ORIGINAL ARTICLE

# Identification of *Calendula* and *Eucalyptus* Alcoholic Tinctures Volatile Compounds in the Compounding Ointment by Gas Chromatography–mass Spectrometry

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## Abstract

**Context:** Alcoholic tinctures of *Calendula officinalis* and *Eucalyptus globulus* are often found in the extemporal dosage forms composition, including ointments. Their pharmacological effect is caused by the presence of biologically active substances, which determine the direction of dosage form action. Therefore, during the stability studies, it is necessary to evaluate their concentration in the dosage form. **Aim:** Development of the gas chromatography–mass spectrometry (GC/MS) method for the determination of the ointment with *C. officinalis* and *E. globulus* tinctures stability. **Materials and Methods:** In this research, the GC/MS method was used for determining the volatile compounds of both tinctures, with their subsequent determination in the compounding ointment containing them. **Results and Discussions:** α-cadinol, δ-cadinene, t-muurolol, γ-cadinene, viridiflorene, and α-muurolene were identified as the *C. officinalis* tincture main components. 1,8-cineole, α-pinene, aromadendrene, ρ-cymene, β-eudesmol, (-)-globulol, α-eudesmol, and β-pinene were isolated in the *E. globulus* tincture. Analysis of the ointment dissolved in chloroform had shown the presence of a small amount of the both tinctures main components. The same technique has been used to analyze the stability of ointment. The results of ointment research after storage at a temperature of 5 ± 3°C for 18 days have been indicated decreasing of the main substances of plant tinctures concentration. **Conclusions:** Thus, developed GC/MS method allows to determine the concentration of active components of both tinctures and can be used to study the ointment chemical stability.

**Key words:** Compounding ointment, gas chromatography–mass spectrometry, herbal tinctures, stability studies

## INTRODUCTION

Tinctures from medicinal plants (*Calendula*, *Eucalyptus*, *Arnica*, valerian, Hawthorn, Mint, Pepper, Sagebrush, Motherwort, etc., Tinctures) are often the main components of compounding preparations for the dermatological diseases treatment. The most popular among them are alcoholic tinctures of *Calendula officinalis* and *Eucalyptus globulus*. *C. officinalis* alcoholic tincture shows antimicrobial, antifungal, antiviral, anti-inflammatory, antioxidant, and wound healing properties due to its content of flavonoids, flavonol glycosides, and anthocyanin's.[1-3] The main pharmacological effect of *E. globulus* tincture is antimicrobial[4,5] due to the presence of an essential oil with 1,8-cineole as the main component.[6] In addition, the principal components of the *E. globulus* essential oil, α-pinene, limonene, β-pinene, p-cymene, γ-terpinene, and aromadendrene also have these properties.[4]

The combination of both tinctures action was used in the compounding ointment: *Calendula* tincture 5 ml; *Eucalyptus* tincture 5 ml; dimexide 2.5 ml; paraffin liquid 12.5 ml; and wool fat substance 12.5.

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Since the effect of ointments with plant tinctures is due to their component composition, it becomes necessary studying the stability of such ointments with the possibility to determine the period during which the ointment will provide the necessary pharmacological action. Great interest, in this case, represents gas chromatography–mass spectrometry (GC/MS) method. It allows identifying the volatile components of the tinctures and determining their concentration and composition changing over time by the peak area of the active components. At the same time, this analysis does not require a lot of time for sample preparation.

Several methods for analyzing the oils components of both tinctures by the GC/MS have been proposed today. The main area of their using is the assessment of the oils components composition changing, depending on different periods and growth conditions, as well as evaluating the efficiency of different extraction types using in the sample preparation process.

GC/MS method of *Calendula* oil analysis was proposed on a capillary GC/MS with split ratio 1:30,[7] GC/MS analysis was done with split mode of Uzbekistan *Calendula*'s essential oils.[8] GC/MS method was used after solid phase microextraction for assessing of the *Calendula*'s oil main components quantitative content changing depending on the application of different types of fertilizers.[9] GC/MS method was developed for studying *Calendula* oil components concentration change with increasing on the period of plant growth.[10] In addition, the GC/MS method was proposed to determine the components content of *Calendula* oil obtained by different methods.[11] GC/MS method was done for *C. officinalis* flowers methanolic extract analysis[12] with split injection (split ratio 1:50) and hydroalcoholic extract[13] by splitless mode.

