Antifungal and anti-adhesion activity of plant extracts and essential oils against *Candida* spp. and *Pichia* spp.

ZORICA TOMIČIĆ – RUŽICA TOMIČIĆ – SONJA SMOLE MOŽINA – FRANZ BUCAR – IVANA TUREK – PETER RASPOR

Summary

The use of natural antifungal agents has gained much attention to extend shelf-life, increase the safety of food products in the food industry and inhibit disease-causing microorganisms. The objective of this study was to evaluate the antifungal and anti-adhesion potential of 15 plant extract, essential oils and chemical compounds against *Candida* spp. and *Pichia* spp. Susceptibility tests indicated that essential oils of *Cinnamomum verum* and *Origanum vulgare* had the highest inhibitory effect. For most extracts and essential oils examined, *Pichia membranifaciens* ZIM 2417 was the most sensitive yeast, while *Candida glabrata* ZIM 2369 proved to be the least sensitive one. Anti-adhesion ability of the plant extracts and essential oils against *C. glabrata* ZIM 2369 was estimated by the standard crystal violet assay. The results showed that the essential oils of *C. verum, O.vulgare, Satureja montana* and *Thymus vulgaris* possessed promising activity against the initial phase of biofilm formation and the pre-formed 24 h biofilm of *C. glabrata*. On the other hand, *Sedum roseum* extract showed the strongest anti-adhesion effect. Results of this study support the use of plant extracts, essential oils and their biologically active components against *C. glabrata*.

Keywords

plant extract; essential oil; antifungal activity; anti-adhesion activity; yeast

The incidence of fungal infections, particularly those caused by *Candida* species (candidiasis) has increased significantly, causing high levels of morbidity and mortality. This fact is mainly due to the increase in antifungal resistance and due to limitation in the number of efficient antifungal drugs, which still have many side effects. *Candida albicans* is the most frequent pathogen responsible for *Candida* infections, followed by *Candida glabrata* [1, 2]. Recent evidence suggests that majority of infections caused by these pathogens is associated with growth of biofilm and its role in human infections is increasingly recognized due to development of resistant or phenotypic adaptation within the biofilm [3, 4]. Biofilm is an additional problem not only on host tissues but it is usually found in medical devices, hindering eradication of e. g. *Candida* infections [5, 6]. New, effective methods are needed to solve this problem [2].

The capacity of yeasts to adhere and to form biofilms has been studied fundamentally in species of medical importance. The biofilm formation by yeasts has gained an increasing importance in the food industry. Of particular importance is that some biofilm-forming species in food factory environments are human pathogens. These pathogens are able to develop biofilm structures on a variety of artificial substrates common in the food industry, leading to contamination of food products, reducing their shelf-life and causing significant economic losses, or resulting in human foodborne diseases [7, 8]. Among them, *Pichia*, *Candida*, *Saccharomyces* and *Rhodotorula* are the genera mainly involved in spoilage of foods and beverages

Zorica Tomičić, Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.
Ružica Tomičić, Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.
Sonja Smole Možina, Peter Raspor, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.
Franz Bucar, Ivana Turek, Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Beethovenstrasse 8, 8010 Graz, Austria.

Correspondence author:
Peter Raspor, tel: +386 41335113, e-mail: peter.raspor@guest.arnes.si; raspor2013@gmail.com

[9, 10]. Food preservation with natural antimicrobial products, such as plant extracts or essential oils, is being popularly used in food industry due to the growing consumer demand for natural food additives and due to concerns regarding synthetic chemical additives [11–13].

Plant extracts and essential oils have been widely used in the food industry as natural antimicrobial agents to control food-borne microorganisms, owing to their chemical diversity [14, 15]. The oils of clove, oregano, rosemary, sage and thyme were found to be most consistently effective against microorganisms [16–18]. While some of the oils and extracts used on the basis of their reputed antimicrobial properties have well documented to inhibit planktonic cell growth [19–21], limited knowledge is available regarding activities of plant extracts and essential oils against *Candida* biofilms. Therefore, the aim of this study was to examine the antifungal activity of 15 different plant extracts, essential oils and their components against *Candida* spp. and *Pichia* spp., as well as their effect on adhesion of *C. glabrata* ZIM 2369 at different times of exposure.

