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Chemical evaluation and thermal analysis of the essential oil from the fruits of the vegetable species *Pimenta dioica* Lindl

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Abstract The vegetal species *Pimenta dioica* Lindl, popularly known as Jamaican pepper, is a 6–15 m tall tree, which belongs to the Mirtaceae family. Its fruits have an essential oil of great economic value in the international market, due to its high level of eugenol (its major compound), which is largely used in chemical and pharmaceutical industries. In this work, the extraction of the essential oil from the fruits of *Pimenta dioica* Lindl was carried out by the hydrodistillation method, using a modified Clevenger system. It was observed that the volume of the extracted oil reaches a maximum at 4 h, with a yield of 2.7% (m/m). The essential oil was characterized by physico-chemical analyses, such as density, refraction index, ethanol solubility, color, and appearance, besides UV–vis and infrared spectroscopy and gas chromatography/mass spectrometry. Thus, eugenol was confirmed as the major component of the essential oil of *Pimenta dioica* Lindl (77%). The technique of differential scanning calorimetry (DSC) was used for the determination of boiling point of the sample of essential oil from the fruits of *Pimenta dioica* (L.).

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Introduction

Pimenta dioica Lindl is a medium-sized tree that belongs to the Mirtaceae family [1]. It is commonly named Jamaican or Jamaica pepper. In Brazil it is called "pimenta de coroa" and "murta pimenta" [2]. The species presents the following taxonomic position: Kingdom: Vegetable; Phylum: Angiosperma; Class: Dicotiledoneae; Order: Mirtales; Familya: Mirtaceae; Genus: Eugenia; Species: *Pimenta dioica* Lindl [1, 3].

Besides essential oils, also phenolic acids, flavonoids, catechins, phenylpropanoids, and galloyl glucosides have been isolated from *Pimenta dioica* (L.). Galloyl glucosides showed radical-scavenging activity and phenylpropanoids exhibited antioxidative activity. Preliminary tests showed that both ethanol and water extracts of *Pimenta dioica* (L.) inhibited HDC activity sufficiently [4].

The essential oil from *Pimenta dioica* (L.) can be extracted from the barks, leaves and fruits, with quite variable yields (1.5–4.5%) [2]. It unites the scent and flavor characteristic of cloves, cinnamon, and nutmeg and it can be used as an important source of eugenol, its main component (70–80%) [2]. It is employed in food industry—mainly meat and canning industries—as well as in perfume compositions and cosmetic products [5]. Besides, essential oils are widely used in medicine due to its therapeutic properties [1, 4, 6–10], as: analgesics, antirheumatics, antimicrobials, antioxidants, antibacterials, antiseptics, acaricidals, carminatives, muscle relaxants, rubefacients, stimulants, and tonics. It can also be helpful for the digestive system, for cramp, flatulence, indigestion, and nausea. Furthermore, it has recently become a focal point of research dealing with seeking new environmentally safe plant pesticides. Many works have proven its insecticide activity and it can be used as a natural repellent [4].

The study of substances extracted from plants has been shown indispensable along the time, either by the great biological diversity in Brazil or by the potential of such extraction. Thus, essential oils represent a feasible alternative in several works involving substances of vegetable origin and thus they should be properly characterized. The characterization of 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, and temperature ranges are often used to express such physical constants instead of a unique temperature [11].

The present work had the purpose of characterizing the essential oil extracted from the fruits of the species *Pimenta dioica* (L.), using the techniques of UV–Vis, infrared spectroscopy, gas chromatography coupled to mass spectrometry and thermal analysis.

Experimental

The fruits from *Pimenta dioica* (L.) were collected at the agribusiness company Cooperativa Agrícola Mista do Projeto Onça Ltd., in Taperoá, state of Bahia, Brazil, in August 2005. The essential oil was extracted from 30 g of fruits with 300 mL of distilled water, by hydrodistillation in a modified Clevenger system, with the temperature being kept at 100 °C. Later the essential oil was dried by percolation through anhydrous Na_2SO_4. These operations were repeated and triplicate samples were taken and were stored in glass containers under refrigeration, in order to avoid possible losses of volatile constituents. The mass/mass yields were calculated using the density measurements and the essential oil volumes obtained in the extraction system.

