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Research Paper

Antimicrobial activity and chemical composition of the essential oil of *Hofmeisteria schaffneri*

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Abstract

Objectives The aims of this study were to establish the antimicrobial potential of *Hofmeisteria schaffneri* essential oil and its chemical composition.

Methods The essential oils of *Hofmeisteria schaffneri* harvested at flowering (batches I and IV) and non-flowering (batches II and III) seasons were prepared by hydrodistillation and analysed by GC and GC-MS. The aqueous and organic (CH_2Cl_2-MeOH 1 : 1) extracts were prepared by using infusion and maceration techniques, respectively. The in-vitro antimicrobial activity of the preparations and compounds against *Candida albicans* and some bacteria (Gram-negative and Gram-positive) was assessed using the broth dilution method in 96-microplate wells.

Key findings Forty-four compounds, representing ~90% of the total constituents, were identified in the essential oil of *Hofmeisteria schaffneri* collected in flowering (batches I and IV) and non-flowering (batches II and III) seasons. In all cases, several thymol analogues were the major components of the oils (~65%); some small differences in the relative proportions of these constituents were observed. The infusion exhibited an antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*, with a MIC value of 64 μg/ml in each case. The essential oil batches were active against *Staphylococcus aureus*, with MIC ranging from 48 to 192 μg/ml. They were, however, inactive against Gram-negative bacteria, including *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* (MIC > 1024 μg/ml). On the other hand, the infusion of the plant as well as the oil from batch I displayed anti-*Candida albicans* activity, with MIC of 128 and 192 μg/ml, respectively. Finally, the organic extract did not displayed significant activity against the tested microorganisms (MIC ≥ 1024 μg/ml). Some of the compounds isolated from the plant were also tested. Compounds 8 and 38, which were present in the essential oils, displayed the best antibacterial effect against Gram-positive bacteria (MIC ranging between 32 and 64 μg/ml). Compounds 6 (present in the infusion) and 10 (present in all preparations) showed higher activity against the yeast (MIC = 128 μg/ml) than the remaining compounds, with MIC values ranging from 256 to 512 μg/ml.

Conclusions The composition and antimicrobial activity of the oils changed slightly from flowering to non-flowering seasons. The results of the present investigation provide in-vitro scientific support for the use of the plant against skin infections in Mexican folk medicine.

Keywords antimicrobial activity; essential oil; GC-FID; *Hofmeisteria schaffneri*; thymol derivatives

Introduction

Hofmeisteria schaffneri (A. Gray) R. M. King & H. Robinson (Asteraceae), commonly known as ambar, is an aromatic evergreen medicinal herb, which grows naturally in the oak and pine–oak forested mountains of the central Mexican states of Jalisco and San Luis Potosi.[1,2] *Hofmeisteria schaffneri* is cultivated in domestic gardens in Mexico City and the states of Mexico and Michoacán. The fresh or dried herb is available in Mexican markets and commonly used by local people for treating stomach aches, skin infections and fevers.[2,3] Previous investigations on the organic (CH_2Cl_2-MeOH 1 : 1) and aqueous extracts from the aerial parts of the plant yielded several thymol analogs, including compounds 1–10, which possess phytotoxic and antinociceptive properties.[1–3]

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As part of our research program to assess the efficacy and quality control procedures of widely used Mexican medicinal plants, the present work was undertaken to establish the antimicrobial potential of *H. scahffneri* infusion and essential oil, together with those of their major components. The relevance of this work stems from the fact that in Mexico there is an urgent need to establish the efficacy and quality control methods useful for identification and standardization of widely commercialized herbal drugs and their preparations.

Materials and Methods

Plant material

Four batches (I–IV) of the aerial parts of *H. schaffneri* were collected on 13 May 2006 (end of flowering season), 30 August 2006 (non-flowering season), 10 October 2006 (non-flowering season) and 16 January 2007 (beginning of flowering season), in Ozumba, Mexico. The plant material was identified by R. Bye and E. Linares and in all cases voucher specimens were deposited at Mexican National Herbarium (MEXU) with accession numbers E. Linares 2021 (May 13 2006), E. Linares 1846 (August 30 2006) and E. Linares 1736 (October 10 2006) and Bye & Linares 34 962 (January 16 2007).

