Revista Brasileira de Ciências Farmacêuticas
Brazilian Journal of Pharmaceutical Sciences
vol. 44, n. 3, jul./set., 2008

RBCF

Analysis of the essential oils from *Calendula officinalis* growing in Brazil using three different extraction procedures

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Terpenes and aroma volatiles from flowers of Calendula officinalis *cultivated in southeastern Brazil were obtained by steam distillation (SD), headspace-cold finger (HS-CF) extraction and headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography and mass spectrometric analysis. The dried flowers contained 0.1% oil. Kovats indices and mass spectra were used to identify 27 individual components in the various volatile fractions. The main components present in the volatile fractions of the* C. officinalis *flowers, obtained by SD, HS-SPME, and HS-CF, were δ-cadinene (22.5, 22.1, and 18.4 %) and γ-cadinene (8.9, 25.4, and 24.9 %) while 20.4 % of α-cadinol was seen only after SD extraction.*

Uniterms
- *Calendula officinalis* L
- Gas chromatography-mass spectroscopy
- Steam distillation
- Headspace solid-phase microextraction
- Headspace-cold finger

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INTRODUCTION

Calendula officinalis (Asteraceae) is an annual herb with yellow to orange flowers, native to Mediterranean region. It is also known as pot marigold, a name historically associated with its use in soups and stews to combat illnesses (Ramos *et al.*, 1988). Nowadays, *C. officinalis* is approved for food use in U.S.A. and appears in the Food and Drug Administration's list of GRAS (Generally Recognized as Safe) substances. Because of its long history of safety as a medicine for the treatment of inflammations and skin wounds (Della Loggia *et al.*, 1994), a number of reports describe its use for innumerable ailments. As a bonus, the beautiful calendula flowers are frequently seen and easily grown in home gardens all over the world (Ramos *et al.*, 1988).

Sesquiterpene glycosides, saponins, xanthophylls, triol triterpenes, flavonoids, and volatiles are observed in its composition. Chalchat *et al*. (1991) studied the essential oil of *C. officinalis* flowers cultivated in the Massif Central, France, and obtained sesquiterpene alcohol and, mainly, α-cadinol using steam distillation. Radulescu *et al*. (2000) analyzed flowers from Romania by headspace and steam distillation, where δ-cadinene plus 1,3,5-cadinatriene and α-muurolol were found as major compounds.

Because of the economic value of *C. officinalis* as an herbal medicine and its use in cosmetics, perfumery, pharmaceutical preparations, and food, we decided to study the composition of essential oil of *C. officinalis* growing in southeastern Brazil. Three different extraction techniques were used to investigate the volatiles, including steam distillation (SD), headspace solid-phase micro extraction (HS-SPME), and headspace-cold finger (HS-CF) extraction, in association with gas chromatography-mass spectrometry (GC-MS and GC-FID).

MATERIAL AND METHODS

Experimental

Plant material

The flowers of *Calendula officinalis* were collected from an experimental plot in the medicinal botanical garden of the Universidade Paranaense in Umuarama, Brazil, at S23º 46.225' and W53º 16.730', and an altitude of 391 m. A voucher specimen, 1311, was deposited at the educational herbarium of the Universidade Paranaense (HEUP). Seeds were planted on 30 April 2004 (during autumn), and collection began on 20 July (winter), three months after planting.

The flowers were dried on mats in the shade and at room temperature, spread into thin layers that were not mixed over the 10-day drying period. After this interval, water loss by both drying and desiccation, according to techniques described in the pharmacopoeia, was determined (Farmacopéia Brasileira, 1988).

Three samples each were used for extraction by steam distillation, HS-SPME, and HS-C, respectively.

