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Antimicrobial activity of a traditionally used complex essential oil distillate (Olbas® Tropfen) in comparison to its individual essential oil ingredients

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ABSTRACT

Plant extracts and essential oils have been widely studied and used as antimicrobial agents in the last decades. In our study we investigated the antimicrobial activities of Olbas® Tropfen (in the following named Olbas), a traditionally used complex essential oil distillate, in comparison to its individual essential oil ingredients. Olbas (10 g) consists of three major components such as peppermint oil (5.3 g), eucalyptus oil (2.1 g), and cajuput oil (2.1 g) and of two minor constituents like juniper berry oil (0.3 g) and wintergreen oil (0.2 g). The composition of Olbas and the five individual essential oils were characterized by GLC–MS. According to GLC–MS analysis 1,8-cineol is the main component of the complex essential oil distillate followed by menthol and menthone. The minimum inhibitory and minimum microbicidal concentrations of Olbas and each of the single essential oils were evaluated in 17 species/strains of bacteria and fungi. Time-kill assay was performed to compare the microbicidal activity of Olbas and peppermint oil during several time intervals. Olbas displayed a high antimicrobial activity against all test strains used in this study, among them antibiotic resistant MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant *Enterococcus*). Its antimicrobial activity was comparable to that of peppermint oil which was the most potent one of all individual essential oils tested. In the time kill assay Olbas as well as peppermint oil demonstrated similar microbicidal activities. Based on its wide antimicrobial properties Olbas can be a useful agent for the treatment of uncomplicated infections of skin and respiratory tract.

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Introduction

Olbas® Tropfen (Olbas) is a complex essential oil distillate traditionally used for the treatment of headaches, colds, cough, mild spasmodic gastrointestinal complaints, and of localized muscle pain. The complex essential oil distillate (10 g) is composed of three major components such as peppermint oil (5.3 g), eucalyptus oil (2.1 g), cajuput oil (2.1 g) and the two minor constituents, juniper berry oil (0.3 g) and wintergreen oil (0.2 g). According to anecdotal reports the origin of Olbas can be traced back to "**Ol**eum **bas**ileum" a complex essential oil distillate very well known in Central Europe in the 16th century (Böhm 2000). Besides other biological effects, the major individual essential oil ingredients of Olbas are also known for their antimicrobial activity (Harkenthal et al. 1999; Pino et al. 2002; Punit and D'Mello 2004; Saeed et al. 2006; Schelz et al. 2006; Lee et al. 2007; Cermelli et al. 2008; Ott and Morris 2008; Rasooli et al. 2009; Warnke et al. 2009; Mulyaningsih et al. 2010; Sadlon and Lamson 2010). Thus, Olbas as a mixture could also be a potent antimicrobial agent. Hitherto, there are no data available on its antimicrobial properties. Therefore, we tested the *in vitro* antimicrobial effects of this interesting essential oil blend in comparison to its individual components against different strains of bacteria and yeasts including multiresistant MRSA and VRE. In addition, we characterized all essential oils involved phytochemically by using GLC/FID and GLC–MS.

Materials and methods

Essential oils

Olbas® Tropfen (Olbas), peppermint oil, eucalyptus oil, cajuput oil, juniper berry oil, and wintergreen oil were provided by Deutsche Olbas GmbH (Germany).

GLC/FID

An OV-5 column (30 m × 0.25 mm ID, film thickness 0.25 μm) was installed in a Varian 3400 gas chromatograph equipped with a flame ionization detector (FID). The column head pressure was 15 kPa helium. Injector temperature was 250 °C in split mode (split ratio 1:40). After an initial isothermal step (2 min at 40 °C) the oven temperature was raised to 300 °C with a rate of 4 °C/min. The

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Table 1
Phytochemical composition of Olbas® Tropfen (Olbas) and the essential oils as determined by GLC–MS.