Preparation of essential oils and extracts

At each seasonal stage, 250 g of the fresh aerial parts cut in small pieces were hydrodistilled (2.5 l of H_2O) for 3 h using a Clevenger-type apparatus. When the condensed material cooled down, the essential oils were separated from the water by liquid–liquid extraction with dichloromethane (CH_2Cl_2; 750 ml × 3). After removal of the solvent, the oils were stored at 4°C until analysis. The aqueous and the CH_2Cl_2-MeOH (1 : 1) extracts were prepared from batch IV as previously described.[2] Briefly, The CH_2Cl_2-MeOH (1 : 1) extract was obtained by macerating air-dried aerial parts of *H. schaffneri* (100 g) at room temperature for 15 days. After filtration, the solvent was eliminated under reduced pressure to yield 25 g of crude extract. The aqueous extract was prepared by treating air-dried aerial parts (9 g) of *H. schaffneri* with hot H_2O (250 ml) for 30 min. The resulting aqueous extract was exhaustively partitioned with CH_2Cl_2 (250 ml × 3); the organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield a brown residue (95 mg).

Solvents and standards

Burdick & Jackson (Honeywell International Inc., Muskegon, MI) supplied dichloromethane. Sigma-Aldrich (Sigma-Aldrich Química, S.A. de C.V, Toluca, México) supplied standards of thymol (10), α-terpinene (11), *m*-cymene (12), *p*-cymene (13), linalol (14), linalyl acetate (22), α-terpinyl acetate (25), geranyl acetate (27), α-copaene (28), aromadendrene (31), alloaromadendrene (32), δ-cadinene (35), *E*-nerolidol (36) and caryophyllene oxide (40), and the *n*-alkanes (C-7–C-40) used for the determination of the retention indexes. Hofmeisterin (1), 3′,4′,4a′,9a′-tetrahydro-6,7′-dimethylspiro[benzofuran-3(2*H*),2′-pyrano[2,3-*b*] benzofuran]-2,4a′-diol (2), hofmeisterins II and III (3 and 4), 2′,3′-dihydroxy-2′-thymyl angelate (6), 8,9-epoxy-10-acetoxythymyl angelate (8) and euparin (50) were available from a previous investigation of *Hofmeisteria schaffneri*[1,2] while eupatoriochromene (49) was obtained from *Helianthella quinquenervis*.[4] Thymyl isobutyrate (33) and thymyl isovalerate (38) were obtained by synthesis as previously described.[3]

Gas chromatography and gas chromatography-mass spectrometry conditions

GC analyses for determining retention indexes were carried out using a Hewlett Packard 5890 GC apparatus equipped with dual flame ionization detector (FID) system and an electronic pressure control injector. Two Supelco fused silica capillary columns (Sigma-Aldrich Química S.A. de CV, Toluca, México) were used: a SPB-1 (dimethylpolysiloxane; 60 m × 0.25 mm I.D.; 0.25 µm film thickness) and a Supelcowax 10 (polyethylene glycol; 30 m × 0.25 mm I.D.; 0.25 µm film thickness). The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 30 min. The injector and detector temperature was 280°C. Samples were injected in the split mode (1/50), using helium as carrier gas (1 ml/min); the injection volume was 1 µl.

For GC-MS/EI, samples were analysed with an Agilent Technologies 5973 mass spectrometer (quadrupole) with electron impact ionization (70 eV), coupled to an Agilent Technologies 6890N gas chromatograph and equipped with a Zebron (Phenomenex Inc. Torrance, CA) fused-silica capillary column ZB-5 (5% phenyl-dimethylpolysiloxane; 30 m × 0.25 mm I.D.; 0.25 µm film thickness). The column temperature was programmed from 40°C to 310°C at 10°C/min and then held isothermally at 310°C for 5 min. Injector and detector temperatures were maintained at 280°C. The carrier gas was helium with a flow rate of 1 ml/min; the injection volume was 1 µl. Mass spectra were acquired over the mass range 50–550 Da.

