

THE ANTIMICROBIAL ACTIVITY OF *THYMUS VULGARIS* AND *ORIGANUM SYRIACUM* ESSENTIAL OILS ON *STAPHYLOCOCCUS AUREUS*, *STREPTOCOCCUS PNEUMONIAE* AND *CANDIDA ALBICANS*

ZEINAB LAKIS¹, DENISA MIHELE¹, ISABELA NICORESCU², VIRGINIA VULTURESCU³, DENISA IOANA UDEANU^{1*}

¹University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, Clinical Laboratory and Food Safety Department, Bucharest

²Institute for Hygiene and Veterinary Public Health, Bucharest

³National Institute of Chemical- Pharmaceutical Research and Development – ICCF, Bucharest

* corresponding author: denisaudeanu@gmail.com

Abstract

The study evaluated the antimicrobial activity of *Thymus vulgaris* essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol) on standard and human wild type strains of *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans* prelevated from the oral cavity. Some possible adverse effects were determined by hematological and blood biochemical routine tests performed on experience animals after the oral treatment with essential oils. The study is part of the preliminary research which aims to obtain new pharmaceutical products with antiseptic activity on oral cavity.

Rezumat

Lucrarea a avut drept scop determinarea activității antimicrobiene a uleiurilor esențiale extrase din speciile de *Thymus vulgaris* (29,74% timol) și *Origanum syriacum* (66,64% carvacrol) asupra unor tulpini atât standard cât și sălbatice de *Staphylococcus aureus*, *Streptococcus pneumoniae* și *Candida albicans*. Un posibil efect secundar al acestor uleiuri volatile administrate în doză terapeutică corespunzătoare efectului antimicrobian a fost determinat în cadrul analizelor biochimice și hematologice la animalele de experiență. Studiul face parte din cercetările preliminare întreprinse cu scopul obținerii unor preparate farmaceutice cu efect antiseptic la nivelul cavității orale.

Keywords: antimicrobial activity, essential oil, *Thymus vulgaris*, *Origanum syriacum*

Introduction

The essential oils extracted from different species of *Lamiaceae* family are very popular traditional herbal medicine products. The genus *Origanum* and *Thymus* are characterized by a large morphological and chemical diversity depending on geographical distribution and on time of plant collection. The essential oils extracted from *Thymus* and *Origanum* species had a very diverse qualitative composition [6,7].

The chemical composition of *Thymus vulgaris* (Romania) and *Origanum syriacum* (Lebanon) essential oils obtained by us and performed by GC/MS (gas chromatography - mass spectrometry) indicated that *Origanum syriacum* essential oil has an increased concentration of carvacrol (66.64%) than *Thymus vulgaris* essential oil which is rich in thymol (29.74%) and p-cymen (30.26%). Thymol and carvacrol are the main phenolic compounds responsible for most of the therapeutic properties [2,9]. *Thymus vulgaris* and *Origanum syriacum* essential oils are known as therapy adjuvants in many diseases especially for the spasmolytic, expectorant, antimicrobial, antioxidant, eupeptic and choleric properties [2,3,6,7].

The study aims the determination of the antibacterial and antifungal activity of *Thymus vulgaris* essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol) on standard and wild type strains of *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans*.

Materials and Methods

a) The antimicrobial activity of *Thymus vulgaris* essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol) was determined using the disc diffusion method (CLSI) [1,8].

The antimicrobial activity of the essential oils was determined using both standard strains and wild type strains of *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans*. The following standard strains were used: *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 6305 and *Candida albicans* ATCC 10231. The wild type strains were isolated from human oral cavity.

The discs with known concentrations of antibiotics were used to evaluate the antimicrobial activity of essential oils on the specified strains. Discs of 6 mm diameter sterile paper were impregnated with 10 μ L essential oils [1,8]. The control antibiotics and their concentrations are presented in Table I.

Table I
The antibiotics concentration used as control

Nr. crt.	Control antibiotics	Abbreviation	The antibiotics concentration
1.	Tetracycline	TE	30 μ g
2.	Ciprofloxacin	CIP	5 μ g
3.	Nalidixic acid	NA	30 μ g
4.	Erythromycin	E	15 μ g
5.	Cephalothin	KF	30 μ g
6.	Gentamicin	CN	10 μ g

Disc-diffusion test

The antimicrobial effects of the essential oils were investigated by the disc diffusion method [1,9]. The bacterial strains were cultured overnight at 37°C in LB medium. The bacterial suspension obtained had a value of 0.5 on McFarland scale. The bacterial suspension was spread on the surface of the plates containing Mueller-Hinton agar. The antimicrobial discs and discs impregnated with *Thymus vulgaris* essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol) were deposited on the plates.

After 24h incubation of the plates at 37°C, the diameters of inhibition area were measured in mm for the tested organism compared to the antibiotic control discs [8].

b) The biochemical and hematological parameters determination

The essential oils used in the study were administrated as oil/water emulsion with a concentration of 10µL essential oil/mL. The emulsions were stabilised with 1% Tween80.