Number	Component	Olbas		Peppermint oil		Eucalyptus oil		Cajuput oil		Juniper Berry oil		Wintergreen oil	
		RI (OV5)	%	RI (OV5)	%	RI (OV5)	%	RI (OV5)	%	RI (OV5)	%	RI (OV5)	%
1	α-Pinene	932	2	–	–	932	1.09	935	0.96	934	39.76	–	–
2	β-Pinene	976	0.72	–	–	976	0.34	977	0.71	976	8.03	–	–
3	β-Myrcene	990	0.71	–	–	988	0.32	990	0.76	990	9.09	–	–
4	α-Phellandrene	1018	0.18	–	–	1010	0.23	–	–	–	–	–	–
5	1,8-Cineol	1035	38.17	1033	12.06	1037	81.93	1036	67.6	–	–	–	–
6	Limonene	1037	4.01	1035	2.11	1037	6.93	1035	4.70	1030	3.68	–	–
7	γ-Terpinene	1061	1.01	–	–	1057	1.62	1061	0.54	1061	3.26	–	–
8	Menthone	1157	12.13	1159	22.24	–	–	–	–	–	–	–	–
9	iso/neo-Menthone	1166	2.3	1166	2.8	–	–	–	–	–	–	–	–
10	iso/neo-Menthol	1167	0.3	1168	0.4	–	–	–	–	–	–	–	–
11	Menthol	1180	26.37	1183	47.29	–	–	–	–	–	–	–	–
12	α-Terpineol	1193	3.54	–	–	–	–	1194	8.22	1191	0.34	–	–
13	Menthylacetate	1296	2.17	–	–	–	–	–	–	–	–	–	–
14	Caryophyllene	1586	0.8	–	–	–	–	1586	2.34	1586	1.45	–	–
15	α-Caryophyllene	1621	0.2	–	–	–	–	1621	0.52	1621	1.04	–	–
16	Menthofuran	–	–	1180	2.03	–	–	–	–	–	–	–	–
17	Pulegone	–	–	1243	0.69	–	–	–	–	–	–	–	–
18	Menthylacetate	–	–	1297	3.28	–	–	–	–	–	–	–	–
19	Terpinolene	–	–	–	–	–	–	1092	2.11	1091	1.59	–	–
20	Linalool	–	–	–	–	–	–	1101	2.11	–	–	–	–
21	Terpinen-4-ol	–	–	–	–	–	–	1180	0.48	1180	4.21	–	–
22	Sabinene	–	–	–	–	–	–	–	–	925	2.16	–	–
23	α-Terpinene	–	–	–	–	–	–	–	–	1016	1.94	–	–
24	O-Cymen	–	–	–	–	–	–	–	–	1025	1.2	–	–
25	Bornylacetate	–	–	–	–	–	–	–	–	1288	0.16	–	–
26	α-Copaen	–	–	–	–	–	–	–	–	1357	0.38	–	–
27	Elemen-Isomer	–	–	–	–	–	–	–	–	1398	1.16	–	–
28	Methylsalicylate	–	–	–	–	–	–	–	–	–	–	1204	87.94

RI, Kovats retention index.

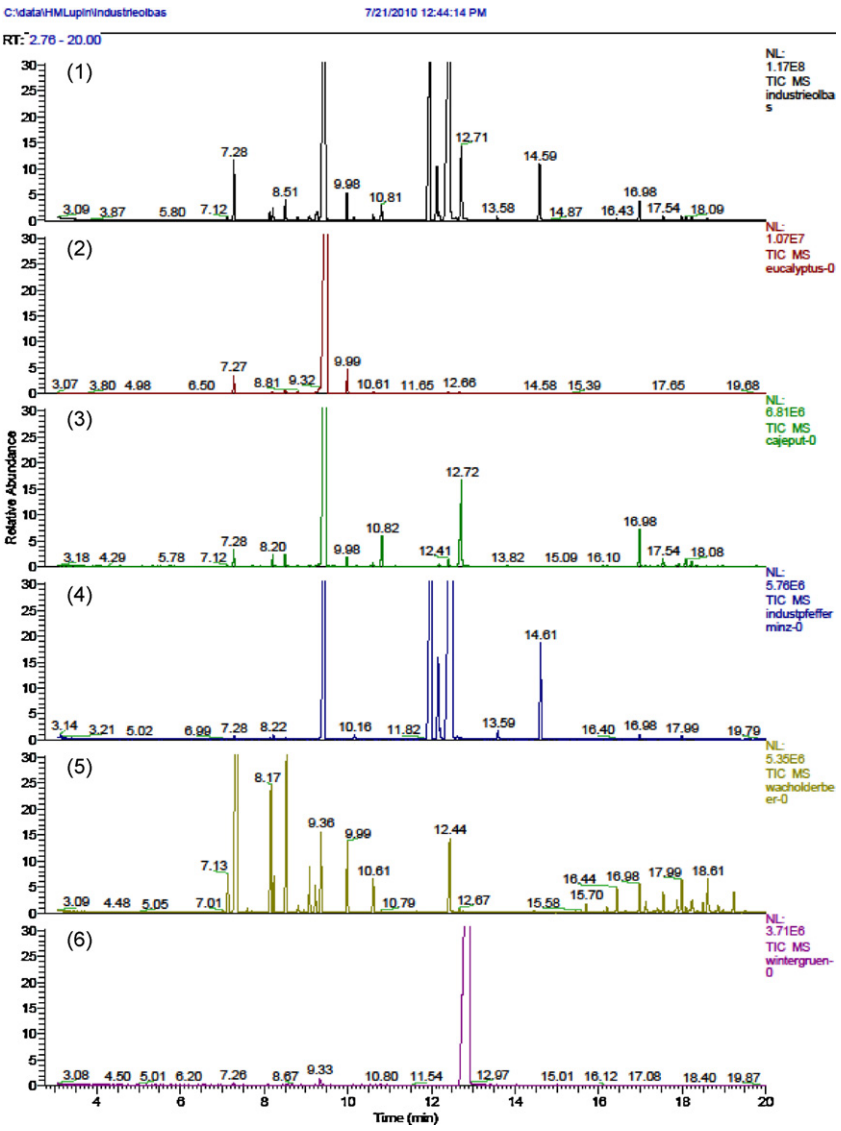

Fig. 1. GLC–MS fingerprinting of Olbas® Tropfen and the essential oils, from top to bottom: 1, Olbas; 2, eucalyptus oil; 3, cajuput oil; 4, peppermint oil; 5, juniper berry oil; 6, wintergreen oil.

temperature of FID was 300 °C. For recording and evaluation the software Peak Simple (SRI instruments) was used.

