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Characterization of the raw essential oil eugenol extracted from *Syzygium aromaticum* L.

Amanda L. Santos · Gilberto O. Chierice · Kenneth S. Alexander · Alan Riga · Ellen Matthews

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Abstract Eugenol is the main volatile compound extracted oil from clove bud, *Syzygium aromaticum* L., and used in traditional medicine, as a bactericide, fungicide, anesthetic, and others. Its extraction was performed using hydrodistillation which is the most common extraction technique. Its components and thermal behavior were evaluated using gas chromatography (GC) and differential scanning calorimetry (DSC), which provide a better characterization of these natural compounds. This extracted product was compared to the standard eugenol results. The GC results suggested ~90% eugenol was found in the total extracted oil, and some of its boiling characteristics were 270.1 °C for peak temperature and 244.1 J g^{-1} for the enthalpy variation.

Keywords *Syzygium aromaticum* L. · Eugenol · Thermal analysis · Differential scanning calorimetry

Introduction

Today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. There is a continuous and urgent need to discover new compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging diseases. Therefore, researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against several health problems [1].

Aroma chemicals present in natural leaves and flowers have been widely used in aroma therapy since ancient times, suggesting that they have some beneficial health effects in addition to their pleasant odor [2].

Essential oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute very potent natural biologically active agents [3].

They have a complex composition, containing from a few to several hundred constituents, especially hydrocarbons and oxygenated compounds. Both hydrocarbons and oxygenated compounds are responsible for the characteristic odors and flavors, which are formed by aromatic plants as secondary metabolites [3].

Essential oils and their components are widely used in medicine as constituents of different medical products, and in the food industry as flavoring additives. Beyond their antimicrobial effects in food systems they may be considered as an additional intrinsic determinant to increase the safety and shelf life of foods and in cosmetics as fragrances [4].

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A. L. Santos (✉) · G. O. Chierice
Instituto de Química de São Carlos, Universidade de São Paulo, 400 Trabalhador São-carlense Avenue, 13560-970 São Carlos, SP, Brazil
e-mail: amandaluizetto@iqsc.usp.br

A. L. Santos · K. S. Alexander
Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, University of Toledo, 2801 W. Bancroft, Toledo, OH 43606, USA

A. L. Santos · A. Riga
Department of Clinical Chemistry, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115, USA

E. Matthews
Buckeye Pharmaceuticals, Beachwood, OH 44122, USA

Eugenol is isolated from the buds of *Syzygium aromaticum* L. It is the principal chemical constituent of the essential oil of the clove, being ~90% of the total oil amount [5]. It is a colorless or light yellowish fluid extracted from dried flower buds by steam distillation. It has a strong phenolic smell and sharp acrid taste [6, 7]. Generally, these major components determine the biological properties of the essential oils. Eugenol chemical structure is represented in Fig. 1.

It has been widely used and well known for its medicinal properties. Traditional uses of clove oil include those in dental and medical care and practice as an analgesic. More recently, additional pharmacological properties of eugenol were demonstrated including potent fungicidal, bactericidal, anesthetic, analgesic, anti-oxidant, anti-inflammatory properties, antiallergic, anticarcinogenic, antimutagenic activity, as well as insecticidal [8, 9].

A range of techniques, varying in cost and level of complexity, may be used for extraction of plant material. The extraction product can vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage. Therefore, in order to obtain essential oils of constant composition, they have to be extracted under the same conditions from the same organ of the plant [4].

Steam distillation has traditionally been applied for essential oil recovery from plant materials. Hydrodistillation is the most common extraction technique used to obtain the volatile fraction from aromatic plants, but it is time-consuming and needs large amounts of sample [10].

Most of the commercialized essential oils are chemotyped by gas chromatography (GC). Gas chromatographic methods are very useful in the determination of essential oils, and offer a significant improvement in sensitivity over previous methods reported [11].

Owing to the widespread use of GC in routine essential oil analysis, it is necessary that good GC methods are developed and that these are thoroughly validated [11].

The characterization of the essential oils involves a series of physical parameters including melting and boiling temperatures and others. The determination of such parameters is not always easy and depends on the technique employed [12].