All animals used in the study were kept in standard laboratory conditions. They received water *ad libitum* and were not fed for 12h before the experiment. All experiments were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from February 2nd, 2002.

Three groups of 10 white male Wistar rats, weighing 160±10g were used for the experiment. The animals from group 1 and 2 were daily treated with essential oil emulsions in an oral dose of 5mL/Kg bw for 14 days. One group was used as control and daily treated with physiological solution in an oral dose of 5mL/Kg bw for 14 days. After 2 hours from the last administration, the animals were ethyl-ether anesthetised and then slaughtered. The blood was different processed depending on the hematological or biochemical assay [10].

The blood collected in tubes with EDTA as anticoagulant was used for the determination of the following hematological parameters: red blood cell count (RBC), hemoglobin, hematocrite, red blood cell indices (mean corpuscular volume – MCV, mean corpuscular hemoglobin concentration – MCHC, mean corpuscular hemoglobin – MCH), white blood cell count (WBC), differential white blood cell count (lymphocytes, monocytes, granulocytes), platelets count. The assay was performed using Abacus Junior Vet hematological multiparameter analyzer for the veterinary use [8]. After collecting the blood, the plasma was separated and used for the determination of the following biochemical parameters: glycaemia, serum lipids, cholesterolemia, serum triglycerides, uric acid, creatinine, total

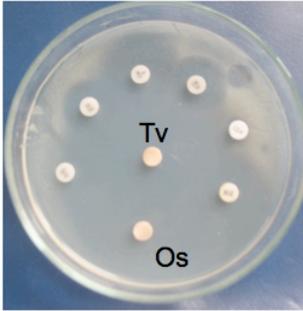


Figure 1
Staphylococcus aureus
ATCC

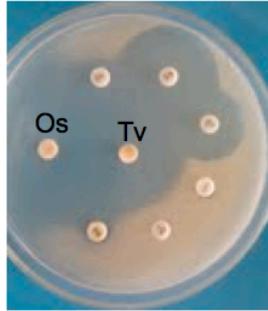


Figure 2
Staphylococcus aureus
wild type strain 1

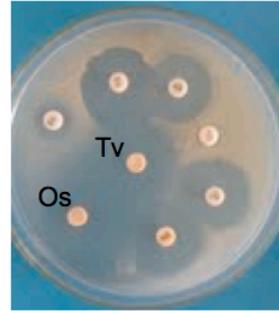


Figure 3
Staphylococcus aureus
wild type strain 2

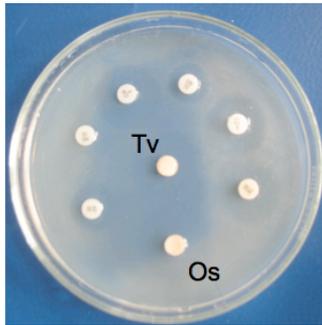


Figure 4
Streptococcus pneumoniae
ATCC

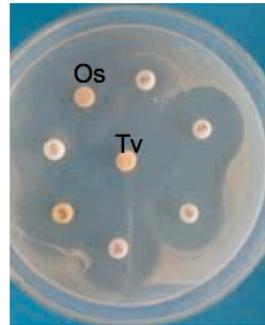


Figure 5
Streptococcus pneumoniae
wild type strain

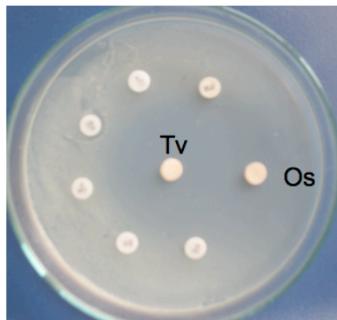


Figure 6
Candida albicans ATCC

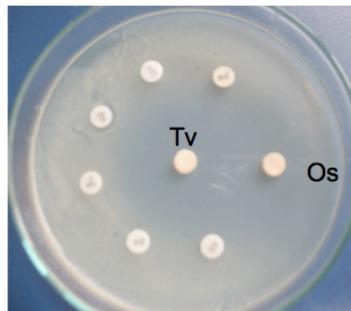


Figure 7
Candida albicans wild type
strain

Where Os = *Origanum syriacum* essential oil (66.64% carvacrol), Tv = *Thymus vulgaris* essential oil (29.74% thymol)

The variation of hematological and biochemical blood parameters on rats after the administration of essential oils.

The hematological and biochemical routine parameters were assayed in order to determine a possible secondary effect after the oral administration of *Thymus vulgaris* and *Origanum syriacum* essential oils. The oral dose used in the study corresponded to 0.03g carvacrol/Kg bw/day in the case of *Thymus vulgaris* essential oil and respectively 0.015g thymol/Kg bw/day in case of *Origanum syriacum* essential oil.

No significant variations of the hematological or biochemical parameters were noticed after the oral treatment with *Thymus vulgaris* essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol) compared to the control group treated with physiological solution (Table III and Table IV).

