

## Article

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# A new approach for quantifying furanodiene and curzerene. A case study on the essential oils of *Eugenia uniflora* (pitangueira) leaves

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**Abstract:** The essential oil obtained from the leaves of *Eugenia uniflora* L., Myrtaceae, which grows in the Brazilian savannah, was studied by gas chromatography mass spectrometry (GC-MS). Furanodiene (1.2%) was thermally rearranged to curzerene (85.1%) to produce a combined content of 86.3%. GC analysis carried out under mild conditions (with a constant temperature of 100 °C) showed that the furanodiene concentration was three-fold greater than the curzerene concentration, *i.e.*, the essential oil contained 64.7% furanodiene and 21.6% curzerene. Germacrene B also rearranged to  $\gamma$ -elemene and the concentration of both was 2.3%. Special care should be taken when conventional gas chromatography analysis is used for quantifying compounds that can rearrange at high temperatures.

## Introduction

*Eugenia uniflora* L., Myrtaceae, known as "pitangueira", is a very common plant in Brazil. It is a native species that is found in coastal areas ranging from the Southern Highlands to the northeast, and in semi-deciduous forests (Lorenzi, 2000). A tea made from the leaves has been used in folk medicine to lower fever and blood pressure and to combat infections (Consolini et al., 1999). There are also reports of strong antibacterial, cytotoxic, molluscicidal, larvicidal and antifungal activities of *E. uniflora* L. against pathogenic agents (Lima et al., 1993; Holetz et al., 2002; Souza et al., 2004; Ogunwande et al., 2005; Leite et al., 2009; Costa et al., 2010).

Several studies have examined the composition of oil from the leaves of *E. uniflora* L. from different regions in Brazil and other countries (Weyerstahl et al., 1988; Morais et al., 1996; Henriques et al., 1996; Maia et al., 1999; Holetz et al., 2002; Melo et al., 2007; Costa et al., 2009; Peixoto et al., 2010). Such studies have shown that in the essential oil of pitangueira leaves the composition and concentration of components are very complex.

Further, there is some controversy regarding furanodiene and curzerene retention times due to the rearrangement of furanodiene to curzerene during the essential oil extraction process and/or during conventional

gas chromatographic analysis in which temperatures over 200 °C are employed. Some authors have reported the same retention time for both components (Melo et al., 2007), while others reported differences (Morais et al., 1996; Mölleken et al., 1998; Baldovini et al., 2001; Baser et al., 2003; Bertoli et al., 2004; Oguwande et al., 2005; Yang et al., 2005, 2007; Raj et al., 2008; Wong et al., 2009; Joshi et al., 2009).

The aim of the present work was thus to evaluate the chemical composition of the essential oil from *E. uniflora* L. leaves from the Brazilian savannah near Uberlândia (Minas Gerais State) by conventional gas chromatography. In addition, in order to elucidate the retention times of both furanodiene and curzerene and the extent of rearrangements occurring during conventional gas chromatography, a gas chromatographic run was carried out under mild conditions (isotherm program at 100 °C) with a long run time (655 min).

## Materials and Methods

### Plant material

Leaves of *Eugenia uniflora* L., Myrtaceae, were collected in August-September 2009, from mature trees growing in the Panga Ecological Reserve (19°09'20"-

19°11'10" S and 48°23'20"-48°24'35" W), at a local altitude of about 800 m, near the city of Uberlândia, Minas Gerais, Brazil. The plants were identified by Prof. Dr. Rosana Romero from the Universidade Federal de Uberlândia and plant collection was authorized by Biology Institute, UFU (IB01/2009). A voucher specimen (56343) of the red fruits biotype has been deposited in the Herbarium Uberlandenses at the Universidade Federal de Uberlândia.

#### Extraction procedure

Essential oils from air-dried leaves (seven days at room temperature, 250 g) of *E. uniflora* L. were submitted to hydrodistillation for 3 h in a Clevenger-type apparatus. The distilled product was extracted three times with 20 mL dichloromethane to obtain the essential oil. The organic layer was dried using anhydrous sodium sulphate and the percentage content was calculated on the basis of the dry weight of the plant material. Prior to further analysis, the oil was stored at -20 °C.