For the characterization of the physical properties of the essential oil the density, the refraction index, the solubility in ethanol at 70% (v/v), color and appearance were determined. For the density calculation, a volumetric flask of 1.0 mL, previously dried and weighted, was used, in which the samples of essential oil were placed. For the ethanol solubility determination, a solution of ethanol in water at 70% (v/v) was utilized, keeping a constant volume of essential oil and adding growing amounts of the alcoholic solution until the complete solubilization of the essential oil was achieved. For the determination of the refraction index, capillary glass tubes were utilized for the addition of essential oil samples directly over the Flint prism of the refractometer, at a temperature of 25 °C. For the determination of color and appearance, a visual comparison of the color of the essential oil with color standards and a visual assessment in terms of transparency and limpidity were performed, respectively.

UV–vis absorption spectra were obtained in the HP 8451A spectrometer, using essential oil samples diluted in a 60% (v/v) ethanol solution in water.

The Nicolet FTIR spectrophotometer model 5SXC, equipped with a TGS detector, was utilized for the analysis of essential oil in KBr, in the 4000–400 cm^{-1} range.

Identification of chemical constituents of essential oils

The essential oil was analyzed on a VARIAN 2100 gas chromatograph/mass spectrometer. The separation was carried out on a VF-1 ms (15 m × 0.25 mm × 0.10 μm) column. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The column temperature was programmed from 60 to 200 °C (heating rate of 8 °C min^{-1}), followed by an increase to 290 °C (rate of 15 °C min^{-1}). The injector temperature was set at 270 °C and the temperature of the mass detector and interface were set at 200 °C. A sample volume of 1.0 μL was injected using split mode (split ratio of 1:50). The essential oil components were identified by comparison of the mass spectra with existing standards of the software library (Mass Spectral Database NIST02). The content of each component was calculated based on the ratio of its peak area to the total peak area.

Thermal analysis

The thermogravimetric curves (TG) were obtained in a Shimadzu TGA-50 thermobalance in a nitrogen atmosphere and a flow rate of 50 mL min^{-1}. The DSC curves were performed in a SDT 2920 thermal analyzer from TA Instruments, temperature range 25–500 °C, with a heating rate of 10 °C min^{-1} in a nitrogen atmosphere and a flow rate of 50 mL min^{-1}.

Results

Characterization of essential oil

The relationship between the essential oil yield and the extraction time, for a constant mass of 30 g of dried powdered fruits is shown in Fig. 1. The maximum yield was obtained for an extraction time of 4 h, in which case a volume of 0.84 mL of essential oil was obtained, being attributed to this case a value of 100%. For extraction times higher than 4 h, the yield was kept unchanged. This was observed due to the growing diminishment of essential oil in the extract, as a consequence of the distillation. Thus,

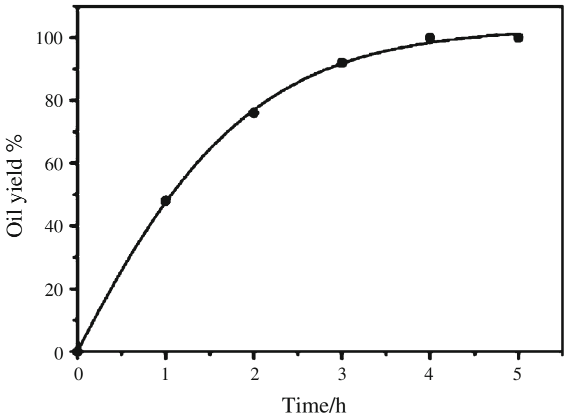

Fig. 1 Extraction kinetics of the essential oil of *Pimenta dioica* (L.)

taking into account the economics of hydrodistillation, extraction times higher than 4 h are not recommended.

The physicochemical properties of the essential oil from the fruits of *Pimenta dioica* (L) are shown in Table 1, as well literature data, for comparison. As essential oils are complex mixtures and always contain a large number of constituents, their physicochemical properties are influenced by factors such as time of collection, different types of soil, and fruit storage conditions, and storage time. Thus, the differences found for the refractive index and density can be attributed to different collection periods and locals, which differ from the experimental conditions adopted by Mouchrek Filho [12].

