Romanian Biotechnological Letters *Vol. 23, No. 6, 2018*

ORIGINAL PAPER

Antifungal activity of some medicinal plant extracts against *Candida albicans* nosocomial isolates

DOI: 10.26327/RBL2018.190

Received for publication, April, 22, 2018
Accepted, July, 23, 2018

HAIFAA NAJEE[1,2], CRINA KAMERZAN[2], LUMINITA MARUTESCU[1,2], IRINA GHEORGHE[1,2], MARCELA POPA[1,2], GRAȚIELA GRĂDIȘTEANU [1,2], VERONICA LAZĂR[1,2]

[1] *University of Bucharest, Faculty of Biology, Department of Botany-Microbiology, Bucharest, Romania;*
[2] *Research Institute of the University of Bucharest – ICUB, Bucharest, Romania*

* ***Corresponding author e-mail address:*** *IRYNA_84@yahoo.com*

Abstract

In this study, we aimed to assess the antimicrobial activity of some essential oils (EOs) from Nigella sativa (NS), Syzygium aromaticum - Thymus vulgaris mixture (M-ST) and Origanum vulgare (OV) commercial oils on Candida albicans clinical strains. Serial two-fold well dilution method was used in order to determine the minimum inhibitory concentration (MIC) and the ranges were: 6.1 - 48.8 mg/ mL for NS, 0.35 – 5.56 mg/ mL for the M-ST, and 0.63 – 2.5 mg/ mL for OV. Our results revealed that the investigated oils are promising solutions for the development of novel antifungal agents.

Keywords: *Candida albicans,* essential oils, antimicrobial activity.

1. Introduction

Candida sp. is the most common yeast that causes worldwide fungal infections (MANOLAKAKI & al., [1]). The dimorphic fungus *Candida* sp. can respond rapidly to environmental changes, and this flexibility could allow *Candida* sp. to use the advantage of immune suppression and facilitate establishment of disease. Although *Candida* sp. is a normal component of skin and mucous membranes microbiota of healthy people, it causes infections that range from superficial to lethal systemic infections, particularly in immunocompromised patients. Various virulence factors are contributing to the colonization and pathogenicity of *Candida* infection, including the expression of adhesins and invasins, yeast-hyphae dimorphims, phenotypic switching, biofilms formation and the secretion of hydrolytic enzymes (FRANCOIS & al., [2]).

The most of soluble virulence factors are extracellular hydrolytic enzymes secreted by the fungus. The most discussed hydrolytic enzymes produced by *C. albicans* are secreted aspartic proteinases (Saps), which play a major role in the overgrowth of the *Candida* sp. since these enzymes pave way to adhere, penetrate and invade host tissues (SCHALLER & al., [3]). Recent evidence suggests that the majority of infections produced by this pathogen are associated with biofilm growth (SARDI & al., [4]).

Nowadays, an increased the number of yeasts resistant to antifungal drugs is recognized worldwide (INGHAM & al*.,* [5]); therefore, there is a need to search for substances with proven antifungal activity as alternatives to the current therapeutic agents. Essential oils (EOs) are found in different parts of plants, including leaves, seeds, flowers, roots and barks and contain anti-bacterial, anti-fungal, and anti-parasitic compounds involved in plants immunity against pathogens (ABDURAHMAN & al., [6]). Therefore, herbal drugs are a promising solution for the effective treatment of diseases caused by particularly resistant microorganisms (Rahman&Hossain, [7]). We aimed to reveal the antifungal and antibiofilm activities for selected EOs against nosocomial *C.albicans* strains.

2. Material and methods

2.1. Clinical strains: A total number of 16 nosocomial *C. albicans* were isolated from patients admitted for surgery in the Institute of Cardiovascular Diseases Prof. C.C. Iliescu, Bucharest, Romania, aged 20–85 years, The fungal strains were isolated from different anatomic sites [i.e. respiratory tract secretions (n=8), other secretions (n=6), and urinary tract infections (n=2) and diagnosed by the automatic identification Vitek II system.

2.2. Essential oils (EOs): commercial plant extracts, i.e.: *Nigella sativa* (from Iraq, Alcaptin company), *Thymus vulgaris* - *Syzygium aromaticum* mixture (M-TS), (Fares) and *Origanum vulgare* (Canada, Provita company) were used in this study.

2.3. Quantitative assay of the antimicrobial activity of vegetal extracts

The antifungal activity of the commercial extracts was assessed by using the quantitative binary serial dilution method in 96 multi well plates, the concentrations ranges being specific depending on the tested EO. Simultaneously, there were achieved serial dilutions for DMSO (dimethylsulfoxide) in the same volume, in order to obtain the negative control. A volume of 20 µL of fungal suspension with 0.5 McFarland density was added in each well. The plates were incubated for 24 h at 37°C, and MICs were read as the lowest essential oils concentration that inhibited the *C. albicans* growth. The microtiter method with violet crystal was used to assess the anti-biofilm activity (PAPARELLA& al., [8]; SAVIUC & al., [9]).