Instrumentation

• GC-MS

Oil qualitative analyses and volatile fractions were carried out using an Agilent 6890 Series II gas chromatograph (Palo Alto, U.S.A.) coupled to an Agilent 5973 quadrupole mass spectrometer with electron ionization mode (EI) generated at 70 eV (ion source at 230 °C and transfer line at 280 °C). The GC was performed using a J&W DB-5 (5% diphenyl- 95% dimethyl silicone) capillary column (30 m x 0.25 mm i.d. x 0.25 μm film), and helium was used as a carrier gas (1 mL min^{-1}). The initial temperature was programmed from 35 °C to 60 °C (at 1 °C min^{-1}), to 170 °C (3 °C min^{-1}), to 200 °C (8 °C min^{-1}), and to 280 °C (15 °C min^{-1}), and maintained at 280 °C for 5 min. The injector port (splitless mode, 0.5 min) was at 250 °C. Retention indexes were calculated with reference to *n*-alkanes. All compounds were identified by comparison of both the mass spectra (Wiley 275 library) and the retention index data found in the literature (Adams, 1995).

• GC-FID

The qualitative analyses of essential oil from *C. officinalis* flowers was carried out using an Agilent 5890 Series II gas chromatograph coupled to an Agilent 3396A integrator equipped with a HP-1 capillary column (12 m X 0.20 mm I.D., 0.33 μm film thickness). Hydrogen was used as the carrier gas (1 mL min^{-1}). Chromatographic conditions were identical to those used for GC-MS.

• Steam distillation (SD)

Plant material (150 g *C. officinalis* flowers) was hydrodistilled in a Clevenger-type apparatus for 3 h. The oil layers obtained were dried over anhydrous Na_2SO_4. The yields (0.1% w/w) were averaged over three experiments, and calculated on the basis of the dry weight of the material. For CG studies, 47 mg of oil dissolved in 1.5 ml of dichloromethane and 1 ml of solution was injected into the GC-MS and the GC-FID spectrometer.

• Headspace solid-phase microextraction (HS-SPME)

The floral scent of *C. officinalis* was trapped on a 100 mm polydimethylsiloxane HS-SPME (PDMS) fiber from flower powder (Lee *et al.*, 1988; Jirovets *et al.*, 2002; Kin *et al.*, 2002). 22 g of finely powdered *C. officinalis* flowers was placed in a 250 ml Erlenmeyer flask at 20 °C and equilibrated for 30 min. Next, the SPME fiber was exposed to this atmosphere for 30 min, and then removed and placed in the GC injector for 5 min at 250 °C.

• Headspace-cold finger (HS-CF) extraction

3620 g of finely powdered *C. officinalis* flowers was placed in a 4000 ml Erlenmeyer flask, which was then closed with a cold finger containing dry ice (Acree and Teranishi 1993). During a 16-hour period at 20 °C, the cold finger was removed every 10 minutes, and the material deposited on the cold glass surface was scraped and washed with 2 mL of dichloromethane (spectroscopic quality) into a beaker. The material was dried with anhydrous Na_2SO_4 and concentrated at 40 °C in a distillation unit with a Claisen head, and cold-finger-cooled to 3 °C to a final volume of 10 ml. A volume of 2 ml were injects in HRGC-MS (Rezende *et al.*, 1999; Rezende *et al.*, 2004).

RESULTS AND DISCUSSION

The yield of oil was determined from dried flowers, in agreement with the methods described in the Farmacopéia Brasileira (1988), in order to provide information useful in future production of a phytomedicine.

The yield found in the literature for the essential oil of *Calendula officinalis* is 0.3% (Chalchat *et al*., 1991) and 0.2% (PDR, 2000). The present experiment yielded an average of 0.1% in each oil extraction. In the experiment by Chalchat *et al*. (1991), calendula cultures from the region of the Massif Central, France, where this plant is native and grows at low temperatures, were evaluated. The likely explanation for this difference in yield is that *Calendula* is a plant native to cold climates, and now acclimated in southern Brazil where autumn and winter temperatures are higher.

Analysis of the *C. officinalis* essential oil extraction techniques by steam distillation, headspace-HS-SPME, or cold-finger analyses and retention indexes, revealed 27 compounds.

Steam-distillation mainly showed sesquiterpene hydrocarbons (68.0 % of total area, compounds 1 to 15 and 22) and sesquiterpenols (27.0 % of total area, compounds 16 to 21) (Figure 1 and Table I). δ-cadinene (22.5%) and α-cadinol (20.4 %) were the main compounds, in agreement with Chalchat *et al.* (1991), who worked with *C. officinalis* from the French Central Massif (δ-cadinene at 12.1 % and α-cadinol at 25.5 %). Radulescu *et al*. (2000) isolated volatile oils of *C. officinalis* from flowers collected in Bucharest, Romania, by steam distillation and HS; these were analyzed by capillary gas-chromatography-mass spectrometry, and had α-muurolol (41.5 % of total area) as the chief component.