GLC–MS

GLC–MS analysis was done on a Hewlett-Packard gas chromatograph (GC 5890 II) coupled to a Finnigan SSQ 7000 Quadrupol mass spectrometer (Thermo-Finnigan). Type of column, carrier gas, head pressure, injector temperature and oven temperature program were the same like in the GLC experiment above. Split ratio was 1:15. Mass spectra were recorded in EI mode with 70 eV electron energy and an ion source temperature of 175 °C with the software Xcalibur 1.3.

The components of the essential oil were identified by comparing their mass spectra and retention indices (relative to co-chromatographed C8–C28 n-alkanes) with those of authentic substances, literature data and data bases.

Microbial strains

Antimicrobial activities of the complex essential oil distillate and its five ingredients were studied separately against 14 bacterial strains and three fungal strains. Gram-positive bacteria: *Staphylococccus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), methicillin-resistant *Staphylococcus aureus* (MRSA ATCC 10442), vancomycin-resistant *Enterococcus* (VRE VanB ATCC 31299), *Streptococcus pyogenes* (ATCC 12344), *Streptococcus agalactiae* (ATCC 27956), *Streptococcus oralis* (ATCC 35037) and *Bacillus subtilis* (ATCC 6051). In addition two clinical isolates of MRSA (MRSA USA 300) and VRE (VRE 902291) were included. Gram-negative bacteria: *Acinetobacter baumanii* (ATCC BAA 747), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603) and *Pseudomonas aeruginosa* (ATCC 27853). Fungi: *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019) and *Candida glabrata* (ATCC MYA 2950). All microorganisms were supplied by Medical Microbiology Lab., Hygiene Institute, Heidelberg University, Germany.

Culture media

The culture media for bacteria included Columbia Agar supplemented with 5% sheep blood (Becton Dickinson, Germany), Mueller–Hinton broth (MHB) (Fluka) and brain heart infusion (BHI) (Merck, Germany). *Candida* species were cultivated on CHROMagar

Table 2
The MIC and MMC values of Olbas® Tropfen (Olbas) and its five individual essential oil ingredients using Tween 80 as an emulsifier. Concentrations of essential oils are given in mg/ml.

	Microorganism	Olbas		Peppermit oil		Eucalyptus oil		Cajuput oil		Wintergreen oil		Juniper Berry oil	
		MIC (mg/ml)	MMC (mg/ml)	MIC (mg/ml)	MMC (mg/ml)	MIC (mg/ml)	MMC (mg/ml)	MIC (mg/ml)	MMC (mg/ml)	MIC (mg/ml)	MMC (mg/ml)	MIC (mg/ml)	MMC (mg/ml)
G^+	*Staphylococcus aureus*	1.25	5	0.6	2.5	10	20	2.5	10	10	>40	10	40
G^+	*Staphylococcus epidermedis*	5	10	1.25	5	10	40	10	40	20	>40	20	>40
G^+	MRSA	1.25	5	0.6	1.25	10	20	5	20	20	>40	20	40
G^+	MRSA (Cl)	2.5	5	0.6	1.25	10	20	2.5	5	40	>40	20	40
G^+	VRE	20	40	10	20	20	>40	40	>40	40	>40	40	>40
G^+	VRE (Cl)	5	10	2.5	2.5	40	>40	5	10	20	>40	20	40
G^+	*Streptococcus pyogenes*	1.25	2.5	5	10	10	>40	5	20	20	40	20	40
G^+	*Streptococcus agalactiae*	2.5	10	2.5	2.5	20	20	5	10	30	40	20	40
G^+	*Streptococcus oralis*	1.25	2.5	1.25	2.5	10	20	1.25	2.5	20	40	10	20
G^+	*Bacillus subtillis*	1.25	1.25	1.25	1.25	20	40	5	5	40	>40	0.6	2.5
G^-	*Acinetobacter baumanii*	0.15	0.3	0.15	0.3	1.25	2.5	0.3	0.3	0.3	0.3	1.25	2.5
G^-	*Escherichia coli*	0.6	1.25	0.6	0.6	10	10	5	5	10	20	20	40
G^-	*Klebsiella pneumoniae*	10	>40	5	>40	20	40	10	10	10	20	40	>40
G^-	*Pseudomonas aeruginosa*	5	20	20	40	10	20	5	20	20	40	20	40
Fungi	*Candida albicans*	0.3	1.25	0.3	1.25	10	20	2.5	5	2.5	5	20	40
Fungi	*Candida parapsilosis*	1.25	2.5	0.6	1.25	5	10	1.25	2.5	2.5	5	10	20
Fungi	*Candida glabrata*	1.25	1.25	0.6	0.6	7.5	30	2.5	5	5	10	20	40

Cl, clinical isolate. All the results are presented as the mean value.

