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Antimicrobial properties of the linalol-rich essential oil of *Hyssopus officinalis* L. var *decumbens* (Lamiaceae)

Gabriela Mazzanti,[1] Lucia Battinelli[1] and Giuseppe Salvatore[2]*

[1]**Facoltà di Farmacia, Institute of Pharmacology and Pharmacognosy, Università di Roma 'La Sapienza', Piazzale Aldo Moro, 5-00185 Roma, Italy**
[2]**Istituto Superiore di Sanità, Department of Comparative Toxicology and Ecotoxicology, Viale Regina Elena 299, 00161 Roma, Italy**

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ABSTRACT: The antimicrobial activity of essential oil of *Hyssopus officinalis* L. var *decumbens* (Jordan & Fourr.) Briq. from France (Banon) and *Hyssopus officinalis* L. from Italy (Piedmont) was studied taking account of their chemical composition determined by GC and GC–MS. Pinocamphone and isopinocamphone are present in *H. officinalis* (4.4% and 43.3%, respectively), according to the ISO 9841 Standard (1991 E) but they are lacking in var. *decumbens*, where linalol (51.7%), 1,8-cineole (12.3%) and limonene (5.1%) instead are predominant. The disc diffusion tests carried out on Gram-positive (*Staphylococcus aureus* and *Enterococcus* spp.) and Gram-negative bacteria (*Klebsiella oxytoca, Escherichia coli*, *Proteus mirabilis*, *Pseudomonas* spp. and two strains of *Salmonella* spp.) showed an antimicrobial activity generally negligible for *H. officinalis*, but broader, and in a few cases more evident (*Enterococcus* spp. and *E. coli*), for var. *decumbens*. All yeasts (seven strains of *Candida albicans*, *C. krusei* and *C. tropicalis*) were strongly inhibited by both species. In liquid medium the MIC of *H. officialis* was always $>1.2\%$ v/v for bacteria and between 0.6 and 1.2% v/v for yeasts, while the MIC of var. *decumbens* was between 0.15 and 0.6% v/v for the Gram-positive bacteria, 0.3 and 1.2% v/v for the Gram-negative bacteria and 0.15 and 0.3% v/v for the yeasts. The effect of var. *decumbens* was generally bactericidal. Linalol, and in a lesser way, 1,8-cineole, may contribute to the greater antimicrobial activity of var. *decumbens* in comparison with *H. officinalis*, while limonene may be responsible for the antimycotic action observed in both oils, as suggested by results of the disc diffusion tests carried out on the pure reference substances. © 1998 John Wiley & Sons, Ltd.

KEY WORDS: *Hyssopus officinalis* L.; *Hyssopus officinalis* L. var *decumbens* (Jordan & Fourr.) Briq.; Lamiaceae; linalol; 1,8-cineole; limonene; antimicrobial activity

Introduction

The essential oils of plants of the genus *Hyssopus* (Lamiaceae) have been studied for different varieties, chemotypes and phenotypes of *Hyssopus officinalis* L. with reference to the parts used (leaves, stems, roots, aerial parts, flowering tops or only flowers, which may be blue, red, white or of mixed colour), to the stages of development of the wild growing or cultivated plants at the time of the harvest, as well as taking account of their different origin.[1–7]

The chemical compositions of these essential oils vary considerably between oils obtained from the same type of hyssop, although the presence of the bicyclic monoterpene ketones, pinocamphones and isopinocamphone, remains peculiar to these products. In fact, there are oils with a higher level of pinocamphone (up to 80%) or isopinocamphone (up to 50%), although the ISO 9841 Standard (1991 E) recommends 5.5–17.5% for pinocamphone and 34.5–50% for isopinocamphone. There are also oils in which other substances may predominate; for example with *Hyssopus officinalis* L., there is a cineole-rich oil (52.9%) from Spain[4] and a methyl eugenol-rich oil (38.3%) from Montenegro.[3]

The essential oil of hyssop may be used as expectorant and antiseptic,[8] although in this regard it should be recalled that toxic effects such as clonic and clonic-tonic convulsions related to the presence of pinocamphone and isopinocamphone in the oil have been reported.[9]

Hyssopus officinalis L. var. *decumbens* (Jordan & Fourr.) Briq. is a plant growing in Europe; it is morphologically different from *Hyssopus officinalis* L. in that it shows non-aristate bracts.[10] Our previous studies[11] have pointed out a peculiar chemical composition of the essential oil of var. *decumbens*, particularly the lack of pinocamphone and isopinocamphone. Considering the remarkable difference of

* Correspondence to: G. Salvatore, Istituto Superiore di Sanità, Department of Comparative Toxicology and Ecotoxicology, Viale Regina Elena 299, 00161 Roma, Italy.

