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1 Chemical composition, antimicrobial activities and odor descriptions of some essential oils with characteristic floral-rosy scent and of their principal aroma compounds

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Summary

The principal aroma compounds (PACs) rose oxides, geraniol, nerol and citronellol as well as some of their derivatives and the composition of these PACs in various essentials oil with floral-rosy scent (citronella,

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3 samples of geranium, Helichrysum, palmarosa, rose and Verbena) were analyzed by GC and GC/MS. Twentyone principal aroma compounds were identified in addition to further more than 50 constituents of all samples. As main PACs (concentration higher than 3%, calculated as %-peak area) in the essential oils, citronellal (in the citronella sample), citronellol (in the citronella, the 3 geranium and the rose samples), geraniol (in the citronella, the 3 geranium, the palmarosa and the rose samples), citronellyl formate (in the 3 geranium samples), geranyl formate (in 2 geranium samples), nerol (in the Helichrysum and rose samples), geranyl acetate (in the palmarosa sample), neryl acetate (in the Helichrysum sample), neryl propionate (in the Helichrysum sample), neral (in the Verbena sample) and geranial (in the Verbena sample) were found. These analytical results were correlated with olfactoric evaluations for reasons of quality control of the aroma compounds and essential oils. Furthermore, all essential oils and the aroma components, a phenolic reference compound and three classic antibiotics were tested against several microorganisms to get informations about the antimicrobial effects of each essential oil. Most of the PACs and the essential oils show medium up to high antimicrobial activities against various gram-(+)- and gram-(-)-bacteria as well as against the yeast Candida albicans.

1. Introduction

In continuation of an international project in the field of combined data interpretation of composition analysis, odor evaluation and antimicrobial activity testings [13,19-21, 36] of various aroma-samples, essential oils with floral-rosy scent (citronella, geranium (3 samples), *Helichrysum*, palmarosa, rose and *Verbena*) and their principal aroma compounds, responsible for this odor-impression (rose oxides, geraniol, nerol and citronellol as well as some of their derivatives) were investigated.

Many papers about the composition, biological effects and use in medicine, food-flavoring, perfumery and cosmetics of these essential oils were published in the last years (e.g. citronella [9,12,17,26,30,34,35,37,39], geranium [5,12,16,17,30,33,37], *Helichrysum* [8,10,12,27,28,37], palmarosa [31,32,37], rose [2,12,17,25,29,30,37,39] and Verbena [4,17,24,37]). Although, these data are of high importance in this field, no systematic investigations of various floral-rosy scented essential oils as to their anti-microbial effects and analyses of principal aroma compounds, responsible for the aroma-impressions as well as possible biological active constituents are known until now. Therefore, the aim of this work was to close this lack of information and to include the results to a developed databank obtained from previous investigations as mentioned above.

2. Materials and methods

2.1. Samples and reference compounds

Citronellal (W23,070-7), citronellol (W23,090-1), citronellyl acetate (W23,111-8), cironellyl butyrate (W23,120-7), citronellyl formate (W23,140-1), citronellyl isobutyrate (W23,130-4), citronellyl propionate (W23,160-6), geranyl tiglate (W50,080-1), eugenol (W24,670-0), hydroxycitronellal (W25,831-8) and tetracycline hydrochloride (achromycine hydrochloride – 25g, T3383-25G) are products from Sigma-Aldrich Austria Co., Vienna; geraniol/nerol and some of their derivatives were obtained from Symrise Co. (former Dragoco Co.), Vienna; the other geraniol/nerol derivatives, rose oxide L and R, the essential citronella leaf oil (*Cymbopogon winterianus* Jowitt ex Bor, *Gramineae*, China: 800213), the geranium leaf oils (*Pelargonium graveolens* L`Hérit. ex Ait., *Geraniaceae*, Africa-Egypt: 800356; Bourbon-Réunion Island: 800357; China: 801745), the *Helichrysum* herb oil (*Helichrysum italicum* (Roth) D. Don, *Compositae*, Bosnia-Hercegovina: 800373), the palmarosa grass oil (*Cymbopogon martinii* (Roxb.) J.F. Wats. var. motia, *Gramineae*, India: 800611), the floral rose leaf oil (*Rosa damascena* Mill., *Rosaceae*, Turkey: 801647) and the verbena leaf oil (*Lippia citriodora* (Lam). Humb. syn. *L. citriodora* Kuntze syn. *Aloysia triphylla* (L`Hér.) Britton, *Verbenaceae*, France: 801557) were purchased from Kurt Kitzing Co., Wallerstein; Ciproxin^{R} 500mg-tablettes (1 tablette = 582 mg ciproflaxoxacine hydrochloride / water) bought from Bayer Austria Co., Vienna and Lidaprim^{R}-infusion-bottle (250 mg containing 0.8 g sulfametrol and 0.16 g trimethoprim) from Nycomed Austria Co., Vienna.