Candida medium (Becton Dickinson, Germany) and Sabouraud Dextrose broth (SDB) (Merck, Germany).

Cultivation of bacteria and fungi

One or two bacterial or fungal colonies from an 18 to 24 h agar plate were suspended in saline to a turbidity matching 0.5 McFarland $\approx 1 \times 10^8$ colony forming unit per ml (cfu/ml) (bacteria) and $\approx 1 \times 10^6$ cfu/ml (yeasts). Then 100 μl of the bacterial suspension was diluted 1:100 with broth to get 1×10^6 cfu/ml and 1000 μl of the yeast suspension was diluted 1:10 with broth to get 1×10^5 cfu/ml.

Determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC)

MICs were determined by micro dilution method according to the German DIN regulation 58940-8 (Deutsches Institute für Normung, 2000). The whole study was repeated twice. Olbas and the five essential oils separately were first dissolved in Tween 80 as emulsifier to the concentration of 40 mg/ml and then diluted twofold with MHB (for bacteria) and SDB (for yeasts) in 96 well plates to obtain a range of concentrations between 40 mg/ml and 0.15 mg/ml. The final Tween 80 concentration in the test system did not exceed 0.5% (v/v). The bacterial and fungal suspensions of 1×10^6 cfu/ml and 1×10^5 cfu/ml, respectively, were then added and the plates were incubated at 37 °C for 24 h (bacteria) and at 25 °C for 48 h (yeasts). The lowest concentration that showed no visible turbidity matching with a negative control was defined as MIC. From each clear well 3 μl was inoculated in appropriate agar medium and incubated under the appropriate condition for bacteria and fungi, as reported previously (Mulyaningsih et al. 2010), MMC was determined as the lowest concentration that yielded no apparent microbial growth on agar after incubation at 37 °C for 24 h. Each plate included a growth control (Tween 80 0.5%) and a sterility control. Ampicillin (Applichem, Germany) and vancomycin (Sigma–Aldrich, Germany) were used as positive controls against bacteria and nystatin (Cellpharm, Germany) against yeasts.

Time kill assay

A time kill assay with Olbas and peppermint oil was performed against *Staphylococcus aureus* MRSA (NCTC 10442), *Streptococcus pyogenes* (ATCC 12344), *Klebsiella pneumoniae* (ATCC 700603), *E. coli* (ATCC 25922), *Acinetobacter baumanii* (ATCC BAA 747), and *Candida albicans* (ATCC 90028) according to the NCCLS guidelines (NCCLS 1999). The essential oils were prepared in duplicate at several concentrations (MIC, 2 × MIC, 4 × MIC and 8 × MIC) in the appropriate medium with 0.5% Tween 80 as an emulsifier and the mixture was inoculated with an overnight culture of the test strains adjusted to approximately 10^6 cfu/ml. Medium with 0.5% Tween 80 was used as growth control. Aliquots of 50 μl were removed after several time intervals (0, 1, 2, 4, 6, and 24 h) and diluted serially (1:10) using 450 μl sterile saline. Three times 20 μl of each dilution was spread onto agar plates and the viable colonies were counted after incubation for 24 h at 37 °C in order to calculate the cfu in the test medium at the corresponding time points (Iten et al. 2009). According to NCCLS an antimicrobial agent is thought to be bactericidal when it causes $\geq 3 \times \log 10$ (99.9%) reduction in colony forming units per ml (cfu/ml) after 18–24 h of incubation in liquid media under a given set of conditions. This definition also applies for essential oils.

Results and discussion

Multi-resistant strains of bacteria and fungi have increased and became widely distributed in the last decades causing life

threatening nosocomial infections. The main cause for this resistance is the excessive use of anti-infective agents in medicine and in agriculture.

Do alternative sources for the treatment of multiresistant microbes exist? Essential oils exhibit a wide range of antimicrobial activity (Reichling et al. 2009). They are mixtures of different compounds with several pharmacophoric groups. This variety enables them to work on several targets and in several modes of actions (Wink 2008a; Reichling et al. 2009). Therefore, essential oils are potential candidates for the treatment of trivial and uncomplicated infections such as skin and wound infections, infections of the upper respiratory tract, cough and sneezes, and herpes labialis (Iten et al. 2009; Reichling et al. 2009; van Vuuren et al. 2009; Reichling 2010). The efficacy of essential oils should not be overestimated and it should be taken into account that in severe cases essential oils cannot replace the established anti-infective agents.

Missing data on the antimicrobial activity of Olbas® Tropfen (Olbas) encouraged us to investigate the antibacterial and antifungal effects of this interesting complex essential oil distillate in comparison to its individual oil components using *in vitro* test methods.