MATERIALS AND METHODS

Strains and growth conditions

Four *Candida* strains (*C. albicans* ATCC 10261, two *C. glabrata* strains and one *C. krusei* strain isolated from clinical samples) and two *Pichia* strains (*P. membranifaciens* ZIM 2417 isolated from white cheese of cows' milk and *Pichia pijperi* ZIM 1368 isolated from must of Refošk) were used to investigate the antifungal activity. The strains were provided from the Collection of Industrial Microorganisms (ZIM) at Biotechnical Faculty, University of Ljubljana (Ljubljana, Slovenia). Yeast cultures were preserved in yeast peptone dextrose (YPD) medium (Sigma-Aldrich, St. Louis, Missouri, USA) supplemented with 40% glycerol at –80 °C and revitalized from frozen stocks by cultivation on malt extract agar (MEA) plates (Merck, Darmstadt, Germany) for 24 h at 37 °C (*Candida* strains) or 27 °C (*Pichia* strains) before performing the assays.

Extracts and essential oils

Plant extracts tested in this study were from herbal parts of *Leontopodium nivale* subsp. *alpinum* (Cass.) Greuter (Asteraceae), roots of *Peucedanum ostruthium* (L.) W. D. J. Koch, and roots and rhizomes of *Sedum roseum* (L.) Scop. (syn. *Rhodiola rosea* L.). *L. nivale* subsp. *alpinum* was the cultivated material provided by Wolfgang Zemanek (Pöllau, Styria, Austria). The roots of *P. ostruthium* were purchased from Kottas (Vienna, Austria) whereas the material from *S. roseum* was collected by Dietmar Vogt (Klagenfurt, Austria), with permission of the Regional Government of Carinthia (decrees SP3-NS-2459/2014 and FE3-NS-1921/2014) at Nockberge (at approximately 1900 m a. s. l.). Extracts were prepared from the dried materials by accelerated solvent extraction using Dionex ASE 200 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with successive extraction by hexane, dichloromethane and 96% ethanol at 60 °C. For the microbiological assays, the 96% ethanolic extracts were used. The extraction yields were 1.9 % (*Leontopodium nivale*), 10.3 % (*Peucedanum ostruthium*) and 30.8 % (*Sedum roseum*), respectively. Essential oils of cinnamon (*Cinnamomum verum* J. Presl), clove (*Syzygium aromaticum* (L.) Merr. & L. M. Perry), juniper (*Juniperus communis* L.), oregano (*Origanum vulgare* L.), red thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), clary sage (*Salvia sclarea* L.) and winter savory (*Satureja montana* L.) were purchased from companies Zuccari (Trento, Italy) and Flora (Lorenzana, Italy), and were stored at the temperature of 4–6 °C in dark conditions immediately for a maximum of 5 days before testing. The main components α-pinene, carvacrol and thymol were purchased from Sigma-Aldrich. All tested plant extracts were prepared at the Institute of Pharmaceutical Sciences, (University of Graz, Graz, Austria).

Minimal inhibitory concentrations

The minimal inhibitory concentrations (*MIC*) of plant extracts, essential oils and compounds were determined using the broth microdilution method according to the M27-A3 protocol of the Clinical and Laboratory Standards Institute (CLSI) [22]. Briefly, the tested antifungal agents were first dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and incorporated into malt extract broth (MEB; Merck) to obtain a concentration of 3 000 $\mu g \cdot ml^{-1}$. Serial dilutions were then performed and extracts, essential oils and compounds were tested at concentrations that varied from 3 000 $\mu g \cdot ml^{-1}$ to 5.9 $\mu g \cdot ml^{-1}$. To each well, 100 μl of inoculum (3×10^3 cells per millilitre) was added. The final concentration of DMSO did not exceed 0.5 %. After inoculation of yeast strains, plates were incubated for 48 h at 35 °C for *Candida* strains and 27 °C for *Pichia* strains. Then, absorbance was measured at 650 nm using using a microplate reader Safire 2 (Tecan, Männedorf, Switzerland). The *MIC* values were determined as

the lowest concentration that inhibited the visible growth of the test microorganisms. Negative control, growth control and DMSO control were included. The experiments were performed with four independent replicates.