2.2. GC analysis

GC/FID analyses were carried out using a GC-14A with SPME sleeve adapted to injector, FID and C-R6A-Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID (Varian, Germany) and C-R1B-Chromatopac integrator (Shimadzu). The carrier gas was hydrogen; injector temperature, 250°C; detector temperature, 320°C. The temperature programme was: 40°C/5 min to 280°C/5 min, with a heating rate of 6°C/min. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 μm (Biorad, Germany) and 30 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 μm (Restek, USA). Quantification was achieved using peak area calculations, and compound identification was carried out partly using correlations between retention times [1,11,13,18-23,36].

2.3. GC-MS analysis

For GC/MS measurements a GC-17A with QP5000 (Shimadzu), SPME sleeve adapted to injector and Compaq-ProLinea data system (class5k-software),

a GC-HP5890 with HP5970-MSD (Hewlett-Packard, USA) and ChemStation software on a Pentium PC (Böhm, Austria), a GCQ (Finnigan-Spectronex, Germany-USA) and Gateway-2000-PS75 data system (Siemens-Nixdorf, Germany, GCQ-software) were used. The carrier gas was helium; injector temperature, 250°C; interface-heating at 300°C, ion-source-heating at 200°C, EI-mode was 70 eV, and the scan-range was 41-450 amu. For other parameters, see description of GC/FID, above. Mass spectra correlations were done using Wiley, NBS, NIST and our own library as well as published data [1,18,22].

2.4. Antimicrobial testings

The essential oil of sage, the key compounds and the reference compound were prepared as 20% solutions of ethanol and dissolved in a 0.9% NaCl solution (ratio of 1:10). As test microorganisms (colony-forming-units=cfu/cm^3), gram-(+)-bacteria *Staphylococcus aureus* ATCC 6538P ($1x10^{13}$) and *Enterococcus faecalis* (clinically isolated, $1x10^{13}$); gram-(-)-bacteria *Escherichia coli* ATCC 8739 ($2x10^{12}$), *Pseudomonas aeruginosa* G 28 ($1.2x10^9$), *Klebsiella pneumoniae* (clinically isolated, $1x10^{13}$), *Proteus vulgaris* (clinical isolated, $3x10^{13}$) and *Salmonella* sp. (clinically isolated, $3x10^{12}$) as well as the yeast *Candida albicans* ATCC 10231 ($1x10^{11}$) – all products from the National Bank of Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria – were used.

The antimicrobial activity was studied by two methods: Agar diffusion disc method using 6 mm paper discs and quantities of 6 μL of the sample. After cultivation of the bacteria and the yeast at 37°C for 24^h the diameter of the inhibition zone (IZ) was measured. Agar serial tube dilution method with results as minimum inhibitory concentration (MIC) as follows: The essential oil, pure and reference compounds were added to brine, containing 1.0% (v/v) Tween 80 at the appropriate volumes to produce final concentrations of the samples in the range of 100-1.000 ppm; the Petri dishes were inoculated by pipetting 0.1cm^3 of the desired culture and 0.6 μL of the samples as well as the reference compounds (the tablettes of Ciproxin^R were added as solution in saline at a quantity of 300 μg) on paper discs (6 mm) and then incubated at 37°C for 24h.

2.5. Olfactoric evaluations

All investigated samples were olfactorically evaluated by a professional perfumer and 2 aroma-chemists using 1 drop of each aroma sample on a commercial odor-strip; the aroma described in Table 1 correlated with odor impressions published elsewhere [3,6,7,14,15,38].

