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Original Article

Antifungal Properties of Some Essential Oils against *Zygosaccharomyces bailii*

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ABSTRACT

Antifungal activities of four essential oils including *Thymus vulgaris*, *Echinacea Angustifolia*, *Rosmarinus officinalis* and *Salvia officinalis* were investigated against *Zygosaccharomyces bailii* at three different concentrations (10, 50 or 100 ppm) using well diffusion method. *Z. bailii* was least susceptible to the essential oils. The diameter of zone inhibition ranged between 0 and 4.3 mm. *T. vulgaris* and *S. officinalis* oils appeared to be the most active, while *E. angustifolia* and *R. officinalis* oils exhibited most weak antifungal activity against *Z. bailii.* Results obtained indicate the possibility of the essential oils to fight these strains responsible for deterioration of some food.

Keywords: essential oils; antifungal activity; *Thymus vulgaris; Salvia officinalis*; *Zygosaccharomyces bailii*.

INTRODUCTION

Yeasts can have positive and negative effects on fermented products consumed by humans and animals. They are used as starter cultures in cheeses and bread, but they can also initiate spoilage in foods, such as yoghurt, fruit juice, salads, and mayonnaise (Lowes *et al*., 2000). Among food spoilage yeasts, those belonging to the genus *Zygosaccharomyces* are considered the most problematic to the food and beverage industries (Mira *et al*., 2014) and that have evolved the ability to grow under difficult environmental conditions (Merico *et al*., 2003). The genus *Zygosaccharomyces* belongs to the hemiascomycetous yeast phylum and includes six previously described species (*Z. bailii*, *Z. bisporus*, *Z. kombuchaensis*, *Z. lentus*, *Z. mellis* and *Z. rouxii*) and six recently proposed novel species (Galeote *et al*., 2013). *Z. bailli* species represent the most significant spoilage yeast within the genus, especially in acidic food products (Mira *et al*., 2014). *Z. bailii* still is a major challenging threat of spoilage in mayonnaise, salad dressings, sauces, pickled or brined vegetables, fruit concentrates and various non-carbonated fruit drinks as well as other acidified foods (Sá-Correia *et al*., 1999).

The widespread in food of *Z. bailii* as a spoilage yeast results from a number of physiological traits of the species, in particular, its remarkable resistance to weak acids used as food preservatives such as acetic, benzoic, propionic, sorbic acids and sulphur dioxide, being able to adapt to high sugar concentrations and high temperatures, vigorously ferment sugar. It is also able to tolerate high concentrations of ethanol and other sanitizers and to grow in a wide range of pH (2.0–7.0) and water activities (0.80–0.99) (Galeote *et al*., 2013; Mira *et al*., 2014; Stratford *et al*., 2013).

In this context, given the need to develop natural means of beverage and food preservation due to consumer demand, efforts developed for controlling this yeast are important and the use of killer toxins could be a profitable way to avoid the presence and activity of *Zygosaccharomyces*.

Apparently, essential oils have been considered as potential solution. There is a growing interest in using EOs by the food industry as natural preservatives against food spoilage and food-borne pathogenic microbes, in order to meet consumer demands for avoiding synthetic components in food (Bagamboula *et al*., 2004). They can be extracted from flowers, buds, seeds, leaves, bark, herbs, fruits, and roots of plants by expression, solvent extraction, steam or hydro distillation (Burt, 2004; Bakkali *et al*., 2008).

Some of the essential oils and their constituents are known to possess biological activity, remarkably anti yeast activity (Conner and Beuchat, 1984; Kamble and Patil, 2008; Sachetti *et al*., 2005; Elgayyar *et al*., 2001; Tserennadmid *et al*., 2011; Araújo *et al*., 2003).
The aim of this study was to evaluate the antifungal effects of four commercial essential oil from *Thymus vulgaris, Echinacea angustifolia*, *Rosmarinus officinalis* and *Salvia officinalis* against *Zygosaccharomyces bailii*.

