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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), tea tree (Melaleuca alternifolia)

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Summary

The oxygenated monoterpenes 1,8-cineole, α-terpineol, terpinen-4-ol and camphor as well as essential oils of pine, rosemary and tea tree, rich in these volatiles, were tested for their antimicrobial activities against some different strains of yeast, Gram-(-)- and Gram-(+)-bacteria by agar diffusion and agar dilution method, respectively. The same was done using the phenolic aroma compound eugenol as a reference-substance. The monoterpene alcohols α-terpineol and terpinen-4-ol were found to be active against all strains used in a wide range. The same result was obtained for the testings of essential oils of pine, rosemary (2 samples) and tea tree. Nearly all aroma chemicals and essential oils did show a

significant high antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

For qualitative and quantitaive investigations of the key aroma compounds and the compositions of the essential oils, gas chromatographic methods (GC-FID and GC-MS with columns of different polarities) were used and the results correlated with that of the antimicrobial testings. The obtained data are discussed to get more insight into the influence of pure aroma compounds on antimicrobial activities of essential oils.

Keywords

Essential pine oil, essential rosemary oils, essential tea tree oil, oxygenated monoterpenes, antimicrobial testing, GC

Introduction

In continuation of our research work on the field of combined data interpretation of antimicrobial testings (agar diffusion and agar dilution methods) and chiral phase gas chromatographic (cyclodextrin) analysis of aroma samples, such as pure compounds, essential oils and extracts [1-6], the oxygenated monoterpenes 1,8-cineole, α-terpineol, terpinen-4-ol and camphor as well as the essential oils of pine (Pinus pinaster), rosemary (Rosmarinus officinalis, 2 samples) and tea tree (Melaleuca alternifolia), known to be rich in these aroma compounds [7-11], were investigated.

Although the medical, pharmaceutical, perfumistic, cosmetic and food flavouring properties [7, 8-13] as well as the compositions [14-21] of essential oils of pine, rosemary and tea tree have been reported in many papers, detailed informations about the influence of the above cited single main compounds of these essential oils on their antimicrobial activities are not available until now.

Therefore, the objectives of this research were to test these essential oils and the cited oxygenated monoterpenes by means of usual agar diffusion and agar dilution methods with various strains of microorganisms [22-40], after improvement of some

parameters [4-6]. Furthermore, gas chromatograpic (GC-FID and GC-MS) analyses and olfactoric evaluations of the target compounds and the essential oils could be used to get informations about the purity as well as the composition of each sample. As result of the combined data interpretation the above mentioned influence of 1,8-cineole, α-terpineol, terpinen-4-ol and camphor on the antimicrobial effects of essential oils of pine, rosemary and tea tree should be ascertained. On basis of these data, a systematic investigation of aroma compounds and odorous samples (essential oils and extracts) will be continued in a running, international project.

Results and discussion

To control the efficiency of the used microbiological testing methods, eugenol as a phenolic compound with well-known antimicrobial activity against many strains of microorganisms was tested. Eugenol shows excellent effects against all strains using both methods, agar diffusion and agar dilution. Therefore, the obtained antimicrobial data (IZ=inhibition zones and MIC=minimum inhibition concentrations) of this component were taken as references for further testings.

The target-compound 1,8-cineole was most active against the Gram-(+)-bacterium *Staphylococcus aureus* (MIC=600) and the yeast *Candida albicans* (IZ=14); α-terpineol against the Gram-(-)-bacterium *Pseudomonas aeruginosa* (MIC=600), the Gram-(+)-bacterium *Staphylococcus aureus* (IZ=25) and the yeast *Candida albicans* (IZ=25); terpinen-4-ol against the Gram-(-)-bacteria *Pseudomonas aeruginosa* (MIC=60) and *Klebsiella pneumoniae* (IZ=27) as well as camphor against the Gram-(-).bacterium *Pseudomonas aeruginosa* (MIC=600) and the yeast *Candida albicans* (IZ=20), as to see in **Table 1**.

The commercially available essential oils did show the following antimicrobial activities (see Table 1):

Pine oil: Most effective against the Gram-(+)-bacterium *Staphylococcus aureus*, the Gram-(-)-bacteria *Escherichia coli* and *Klebsiella pneumoniae* as well as the yeast

Candida albicans (IZ=25, each), but also against the Gram-(-)-bacterium *Pseudomonas aeruginosa* (MIC=600).

