

Assessment of *in vitro* antifungal activity of preparation “fin Candimis” against *Candida* strains

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The aim of the study was to assess the antifungal activity of preparation „fin Candimis” (oregano essential oil) against yeast-like strains belonging to the genus *Candida*.

During the investigation, there were used up nine *Candida albicans* strains and ten *C. glabrata* strains isolated from different clinical material, along with one *C. albicans* demonstration strain ATCC 90028. The oregano essential oil, utilized in the study, was obtained from fresh leaves of *Origanum vulgare* L. and bore a trade name „fin Candimis”. According to data yielded by its manufacturer, concentration of pure oregano essential oil in preparation „fin Candimis” totals up to 210 mg/ml. The susceptibility of the *Candida* strains to preparation „fin Candimis” was assessed by means of the disc-diffusion method, upon the *Sabouraud* solid medium (after a 24-hour incubation of the cultures at temperature of 37 degrees centigrade); the oregano essential oil had been diluted in 1 ml of DMSO, according to the geometrical progression. A measure of the antifungal activity of preparation „fin Candimis” was the minimal inhibitory concentration (MIC), in terms of the fungus growth.

Preparation „fin Candimis” is capable of being applied in the prevention and treatment of candidiasis – alone, or as a natural adjunctive agent. The *C. albicans* strains are more susceptible to preparation „fin Candimis” in comparison to the *C. glabrata* ones.

Key words: *Candida albicans*, *C. glabrata*, oregano essential oil, susceptibility

INTRODUCTION

According to epidemiological data spreading over the past two decades, there has been observed in the world a significant increase in the number of infections caused by fungi belonging to the genus *Candida*, referring particularly to patients admitted to intensive care units, undergoing immunosuppression or taking broad-spectrum antibiotics, and after having received a graft (Rex 1995; Eggimann, Gabrino and Pitter 2003; Krutkiewicz 2010).

The epidemiological situation impels scientists to search for new therapeutic agents – among the others, natural and vegetal ones – which bear the stamp of high effectiveness and low toxicity. These compounds could be useful in the field of antifungal prophylaxis and treatment. To this group there belong essential oils which present antifungal properties – inclusive of *Origanum vulgare* essential oil, popularly named *oregano*.

The name *oregano* has been derived from two Greek words: *oros* – „a mountain”, and *ganos* – „an adornment”; it stands for „the mountain’s joy”, and is connected with the area the plant overgrows in Central and Southern Europe. For many ages, *Origanum vulgare* has been known for its healing properties. By the first century A.D., Greek physician Pedanius Dioscorides, and, before him, Hippocrates (460–370 B.C.), recognized the Father of Contemporary Medicine, along with philosopher and erudite Aristotle (384–322 B.C.), had appreciated oregano as a powerful antidote to various sorts of maladies. The ancient medicals customarily used *O. vulgare* as an analgesic, carminative, disinfecting, and detoxifying drug. *O. vulgare* was widely used also in folk medicine; it was administered in combination with wine, e.g., to individuals who had been bitten by animals. In the Middle Ages, oregano was an ingredient of the infusion that was dispensed in order to protect from wizardries.

Nowadays, *O. vulgare* did not forfeit its remedial purport, none the less the plant is predominantly associated with a condiment which is widely used within the Mediterranean cuisine. In support of the oregano’s numerous beneficial properties, which had been observed in the past ages, there were adduced reliable results due to the plentiful scientific investigations.

The biological activity of the oregano essential oil (obtained from its fresh leaves) is closely connected with its phenol compound content (it gives a distinctive taste and flavour). *O. vulgare* comprises up to 3% of the essential oil which is fruitful in phenols such as carvacrol and thymol. The phenolic content comes up even to 60%. Oregano contains also sesquiterpenes, catechol, phenolic acids and flavonoids (Hołderna-Kędzia 2010).

The aim of the study was to assess the antifungal activity of preparation „fin Candimis” (oregano essential oil) against yeast-like strains belonging to the genus *Candida*, isolated from different clinical materials, and against a *Candida albicans* demonstration strain ATCC 90028.

MATERIAL AND METHODS

In the present study, there were subjected to investigation 20 strains of fungi belonging to the genus *Candida*: 10 *C. albicans* strains and 10 *C. glabrata* ones. The strains derived from patients who had been hospitalized in the Ludwik Rydygier Specialistic Hospital in Kraków in the following departments: intensive care unit, cardiologic dept., haematologic dept., orthopaedic dept., internal diseases dept., and rehabilitation unit. The investigated strains were isolated from aspirate of the upper airways (18 strains), from urine (one strain), and one strain constituted a reference pattern, i.e., *C. albicans* ATCC 90028.