Table 1. Investigated principal aroma compounds.

Sample	Purity (GC)	Odor impressions (evaluation by professional perfumers and aroma chemists)
Geranial[1]	99.2%[a]	pleasant floral (rose-like)
Geraniol[2]	98.3%[a]	typical geraniol, floral (rose-like), weak fruity-sweet
Geranyl formate[2]	97.8%[a]	pleasant geraniol-rose notes
Geranyl acetate[2]	98.1%[a]	rose-like, weak fruity-sweet
Geranyl butyrate[2]	98.2%[a]	rose-like (geraniol), sweet-fruity side-note (apple-like)
Geranyl tiglate[3]	96.4%[b]	floral (geranium-rose notes), sweet-fruity, herbal notes
Neral[1]	97.4%	floral (rose-notes)
Nerol[1]	98.7%[a]	floral (geraniol-like with metallic side-note), weak fruity
Neryl acetate[2]	98.9%	floral (sweet rose-orange blossom notes), fruity (apple notes)
Neryl butyrate[1]	99.1%[a]	floral (rose-like), sweet-fruity (strawberry-like)
Neryl propionate[1]	97.1%[b]	floral (mild rose-like), fruity-honey notes
(*Z*)-Rose oxide[2]	94.6%[a]	floral (rose-like)
(*E*)-Rose oxide[2]	93.9%[a]	floral (rose-like)
Citronellal[3]	97.4%[b]	fresh-green, floral, strong citrus-lemon notes
Citronellol[3]	99.1%[b]	floral (intense rose-like)
Citronellyl formate[3]	96.4%[b]	floral (rose notes), fruity (apricot-peach-plum notes)
Citronellyl acetate[3]	98.7%[b]	floral (rose notes), citrus-lemon peel notes
Citronellyl butyrate[3]	97.9%[b]	floral (rose notes), sweet-fruity
Citronellyl isobutyrate[3]	97.5%[b]	weak floral (rose notes), intense fruity
Citronellyl propionate[3]	98.6%[b]	floral (rose notes), fruity
Hydroxycitronellal[3]	98.5%[b]	floral (sweet rose-lily notes)
Eugenol[3]	99.3%[a]	strong spicy (clove-cinnamon-like)

[1]Symrise Co. [2]Kurt Kitzing Co., [3]Sigma-Aldrich [a]60m CW [b]30mHP-5

3. Results and discussions

The essential oils were olfactorically evaluated as follows: *Cymbopogon winterianus*: Floral-fresh (rose- and lily-of-the-valley-like, weak sweet-fruity (citrus note); *Pelargonium graveolens* 1 (Africa): Intense floral-rosy and geranium-like, fruity side-notes; *P. graveolens* 2 (Bourbon): Floral (rose-geranium-like), fruity (apricot-peach notes); *P. graveolens* 3 (China): Intense rose-geranium-like, weak fruity (citrus-apricot notes); *Helichrysum italicum*: Fresh-piney, floral (rose- and orange-blossom-like), fruity (lemon notes), weak herbal-woody side-notes; *Cymbopogon martini*: Intense floral (geraniol-rose-like); *Rosa damascena*: Intense floral (rose-like), weak fruity; *Lippia citriodora*: Fresh-floral (rose notes), green-fruity (lemon notes), spicy-herbal-woody in the background.

By means of gas chromatographic-spectroscopic analysis (GC/FID and GC/MS) the identity and purity of totally 21 principal aroma compounds were investigated and the odor impressions evaluated by professional perfumers and aroma chemists. All PACs were found in high purity-values with characteristic aromas (see Table 1).

