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Chemical composition and biological activities of the essential oils of *Salvia canariensis*

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ABSTRACT: Comparative studies of the chemical composition of steam-distilled essential oils from cultivated *Salvia canariensis*, collected at different seasons of the year, were studied. The essential oils were analysed by gas chomatography–mass spectrometry: the major components were bornyl acetate (17.8–28.6%), β-caryophyllene (12.7–30.2%), α-pinene (4.6–9.5%) and viridiflorol (13.9–17.3%) in all samples. The essential oils were evaluated for antimicrobial and cytostatic activities and enzymatic inhibitions of xanthine oxidase, β-glucosidase and β-glucuronidase. Concerning the antimicrobial and cytotoxic tests, the oils showed interesting activities towards different Gram-positive bacteria (MIC 45–35 μg/ml), but had no effect against eukaryotic cells. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Salvia canariensis*, Lamiaceae; essential oil; chemical composition; antimicrobial activity; cytotoxic activity, enzymatic inhibitions

Introduction

The genus *Salvia* (tribe Menthae) is included in the widespread and diversified family of Labiatae (Lamiaceae) and consists of about 900 different species organized in five subgenera (*Sclarea*, *Audibertia*, *Jungia*, *Leonia* and *Salvia*) present in such diverse areas as the Mediterranean, Central Asia, the Pacific Islands, tropical Africa and America.[1] Based on their well-characterized antioxidant, aromatic and antimicrobial properties, it is not surprising that members of this genus have been used since ancient times in the cosmetic industry, popular medicine and as food flavouring and preservation products.[2,3] Despite the large availability of useful synthetic products for the cosmetic, pharmaceutical and food industry, the demand for aromatic plants is increasing due to the growing preference of consumers for natural products.[2,4,5]

The Canary sage (*Salvia canariensis* L.) is a protected endemism widely used in the popular medicine of the Canary Archipelago due to its anti-inflammatory, wound healing and antiseptic properties, being particularly recommended its infusion for all kinds of stomach complaints.[6] In contrast, with other species of genus *Salvia*, the Canary sage is a shrub that can grow up to 2 m high and whose long branches with long lanceolate leaves show a characteristic dense arrangement. Therefore, this species has been considered as a likely link between Old and New World sages.[7]

In general, the chemical composition of plant extracts depends on the genetic background of the species but also on the harvest time, the type of extraction and processing and other such significant factors as the environmental and ecological characteristics of the growing region.[8] We have found an important variability in the chemical composition of the essential oil of the Spanish sage (*Salvia lavandulifolia* Vahl.) that could be attributed to the existence of various chemotypes in some subspecies.[9]

Although the chemical composition of essential oils of *Salvia canariensis* has been reported,[10–13] the seasonal variation of its composition is not known. As part of our systematic phytochemical research on *Salvia* species,[14,15] we have investigated the differences in essential oil composition of cultivated *S. canariensis* collected in different seasons and their biological activities.

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Material and methods

Plant material

Plants of the cultivated endemism *Salvia canariensis* L. (2 years old) were harvested in the experimental fields of Monteflor S.A. (Guía, Gran Canaria). The plant was collected in three different periods: after flowering, in November 2000 (leaves and some flowers, sample A); before flowering, in March 2001 (leaves only, sample B) and at the flowering stage, in September 2001 (flowers and leaves, sample C).

Isolation of essential oils

The three different air-dried samples (100 g each) were distilled in a Clevenger-type apparatus, according to the method recommended by European Pharmacopoeia. The yield of oils (v/w) was 4% for each sample.

Gas chromatography–mass spectrometry

The essential oils were analysed by gas chromatography–mass spectrometry (GC-MS) using a Hewlett-Packard gas-chromatograph, model 5890A, connected to a Hewlett-Pakard mass detector (EI, 70 eV), model 5971A, and equipped with a 30 m × 0.25 mm i.d. PTE-5 capillary column (0.25 μm film thickness). A Carbowax 20 M column was used as well for determining the retention times on a polar column. The working conditions were: injector temperature, 260 °C; detector temperature, 300 °C; column temperature, 60 °C, for 5 min, and then heated to 270 °C, at 4 °C/min. Helium flow was adjusted to 0.5 ml/min.