Differential scanning calorimetry (DSC) is an appropriate instrument for the measurement and characterization of the thermal properties of materials [13]. The application of DSC to the analysis of oils and fats is well known. It also has been suggested as a valuable tool for the characterization of oil from vegetable resources [14].

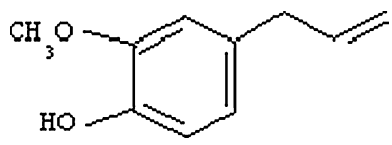

Fig. 1 Chemical structure of eugenol

Owing to the new attraction for natural products, such as essential oils, despite their wide use and being familiar to us as fragrances, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment [4].

Based on these applications, the main purpose of this study was to determine the conditions necessary for essential oil extraction and to characterize it using GC. Then, using the DSC technique to determine its thermal behavior and compare with the eugenol standard results.

Experimental

After the extraction parameters were optimized, the dried bud cloves from *S. aromaticum* L. were water-distilled for 5 h, using a modified Clevenger-type apparatus, at 100 °C. The total amount of clove used was 20 g, using 1:2 (w/w) of solvent proportionally. The extracted oil was kept under refrigeration and used without previous purification. Standard eugenol was purchased from Fluka (assay 99%).

A Shimadzu model GC-2010 GC equipped with a flame ionization detector (FID) and an auto injector AOC-2OI (Shimadzu) was used to analyze all the samples. Separation was achieved using a middle polar capillary column RTx-5MS with length 30 m, diameter 0.25 mm and film thickness 0.25 μm.

The injector and detector temperature conditions were at 280 and 300 °C, respectively. The oven temperature program was started from 100 to 230 °C at 8 °C min^{-1}, then from 230 until 280 °C at 15 °C min^{-1}, and kept constant at 300 °C during 2 min. Hydrogen was used as the carrier gas with a flow rate of 30 mL min^{-1}.

The GC–FID parameters used in the method development were based on the boiling point. The identification of the oil components was established from their GC retention indices, by comparison to their standard.

A standard solution at 2.6×10^{-2} mol L^{-1} was prepared for the standard eugenol, then the linear proportionality was established between 5.2×10^{-3} mol L^{-1} and 1.1×10^{-3} mol L^{-1}.

The standard solution concentration for the extracted eugenol was prepared and used to determine its concentration, by associating its peak area and the standard eugenol analytical curve.

Thermal behavior studies were developed for the eugenol standard and extracted oil samples using the same experimental conditions. All experiments were performed

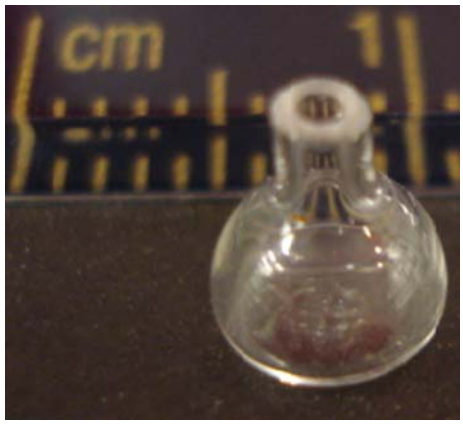

Fig. 2 Photography of the glass sample holder

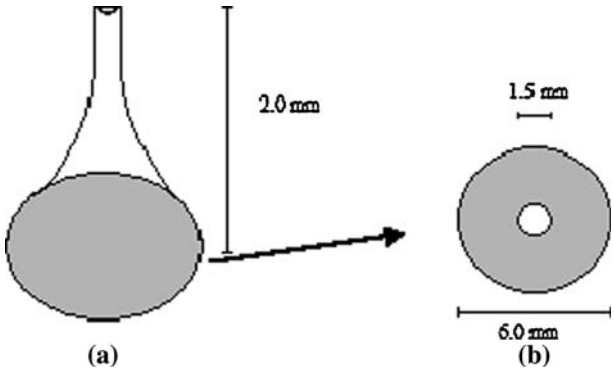

Fig. 3 **a** Side view and **b** top view drawing of the glass sample holder

using a DSC-910 Calorimetric Module attached to Liquid Nitrogen Cooling System (LNCA) (TA Instruments).

The optimized conditions for this study were 100 °C for the initial and 350 °C for the final temperatures, at 10 °C min^{-1} heating rate and using 17 mg as the sample mass.