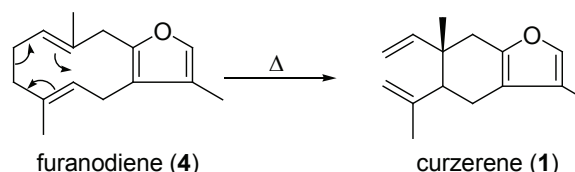
#### Gas chromatography mass spectrometry (GC-MS) analysis

The volatile compounds were analysed using a gaseous chromatograph system coupled to a mass spectrometer (Shimadzu GC-MS, GC-17A/QP-5000). The operating conditions were: capillary column J&W, DB-5, 30 m, 0.25 mm i.d., film 0.25 µm; the temperature program used was: 60 °C to 240 °C (3 °C min<sup>-1</sup>), injector at 220 °C, interface at 240 °C; electronic impact at 70 eV (Adams, 2001). The fragments were collected from 40 to 450 u. Identification of oil components was based on the Kovats index, comparison of their mass spectral fragmentation patterns with the Mass Spectra libraries (Wiley 139, 275 and 7 and Nist 127), published data (Weyerstahl et al., 1988; Henriques et al., 1993; Lima et al., 1993; Morais et al., 1996; Maia et al., 1999; Ogunwande et al., 2005; Melo et al., 2007; Costa et al., 2009, 2010; Peixoto et al., 2010) and myrrh essential oil injection (Mölleken et al., 1988). The GC-MS quantification was obtained using the total ions chromatogram (TIC) and was expressed as an average of the data obtained for three samples of extracted leaf oil. For gas chromatography analysis under mild conditions, the same parameters as above were used excepting the temperature which was maintained at 100 °C; the run time was delayed to 655 min.

#### Results and Discussion

The gas chromatogram of the essential oil of *E. uniflora* L. leaves from the Uberlândia savannah is shown in Figure 1. The main component is curzerene (1), which presents a sharp peak (n° 10 in Figure 1) and a broad one between 39 and 44 min, whose

mass spectrum is identical to the mass spectrum of curzerene. This broad peak results from the thermal rearrangement of furanodiene (4) to give curzerene through a [3.3] sigmatropic reaction, also known as the Cope rearrangement, during the chromatographic run (Mölleken et al., 1988; Weyerstahl et al., 1988; Baldovini et al., 2001). As curzerene is formed in the column, its retention time varies continuously.

