CHEMICAL COMPOSITION, ANTIMICROBIAL ACTIVITIES AND ODOR DESCRIPTION OF SOME ESSENTIAL OILS WITH CHARACTERISTIC FLORAL-ROSY SCENT AND OF THEIR PRINCIPAL AROMA COMPOUNDS

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INTRODUCTION

In continuation of an international project in the field of combined data interpretation of composition analysis, odor evaluation and antimicrobial activity testings of various aroma-samples, some 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.

The chemical composition, biological effects and use in medicine, food-flavoring, perfumery and cosmetics of essential oils have been already published, but no systematic investigations of antimicrobial effects, analyses of principal aroma compounds and possible biological active constituents are known.

It was our aim to append these data obtained from various floral-rosy scented essential oils into a developed database acquired from previous investigations.

MATERIALS AND METHODS

Samples & reference compounds:

Citronella leaf oil: *Cymbopogon winterianus* Jowitt, Poaceae, China.
Geranium leaf oils:*Pelargonium x ssp.* Geraniaceae.
a) Africa-Egypt
b) Bourbon-Réunion Island
c) China
Helichrysum oil: *Helichrysum italicum* (Roth) G. Don, syn. *Helichrysum angustifolium* (Lam.) *DC.*, Asteraceae, Bosnia-Hercegovina.
Palmarosa oil: *Cymbopogon martinii* (Roxb.) Will. Watson var. m*otia*, Poaceae, India.
Floral rose leaf oil: *Rosa damascena* Mill, Rosaceae, Turkey.
Verbena oil: *Aloysia citriodora* Palau syn. *Aloysia triphylla* (L'Hérit.) Britton syn. *Lippia triphylla* (L'Herit.) Kunze, syn. *Verbena triphylla* L'Herit. syn. *Lippia citriodora* Kuntze, Verbenaceae, France.

Geranial, geraniol, geranyl formate, geranyl acetate, geranyl butyrate, geranyl tiglate, neral, nerol, neryl acetate, neryl butyrate, neryl propionate, (*Z*)-rose oxide, (*E*)-rose oxide, citronellal, citronellol, citronellyl formate, citronellyl acetate, citronellyl butyrate, citronellyl isobutyrate, citronellyl propionate and hydroxycitronellal

Eugenol
Ciproxin ^{R} 500mg tablet
Lidaprim ^{R} infusion bottle
Tetracycline hydrochloride (achromycine hydrochloride)

Qualitative and quantitative determination:

GC/FID: GC-14A with FID and C-R6A-Chromatopac integrator (Shimadzu Co., Japan); GC-3700 with FID (Varian Co., Germany) and C-R1B-Chromatopac integrator (Shimadzu).

GC/MS: GC-17A with QP5050-MS and HP-Compaq data system (class5k-software), a GC-HP5890 with HP5970-MSD (Hewlett-Packard Co., USA), ChemStation software on a Pentium PC (Böhm Co., Austria), GCQ (Finnigan-Spectronex Co., Germany-USA) and Gateway-2000-PS75 data system (Siemens-Nixdorf Co., Germany, GCQ-software).

Antimicrobial activity:

Gram-(+)-bacteria: *Staphyllococcus aureus* and *Enterococcus faecalis*.

Gram-(-)-bacteria: *Escherichia coli*, *Proteus vulgaris*:, *Pseudomonas aeruginosa*, *Salmonella* sp. and *Klebsielle pneumonia*.

Yeast: *Candida albicans*.

Antimicrobial testing methods:

Agar diffusion disc method:-> Diameter of Inhibition Zone (IZ)

Agar serial dilution method:-> Minimum Inhibitory Concentration (MIC)

Olfactoric evaluation:

One drop of sample on commercial odor strip.

RESULTS AND DISCUSSION (Part 1.)

Table 1. Investigated principal aroma compounds

Sample	Purity (GC)	Odor impression (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]	99:35[a]	strong spicy (clove-cinnamon-like)

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

Table 2.a) Investigated essential oil samples and their olfactoric evaluation

Sample	Main compounds	Olfactoric evaluation
Citronella oil:	Citronellal (37.4%), geraniol (23.6%) and citronellol (10.8%) – further main components (not presented): Elemol (3.9%) and limonene (3.4%);	Floral-fresh (rose- and lily-of-the-valley-like, weak sweet-fruity (citrus note);
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;	Intense floral-rosy and geranium-like, fruity side-notes;
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%);	Floral (rose-geranium-like), fruity (apricot-peach notes);
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%);	Intense rose-geranium-like, weak fruity (citrus-apricot notes);

RESULTS AND DISCUSSION (Part 2.)

Table 2.b) Investigated essential oil samples and their olfactoric evaluation

Sample	Main compounds	Olfactoric evaluation
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%);	Fresh-piney, floral (rose- and orange-blossom-like), fruity (lemon notes), weak herbal-woody side-notes;
Palmarosa oil:	Geraniol (79.3%) and geranyl acetate (6.2%) – further main component: Linalool (3.4%);	Intense floral (geraniol-rose-like);
Rose oil:	Citronellol (38.7%), geraniol (17.2%) and nerol (8.3%) – further main component: Nonadecane (7.2%);	Intense floral (rose-like), weak fruity;
Verbena oil:	Geranial (13.1%) and neral (9.3%) – further main components: Limonene (17.3%), methyl-heptenone (5.4%), β-caryophyllene (5.3%), germacrene D (3.6%) and *trans*-β-ocimene (3.1%).	Fresh-floral (rose notes), green-fruity (lemon notes), spicy-herbal-woody in the background;

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

Compounds	Inhibition zone (IZ) in mm and Minimum Inhibitory Concentrations (MIC) in ppm of test organisms									
	Staphyllococcus 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	05/12	60/60	11/10	60/60	10	600	15	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	600/600	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	/	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 Borubon	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/-	600/0	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	600/600	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	-	-

RESULTS OF THE ANTIMICROBIAL TESTINGS

✓Gram-(+)- bacterium *Staphylococcus aureus*: High antimicrobial activity of all samples.

✓Gram-(+)- bacterium *Enterococcus faecalis*: Medium to high activity of all samples without neryl acetate.

✓Gram-(-)- bacterium *Escherichia coli*: Medium to high activity of most of the samples, without 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, citronallal, 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 *Klebsielle pneumonia*: 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.

✓Geranial, geraniol, geranyl formate, geranyl tiglate, neral, nerol, hydroxycitronellal, citronella oil, all 3 samples of geranium, palmarosa, rose and *Verbena* oil are effective against all tested microorganisms.

✓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.

CONCLUSION

We can state that the antimicrobial activities of essential oils with characteristic floral-rosy scent and of essential oils with principal aroma compounds geraniol, nerol, citronellol as well as some of their derivatives show antimicrobial activities against gram-(+)-, gram-(-)-bacteria and yeast. These activities 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.

These effects are mainly the result of a combination of some active principal aroma compounds (geraniol, nerol, citronellol and many of their derivatives) in a medium up to high concentration.

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