Adhesion assay

Adhesion assays were performed as previously described by TOMIČIĆ et al. [2] with a few modifications. Prior to testing, the selected *C. glabrata* ZIM 2369 strain was grown on MEA plates at 37 °C for 48 h. After the incubation, a loopful of actively growing cells was suspended in the MEB medium. The concentration of cells was determined and adjusted to 1×10^7 cells per millilitre by using the Bürker-Türk counting chamber (Brand, Wertheim, Germany), a microscope with a camera (Leica DFC290, Leica Microsystems, Wetzlar, Germany) and image processing software ImageJ (National Institutes of Health, Bethesda, Maryland, USA) as described by ZUPAN et al. [23]. The assay was initiated by the addition of 200 μl cell suspension, with or without the presence of plant extracts, essential oils and compounds into wells of a 96-well polystyrene microtiter plate (Nunc, Roskilde, Denmark), which were then incubated for 24 h at 37 °C. In the first part of the experiment, *C. glabrata* ZIM 2369 cells were exposed to various concentrations (3/4 × *MIC* and 1/4 × *MIC*; average *MIC* and 2 × average *MIC*) of extracts, essential oils and compounds during 24 h of adhesion. In the second part of experiment, following the biofilm formation after 24 h of incubation, the medium was aspirated and non-adherent cells were removed by washing three times with sterile phosphate buffered saline (PBS, Oxoid, Basingstoke, England). Adherent cells were then exposed to various concentrations (3/4 × *MIC* and 1/4 × *MIC*; average *MIC* and 2 × average *MIC*) of extracts, essential oils and compounds for 3 h. The experiments were performed with eight replicates.

The amount of yeast cells that adhered to the polystyrene plates was measured using the crystal violet (CV) staining method [2]. The amount of adhered cells, that is, the concentration of the released crystal violet, was determined by measuring absorbance at 584 nm using a microplate reader.

Statistical analysis

Descriptive statistical analyses were performed using Microsoft Excel software (Microsoft Office 2013, Microsoft, Redmond, Washington, USA). Results were expressed as mean ± standard deviation (*SD*) of eight replicates for adhesion assay. Analysis of variance (ANOVA) for comparison of means was carried out using the software package StatSoft Statistica, ver. 10 (IBM, Armonk, New York, USA). A *p*-value of 0.05 was used to consider statistical significance.

RESULTS AND DISCUSSION

The resurgence of interest in natural therapies and an increase in consumer demand for effective, safe, natural products means that it is important to investigate those plants that will be used in the food industry and medicine as potential sources of new antifungal agents. A survey of literature reveals that there are many plant extracts and essential oils which possess antifungal activity [14, 24, 25]. The species of yeasts used in this study were selected primarily on the basis of their pathogenicity and insufficient knowledge of their susceptibility to natural antifungal agents.

Antifungal activity

Fifteen plant extracts, essential oils and compounds were screened for their antifungal activity against *Candida* spp. and *Pichia* spp. as shown in Tab. 1. The results showed that the tested plant extracts and essential oils exhibited quite different degrees of antifungal activity against *Candida* spp. and *Pichia* spp. For the most plant extracts and essential oils examined, strain *P. membranifaciens* ZIM 2417 was the most sensitive yeast with *MIC* values from 93.75 $\mu g \cdot ml^{-1}$ to $> 3\,000$ $\mu g \cdot ml^{-1}$, while *C. glabrata* ZIM 2369 proved to be the least sensitive (*MIC* values of 187.5 $\mu g \cdot ml^{-1}$ to $> 3\,000$ $\mu g \cdot ml^{-1}$). The strongest antifungal activity was shown by *C. verum* essential oil. This was in agreement with RANGEL et al. [26] who stated that *C. verum* essential oil had powerful antifungal activity on both reference and clinical *Candida* strains. When the *MIC* is equal to or lower than 500 $\mu g \cdot ml^{-1}$, natural products are considered powerful inhibitors of microorganisms [27]. Consistently, our results showed that essential oils of *J. communis, O. vulgare, S. montana, S. aromaticum* and *T. vulgaris* possessed strong activity against all the tested strains. However, FU et al. [16] reported that the essential oils of clove and rosemary were effective to inhibit *C. albicans*, which is not consistent with the results obtained in our study where even the highest tested concentration of rosemary did not exhibit an antifungal effect. The antifungal effect of these oils and extracts correlated with occurrence of compounds such as carvacrol, thymol, cinnamic aldehyde, eugenol and α-pinene, which are known to disrupt fungal cell membranes [19, 24]. Previous studies corroborated the antifungal activity of the main compounds used in

Tab. 1. Minimum inhibitory concentrations of the plant extracts, essential oils and compounds against yeasts.