Spectrophotometric analyses

The UV–vis absorption spectra for the (A) 60% v/v ethanol/water solution, (B) essential oil, and (C) for the eugenol standard are illustrated in Fig. 2.

UV–vis absorption spectra of essential oil and eugenol showed maximum absorption peaks at 230 and 280 nm attributed to $\pi \rightarrow \pi^*$ transitions, characteristic of unsaturated functional groups [13]. The overlapping of the spectra of eugenol and the essential oil showed similar absorptions, thus constituting an indication of the presence of eugenol in the essential oil.

Table 1 Physicochemical properties of the essential oil extracted from fruits of the species *Pimenta dioica* (L.)

Physicochemical properties	Essential oil	
	Present work	Reference [12]
Density/g mL^{-1}	0.949	0.997
Refraction index (N_D 25°)	1.332	1.521
Solubility in alcohol at 70% (v/v)	1:2	1:2
Yield/%	2.8	1.6
Color	Yellow	Yellow
Appearance	Limpid	Limpid

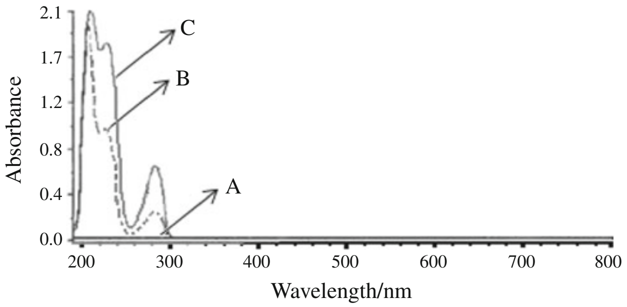

Fig. 2 UV–vis absorption spectra of (*A*) 60% v/v ethanol/water solution; (*B*) essential oil; (*C*) eugenol standard

FTIR infrared spectroscopy analyses

The absorption spectra in the infrared region of the eugenol standard and of the essential oil extracted from the fruits of species Pimenta *dioica* (L.) showed that the absorption bands quite coincide. Strong absorption bands at the regions of 3500 and 1370 cm^{-1} were ascribed to the stretching of OH groups. The bands at 2980 and 2920 cm^{-1} were attributed to the aliphatic C–H stretching and the band at 3100 cm^{-1} to the aromatic C–H stretching. The bands at 1270 and 1615 cm^{-1} are attributed to the C–O stretching of the C=C bond, and to the C–C aromatic stretching [13]. These observations for the essential oil of *Pimenta dioica* (L.) are quite justifiable, as eugenol is the main component of this essential oil, according to the chromatographic data.

Gas chromatograph/mass spectrometry analyses

Figure 3 shows the chromatogram of the essential oil extracted from the fruits of species *Pimenta dioica* (L.). The selection of the main peaks and the determination of their mass spectra allowed the identification of seventeen compounds by comparison with the mass spectra from the NIST02 mass spectral library. For instance, peak 10 with a retention time of 7.2 min corresponds to eugenol.

By normalizing the peak areas of the chromatogram (Fig. 3) were calculated the amounts of the components contained in the essential oil, of which eugenol was the main constituent (Table 2).

Thermal analysis of the essential oil

Thermogravimetric analyses

The thermogravimentric curve of the essential oil from the fruits of *Pimenta dioica* (L.) showed that the mass loss of the sample in 1 h was of approximately 25%, with the temperature varying from 25 to 40 °C. After 10 h of exposition at a temperature about 40 °C, the overall mass