For GC-MS/CI, samples were analysed with a Hewlett Packard (5890 series II) gas chromatograph equipped with a Agilent (Agilent Tecnologies, Santa Clara, CA) fused silica capillary column Ultra-2 (5% phenyl-dimethylpolysiloxane; 25 m × 0.20 mm I.D.; 0.33 µm film thickness) coupled to an Agilent Technologies 5973 mass spectrometer with specific ionization chemical source, using helium (1 ml/min) as carrier gas and methane as ionizing gas. The oven temperature was programmed from 40°C to 310°C at 10°C/min and then held isothermally at 310°C for 5 min. The injector and detector temperature was 280°C. The carrier gas was helium with a flow rate of 1 ml/min; the injection volume was 1 µl. Mass spectra were acquired over the mass range 50–550 Da.

Identification of essential oil constituents

Most of the essential oils components were identified by comparing the experimental retention indexes (*Ia* and *Ip*), relative to C-7–C-40 *n*-alkanes on Supelcowax 10 and SPB-1 columns, and mass spectra with those of authentic reference standards. Other components were characterized by comparing their experimental retention indexes and mass spectra with literature data[5,6] as well as the spectra generated by the instrument software (NIST Mass Spectral Search Program version 2.0f). The proportion of the components was obtained from the electronic integration of the FID peak areas in the total ion

chromatogram (TIC) resulting from the analysis with the SPB-1 column.

Antibacterial and antifungal studies

Bacillus subtilis ATCC6633 and *Staphylococcus aureus* ATCC25923 (Gram-positive bacteria) as well as *Escherichia coli* ATCC10536, *Salmonella typhi* ATCC9992 and *Pseudomonas aeruginosa* ATCC27853 (Gram-negative bacteria) were used for the antimicrobial susceptibility test. The bacteria were cultured on nutrient agar (Bioxon, Becton Dickinson de Mexico) before determination of MIC (Minimum Inhibitory Concentration) values.[7] Mueller–Hinton broth (MHB, Bioxon, Becton Dickinson de Mexico, México City) containing 20 and 10 mg/l of Ca^{2+} and Mg^{2+}, respectively, was used for bacterial growth. The yeast (*Candida albicans* ATCC10231) was cultured on Sabouraud-4% glucose agar (MERCK, S.A. DE C.V. Naucalpan, México),[7] and RPMI-1640 (Sigma-Aldrich Química S.A. de CV, Toluca, México) was used for MIC determinations.

Stock solutions of all tested materials were prepared by dissolving 4 mg of the oil in 80 µl DMSO (Dimethyl Sulfoxide). Sterile water was added to make the volume up to 2 ml. The stock mixtures were serially diluted to obtain final concentrations between 1024 and 2 µg/ml. The inocula of the microorganisms were prepared from broth cultures in Mueller–Hinton medium or RPMI-1640; serial dilutions were made to achieve a suspension of approximately 10^5 CFU/ml. For every experiment, a sterility check (2% DMSO and inoculum), negative control (2% DMSO, medium and inoculum) and positive control (2% DMSO, media, inoculum and antibiotics) were included. The positive control was ampicillin (bacteria) or fluconazole (yeast) (Sigma-Aldrich Química S.A. de CV, Toluca, México). In general, the 96-well plates were prepared by dispensing into each well 100 µl each of an appropriate medium, test extracts and 20 µl of the inoculum. The contents of each well were mixed thoroughly with a multi-channel pipette and the microtitre plates incubated at temperatures and for periods appropriate to the organism under study. Bacterial growth was determined by adding 20 µl of a 5% solution of tetrazolium salt and incubating for a further 10 min. Clear wells indicated inhibition of the growth of organism, while dark-colored wells indicated absence of inhibition. The temperature used for incubation of bacteria was 37°C, maintained for 24 h; for yeast the temperature was 35°C, maintained for 48 h. After incubation, plates were observed for bacterial or yeast growth and MIC (the lowest concentration of sample that completely inhibited microorganism visible growth) calculated. All experiments were performed in triplicate.[7]