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the chemical composition of the hyssop essential oils, we aimed to compare the antimicrobial activity of two different types of oil, one of *Hyssopus officinalis* L. var. *decumbens* (Jordan & Fourr.) Briq. from France (Banon), the other of *Hyssopus officinalis* L. of Italian origin (Piedmont). For this purpose the capability of the oils to inhibit the growth of Gram-positive (two strains) and Gram-negative bacteria (six strains) and yeasts (nine strains) was studied. The tests were carried out on solid medium, measuring the growth inhibition zone, and in liquid medium, establishing the MIC and the MBC. The main constituents of the oil of var. *decumbens*, linalol, 1,8-cineole and limonene, were also studied.

Material and Methods

Plant Material and Corresponding Essential Oils

Hyssopus officinalis L. var. *decumbens* (Jordan & Fourr.) Briq. was collected and identified at Banon (France). The fresh aerial material was distilled in October 1995 by Société Civile d'Exploitation Agricole (SCEA) Petit-chêne de Rosalyne Dubois (Les Agreniers, 04150 Banon), giving six distinct batches of essential oil. These oils were singly examined for their chemical composition and found to be very similar; consequently, only one of them (var. *decumbens*) was investigated for its antimicrobial activity. A sample of essential oil of *Hyssopus officinalis* L. produced in Italy (Piedmont) by Agronatura, was examined for comparison.

Reference Substances

The characteristics of pure reference substances were as follows: Linalol (97% pure, Sigma-Aldrich); 1,8-cineole (99% pure, Sigma-Aldrich); (*R*)-(+)-limonene (97% pure, Sigma-Aldrich). A mixture of pinocamphone (83.5%, GC) and isopinocamphone (13.6%, GC) was kindly provided by A. D'Andrea (ENEA, Rome, Italy).

Gas Chromatography (GC–FID) and Gas Chromatography–Mass Spectrometry (GC–MS)

Gas chromatographic equipment: a Perkin Elmer AutoSystem GC equipped with two fused-silica SPB 5 columns (60 m × 0.25 mm i.d., film thickness 0.25 mμ), mounted in parallel in the same oven, and with two detectors: FID and Q-Mass 910 (electron ionization 70 eV electron energy, transfer line 220°C). Carrier gas: oxygen and moisture-free helium obtained from SUPELCO™ High Capacity Heated Carrier Gas Purifier, provided with OMI-2 indicating tube, at the average flow rate of 1 ml/min. Oven temperature programme: 60°C for 4 min, then 2°C/min to 180°C, then 3°C/min to 250°C. Detector temperature: 280°C. Injector temperature: 280°C. Volume of injected essential oil: 0.1 μl. Split ratio: 1:50. Two distinct data systems are connected to the GC–FID or GC–MS: Turbochrom and Q-Mass Analytical Workstation Software with NIST/EPA/MSDC Mass Spectral Database, respectively.

Chemical Identification and Quantitative Estimation

Chemical components were identified by comparing the retention time of the GC peaks and GC–MS of pure reference substances added to the test oils. Quantitative data were based on peak area normalization without use of correction factors.

Micro-organisms

Two Gram-positive bacteria (*Staphylococcus aureus* 484 and *Enterococcus faecalis* 413), six Gram-negative bacteria (*Klebsiella oxytoca* 487, *Escherichia coli* 910, *Proteus mirabilis* 608, *Pseudomonas aeruginosa* 514 and *Salmonella typhi* 839 and 769), and nine yeasts (seven strains of *Candida albicans*: 136, 308, 323, 603, 615, 616 and 829, *Candida krusei* 324 and *Candida tropicalis* 365) were used. Micro-organisms, obtained by clinical isolation, were grown in nutrient broth (CM001B; Unipath Milan, Italy) and nutrient agar (CM003B, Unipath, Milan, Italy) at pH 7.4.