MATERIALS AND METHODS

Essential Oils

The essential oil of *Rosmarinus officinalis, Thymus vulgaris, Echinacea angustifolia* and *Salvia officinalis* was of commercial origin and purchased from Farmalabor (Canosa di Puglia, Italy) as liquid extract. The essential oil samples were stored in dark amber bottles with teflon-sealed caps.

Yeast Strain

The antifungal activity of the four commercial essential oils was investigated against *Zygosaccharomyces bailii* DSM 70492 obtained from German Collection of Microorganisms and Cell Cultures (Deutsche SammLung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Germany).

Evaluation of Antifungal Activity

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of antifungal activities of the four commercial essential oils.
The overnight culture of the microorganisms cultures were inoculated on Nutrient agar plates using sterilized cotton swabs. After media were solidified, two holes were made by using a sterilized cork borer each hole was filled with 10, 50 or 100 ppm of plant extract. The control was cultured without essential oil. Plates were incubated at 25°C for 24 hours. The zones of inhibition were then recorded in millimeters.

RESULTS

Table 1 summarises the antifungal properties of the four essential oils (*T. vulgaris*, *E. angustifolia*, *R. officinalis* and *S. officinalis*). The yeast susceptibility to the essential oils, as determined by the well diffusion method, showed that oils with the weak inhibitory effects produced inhibition zones of 0–4.3 mm diameter.

Among the four essential oils, *T. vulgaris* and *S. officinalis* oils showed the highest activity, inhibiting the tested yeast with diameter of zone inhibition ranged between 1.5 to 4 mm for the first and 0-4.3 mm for the second. *E. angustifolia* and *R. officinalis* oils were weakest in activity with diameter of zone inhibition ranged between 2 to 3mm for the first and 2.25 to 2.5 mm for the Rosemary. The highest inhibition zone values (4.3 mm) observed against *Z. baillii* with 10 ppm *S. officinalis* oils while 100 ppm of the same oil did not show any antifungal activity.

Table 1: Antifungal activity as diameter of inhibition zone (mm) of the tested essential oils against *Z. baillii*

Plant Species	Concentration of added EO in ppm	Diameter of the zone of inhibition of Z. baillii in mm
Thymus vulgaris	10	4
	50	3
	100	1.5
Echinacea angustifolia	10	3
	50	1.5
	100	2
Rosmarinus officinalis	10	2.3
	50	2.25
	100	2.5
Salvia officinalis	10	4.3
	50	3
	100	NI

NI: No Inhibition

DISCUSSION

Plant oils as well as their extracts have been used for many significant purposes for many thousands of years (Ben Yeoshua and Mercier, 2005). It is necessary to investigate those plants scientifically which have been used traditionally in food preservation. Essential oils are a rich source of biologically active compounds and they are potential sources of novel antimicrobial compounds (Mitscher *et al*., 1987; Pereira *et al*., 2010) especially against pathogens. Results from in vitro studies in this work showed that the essential oils inhibited fungal growth but their effectiveness varied. The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak (Zaika, 1988).

In our study, *T. vulgaris, E. angustifolia, R. officinalis,* and *S. officinalis* oils exhibited least inhibitory activity against the selected yeast strain. A few publications have documented the antifungal activity of essential oils against *Z. bailii*, which the majority is very recent (Tyagi *et al*., 2014; Gkogka *et al*., 2013; Araújo *et al*., 2013; Wallis, 2013; Sagdic *et al*., 2011; Lis-Balchin *et al*., 1998).

Recently, Tyagi *et al*. (2014) studied the antimicrobial potential of eucalyptus essential oil *in vitro* against 8 different food spoilage yeasts *(Saccharomyces cerevisiae* SPA, *Zygosaccharomyces bailii* 45, *Aureobasidium pullulans* L6F, *Candida diversa* T6D, *Pichia fermentans* T2A1, *Pichia kluyveri* T1A, *Pichia anomala,* and *Hansenula polymorpha* CBS 4732*)* by disc diffusion, disc volatilization, and microdilution methods. In the first technique and after addition of three different concentrations of this oil (10, 20, and 30 µL), *Z. bailii* presented the intermediate inhibition zone (10, 14 and 22 mm). By disc volatilization method, the inhibition zones observed was between 10 mm and 38 mm. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) obtained varied from 2.25 mg/mL and 4.5 mg/mL respectively.