Results

Antimicrobial activity of extracts and essential oils

Table 1 summarizes the in-vitro antimicrobial activity of two extracts (aqueous and organic) and essential oils from four different collections of *H. schaffneri* against five bacterial and one yeast species. According to the data in Table 1, the infusion and oils were active against Gram-positive bacteria (*S. aureus* and *B. subtillis*) and *C. albicans*, but the Gram-negative bacteria (*E. coli*, *S. typhi* and *P. aeruginosa*) were not sensitive to any of the treatments (MIC > 1024 µg/ml). Among the Gram-positive bacteria, *S. aureus* was more susceptible. The MIC value of the infusion against *S. aureus* was 64 µg/ml, while the oils exhibited MIC values in the range 48–192 µg/ml. The most active sample was the essential oil from batch IV, with a MIC value of 48 µg/ml. The infusion (MIC = 128 µg/ml) and essential oil from batch I (MIC = 192 µg/ml) displayed the best effect against the yeast, *C. albicans*.

Table 1 Susceptibility of *S. aureus, B. subtilis* and *C. albicans* to essential oils, extracts and compounds from *H. schaffneri*

Oil batch, extract or compound	MIC (µg/ml)		
	Staphylococcus aureus (ATCC25932)	*Bacillus subtilis* (ATCC6633)	*Candida albicans* (ATCC10231)
I	96	256	192
II	192	256	512
III	192	512	512
IV	48	768	512
1	>1024	>1024	256
2	512	>1024	>1024
3	>1024	>1024	>1024
4	>1024	>1024	512
6	>1024	>1024	128
8	32	64	>1024
10	512	256	128
33	>1024	>1024	256
38	64	32	512
Infusion	64	64	128
Organic extract (CH_2Cl_2-MeOH 1 : 1)	>1024	>1024	1024
Ampicillin	<2	<2	–
Fluconazole	–	–	2

Composition of the essential oils

GC and GC-MS analyses established the chemical composition of the active essentials oils (batches I–IV) from the aerial parts of *H. schaffneri* collected during flowering and non-flowering stages. Forty-four components were identified in the four batches, representing ~90% of the total amount. Table 2 summarizes chemical profiles and the percentage content of the individual components of the oils (Figure 1).

In general, the most abundant compounds were hofmeisterin III (4) and thymyl isovalerate (38) (Figure 2). It should be noted that batches I and IV contained significant amounts of 8,9-epoxy-10-acetoxythymyl angelate (8) (Figure 2). The sesquiterpenoid content was more variable and ranged from 3.92 (batch I) to 15.34% (batch IV), with α-caryophyllene oxide (40) the most important component.

Antimicrobial effect of compounds 1–4, 6, 8, 10, 33 and 38

The antimicrobial activity of compounds 1–4, 6, 8, 33 and 38, available from previous investigations,[1–3] were also tested and compared with that of thymol (10), a well-known antiseptic agent also present in the preparations analysed, and standard antibiotics. Table 1 shows the results of these assays.

Table 2 Composition of the essential oils from *H. schaffneri* collected in flowering and non-flowering seasons