To ensure the identity of the test oils, the main components of Olbas and each of its individual essential oil ingredients were qualitatively and quantitatively analyzed by high-resolution GLC and GLC–MS. The results of the phytochemical analyses are presented in Table 1 and Fig. 1. The main oil components were identified by comparing their mass spectral data and retention indices (relative to co-injected n-alkanes) with those of authentic reference substances as well as literature data. Menthol and menthone are the main compounds in peppermint oil followed by 1,8-cineol, menthylacetate, limonene and menthofuran (Punit and D'Mello 2004). The main compound in eucalyptus oil is 1,8-cineol followed by limonene, γ-terpinene and α-pinene (Sadlon and Lamson 2010). Cajuput oil consists of the monoterpene 1,8-cineol, followed by α-terpineol, limonene, caryophyllene, terpineolene, linalool and α-caryophyllene (Farag et al. 2004). While α-pinene is the main compound in juniper berry oil (Filipowicz et al. 2003), methylsalicylate is the predominant substance (96–98%) in wintergreen oil (Jänicke et al. 2003). Monoterpenes as 1,8-cineol, menthol and menthone are the main constituents in Olbas since peppermint, cajuput and eucalyptus oils dominate the complex essential oil distillate. Monoterpenes are highly hydrophobic substances and that enable them, for instance, to increase cytoplasmic membrane fluidity and permeability, disturb the conformation of membrane-embedded proteins, inhibit cell respiration, and alter ion transport processes, resulting in cell death (Reichling et al. 2006, 2009; Wink 2008a,b; Reichling 2010).

Minimum inhibitory concentrations (MIC) and minimum microbicidal concentrations (MMC) of Olbas and its individual essential oil ingredients are given in Table 2. According to our findings, the solubility of the test oils in culture media was very adequate when using Tween 80 as an emulsifier. Furthermore, 0.5% Tween 80 did not exhibit any antimicrobial effect *in vitro*. Olbas showed a substantial antimicrobial activity against all test strains used in this study including clinical isolates of multi-resistant MRSA and VRE, exhibiting MIC values of 0.15–20 mg/ml. In most cases, MMC values were one to two times higher than MIC values, demonstrating a dose dependent effect. The prominent activity of Olbas against the bacteria *Acinetobacter baumanii* (MIC = 0.15 mg/ml), *Escherichia coli* (MIC = 0.6 mg/ml), *Pseudomonas aeruginosa* (MIC = 5 mg/ml) and the yeast *Candida albicans* (MIC = 0.3 mg/ml) is worth to be emphasized in particular. *A. baumanii*, *S. aureus*, *E. coli* and *P. aeruginosa* cause wound and nosocomial infections and often they are multiresistant against different antibiotics, especially *Pseudomonas aeruginosa* is strongly resistant to many essential oils (Reichling et al. 2009). The yeast *Candida albicans* colonizes mucous membranes like pharyngo-oral cavity and genital organs.

Regarding the antibacterial and antifungal effects of the essential oils which constitute Olbas it is obvious that peppermint oil and cajuput oil are the most active ones. In many cases peppermint oil seems to be a little bit more active in comparison to Olbas and cajuput oil. On the other hand, it is worth to be mentioned that besides Olbas only cajuput oil exhibited a relatively high antibacterial property against *Pseudomonas aeruginosa*. All other essential oils tested were more or less ineffective (MIC values: 10–40 mg/ml) against this extremely problematic germ. Against this background it should be mentioned that in our experiments *P. pseudomonas* proved to be resistant against the antibiotics ampicillin and vancomycin (Table 3). According to our findings, the antimicrobial activity of Olbas and its essential oil ingredients can be ranked in the following ascending order: juniper berry oil < wintergreen oil < eucalyptus oil < cajuput oil < Olbas < peppermint oil.

MIC is an endpoint determination which allows no deeper insight into the toxicological process taking place during a 24 h incubation period in the presence of an antimicrobial agent. Besides MICs, reliable information on the microbicidal property of an antimicrobial agent is essential for a successful treatment of infections. As demonstrated in animal models and with human patients,

Table 3
The MIC and MMC values of the positive controls.

	Microorganism	Ampicillin		Vancomycin		Nystatin	
		MIC (μg/ml)	MMC (μg/ml)	MIC (μg/ml)	MMC (μg/ml)	MIC (μg/ml)	MMC (μg/ml)
G^+	*Staphylococcus aureus*	0.2	3.125	3.125	12.5	NT	NT
G^+	*Staphylococcus epidermedis*	0.8	4.5	1.56	4.5	NT	NT
G^+	MRSA	25	>25	0.8	12.5	NT	NT
G^+	VRE	1	7	25	>50	NT	NT
G^+	MRSA (CI)	50	>50	7	12.5	NT	NT
G^+	VRE (CI)	NA	NA	NA	NA	NT	NT
G^+	*Streptococcus pyogenes*	0.05	0.1	0.1	0.4	NT	NT
G^+	*Streptococcus agalactiae*	0.4	0.4	0.4	50	NT	NT
G^+	*Streptococcus oralis*	0.05	0.1	0.4	0.4	NT	NT
G^+	*Bacillus subtillis*	0.05	1.5	0.2	6	NT	NT
G^-	*Acinobactr baumanii*	6.25	12.5	50	50	NT	NT
G^-	*Escherichia coli*	12.5	25	NA	NA	NT	NT
G^-	*Klebsiella pneumoniae*	25	25	25	50	NT	NT
G^-	*Pseudomonas aeruginosa*	NA	NA	NA	NA	NT	NT
Fungi	*Candida albicans*	NT	NT	NT	NT	0.2	0.4
Fungi	*Candida parapsilosis*	NT	NT	NT	NT	0.2	0.2
Fungi	*Candida glabrata*	NT	NT	NT	NT	0.2	0.2

NA, not active; NT, not tested; CI, clinical isolate. All the results are presented as the mean value.