Plant extracts and essential oils	*MIC* [$\mu g \cdot ml^{-1}$]					
	Candida albicans ATCC 10261	*Candida glabrata* ZIM 2344	*Candida glabrata* ZIM 2369	*Candida krusei* ZIM 548	*Pichia membranifaciens* ZIM 2417	*Pichia pijperi* ZIM 1368
Cinnamomum verum	93.75	187.5	187.5	93.75	93.75	93.75
Juniperus communis	1 000	400	200	800	200	100
*Leontopodium nivale**	> 1200 +	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +
Origanum vulgare	375	375	375	375	187.5	187.5
*Peucedanum ostruthium**	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +
*Sedum roseum**	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +	> 1 200 +
Rosmarinus officinalis	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +
Salvia officinalis	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +
Salvia sclarea	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +	> 3 000 +
Satureja montana	375	750	375	750	187.5	375
Syzygium aromaticum	375	750	750	750	187.5	375
Thymus vulgaris	750	375	375	750	750	187.5
Compounds						
α-Pinene	750	375	375	750	187.5	375
Carvacrol	187.5	187.5	375	375	93.75	93.75
Thymol	100	100	100	200	100	50

MIC – minimum inhibitory concentration, * – plant extracts, (+) – no inhibition at the maximum concentration used.

the present study on *Candida* spp. and *Pichia* spp. planktonic cells [17, 28, 29].

Anti-adhesion activity

In addition to the ability of the plant extracts, essential oils and compounds to act on *Candida* spp. planktonic cells, an interesting anti-adhesion potential was detected relative to *C. glabrata* ZIM 2369 strain at various exposure times. The effect of plant extracts, essential oils and compounds at different concentrations ($1/4 \times MIC$ and $3/4 \times MIC$; average *MIC* and $2 \times$ average *MIC*) on the initial phase of biofilm formation of *C. glabrata* ZIM 2369 is shown in Tab. 2. The biofilm inhibition ability was estimated by the standard crystal violet assay and calculated by their percentage of biofilm inhibition. All tested plant extracts, essential oils and compounds had the ability to interfere with the initial adhesion (Tab. 2.). The results indicated that after 24 h of adhesion, the essential oils of *C. verum, O. vulgare, S. montana* and *T. vulgaris* significantly reduced the adhesion of *C. glabrata* ($p < 0.05$) with an inhibition range of 30–45 %. The best results concerning reduction of *C. glabrata* ZIM 2369 adhesion were observed after 24 h exposure to *S. roseum* extract at concentrations of average *MIC* and $2 \times$ average *MIC*, despite the fact that antifungal activity was not detected. These findings suggest that the potential of *S. roseum* extract to prevent initial phase of biofilm formation is superior to its ability to inhibit the planktonic growth. To our knowledge, we have shown for the first time the effect of this extract on adhesion of *C. glabrata*. The results also revealed a strong anti-adhesive effect of carvacrol at a concentration of $3/4 \times MIC$. Carvacrol is one of the main compounds of the essential oil of *O. vulgare* and is also present in the essential oils of *T. vulgaris* and *S. montana* [30], which showed equal and strong anti-adhesive effect on *C. glabrata* ZIM 2369 in our study. On the other hand, it is interesting to note that in spite of the strong antifungal effect of *J. communis* essential oil, adhesion of *C. glabrata* was significantly induced when compared to control ($p < 0.05$). A similar effect was observed with the *P. ostruthium* extract. The mechanisms by which biofilm cells have elevated antifungal tolerance are complex and likely multifactorial. These include i) expression of resistance genes induced by contact with a surface; ii) a decreased growth rate or nutrient limitations and iii) the presence of extracellular polymeric substances that impedes penetration of agents into the biofilm [31–33]. Additionally, biofilm formation of *C. glabrata* was resistant to the tested compounds despite of their antifungal activity according to CLSI test. This

Tab. 2. The effect of plant extracts, essential oils and compounds on the initial phase of biofilm formation of *Candida glabrata* ZIM 2369.