No.	Compounds	*Ia*[a]	*Ip*[b]	Composition (%)			
				Batch			
				I May	II August	III October	IV January
11	α-Terpinene	1009	1175	0.1	0.09	0.23	0.10
12	*m*-Cymene	1015		0.15	0.28	0.35	0.06
13	*p*-Cymene	1017	1237	0.34	–	0.32	0.22
14	Linalol	1081	1519	0.25	1.38	0.56	0.40
15	Undecane	1100	1100	0.08	–	–	0.13
16	*cis*-Pinene hydrate	1102	–	0.2	–	–	0.60
17	*trans*-*p*-Menth-2-ene-1-ol	1122	1541	0.58	tr	0.10	0.11
18	*p*-Cymen-7-ol	1166	–	0.36	1.36	0.80	0.89
19	*cis*-Piperitol	1184	–	0.07	–	–	0.22
20	8,9-Dehydrothymol	1192	1965	1.52	0.58	0.13	0.13
21	Thymol methyl ether	1212	–	0.21	2.23	0.24	0.38
22	Linalyl acetate	1237	1522	0.12	0.57	0.47	0.17
10	Thymol	1267	2160	1.23	0.27	0.37	0.42
23	Tridecane	1300	1300		0.11	0.08	0.20
24	2,3-Epoxycinnamyl alcohol	1308	–		–	0.13	0.14
25	α-Terpinyl acetate	1331	1681	0.45	0.23	0.24	0.18
26	α-Cubebene	1355	1460	0.23	1.90	0.96	0.76
27	Geranyl acetate	1357	1741	0.51	–	–	0.52
28	α-Copaene	1365	1495		–	–	0.12
29	β-Cubebene	1392	1535		0.32	0.32	0.72
30	Thymohydroquinone dimethyl ether	1420	–	0.2	0.10	–	0.17
31	Aromadendrene	1435	1565	tr	0.83	0.65	0.21
32	Alloaromadendrene	1452	1597		-	0.27	0.47
33	Thymyl isobutyrate	1456	1867	3.41	1.54	2.41	2.66
34	4-*epi*-Cubebol	1505	–		–	–	0.26
35	δ-Cadinene	1522	–	tr	0.19	0.26	0.32
36	*E*-Nerolidol	1545	1962	1.31	1.38	1.76	3.11
37	8,9-Dehydrothymyl isovalerate	1548	1950	0.89	–		0.94
38	Thymyl isovalerate	1551	1921	30.97	17.28	18.63	14.12
39	Maaliol	1558	1918		0.39	0.25	0.26
40	Caryophyllene oxide	1574	1926	1.54	2.19	3.08	6.63
4	Hofmeisterin III	1577	2037	24.12	33.55	34.85	30.20
41	Dehydrothymyl angelate	1581	–	3.01	2.78	2.54	2.00
42	Humulene 6,7-epoxide	1596	–	–	0.14	–	0.09
43	1,10-*epi*-Cubenol	1610	–	–	–	–	0.64
44	γ-Eudesmol	1617	–	–	0.88	0.32	0.58
45	Cubenol	1629	2025	0.09	1.91	1.41	0.57
46	γ-Bisabolol	1667	–	0.65	1.09	0.87	0.22
47	14-Hydroxy-4,5-dihydro-β-caryophyllene	1690	–	0.10	0.36	0.40	0.37
48	Heptadecane	1700	1700	2.12	2.38	1.96	0.57
49	Eupatoriochromene	1726	–	0.66	0.84	tr	0.70
50	Euparin	1860	2803	0.11	tr	tr	0.17
51	9-Acetoxy-8,9-dehydrothymyl angelate	1875	2930	2.36	5.23	4.76	2.50
8	8,9-Epoxy-10-acetoxythymyl angelate	1982	2901	2.31	0.41	0.42	15.00
	Monoterpene hydrocarbons			0.59	0.38	0.90	0.38
	Oxygen-containing monoterpenes			72.77	67.50	66.52	71.62
	Sesquiterpene hydrocarbons			0.23	3.25	2.47	2.61
	Oxygen–containing sesquiterpenes			3.69	8.35	8.09	12.73
	Others			2.97	3.33	2.17	1.90
	Total identified			**80.25**	**82.80**	**80.15**	**89.23**
	Oil yield (% w/w – dry basis)			1.1	1.0	0.9	0.9

[a]*Ia* is the retention index on the non-polar column (SPB-1). [b]*Ip* is the retention index on the polar column (Supelcowax 10); tr = trace (<0.05%).

