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Full Length Research Paper

Screening of antimicrobial and antioxidant activity of commercial *Melaleuca alternifolia* (tea tree) essential oils

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The paper deals with the investigation of the chemical composition, antimicrobial and antioxidant activity of four commercial products of *Melaleuca alternifolia* essential (TTO). The chemical composition was determined using gas chromatography mass spectrometry (GC/MS) analysis. The broth-microdilution method was used to test antimicrobial activity. The antioxidant activity was tested by stable free radical of the 2,2-diphenyl-1-picrylhydrazyl (DPPH). Results of the chemical analysis identified over 30 components. The composition of the four samples varied and the major components included the following: terpinen-4-ol, γ-terpinene and α-terpinene, respectively. The samples were compliant to the ISO Standard 4730. All TTOs presented similar degrees of antimicrobial activity. A slightly higher activity was shown by TTO[4], having a balanced level of the three main components: terpinen-4-ol (34.58%) γ-terpinene (9.89%) and α-terpinene (12.59%). Fungi were more sensitive than bacteria and yeast. All TTOs tested possessed the concentration-dependent antioxidant activity. Antioxidant activity was based on a balanced chemical composition between terpinen-4-ol and terpenic compounds. Biological testing confirmed the TTO antimicrobial activity against a wide range of the Gram-positive and Gram-negative bacteria, and fungi and yeasts. The correlation between the chemical composition and biological activity was quite evident. In conclusion, this study's results reveal not only the biological activity of TTO, but also its potential as an antioxidative agent.

Key words: *Melaleuca alternifolia*, TTO, gas chromatography mass spectrometry (GC/MS), antimicrobial, antioxidant activity.

INTRODUCTION

The products of secondary metabolism in plants, essential oils (EOs), have been used for a long time, primarily because they possess a wide range of biological activities, some of them being the antimicrobial, antioxidant, anti-inflammatory, anticancer, etc. EOs is in focus of scientific research as they are broadly used in pharmaceutical, cosmetic and food industry (Kalemba and Kunicka, 2003).

Interest in the therapeutic use of non-conventional, over-the-counter drugs or alternative medicines and herbs for treating infectious diseases has grown remarkably in the recent years. It is mostly because of well-known side effects of conventional drugs, as well as by the spread of antimicrobial resistance to them, although they are highly efficient and well-tolerated (Carson and Riley, 1993). Treatment with synthetic antimicrobial drugs has caused the rise and improvement

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of quality of an average life expectancy in the 20th century. However, a great number of pathogenic bacteria and fungi that cause infections have become resistant to the commonly used drugs. The bacteria, such as *Escherichia coli* and *Staphylococcus aureus,* can cause food poisoning and infect wounds. The infections and diseases caused by resistant bacteria can be treated only with experimental and potentially toxic drugs. Candidiasis is often associated with conditions such as diabetes mellitus, antibiotherapy, corticotherapy, but also with pregnancy. *Candida* species resistant to azole antifungal drugs are increasingly isolated from the mouths of patients suffering from oral fungal infections.

Tea tree oil is an agent possessing antimicrobial properties that may prove useful in the prevention and management of infections caused by these organisms (Cowan, 1999). EO from Australian native *Melaleuca alternifolia*, known as tea tree oil (TTO), is one of most used native products. TTO is composed of over 100 components, mainly terpene hydrocarbons, usually mono and sesquiterpenes, and their related alcohols (Brophy et al., 1989). The main constituents of the TTO are: terpinen-4-ol, γ-terpinene and α-terpinene comprising ~70% of whole oil. Researchers revealed that it showed an inhibitory effect against a number of Gram-negative and Gram-positive bacteria and fungi (Hammer et al., 2006). It has been used in treatment of various infections (Carson et al., 2001, Satchell et al., 2002, Buck et al., 1994, Vazquez and Zawawi, 2002) and in various commercial products, such as creams, shampoos, oral rinses and others.

In contrast to its antimicrobial activity, the antioxidant properties of TTO have not been sufficiently investigated. It would be of great importance to explore the antioxidant potential of TTO, and sum the biological activity of this intriguing preparation.