Compounds and Essential Oils	Inhibition Zones (IZ) in mm and Minimum Inhibition Concentrations (MIC) in ppm of test-microorganisms									
	Staphylococcus aureus		*Escherichia coli*		*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*		*Candida albicans*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
1,8-Cineole	10	600	-	-	10	60	12	6	14	60
α-Terpineol	25	60	21	60	11	600	23	6	25	6
Terpinen-4-ol	25	6	25	6	8	60	27	6	25	0.6
Camphor	13	60	-	-	8	600	-	-	20	6
Rosemary oil "Spain"	19	60	9	6	8	600	9	60	16	60
Rosemary oil "Tunesia"	13	600	10	600	9	0.6	12	60	14	600
Pine oil	25	60	25	6	10	600	25	6	25	0.6
Tea tree oil	22	60	23	6	8	600	13	6	13	60
Eugenol	30	600	28	600	25	600	28	600	32	600

- no inhibition observed

Table 1. Antimicrobial activities of oxygenated monoterpenes, rosemary, pine and tea tree essential oils

Rosemary oil: Highest effects of the sample from Spain were found against *Staphylococcus aureus* (IZ=19) and *Pseudomonas aeruginosa* (MIC=600), while the oil from Tunesia showed the best activity against *Candida albicans* (IZ=14, MIC=600), *Staphylococcus aureus* and *Escherichia coli* (MIC=600, each).

Tea tree oil: The oil was most effective against *Escherichia coli* (IZ=23) and *Pseudomonas aeruginosa* (MIC=600).

Using gas chromatography (GC-FID and GC-MS with 2 columns of different polarity) in combination with olfactoric evaluations, the purity of the target compounds as well as the compositions of the essential oils were investigated.

In general, the purity of all tested oxygenated monoterpenes was found to be higher than 96% (calculated by %-peak area of GC with an apolar OV-5-type column) with a range from 96.3 for terpinen-4-ol upto 99.9% for 1,8-cineole. The

olfactoric evaluation with characteristic attributes for each sample proves the high purity and quality of these compounds (see **Table 2**).

Sample	Concentrations in %-peak area	Odor
1,8-Cineole	99.9	Eucalyptus, fresh
α-Terpineol	99.2	Floral, liliac-like
Terpinen-4-ol	96.3	Floral, fruity
Camphor	97.5	Camphoraceous

Table 2. GC analyses and olfactoric evaluations of target compounds

The gas chromatographic investigations of the essential oils gave following results (see **Table 3**): The pine oil is rich (higher than 3%) in the target compound α-terpineol (67.3%), further terpineols (γ: 8.1%, *cis*-β: 6.4%, *trans*-β: 5.0%) and α-2,5-dimethyl styrene (3.9%).

The main compounds of the rosemary oil from Spain are the target compounds 1,8-cineole (45.9%) and camphor (9.2%), the monoterpene hydrocarbons α-pinene (10.2%), β-pinene (8.0%), camphene (4.6%) and limonene (3.9%) as well as the sesquiterpene hydrocarbon caryophyllene (3.2%), and those from the Tunesian essential oil 1,8-cineole (46.7%), α-pinene (10.3%), β-pinene (10.1%) camphor (9.7%), camphene (4.5%) and caryophyllene (3.1%).

Major constituents of the tea tree oil are the target compound terpinen-4-ol (38.1), γ-terpinene (23.0%), α-terpinene (9.0%), *p*-Cymene (5.3%), 1,8-cineole (4.4%) and terpinolene (3.6%).

Also in the case of the essential oils the gas chromatographic data are in accordance to that one of olfactoric evaluations (using correlations with elsewhere published odor attributes [41-45]) and proves the high quality of each sample.