The GC/MS method was used to determine the *Eucalyptus* essential oil components after hydrodistillation. The samples of oil were dissolved in hexane (split ratio 1:20)[6] and by GC/MS with split ratio 1:4.[14] *Eucalyptus* oil analysis by GC/MS with the split mode 1:100[15,16] was also described in the literature.

GC/MS method with split injection mode for the ointment with *Eucalyptus* oil stability analysis was proposed. The stability of three ointments was studied within 6 months including the determination of percentage of *Eucalyptus* oil in them.[17]

## MATERIALS AND METHODS

### Materials

For the ointment preparation and further researchers were used *C. officinalis* alcoholic tincture (series 30416, PJSC "Phytopharm," Ukraine), *E. globulus* alcoholic tincture (series 81115, PJSC "Phytopharm," Ukraine), dimethylsulfoxide (series PHS130916, Gaylord Chemical Company LLS, USA), paraffin liquid (series 0000022579, Sasol Wax GmbH, Germany), and wool fat substance (series 4039, Imperial-Oel-Import, Germany). The investigations were carried out on a gas chromatograph with a mass detector GC/MS-QP2010 Ultra Shimadzu with autosampler AOC 5000 Plus and column Rxi-5MS (30.0 m × 0.25 µm). The electronic balances Shimadzu UniBloc AUW 120D were used for weighing of the ointment samples.

### Chromatographic conditions

The column oven temperature with gradient method: initial temperature was 50°C (held for 5 min) and final temperature was 200°C by increasing rate of 5°C/min (held for 8 min). Flow rate: 1.22 ml/min. Injection temperature: 270°C. Injection mode: splitless, sampling time 1.5 min (for the ointment analysis) and split 1:50 (for the tinctures analysis). Carrier gas: helium. Total analysis time: 43 min.

### Sample preparation

About 0.500 g of ointment was dissolved in 50.0 ml volumetric flask in chloroform. The solution was filtered through a Q-MAX RR Syringe Filters filter (filter diameter 25 mm, membrane 0.22 µm PTFE hydrophobic) into the vials. *C. officinalis* and *E. globulus* tinctures were placed in vials in an amount corresponding to their content in the ointment samples (0.067 ml).

## RESULTS AND DISCUSSION

After analyzing the literature information on the analysis of *C. officinalis* and *E. globulus* oils, we have developed the GC/MS method for analyzing the test ointment. We have used splitless injection mode, based on the small concentrations of the tinctures active ingredients in the ointment. *C. officinalis* and *E. globulus* tinctures, which are part of the ointment, were used as standards. To determine the both tinctures markers, they were first analyzed by the developed method but using split mode.

Due to the *C. officinalis* alcoholic tincture analysis [Figure 1 and Table 1], 16 biologically active substances were isolated. Six compounds are the main ones: α-cadinol, δ-cadinene, t-muurolol, γ-cadinene, viridiflorene, and α-muurolene.

The results obtained match up with the data of earlier studies on the components *Calendula* oil content. In different studies, α-cadinol, t-cadinol,[9,13,18] α-thujene,[9,11,13] α-muurolene, γ-muurolene,[9,18] γ-cadinene, δ-cadinene, α-cadinene,[8,11] viridiflorol, β-cubebene,[8,18] epi-α-muurolol, β-caryophyllene,[11] and α-humulene[18] were isolated in it.

**Table 1:** Components of volatile fraction of *C. officinalis* alcoholic tincture

| Compound name | $t_R$ (min) | Area (%) |
|---|---|---|
| α-thujene | 8.463 | 2.76 |
| α-pinene | 8.688 | 1.25 |
| α-humulene | 24.723 | 1.67 |
| 9-epi-(E)-caryophyllene | 24.917 | 2.33 |
| α-muurolene | 25.282 | 1.83 |
| α-guaiene | 25.615 | 1.57 |
| viridiflorene | 25.767 | 7.53 |
| α-muurolene | 25.870 | 5.09 |
| γ-cadinene | 26.221 | 9.81 |
| δ-cadinene | 26.430 | 21.49 |
| cubenene | 26.658 | 2.00 |
| α-cadinene | 26.785 | 3.00 |
| viridiflorol | 28.116 | 1.74 |
| t-muurolol | 29.235 | 11.32 |
| α-muurolol | 29.328 | 1.58 |
| α-cadinol | 29.528 | 25.03 |

*C. officinalis: Calendula officinalis*

The results of the *E. globulus* alcoholic tincture analysis [Figure 2 and Table 2] have shown the presence of 19 biologically active substances.