Gkogka *et al.*, (2013) tested *in vitro* essential oil of the resin of *Pistacia lentiscus* var. *Chia* (mastic oil) against a wide range of foodborne pathogenic and spoilage microorganisms (including *Z. bailii*) with diffusion and a dilution method. Both methods showed that *Z. bailii* was most susceptible to this essential oils with 31 mm of the diameter of the zone inhibition and 0.5 (% v/v) of Minimal Inhibitory Concentration (MIC).

Araújo *et al.,* (2013) in their study assessed the inhibitory effect of essential oils from *Melissa officinalis, Lavandula angustifolia, Salvia officinalis,* and *Mentha piperita* against five food spoilage yeasts: *Torulaspora delbrueckii, Zygosaccharomyces bailii, Pichia membranifaciens, Dekkera anomala,* and *Yarrowia lipolytica* by a disc diffusion technique and broth dilution method. *Melissa officinalis oils* are the most active against *Z. bailii* (close to 45 mm), while *Salvia officinalis* oil (leaves and flowers) exhibited a weak activity against *Z. bailii* with diameter zone of inhibition less than 5 mm, which (those of *S. officinalis*) are in agreement to those obtained in our study. The MIC of the tested oils are between 500 and 2.000 µg/ml.

Wallis (2013) in his thesis evaluated the antimicrobial activity of three commercial oils (cinnamon bark oil, clove oil, and thyme oil) against *Z. bailii*. On the basis of results of minimum inhibitory concentrations determination, cinnamon bark oil inhibited growth of the tested *Z. bailii* at 50 mg/l, clove oil at 200 mg/l, while thyme oil required 400 mg/l.

The pomaces and extracts from five commercial grape (*V. vinifera* L) cultivars

(Emir, Gamay, Kalecik karasi, Narince and Okuzgozu) grown in Turkey were assessed at different concentrations of 1%, 2%, 5% and 10% for their antifungal activity against *Zygosaccharomyces rouxii* and *Z. bailii* using agar-well diffusion assay by a group of Sagdic *et al*. (2011). This study has demonstrated that the pomaces and extracts of Gamay and Kalecik karasi could be more effective antifungal agents than those of Emir, Narince and Okuzgozu grape cultivars and revealed also that *Z. rouxii* was more sensitive to extracts than *Z. bailii*.

In other study, eight essential oils obtained by steam distillation from the scented leaves of *Pelargonium* species and cultivars were added at 250 and 500 ppm to a quiche filling, inoculated with either *Saccharomyces ludwigii* or *Zygosaccharomyces bailii* (at 10^8 cfu/g), *Salmonella enteriditis* or *Listeria innocua* (at 10^9 cfu/g). Commercial oils of cinnamon, clove, coriander, geranium and thyme were used as controls. At 250 ppm, 'Sweet Mimosa' oil had the greatest log cfu/g reduction against *Z. bailii*. At 500 ppm, thyme, 'Madam Nonin', 'Paton's Unique' and 'Sweet Mimosa' oils were very active against *Z. baillii* (Lis-Balchin *et al*., 1998).

The antimicrobial activity of essential oils depends on its chemical composition (Rai and Mares, 2003). In general, the inhibitory effect has been attributed to the most abundant components and not to the other associated substances of the oil (Farag *et al*., 1989). However, some workers found more antifungal activity when adding the whole extract to the medium than when adding only the principal compound, suggesting a synergistic effect of some minor constituents of the oil (Bullerman *et al*., 1977; Paster *et al*., 1995; Chang *et al*., 2001). Carriles *et al*., (2005) has demonstrated synergistic effects on fungi *Zygosaccharomyces bailii* inhibition when citral was used in combination with vanillin, thymol, carvacrol, or eugenol.