Since the commercial TTO preparation and possible differences in its biological activity have not yet been researched, the aim of this study was to compare the chemical composition of some TTO commercial preparations, and also to test for the existance of the differences between antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Essential oils

We purchased four commercial TTOs: TTO[1]-Tea tree oil from the Thursday plantation, TP Health Ltd. Pacific Highway, Australia; TTO[2]-Teebaum 100% pure aus Bio-Anbau and TTO[3]-Tee baum 100% pure both from Bergland-Pharma GmbH and Co. KG and TTO[4]-TTO 100% pure from Dr. Müller Pharma, Czech Republic.

Essential oil analyses procedure

All EOs samples were diluted in ethanol (1µl) and injected in split-mode (1:30). The GC was performed on the GC Agilent Technologies 7890A apparatus, equipped with the split-splitless injector attached to HP-5 column (30 m × 0.32 mm, film thickness 0.25 µm) and fitted to flame-ionization detector (FID). The operating conditions were as follows: carrier gas was H_2 (1 ml/min/210℃); the temperatures were set as follows: injector at 250℃ and detector at 280℃, while the column temperature was linearly programmed 40 to 260℃ at 4℃/min. The percentage compositions of each sample were computed from the peak areas, without correction factors.

The GC/MS was performed on HP G 1800C Series II GCD analytical system equipped with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). Carrier gas was He (1 ml/min). Other chromatographic conditions were as those for GC-FID. The transfer line was heated at 260℃. The mass spectra were recorded in electron ionization (EI) mode (70 eV), in a range of m/z 40 to 450.

The identification of individual constituents was accomplished by comparison of their spectra with those from available MS libraries (NIST/Wiley), and by comparison of their experimentally determined retention indices (calibrated AMDIS), with the data from the literature (Adams, 2001).

Antibacterial activity

The following Gram-negative bacteria were used in the research: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterococcus cloacae* (human isolate), and the following Gram-positive bacteria: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973) and *Staphylococcus aureus* (ATCC 6538). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute of Biological Research “Sinisa Stankovic”, Belgrade, Serbia. All bacterial species were maintained on the Mueller Hinton agar and subcultured once a month. The antibacterial assay was carried out by a microdilution method (Douk et al., 1995). The bacterial suspensions were adjusted to sterile saline to a concentration of 1.0×10^5 CFU/ml. The inocula were prepared daily and stored at +4℃ until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined by using 96-well microtitre plates. The bacterial suspension was adjusted to sterile saline to the concentration of 1.0×10^5 CFU/per well. The TTO samples were added in Tripticase Soy Broth (TSB; Merck, Germany) medium containing 2% dimethyl sulfoxide (DMSO) in final concentration. It did not influence the growth of bacteria; it, however, enhanced the solubility of the TTO. Medium, together with bacterial inoculum, made desired concentrations of TTO (0.01 to 4% v/v be achieved. The microplates were incubated for 24 h at 48℃. The MBCs were determined by serial sub-cultivation of 10 µl into microtitre plates containing 100 µl of broth per well and further incubation for 72 h at 28℃. The lowest concentrations with no visible growth were defined as the MBCs, indicating 99.5% killing of the original inoculum. Streptomycin (Sigma P 7794) was used as a positive control. The DMSO was used as a negative control. All experiments were done in triplicate.

Antifungal activity

In antifungal assay, eight fungi were used: *Aspergillus ochraceus* (ATCC 12066), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus flavus* (ATCC 9643), *Penicillium funiculosum* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Fusarium verticiloides* (plant isolate) and *Trichoderma viride* (IAM 5061). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute of Biological Research “Sinisa Stankovic”, Belgrade, Serbia. The micromycetes were maintained on malt agar (MA) and stored at +4℃ and

subcultered once a month (Booth, 1971). The microdilution technique was used to determine the antifungal activity of the TTO samples. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spores suspension was adjusted to the sterile saline to the concentration of approximately 1.0×10^5 in the final volume of 100 µl per well. The inocula were stored at 4℃ for furt her use.

