

## The Inhibitory Effect of Some Natural Essential Oils upon *Prototheca* Algae *in vitro* Growth

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**Abstract.** The aim of this paper was to present the *in vitro* evaluation of the inhibitory effect of some natural essential oils against unicellular algae from *Prototheca* genus.

Ten *P. zopfii* isolates from cows mastitic milk samples and one *P. wickerhamii* referent strain (RE-4608014ATCC16529), from American Type Collection, were submitted to antifungal susceptibility testing by classical diffusimetric method. The natural products tested were represented by fir (*Abies alba*), savory (*Satureja hortensis*), peppermint (*Mantha piperita*), tea tree (*Maleleuca alternifolia*), grape seed and oregano (*Oreganum compactum*) essential oils. Comparative the efficiency of antimicrobial drugs (Itraconazole) was tested.

For *Prototheca zopfii* tested isolates the highest efficiency were registered for: savory, mint and tea tree oils while *P. wickerhamii* proved to be sensitive for all natural products.

Difficulties in treating protothecosis in humans and animals with conventional drugs and the potent *in vitro* activity of essential oils demonstrated here raise the interest in further investigations on the therapeutic use of these non-conventional natural products.

**Key words:** *Prototheca*, essential oils, inhibitory effect, therapy

### INTRODUCTION

*Prototheca* genus was first described by Krüger in 1894, and designates a group of unicellular, anphotosynthetic algae, descendent from *Chlorella* genus. Over time genus include numerous species, but currently recognized these: *Prototheca zopfii*, *Prototheca wickerhamii*, *Prototheca stagnora*, *Prototheca ulmea* and *Prototheca blaschkeae*. Regarding taxonomic position *Prototheca* algae are included in *Eucariota* domain, kingdom *Viridiplantae*, phylum *Chlorophyta*, class *Trebouxiophyceae*, order *Chlorellales*, family *Chlorellaceae*, genus *Prototheca* (Roesler *et al.*, 2006). In addition, three distinct biotypes of *P. zopfii* have been defined on the basis of phenotypic characteristics, and recently by molecular characterization (Roesler *et al.*, 2003).

*Prototheca* are ubiquitous microorganisms, which can be isolated from plants, soil, sewage, mud, feces of wild and domestic animals and many water sources including lakes and domestic waste water (Hollingsworth, 2000; Huerre, 1993). It is mentioned that these microorganisms were isolated from fresh vegetables, cool, clams, from various dairy products (ice cream and butter) and beef, pork and poultry (Alboudi, 1990; Pore, 1983). A pathogenic potential has been indicated for *P. wickerhamii* and *P. zopfii*. Although the former is the predominant cause of human infections, the latter causes infections in animals, particularly in cows and dogs (Lass Florl *et al.*, 2007).

*P. zopfii* has been identified as inducing a therapy-resistant inflammation of the mammary gland in dairy cows leading to severe losses in an infected herd. These algae do not respond to routine mastitis therapy and the only control measure to date has been the elimination of the infected animals (Buzzini *et al.*, 2004; Marques *et al.*, 2006). The increased number of algae infections, especially in immunodepressed subjects, and of the development of resistance towards antifungal agents (Hazen, 1995), there is a considerable scientific and commercial interest in the discovery of novel classes of antimycotic compounds. Plants represent a good sources of a novel antimicrobial molecules (Buzzini *et al.*, 2003), due to its high content in active principles such as: poliphenols, catechins, isoflavones such as genistein and daidzein glycosides (Romani *et al.*, 2003).

Recently, the potential antimicrobial effects of essential plant oils have attracted serious attention within the scientific community. Several reports have documented the antimicrobial effects of essential oils extracted from various plant species, such as *Melaleuca alternifolia* (tea tree) and, more recently, from *Citrus bergamia* (bergamot) (Mondello *et al.*, 2003; Romano *et al.*, 2005).

The aim of this study was to investigate the *in vitro* susceptibility of *Prototheca* isolates to conventional antifungal agents and to essential oils.

