so the solvent chosen was ethanol. A time of 4 h gave the best yield of EO (31.9 %). However the yield of eugenol was only of 3.68 %, this is because ethanol has an intermediate polarity, so that decreased the purity of extract obtained (and extracted others compounds).

Figure 6 shows chromatogram obtained for the clove oil with ethanol for 4 h and 30 g of sample.

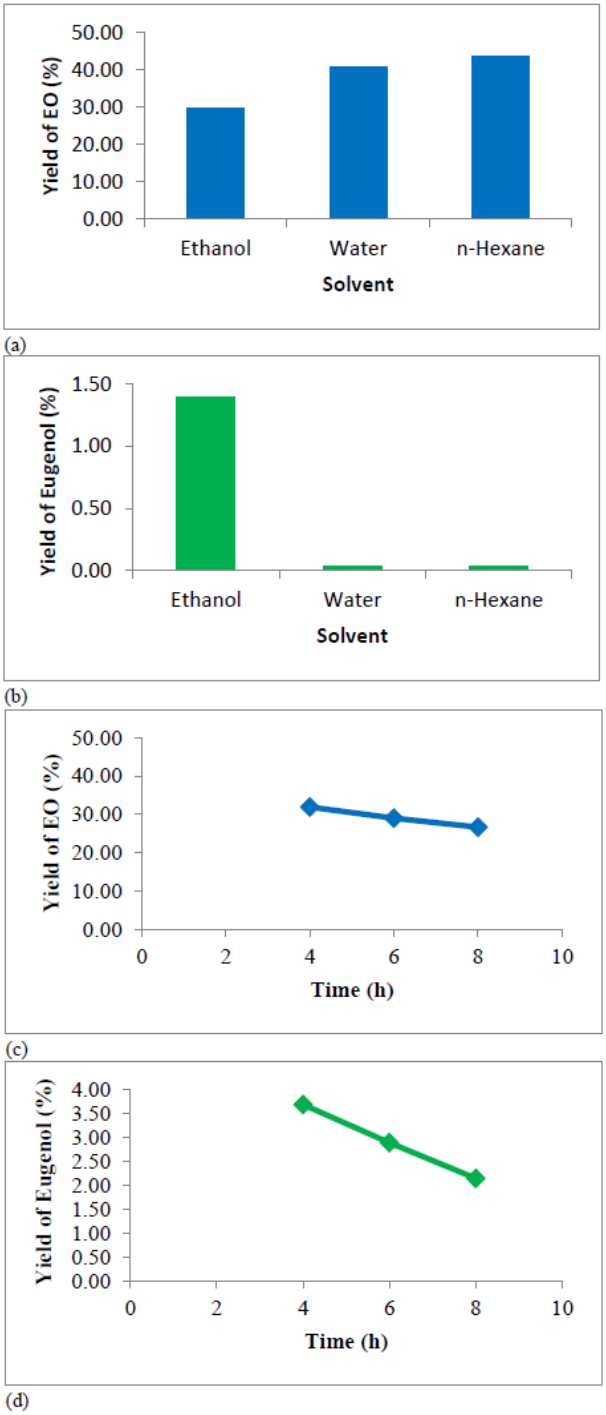

Figure 5. (a) Yield of EO and (b) Yield of Eugenol obtained by different solvents (c) Yield of EO and (d) Yield of Eugenol obtained at different times with ethanol

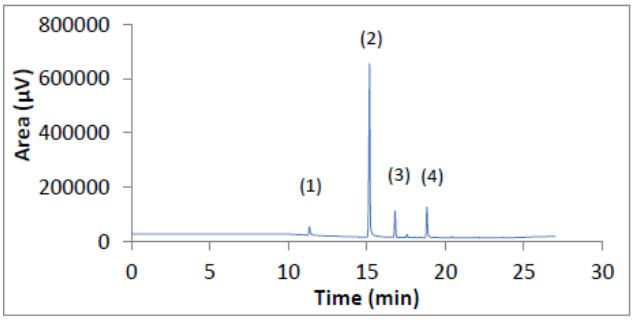

Figure 6. Chromatogram of clove oil. Internal patron: Methyl salicylate (1). Components: Eugenol (2), β-Caryophyllene (3), Eugenol acetate (4)