A 20 µL homemade capillary long neck glass sample holder was used. The capillary neck is 2 mm long with an internal diameter of around 1.5 mm.

These capillaries were fabricated in the glass shop of the University Federal of São Carlos (São Carlos/SP, Brazil), and grouped as pairs by their mass capacity similarity. An empty capillary was used as a reference and another was filled with the desired amount of essential oil with the help of a 1.0 mL syringe.

The photography of the glass sample holder is shown in Fig. 2. A side (a) and top (b) view drawing of this glass holder is in Fig. 3.

Results and discussion

The parameters for the eugenol extraction was studied through some conditions variation, such as the time, sample mass, temperature, and others. The solvent choice was water due to be the universal solvent, which avoids some purification steps during its next utilization. The best response was obtained employing 20 g of clove mass, 200 mL of water at 100 °C, and 5 h of extraction. The tested conditions can be seen in Table 1.

Table 1 Parameters' optimization for eugenol extraction

Clove mass	Extraction time	Volume measured	Volume desired
20 g	2 h	0.47 mL	0.44 mL
20 g	3 h	1.54 mL	1.51 mL
20 g	4 h	0.75 mL	0.73 mL
20 g	5 h	2.39 mL	2.26 mL
20 g	6 h	2.03 mL	1.96 mL

The oil volume measured was established using the total extraction volume. The volume desired is the theoretical, which does not match exactly with the experimental results due to the presence of others constituents in the total oil extracted.

Gas chromatographic data for the extracted oil sample and standard eugenol were compared. Chromatogram for standard eugenol showed one sharp peak with approximately 8.121 min retention time. The extracted essential oil chromatogram displayed three well defined peaks, which is seen in Fig. 4. The first peak at 8.117 min is associated with the eugenol, and the others at 9.191 and 10.520 min are the impurities, which may result from different compounds extracted together with the eugenol from *S. aromaticum* L.

The standard eugenol analytical curve and the statistical parameters found in the GC quantification can be seen in Fig. 5.

Assessing the obtained results, the extracted essential oil concentration found using the analytical curve was 2.7×10^{-3} mol L^{-1}. This result is well adapted in the concentration range, since the elements do not divert from the analytical curve.