Plant extract and essential oils	Anti-adhesion activity [%]			
	1/4 × *MIC*	3/4 × *MIC*	average *MIC*	2 × average *MIC*
Cinnamomum verum	5.45 ± 2.07 ^{Aa}	40.17 ± 7.26 ^{Ba}	–	–
Juniperus communis	–33.90 ± 3.22 ^{Ab}	–35.78 ± 2.42 ^{Ad}	–	–
*Leontopodium nivale**	–	–	8.12 ± 2.52 ^{Aacg}	34.98 ± 2.90 ^{Bd}
Origanum vulgare	32.56 ± 2.91 ^{Ac}	43.17 ± 4.05 ^{Ba}	–	–
*Peucedanum ostruthium**	–	–	–15.00 ± 4.60 ^{Ad}	–14.86 ± 3.84 ^{Ac}
*Sedum roseum**	–	–	73.89 ± 10.06 ^{Af}	86.88 ± 13.10 ^{Bf}
Rosmarinus officinalis	–	–	–4.69 ± 3.23 ^{Aadg}	–12.38 ± 5.14 ^{Aac}
Salvia officinalis	–	–	–12.37 ± 5.86 ^{Ad}	6.62 ± 4.25 ^{Bag}
Salvia sclarea	–	–	26.52 ± 3.12 ^{Ah}	–51.53 ± 7.27 ^{Bb}
Satureja montana	38.85 ± 8.54 ^{Ac}	32.87 ± 6.73 ^{Ae}	–	–
Syzygium aromaticum	8.64 ± 4.32 ^{Aa}	9.00 ± 3.57 ^{Af}	–	–
Thymus vulgaris	27.36 ± 6.06 ^{Ac}	42.96 ± 8.22 ^{Ba}	–	–
Compounds				
α-Pinene	–34.30 ± 8.82 ^{Ab}	–65.81 ± 9.71 ^{Bb}	–	–
Carvacrol	–30.59 ± 10.11 ^{Ab}	50.68 ± 4.79 ^{Bg}	–	–
Thymol	–57.38 ± 7.44 ^{Ad}	–1.21 ± 5.64 ^{Bh}	–	–

Anti-adhesion activities are presented as mean ± standard deviation. Groups with different lowercase letters in superscript within a column are significantly different ($p < 0.05$), and groups with different uppercase letters in superscript within a row are significantly different ($p < 0.05$), with negative values included.
MIC – minimum inhibitory concentration, * – plant extracts, (–) – negative values indicate stimulant effects.

Tab. 3. The effect of plant extracts, essential oils and compounds on the preformed 24h biofilm of *Candida glabrata* ZIM 2369 with 3 h of exposure.

Plant extract and essential oils	Anti-adhesion activity [%]			
	1/4 × *MIC*	3/4 × *MIC*	average *MIC*	2 × average *MIC*
Cinnamomum verum	46.20 ± 5.05 ^{Aa}	42.70 ± 5.93 ^{Aae}	–	–
Juniperus communis	56.21 ± 7.12 ^{Ad}	56.23 ± 5.88 ^{Bc}	–	–
*Leontopodium nivale**	–	–	11.98 ± 4.04 ^{Ac}	7.95 ± 3.54 ^{Aa}
Origanum vulgare	33.94 ± 4.80 ^{Ae}	57.80 ± 6.94 ^{Bc}	–	–
*Peucedanum ostruthium**	–	–	16.45 ± 3.55 ^{Ac}	13.28 ± 4.45 ^{Aad}
*Sedum roseum**	–	–	–42.55 ± 8.12 ^{Ae}	23.91 ± 5.87 ^{Bcd}
Rosmarinus officinalis	–	–	53.61 ± 10.13 ^{Ab}	47.66 ± 8.33 ^{Ae}
Salvia officinalis	–	–	–18.47 ± 3.74 ^{Af}	–34.61 ± 5.64 ^{Af}
Salvia sclarea	–	–	–25.44 ± 5.26 ^{Af}	–4.55 ± 4.60 ^{Bg}
Satureja montana	44.85 ± 7.14 ^{Aae}	74.57 ± 10.49 ^{Bd}	–	–
Syzygium aromaticum	34.43 ± 5.76 ^{Ae}	47.02 ± 6.20 ^{Bce}	–	–
Thymus vulgaris	37.91 ± 6.00 ^{Ae}	72.18 ± 7.05 ^{Bd}	–	–
Compounds				
α-Pinene	32.87 ± 5.62 ^{Ae}	46.98 ± 4.66 ^{Be}	–	–
Carvacrol	11.83 ± 3.10 ^{Abf}	44.36 ± 4.81 ^{Be}	–	–
Thymol	17.37 ± 4.22 ^{Af}	39.55 ± 5.09 ^{Bbe}	–	–

Anti-adhesion activities are presented as mean ± standard deviation. Groups with different lowercase letters in superscript within a column are significantly different ($p < 0.05$), and groups with different uppercase letters in superscript within a row are significantly different ($p < 0.05$), with negative values included.
MIC – minimum inhibitory concentration, * – plant extracts, (–) – negative values indicate stimulant effects.

was in accordance with previous findings that the concentrations of antimicrobial agents required to reduce biofilms were up to 1 000-fold higher than the corresponding *MIC* values [2, 34, 35].