MIC and minimum fungicidal concentration (MFC) values were determined by the dilution technique using 96-well microtitre plate. Different concentrations of commercial TTOs (0.01 to 2% v/v) were added to Malt broth medium containing 2% DMSO in final concentration. It did not influence the growth of bacteria; it, however, enhanced the solubility of the TTO. Medium, together with bacterial inoculum, made desired concentrations of TTO (2 to 0.01% v/v). A commercial fungicide, Fluconazole, was used for control. The microplates were incubated for 72 h at 28℃. The lowest concentrations without visible growth were defined as MIC. MFCs were determined by serial subcultivation of 10 µl into microtiter plates containing 100 µl of broth per well and further incubation of 72 h at 28℃. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. The commercial fungicide Fluconazole (Pfizer PGM, France) was used as the control one.

Anticandidal activity

In this assay, total of 7 clinical isolates of *Candida albicans* species and one (ATCC 10231) *C. albicans* were used. Clinical isolates were obtained from oral cavities of healthy individuals who attended the Teaching Hospital at the Faculty of Dentistry in Belgrade, Serbia, for regular dental treatment. Identification of isolates was done by standard mycological methods. The MICs and MFCs were determined using 96-well microtitre plates. The yeast was cultured in Sabourand dextrose agar (SDA; Merck, Germany) plates at 37℃. The following incubation cell suspension was adjusted spectrophotometrically to the concentration of 1.0×10^5 CFU/ml. The TTO was added to the TSB medium containing 2% DMSO in final concentration. Medium, together with bacterial inoculum, made desired concentrations of TTO (0.06 to 2%). The microplates were incubated for 24 h at 35℃. The MIC and MFC values were determined as described earlier. Nystatin (Hemofarm, Serbia) was used as the control one.

Antioxidant activity

The antioxidant activity of the commercial of TTO samples was determined by using the stable radical DPPH method (Blois, 1958) with modifications. 100 µl of various concentrations of TTOs in methanol mixed with 900 µl of 0.04 mg/ml methanolic solution of DPPH. The absorbance at 517 nm was measured after 30 min in dark at room temperature. The Methanol (900 µl) plus sample solution (100 µl) were used as a blank. The DPPH (900 µl) solution and methanol (100 µl) were used as control ones. The inhibition percentage was calculated using the following formula:

$$\% \text{ of Inhibition} = \left[\frac{Ac - As}{Ac}\right] \times 100$$

Where Ac is the absorbance of the negative control and As is the absorbance of the samples.

RESULTS AND DISCUSSION

This study proved that different TTOs with different chemical composition have shown great biological potential, not only in antimicrobial, but also in antioxidant screening.

This is the first study involving both testing different TTOs and all groups of microorganisms together. According to the available data from the literature, the before-mentioned bacteria and fungi are for the first time tested on the TTOs.

Chemical composition

The results of chemical analysis of different TTOs samples are presented in Table 1. All four commercial TTOs samples had a similar composition. Chemical analysis of commercial TTO samples had over 30 components identified. The main components were terpinen-4-ol, γ-terpinene and α-terpinene respectively. These three compounds accounted for about > 60% of total amount of EOs. The TTO^3 has highest content of terpinen-4-ol (44.17%) than the TTO^2 (38.58%) > TTO^4 (34.57%) > TTO^1 (30.03%). In the $TTO^{2,}$ the γ-terpinene was the most prevalent (21.74%), than in the TTO^1 (21.46%) > TTO^4 (17.87%), while the lowest was in TTO^3 (16.70%). The third most representative component is α-terpinene, highest in TTO^4 (12.59%), in comparison to TTO^1 (9.89%) > TTO^2 (9.11), while the TTO^3 has the lowest content (6.33%). The amount of other compounds, terpinolene, p-cymen and 1,8-cineole, made up less than 5% of the whole amount of the oil, except the neo-*allo*-O-cimene in TTO^1 which amounted 9.51%.

The results of the chemical analysis of the TTO samples that we obtained are similar to the findings of previous investigations (Brophy et al., 1989). All four commercial TTOs samples are in compliance with the ISO 4730 international standard (ISO 4730, 2004) which requires that the terpinen-4-ol content should be greater than 30%, while 1,8-cineole can be less than 15%. This is explained by the fact that the terpinen-4-ol is an active antimicrobial constituent and because of the 1,8-cineole's potential as a skin irritant (Hammer et al., 1996).

