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OCIMUM BASILICUM AND *MENTHA PIPERITA* ESSENTIAL OILS INFLUENCE THE ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCUS AUREUS STRAINS

Alexandru Mihai Grumezescu[1*], Carmen Mariana Chifiriuc[2], Ioana Marinaş[2], Crina Saviuc[2], Dan Mihaiescu[1], Veronica Lazăr[2]

[1]*Faculty of Applied Chemistry and Materials Science, University Politehnica of Bucharest, Polizu Street 1-7, Romania*
[2]*Faculty of Biology, University of Bucharest, Aleea Portocalelor 1-3, Romania*

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Abstract

Essential oils extracted by microwave assisted hydrodistillation technique from Ocinum basilicum and Mentha piperita were characterized by GC-MS. An adapted diffusion method was used in order to assess the potentiator effect of the essential oils on the susceptibility of Staphylococcus aureus clinical and reference strains to some of the currently used antibiotics, i.e. clindamycin, ciprofloxacin, tetracycline, gentamicin, penicillin and erythromycin.. The Ocimum basilicum and Mentha piperita essential oils, exhibited a strong, strain specific influence on the antibiotic susceptibility of the tested strains.

Keywords

Ocimum basilicum, Mentha piperita, Staphylococcus aureus, antimicrobial activity

*Corresponding author e-mail address:**grumezescu@yahoo.com**

Introduction

Aromatic plants are used in folk medicine as antimicrobial agents and their essential oils have been known to have antibacterial and antifungal proprieties [1]. Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [23]. *Mentha piperita*, commonly called peppermint, is a well-known herbal remedy used for a variety of symptoms and diseases (recognized for its carminative, stimulant, antispasmodic, antiseptic, antibacterial and antifungal activities) [4]. Traditionally, basil (*O. basilicum*) has been extensively used in food as a flavouring agent, and in perfumery and medical industries [5]. Recently the potential uses of *O. basilicum* essential oil, particularly as antimicrobial and antioxidant agents have also been investigated [6].

Bacteria can develop antibiotic resistance by altering the expression or function of their own genes or by acquiring new genes. The rate of resistance development by mutation depends upon the number of events required for resistant clones to reach clinically relevant levels. The larger a bacterial population is, the higher the probability that antibiotic pressure will select spontaneous or preexisting mutants with reduced susceptibility. The exchange and acquisition of new genetic material, by transduction, transformation or conjugation, contributes to the rapid horizontal dissemination of resistance determinants [7]. The emergence of multidrug resistant bacterial strains has become a major challenge in the treatment of infectious diseases and a top public health problem. The antibiotic-resistance of *S. aureus* strains is a serious concern besideits pathogenicity. Strains of *S. aureus* have been observed to show resistance against multiple antimicrobials. Various genetic determinants such as MecA (methicillin), TetK/M (tetracyclines), MsrA/B (macrolides), AacA-D (aminoglycosides), ErmA/B/C (macrolides, lincosamides and streptogramin B), and LinA (lincosamides) have been reported in human *S. aureus* isolates[8,9]. The emergence of methicillin resistant *Staphylococcus aureus* (MRSA) as a major nosocomial pathogen lead to the endemic spread of resistant strains in many hospitals [10]. In the present paper, we established the chemical composition of essential oils from *Ocimum basilicum* and

Mentha piperita by GC–MS method, and evaluated the influence of the essential oils on the susceptibility fo *S. aureus* strains to different antibiotics. it is to be notedthe fact that is the first report on the influence of these essential oils on the antimicrobial effect of drugs on clinical strains.

Experiment Details

Extraction and characterization of the essential oils. *Ocimum basilicum* and *Mentha piperita* plant materials were purchased from a local supplier and subjected to essential oil extraction. A Neo Clevenger type apparatus according to European Pharmacopoeia 6 was used performing two microwave assisted extractions from 225g plant material [11]. Subsequently, DMSO was added to form a stock solution (1:1 with essential oil v/v) which was kept in a cool place before use. Chemical composition was settled by GC-MS analysis. Gas chromatographic analysis was performed by using an Agilent 6890 Series GC System. Detection was carried out with a 5973 mass-selective single quadrupole detector (Agilent technologies). Operation control and data process were carried out by Agilent Technologies ChemStation software (Santa Clara, CA, USA). The mass spectrometer was calibrated before use with perfluorotributylamine (PFTBA) as a calibration standard. The working conditions were: H_2-carrier gas, flow: 1,2 ml/min, temperature program 50/300^{o}C with a ramp rate of 5^{o}C/min; the temperature of the injector and of the detector was 250^{o}C, and a DB5-MS (30m; 0.25 mm id; 0.25 µm) column.

Microbial strains. Four *S. aureus* strains of clinical origin (two wound secretions and two blood cultures), as well as the *S. aureus* ATCC 25923 reference strain were tested. Isolates were identified by using an automatic Vitek II system [12].

The antibiotic potentiator effect of the essential oils. An adapted diffusion method was used in order to assess the potentiator effect of the essential oils and of ther analytical standards on the antibiotic susceptibility of the tested *S. aureus* strains to some of the currently used antibiotics, chosen according to CLSI reccomandations, i.e. clindamycin, ciprofloxacin, tetracycline, gentamicin, penicillin and erythromycin. Standardized antibiotic discs have been placed on the Mueller Hinton agar medium distributed in Petri dishes, previously seeded with a bacterial inoculum with a density adjusted by the aid of the 0.5 McFarland standard. Stock solutions of the *R. officinalis* essential oil and eucalyptol were spoted on the antibiotic discs. The plates were incubated 24h at 37°C, and the differences between inhibition zones diameters were quantified and compared with the growth inhibition zones obtained for the respective antibiotics.