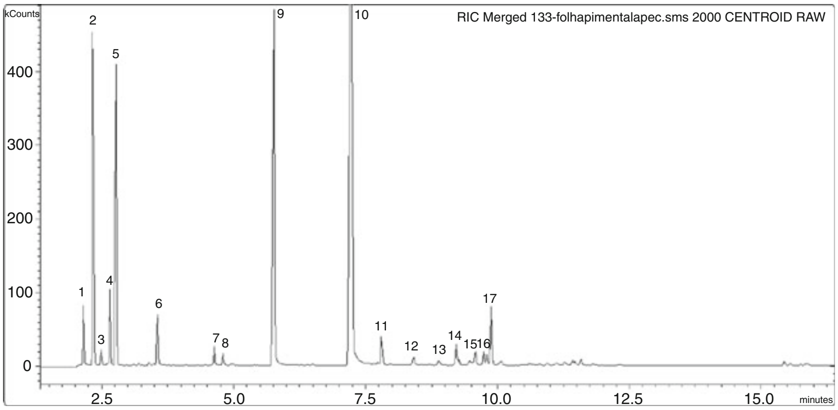

Fig. 3 Chromatogram of the sample of essential oil from the fruits of *Pimenta dioica* (L.)

Table 2 Contents of the components identified by the mass spectra of the fruits of *Pimenta dioica* (L.)

Peak	T_r/min	Percentage/%
1 (1-octen-3-ol)	2.15	1.40
2 (β-pinene)	2.33	6.52
3 (α-pinene)	2.49	0.28
4 (*o*-cymene)	2.66	1.94
5 (limonene)	2.77	4.09
6 (linalool)	3.56	0.64
7 (sabinene)	4.64	0.22
8 (α-terpineol)	4.80	0.13
9 (5-indanol)	5.76	5.88
10 (eugenol)	7.23	76.98
11 (α-cubebene)	7.80	0.35
12 (cariofilene)	8.41	0.09
13 (α-cariofilene)	8.90	0.08
14 (γ-murolene)	9.22	0.25
15 (α-candidene)	9.58	0.19
16 (α-murolene)	9.74	0.22
17 (δ-candidene)	9.88	0.76

T_r = retention time

loss was of around 47% (Fig. 4). This is possibly due to the volatilization of some components of the essential oil.

The study of the isothermal TG curve of the essential oil from the fruits of *Pimenta dioica* (L.) was shown to be important when scent essences are used as bee baits, as the results point out the percentage of mass loss as a function of time. This allows for the refilling of the proper amount of the essential oil, as nowadays the refill of the essence is randomly made. Thus, the data obtained in the present work will be helpful for future studies.

Differential scanning calorimetry

The DSC curves obtained in closed aluminum crucibles are shown in Fig. 5. The DSC curve for the eugenol standard displayed one only endothermic peak, at 258 °C

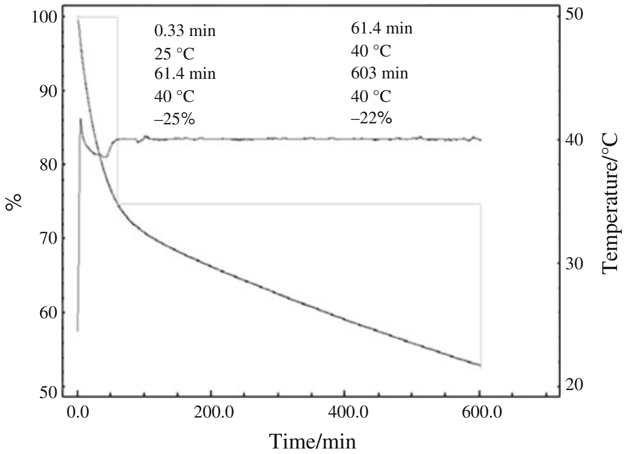

Fig. 4 Isothermal thermogravimetric curve, at 40 °C, of the essential oil from *Pimenta dioica* (L.)

(314 J g^{-1}), attributed to the eugenol volatilization. On the other hand, the DSC curve of the essential oil displayed two endothermic peaks at the temperatures (T_P) of 109 °C (9.5 J g^{-1}) and 256 °C (219 J g^{-1}), ascribed to the volatilization of water and eugenol, the major component of the essential oil, respectively.

The similarity between the DSC curves of eugenol and essential oil, for temperatures higher than 139 °C, allowed correlating the value of T_P of the eugenol standard and of the essential oil with the boiling point of eugenol, 254 °C [14]. The differences between the boiling points and the enthalpies, hence, may be justified by the presence of other components at their respective concentrations in the essential oil. The DSC curves also show evidences of the eugenol decomposition, due to the broadening of peak, but without impairing the determination of the boiling point of the essential oil from *Pimenta dioica* (L).