Using GC/FID- and GC/MS-analyses about 80 constituents could be identified in the 8 essential oils with concentrations of the principal aroma compounds (floral-rosy scent) as follows (concentration higher than 3.0%, calculated as relative %-peak-area using GC-FID with an apolar column, see Table 2): Citronella oil: Citronellal (37.4%), geraniol (23.6%) and citronellol (10.8%) – further main components (not presented): Elemol (3.9%) and limonene (3.4%); geranium oil 1: Citronellol (30.9%), geraniol (15.7%), citronellyl formate (6.4%) and geranyl formate (3.1%) – further main components: Linalool (6.2%), *iso*-menthone (6.1%) and *epi*-γ-eudesmol; geranium oil 2: Citronellol (22.8%), geraniol (17.3%), citronellyl formate (8.7%) and geranyl formate (5.8%) – further main components: Linalool (9.4%), *iso*-menthone (7.2%) and guai-6,9-diene (6.4%); geranium oil 3: Citronellol (37.8%), citronellyl formate (11.7%) and geraniol (8.3%) – further main components: *iso*-Menthone (5.8%), guai-6,9-diene (5.6%) and linalool (3.1%); *Helichrysum* oil: Neryl acetate (12.2%), nerol (9.6%) and neryl propionate (4.8%) – further main components: α-Curcumene (20.7%), α-pinene (17.8%), β-caryophyllene (5.9%), γ-elemene (5.1%), limonene (3.2%), β-selinene (3.1%) and italicene (3.1%); palmarosa oil: Geraniol (79.3%) and geranyl acetate (6.2%) – further main component: Linalool (3.4%); rose oil: Citronellol (38.7%), geraniol (17.2%) and nerol (8.3%) – further main component: Nonadecane (7.2%); *Verbena* oil: Geranial (13.1%) and neral (9.3%) – further main components: Limonene (17.3%), methylheptenone (5.4%), β-caryophyllene (5.3%), germacrene D (3.6%) and *trans*-β-ocimene (3.1%).

Table 2. Principal aroma compounds (floral-rosy odor) composition of the essential oils in order of their retention-indices[*] and percentage[#].

Component	RI	Co[1]	Go1[2]	Go2[3]	Go3[4]	Ho[5]	Po[6]	Ro[7]	Vo[8]
(*Z*)-Rose oxide	1108	nd	1.5	0.9	1.9	nd	nd	0.7	nd
(*E*)-Rose oxide	1126	nd	1.0	0.4	0.8	nd	nd	0.5	nd
Citronellal	1153	37.4	0.3	0.2	0.2	nd	tr	0.4	tr
Citronellol	1226	10.8	30.9	22.8	37.8	0.1	tr	38.7	0.3
Nerol	1230	0.5	0.1	0.5	1.0	9.6	0.2	8.3	2.5
Neral	1238	0.4	0.5	0.4	0.5	0.8	0.2	0.4	9.3
Geraniol	1253	23.6	15.7	17.3	8.3	0.2	79.3	17.2	2.0
Geranial	1267	0.6	0.9	1.2	0.6	0.1	0.5	0.8	13.1
Citronellyl formate	1274	0.1	6.4	8.7	11.7	nd	nd	0.1	tr
Hydroxycitronellal	1288	tr	0.6	0.2	0.3	nd	nd	tr	tr
Geranyl formate	1299	nd	3.1	5.8	2.1	tr	0.2	0.1	0.1
Citronellyl acetate	1363	2.2	0.6	0.3	0.5	nd	nd	nd	nd
Neryl acetate	1364	0.1	tr	0.1	tr	12.2	tr	0.1	0.6
Geranyl acetate	1381	2.9	0.7	0.5	0.3	0.3	6.2	1.6	1.8
Citronellyl propionate	1446	0.1	0.4	0.4	0.9	nd	nd	nd	nd
Neryl propionate	1455	nd	tr	0.1	0.2	4.8	0.5	0.1	tr
Citronellyl isobutyrate	1483	nd	0.1	0.1	0.2	nd	nd	nd	nd
Neryl butyrate	1516	nd	nd	tr	0.1	1.3	nd	0.1	0.8
Citronellyl butyrate	1532	tr	1.3	0.6	0.8	nd	nd	nd	nd
Geranyl butyrate	1564	tr	1.5	1.1	0.91	tr	0.3	0.3	1.2
Geranyl tiglate	1696	nd	1.1	0.7	0.6	nd	nd	nd	nd

[*]apolar column, [#]in relative %-peak area using GC with an apolar column (meanvalue of 3 analyses)
Co[1] Citronella oil, Go1[2] Geranium oil 1 (Africa), Go2[3] Geranium oil 2 (Bourbon), Go3[4] Geranium oil 3 (China),
Ho[5] Helichrysum oil, Po[6] Palmarosa oil, Ro[7] Rose oil, Vo[8] Verbena oil
[$]not detected, [&]trace compound (less than 0.01%)

A correlation of the odor of the essential oils with data from the olfactoric evaluations and published aroma-impression descriptions of pure compounds from elsewhere (see experimental part – olfactoric evaluations) shows that the characteristic floral-rosy odor of each samples can also be correlated to citronellol, geraniol and nerol as well as some of their derivatives in higher concentrations.