Identification of the components

The identification of the chemical constituents was assessed by their retention times (RT) on a non-polar (PTE-5) and on a polar (Carbowax 20 M) column, and their EI mass spectra, by comparing them with those in the Wiley Mass Spectral Database, 1986.

Antimicrobial assay

The activity of the essential oils was tested against Gram-positive (*Staphylococus aureus* ATCC 6538, *S. saprophyticus* CECT 235, *S. epidermidis* CECT 232, *Bacillus subtilis* CECT 39, *B. cereus* CECT 496, *B. pumilus* CECT 29, *Mycobacterium smegmati* CECT 3032, *Enterococcus faecalis* CECT 481) and Gram-negative (*Escherichia coli* CECT 99, *Pseudomonas aeruginosa* AK 958, *Proteus nirabilis* CECT 170, *Salmonella* sp CECT 450) bacteria and the yeast *Candida albicans* UBC2. The bacteria cultures were developed in nutrient broth or brain–heart infusion broth (for *E. faecalis*, and *M. smegmatis* containing 0.06% Tween 80), and the yeast was cultured in Sabouraud liquid medium. All medium was purchased from Oxoid.

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were performed following the two-fold dilution method.[16] The initial cellular densities were about 10^5 CFU/ml. The essential oils were added in a solution of dimethysulphoxide (DMSO), and wells with the same proportions were used as controls. After 24 h, except for *M. smegmatis* (48 h), in agitation at 37 °C the optical density was measured using a microELISA reader (Multiskan Plus II) at 600 nm.

The MIC was defined as the lowest concentration of essential oil that completely inhibited the growth of the organism as detected by them. All wells with no visible growth were subcultured by transfering them in duplicate (100 μl) to nutrient or brain–heart infusion agar plates. After overnight incubation, colony counts were performed and the MBC was defined as the lowest essential oil concentration that produced ≥99.9% killing of the initial inoculum.

Cytotoxic activity

HeLa (human carcinoma of the cervix) and Hep-2 (human carcinoma of the larynx) cell lines were grown as a monolayer in Dulbecco's modified Eagle's medium, DMEM (Sigma), supplemented with 10% fetal calf serum (Gibco) and 1% of penicillin–streptomycin mixture (10 000 UI/ml). The cells were maintained at 37 °C in 5% CO_2 and 90% humidity. The cytotoxic activity was assessed using colorimetric MTT 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide reduction assay.[17] Approximately 2×10^4 (in 50 μl) cells were added to each well and the optical density was measured using a microELISA reader (Multiskan Plus II) at 550 nm after solubilizing the MTT formazan with DMSO (100 μl).

The percentage viability was plotted against the essential oil concentrations, and the 50% cell viability (IC_{50}) was calculated from the curve. All experiments were repeated three times.

Xanthine oxidase activity

Xanthine oxidase (XO) activity was measured spectroscopically as previously described.[18] Xanthine oxidase from cow's milk, xanthine and the standard inhibitor allopurinol were purchased from Sigma Chemical Co. (St Louis, MO, USA). Essential oils were tested at 50 μg/ml

dissolved in DMSO, and those inhibiting the enzyme >50% were further tested for IC_{50}.

Assay of β-glucuronidase

β-Glucuronidase activity was measured, using *p*-nitrophenyl β-D-glucuronide as substrate.[19] The inhibitor glucosaccharo-1:4 lactone was used as control. All reagents were obtained from Sigma Chemical Co. Essential oils were tested at 50 µg/ml dissolved in DMSO, and those inhibiting the enzyme >50% were assayed for IC_{50}.

Assay of β-glucosidase inhibition

β-Glucosidase activity was measured, using *p*-nitrophenyl β-glucopyranoside as substrate.[20] The assay involves hydrolysis of substrate by β-glucosidase (bitter almonds emulsin) and measurement of the liberated *p*-nitrophenol at 410 nm. Essential oils were tested at 100 µg/ml dissolved in DMSO, and those inhibiting the enzyme >50% were assayed for IC_{50}. The castanospermine was used as control.