The DSC curves of the samples, when they were obtained in holed aluminum crucibles, showed the endothermic transitions shifted toward lower temperatures, owed to the decrease of the vapor pressure (Fig. 6). For the eugenol standard was observed an endothermic transition

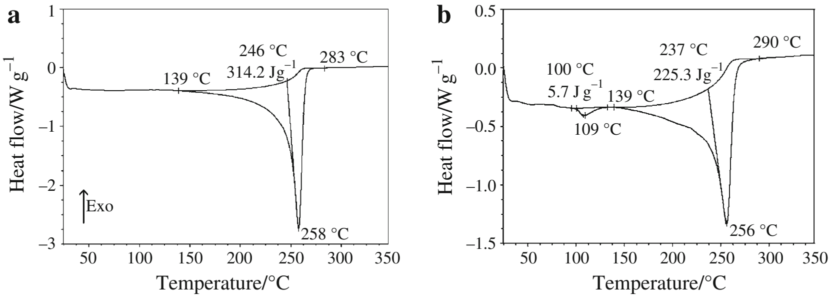

Fig. 5 Thermoanalytical DSC curves obtained in closed aluminum crucibles for: **a** eugenol standard and **b** essential oil of *Pimenta dioica* (L.)

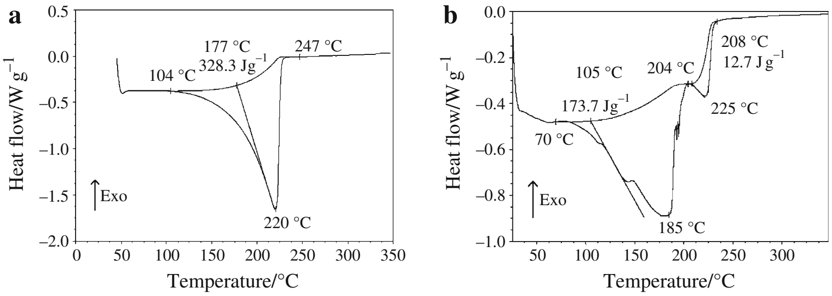

Fig. 6 Thermoanalytical DSC curves obtained in holed aluminum crucibles for: **a** eugenol standard and **b** essential oil of *Pimenta dioica* (L.)

with a peak temperature of 220 °C (328 J g^{-1}) with the same profile of the DSC curve obtained with the closed aluminum crucible. However, for the essential oil the curve profile was different, being noticed five endothermic transitions; the first was attributed to the water volatilization and the transitions between 105 and 203 °C were ascribed to the decomposition of the essential oil. It is believed that minor components of the essential oil contribute to the decomposition, once the transition between 204 and 237 °C is owed to the eugenol volatilization.

New studies should be carried out aiming at increasing the efficiency of DSC as a tool for the scientific investigation of other essential oils. The data obtained can also be used for the certification of essential oils, once in many instances essential oils of high economic values are adulterated.

The comparison between the DSC curves of Figs. 5 and 6 shows that the best condition to attribute the boiling point for an essential oil is using a closed aluminum crucible. The good correlation between the T_P values of the essential oil of *Pimenta dioica* (L.) and the eugenol standard may be justified by the fact that eugenol be the main component of the essential oil. Nevertheless, the DSC curves obtained with holed aluminum crucibles may be useful for the certification of essential oils, once different behaviors were noticed for the eugenol standard and the essential oil, what was not observed by the techniques of UV–vis and infrared spectroscopies.

Conclusions

Essential oils extracted from the fruits of *Pimenta dioica* (L.), using a Clevenger apparatus yielded 0.82 g (2.7% m/m). Eugenol was the major component (77%) among the seventeen compounds identified from the volatile chemical constituents. DSC can be used to determine the boiling point of the major component of the oil essential, as the DSC peak variation between the essential and standard eugenol was only 1.7 °C and these value correlated with the boiling point of eugenol reported in the literature.

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