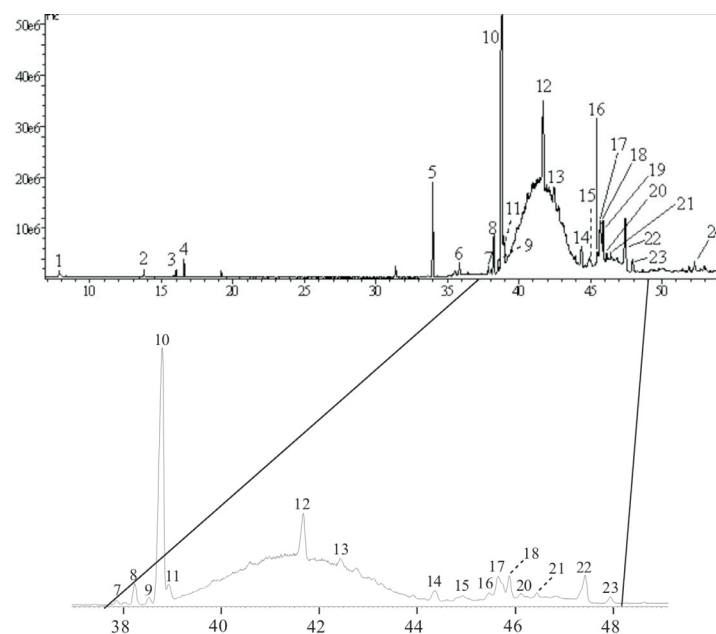


The identified compounds in Figure 1 and their concentrations are listed in Table 1. Thus, the main components are: curzerene (2, 85.1%), germacrene B (3, 2.0%), β-elemene (1, 1.9%), furanodiene (4, 1.2%), selin-11-en-4-alfa-ol (1.0%), germacrene D (0.9%), bicyclogermacrene (0.9%), and atractilone (0.8%). Monoterpenes (0.5%), sesquiterpenes (6.5%) and oxygenated sesquiterpenes (90.3%) totalled 97.3% of the essential oil.

The great difference displayed by the Uberlândia pitangueira essential oil was in terms of on the high curzerene concentration (85.1%), as such a value has been not reported so far. Excepting this, the minor components present in the sample are well known in essential oils from pitangueira leaves, from South to North Brazil and are also similar to oils from Nigeria. Curzerene is also the principal component in essential oils from pitangueira leaves from Rio de Janeiro (50.2%, Melo et al., 2007), Goiás (42.6%, bright red fruit pitangueira, Costa et al., 2010), Goiás (34.8%, Peixoto et al., 2010) and Nigeria (19.7%, Ogunwande et al., 2005).

Comparing the essential oil composition of Uberlândia pitangueira leaves with those from Brazil and Nigeria, we can conclude that the composition of the essential oil and the concentrations of the components are very complex and, thus, the variability could be due to biotypes and environmental factors of each region (Costa et al., 2009).

As furanodiene was rearranged during the gas chromatographic assay, the real concentrations of furanodiene and curzerene in the *E. uniflora* L. leaf essential oil are not those shown in Table 1. To correctly determine these concentrations, gas chromatographic analysis was carried out using mild heating conditions (isotherm program at 100 °C, the steam temperature used in essential oil extraction) and a long run time (655 min) to assure that both compounds would be

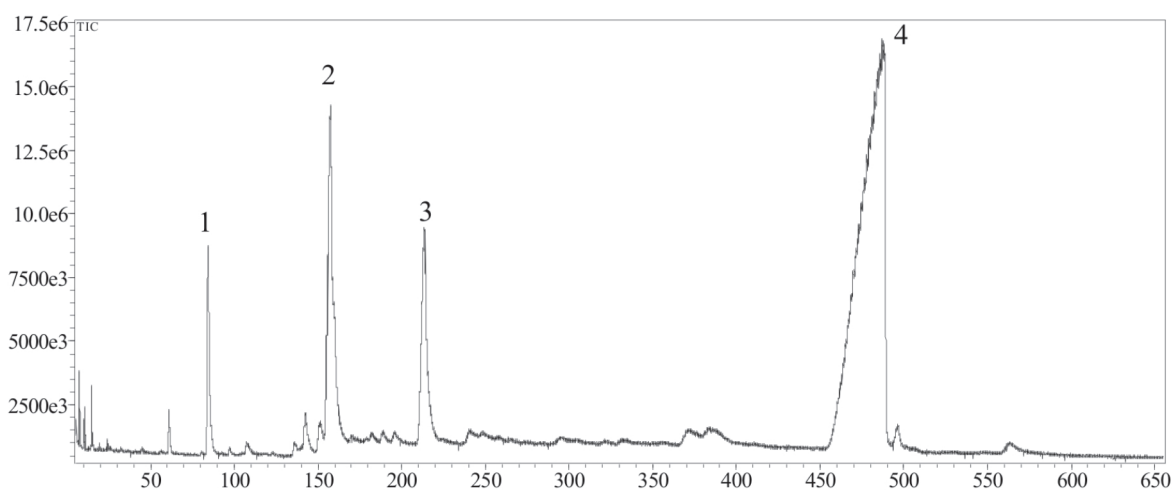


**Figure 1.** Chromatogram of *Eugenia uniflora* L. leaf essential oil from Uberlândia using conventional temperature program (60 °C - 240 °C, 3 °C/min).