Table III

The hematological parameters variation on Wistar rats treated with *Thymus vulgaris* essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol)

Parameter Mean±SD	Control group	Group 1	Group 2	ANOVA
	Physiological solution	<i>Thymus vulgaris</i> essential oil	<i>Origanum syriacum</i> essential oil	
Red blood cell ($\times 10^6/\text{mm}^3$)	8.46±0.24	8.58±0.79	8.73±0.69	F=2.086 p>0.05
Hemoglobin (g/dL)	14.56±0.35	16.08 ±1.11	16.16±0.63	F=6.51 p=0.001
Hematocrit (%)	40.34±1.51	44.48±4.6	43.62±2.63	F=2.35 p>0.05
Mean corpuscular volume (fl)	46.5±3	51.4±2.07	49.8±1.3	F=5.074 p<0.01
Mean corpuscular hemoglobin (pg)	17.08±0.7	18.48±0.32	18.4±0.94	F=5.386 p<0.01
Mean corpuscular hemoglobin concentration (g/dL)	35.38±0.27	36.22±1.39	37.04±1.15	F=2.58 p>0.05
Red cell distribution width (%)	21.02±0.43	19.64±1.3	20.84±0.54	F=5.911 p<0.01
White blood cell ($\times 10^3/\text{mm}^3$)	14.18±1.648	14.06±2.358	15.58±3.304	F=0.794 p>0.05
Lymphocyte ($\times 10^3/\text{mm}^3$)	10.79±1.91	10.71±2.02	12.12±2.64	F=0.458 p>0.05
Granulocyte ($\times 10^3/\text{mm}^3$)	20.82±4.18	22.32±0.99	20.1±4.3	F=6.41 p=0.001

SD = Standard Deviation

Table IV

The variation of biochemical parameters on rats treated with essential oils extracted from *Thymus vulgaris* and *Origanum syriacum* (*Lamiaceae*)

Parameter Mean±SD	Control group	Group 1	Group 2	ANOVA
	Physiological solution	<i>Thymus vulgaris</i> essential oil	<i>Origanum syriacum</i> essential oil	
Glycaemia (mg/dL)	131.8±1.48	121.2±9.2	134.2±8.52	F = 33.03 p < 0.001
Aspartate amino- transferase(U/L)	135.4±28.34	166.32±13.49	168.68±14.25	F = 2.838 p = 0.05
Alanine amino- transferase (U/L)	37.2±2.58	42.2±3.11	40.2±4.86	F = 1.252 p < 0.05
Alkaline phosphatase (U/L)	144.2±6.26	141.8±21.18	156.8±15.77	F= 10,212 p < 0.001
Cholesterol (mg/dL)	64±2.91	53.4±4.82	59.4±4.03	F = 9.813 p < 0.001
Triglycerides (mg/dL)	88.2±8.61	126.4±17.21	92.8±26.78	F= 6.4 p = 0.001
Creatinine (mg/dL)	0.448±0.01	0.5±0.01	0.47±0.02	F = 3.462 p < 0.05
Albumin (g/L)	3.64±0.11	3.62±0.19	3.72±0.08	F = 2,559 p = 0.05
Uric acid (mg/dL)	1.55±0.06	2.046±0.15	1.824±0.17	F = 15,92 p < 0.001
Total proteins (g%)	6.738±0.13	7.112±0.24	7.116±0.15	F = 3,173 p < 0.05

SD = Standard Deviation

Thymus vulgaris essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol) demonstrated a strong antibacterial activity. The inhibitory activities were similar and in some cases higher than the control antibiotics in both standard and wild type strains used in the study. The main components responsible for the antimicrobial effects are thymol and carvacrol, both being present in high concentrations in the studied essential oils. The main mechanism of action

proposed for thymol and carvacrol was the membrane perforation of bacteria present in oral cavity [9,2].

Thymol and carvacrol are the main phenolic compounds which inhibit the formation and viability of hyphae and produce morphological alteration of the *Candida albicans* envelope. The high concentration of these compounds is favourable for the strong antifungal activity on standard and wild type strains of *Candida albicans* [9,4].

No significant variations of the hematological and biochemical routine parameters were noticed after the treatment with the studied essential oils on experience animals. The biochemical and hematological tests are part of the preliminary toxicological profile performed for the potential oral use of pharmaceutical products based on *Thymus vulgaris* essential oil (29.74% thymol) and *Origanum syriacum* essential oil (66.64% carvacrol).

Conclusions

Thymus vulgaris and *Origanum syriacum* essential oils demonstrated a significant antimicrobial effect on *Staphylococcus aureus* and *Streptococcus pneumoniae* on both standard and wild type strains isolated from human oral cavity. The antimicrobial activity was significantly increased in comparison with control antibiotics used in the study. A strong antifungal effect was noticed in the case of *Candida albicans* standard strains and wild type. It is important to notice that the antimicrobial and antifungal therapeutic dose demonstrated no variations of the routine hematological and biochemical parameters on experience animals treated with essential oils. Both *Thymus vulgaris* (Romania) and *Origanum syriacum* (Lebanon) essential oils obtained by us could be incorporated in different pharmaceutical formula for treating oral cavity infections.

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