Results of antimicrobial testings (agar diffusion and agar dilution method in accordance to [13,19-21,36]) of the different PACs and essential oils against various microorganisms were as follows (see Table 3): Gram-(+)-bacterium *Staphyllococcus aureus*: High antimicrobial activity of nearly all samples; gram-(+)-bacterium *Enterococcus faecalis*: Medium up to high activity of all samples without neryl acetate; gram-(-)-bacterium *Escherichia coli*: Medium up to high activity of most of the samples without the pure compounds neryl propionate, citronellal, citronellol, citronellyl acetate, butyrate and propionate as well as (*Z*)- and (*E*)-rose oxide; gram-(-)-bacterium *Proteus vulgaris*: Medium up to high activity of many of the samples without geranyl acetate and butyrate, citronellyl acetate, butyrate and propionate as well as the *Helichrysum* oil; gram-(-)-bacterium *Pseudomonas aeruginosa*: Medium up to high activity of most of the samples without geranyl acetate, neryl butyrate, citronellal, citronellol, citronellyl acetate and isobutyrate and (*E*)-rose oxide; gram-(-)-bacterium *Salmonella* sp.: Weak up to high activity of nearly all of the samples without citronellyl acetate and isobutyrate, (*E*)-rose oxide and the *Helichrysum* oil; gram-(-)-bacterium *Klebsiella pneumoniae*: Medium up to high activity of many of the samples without citronellol, citronellyl formate and acetate, (*Z*)- and (*E*)-rose oxide; yeast *Candida albicans*: Medium up to high activity of most of the samples without neryl acetate, citronellal, citronellol and citronellyl isobutyrate.

Correlations with the data of the high antimicrobially active phenolic compounds eugenol and the synthetic antibiotics tetracycline hydrochloride and Ciproxin^{R} (these products and Lidaprim^{R} are known to have no effects against the yeast *C. albicans*) prove the effectivity of the chosen testing methods, while Lidaprim^{R} (no effects against *P. aeruginosa* and *K. pneumoniae*) was found to have not the antimicrobial activity as expected.

The comparison of the single results of antimicrobial testings of the group of principal aroma compounds and essential oils with floral-rosy odor showed effects against all microorganisms in the case of geranial, geraniol, geranyl formate, geranyl tiglate, neral, nerol, hydroxycitronellal, citronella oil, all 3 samples of geranium, palmarosa, rose and *Verbena* oil.

To compare the analytical and antimicrobial results of this investigation, it can be seen that in the most cases the essential oils with more than 1 principal aroma compound as main constituents possess much more antimicrobial activity against the different strains of microorganisms as a single component shows.

Table 3. Antimicrobial activities of principal odor compounds, essential oils with floral-rosy scent and reference compounds.