## MATERIALS AND METHODS

Research carried out for testing the natural products, with possible application in protothecosis therapy were conducted during August-September 2010, within Microbiology Laboratory, Faculty of Veterinary Medicine Cluj-Napoca.

A total of 10 *P. zopfii* isolates collected from cows mastitic milk samples, and 1 *P. wickerhamii* referent strain (RE-4608014ATCC16529), from American Type Collection, were used for the study. The isolates were identified on the basis of the presence of daughter cells and on biochemical features (assimilation of glucose, galactose, glycerol, sucrose and trehalose, and growth at 28 and 37°C).

*In vitro* susceptibility testing was performed by classical diffusimetric method. Six essentials oils: fir (*Abies alba*), savory (*Satureja hortensis*), peppermint (*Mantha piperita*), tea tree (*Maleleuca alternifolia*), grape pips and oregano (*Oreganum compactum*) - acquired in trade as standardized products – and one antifungal drug (Itraconazole) (Janssen Research Foundation, Bersee, Belgium) – were investigated.

Algae were inoculated into glucose-agar and after 48 hours growth, colonies were directly suspended in saline solution so that turbidity matched the turbidity corresponding of 2 tube McFarland standard, which is  $600 \times 10^6$  CFU/ml. Kirby Bauer diffusimetric method was performed by using sterile glucose agar plates with seven wells. In each well was distributed 50 µl for each products tested.

The plates were incubating in aerobiotic condition at 37° for 48 hours and the results were appreciated according the inhibition diameter area.

## RESULTS AND DISCUSSION

### *Kirby Bauer diffusimetric method*

Tests carried out in the direction of establishing the antimicrobials effect of plant oils extracts showed different aspects according from the plants from which extract was obtained and also according to the species of *Prototheca* tested.

Table 1 summarizes the *in vitro* susceptibility profile of *Prototheca* species to natural essential oils comparative with synthetic drug (Itraconazole).

Table 1

*In vitro* susceptibility profile of two *Prototheca* species to natural products tested

Isolates tested		Products tested						
		Fir	Savory	Mint	Tea tree	Grape seed	Oregano	Itraconazole
<i>P. zopfii</i>	1.	R	36	37	34	R	R	30
	4.	R	28	25	35	R	R	32
	5.	12	20	20	32	R	R	18
	8.	R	14	22	27	R	R	22
	9.	R	32	35	36	R	R	26
	16.	R	25	36	34	R	R	30
	18.	R	24	26	30	R	R	20
	19.	R	16	25	28	R	R	R
	20.	R	R	30	22	R	R	22
28.	R	15	24	22	R	R	R	
Average of inhibition area (mm in diameter)		<b>R</b>	<b>21</b>	<b>28</b>	<b>30</b>	<b>R</b>	<b>R</b>	<b>20</b>
<i>P. wickerhamii</i>		<b>20</b>	<b>24</b>	<b>30</b>	<b>32</b>	<b>20</b>	<b>18</b>	<b>28</b>

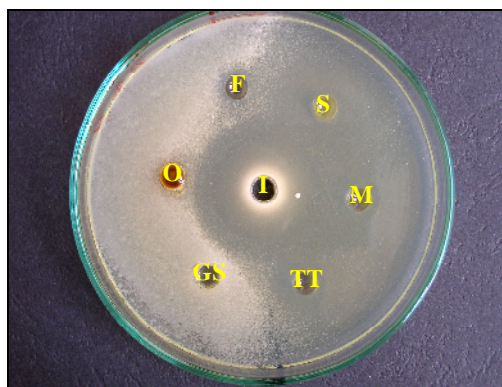


Fig. 1, *P. zopfii* (4 isolates) the efficiency of natural essential oils compared with Itraconazole. Sensitivity to savory (S), mint (M) and tea tree (TT) essential oils; resistance to grape seed (GS), oregano (O) and fir (F) essential oils