Only sesquiterpene hydrocarbons were identified by HS-SPME at room temperature using a PDMS fiber and analyzed by GC-MS, as shown in Figure 1 and Table II. The HS-CF extraction showed similar compositions as when using HS-SPME (Figure 1 and Table III).

In the present experiment, three different techniques were used, with different conditions of time and temperature, resulting in the identification of more compounds in steam distillation compared with the HS-SPME and HS-CF methods. By analyses of chromatograms (Figure 1), δ-cadinene appeared as one of the major compounds in all three techniques.

The absence of the sesquiterpene alcohols in the HS-SPME product suggests that polar alcohols and low-molecular-mass terpenes are not well adsorbed by the PDMS fiber used.

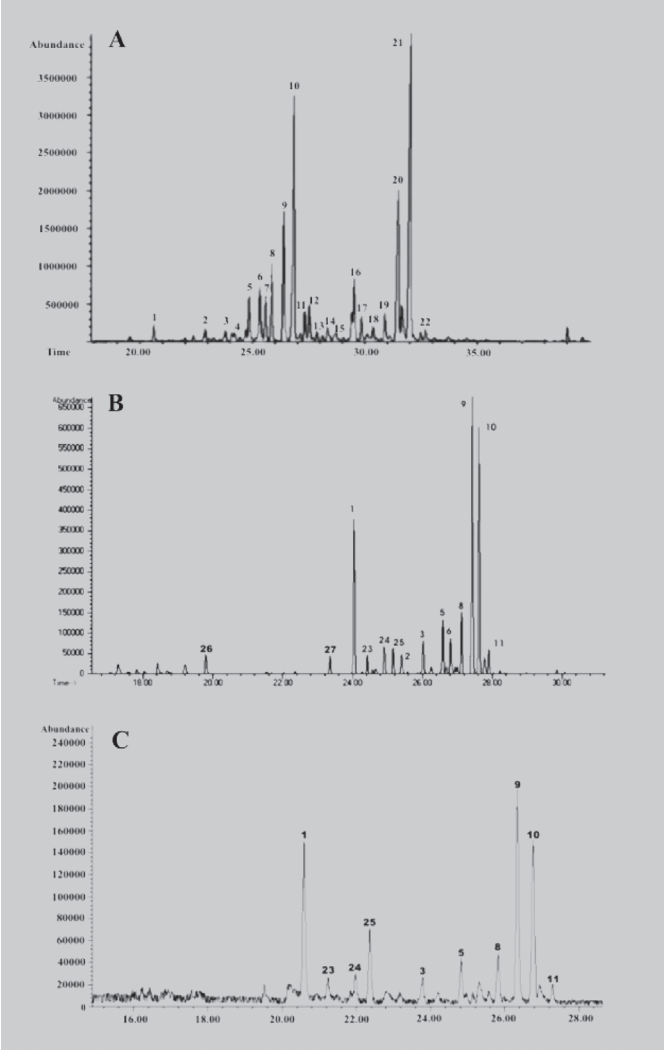

FIGURE 1 - Total ion chromatogram of the *Calendula officinalis* flowers: (**A**) volatile oil obtained by Steam distillation (SD); (**B**) after headspace analysis with HS-SPME; (**C**) extracted by headspace-cold finger (HS-CF).

TABLE I - Identification of essential oil compounds from *Calendula officinalis* flower by steam distillation with Kovats' index (*Ki*)