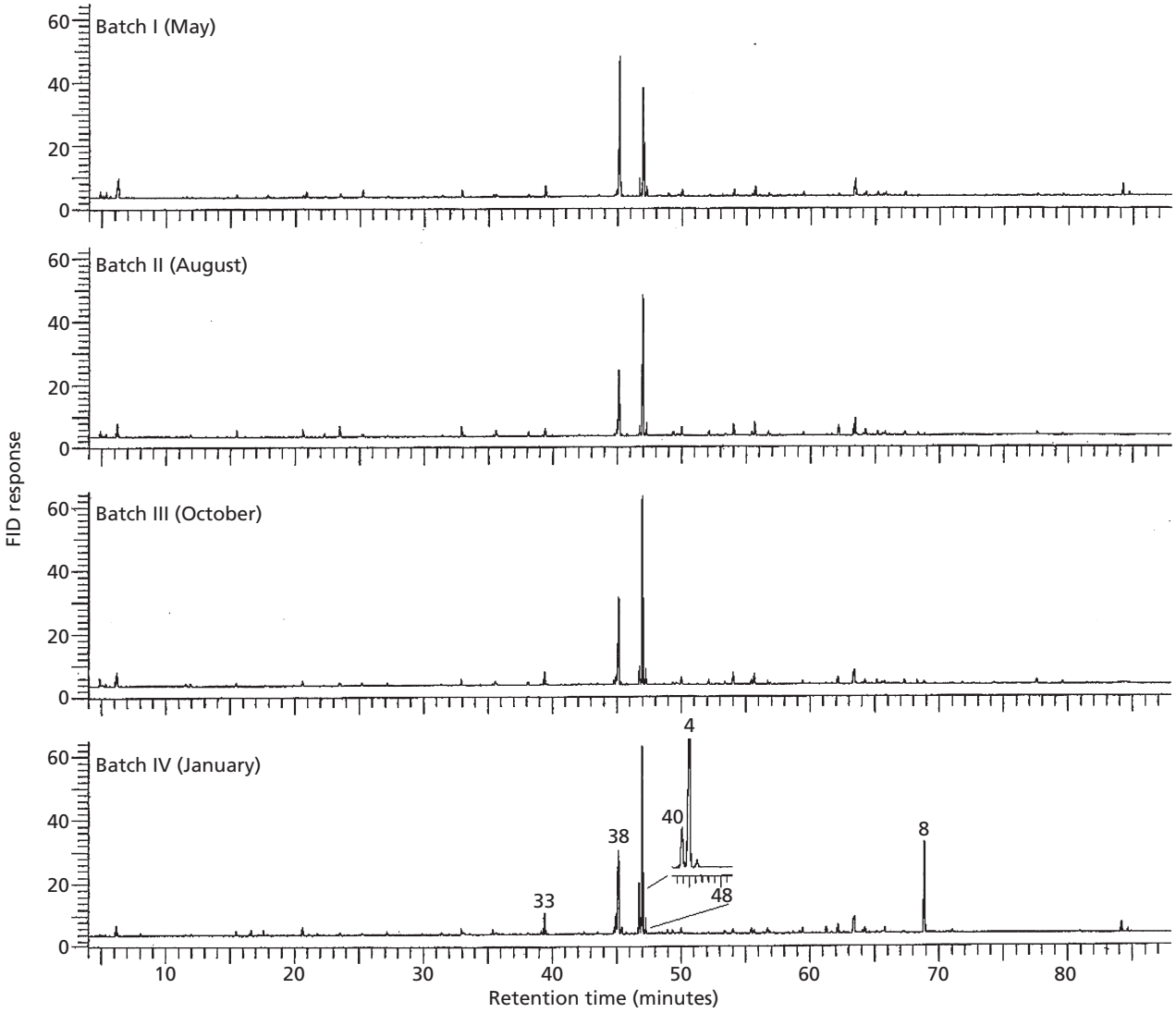

Figure 1 GC-FID chromatograms of essential oils analysed with a non-polar SPB-1 capillary column, showing profiles of four batches of *H. schaffneri*. Peak numbers are according to Table 2. For chromatographic conditions, see Materials and Methods section.