The main Eucalyptus oil component was 1,8-cineole (30.85%). In addition, seven substances were identified in it as markers: α-pinene, aromadendrene, ρ-cymene, β-eudesmol, (-)- globulol, α-eudesmol, and β-pinene.

The results obtained coincide with the data of other studies on the component content of *E. globulus* oil. Other authors in the analysis of *E. globulus* oil or tincture identified α-pinene,[6,14,16,17,19] β-pinene, 1,8-cineole,[6,14-17,19] limonene,[6,16] cymene,[6,15-17] globulol, α-terpineol,[15-17,19] α-phellandrene, γ-terpinene, α-terpineol,[6,15] α-terpinyl acetate, α-gurjunene, viridiflorol, and (-)-globulol.[15]

After this, to analyze the content of plant components, an analysis of the ointment dissolved in chloroform has been performed [Figure 3].

Among the plant components, nine marker compounds were identified [Table 3].

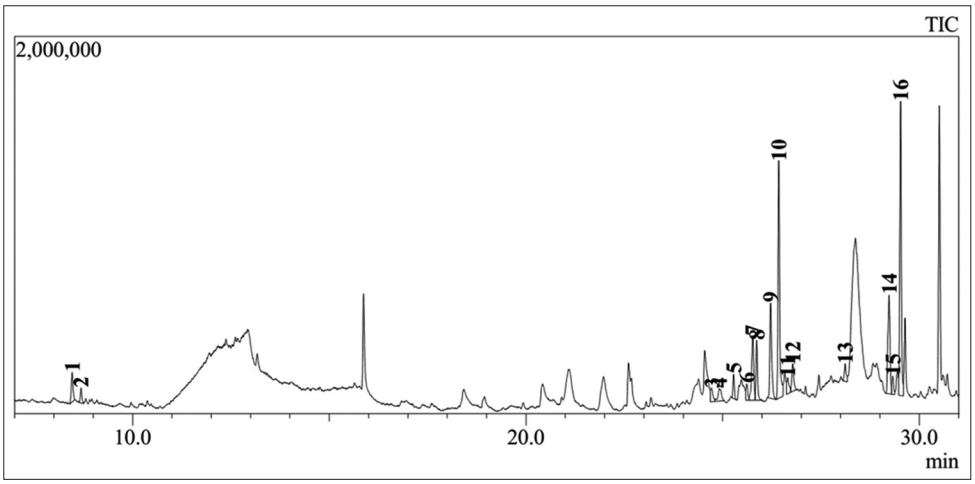


**Figure 1:** Chromatogram of the *Calendula officinalis* alcoholic tincture analysis by gas chromatography–mass spectrometry