The oregano essential oil, which was employed in the study, had been obtained from the *O. vulgare* fresh leaves, and bore a trade name „fin Candimis”. The oregano essential oil issued from the Fin Club Poland Co. Ltd., and was elaborated by the business concern named Hankintatukku Oy in Karkkila (Finland). According to the manufacturer’s data, concentration of the *O. vulgare* pure essential oil in preparation „fin Candimis” amounts to 210 mg/ml; the essential oil contains from 75% to 85% of carvacrol (chemical name: 5-isopropyl-2-methylphenol; molecular formula: C₁₀H₁₄O).

The investigated *Candida* strains’ susceptibility to preparation „fin Candimis” was assessed by means of the disc-diffusion method on an agar medium (agar gel). The isolated fungal strains were inoculated upon *Sabouraud* medium and exposed to a 24-hour incubation at temperature 37 degrees centigrade. From the cultures, there was prepared an *inoculum* in sterile physiological saline, given density 0,5 on the *McFarland* scale. The suspensions of the investigated *Candida* strains, prepared as above, in a volume of 100 µl, were inoculated upon *Sabouraud* medium, whereafter they were fixed in the medium discs made of blotting-paper, each one comprising 15 µl of preparation „fin Candimis” (oregano essential oil) in a required concentration (the oregano essential oil had been diluted in 1 ml of dimethyl sulfoxide – DMSO, according to the geometrical progression). The *Petri* dishes were incubated over a 24-hour period in a thermostat at temperature of 37 degrees centigrade, whereupon there were measured diameters of the growth inhibition zones around the discs, N = [mm], in case of each investigated fungal strain, assuming a mean value from two diameters being perpendicular to each other.

The minimal inhibitory concentration, MIC = [mg/ml], was obtained from a growth curve that had been drawn in a semi-logarithmic Cartesian coordinate system, i.e., apposing: on the abscissa – a common logarithm of concentration of preparation „fin Candimis”, and on the ordinate – a mean diameter of the growth inhibition zone, N = [mm], after the 24-hour incubation.

A measure of the antifungal activity of preparation „fin Candimis” was its minimal inhibitory concentration (MIC), in terms of the fungus growth, which was computed after the Kadłubowski’s (1971) modified formula:

$$\log \text{MIC} = \overline{\log^1 C} + \frac{\overline{\log^2 C} - \overline{\log^1 C}}{N_2 - N_1} (6 - \overline{N_1})$$

where:

$\overline{N_1}$, $\overline{N_2}$ – arithmetical means, obtained for two groups of diameters of the growth inhibition zones,

$\overline{\log^1 C}$, $\overline{\log^2 C}$ – arithmetical means for the common logarithm, computed for two groups of concentrations of the preparation.

In order to estimate statistically the diameters of the growth inhibition zones there were computed: mean values, standard errors of mean, standard deviations, confidence intervals, along with minimal and maximal values (Petrie, Sabin 2009).

RESULTS

The antifungal activity of preparation „fin Candimis” was assessed *in vitro* by means of the disc-diffusion method against 19 fungi strains belonging to the genus *Candida*, which had been isolated from aspirate of the upper airways (18 strains) and from urine (one strain). From amongst 19 *Candida* strains, nine strains belonged to the species *C. albicans*, and ten did to the species *C. glabrata*. Moreover, there was determined the antifungal activity of preparation „fin Candimis” against the *C. albicans* demonstration strain ATCC 90028.

Diameters of the growth inhibition zones, N = [mm], in case of the *C. albicans* clinical strains exposed to the activity of five concentrations of the oregano essential oil, according to the concentration adhibited, amounted to 44-55 mm at the outmost, given concentration 210 mg/ml (on the average $48.78 \pm SD = 4.12$ mm), and minimally to 8-10 mm, given concentration 13.125 mg/ml (on the average $9.11 \pm SD = 0.93$ mm). Diameters of the growth inhibition zones, N = [mm], for the *C. albicans* demonstration strain ATCC 90028 exposed to the activity concentrations of the oregano essential oil amounted to 47 mm and 10 mm, respectively (Tab. 1).

Diameters of the growth inhibition zones, N = [mm], in case of *Candida glabrata* strains exposed to the activity of five concentrations of the oregano essential oil, according to the concentration, amounted to 22-45 mm at the outmost (on the average $36.00 \pm SD = 7.72$ mm), given concentration 210 mg/ml, and minimally to 6-10 mm (on the average $7.60 \pm SD = 1.07$ mm), given concentration 13.125 mg/ml (Tab. 2).