Compounds	Inhibition Zones (IZ) in mm and Minimum Inhibitory Concentrations (MIC) in ppm of test-microorganisms									
	Staphylococcus aureus/ Enterococcus faecalis		*Escherichia coli/ Proteus vulgaris*		*Pseudomonas aeruginosa/ Salmonella sp.*		*Klebsiella pneumoniae*		*Candida albicans*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
Geranial	15 / 23	60 / 60	11 / 9	60 / 600	10 / 8	60 / 600	9	600	28	60
Geraniol	15 / 12	60 / 60	15 / 12	60 / 60	11 / 10	60 / 60	10	600	25	60
Geranyl formate	10 / 9	600 / 600	7 / 8	600 / 600	8 / 7	600 / 600	7	600	15	600
Geranyl acetate	10 / 8	600 / 600	7 / -	600 / -	- / 7	- / 600	-	-	9	600
Geranyl butyrate	10 / 11	600 / 60	7 / -	600 / -	9 / 7	600 / 600	7	600	10	600
Geranyl tiglate	17 / 10	600 / 600	11 / 9	600 / 600	8 / 8	600 / 600	15	600	15	600
Neral	15 / 20	60 / 60	10 / 6	60 / 60	12 / 10	60 / 60	10	600	25	60
Nerol	11 / 8	60 / 600	10 / 10	60 / 600	10 / 7	600 / 600	7	600	27	60
Neryl acetate	8 / -	600 / -	7 / 7	600 / 600	7 / 8	600 / 600	7	600	-	-
Neryl butyrate	25 / 8	6 / 600	8 / 8	600 / 600	- / 8	- / 600	8	600	10	600
Neryl propionate	17 / 10	600 / 600	- / 7	- / 600	8 / 9	600 / 600	10	600	14	60
Citronellal	25 / 18	600 / 600	- / 9	- / 600	- / 7	- / 600	14	600	-	-
Citronellol	25 / 18	600 / 60	- / 8	- / 600	- / 7	- / 600	-	-	-	-
Citronellyl formate	18 / 20	60 / 600	10 / 8	60 / 60	9 / 7	600 / 60	-	-	13	600
Citronellyl acetate	10 / 6	60 / 60	- / 6	- / -	7 / 6	- / -	7	-	9	60
Citronellyl butyrate	8 / 8	60 / 60	- / -	- / -	8 / 7	60 / 6	8	60	10	60
Citronellyl isobutyrate	8 / 10	60 / 60	9 / 7	60 / 600	- / -	- / -	7	60	-	-
Citronellyl propionate	15 / 20	600 / 600	- / -	- / -	10 / 15	60 / 600	11	60	15	600
Hydroxycitronellal	20 / 20	600 / 60	23 / 16	600 / 60	17 / 15	60 / 60	14	600	25	60
(Z)-Rose oxide	8 / 10	600 / 600	- / 11	- / 600	7 / -	600 / -	-	-	28	600
(E)-Rose oxide	7 / 8	600 / 600	- / 10	- / 600	- / -	- / -	-	-	28	600
Citronella oil	10 / 10	600 / 600	7 / 10	600 / 60	7 / 7	600 / 600	7	600	20	600
Geranium oil Africa	16 / 12	60 / 600	10 / 10	600 / 600	10 / 9	600 / 600	11	600	28	600
Geranium oil Bourbon	13 / 12	600 / 600	8 / 12	600 / 60	10 / 10	600 / 600	10	600	25	600
Geranium oil China	20 / 13	60 / 600	14 / 9	60 / 60	9 / 9	60 / 600	10	60	25	600
Helichrysum oil	20 / 13	600 / 600	8 / -	600 / -	9 / -	600 / -	7	600	7	600
Palmarosa oil	8 / 13	600 / 60	12 / 9	60 / 600	11 / 10	600 / 600	10	60	20	60
Rose oil	20 / 15	60 / 60	10 / 10	600 / 600	8 / 9	600 / 600	10	600	20	600
Verbena oil	27 / 25	600 / 60	10 / 13	600 / 600	10 / 12	600 / 600	10	600	25	600
Eugenol	30 / 30	600 / 600	28 / 28	600 / 600	25 / 25	600 / 600	28	600	32	600
Ciproxin^{R}	35 / 33	600 / 600	22 / 25	600 / 600	32 / 10	600 / 600	25	600	-	-
Lidaprim^{R}	27 / 27	600 / 600	11 / 23	60 / 600	- / 8	- / 60	-	-	-	-
Tetracycline hydrochloride	15 / 22	600 / 600	11 / 13	600 / 600	15 / 10	600 / 600	20	600	-	-

- no inhibition observed

Therefore, we can state that also the antimicrobial activities of essential oils with characteristic floral-rosy scent and of geraniol, nerol, citronellol as well as some of their derivatives as principal aroma compounds against gram-(+)-, gram-(-)-bacteria and yeast are determined by many constituents with synergistic and antagonistic effects and cannot be attributed to a single compound.