3.3. Extraction of Essential Oil Assisted by Microwave

In this study we focused to the Taguchi experimental design, which uses orthogonal arrangements. The orthogonal arrangement allows estimate the effects of factors in the mean and the variation of response. Each factor can be evaluated independently, so that the effect of one factor does not affect the estimate of the other. The extraction yields for the corresponding factors at each level were calculated. The mean values of the levels of each factor indicate how the extraction yield will change when the level of that factor is changed. Figure 7 can be seen the yield of EO for the corresponding factors at each level. With respect to ethanol solution, yield decreased from a solution of 40 %, indicating an optimum in this concentration. This behavior can be attributed that presence of water can improve the penetration of the solvent into the sample matrix and therefore the efficiency of the heating but an excessive amount of solvent can be insufficient heating. The highest yield was obtained at a temperature of 50 °C. The results show that an additional increase in extraction temperature reduced extraction yield. The stirring optimal was clearly at 720 rpm. With respect to time it presents a maximum yield to 15 minutes. The relation liquid:solid optimum was 7 ml/mg. We can see that relation superior to this decrease the yield, which can be explained through that the excess of solvent causes a poor heating.

On the other hand, yield of eugenol obtained for each run is shown in Figure 8. The maximum yield of eugenol was 47.5 % under the conditions of experiment number of 22.

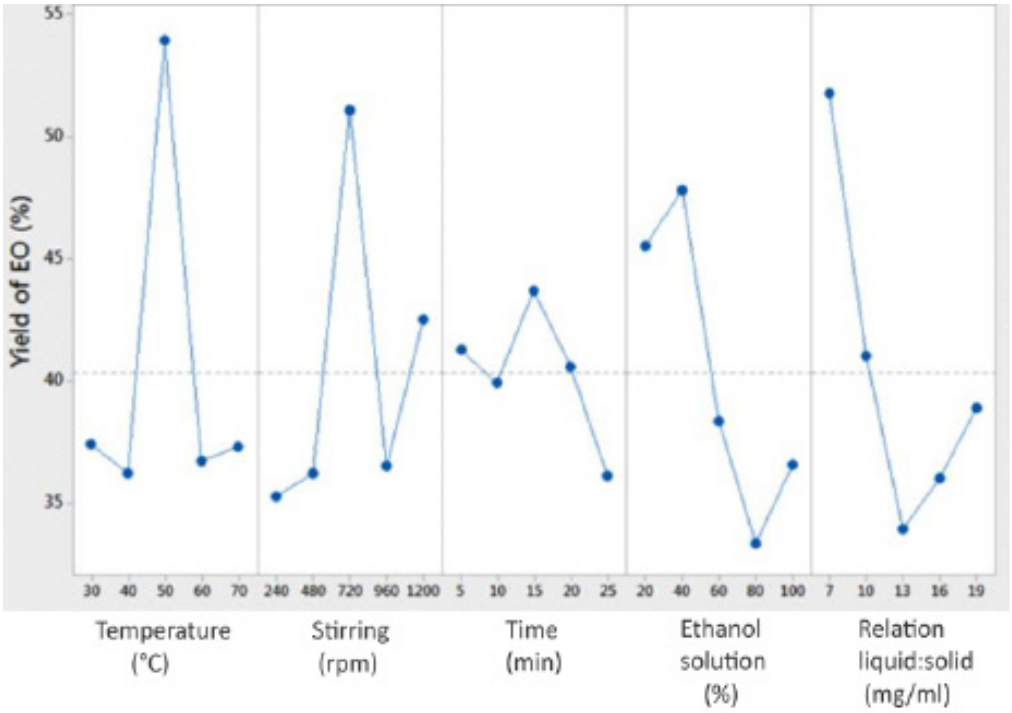

Figure 7. Effects of temperature, stirring, time, ethanol solution and relation liquid:solid on the extraction yield