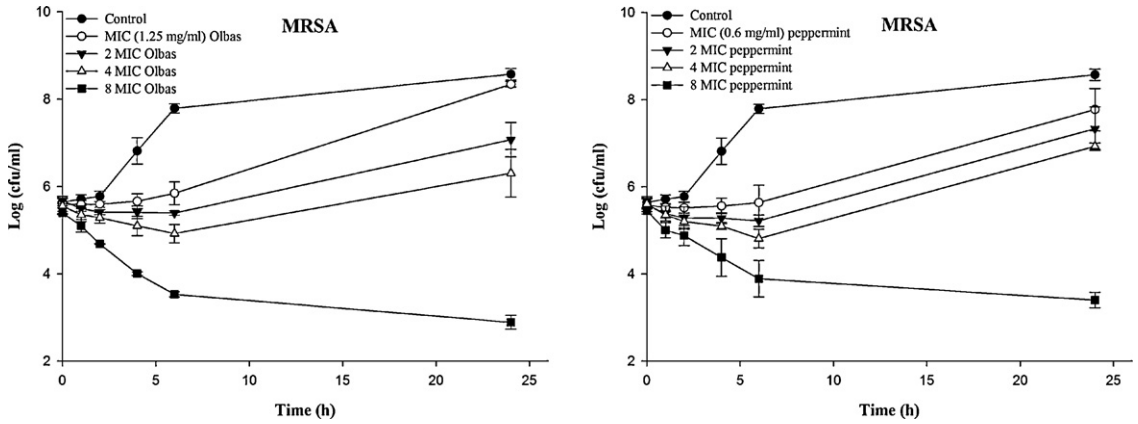

Fig. 2. Time-kill curves of Olbas® Tropfen (Olbas) and peppermint oil against MRSA.

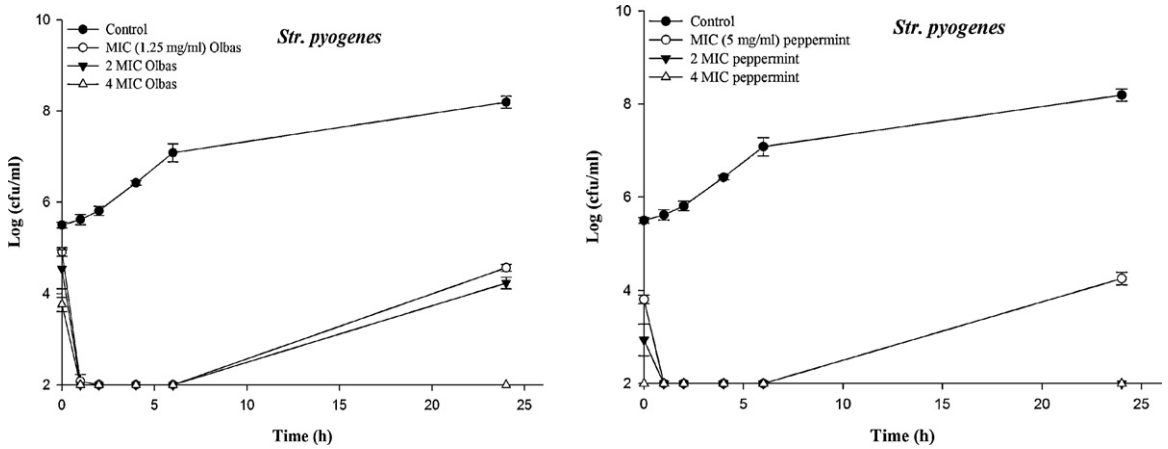

Fig. 3. Time-kill curves of Olbas® Tropfen (Olbas) and peppermint oil against *S. pyogenes*.

microbicidal data collected by time-kill assay have the best correlation with clinical efficacy (Drake and Sande 1983; Chandrasekar et al. 1987).