Compound	RI	Rosemary I*	Rosemary II*	Pine	Tea tree	Odor in accordance to [41-45]
cis-3-Hexenol	859	tr[1]	tr	nd[2]	0.1	green, grassy, weak fatty
Tricyclene	927	0.1	0.2	tr	nd	terpenic
α-Thujene	930	0.1	0.3	tr	0.5	camphoraceous, herbal
α-Pinene	939	10.2	10.3	0.1	2.3	pine-like, warm, weak herbal
α-Fenchene	953	0.6	tr	nd	nd	fresh, herbal, camphoraceous
Camphene	955	4.6	4.5	tr	0.1	camphoraceous, fresh, clean
Sabinene	975	0.1	0.1	tr	0.1	warm, oily-peppery, woody, spicy, weak herbal
β-Pinene	979	8.0	10.1	tr	1.1	resinous-piney, dry-woody
1-Octen-3-ol	981	0.1	0.2	nd	nd	mushroom-like, moody, earthy
Myrcene	991	1.8	1.6	nd	0.9	weak citrus- and lime-like
α-Phellandrene	1003	0.3	0.4	nd	0.4	citrus-like, weak peppery
1,4-Cineole	1015	nd	nd	0.2	tr	fresh, eucalyptus-like, camphoraceous
α-Terpinene	1017	0.2	0.6	tr	9.0	weak lemon- and citrus-like
p-Cymene	1025	1.4	0.5	0.1	5.3	weak citrus-, lemon- and bergamot-like
Limonene	1029	3.9	2.2	0.7	1.3	lemon-like
β-Phellandrene	1031	nd	nd	nd	0.2	peppery, minty, weak citrus-like
δ-3-Carene	1033	0.1	0.1	nd	tr	sweet, limonene-like
1,8-Cineole	**1035**	**45.9**	**46.7**	**0.2**	**4.4**	**fresh, eucalyptus-like**
γ-Terpinene	1060	1.1	1.3	0.2	23.0	citrus-like, herbal
cis-Sabinene hydrate	1071	0.1	0.1	tr	0.1	warm, spicy, peppery, weak woody
Terpinolene	1089	0.5	0.4	0.3	3.6	pleasant, sweet-piney
trans-Sabinene hydrate	1095	0.1	0.1	tr	0.1	warm, herbal, spicy, woody
Linalool	1097	1.5	0.7	tr	0.2	fresh, floral
α-2,5-Dimethylstyrene	1099	nd	nd	3.9	0.1	sweet, balsamic, floral
cis-Menth-2-en-1-ol	1122	nd	nd	nd	0.1	fresh, minty
trans-Pinocarveol	1139	0.2	0.1	tr	tr	warm, woody-balsamic, pine-like
trans-Menth-2-en-1-ol	1141	nd	nd	nd	0.1	fresh, minty
cis-β-Terpineol	1144	tr	tr	6.4	nd	woody-earthy
Camphor	**1146**	**9.2**	**9.7**	**tr**	**tr**	**fresh, camphoraceous**
Isoborneol	1161	0.1	tr	0.3	nd	camphoraceous, weak piney

trans-β-Terpineol	1163	nd	nd	5.0	nd	woody, herbal-earthy
δ-Terpineol	1166	0.3	0.4	0.6	tr	sweet, floral
Borneol	1169	1.4	1.4	2.5	tr	camphoraceous, peppery, earthy
Terpinen-4-ol	**1177**	**0.3**	**0.8**	**1.5**	**38.1**	**warm, peppery, woody, weak liliac-like**
α-Terpineol	**1189**	**2.0**	**1.4**	**67.3**	**2.9**	**sweet, floral, liliac-note**
γ-Terpineol	1199	0.2	tr	8.1	nd	sweet, weak floral
trans-Piperito	1209	tr	tr	nd	0.1	fresh, minty, camphoraceous
Linalyl acetate	1257	0.1	0.1	nd	nd	floral, weak fruity
Bornyl acetate	1289	1.1	1.1	0.3	tr	piney, sweet-balsamic, herbal
Isobornyl acetate	1286	0.1	tr	tr	nd	mild piney, oily-camphoraceous, balsamic
Verbenone	1205	nd	0.2	nd	nd	warm-resinous, minty-camphoraceous, spicy
α-Copaene	1377	0.2	0.1	nd	nd	weak woody, herbal
Methyl eugenol	1404	nd	nd	nd	0.1	spicy, warm, clove-like
α-Gurjunene	1410	nd	nd	nd	0.5	woody, herbal, spicy
Caryophyllene	1419	3.2	3.1	nd	0.4	dry, woody-spicy
Aromadendrene	1441	nd	nd	tr	1.2	woody, spicy
α-Humulene	1455	0.3	0.4	nd	tr	woody, spicy
allo-Aromadendrene	1460	nd	nd	nd	0.5	spicy, herbal, woody
Viridiflorene	1497	nd	nd	nd	0.9	herbal, floral
β-Bisabolene	1506	0.1	0.1	nd	tr	warm, spicy, sweet-balsamic
δ-Cadinene	1514	0.1	0.2	nd	0.9	dry-woody, mild
Ledol	1569	nd	nd	nd	0.1	warm, woody, balsamic
Spathulenol	1578	nd	nd	nd	0.1	woody, herbal
Caryophyllene oxide	1583	0.1	0.1	tr	tr	warm, spicy, woody
Globulol	1585	nd	nd	nd	tr	woody, spicy
Viridiflorol	1593	nd	nd	nd	0.4	herbal, floral, woody
α-Eudesmol	1654	nd	nd	nd	0.3	sweet-woody, weak floral