Antimicrobial activity

The results of the antibacterial activity of four TTOs samples are presented in Table 2. All commercial samples of TTOs show similar antibacterial activity with exception of the TTO^4 which showed slightly better antibacterial activity than the others. The MIC values ranged from 2 to 9 mg/ml, and MBCs ranged from 4.5 to 36.5 mg/ml. The most sensitive bacteria was *Enterobacter cloacae* with MIC of 2 to 4.5 mg/ml and MBC of 4.5 to 9 mg/ml, while the most resistant was *L. monocytogenes* with MIC range from 9 to 18 mg/ml and MBC values of 35 to 36.5 mg/ml. All commercial TTOs samples showed lower antibacterial activity in comparison to the positive control of

Table 1. Chemical composition of four commercial samples of *M. alternifolia* essential *Retention index.

No	Components	RI*	(%)			
			TTO[1]	TTO[2]	TTO[3]	TTO[4]
1	α-thujene	926	0.95	0.76	1.00	0.83
2	α-pinene	931	2.63	2.61	2.75	3.05
3	sabinene	973	0.18	-	0.18	0.41
4	β-pinene	974	0.78	0.69	0.82	0.74
5	myrcene	993	0.93	0.87	0.66	2.15
6	α-phellandrene	1004	0.48	0.37	0.30	0.54
7	α-terpinene	1017	9.89	9.11	6.33	12.59
8	*p*-cymene	1024	3.06	3.52	4.88	2.83
9	β-phellandrene	1028	1.66	1.93	1.10	3.76
10	1,8-cineole	1031	3.99	3.48	3.08	2.57
11	γ-terpinene	1060	21.46	21.74	16.70	17.87
12	terpinolene	1088	3.64	3.50	2.93	4.94
13	allo-Ocimene	1124	0.17	0.13	0.39	0.19
14	neo-*allo*-O-cimene	1143	9.51	1.22	2.31	0.99
15	terpinen-4-ol	1183	30.03	38.58	44.17	34.57
16	α-terpineol	1192	2.80	2.75	3.59	3.32
17	γ-terpineol	1195	0.11	0.04	0.57	0.55
18	α-gurjunene	1408	0.34	0.33	0.32	0.19
19	β-caryophyllene	1417	0.37	0.42	0.36	0.49
20	β-gurjunene	1426	0.06	0.07	0.07	0.10
21	β-copaene	1430	1.37	1.55	1.54	1.54
22	aromadendrene	1442	0.11	0.08	0.18	0.06
23	*cis*-muurola-3,5-diene	1449	0.11	0.12	0.11	0.05
24	allo-aromadendrene	1459	0.49	0.55	0.55	0.25
25	dauca-5,8-diene	1473	0.37	0.36	0.26	0.32
25	viridiflorene	1494	2.04	1.70	1.85	1.11
27	δ-cadinene	1523	1.34	1.49	1.41	1.07
28	*trans*-cadina-1,4-diene	1532	0.16	0.18	0.16	0.22
29	globulol	1586	0.24	0.49	0.32	0.26
30	viridiflorol	1593	0.19	0.56	0.24	1.00
31	cubeban-11-ol	1596	0.21	0.42	0.25	0.31
32	1-epi-cubenol	1630	0.13	0.20	0.40	0.14
	Total		99.81	99.84	99.80	99.81

Streptomycin (MIC range of 0.006 to 0.1 mg/ml and MBC values of 0.03 to 0.2 mg/ml) against all bacteria species. Some previous reports have pointed to the resistance of *P. aeroginosa* to TTO (Hammer et al., 1996). In our study, the *L. monocytogenes* was the most resistant species.