Antimicrobial Tests

Disc Diffusion Method. Inhibitory activity of oils and pure substances on micro-organism growth was determined by the method of Bauer *et al.*[12] Agar plates (120 mm diameter) were seeded with micro-organism suspension (10^5 cells/ml) using a sterile cotton swab. Cellulose discs (PAR-Test Blank Discs, 6 mm diameter, Unipath, Milan, Italy) were impregnated with 20 μl of essential oil or pure reference substance (linalol, limonene and 1,8-cineole). The test discs and blank were placed on the plates (four discs for each plate) and incubated at 37°C for 24 h; the incubation time for yeasts was 48 h. During the incubation the plates were inverted. The antimicrobial activity was estimated by measuring the radius of the zone of growth inhibition. In these conditions the maximal detectable radius was 25 mm. Oil samples and reference substances were tested in triplicate.

Determination of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC). The MIC was determined according to Wilkinson and Gentry.[13] Serial twofold dilutions of the oil solubilized in DMSO (maximum 5%) were made and the tubes were inoculated with different micro-organisms at a density of 10^5 cells/ml. After incubation for 24 h (or 48 h for the yeasts) at 37°C, the tubes were visually examined for growth. The MIC was the lowest concentration of essential oil which prevented visible turbidity. For determination of MBC the cultures that did not present growth were used to inoculate plates of solid medium. After the prescribed time of incubation the plates were observed: the lack of colonies denoted a bactericidal action. Control tests were run simultaneously by adding DMSO without essential oil. Oil samples were tested in triplicate.

Results and discussion

Chemical Composition

The chemical composition of the six batches of *Hyssopus officinalis* L. var. *decumbens* (Jordan & Fourr.) Briq. essential oil was very similar, consequently only one of them was used for the biological investigations. In Table 1, the components of the two species are reported and listed in order of their elution on the SPB5 column with their percentage. The peculiarity of the var. *decumbens* is the lack of bicyclic monoterpene ketones (*cis*- and *trans*-pinocamphone), while linalol (51.7%), 1,8-cineole (12.3%) and limonene (5.1%) proved to be the predominant components. This chemical composition is very different from those of hyssop oils published by other authors[1–7] and from the one here reported for the Italian *H. officinalis*, which is according to the ISO 9841 Standard (1991 E). Figure 1 clearly shows these diversities.

Antimicrobial Activity

In agar diffusion tests, both species showed a similar behaviour (Table 2): maximal inhibition of the growth of *Staphylococcus aureus* 484 and all the yeasts (in all cases, inhibition zone > 25 mm) and inactivity against *Pseudomonas aeruginosa* 514. On the other bacteria, var. *decumbens* was generally more active (inhibition zone 2 or 3 mm and >25 mm for *Escherichia coli* 910) than the *H. officinalis*, which was inactive on *Proteus mirabilis* 608, *Salmonella typhi* 839 and *Salmonella typhi* 769.

Table 3 shows that the pure limonene was inactive against all Gram-negative bacteria and *Enterococcus faecalis* 413, but active against *Staphylococcus aureus* 484 and *Candida albicans* 308 (inhibition zone > 25 mm), *Candida albicans* 615 being the most resistant (inhibition zone 2 mm).

1,8-Cineole was inactive against *Pseudomonas aeruginosa* 514, but it inhibited the growth of all the other micro-organisms, even if to a different degree (inhibition zones between 2 and 17 mm); maximal inhibition was observed against *Staphylococcus aureus* 484 (inhibition > 25 mm).

Linalol proved to be the most active compound. The substance inhibited completely the growth of all the yeasts plus *Staphylococcus aureus* 484 and *Escherichia coli* 910 (inhibition zone > 25 mm); other micro-

Table 1. Chemical composition of the essential oil of *Hyssopus officinalis* L. (Piedmont, Italy) and *Hyssopus officinalis* L. var *decumbens* (Banon, France) in comparison with the ISO 9841 Standard (1991 E)

Components	*H. officinalis* var. *decumbens*		*H. officinalis*		ISO 9841 Standard (1991 E) (%)
	$R_T$min[1]	(%)	$R_T$min[1]	(%)	
α-Pinene	18.89	2.2	18.94	0.6	1–1.5
Camphene	19.93	1.9	–	–	
Sabinene	21.57	0.8	21.61	1.5	2–3
β-Pinene	21.87	3.0	21.97	11.1	13.5–23
Myrcene	22.62	1.3	22.66	2.1	1–2
Limonene	25.63	5.1	25.70	12.2	1–4
1,8-Cineole	25.83	12.3	–	–	
Linalol	31.12	51.7	–	–	
Pinocamphone	35.65	1.0	35.73	4.4	5.5–17.5
Isopinocamphone	36.72	1.4	37.11	43.3	34.5–50
β-Bourbonene	52.39	1.0	52.46	1.4	1.5–2
Methyl eugenol	–	–	53.11	4.0	
β-Caryophyllene	54.85	2.4	54.91	1.5	1–3
Unknown $C_{15}H_{24}$	58.91	1.2	59.00	2.2	
Elemol[2]	–	–	63.15	1.7	
Caryophyllene oxide	65.58	2.6	65.60	0.5	

[1] $R_T$min = retention time in min.
[2] Tentative identification from MS library search data.