In a time-kill study the death kinetics of Olbas and peppermint oil as the most active agents (Table 2) against six strains of bacteria as well as the yeast *Candida albicans* were investigated in more detail. Results are presented as $\log_{10}$ cfu/ml change in the viable colony number. The results obtained are shown in (Figs. 2–7). Against MRSA both oils exhibited a bactericidal activity (reduction of $3 \times \log_{10}$ cfu/ml) at the concentration of $8 \times$ MIC (10 mg/ml Olbas and 5 mg/ml peppermint oil) after 24 h of incubation (Fig. 2). Lower concentrations demonstrated only a weak bacteriostatic effect within the first 6 h followed by a significant re-growth. In case of *Streptococcus pyogenes* a rapid initial decrease in the number of surviving bacteria (reduction of $3 \times \log_{10}$ cfu/ml) was observed within the first 6 h (Fig. 3). A bactericidal effect after 24 h of incubation was recorded for Olbas at $4 \times$ MIC (5 mg/ml Olbas) whereas peppermint oil displayed the same effect at $2 \times$ MIC (2.5 mg/ml peppermint oil). Only Olbas exhibited a strong bacteriostatic effect against *Klebsiella pneumoniae* at all concentrations

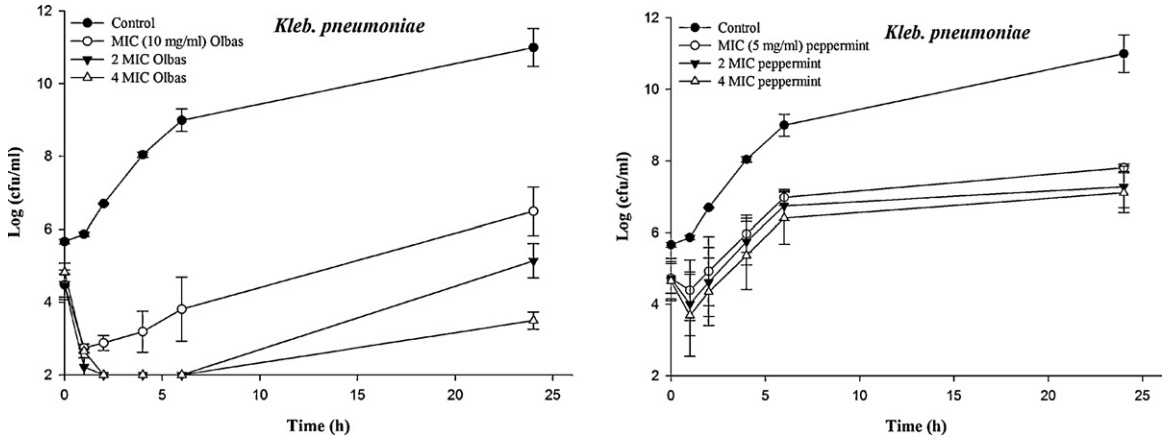

Fig. 4. Time-kill curves of Olbas® Tropfen (Olbas) and peppermint oil against *K. pneumoniae*.

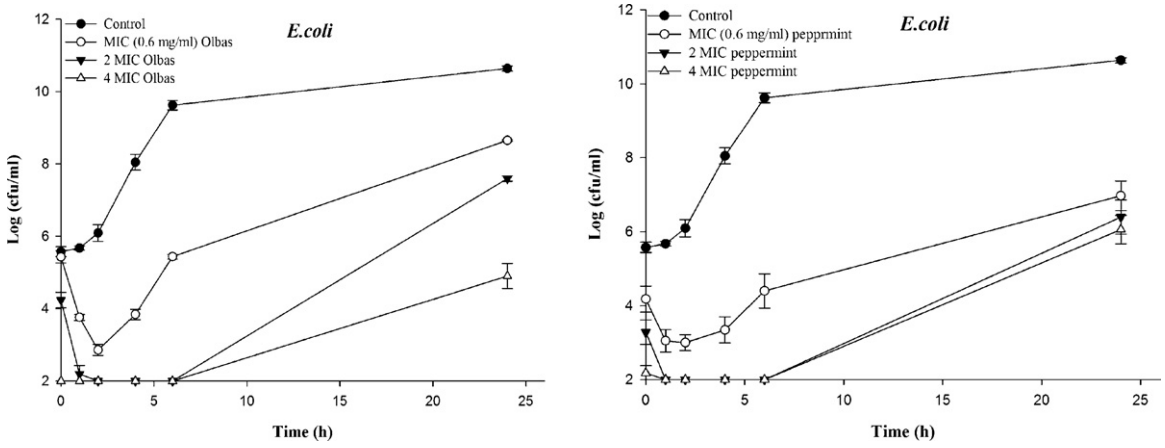

Fig. 5. Time-kill curves of Olbas® Tropfen (Olbas) and peppermint oil against *E. coli*.