Compound 8, the major component of the infusion and present in all oils batches analysed, particularly batches IV and I, turned out to be more active than thymol (10) against *S. aureus* and *B. subtilis*, and was inactive against *C. albicans* (MIC > 1024 µg/ml). However, hofmeisterin III (4), the major component of the oils and the organic extract,[2] was inactive against all microorganisms tested. Compound 38 displayed good antibacterial activity against both Gram-positive bacteria, although it showed low activity against *C. albicans*. Compound 6 (MIC = 128 µg/ml), one of the components of the infusion,[2] also showed an antifungal activity comparable with that of thymol (10) (MIC = 192 µg/ml). The remaining compounds did not exhibit significant activity.

Discussion

Mexico, where a large proportion of the population relies on traditional medicine for primary health care, possesses a long tradition in the use of medicinal herbs. Despite their continued use, few herbal drugs have been properly studied to provide an accurate assessment of their quality, efficacy and safety. Such studies are very important for promoting proper use of traditional medicine. In this scenario we evaluated the preclinical antiseptic activity of *Hofmeisteria schaffneri*, considering its extensive use by local people, mestizos or not, for treating skin infections such as impetigo, ringworm and pimples. In addition, the analysis of the antimicrobial activity of extracts, essential oils or compounds isolated from plants used in folk medicine could lead to the discovery of potential therapeutic agents.[8–11]

The results of the analysis of two extracts (aqueous and organic) and essential oils from four different collections of *H. schaffneri* against five bacteria and one yeast species revealed that the plant showed antibacterial effects against *S. aureus* and *C. albicans*. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impe-