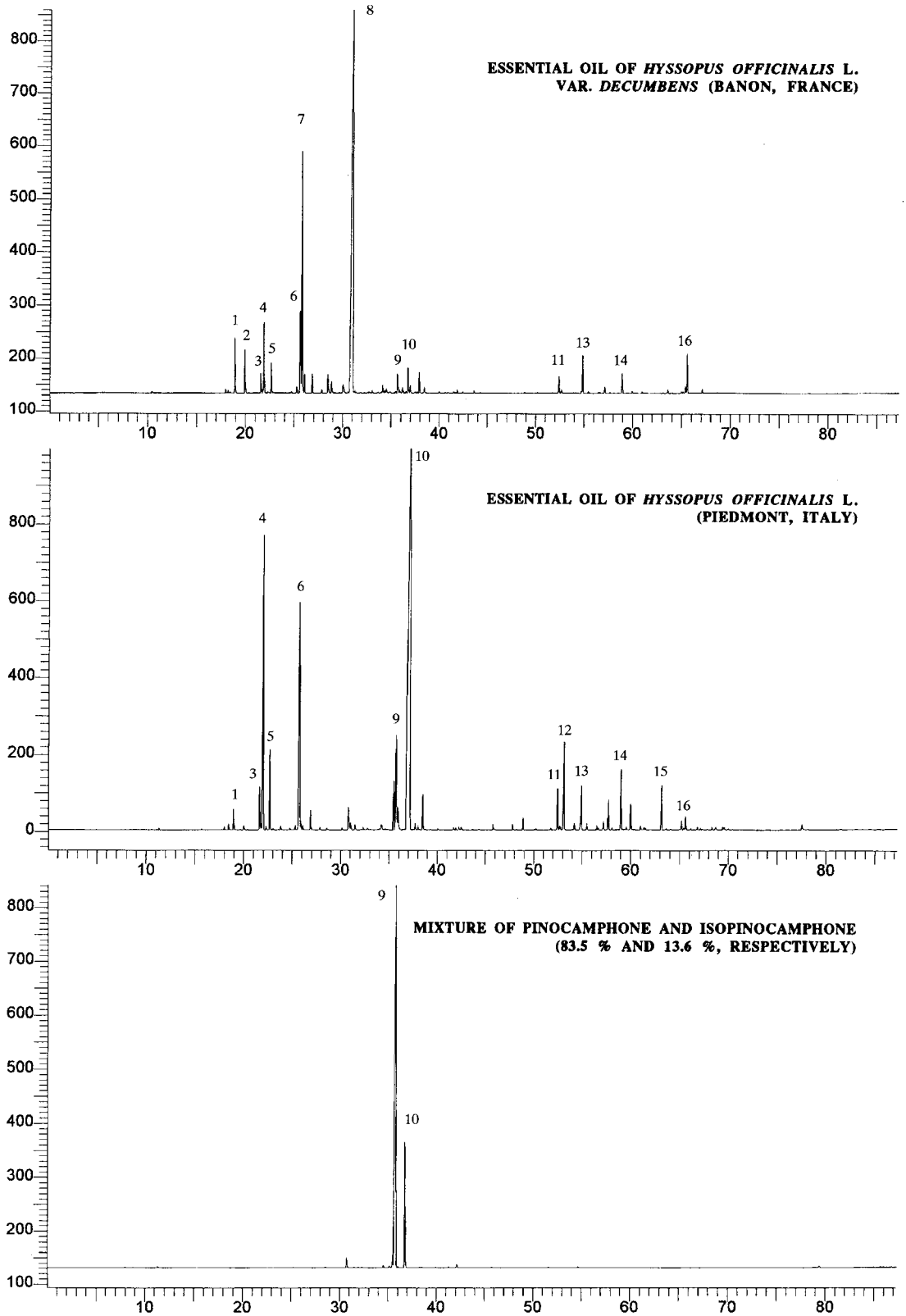

Figure 1. Gas chromatographic profiles (abscissa: time, min; ordinate: response, mV) of: (a) the essential oil of *Hyssopus officinalis* L. var. *decumbens* (Jordan & Fourr.) Briq. from France (Banon); (b) the essential oil of *Hyssopus officinalis* L. from Italy (Piedmont); (c) the mixture of pinocamphone and isopinocamphone, 83.5% and 13.6% respectively. 1 = α-pinene; 2 = camphene; 3 = sabinene; 4 = β-pinene; 5 = myrcene; 6 = limonene; 7 = 1,8-cineole; 8 = linalol; 9 = pinocamphone; 10 = isopinocamphone; 11 = β-bourbonene; 12 = methyl eugenol; 13 = β-caryophyllene; 14 = unknown $C_{15}H_{24}$; 15 = elemol; 16 = caryophyllene oxide

Table 2. *Hyssopus officinalis* L. (Piedmont, Italy) and *Hyssopus officinalis* L. var. *decumbens (Jordan & Fourr.) Briq. (Banon, France) essential oils: antimicrobial activity by disc diffusion method*

Micro-organisms	*H. officinalis* var. *decumbens*	*H. officinalis*
Gram-positive		
Staphylococcus aureus 484	>25	>25
Enterococcus faecalis 413	>25	4
Gram-negative		
Klebsiella oxytoca 487	3	1
Escherichia coli 910	>25	2
Proteus mirabilis 608	2	0
Salmonella typhi 839	2	0
Salmonella typhi 769	3	0
Pseudomonas aeruginosa 514	0	0
Yeasts		
Candida albicans 603	>25	>25
Candida albicans 829	>25	>25
Candida albicans 615	>25	>25
Candida albicans 616	>25	>25
Candida albicans 308	>25	>25
Candida albicans 136	>25	>25
Candida albicans 323	>25	>25
Candida krusei 324	>25	>25
Candida tropicalis 365	>25	>25

Values are the mean zone size (radius; mm) after the deduction of the disc radius. Cellulose discs (6 mm diameter) were impregnated with 20 µl of test essential oil.