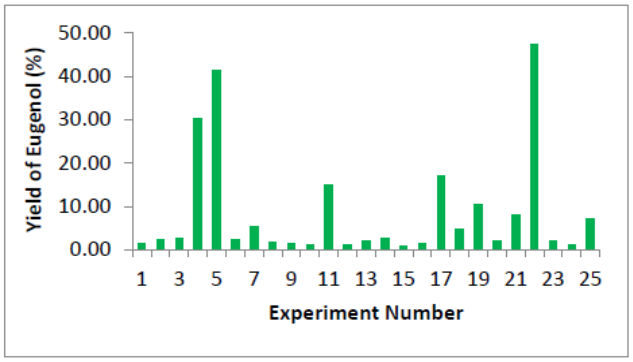

Figure 8. Yield of Eugenol according experiment number

4. Conclusion

This practical work proved to be an interesting in experimental terms and it represents a good manner to introduce the students in different aspects of knowledge in organic chemistry experimental methodology. The students carried out the extraction of active ingredients from cloves by several methods. SD, Soxhlet extraction and MAE have been successfully used for obtaining the corresponding EO. Analysis of the oil revealed differences between SD and Soxhlet extraction. For the same quantity of sample the yield of eugenol obtained by SD was notably higher compared to Soxhlet method. However, the

yield of EO was higher using the Soxhlet methodology. This shows that the yield of EO could be higher in one method but not the yield of one component as a consequence of the type of extraction solvent.

Moreover, it was proposed as alternative method MAE. Results obtained by MAE show the conditions that ensure a maximum yield of eugenol are 70°C, 480 rpm, 5 min, 100 % ethanol solution and a relation liquid:solid of 16 ml/mg. This shows that a reduction of time is considerable compared with SD and soxhlet extraction. Besides MAE and SD produce similar yield of eugenol.

Acknowledgements

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References

[1] Flaherty, A., O'Dwyer, A., Mannix-McNamara, P., and Leahy, J.J., "The influence of psychological empowerment on the enhancement of chemistry laboratory demonstrators' perceived teaching self-image and behaviours as graduate teaching assistants", *Chem. Educ. Res. Pract.*, 2017.

[2] Cooper, M.M., "Chemistry and the Next Generation Science Standards", *J. Chem. Educ.*, 90, 679-680, 2013.

[3] Canelas, D.A., Hill, J.L., and Novicki, A., "Cooperative learning in organic chemistry increases student assessment of learning gains in key transferable skills", *Chem. Educ. Res. Pract.*, 2017.

[4] Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M., "Biological effects of essential oils – A review", *Food Chem. Toxicol.*, 46, 446–475, 2008.

[5] García, A., and Carril, E., "Metabolismo secundario de plantas", *Reduca Biol. Ser. Fisiol. Veg.*, 2(3), 119-145, 2009.

[6] Ali, B., Al-Wabel, N., Shams, S., Ahamad, A., Khan, S.A., and Anwar, F., "Essential oils used in aromatherapy: A systemic review", *Asian Pac. J. Trop. Biomed.*, 5(8), 1-11, 2015.

[7] Conde-Hernández, L.A., Espinosa-Victoria, J.R., Trejo, A., and Guerrero-Beltrán, J.A., "CO_2 Supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (Rosmarinus officinalis)", *J. Food Eng.*, 200, 81-86, 2017.

[8] Calo, J.R., Crandall, P.G., O'Bryan, C.A., and Ricke, S.C., "Essential Oils as Antimicrobials in Food Systems – A Review", *Food Control*, 54, 111-119, 2015.

[9] Hakkı Alma, M., Ertaş, M., Nitz, S., and Kollmannsberger, H., "Chemical composition and content of essential oil from the bud of cultivated turkish clove", *BioResources*, 2(2), 265-269, 2007.

[10] Sebaaly, C., Jraij, A., Fessi, H., Charcosset, C., and Greige-Gerges, H., "Preparation and characterization of clove essential oil-loaded liposomes", *Food Chem.*, 178, 52-62, 2015.

[11] Deng, J., Yang, B., Chen, C., and Liang, J., "Renewable Eugenol-Based Polymeric Oil-Absorbent Microspheres: Preparation and Oil Absorption Ability", *ACS Sustain. Chem. Eng.*, 3, 599-605, 2015.

[12] Aguilar-González, A.E., López-Malo, A.A., "Extractos y aceite esencial del clavo de olor (Syzygium aromaticum) y su potencial aplicación como agentes antimicrobianos en alimentos", *Temas Sel. Ing. los Aliment.*, 7(2), 35-41, 2013.