Results and Discussion

A typical chromatogram (GC-MS) of the essential oil of *S. canariensis* is shown in Fig. 1, and the chemical compositions of the three samples studied (A) after flowering, (B) before flowering and (C) at flowering are shown in Table 1. Thirty compounds were identified representing 95.4, 82.0 and 92.4%, respectively, of the total (Table 1).

Samples of *S. canariensis* after (sample A) and during flowering (sample C) contained high quantities of monoterpene hydrocarbons (24.2, 19.9%) and oxygenated monoterpenes (34.3, 35.7%). The main constituents of these samples were α-pinene (9.5 and 7.3%) and bornyl acetate (26.9 and 28.6%), respectively. However, the oil of sample B (collected before flowering) was richer in sesquiterpene hydrocarbons (42.1%), than samples A (21.8%) and C (19.0%), β-caryophyllene being the dominant constituent. On the other hand we think that the major content of oxygenated monoterpenes in samples A and C with respect to sample B could be due to the response of the plant at the high temperature and luminic stress. As far as the oxygen sesquiterpene derivatives are concerned, no significant differences were found in the three samples, viridiflorol being the principal component.

In conclusion, the chemical composition of essential oils of *S. canariensis* showed seasonal variations and they have significant differences compared with other *Salvia* species,[13,21] and only the oil of *S. multicaulis* Vahl from Iran has been found to have a similar composition.[22]

Tests carried out to show the inhibitory effect of the essential oils against xanthine oxidase, β-glucosidase and β-glucuronidase were negative (IC_{50} > 50 µg/ml).

MIC and MBC values, listed in Table 2, clearly show that the effect of essential oils is limited to Gram-positive bacteria and they are inactive against Gram-negative bacteria and the yeast *Candida albicans* (MICs > 200 µg/ml). In general we could say that sample A is more active that samples B and C. A comparison among the chemical composition of these samples shows that the antibiotic activity must be related to the highest content of monoterpene hydrocarbons.

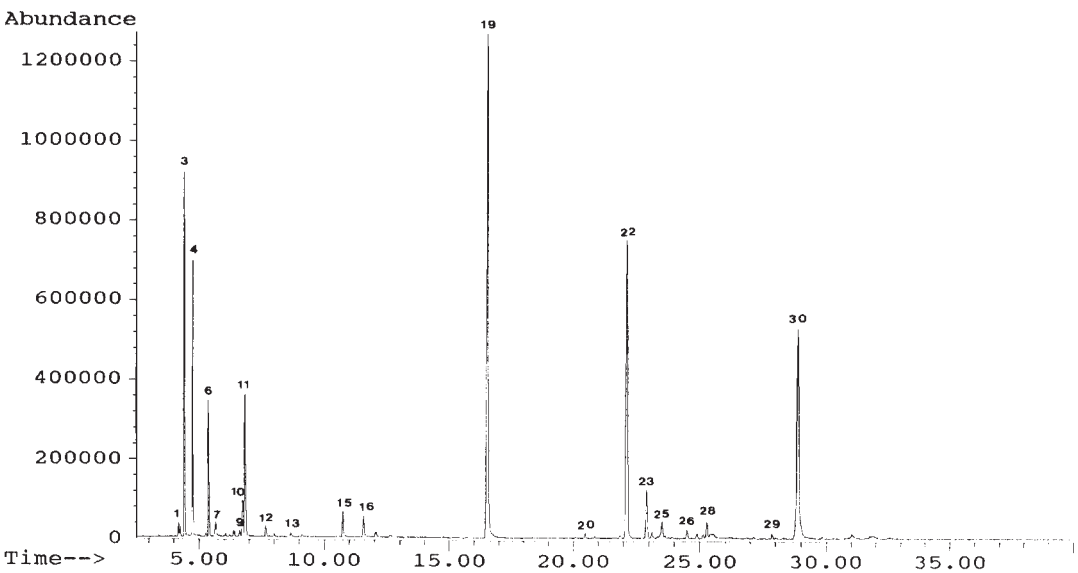

Figure 1. GC-MS chromatogram of a essential oil of *Salvia canariensis* (see peak identifications in Table 1).