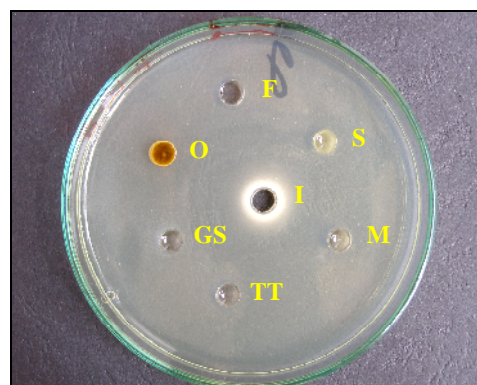


Fig. 2, *P. wickerhamii* – the efficiency of nonconventional antimicrobial products: fir (F), savory (S), mint (M), tea tree (TT), grape seed (GS) and oregano (O), comparing with synthetic drug (Itraconazole).

All 10 *P. zopfii* isolates showed a high sensitivity to tea tree, mint and savory essential oils. In which concern *P. wickerhamii* species all six essential oils tested proved to be effective (fig. 1, 2).

A graphic representation of the average of diameters of inhibition area for each plant extract tested compared to synthetic drug is depicted in the fig. no 3.

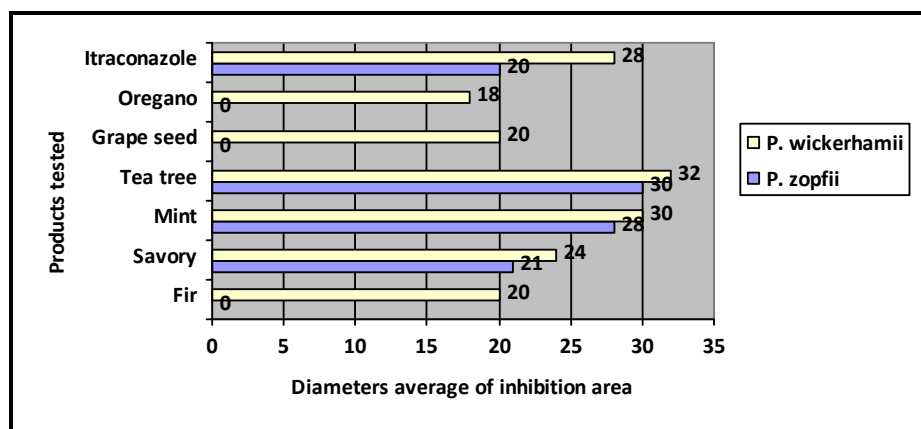


Fig. 3 Average efficiency of natural products tested compared with Itraconazole for both *Prototheca* species.

Our research performed in order to select a new vegetable antimycotic compound, with possible application in protothecosis therapy, allow us to assert that the results obtained are consisted with literature data. As an alternative to using conventional antifungals, a study of Italian researchers mention the *in vitro* efficiency of *Maleleuca alternifolia* (tea three) and *Citrus bergamota* (bergamot) essentials oils (9, 13). Although our date regarding the effectiveness of essential oil obtained from tea tree (*Maleleuca alternifolia*) are consistent with the literature, in wich concern the assesement of antifungal effect of peppermint (*Mentha piperita*), savory (*Satureja hortensis*), fir (*Abies alba*), oregano (*Oreganum compactum*) and essential oils obtained from grape pips, we can say that the study undertaken have global priority us there are no similar studies.

It may be concluded plants extract essential oils antimicrobials and antioxidant activities is due to a wide variety of compounds specially including poliphenols and flavones with different mechanisms of action.

## CONCLUSIONS

- *P. zopfii* tested isolates were shown to be susceptible to tea tree (*Maleleuca alternifolia*), peppermint (*Mentha piperita*), savory (*Satureja hortensis*), and resistant to fir (*Abies alba*), grape pips and oregano (*Oreganum compactum*) essential oils.
- *P. wickerhamii* referent strain proved to be suscentible to all non conventional antifungals tested.
- Both *Prototheca* tested species were shown to be resistant to synthetic antifungal drug (Itraconazole).
- The results obtained encourage further research towards determining the degree of correlation *in vitro* - *in vivo* and to determine the appropriate dose for the commencement of *in vivo* experiental studies.

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