Table 1
Growth inhibition zones N = [mm] for nine *Candida albicans* strains isolated from the patients and one *Candida albicans* demonstration strain under the influence of different concentrations of preparation “fin Candimis”

SN	Species	Strain number	Clinical material	Concentration of the oregano essential oil [mg/ml]				
				210	105	52.5	26.25	13.125
				Growth inhibition zone, N = [mm]				
1.	<i>C. albicans</i>	ATCC 90028	demonstration strain	47	30	28	14	10
2.	<i>C. albicans</i>	724/09	aspirate	45	30	23	12	8
3.	<i>C. albicans</i>	733/09	aspirate	45	30	28	11	10
4.	<i>C. albicans</i>	765/09	aspirate	45	33	22	10	8
5.	<i>C. albicans</i>	893/09	aspirate	50	32	27	15	10
6.	<i>C. albicans</i>	956/09	aspirate	53	35	30	17	9
7.	<i>C. albicans</i>	1164/09	aspirate	44	33	20	11	9
8.	<i>C. albicans</i>	340/10	aspirate	52	35	30	15	10
9.	<i>C. albicans</i>	369/10	aspirate	55	40	30	10	8
10.	<i>C. albicans</i>	21/11	urine	50	40	30	12	10

Table 2
Growth inhibition zones N = [mm] for ten *Candida glabrata* strains isolated from the patients under the influence of different concentrations of preparation “fin Candimis”

SN	Species	Strain number	Clinical material	Concentration of the oregano essential oil [mg/ml]				
				210	105	52.5	26.25	13.125
				Growth inhibition zone, N = [mm]				
1.	<i>C. glabrata</i>	372/09	aspirate	42	28	23	12	8
2.	<i>C. glabrata</i>	523/09	aspirate	35	25	20	8	6
3.	<i>C. glabrata</i>	627/09	aspirate	28	32	22	10	7
4.	<i>C. glabrata</i>	669/09	aspirate	45	33	22	10	8
5.	<i>C. glabrata</i>	762/09	aspirate	40	30	23	10	8
6.	<i>C. glabrata</i>	768/09	aspirate	22	30	22	12	8
7.	<i>C. glabrata</i>	981/09	aspirate	45	37	17	8	7
8.	<i>C. glabrata</i>	1041/09	aspirate	33	28	22	10	7
9.	<i>C. glabrata</i>	10/11	aspirate	40	30	23	15	10
10.	<i>C. glabrata</i>	393/10	aspirate	30	25	23	14	7

Table 3
Statistical computations for the growth inhibition zones N = [mm] for nine *Candida albicans* strains isolated from the patients under the influence of different concentrations of preparation “fin Candimis”

Concentration [mg/ml]	Mean	Standard deviation	Standard error	95% Confidence interval	Minimum	Maximum
S.N.						
1. 210.000	48.78	4.12	1.37	45.61–51.94	44	55
2. 105.000	34.22	3.73	1.24	31.35–37.09	30	40
3. 52.500	26.67	3.97	1.32	23.62–29.72	20	30
4. 26.250	12.56	2.51	0.84	10.63–14.48	10	17
5. 13.125	9.11	0.93	0.31	8.40–9.82	8	10

Table 4
Statistical computations for the growth inhibition zones N = [mm] for ten *Candida glabrata* strains isolated from the patients under the influence of different concentrations of preparation “fin Candimis”

Concentration [mg/ml]	Mean	Standard deviation	Standard error	95% Confidence interval	Minimum	Maximum
S.N.						
1. 210.000	36.00	7.72	2.44	30.48–41.52	22	45
2. 105.000	29.80	3.65	1.15	27.19–32.41	25	37
3. 52.500	21.70	1.89	0.60	20.35–23.05	17	23
4. 26.250	10.90	2.33	0.74	9.23–12.57	8	15
5. 13.125	7.60	1.07	0.34	6.83–8.37	6	10

The minimal inhibitory concentration (MIC) values for preparation „fin Candimis” in case of nine investigated *C. albicans* and ten *C. glabrata* clinical strains, isolated from the hospitalized patients, figured out at 13.38 mg/ml and 13.58 mg/ml, respectively.

The statistical computations for the growth inhibition zones N = [mm] in case of nine *C. albicans* and ten *C. glabrata* strains exposed to different concentrations of preparation „fin Candimis” were submitted to Tables 3 and 4. The mean diameters

of the growth inhibition zones for the *C. albicans* clinical strains are larger than the corresponding mean values in case of the *C. glabrata* strains, given all the five adhibited concentrations of the investigated oregano essential oil.

DISCUSSION

The susceptibility of microorganisms to essential oils is an outcome of the microorganisms' peculiarities, and the essential oils' properties. Within the antifungal activity of the essential oils, two factors play the leading role: lipophilous character of the hydrocarbon skeleton, and hydrophilous nature of the functional groups. The sequence of the essential oils' properties appears as follows:

phenols > aldehydes > ketones > alcohols > esters > ethers > hydrocarbons.