In conclusion, we can report that essential oils with floral-rosy scent, such as citronella, geranium, *Helichrysum*, palmarosa, rose and *Verbena* possess high antimicrobial activities against various microorganisms and these effects are mainly the result of a combination of some also biologically active principal aroma compounds (geraniol, nerol, citronellol and many of their derivatives) in a medium up to high concentration.

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References

1. *Adams R.P.*: Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured, Carol Stream, 2001.
2. *Agarwal S.G., Gupta A., Kapahi B.K., Baleshwar A., Thappa R.K., Suri O.P.*: Chemical composition of rose water volatiles. J Essent Oil Res 2005; 17: 265-267.
3. *Arctander St.*: Perfume and Flavor Chemicals (Aroma Chemicals). Arctander Publ., Montclair, 1969.
4. *Ardakani M.S., Mosaddegh M., Shafaati A.*: Volatile constituents from the aerial parts of *Verbena officinalis* L. (Vervain). Iranian J Pharm Res 2003; 2: 39-42.
5. *Babu K.G.D., Kaul V.K.*: Variation in essential oil composition of rose-scented geranium (*Pelargonium sp.*) distilled by different distillation techniques. Flavour Fragr J 2005; 20: 222-231.
6. *Bauer K., Garbe D., Surburg H.*: Common Fragrance and Flavor Materials, 3rd Ed., VCH, Weinheim, 1997.
7. *Boelens M.H.*: Chemical and sensory evaluation of three sage oils. Perfum Flavor 1997; 22: 19-40.
8. *Bougatsos Ch., Ngassapa O., Runyoro D.K.B.*: Chemical composition and in vitro antimicrobial activity of the essential oils of two *Helichrysum* species from Tanzania. Z Naturforsch C 2004; 59: 368-372.
9. *Chagonda L.S., Makanda Ch., Chalchat J.-C.*: Essential oils of cultivated *Cymbopogon winterianus* (Jowitt) and *C. citratus* (DC) (Stapf) from Zimbabwe. J Essent Oil Res 2000; 12: 478-480.
10. *Chinou I.B., Bougatsos Ch., Perdetzoglou D.*: Chemical composition and antimicrobial activities of *Helichrysum amorginum* cultivated in Greece. J Essent Oil Res 2004; 16: 243-245.

11. *Davies N.W.*: Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J Chromatogr 1990; 503: 1-24.
12. *Duke J.A., Bogenschutz-Godwin M.J., duCellier J., Duke, P.-A.K.*: Handbook of Medicinal Herbs, 2nd Ed., 642-643, CRC Press, Boca Raton, 2002.
13. *Edris A.E., Jirovetz L., Buchbauer G., Denkova Z., Slavchev A., Stoyanova A.*: Chemical composition, antimicrobial activities and olfactoric evaluation of *Salvia officinalis* L. (sage) essential oil from Egypt. J Essent Oil Res 2005, paper submitted.
14. *Fazzalari F.A.*: Compilation of Odor and Taste Threshold Values Data, American Society for Testing and Materials, Philadelphia, 1978.
15. *Furia T.E., Bellanca N.*: Fenaroli´s Handbook of Flavor Ingredients, 2nd Ed., CRC Press, Cleveland, 1975.
16. *Gauvin A., Lecomte H., Smadja J.*: Comparative investigations of the essential oils of two scented geranium (*Pelargonium spp.*) cultivars grown on Reunion Island. Flavour Fragr J 2004; 19: 455-460.
17. *Hiller K., Melzig M.*: Lexikon der Arzneipflanzen und Drogen, Spektrum Akademischer Verlag GmbH, Heidelberg, 2000.
18. *Jennings W., Shibamoto T.*: Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography. Academic Press, New York, 1980.
19. *Jirovetz L., Buchbauer G., Denkova Z., Stoyanova A., Murgov I.*: Antimicrobial Testings and Chiral Phase Gas Chromatographic Analysis of Lavandula Oils and Related Key Compounds. EURO COSMET 2004; 12(1): 30-33.
20. *Jirovetz L., Buchbauer G., Denkova Z., Stoyanova A., Murgov I., Schmidt E., Geissler M.*: Antimicrobial testings and gas chromatographic analysis of pure oxygenated monoterpenes 1,8-cineole, α-terpineol, terpinen-4-ol and camphor as well as target compounds in essential oils of pine (*Pinus pinaster*), rosemary (*Rosmarinus officinalis*) and tea tree (*Melalaeuca alternifolia*). Sci Pharm 2005; 73: 27-39.
21. *Jirovetz L., Buchbauer G., Denkova Z., Stoyanova A., Murgov I., Schmidt E., Geissler M.*: Comparative Investigations of Antimicrobial Activities and Compositions of Various Aroma Samples – A Short Review. Rec Progr Med Plants (J.N. Govil, ed.), Chapter 26, 461-484, STUDIUMPRESS, New Delhi, 2005.
22. *Joulain D., König W.A.*: The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E.B.-Verlag, Hamburg, 1998.
23. *Kondjoyan N., Berdaqué J.-L.*: A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds. Edition du Laboratoire Flaveur. Saint Genes Champanelle, 1996.
24. *Lawrence B.*: Progress in essential oils. Perfum Flavor 2004; 29: 48-58.
25. *Lawrence B.*: Progress in essential oil. Rose oil and extracts. Perfum Flavor 2005; 30: 60-74.
26. *Lorenzo D., Dellacassa E., Atti-Serafini L., Santos A.C., Frizzo C., Paroul N., Moyna P., Mondello L., Dugo G.*: Composition and Stereoanalysis of *Cymbopogon winterianus* Jowitt Oil from Southern Brazil. Flavour Fragr J 2000; 15: 177-181.
27. *Lourens A.C.U., Reddy D., Baser K.H.C., Viljoen A.M., Van Vuuren, S.F.*: In vitro biological activity and essential oil compositions of four indigenous South African *Helichrysum* species. J Ethnopharmacol 2004; 95: 253-258.