**Table 1.** Chemical composition of *Eugenia uniflora* L. leaves essential oil from Uberlândia.

Nº	Compound	Kovats Index	TIC (%)
1	Hexenal	855	0.1
2	β-mircene	951	0.1
3	β-cis-ocimene	1037	0.1
4	β-trans-ocimene	1050	0.3
5	β-elemene	1391	1.9
6	γ-elemene	1433	0.3
7	γ-gurjunene	1477	0.2
8	Germacrene D	1485	0.9
9	α-selinene	1498	0.3
10	Curzerene	1499	85.1
11	Bicyclogermacrene	1500	0.9
12	Germacrene B	1561	2.0
13	Spatulenol	1578	0.3
14	Copaen-4-alfa-ol <alfa>	1642	0.7
15	α-cadinol	1654	0.3
16	N.i.	1657	0.5
17	Selin-11-en-4-alfa-ol	1660	1.0
18	Atractilone	1662	0.8
19	Furanodiene	1666	1.2
20	Germacrone	1682	0.2
21	Heptadecane	1700	0.2
22	N.i.	1720	1.6
23	Ledene II oxide	1734	0.2
24	N.i.	1853	0.2
Total			99.4

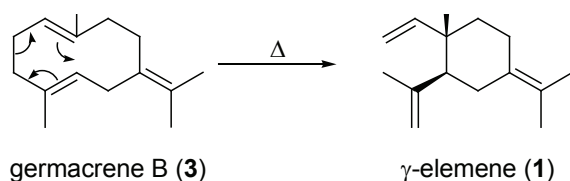
N.i.: Not identified



**Figure 2.** Chromatogram of *Eugenia uniflora* L. leaf essential oil from Uberlândia using isothermal temperature program (100 °C):  $\beta$ -elemene (1), curzerene (2), germacrene B (3) and furanodiene (4).

detected (Figure 2).

Under these conditions, curzerene was detected at 155 min (peak n° 2) and did not appear as the broad peak seen in Figure 1; its isomer furanodiene (peak n° 4) appeared approximately 5 h later, at 460 min. The area integration showed that the furanodiene concentration was about three-fold higher than that of curzerene.  $\gamma$ -Elemene (1) was not detected under these chromatographic conditions, suggesting that this compound could be formed during conventional gas chromatography by rearrangement of germacrene B (3) (peak n° 3 from Figure 2 and Table 1).



$\beta$ -Elemene was detected under mild gas chromatography conditions (at about 83 min, peak n° 1) and under conventional conditions (at 34 min, peak n° 5). Germacrene can be seen in Figure 1 (peak n° 20) and, surprisingly, was not detected under mild conditions (Figure 2), suggesting that its retention time exceeds 655 min.

Figures 1 and 2 demonstrate that special attention is required when working with thermo-sensitive samples. On the other hand, low temperature chromatography should not be used indiscriminately because many low volatility substances with a high Kovats index are unable to volatilise under such conditions.

The sum of the furanodiene and curzerene concentrations in the oil, obtained using the conventional technique, was 86.3%, which exceeds levels reported to date. Quantification at 100 °C showed that the concentration of furanodiene was three-fold higher than that of curzerene, therefore, the concentration of furanodiene was 64.7% and that of curzerene was 21.6%.

## Conclusions

The essential oil from *E. uniflora* L. leaves from the Uberlândia savannah has a distinctive concentration on curzerene (85.1%). Furanodiene (1.2%) rearranged to curzerene and germacrene B rearranged to  $\gamma$ -elemene during conventional gas chromatography analysis (temperature program up to 240 °C). Essential oil gas chromatography under mild conditions (constant temperature at 100 °C) showed unequivocally that the concentration of furanodiene in the extracted essential oil was threefold higher than that of curzerene (64.7 and 21.6%, respectively). The identification and quantification of essential oils obtained via steam distillation and analysed by conventional gas chromatography should be carried out carefully if thermo-sensitive compounds are present.

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