[13] Anand, A., Jayaramaiah, R.H., Beedkar, S.D., Singh, P.A., Joshi, R.S., Mulani, F.A., Dholakia, B.B., Punekar, S.A., Gade, W.N., Thulasiram, H.V., and Giri, A.P., "Comparative functional characterization of eugenol synthase from four different Ocimum species : Implications on eugenol accumulation", *Biochimica et Biophysica Acta*, 1864, 1539-1547, 2016.

[14] Mahapatra, S.K., Chakraborty, S.P., Majumdar, S., Bag, B.G., and Roy, S., "Eugenol protects nicotine-induced superoxide mediated oxidative damage in murine peritoneal macrophages in vitro", *Eur. J. Pharmacol.*, 623, 132-140, 2009.

[15] Xavier, V.B., Vargas, R.M.F., Cassel, E., Lucas, A.M., Santos, M.A., Mondin, C.A., Santarem, E.R., Astarita, L.V., and Sartor, T., "Mathematical modeling for extraction of essential oil from Baccharis spp. by steam distillation", *Ind. Crop. Prod.*, 33, 599-604, 2011.

[16] Yang, Y., Song, X., Sui, X., Qi, B., Wang, Z., Li, Y., and Jiang, L., "Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability", *Ind. Crop. Prod.*, 80, 141-147, 2016.

[17] Wollinger, A., Perrin, É., Chahboun, J., Jeannot, V., Touraud, D., and Kunz, W., "Antioxidant activity of hydro distillation water residues from Rosmarinus of fi cinalis L. leaves determined by DPPH assays", *Comtes Rendus Chim.*, 19(6), 754-765, 2016.

[18] Khajeh, M., Yamini, Y., and Shariati, S., "Food and Bioproducts Processing Comparison of essential oils compositions of Nepeta persica obtained by supercritical carbon dioxide extraction and steam distillation methods", *Food Bioprod. Process.*, 88, 227-232, 2008.

[19] Wenqiang, G., Shufen, L., Ruixiang, Y., Shaokun, T., and Can, Q., "Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods", *Food Chem.*, 101, 1558-1564, 2007.

[20] Kim, S.J., Vassão, D.G., Moinuddin, S.G.A., Bedgar, D.L., Davin, L.B., and Lewis, N.G., "Allyl/propenyl phenol synthases from the creosote bush and engineering production of specialty/commodity chemicals, eugenol/isoeugenol, in Escherichia coli", *Arch. Biochem. Biophys.*, 541, 37-46, 2014.

[21] Subramanian, R., Subbramaniyan, P., Ameen, J., and Raj, V., "Double bypasses soxhlet apparatus for extraction of piperine from Piper nigrum", *Arab. J. Chem.*, 9, 537-540, 2016.

[22] Lee, K.G., and Shibamoto, T., "Antioxidant property of aroma extract isolated from clove buds [Syzygium aromaticum (L.) Merr. et Perry]", *Food Chem.*, 74, 443-448, 2001.

[23] Li, Y.Q., Kong, D.X., and Wu, H., "Analysis and evaluation of essential oil components of cinnamon barks using GC – MS and FTIR spectroscopy", *Ind. Crop. Prod.*, 41, 269-278, 2013.

[24] Chan, C.H., Yusoff, R., Ngoh, G.C., and Kung, F.W.L., "Microwave-assisted extractions of active ingredients from plants," *J. Chromatogr. A*, 1218, 6213-6225, 2011.

[25] Kizer, P.E., "Fundamentals of gas chromatography", *International School of Hydrocarbon Measurement*, Oklahoma City, OK, 1995.

[26] Tramel, T.Y., "Fundamentals of gas chromatography", *International School on Hydrocarbon Measurement*, Norman, OK, USA, 1984.

[27] Minitab, "http://www.minitab.com/es-mx/".

[28] Zhang, H.F., Yang, X.H., and Wang, Y., "Microwave assisted extraction of secondary metabolites from plants: Current status and future directions", *Trends Food Sci. Technol.*, 22, 672-688, 2011.

[29] Guntero, V.A., Kneeteman, M.N., and Mancini,P.M., "Preparation of Gel Alcohol Flavored with Essential Oils. An Employ of Laboratory Techniques in the Organic Chemistry Study", *World J. Chem. Educ.*, 5(3), 86-90, 2017.