The phenols – thymol, carvacrol and eugenol – demonstrate the strongest antimicrobial activity. The explanation of this phenomenon is the alkaline character of the hydroxylic group that may set up hydrogen bonds with the enzyme's active site (Knobloch et al. 1989). Therefore the essential oils, which are mostly composed of phenols, exert themselves most powerfully upon the microorganisms, and possess the broadest spectrum in terms of their antimicrobial activity.

Various studies conducted in order to uncover the correlation between the composition of the essential oils and their specific activity has as yet come to naught (Pattnaik 1997). It is possible that not the essential oil particular contents, but their mutual proportions do determine the essential oil's activities (Kalemba 1999). Concerning the practical use of many essential oils, it is crucial to attain a fast effect, and to protract the essential oil's antimicrobial activity (Akgil, Kivanc 1989).

Inconsistent ways in which various scientific investigators tend to present the MIC values do trammel their likening. A similar hitch is the diversity regarding a range of concentrations of the essential oils, along with discrepant methods of measurement. When comparing outcomes of various studies, it occurs that one ought to standardize the units (Kalemba 1999). In the present paper, in order to preserve the integrity of results, the MIC values are expressed in the units which have been stated by the authors of cited articles.

Kursat and Erecevit (2008) proved the germicidal activity of oregano essential oil against bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus megaterium*, and fungi belonging to the genus *Epidermophyton*. Yet they did not demonstrate the antifungal activity against *Candida* and *Trichophyton* strains. During their research work, the authors made use of essential oil obtained from the plant named *Origanum vulgare* L. subsp. *gracile* (C. Koch). This essential oil, diluted in 98.1% solution of methyl alcohol, showed the germicidal activity against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus megaterium*.

The study conducted in Spain by López et al. (2007) showed that the oregano essential oil displayed its antifungal properties. It exerted an effect upon *Candida albicans*, *Apsergillus flavus* and *Penicillium islandicum* strains, along with Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*) and Gram-negative ones (*Escherichia coli*, *Pseudomonas aeruginosa*,

Yersinia enterocolitica i *Salmonella choleraesuis*). The MIC value in case of the *Candida albicans* strains amounted to 17.5 µl/l (López et al. 2007).

Consecutive studies, aiming at estimating the susceptibility of *Candida* strains, were carried out in the United States by Manohar et al. (2001). The researchers proved that the oregano essential oil inhibited the growth of the investigated *Candida albicans* strains. The MIC value, provided by the authors, figured out at 0.125 mg/ml. The discussed study was conducted by the use of one *Candida albicans* demonstration strain ATCC 48274, and strains that stemmed from Manohar et al.'s own culture. The authors administered the oregano essential oil to mice infected with candidiasis; the essential oil was prepared in controlled doses: the higher was the dose, the more potent was the antifungal activity of the oregano essential oil (Manohar et al. 2001).

CONCLUSIONS

1. Preparation „fin Candimis” (oregano essential oil) is capable of being applied in the prevention and treatment of candidiasis – alone, or as a natural adjunctive agent.
2. The investigated *Candida albicans* strains are more susceptible to preparation „fin Candimis” in comparison to the *Candida glabrata* ones.

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Ocena *in vitro* aktywności przeciwgrzybiczej preparatu „fin Candimis” wobec szczepów grzybów z rodzaju *Candida*

Streszczenie

Celem pracy była ocena aktywności przeciwgrzybiczej preparatu „fin Candimis” (olejku eterycznego oregano) wobec szczepów grzybów z rodzaju *Candida*.

Do badań wykorzystano 9 szczepów *Candida albicans* oraz 10 szczepów *Candida glabrata*, które wyizolowano z różnych materiałów klinicznych, i 1 szczep wzorcowy *Candida albicans* ATCC 90028 oraz preparat „fin Candimis”, zawierający olejek eteryczny otrzymany ze świeżych liści oregano. Według danych producenta, stężenie czystego olejku eterycznego oregano w produkcie „fin Candimis” wynosi 210 mg/ml. Wrażliwość szczepów z rodzaju *Candida* na preparat „fin Candimis” oznaczano metodą dyfuzyjno-krażkową na podłożu Sabouraud’a (po 24-godzinnej inkubacji hodowli w temperaturze 37°C); olejek eteryczny oregano rozcieńczano w 1 ml DMSO według postępu geometrycznego. Miarą przeciwgrzybiczego działania preparatu „fin Candimis” było najmniejsze stężenie hamujące wzrost grzyba (MIC).

Preparat „fin Candimis” (olejek eteryczny oregano) może mieć zastosowanie w profilaktyce i leczeniu kandydoz – samodzielnie lub jako naturalny środek wspomagający. Szczepy *Candida albicans* są bardziej wrażliwe niż szczepy *Candida glabrata* na preparat „fin Candimis”.