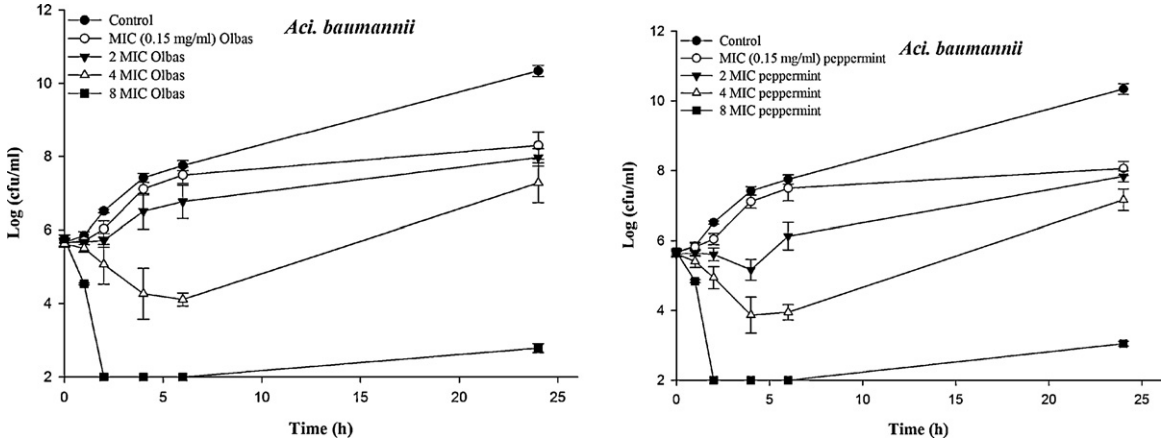

Fig. 6. Time-kill curves of Olbas® Tropfen (Olbas) and peppermint oil against *A. baumanii*.

tested (Fig. 4). Using peppermint oil only a moderate bacteriostatic activity could be demonstrated. In case of *Escherichia coli* Olbas as well as peppermint oil revealed a bactericidal activity within the first 6 h for 2 × MIC and 4 × MIC (Fig. 5). As shown for other bacteria, a first strong bactericidal effect was followed by re-growth of bacteria whereby the extent of re-growth depended on the oil concentrations. Regarding *Acinetobacter baumanii* concentrations of 8 times greater than MIC Olbas (2.5 mg/ml) as well as peppermint oil (2.5 mg/ml) were rapidly (even after 2 h) bactericidal and exhibited a maximal bactericidal effect after 24 h of incubation (Fig. 6).

Concerning the yeast *Candida albicans*, time-kill curves of both Olbas and peppermint oil exhibited a strong decrease of cell viability of about 3 × $\log_{10}$ cfu/ml within 6 h using the highest concentration of 8 × MIC (2.5 mg/ml Olbas and peppermint oil). After 6 h only a moderate re-growth was noted indicating a fungicidal effect of both volatile agents (Fig. 7).

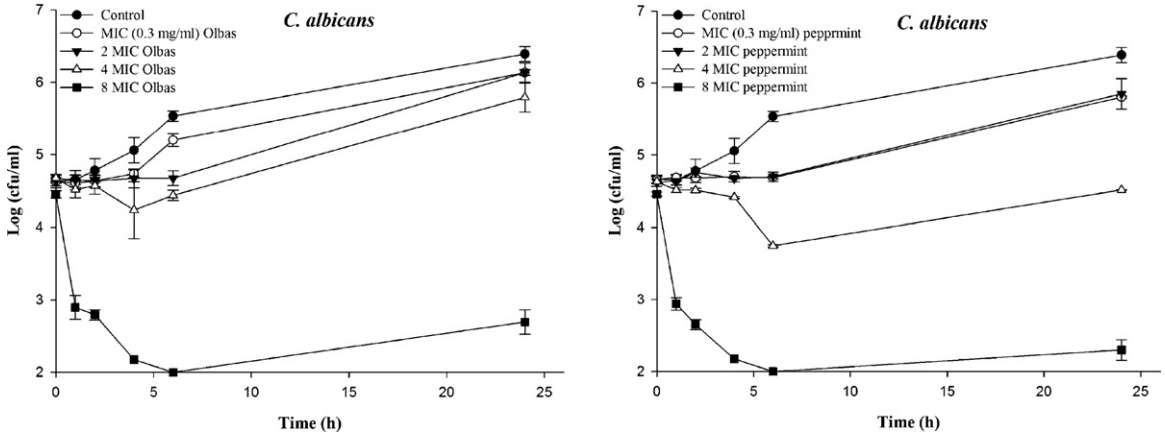

Fig. 7. Time-kill curves of Olbas® Tropfen (Olbas) and peppermint oil against *C. albicans*.

The maximal bactericidal effects were obtained by Olbas and peppermint against *A. baumanii*, *MRSA*, *S. pyogenes*, and *C. albicans* in concentrations 4–8 times greater than the respective MICs. The antimicrobial effect was clearly time and concentration dependent. It is known from literature that for appropriate treatment of bacterial and fungal infections the drug concentration should not only reach MIC level but should exceed it several times to ensure maximal efficacy (Aiyegoro et al. 2008; Ogunmwonyi et al. 2010). This means that the drug concentration used was not high enough to kill all bacteria. In addition, essential oils are volatile agents and evaporate over time. But the killing process continues only as long as the concentration of the essential oil is in excess of the MIC. These findings are also of interest for the application of Olbas and other essential oils *in vivo*. To ensure a successful treatment of bacterial and fungal infections of skin or upper respiratory tract essential oils should be administered in a proper concentration several times a day.

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