Results and Discussions

There are many commercial basil varieties having different chemical properties. Basil has been clasiffied according to different geographical origins. They are the European chemotype, from Italy, France, Bulgaria, Egypt, and South Africa, having linalool and methyl chavicol as main components; Tropical chemotype, from India, Pakistan and Guatemala, being rich in methyl cinnamate; Reunion chemotype, from Thailand, Madagascar and Vietnam, being characterized by high concentrations of methyl chavicol [13, 14]. There is also a eugenol-rich chemotypefrom North Africa and Russia. The chemistry of peppermint oil is very complex and highly variable. The relative concentrations vary depending on climate, cultivar, and geographic location [15, 16, 17]. As a result of GC–MS analyses, the essential oil composition is listed in Table 1 for *Mentha piperita* and Table 2 for *Ocimum basilicum***.** *Mentha piperita* essential oil proved to be rich in β-pinene, limonene, menthone, isomenthol, menthol while *Ocimum basilicum* in estragole, O-methyleugenol, cis-geraniol and eucalyptol.

Table 1: *The main compounds and their percentage in* Mentha *piperita essential oil*

Peak	R.T.	%	compounds
1	4.882	3.57	α-Pinene
2	5.24	0.09	Camphene
3	6.046	4.277	β-Pinene
4	6.684	0.211	Ethylamylcarbinol
5	6.841	0.04	α -Phellandrene
6	7.21	0.161	α -Terpinen
7	7.613	5.823	Limonene
8	8.486	0.061	τ -Terpinen
9	9.36	0.14	m-Cymene
10	9.83	0.322	Geraniol
11	11.162	1.456	Isopulegol
12	11.476	21.009	Menthone
13	11.733	12.437	Isomenthol
14	12.248	34.825	Menthol
15	12.573	3.365	α -Terpineol
16	12.785	0.108	Carveol
17	13.827	0.896	Pulegone
19	15.427	3.461	Menthol, acetate
20	17.722	0.535	β -Bourbonene
21	17.946	0.225	β -Copaene
22	18.618	1.579	α -Caryophyllene
23	19.245	0.08	τ-Muurolene
24	21.285	0.228	δ -Cadinene

Table 2: *The main compounds and their percentage in Ocimum basilicum essential oil*

Peak	R.T.	%	compounds
1	5.867	2.077	Eucalyptol
2	8.839	6.254	cis-Geraniol
3	10.613	67.291	Estragole
4	14.968	1.439	Eugenol
5	15.618	1.898	β -Isosafrole
6	16.256	7.36	O-Methyleugenol
7	16.983	1.96	α -Farnesene
8	17.375	0.385	Guaiene
9	18.615	1.06	Caryophyllene
10	21.282	0.591	δ-Cadinene
11	21.876	2.43	cis- α -Bisabolene

All the isolates were susceptible to clindamycin, ciprofloxacin and tetracycline, except for the *S. aureus* 5W, a tetracycline resistant strain. The four tested strain proved to be resistant to penicillin, erytromycin and oxacillin, irrespective to their isolation source (table 3). The results presented in Table 3 are demonstrating that the tested essential oils are representing antibiotic potentators, with a notable effect on the oxacillin susceptibilityin the presence of *O. basilicum* stock solution supplementation.

Table 3: *The growth inhibition zone diameters obtained for the tested antibiotics in the presence of Menta piperita and Ocinum basilicum Staphylococcus aureus strains*

Staphylococcus aureus 2

	MP	OB	Control
Clindamycin	36	36	32
Ciprofloxacin	23	32	28
Tetracycline	17	23	22
Gentamicin	21	25	19
Penicillin	11	12	0
Erythromycin	23	28	22

Staphylococcus aureus 3

	MP	OB	Control
Clindamycin	36	40	38
Ciprofloxacin	27	27	25
Tetracycline	22	22	20
Gentamicin	22	22	19
Penicillin	12	0	0
Erythromycin	11	12	10

Staphylococcus aureus 4

	MP	OB	control
Clindamycin	40	36	34
Ciprofloxacin	22	26	24
Tetracycline	26	20	20
Gentamicin	23	23	20
Penicillin	18	16	15
Erythromycin	21	12	11

Staphylococcus aureus 5

Antibiotics	MP	OB	Control
Clindamycin	28	26	25
Ciprofloxacin	25	24	23
Tetracycline	28	8	0
Gentamicin	19	19	18
Penicillin	8	0	0
Erythromycin	0	0	0

MP= *M. piperita,* OB =*O. basilicum* essential oil;

However, the modulation of the inhibition zone diameter varied with the tested strain and antibiotic, demonstrating the necessity of the study extension on a statistically significant number of strains belonging to different species, with different antimicrobial

susceptibility profiles, in order to confirm the potentiating effect of these oils on the antimicrobial activity of the existent drugs and indepth research for the identitification of the molecular mechanisms of this synergic action.

Conclusions

The results of the present study are demonstrating that the *Ocimum basilicum* and *Mentha piperita* essential oils could potentiate the antimicrobial activity of the existent antibiotics, representing a possible solution to the perpetual need for effectiveantibiotics and to the lack of new structures with potent microbicidal activity.

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