[+]Rosemary oil from Spain
[*]Rosemary oil from Tunesia
[1]trace compound less than 0.1%
[2]not detected

Table 3. Compositions of essentials oils (%-peak area of GC using an apolar fused silica OV-5-type column)

In conclusion of this combined investigation of antimicrobial activities and chiral qualities of linalools and linalool-rich essential oils, we can state:

1.) Eugenol as the reference component with well-known antimicrobial activities was found to possess the highest antimicrobial potential of all samples tested. This result proves that both, agar diffusion and agar dilution methods furnish reproducible antimicrobial data in testing aroma samples.

2.) The antimicrobial effects found in tests with α-terpineol are significantly compareable with these in tests with the pine oil, rich in terpineols.

3.) The same statement is possible in the case of terpinen-4-ol and tea tree oil, with a concentration of this monoterpene alcohol higher than 38.0% (further terpinenes in higher concentrations in this tea tree oil may be responsible for minor variations of the antimicrobial activity against some used microorganisms).

4.) A comparison of antimicrobial effects of 1,8-cineole and the rosemary oils with a higher amount of this monoterpene ether (more than 45.0%, each) shows only in tests against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Candida albicans* as well as partly against *Pseudomonas aeruginosa* similar results, while 1,8-cineole, in contrary to the rosemary oils, is not active against *Escherichia coli*. It seems that the relatively high concentrations of the monoterpene hydrocarbons α-pinene, β-pinene, camphene and limonene are responsible for this single different result.

Further combined investigations, antimicrobial testings and GC (in combination with olfactoric evaluations), will be done to get more data for a satisfying answer of the question, how effective pure aroma chemicals in complex aroma samples (e.g. essential oils and extracts) really are.

Experimental

Samples

The essential oils are products from Kurt Kitzing Co., Germany as follows: Pine oil (Pinus pinaster Aiton, Pinaceae: branch gum + oleoresin) – product-no 800676 (Térébenthine ISO 11020); rosemary oil (Rosmarinus officinalis L.,

Lamiaceae: flowering top + leaf) – product-no 2106040 (Spain, ISO1342) and 859789 (Tunesia, ISO 1342) as well as tea tree oil (Melaleuca alternifolia (Maiden et Betsche) Cheel, Myrtaceae: leaf & terminal branchlets) – product-no 801469 (Australien, ISO 4730).

The target and reference compounds are products from Sigma-Aldrich, such as 1,8-cineole (eucalyptol) – W24,650-6, camphor – W52,660-6, α-terpineol – W30,452-2 and eugenol – W24,670-0, while , terpinen-4-ol was also from Kurt Kitzing Co. with product-no 800760.

Antimicrobial testings

As test microorganisms, the Gram-(+)-bacterium *Staphylococcus aureus* ATCC 6538P, the Gram-(-)-bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* G 28 and *Klebsiella pneumoniae* as well as the yeast *Candida albicans* ATCC 10231 – 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 Whatman No. 1 filter paper discs (6mm) and quantities of 6µl of the sample, each. After cultivation of the bacteria and the yeast at 37°C for 24^h the diameter of the inhibition zone (IZ) was measured [4-6] as well as agar serial tube dilution method with results as minimum inhibitory concentration (MIC) in accordance to [4-6] as follows: The essential oils 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 100-1000ppm; the Petri dishes were inoculated by pipetting $0.1cm^3$ of the desired culture and 6.0µL of the samples as well as the reference compounds on paper discs (6mm) and then incubated at 37°C for 24h.

Olfactoric evaluations

All investigated samples were olfactorically evaluated by professional perfumers and/or olfactive trained chemists and the aroma described as mentioned

in Table 1 and 2 as well as correlated with odor impressions published elsewhere [41-45].

GC/FID

A GC-14A with FID and integrator C-R6A-Chromatopac (Shimadzu Co.) resp. a GC-3700 with FID (Varian Co.) and integrator C-R1B-Chromatopac (Shimadzu Co.) were used; carrier gas: hydrogen; injector-temp.: 250°C; detector-temp.: 300°C; temp.-progr.: 40°C/5 min. to 280°C/5 min. with a heating-rate of 6°C/min.; columns: 30m x 0.32mm bonded FSOT-RSL-200 (OV-5-type) fused silica (film thickness: 0.25 micron; Biorad Co.) and 50m x 0.32mm bonded Stabilwax (film thickness: 0.50micron; Restek Co.); quantification by %-peak-area-calculation.