Antifungal activity results are summarised in Table 3. Fungi appear to be more sensitive to the effect of all four TTOs tested. MIC and MFC values range from 0.5 to 4.5 mg/ml and 2 to 9 mg/ml, respectively. Generally, all TTOs samples showed similar results for antifungal activity. The most sensitive were *P. funiculosum* and *A. versicolor* and the most resistant were *A. niger and A. flavus*. All TTOs tested showed the same antifungal effect as the commercial drug Fluconazole (MIC and MFC 0.5 to 6 mg/ml). According to the literature data, *A. ochraceus* was tested for the first time for susceptibility to TTO. The MIC and MFC values for other fungi tested are in accordance with previous studies (Hammer et al., 2002, Hammer et al., 2003a).

The ATCC isolate of *C. albicans* and all clinical isolates of *C. albicans* were susceptible to TTO (Table 4). The MIC's range from 2 to 9 mg/ml and for MFC's from 4.5 to 18 mg/ml. The obtained testing results of the commercial TTO were similar, although the TTO[4] showed slightly better results in comparison to all four TTOs. The TTO showed lower anticandidal activity than Nystatin in all cases with the MIC and MFC values of 0.06 to 0.12 mg/ml and 0.12 to 0.25 mg/ml, respectively. It has been proved that the TTO alters the membrane properties and

Table 2. Antibacterial activity of commercial samples of *M. alternifolia* essential oils and streptomycin.

Bacteria	mg/ml								Streptomycin (mg/ml)	
	TTO[1]		TTO[2]		TTO[3]		TTO[4]			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	9 ± 2.59	18 ± 5.19	9 ± 2.59	18 ± 5.19	9 ± 0	18 ± 0	4.5 ± 0	9 ± 0	0.03 ± 0.01	0.06 ± 0.03
B. cereus	4.5 ± 1.44	9 ± 0	4.5 ± 1.44	9 ± 2.59	4.5 ± 1.44	9 ± 2.59	4.5 ± 0	4.5 ± 1.44	0.01 ± 0	0.03 ± 0.01
M. flavus	4.5 ± 1.44	18 ± 5.19	4.5 ± 1.44	9 ± 2.59	4.5 ± 1.44	18 ± 2.59	2 ± 0.50	9 ± 2.59	$6x10^{-3}$ ± 0	0.03 ± 0.01
L. monocytogenes	9 ± 2.59	35 ± 9.18	18 ± 0	35 ± 0	9 ± 2.59	35 ± 9.81	9 ± 0	36.5 ± 0	0.01 ± 0	0.03 ± 0.01
P. aeruginosa	4.5 ± 1.44	9 ± 2.59	9 ± 2.59	18 ± 5.19	9 ± 2.59	18 ± 5.19	4.5 ± 1.44	18 ± 5.19	0.05 ± 0.01	0.10 ± 0
E. cloacae	2 ± 0.50	9 ± 0	4.5 ± 0	9 ± 0	4.5 ± 0	4.5 ± 0	2 ± 0. 50	4.5 ± 1.44	0.05 ± 0.01	0.10 ± 0
E. coli	4.5 ± 1.44	9 ± 2.59	9 ± 2.59	18 ± 5.19	9 ± 2.59	18 ± 5.19	4.5 ± 1.44	18 ± 5.19	0.10 ± 0	0.20 ± 0.01
S. typhimurum	4.5 ± 0	9 ± 0	4.5 ± 1.44	9 ± 2.59	4.5 ± 0	9 ± 2.59	2 ± 0	4.5 ± 0	0.10 ± 0	0.20 ± 0.01

Values ± St. Dev of triplicate samples.

Table 3. Antifungal activity of commercial samples of *M. alternifolia* essential oils and fluconazole.