In the present study, for the four concentrations assessed (1/4 × *MIC*, 3/4 × *MIC*, average *MIC* and 2 × average *MIC*), the effect of the plant extract, essential oils and compounds on *C. glabrata* preformed biofilm was perceptible after 3 h of exposure (Tab. 3). Notably, the essential oils of *S. montana* and *T. vulgaris* induced almost equal and strong reduction of the preformed 24 h biofilm at concentrations of 1/4 × *MIC* and 3/4 × *MIC*. In addition, the percentage of adherent cells decreased from 10 % to 24 % after exposure to plant extracts of *L. nivale, P. ostruthium* and *S. roseum*. It was been claimed that monoterepenes contained in plant extracts and essential oils affect the biofilm formation of *Candida*, since these compounds interact with lipid components in the cellular structure, thereby increasing permeability of the membrane and cause electrolyte imbalance [31]. However, specific mechanisms involved in the antimicrobial action of monoterpenes remain poorly characterized. In contrast, essential oils of *S. officinalis* and *S. sclarea* were inactive against *C. glabrata* biofilm. Our findings are consistent with the recent work of Pušková et al. [36], who reported that the low antimicrobial efficancy of essential oils of *Salvia* species may be due to the relatively low phenolics content of these oils. Moreover, there was a significant anti-adhesion activity of the tested compounds in the range of 11.8 % to 47.0 % ($p < 0.05$). Carvacrol was assessed in this study for its ability to inhibit the initial phase of biofilm formation of *C. glabrata* and to reduce the previously formed 24 h biofilm by up to 50 % at a concentration of 3/4 × *MIC*.

CONCLUSION

Our findings demonstrated the potential antifungal and anti-adhesion activity of fifteen plant extracts, essential oils and compounds against *Candida* spp. and *Pichia* spp. Plant oils from cinnammon, clove, oregano, red thyme and savory showed significant inhibitory effects, while lower activity was shown by other plant materials. Understanding more about the antifungal performance and possible mechanism of the tested plant extracts and essential oils will be helpful for its application against *Candida* spp. and *Pichia* spp. in the food industry and medicine in the future.

Acknowledgements

Authors thank doc. dr. Neža Čadež, the curator of the Collection of Industrial Microorganisms (ZIM, Ljubljana, Slovenia), for providing us with the yeast strains.

Z. T. thank Ministry of Education, Science and Technological Development, Republic of Serbia (Agreement on realization and financing of scientific research work: 451-03-68/2022-14/200222) and FEMS Research Grant (FEMS-RG-2015-0094) for financial support during study stay at Biotechnical faculty in University of Ljubljana (Ljubljana, Slovenia).

I. T. acknowledges financial support for a research stay at the Biotechnical faculty (University of Ljubljana, Ljubljana, Slovenia) by bilateral project ARRS-BI-AT-JR-Prijava/2015/47.

The authors would like to thank Dietmar Vogt and Wolfgang Zemanek for providing plant materials.

REFERENCES

1. Rodrigues, C. F. – Silva, S. – Henriques, M.: *Candida glabrata*: a review of its features and resistance. European Journal of Clinical Microbiology and Infectious Diseases, *33*, 2014, pp. 673–688. DOI: https://doi.org/10.1007/s10096-013-2009-3.
2. Tomičić, Z. – Zupan, J. – Matos, T. – Raspor, P.: Probiotic yeast *Saccharomyces boulardii* (nom. nud.) modulates adhesive properties of *Candida glabrata*. Medical Mycology, *54*, 2016, pp. 835–845. DOI: 10.1093/mmy/myw026.
3. Nett, J. E. – Andes, D.: Fungal biofilms: *In vivo* models for discovery of anti-biofilm drugs. Microbiology Spectrum, *3*, 2015, E30. DOI: 10.1128/microbiolspec.MB-0008-2014.
4. Taff, H. T. – Mitchell, K. F. – Edward, J. A. – Andes, D. R.: Mechanisms of *Candida* biofilm drug resistance. Future Microbiology, *8*, 2013, pp. 1325–1337. DOI: 10.2217/fmb.13.101.
5. Cavalheiro, M. – Teixeira M. C.: *Candida* biofilms: Threats, challenges, and promising strategies. Frontiers in Medicine, *5*, 2018, article 28. DOI: 10.3389/fmed.2018.00028.
6. Ramage, G. – Rajendran, R. – Sherry, L. – Williams, C.: Fungal biofilm resistance. International Journal of Microbiology, *2012*, 2012, article ID 528521. DOI: 10.1155/2012/528521.
7. Galié, S. – García–Gutiérrez, C. – Miguélez, E. M. – Villar, C. J. – Lombó, F.: Biofilms in the food industry: health aspects and control methods. Frontiers in Microbiology, *9*, 2018, article 898. DOI: 10.3389/fmicb.2018.00898.
8. Tomičić, R. – Raspor, P.: Influence of growth conditions on adhesion of yeast *Candida* spp. and *Pichia* spp. to stainless steel surfaces. Food Microbiology, *65*, 2017, pp. 179–184. DOI: 10.1016/j.fm.2017.02.008.
9. Loureiro, V. – Malfeito-Ferreira, M.: Spoilage yeasts in the wine industry. International Journal of Food Microbiology, *86*, 2003, pp. 23–50. DOI: 10.1016/s0168-1605(03)00246-0.