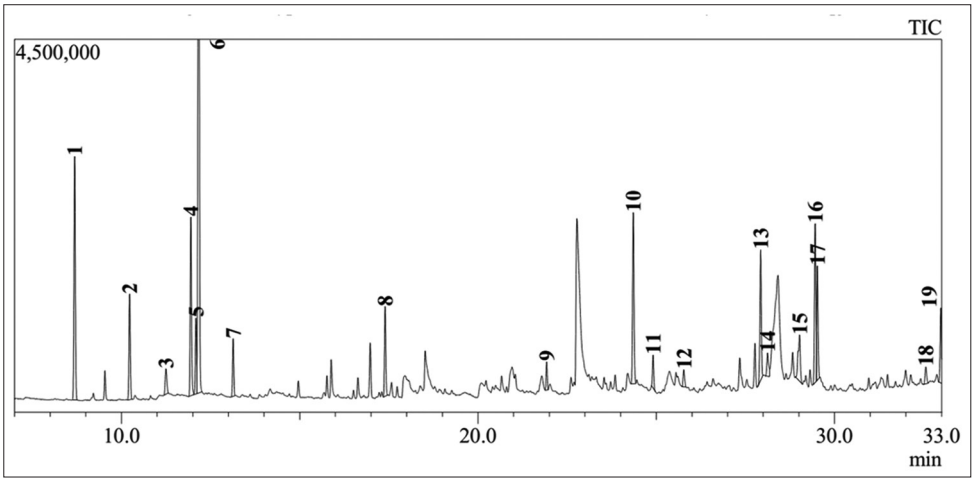


**Figure 2:** Chromatogram of *Eucalyptus globulus* alcoholic tincture analysis by gas chromatography–mass spectrometry

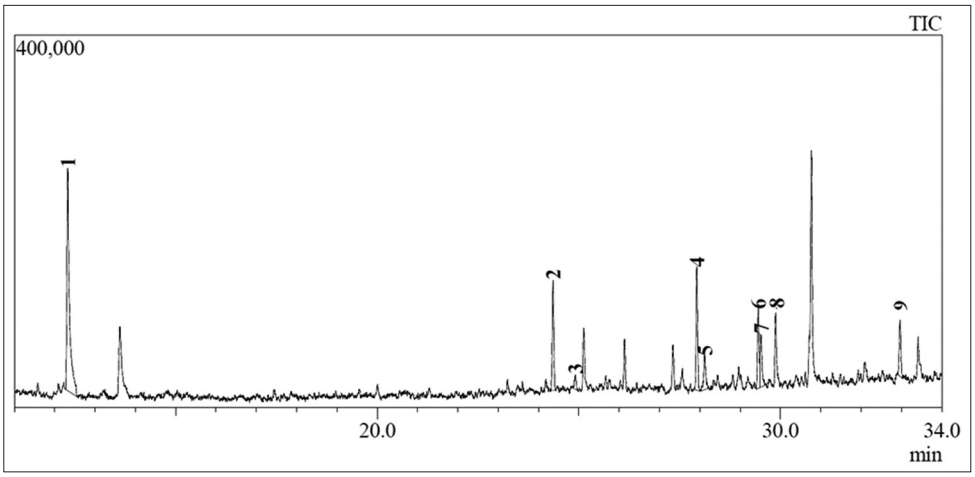


**Figure 3:** Chromatogram of the ointment chloroform solution analysis by gas chromatography–mass spectrometry

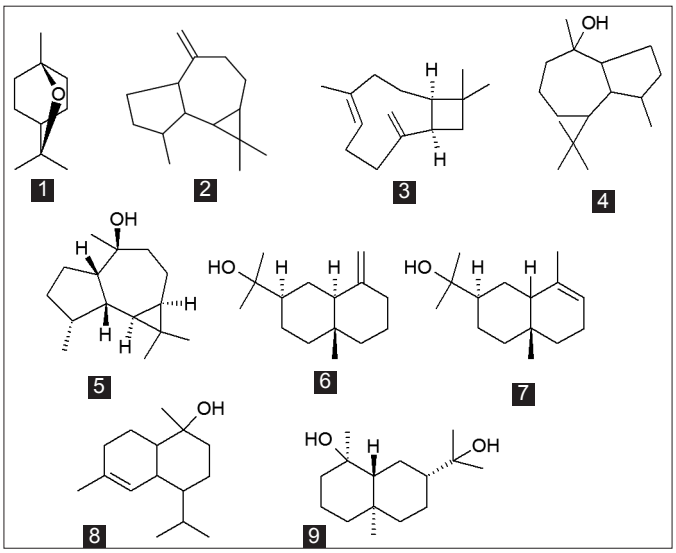


**Figure 4:** Chemical structure of compounds identified by gas chromatography–mass spectrometry in the compounding ointment with tinctures. (1) 1,8-cineole, (2) aromadendrene, (3) 9-epi-(E)-caryophyllene, (4) (-)-globulol, (5) viridiflorol, (6) β-eudesmol, (7) α-eudesmol, (8) α-cadinol, (9) cryptomeridiol

1,8-cineole, aromadendrene, (-)-globulol, β-eudesmol, α-eudesmol, and cryptomeridiol are the components of the *E. globulus* essential oil; α-cadinol is the main component of *C. officinalis* essential oil; 9-epi-(E)-caryophyllene and viridiflorol are contained in both plants essential oils. Figure 4 shows the formulas of marker substances that were determined in ointment samples using this method.