Table 3. Antimicrobial activity of limonene, linalol and 1,8-cineole by disc diffusion method

Micro-organisms	Limonene	Linalol	1,8-Cineole
Gram-positive			
Staphylococcus aureus 484	>25	>25	>25
Enterococcus faecalis 413	0	10	4
Gram-negative			
Klebsiella oxytoca 487	0	5	3
Escherichia coli 910	0	>25	6
Proteus mirabilis 608	0	4	4
Salmonella typhi 839	0	5	3
Salmonella typhi 769	0	5	4
Pseudomonas aeruginosa 514	0	8	0
Yeasts			
Candida albicans 603	16	>25	17
Candida albicans 829	9	>25	9
Candida albicans 615	2	>25	2
Candida albicans 616	10	>25	15
Candida albicans 308	>25	>25	5
Candida albicans 136	5	>25	3
Candida albicans 323	2.5	>25	5
Candida krusei 324	5	>25	5
Candida tropicalis 365	11	>25	8

Values are the mean zone size (radius; mm) after the deduction of the disc radius. Cellulose discs (6 mm diameter) were impregnated with 20 µl of test substance.

organisms were inhibited at a lesser degree (inhibition zones between 4 and 10 mm).

In liquid medium tests (Table 4) *H. officinalis* was ineffective against all Gram-negative and Gram-positive bacteria (MIC and MBC > 1.2% v/v) and poorly inhibited yeasts (*Candida albicans*, 603, 829, 615 and 616: MIC between 0.6 and 1.2%, v/v; MBC > 1.2%, v/v). By contrast, var. *decumbens* inhibited almost all the micro-organisms to a greater or lesser degree. Generally, MIC and MBC values were equal (0.3–1.2%, v/v), except for *Candida albicans* 615 (MIC = 0.15%, v/v; MBC = 0.6%, v/v) and *Staphylococcus aureus* 484 (MIC = 0.075%, v/v; MBC = 0.3%, v/v). The latter was the most sensitive micro-organism, while *Klebsiella oxytoca* 487 and *Proteus mirabilis* 608 were sensitive only at the maximal concentration of the oil (MIC and MBC = 1.2%, v/v).