GC/MS

A GC-17A with QP5000 (Shimadzu Co.) and data-system Compaq-ProLinea (Class5k-software, Shimadzu Co.), a GC-17A with QP5050 (Shimadzu Co.) with data-system PentiumII (Class5k-software, Shimadzu Co.), a GC-HP5890 with HP5970-MSD (Hewlett-Packard Co.) and Pentium-PC (Böhm Co., ChemStation-software) resp. a GCQ (Finnigan-Spectronex Co.) and Gateway-2000-PS75 (Siemens-Nixdorf Co.; GCQ-software) were used; carrier gas: helium; injector-temp.: 250°C; interface-heating: 300°C; ion-source-heating: 200°C; EI-mode, 70 eV; scan-range: 41-550 amu; other parameters see GC/FID-part. Mass spectra correlations with Wiley-, NBS-, NIST- and private library spectra on-line.

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References

[1] Jirovetz, L., *34th International Symposium on Essential Oils*, September 10th,

2003, Lecture 18, Würzburg, Germany.
[2] Jirovetz, L., Buchbauer, G., Stoyanova, A., Denkova, Z., Murgov, I., *EURO COSMETICS* **12** (1), 30-33 (2004).
[3] Jirovetz, L., Buchbauer, G., Stoyanova, A., Denkova, Z., Murgov, I., *Ernährung/Nutrition* **28** (6), 257-260 (2004).
[4] Jirovetz, L., Buchbauer, G., Stoyanova, A., Denkova, Z., Murgov, I., Schmidt, E., Geissler, M., *J. Essent. Oil-Bearing Plants*, in press (2004).
[5] Jirovetz, L., Buchbauer, G., Stoyanova, A., Denkova, Z., Murgov, I., Schmidt, E., Geissler, M., *III International Symposium Breeding Research on Medicinal and Aromatic Plants (ISMAP)*, July 5-8, 2004, Poster 136, Campinas, Brasil.
[6] Schmidt, E., Jirovetz, L., Buchbauer, G., Stoyanova, A., Denkova, Z., Murgov, I., Geissler, M., *35th International Symposium on Essential Oils*, September 8-10, 2004, Poster 76, Messina, Italy.
[7] Bauer, K., Garbe, D., Surburg, H. *Common Fragrance and Flavor Materials*, 3rd Ed., VCH, Weinheim (1997).
[8] Connolly, J.D., Hill, R.A., *Dictionary of Terpenoids*, Vol. 1: *Mono- and Sesquiterpenoids*, Chapman & Hall, London (1991).
[9] Finnemore, H., *The Essential Oils*, Ernest Benn Ltd., London (1927).
[10] Furia, T.E., Bellanca, N. (Eds.), *Fenaroli´s Handbook of Flavour Ingredients*, 2nd Edition, Vol. I, CRC Press, Cleveland (1975).
[11] Gildemeister, E., Hoffmann, Fr., *Die Ätherischen Öle*, Vol. IV, Akademie-Verlag, Berlin (1956).
[12] Österreichisches Arzneibuch (Pharmacopoae Austriaca) 1981, 1. Nachtrag, 213-215, *Aetheroleum Rosmarini*, Staatsdruckerei, Wien (1981).
[13] Österreichisches Arzneibuch (Pharmacopoae Austriaca) 1990, 12. Nachtrag, *Aetheroleum Terebinthinae rectificatum*, Verlag Österreich, Wien (2004).
[14] Macchioni, F., Cioni, P.L., Flamini, G., Morelli, I., Maccioni, S., Ansaldi, M., *Flavour Fragr. J.* **18**, 139-143 (2003).
[15] Reichling, J., Harkenthal, M., *Dtsche. Apoth.-Ztg.* **138** (38), 47-54 (1998).
[16] Boutekedjiret, C., Bentahar, F., Belabbes, R., Bessiere, J.M., *Flavour Fragr. J.* **18**, 481-484 (2003).
[17] Salido, S., Altarejos, J., Nogueras, M., Sánchez, A., Luque, P., *J. Essent. Oil Res.* **15**, 10-14 (2003).
[18] Galle-Hoffmann, U., König, W.A., *Dtsche. Apoth.-Ztg.* **139** (3), 64-72 (1999).
[19] Kreck, M., Scharrer, A., Bilke, St., Mosandl, A., *Flavour Frgr. J.* **17**, 32-40 (2002).
[20] Mori, M., Ikeda, N., Kato, Y., Minamino, M., Watabe, K., *YAKUGAKU ZASSHI* **122** (3), 253-261 (2002).
[21] Shellie, R., Marriott, Ph., Zappia, G., Mondello, L., Dugo, G., *J. Essent. Oil Res.* **15**, 305-312 (2003).
[22] Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M., *J.Agric.Food Chem.***46**, 1739-1745 (1998).
[23] Bär, B., *Analytik und Mikrobiologie des ätherischen Öls verschiedener Kamillenarten*, Thesis, Freie Universität Berlin (1995).