Table 1. Chemical composition of essential oil of *Salvia canariensis*

Peak no.[a]	Components	RI_{n-p}[b]	RI_p[c]	Area (%)		
				Sample A	Sample B	Sample C
Monoterpene hydrocarbons				*24.2*	*12.9*	*19.9*
1	Tricyclene	921	1014	0.3	0.2	0.3
2	α-Thujene	923	1018	0.2	0.2	0.4
3	α-Pinene	931	1026	9.5	4.6	7.3
4	Camphene	947	1071	7.4	3.9	5.6
5	Sabinene	970	1124	0.1	0.2	0.2
6	β-Pinene	976	1113	3.8	2.6	3.8
7	Myrcene	987	1164	0.6	0.1	0.2
8	α-Terpinene	1015	1182	0.2	0.1	0.2
9	*p*-Cymene	1022	1271	0.2	0.1	0.1
10	Limonene	1026	1203	1.3	0.6	1.0
12	γ-Terpinene	1054	1244	0.4	0.2	0.6
13	Terpinolene	1082	1283	0.2	0.1	0.2
Oxygen monoterpene derivatives				*34.3*	*22.5*	*35.7*
11	1,8-Cineole	1030	1210	4.7	3.4	2.9
14	Linalool	1099	1556	0.1	0.2	0.2
15	Camphor	1143	1503	1.1	0.3	0.8
16	Borneol	1168	1696	1.1	0.3	2.4
17	Terpinen-4-ol	1177	1599	0.3	0.3	0.5
18	α-Terpineol	1192	1694	0.1	0.2	0.3
19	Bornyl acetate	1281	1570	26.9	17.8	28.6
Sesquiterpene hydrocarbons				*21.8*	*42.1*	*19.0*
20	α-Copaene	1362	1469	0.3	0.1	t[d]
21	Longifolene	1399	1522	0.2	0.2	0.2
22	β-Caryophyllene	1411	1578	15.9	30.2	12.7
23	Aromadendrene	1429	1585	2.4	2.1	2.7
24	3,7-Guayadiene	1434	1590	0.3	1.1	0.5
25	α-Humulene	1446	1645	1.1	1.6	0.8
26	Germacrene-D	1468	1642	0.4	1.7	0.5
27	β-Selinene	1479	1674	0.3	0.9	0.4
28	Viridiflorene	1482	1772	0.9	4.2	1.2
Oxygen sesquiterpene derivatives				*15.1*	*14.5*	*17.8*
29	Ledol	1552	1946	0.2	0.6	0.5
30	Viridiflorol	1575	2056	14.9	13.9	17.3
Total identified				95.4	82.0	92.4

[a] Numbering refers to the elution order PTE-5 capillary column.
[b] RI_{n-p}, retention index on a non-polar column (PTE-5).
[c] RI_p, retention index on a polar column (Carbowax 20 M).
[d] t = traces (<0.1).

Table 2. Antimicrobial activity expressed in μg/ml of essential oils against Gram-positive bacteria

Microorganism	A		B		C		Control[a]	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	75–50	75	125–100	175–150	100–75	150	2.5–1.25	4
S. epidermidis	100–75	175–150	150–100	>200	150	>200	5–2	8
S. saprophyticus	>200	>200	>200	>200	200	>200	0.6–0.3	1
B. subtilis	80–75	80	45–35	55	200–100	200	8	>10
B. cereus	80–75	80	110–100	150–140	200–100	200	>10	>10
B. pumilus	75–50	>200	90–80	90–80	150–100	200	>10	>10
M. smegmatis	>200	>200	200–150	>200	>200	>200	>10	>10
E. faecalis	175–150	175	>200	>200	>200	>200	>10	>10

[a] Positive control was cephotaxime.
All essential oils were inactive against the Gram-negative bacteria and the yeast assayed at 200 μg/ml.

Table 3. Cytotoxic activity (IC_{50} μg/ml) of the essential oils from *S. canariensis* against HeLa and Hep-2 cells

Samples	HeLa	Hep-2
A	150.7	>200
B	>200	>200
C	96.2	99.2
Control[a]	0.001	1.5

[a] Actinomycin was used as positive control.

None of them showed significant cytotoxic activity against HeLa and Hep-2 cells lines in culture (Table 3). In this case, it seems that the highest content of oxygenated compounds (sample C) could be related to the activity against the two cells lines assayed.

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