These results show that both species possess an antimicrobial activity *in vitro*, *H. officinalis* var. *decumbens* generally being more effective than the parent species. Regarding to the contribution of pure components to the antimicrobial activity of the oils,

Table 4. MIC and MBC (expressed in percentages, v/v) in liquid medium of *Hyssopus officinalis* L. (Piedmont, Italy) and *Hyssopus officinalis* L. var. *decumbens* (Jordan & Fourr.) Briq. (Banon, France) essential oils

Micro-organisms	*H. officinalis* var. *decumbens*		*H. officinalis*	
	MIC (%)	MBC (%)	MIC (%)	MBC (%)
Gram-positive				
Staphylococcus aureus 484	0.075	0.3	>1.2	>1.2
Enterococcus faecalis 413	0.6	0.6	>1.2	>1.2
Gram-negative				
Klebsiella oxytoca 487	1.2	1.2	>1.2	>1.2
Escherichia coli 910	0.3	0.3	>1.2	>1.2
Proteus mirabilis 608	1.2	1.2	>1.2	>1.2
Salmonella typhi 839	0.6	0.6	>1.2	>1.2
Salmonella typhi 769	0.6	0.6	>1.2	>1.2
Pseudomonas aeruginosa 514	0.3	0.3	>1.2	>1.2
Yeasts				
Candida albicans 603	0.3	0.3	0.6	>1.2
Candida albicans 829	0.3	0.6	0.6	>1.2
Candida albicans 615	0.15	0.6	1.2	>1.2
Candida albicans 616	0.3	0.3	1.2	>1.2

limonene plays some role in the antimycotic action of both species, inhibiting all the tested yeasts and being present in both the oils. 1,8-Cineole and mostly linalol could be responsible for the stronger antimycotic action, observed in liquid medium, of var. *decumbens* in comparison with that of the parent species; moreover, they could contribute to the inhibitory action of var. *decumbens* against Gram-negative bacteria. These components are contained only in var. *decumbens* and have been shown to possess a broad spectrum of antimicrobial action. However, it has to be considered that minor components, as well as possible interactions between the substances, could also affect the microbiological properties of the essential oils in some way.

Finally, if we consider that *Hyssopus officinalis* L. var. *decumbens* (Jordan & Fourr.) Briq. essential oil is lacking in pinocamphone and isopinocamphone, bicyclic monoterpene ketones reported as toxic,[9,14] the antimicrobial activity of this oil appears interesting and worthy to be considered for practical uses.

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References

1. B. M. Lawrence, *Chemical components of Labiatae oils and their exploitation, in Advances in Labiate Science*, ed. R. M. Harley and T. Reynolds, pp. 399, Royal Botanic Gardens, Kew (1992).
2. B. M. Lawrence, *Perfumer & Flavorist*, **20**, 96 (1995).
3. M. S. Gorunovic, P. M. Bogavac, J. C. Chalchat and J. L. Chabard, *J. Essent. Oil Res.*, **7**, 39 (1995).
4. M. J. Garcia Vallejo, J. Guijarro Herraiz, M.J. Perez-Alonso and A. Velasco-Negueruela, *J. Essent. Oil Res.*, **7**, 567 (1995).
5. E. T. Tsankova, A. N. Konaktchiev and E. M. Genova, *J. Essent. Oil Res.*, **5**, 609 (1993).
6. G. Schultz and E. Stahl-Biskup, *Flavour and Fragr. J.*, **6**, 69 (1991).
7. K. Kerrola, B. Galambosi and H. Kallio, *J. Agric. and Food Chem.*, **42**, 776 (1994).
8. J. Bruneton, in *Pharmacognosie Phytochimie Plantes Médicinales*, 2nd edn, p. 428, Lavoisier TEC & DOC, Paris (1993).
9. M. D. Steinmetz, P. Tognetti, M. Mourgue, J. Jouglard and Y. Millet, *Plantes Médicinales et Phytothérapie*, **14**, 34 (1980).
10. T. G. Tutin and V. H. Heywood (eds), *Flora Europaea*, Vol. 3, pp. 170–71, Cambridge University Press (1972).
11. G. Salvatore, M. Nicoletti, V. Di Gioia, R. Ciccoli and A. D'Andrea, *Rivista Italiana EPPOS*, Numero Speciale, 672 (1997).
12. A. W. Bauer, W. M. M. Kirby, J. C. Sherris and M. Turck, *Amer. J. Clin. Path.*, **45**, 493 (1966).
13. I. D. Wilkinson and L.O. Gentry, *J. Antimicrobial Chemotherapy*, **8**, 53 (1981).
14. M. D. Steinmetz, P. Joanny, Y. Millet and F. Giannellini, *Plantes Médicinales et Phytothérapie*, **19**, 35 (1985).