Fungi	mg/ml								Fluconazole (mg/ml)	
	TTO[1]		TTO[2]		TTO[3]		TTO[4]			
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. flavus	4.5 ± 1.44	9 ± 2.59	2 ± 0.50	4.5 ± 0	4.5 ± 1.44	9 ± 2.59	2 ± 0	4.5 ± 0	5 ± 2.88	6 ± 1.73
A. fumigatus	2 ± 0.50	4.5 ± 1.44	1 ± 0.28	2 ± 0.50	2 ± 0.50	4.5 ± 1.44	2 ± 0.50	4.5 ± 1.44	0.5 ± 0	2 ± 0.50
A. niger	4.5 ± 1.44	9 ± 2.59	2 ± 0.50	4.5 ± 1.44	4.5 ± 0	9 ± 0	2±0.50	4.5 ± 1.44	1 ± 0.28	2 ± 0.50
T. viride	2 ± 0.50	4.5 ± 0	2 ± 0	4.5 ± 0	4.5 ± 1.44	9 ± 2.59	2±0	4.5 ± 1.44	0.5 ± 0	1 ± 0
F. verticiloides	2 ± 0	4.5 ± 1.44	2 ± 0.50	4.5 ± 1.44	4.5 ± 1.44	9 ± 2.59	2±0.50	4.5 ± 1.44	2 ± 0.50	3 ± 0
P. funiculosum	0.5 ± 0	1 ± 0	2 ± 0.50	4.5 ± 0	2 ± 0	4.5 ± 1.44	1±0 .28	2 ± 0.50	1 ± 0.28	2 ± 0.50
A. versicolor	1 ± 0.28	2 ± 0.50	1 ± 0.28	2 ± 0.50	2 ± 0.50	4.5 ± 1.44	1±0.28	2 ± 0.50	2 ± 0	3 ± 0
P. ochrohloron	2 ± 0	4.5 ± 0	2 ± 0	4.5 ± 0	2 ± 0.50	4.5 ± 0	2±0.50	4.5 ± 0	5 ± 2.88	6 ± 1.73

Values ± St. Dev of triplicate samples.

permeability of *C. albicans* cells (Hammer et al., 2004). It is used in preparations for oral hygiene and vaginal candidosis, and can be found on the market of the USA and Europe (Hammer et al., 1998). There have been reports of *in vivo* studies of the TTO against *Candida*-related infection which are very efficient, and in positive correlation with *in vitro* results (Groppo et al., 2002).

Antioxidant activity

Antioxidant activity of different TTOs is shown in Table 5. All commercial samples of the TTOs showed very similar antioxidant capacity. The antioxidant activities of the TTOs samples were dose dependent. At the concentration of 3 µl/ml, saturation occurred, while 1 µl/ml concentration had from 27.74 to 34.45% of the DPPH free stable radical inhibition. Tested samples showed antioxidant activity in the following order:

Table 4. Anticandidal activity of commercial samples of *M. alternifolia* essential oils and nystatin.

Yeast	mg/ml								Nystatin (mg/ml)	
	TTO[1]		TTO[2]		TTO[3]		TTO[4]			
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans clin.	9 ± 2.59	18 ± 0	9 ± 0	18 ± 2.59	9 ± 0	18 ± 2.59	4.5 ± 0	9 ± 0	0.06 ± 0.03	0.12 ± 0.03
C. albicans clin.	9 ± 2.59	18 ± 0	9 ± 2.59	18 ± 0	9 ± 2.59	18 ± 2.59	9 ± 0	18 ± 2.59	0.12 ± 0	0.25 ± 0.01
C. albicans clin.	9 ± 0	18 ± 2.59	9 ± 0	18 ± 0	9 ± 2.59	18 ± 0	4.5 ± 1.44	9 ± 0	0.12 ± 0	0.25 ± 0
C. albicans clin.	9 ± 0	18 ± 0	9 ± 0	18 ± 0	4.5 ± 0	9 ± 0	2 ± 0.50	4. 5 ± 1.44	0.12 ± 0	0.25 ± 0.01
C. albicans clin.	9 ± 2.59	18 ± 2.59	9 ± 0	18 ± 2.59	9 ± 0	18 ± 2.59	4.5 ± 1.44	9 ± 2.59	0.12 ± 0	0.25 ± 0.01
C. albicans clin.	2 ± 0.50	4.5 ± 1.44	4.5 ± 1.44	9 ± 2.59	2 ± 0.50	4. 5 ± 0	4.5 ± 1.44	4.5 ± 1.44	0.12 ± 0	0.25 ± 0.01
C. albicans clin.	9 ± 2.59	18 ± 2.59	9 ± 0	18 ± 0	9 ± 2.59	18 ± 2.59	9 ± 0	18 ± 0	0.12 ± 0	0.25 ± 0
C. albicans ATCC 10231	9 ± 2.59	18 ± 0	9 ± 2.59	18 ± 0	4.5 ± 1.44	9 ± 2.59	4.5 ± 1.44	9 ± 2.59	0.12 ± 0	0.25 ± 0

Values ± St. Dev of triplicate samples.