Since today, the problem of extemporal dosage forms stability is acute in Ukraine and foreign countries; it was interesting to check the possibility of the method using to assess the ointment stability by changing the areas of plant components peaks values. This method does not require a long sample preparation, which ensures the analysis rapidity. The studied ointment has been stored after opening the package at a temperature of 5 ± 3°C for 18 days. After this,

**Table 2:** Components of volatile fraction of *E. globulus* alcoholic tincture

| Compound name | $t_R$ (min) | Area (%) |
|---|---|---|
| α-pinene | 8.687 | 10.26 |
| β-pinene | 10.231 | 4.37 |
| α-phellandrene | 11.247 | 1.33 |
| ρ-cymene | 11.946 | 7.39 |
| D-limonene | 12.090 | 3.01 |
| 1,8-cineole | 12.165 | 30.85 |
| γ-terpinene | 13.134 | 2.19 |
| L-α-terpineol | 17.394 | 3.65 |
| α-terpinyl acetate | 21.927 | 1.05 |
| aromadendrene | 24.353 | 7.47 |
| 9-epi-(E)-caryophyllene | 24.912 | 1.30 |
| γ-gurjunene | 25.772 | 0.82 |
| (-)-globulol | 27.928 | 5.93 |
| viridiflorol | 28.118 | 1.25 |
| γ-eudesmol | 29.019 | 3.12 |
| β-eudesmol | 29.457 | 6.94 |
| α-eudesmol | 29.521 | 4.86 |
| α-phellandrene dimer | 32.561 | 0.84 |
| cryptomeridiol | 32.981 | 3.36 |

*E. globulus: Eucalyptus globulus*

the ointment repeated analysis was performed using the developed GC/MS method [Figure 5]. In the figure below, ointment 1 is the freshly prepared ointment, and ointment 2 is the ointment after 18 days of storage.

The obtained results indicate the concentration decreasing of the main active ingredients of both tinctures essential oils in the second ointment. Some components (9-epi-(E)-caryophyllene, viridiflorol, β-eudesmol, and α-eudesmol) were not identified in it. The conducted researchers confirmed the possibility of using this method for stability analysis of ointment.

**Table 3:** Components of volatile fraction of the ointment chloroform solution

| Compound name | $t_R$ (min) | Area (%) |
|---|---|---|
| 1,8-cineole | 12.321 | 35.76 |
| aromadendrene | 24.360 | 10.46 |
| 9-epi-(E)-caryophyllene | 24.915 | 1.76 |
| (-)-globulol | 27.926 | 11.03 |
| viridiflorol | 28.120 | 5.20 |
| β-eudesmol | 29.452 | 8.40 |
| α-eudesmol | 29.518 | 6.07 |
| α-cadinol | 29.539 | 14.63 |
| cryptomeridiol | 32.969 | 6.68 |

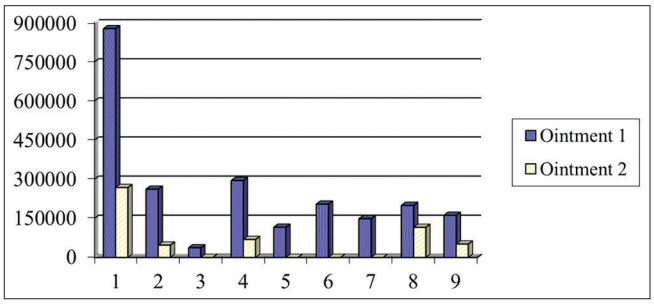


**Figure 5:** (1) 1,8-cineole, (2) aromadendrene, (3) 9-epi-(E)-caryophyllene, (4) (-)-globulol, (5) viridiflorol, (6) β-eudesmol, (7) α-eudesmol, (8) α-cadinol, (9) cryptomeridiol

## CONCLUSIONS

During the analysis of volatile components of *Calendula* and *Eucalyptus* alcoholic tinctures, and ointment containing them, by GC/MS method the substances - markers of tinctures were determined. They can be used to analyze the stability of the ointment. Developed method was used for the stability analysis of the ointment, which has been stored for 18 days at a temperature of 5 ± 3°C. The obtained results indicate decreasing of main ointment components concentration and prove the possibility of using the developed method for studying the ointment stability.