Peak	Compound	Area %[a]	*Ki*	*Ki*[*]
1	α-copaene	0.9	1365	1376
2	α-ionone	1.5	1421	1426
3	α-humulene	1.2	1444	1454
4	geranylacetone	1.6	1452	1453
5	γ-muurolene	2.3	1472	1477
6	β-ionone	3.2	1484	1485
7	Ledene	2.3	1488	1493
8	α-muurolene	5.6	1497	1499
9	γ-cadinene	8.9	1511	1513
10	δ-cadinene	22.5	1522	1524
11	α-cadinene	0.9	1533	1538
12	α-calacorene	2.3	1539	1542
13	caryophyllene oxide	0.5	1547	1581
14	copaen-4-α-ol	0.6	1566	1584
15	β-oplopenone	1.7	1568	1606
16	viridiflorol	2.2	1585	1590
17	ledol	1.3	1595	1565
18	1,10-di-epi-cubenol	0.9	1608	1614
19	1-epi-cubenol	1.6	1621	1627
20	epi-α-muurolol	12.9	1639	1641
21	α-cadinol	20.4	1654	1653
22	cadalene	0.8	1671	1674

[a] Relative percentages of the compounds were obtained electronically from the GC-FID area percent data.
Ki- was calculated from the GC-MS chromatograms,
Ki[*]-calculated using Adams data.

TABLE II - GC-MS identification of the volatile fraction from *Calendula officinalis* flowers obtained by head space-HS-SPME with Kovats' index (*Ki*)

Peak	Compound	Area %	*Ki*	*Ki**
26	β-cyclocitral	2.1	1200	n.f
27	α-cubebene	1.8	1338	1351
1	α-copaene	15.1	1364	1376
23	β-cubebene	1.8	1378	1390
24	α-gurjunene	2.7	1396	1409
25	β-cariophyllene	2.7	1400	1418
2	α-ionone	2.3	1420	1426
3	α-humulene	3.9	1448	1454
5	γ-muurolene	5.3	1474	1477
6	β-ionone	3.9	1485	1485
8	α-muuronele	6.2	1500	1499
9	γ-cadinene	25.5	1516	1513
10	δ-cadinene	22.1	1525	1524
11	α-cadinene	2.3	1538	1538

The percents were calculated from the GC-FID chromatograms, *Ki*- was calculated from the GC-MS chromatograms,
*Ki**-calculated using Adams data.
n.f. = not found.

TABLE III - GC-MS identification of the volatile fraction from *Calendula officinalis* flowers obtained by headspace-cold finger with Kovats index (*Ki*)

Peak	Compound	Area %	*Ki*	*Ki**
1	α-copaene	18.4	1398	1376
23	β-cubebene	3.7	1343	1390
24	α-gurjunene	4.2	1394	1409
25	β-caryophyllene	8.6	1407	1418
3	α-humulene	3.9	1445	1454
5	γ-muurolene	4.7	1471	1477
8	α-muurolene	5.8	1495	1499
9	γ-cadinene	24.9	1507	1513
10	δ-cadinene	18.6	1519	1524
11	α-cadinene	2.3	1531	1538

The percents were calculated from the GC-FID chromatograms, *Ki*- was calculated from the GC-MS chromatograms,
*Ki**-calculated using Adams data.

The experiment demonstrated that the HS-SPMS and HS-CF techniques did not replace the traditional technique of steam distillation in the analytical conditions used, because these techniques have different purposes and applications.

RESUMO

Análise por CG-EM do óleo essencial de *Calendula officinalis* cultivado no Brasil utilizando-se três diferentes processos de extração

Terpenos e aromas voláteis das flores de Calendula officinalis *cultivados no sudoeste do Brasil foram isolados por arraste a vapor (SD), dedo frio (HS-CF) e micro extração em fase sólida (HS-SPME) acoplada à espectrometria de massas. As flores secas da* C. officinalis *contêm 0,1% de óleo essencial e foram identificadas 27 substâncias químicas através do cálculo do índice de Kováts e interpretação dos espectros de massas. As substâncias majoritárias presentes no óleo essencial das flores de* C. officinalis*, obtido por SD, HS-SPME e HS-CF foram δ-cadinene (22,5; 22,1 e 18,4 %) γ-cadinene (8,9, 25,4 e 24,9 %) e 20.4 % de α-cadinol foi observado apenas na extração por arraste a vapor.*

UNITERMOS: Calendula officinalis L. *Cromatografia a gás acoplada a espectrometria de massas. Destilação por arraste a vapor. Dedo frio "cold finger". Microextração em fase sólida*.

ACKNOWLEDGEMENTS

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support.

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Recebido para publicação em 06 de dezembro de 2006
Aceito para publicação em 06 de julho de 2008