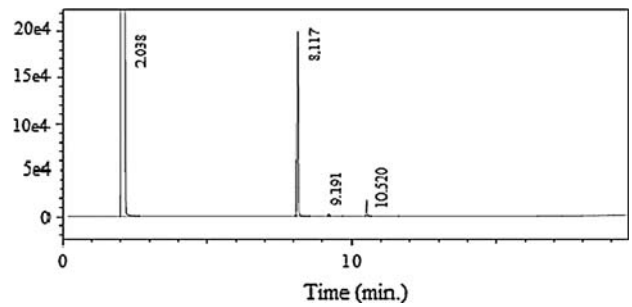

Fig. 4 Chromatogram of extracted essential oil from *Syzygium aromaticum* L

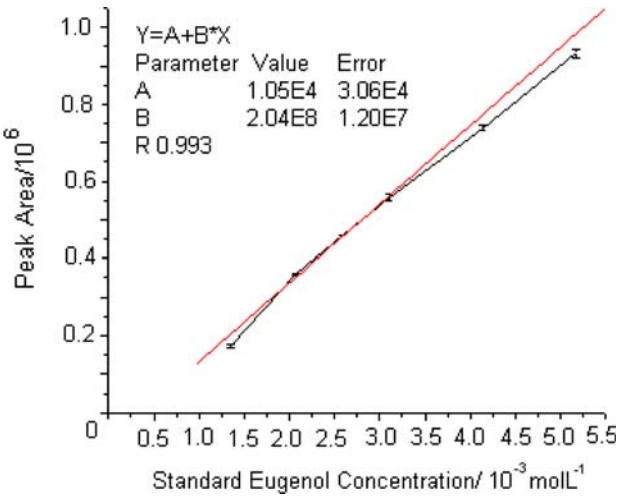

Fig. 5 Standard eugenol analytical curve

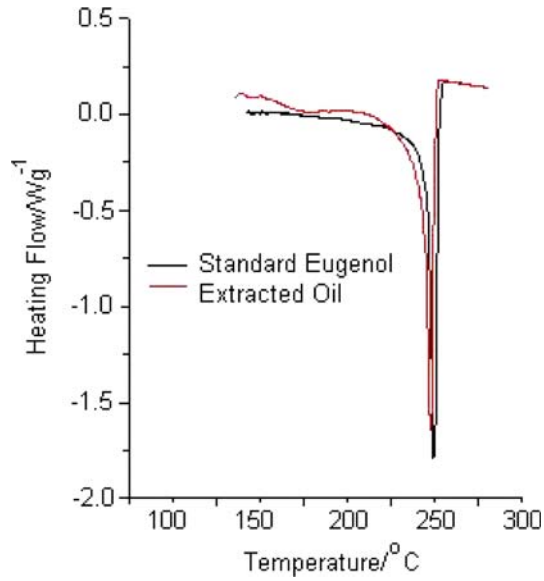

Fig. 6 DSC curves for standard eugenol and extracted oil from *Syzygium aromaticum* L

The repeatability and middle accuracy results obtained for the instrument are acceptable, since they are within satisfactory range for chromatographic methods that is 5%.

In the thermal behavior study pays attention to the sample holder geometry used in the essential oil analysis which plays an important role in the shape of the DSC curve, and is considered one parameter that can influence a DSC curve profile [12].

The glass holder was chosen due to the comparative results found in studies using the same DSC conditions and sample, but with different pans, such as glass and aluminum. The sample holder is relevant as it improved the definition and shape of DSC peak profiles [12].

This technique was employed by Chaar et al. in the IQSC laboratory and used in studies of another natural product, for e.g. linalool. In this study, the peak temperature and enthalpy of boiling were 195 °C ± 1 and 172 J g^{-1} ± 1, respectively for five successive runs [12].

In the DSC curve obtained with the glass sample holder it is clear that there is a well-defined sharper peak than for analysis developed with the aluminum pan, which produced a broad peak for the same thermal event. The well defined peak is related to a more accurate temperature result for samples with specific characteristics, for example volatile samples. The baseline changes were observed in both experiments for the volatile characteristics of the sample.

The DSC thermograms for the standard and extracted eugenol can be seen in Fig. 6. Both DSC curves showed a well defined and sharp endothermic event, which happens almost within the same temperature range. These events are attributed to the essential oil boiling process. One can observe that the entire standard eugenol boiling process is over a narrow temperature range and demonstrates a sharper event than that observed for the extracted eugenol.

The peak area, the temperature and the enthalpy of boiling were obtained with the utilization of the integrate peak linear option in the program, Universal Analysis (2000) V4.5A (TA Instruments).

As can be observed, the proposed glass sample holder produces excellent results with a sharp well defined peak that allows a more accurate boiling temperature determination.

The data values for the standard eugenol boiling process were 274.5 °C for peak temperature (T_p), 113.5 °C onset temperature (T_o), and 284.0 J g^{-1} for the enthalpy of boiling (ΔH_b). The data for the extracted eugenol was similar to the standard. It shows the same characteristic peak, but with some important changes in the thermal properties, such as its boiling temperature range and energy requirement. Its boiling data were 270.2 °C for T_p, 156.2 °C T_o and 244.1 J g^{-1} ΔH_b.

This temperature range difference between the standard and extracted eugenol is due to the utilization of the extracted essential oil without previous purification, it means that the impurities present can cause some physical chemical variations, such as a variation in the boiling temperatures and the process energy.

Therefore, the essential oil impurities have an important influence on the total oil physical chemical characteristics, even if it is just a small percentage, such as 2%. The other compounds behave as second oil compound, which change the pure oil properties, resulting in a different product from the natural *S. aromaticum* L.

This characteristic is an important feature since the particularity is conferred for each essential oil, by the

difference between each process using the natural resources, such as its composition, then resulting in significant differences in the results.

Conclusions

Therefore, it is possible to conclude that there is a higher concentration of Eugenol in the extracted oil from *S. aromaticum* L., around 93% of the total extracted amount. From the GC and DSC results obtained it is clear that the presence of impurities do not have significant effects on the thermal behavior. It is confirmed with profile analysis which presents the peak temperature and enthalpy of boiling were very similar, with 11% and 14% the differences respectively.

The glass holder proved is a useful tool for determination of thermal behavior in essential oils, especially due to their volatile characteristics.

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