10. Tomičić, R. – Tomičić, Z. – Raspor, P.: Adhesion of *Candida* spp. and *Pichia* spp. to wooden surfaces. Food Technology and Biotechnology, *55*, 2016, pp. 138–142. DOI: 10.17113/ftb.55.01.17.4514.
11. Bulut, S. – Popović, S. – Hromiš, N. – Šuput, D. – Lazić, V. – Kocic-Tanackov, S. – Dimić, G. – Kravić, S.: Antibacterial activity of biopolymer composite materials obtained from pumpkin oil cake and winter savory or basil essential oil against various pathogenic bacteria. Journal of Food and Nutrition Research, *59*, 2020, pp. 250–258. ISSN: 1336-8672. <https://www.vup.sk/download.php?bulID=2074>
12. Mutlu-Ingok, A. – Devecioglu, D. – Dikmetas, D. N. – Karbancioglu-Guler, F. – Capanoglu, E.: Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review. Molecules, *25*, 2020, article 4711. DOI: 10.3390/molecules25204711.
13. Rossi, C. – Chaves-López, C. – Smole Možina, S. – Di Mattia, C. – Scuota, S. – Luzzi, I. – Jenič, T. – Paparella, A. – Serio, A.: *Salmonella enterica* adhesion: Effect of *Cinnamomum zeylanicum* essential oil on lettuce. LWT – Food Science and technology, *111*, 2019, pp. 16–22. DOI: 10.1016/j.lwt.2019.05.026.
14. Gyawali, R. – Ibrahim, S. A.: Natural products as antimicrobial agents. Food Control, *46*, 2014, pp. 412–429. DOI: 10.1016/j.foodcont.2014.05.047.
15. Klančnik, A. – Šimunović, K. – Sterniša, M. – Ramić, D. – Smole Možina, S. – Bucar, F.: Anti-adhesion activity of phytochemicals to prevent *Campylobacter jejuni* biofilm formation on abiotic surfaces. Phytochemical Review, *20*, 2020, pp. 55–84. DOI: 10.1007/s11101-020-09669-6.
16. Fu, Y. – Zu, Y. – Chen, L. – Sh, X. – Wang, Z. – Sun, S. – Efferth, T.: Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytotherapy Research, *21*, 2007, pp. 989–994. DOI: 10.1002/ptr.2179.
17. Nazzaro, F. – Fratianni, F. – Coppola, R. – Feo, V. D.: Essential oils and antifungal activity. Pharmaceuticals, *10*, 2017, article 86. DOI: 10.3390/ph10040086.
18. Solgi, M. – Ghorbanpour, M.: Application of essential oils and their biological effects on extending the shelf-life and quality of horticultural crops. Trakia Journal of Sciences, *2*, 2014, pp. 198–210. ISSN: 1313-7050. <http://www.uni-sz.bg/tsj/Vol.%2012,%20N2,%202014/M.Solgi.pdf>
19. Holley, R. A. – Patel, D.: Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology, *22*, 2005, pp. 273–292. DOI: 10.1016/j.fm.2004.08.006.
20. Ibrahim, S. Y.: Essential oils antagonism against three hygiene significant yeasts and juice spoilage by *Saccharomyces cerevisiae*. Journal of Human Health Research, *1*, 2017, pp. 1–10. DOI: 10.14302/issn.2576-9383.jhhr-17-1768.
21. Potente, G. – Bonvicini, F. – Gentilomi, G. A. – Antognoni, F.: Anti-*Candida* activity of essential oils from *Lamiaceae* plants from the Mediterranean area and the Middle East. Antibiotics (Basel), *9*, 2020, article 395. DOI: 10.3390/antibiotics9070395.
22. M27-A3. Reference method for broth dilution antifungal susceptibility testing of yeasts. 3rd ed. Wayne : Clinical and Laboratory Standards Institute, 2008.
23. Zupan, J. – Avbelj, M. – Butinar, B. – Kosel, J. – Šergan, M. – Raspor, P.: Monitoring of quorum-sensing molecules during minifermentation studies in wine yeast. Journal of Agricultural and Food Chemistry, *61*, 2013, pp. 2496–2505. DOI: 10.1021/jf3051363.