Table 5. Percentage inhibition of DPPH by different concentrations of *M. alternifolia* essential oils (µg/ml).

Concentration of TTO	TTO[1] (%)	TTO[2] (%)	TTO[3] (%)	TTO[4] (%)
1	29.08	32.31	27.74	34.45
2	57.55	60.22	59.11	54.01
3	76.21	77.08	75.94	62.33
4	79.71	79.82	75.98	65.09

TTO[1]>TTO[3]>TTO[4]. Kim et al. (2004) found out that the main components responsible for the antioxidant activity of the TTO are α-terpinen, α-terpinolene and γ-terpinene, while the weakest component, terpinen-4-ol, and is found in proportionally greatest amount. This explained how the TTO[2] highest antioxidant activity contributs to its balanced chemical composition between terpinen-4-ol and terpenic compounds. It is also stated that synergistic and antagonistic properties of compounds play significant role in antioxidant activity of the TTO.

The significant increase in the prevalence of resistance and side effect of synthetic drugs used in food and medicinal industry has driven research aimed at using natural medicines to battle bacterial infections. The EOs in plants are the focus of the research, as they have been used since the ancient times, and today there are lots of available data proving their biological potential. Obviously, the TTOs have the potential to be used as antibacterial and antioxidative substances, and it is the question of our willingness and readiness if we are going to exploit them or not. Antioxidants are those that maintain the cellular structures and the DNA molecules; the nature uses them as defence against the damaging effects of free radicals. Antioxidants have been widely used to provide protection against reactive oxygen species and oxidative degradation of foods by free radicals.

Among all commercial samples tested, the TTO[4] showed slightly better antimicrobial activity than the others. The fact that TTO[4] does not have the highest terpinen-4-ol content, on the other hand, has the highest percentage of α-terpinene (12.59), suggests that a synergy of combined substances is responsible for its antimicrobial activity. Cox et al. (2000) showed that the interaction of terpinen-4-ol with terpenes can result in an antagonistic effect. It is indicated that terpinen-4-ol has the main role in antimicrobial

activity, while the monoterpens content in the TTOs has its role in it, as well (Hammer et al., 2003b). However, it is not clear whether terpinen-4-ol is the only active compound in the mixture, or whether other components, even in trace amounts, add synergically to the activity. Our TTOs samples were fully characterized by gas chromatography and mass spectrometry. It was shown that they contain another monoterpene compounds in addition to terpinen-4-ol and cineole, constituting >60% of the whole composition, and it can be safely anticipated that the antimicrobial activity of terpinen-4-ol is somehow modulated by their presence. The TTOs samples used in our investigation were predominantly composed of terpinen-4-ol, which is considered to be an active antimicrobial compound, whereas the percentage of 1,8-cineole, generally considered to affect the therapeutic performance of the oil mixture negatively, was found to be well below the established standard. However, the use of whole EOs does not allow the determination of the active principle, because of the complexity of their components. Furthermore, these natural components have the advantage of being volatile molecules that can penetrate inaccessible areas. This, however, is not the case with all antimicrobial drugs. This work suggests that EOs occur in nature as antimicrobial and antioxidant agents which could be promising drugs for treating and preventing various bacteria and fungi-related infections.

Our study confirmed the antimicrobial activity of TTO against a wide range of Gram-positive and Gram-negative bacteria and fungi, as well as good antioxidant potential. Although TTO is one of the best investigated EOs and is used in practice, it is evident from this study of chemical composition and interaction between them that EO is essential for the TTO biological potential. The interactions of compounds in the EOs of TTO suggest that synergistic mechanisms of action are taking place and should be further studied. In particular, the data from the present study illustrate the ways in which TTO inhibits and kills bacteria and fungi, the fact that may ultimately be useful in developing TTO therapies and in searching for new antimicrobial agents.

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