28. *Mastelic J., Politeo O., Jerkovic I., Radosevic N.*: Composition and antimicrobial activity of *Helichrysum italicum* essential oil and its terpene and terpenoid fractions. Chem Nat Comp 2005; 41: 35-40.
29. *Nowak R.*: Chemical composition of hip essential oils of some *Rosa* L. species. Z Naturforsch C 2005; 60: 369-378.
30. *PDR^R for Herbal Medicines* (LaGow, B., Chief-Editor), 3rd Ed., Thomson PDR, Montvale, 2004.
31. *Prashar A., Hili P., Veness R.G., Evans Ch.S.*: Antimicrobial action of palmarosa oil (*Cymbonpogon martini*) on *Saccharomyces cerevisiae*. Phytochemistry 2003; 63: 569-575.
32. *Raina V.K., Srivastava S.K., Aggarwal K.K., Syamasundar K.V., Khanuja S.P.S.*: Essential oil composition of *Cymbopogon martini* from different places in India. Flavour Fragr J 2003; 18: 312-315.
33. *Ram P., Kumar B., Naqvi, A.A., Verma R.S., Patra N.K.*: Post-harvest storage effect on quantity and quality of rose-scent geranium (*Pelargonium sp.* cv. "Bourbon") oil in Uttaranchal. Flavour Fragr J 2005; 20: 666-668.
34. *Rao B.R.B., Bhattacharya A.K., Mallavarapu G.R., Ramesh S.*: Yellowing and crinkling diseases and its impact on the field and composition of the essential oil of citronella (*Cymbopogon winterianus* Jowitt.). Flavour Fragr J 2004; 19: 344-350.
35. *Rao Y.R., Rout P.K., Jena K.S., Sahu S.B.*: Composition of essential oil of citronella (*Cymbopogon winterianus* Jowitt.) grown in coastal Orissa. PAFAI J 2000; 2: 29-31.
36. *Schmidt E., Jirovetz L., Buchbauer G., Denkova Z., Stoyanova A., Murgov I., Geissler M.*: Antimicrobial Testings and Gas Chromatographic Analyses of Aroma Chemicals. J Essent Oil-Bearing Plants 2005; 8(1): 99-106.
37. *Seidemann J.*: World Spice Plants, Springer-Verlag, Heidelberg, 2005.
38. *Sigma-Aldrich*: Flavors & Fragrances, The Essence of Excellence. Sigma-Aldrich Co., Milwaukee, 2003.
39. *Wichtl M.* (ed.): Teedrogen. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1989.