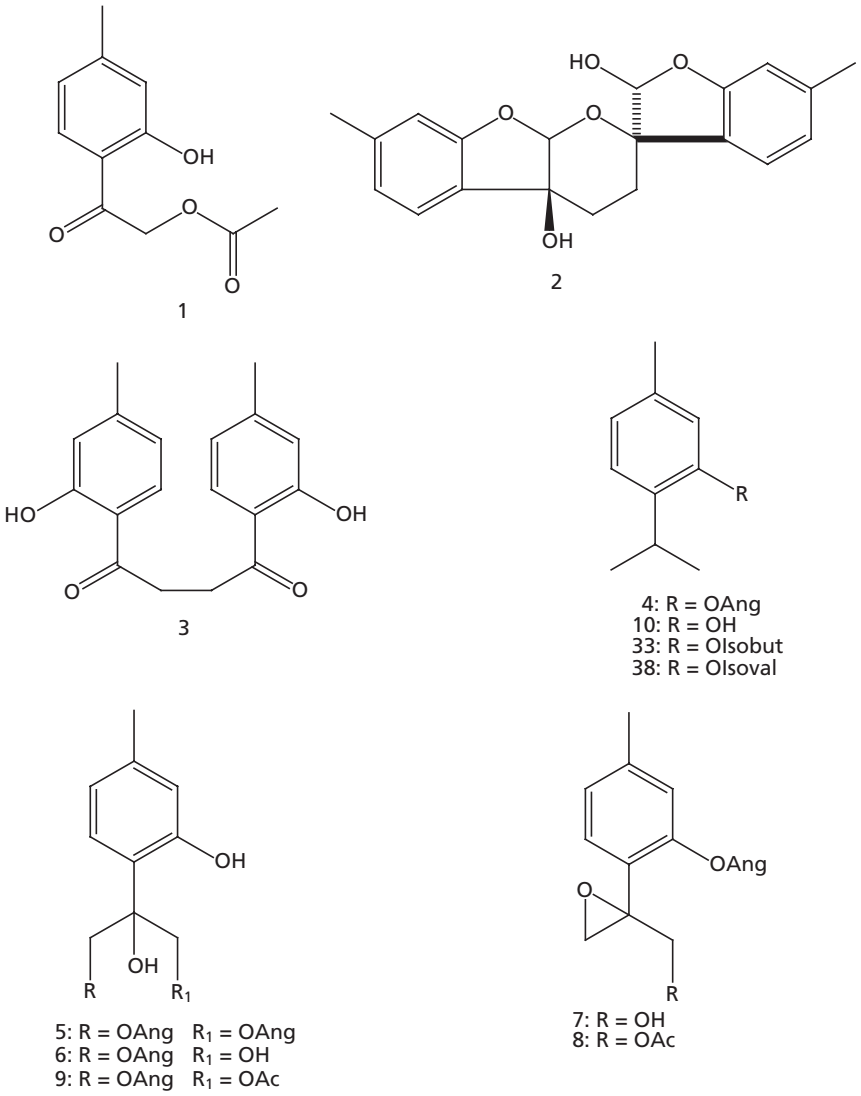

Figure 2 Structure of thymol derivatives from *H. schaffneri.*

tigo, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, bacteremia and sepsis, among others.[12] Furthermore, *S. aureus* is extremely prevalent in atopic dermatitis patients, who usually suffer more severe skin infections. *C. albicans*, on the other hand, causes vaginal infections, thrush, skin rash and nailbed infections, to mention the most common.[13] Thus, the activity of the preparations of the plant supported, at least *in vitro*, the popular use of *H. schaffneri* as a topical anti-infective agent. Furthermore, since infectious diseases account for one-third of all deaths worldwide, the present results could be of benefit from the phytotherapeutic point of view.

Although, the plant is harvested throughout the year for medicinal purposes, the oils analysed in this work were prepared from both flowering and non-flowering seasons to determine the variability, if any, in chemical and biological profiles due to ontogenic variations. Identification of the constituents of the oils using GC (FID detection) was carried out according to the Kovats methodology. The retention indices (*Ia* and *Ip*) using two different columns (polar and non-polar) were calculated by means of the Van Den Dool equation.[14,15] The use of the polar and non-polar columns makes the identification process more certain.[16] Compound concentrations (as percentage content), however, were established with the non-polar column (SPB-1) from the FID response without application of correction factors. The identity of some compounds was corroborated by co-elution with available standards. On the other hand, when standard references were not available identification of the components was confirmed on the basis of their spectral data, obtained by GC-MS analysis using both EI and CI ionization modes. The latter technique was used to verify the molecular ions. The most important chemical feature of the oils was the presence of a high

percentage of oxygenated monoterpenes, mainly thymol esters of different short-chain fatty acids. These constituents showed synchronized patterns of variation during the different collection periods.

The plant material obtained from the beginning of the flowering season (batch IV) yielded the high antibacterial essential oil. Comparative analysis of the chemical profiles of the oils from batches I–IV indicated that the most important differences were the content of 8,9-epoxy-10-acetoxythymyl angelate (8) and thymyl isovalerate (38), which showed the best antibacterial effect in this study. These results are also consistent with the activity observed for the oils from batches I and IV.

Compound 6, which showed the highest anti-*Candida* activity, is present in the infusion, along with compounds 8 and 10, supporting the use of this phytopreparation in folk medicine as an antiseptic agent.

As thymol itself, the active compounds found in this work may destroy microbial cell integrity by different mechanisms.[17–21] The essential oils from several Lamiaceae and Asteraceae species, which are also rich in thymol analogs, possess similar high antibacterial action to the oil batches from *H. schaffneri* analysed in the present study.[22–31]

Conclusions

The results of this work show that *H. schaffneri* infusion and essential oils (batch IV) have antimicrobial activity against *S. aureus*, *B. subtillis* and *C. albicans*, the microorganisms responsible for several skins infections. A series of thymol esters of different short-chain fatty acids were the active principles of the different preparations of *H. schaffneri* analysed. The composition and antimicrobial activity of the essential oil of *H. schaffneri* changes slightly over the year, showing the relevance of the harvest season to biological activity. The chemical profile of the essential oils obtained in this investigation will also be useful for quality control of the plant.

From the chemotaxonomic point of view it is noteworthy that many species of the Eupatorieae tribe of the Asteraceae are used as anti-infective agents in folk medicine and contain thymol and thymol ester derivatives.[22] So far these compounds have been reported in *Doronicum corsicum*,[23] *Laggera pterodonta*,[24] *Schizogyne glaberrima*,[25] *Helichrysum arenarium*[26] and some *Eupatorium* species.[27,28]

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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