[24] Baratta, T., Dorman, S., Deans, S., Figueiredo, A., Barroso, J., Ruberto, G., *Flavour Fragr.J.* **13**, 235-244 (1998).
[25] Boesel, R., *Pharmakologische, phytochemische und mikrobiologische Untersuchungen von Queckenwurzelstock Rhizoma Graminis (Erg.-B. 6) (Agropyron repens (L.) PALISOT DE BEAUVOIS, Poaceae)*, Thesis, Freie Universität Berlin (1991).
[26] Bourrel, C., Vilarm, G., Michel, G., Gaset, A., *Rivista Italiana* **16/17**, 1-15 (1995).
[27] Davidson, P., Parish, M., *Food Technol.* **1989**, 148-155.
[28] Gabrielli, G., Loggini, F., Cioni, P., Giannaccini, B., Mancuso, E., *Pharm. Res. Commun.* **20**, 37-40 (1988).
[29] Griffin, S., Grant, W., Markham, J., Leach, D., *Flavour Fragr.J.* **14**, 323-332 (1999).
[30] Hammer, K., Carson, C., Riley, T., *J.Appl.Microbiol.* **86**, 985-990 (1996).
[31] Hood, J.R., Wilkinson, J.M., Cavangh, M.A., *J.Essent.Oil Res.* **15**, 428-433 (2003).
[32] Illiev, S., Papanov, G., Malakov, P., Hristeva, P., Tomova, K., University of Plovdiv, *Scientific works* **21**, No. 3, 105-112 (1983).
[33] Janssen, A.M., Scheffer, J.J.C., Baerheim Svendsen, A., *Planta Med.* **1987**, 395-398.
[34] Jirovetz, L., Buchbauer, G., Stoyanova, A.S., Georgiev, E.V., Damianova, St.T., *J. Agric. Food Chem.* **51**, 3854-3857 (2003).
[35] Johnson, T.R., Case, Ch.L., *Laboratory Experiments in Microbiology – Brief Edition*, 3rd Ed., 153-155, The Benjamin/Cummings Publishing Company, Inc., Redwood City (1992).
[36] Lis-Balchin, M., Deans, A., Eaglesham, E., *Flavour Fragr.J.* **13**, 98-104 (1998).
[37] Neunaber, E., *Phytochemische und mikrobiologische Untersuchungen von Propolis verschiedener Provenienz als Beitrag zur Kenntnis der Wirkprinzipien in Propolis*, Thesis, Freie Universität Berlin (1995).
[38] Paukova, T., Stoichev, I., Shabanova, R., Higher Institute of Flavor and Fragrance Industries, Plovdiv, *Scientific works* **20**, No. 3, 305-314 (1973).
[39] Stendel, H., *Seifen-Öle-Fette-Wachse* **97**, 736-738 (1971).
[40] Zaika, L., *J.Food Safety* **9**, 97-118 (1988).
[41] Arctander, St., *Perfume and Flavor Chemicals*, Vol. I, II & III, Arctander Publication, Montclair (1969).
[42] Fazzalari, F.A., *Compilation of Odor and Taste Threshold Values Data*, American Society for Testing and Materials, Philadelphia (1978).
[43] Bauer, K., Garbe, D., Surburg, H., *Common Fragrance and Flavor Materials*, 3rd Ed., VCH, Weinheim (1997).
[44] Furia, T.E., Bellanca, N. (Eds.), *Fenaroli´s Handbook of Flavor Ingredients*, 2nd Edition, Vol. I & II, CRC Press, Cleveland (1975).
[45] Sigma-Aldrich, *Flavors & Fragrances, The Essence of Excellence*, Sigma-Aldrich Co., Milwaukee (2003).

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