24. Swamy, M. K. – Akhtar, M. S. – Sinniah, U. R.: Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Evidence-Based Complementary and Alternative Medicine, *2016*, 2016, article ID 3012462. DOI: 10.1155/2016/3012462.
25. Tomičić, R. – Tomičić, Z. – Dodić, S – Raspor, P.: Influence of various factors on adhesion of yeast *Candida* spp. and *Pichia* spp. to abiotic surfaces. Acta Microbiolica Bulgarica, *35*, 2019, pp. 19–28. ISSN: 0204-8809. <https://actamicrobio.bg/archive/march-2019/amb-march-2019-article-3.pdf>
26. Rangel, M. L. – de Aquino, S. G. – de Lima, J. M. – Castellano, L. R. – de Castro, R. D.: *In vitro* effect of *Cinnamomum zeylanicum* blume essential oil on *Candida* spp. involved in oral infections. Evidence-Based Complementary and Alternative Medicine, *2018*, 2018, article ID 4045013. DOI: 10.1155/2018/4045013.
27. Duarte, M. C. – Leme, E. E. – Delarmelina, C. – Soares, A. A. – Figueira, G. M. – Sartoratto, A.: Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. Journal of Ethnopharmacology, *111*, 2007, pp. 197–201. DOI: 10.1016/j.jep.2006.11.034.
28. Marchese, A. – Arciola, C. R. – Barbieri, R. – Silva, A. S. – Nabavi, S. F. – Tsetegho Sokeng, A. J. – Izadi, M. – Jafari, N. J. – Suntar, I. – Daglia, M. – Nabavi, S. M.: Update on monoterpenes as antimicrobial agents: A particular focus on *p*-cymene. Materials (Basel), *10*, 2017, article 947. DOI: 10.3390/ma10080947.
29. Silva, A. C. R. – Lopes, P. M. – Azevedo, M. M. B. – Costa, D. C. M. – Alviano, C. S. – Alviano, D. S.: Biological activities of α-pinene and β-pinene enantiomers. Molecules, *17*, 2012, pp. 6305–6316. DOI: 10.3390/molecules17066305.
30. Trevisan, D. A. C. – da Silva, A. F. – Negri, M. – de Abreu Filho, B. A. – Machinski Junior, M. – Patussi, E. V. – Zanetti Campanerut-Sá, P. A. – Graton Mikcha, J. M.: Antibacterial and antibiofilm activity of carvacrol against *Salmonella enterica* serotype Typhimurium. Brazilian Journal of Pharmaceutical Sciences, *54*, 2018, e17229. DOI: 10.1590/s2175-97902018000117229.
31. Braga, P. C. – Culici, M. – Alfieri, M. – Dal Sasso, M.: Thymol inhibits *Candida albicans* biofilm formation and mature biofilm. International Journal of Antimicrobial Agents, *31*, 2008, pp. 472–477. DOI: 10.1016/j.ijantimicag.2007.12.013.
32. Douglas, L. J.: *Candida* biofilms and their role in infection. Trends in Microbiology, *11*, 2003, pp. 30–36. DOI: 10.1016/s0966-842x(02)00002-1.

33. Serra, E. – Hidalgo-Bastida, L. A. – Verran, J. – Williams, D. – Malić, S.: Antifungal activity of commercial essential oils and biocides against *Candida albicans*. Pathogens, *7*, 2018, article 15. DOI: 10.3390/pathogens7010015.
34. Alves, M. – Gonçalves, M. J. – Zuzarte, M. – Alves-Silva, J. M. – Cavaleiro, C. – Cruz, M. T. – Salgueiro, L.: Unveiling the antifungal potential of two Iberian thyme essential oils: effect on *C. albicans* germ tube and preformed biofilms. Frontiers in Pharmacology, *10*, 2019, article 446. DOI: 10.3389/fphar.2019.00446.
35. Olsen, I.: Biofilm-specific antibiotic tolerance and resistance. Europian Journal of Clinical Microbiology and Infection Disease, *34*, 2015, pp. 877–886. DOI: 10.1007/s10096-015-2323-z.
36. Puškárová, A. – Bučková, M. – Kraková, L. – Pangallo, D. – Kozics, K.: The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. Scientific Reports, *7*, 2017, article 8211. DOI: 10.1038/s41598-017-08673-9.

Received 23 September 2021; 1st revised 15 December 2021; accepted 20 December 2021; published online 18 February 2022.