Many publications have documented the antifungal activity of thyme oil against different microbial species (Tullio *et al*., 2007; Mota *et al*., 2012; Segvić *et al*., 2007; Soković *et al*., 2008; Moghtader, 2012). The inhibition of the fungal growth is attributed to the presence of phenolic compounds, namely thymol and carvacrol (Tullio *et al*., 2007; Moghtader, 2012). Thymol is lipophilic, enabling it to interact with the cell membrane of fungus cells, altering cell membrane permeability by permitting the loss of macromolecules (Segvić *et al*., 2007). Carvacrol has biocidal properties, which lead to bacterial membrane perturbations. Moreover, it may cross cell membranes, reaching the interior of the cell and interacting with intracellular sites vital for antibacterial activities (Cristani *et al*., 2007; Moghtader, 2012).

Recently Chavan and Tupe (2014) demonstrated in the study to mechanism of action of carvacrol and thymol against grapes spoilage yeasts that Carvacrol and thymol exerted their antifungal action through membrane damage, leakage of cytoplasmic content and ergosterol depletion. Similarly to thyme oil, many scientists have demonstrated the antimicrobial activity *Rosmarinus officinalis* essential oils of against many pathogens (Angioni *et al*., 2004; Pintore *et al*., 2002; Genena *et al*., 2008; Özcan and Chalchat, 2008; Tavassoli *et al*., 2011; Matsuzaki *et al*., 2013; Tahri *et al*., 2015). The major constituent of rosemary are alpha-pinene (24.1%), 1,8-cineole (23.5%) and camphor (19.7%), within these compounds assumed to be a major attributor to the antimicrobial activities (Inouye *et al*., 2001). In agreement with our results, Angioni *et al*., (2004) and Felšöciová *et al*. (2015) found that the antimicrobial tests showed a weak activity of rosemary oils.

The antimicrobial properties of the essential oils have been well recognized for many years, and as naturally occurring antimicrobial agents, they have been applied to pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, and food preservation (Fu *et al*., 2013). Furthermore, the essential oils of *S. officinalis* have recently been investigated by a number of researchers worldwide (Bozin *et al*., 2007; Jirovetz *et al*., 2007; Damjanoviae-Vratnica *et al*., 2007; Khalil and Li , 2011; Miguel *et al*., 2011; Abu-Darwish *et al*., 2013; Mahmoudi and Ahmad, 2013; Sookto *et al*., 2013; Felšöciová *et al*., 2015), showing antifungal activity. Regarding the chemical analysis of this oil, the major compounds identified were α-thujone, 1,8- cineole and camphor are well known for their antimicrobial activity (Ben Hsouna *et al.*, 2014; Li *et al.*, 2014; Vilela *et al.*, 2009; Viljoen *et al.*, 2006). A study by Miguel *et al*. (2011) and Felšöciová *et al*., (2015) demonstrated lowest level of antifungal activity of the essential oil of *S. officinalis* in line with results of the current study. Contrary to the previous three plants, few studies have focused on the antimicrobial activities of *Echinacea angustifolia* essential oils (Binns *et al*., 2000; Sharma *et al*., 2008; Bírošová *et al*., 2012; Mir-Rashed *et al*., 2010; Dahui *et al*., 2011). The antimicrobial activity was related to echinacoside and caffeic acid derivative (Bergeron *et al*., 2000).

The observed weak antimicrobial activity of the tested essential oils founded in our study could be explained by the absence of the active compounds or they are presented in low amount.

CONCLUSION

In this study, the commercial essential oils from thyme, narrow-leaved purple coneflower, rosemary and sage showed weak and promising antifungal effects against food spoilage yeasts (*Z. baillii*). These investigated essential oils could be a candidates to be used as natural alternatives for further application in food preservation to retard or inhibit this food spoilage yeasts growth and for safety and to extend the shelf life of the food products. Further studies are needed to investigate the oils incorporation into appropriate food formulations, and evaluate organoleptic impact, chemical changes and antifungal effect in the whole food system.

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