## REFERENCES


1. Al-Snafi AE. The chemical constituents and pharmacological effects of *Calendula officinalis*-a review. Ind J Pharm Sci Res 2015;5:172-85.
2. Elzorba H, Banna EL, Derbala D. Some toxicological and pharmacological activities of *Calendula officinalis* Linn. flower 70 % ethanolic extract. Anim Vet Sci 2016;4:26-31.
3. Farahpour MR. Antioxidant activity, antinociceptive and anti-inflammatory effects of *Pot marigold* hydroalcoholic extract on experimental animals. Int J Pharm Tech Res 2014;6:1640-6.
4. Nezhad FM, Zeighami H, Mota A, Sattari M, Yadegar A. Antibacterial activity of *Eucalyptus* extracts on meticillin resistance *Staphylococcus aureus*. Res J Biol Sci 2009;4:905-8.
5. Rekha S, Chandrashekhara S, Murugan V. Comparitive study and antimicrobial activities of alcoholic and aqueous extracts of *Eucalyptus globulus*. World J Pharm Pharm Sci 2016;5:1678-84.
6. Damjanovic-Vratnica B, Dakov T, Sukovic D, Damjanivic J. Antimicrobial effect of essential oil isolated from *Eucalyptus globulus Labill.* from Montenegro. Czech J Food Sci 2011;29:277-84.
7. Mishra AK, Mishra A, Chattopadhyay P. Assessment of *in vitro* sun protection factor of *Calendula Officinalis* L. *(Asteraceae)* essential oil-formulation. J Young Pharm 2012;4:17-21.
8. Abdullabekova V, Juraeva A, Azizov O, Yunusxodjaeva N. Essential oil of *Calendula officinalis*. Eur Med Health Pharm J 2014;7:34-7.
9. Lelesz JÉ, Csajbók J. Relationship investigation between the *Marigold* (*Calendula officinalis L.*) essential oil agents andquantitative presences change under different fertilization settings. Nat Resour Sustain Dev 2016;2016;93-9.
10. Okoh OO, Sadimenko AA, Afolayan AJ. The effects of age on the yield and composition of the essential oils of *Calendula officinalis*. J Appl Sci 2007;7:3806-10.
11. Salome-Abarca LF, Soto-Hernandez RM, Cruz-Huerta N, Gonzalez-Hernandez VA. Chemical composition of scented extracts obtained from *Calendula officinalis* by three extraction methods. Bot Sci 2015;93:633-8.
12. Raghad DH. GC-MS analysis of *Calendula officinalis* and cytotoxic effects of its flower crude extract on human epidermoid larynx carcinoma (HEP-2). World J Pharm Pharm Sci 2014;3:237-75.
13. Caamal-Herrera IO, Muñoz-Rodríguez D, Madera-Santana T, Azamar-Barrios JA. Identification of volatile compounds in hydro-alcoholic extracts of *Calendula officinalis* L. flowers and mimosae tenuiflorae bark using GC/MS. Int J Appl Res Nat Prod 2016;9:20-30.
14. Jimnez-Carmona MM, Luque de Castro MD. Isolation of *Eucalyptus* essential oil for GC-MS analysis by extraction with subcritical water. Chromatographia 1999;50:578-82.
15. Bachheti RK. Chemical composition and antibacterial activity of the essential oil from the leaves of *Eucalyptus globulus* collected from Haramaya University, Ethiopia. Der Pharm Chem 2015;7:209-14.
16. Maciel MV, Morais SM, Bevilaqua CM, Silva RA, Barros RS, Sousa RN *et al.* Chemical composition of *Eucalyptus spp*. essential oils and their insecticidal effects on Lutzomyia longipalpis. Vet Parasitol 2010;167:1-7.
17. Dawar N, Arora M, Naved T, Tyagi VK. Stabilities studies of formulations containing *Eucalyptus* oil. Indo Glob J Pharm Sci 2013;3:174-80.
18. Gazim ZC, Rezende CM, Fraga SR, Filho BP, Nakamura CV, Cortez DA. Analysis of the essential

oils from *Calendula officinalis* growing in Brazil using three different extraction procedures. Braz J Pharm Sci 2008;44:391-5.
19. European Medicines Agency. EMA/HMPC/892615/2011. Assessment Report on *Eucalyptus globulus Labill.*, Folium. Great Britain, London: Committee on Herbal Medicinal Products (HMPC); 2012.

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