3. Results and Conclusion

Quantitative assay of the antifungal activity of vegetal extracts

The increasing resistance of *C. albicans* towards the existent antifungal compounds and the reduced number of available drugs led to the search of new therapeutic alternatives (SARDI & al., [10]). Plants and their EOs, which were empirically used since longtime due to their broad spectrum antimicrobial properties for domestic and therapeutic purposes. EOs have been suggested to have potent therapeutic activity, including anthelmintic, skin infections and insect bites, chicken pox, colds, flu and measles sinus congestion, asthma, bronchitis, pneumonia, tuberculosis, and cholera, due to their phenolic, alcoholic, and terpenoid constituents (CHIFIRIUC & al., [11]).

The inhibitory effect of some commercial extracts was tested against clinical *C. albicans* strains, the most effective EOsbeing M-ST and *O. vulgare*. (fig.1). The MIC ranges were: 6.1 - 48.8 mg/ mL for *N. sativa*, 0.35 – 5.56 mg/ mL for the M-ST and 0.63 – 2.5 mg/ mL for *O. vulgare.* At the recorded MIC, the final EOs concentrations were below 5% extract in the culture medium. *N. sativa* seed oil consists of oleoresins and essential oil components, including thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, carvacrol, 4-terpineol, α-thujene, t-anethol, longifolene, thymol, and pinene (SINGH & al., [12]). Thymoquinone (found in concentrations of 30–52.6%) followed by its related compounds, such as thymohydroquinone, dithymoquinone, thymol along with carvacrol and p-cymene (found in concentrations of 7–25.8%) have been shown to be mostly related to the antimicrobial activity of *N. sativa* seeds oil (Singh & al., [12]; SHAABAN & al., [13]; HALAWANI, [14]). Other constituents, oleoresins, linoleic acid, and oleic acid, may also have minor antimicrobial activity (Singh & al., [12]). Bakathir and Abbas previously reported in 2011, that the MIC value of 12.5 mg/mL of the *N.sativa* seed extract was the lowest inhibitory concentration for the tested *C.albicans* strains.

The main compounds present in oregano essential oil are phenolic monoterpenes, carvacrol (64.5%) and thymol (BAKKALI & al., [15]; SOKOVIĆ & al, [16]).

T. vulgaris is an important source of phenolic compounds, and the result of the present study showed that the extract of this plant contains a high amount of flavonoids, responsible for the antioxidant and antibacterial activity (ZEGHAD and MERGHEM, [17]). The most

dominant of all identified compounds of thyme EO were thymol (50.48%), followed by p-cymene (24.79%), linalool (4.69%), γterpinene (4.14%) and 1,8-cineole (4.35%). Similar results were obtained by (NIKOLIĆ & al., [18]). Clove (*Syzygium aromaticum*) antimicrobial activity was established against many Gram positive and Gram negative microorganisms, including some fungi. The antimicrobial activity of clove is attributable to eugenol, oleic acids and lipids found in its essential oils (EL-ABED & al., [19]). Our study also revealed a broad spectrum inhibitory activity of the EOs of *T. vulgaris*, *Syzygium aromaticum* and *Origanum vulgare* against *C.albicans* strains. The M-ST oil and oregano essential oil inhibited the growth of all strains at very low MICs.

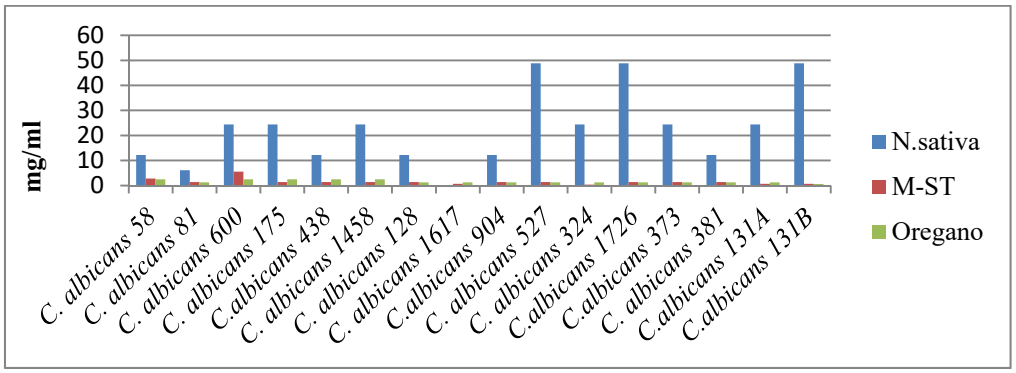

Figure 1. MIC values obtained against *C. albicans* strains for *Nigella sativa*, *Thymus vulgaris* - *Eugenia aromaticum* mixture (M-TS) and *Origanum vulgare* EOs.

Biofilms formed by fungal organisms are associated with drastically enhanced resistance against most antimicrobial agents, contributing to the persistence of the fungi despite antifungal therapy (CHIFIRIUC & al., [11]). The minimum biofilm eradication concentrations (MBECs) ranges were: 0.38 – 24.4 mg/ mL for *N. sativa*, 0.09 – 2.78 mg/ mL for M-ST and 0.16– 1.25 mg/ mL for *O. vulgare*. The M-ST and *O. vulgare* EOs proved to be more potent inhibitors than *N.sativa* of *C. albicans* biofilm formation (fig. 2).

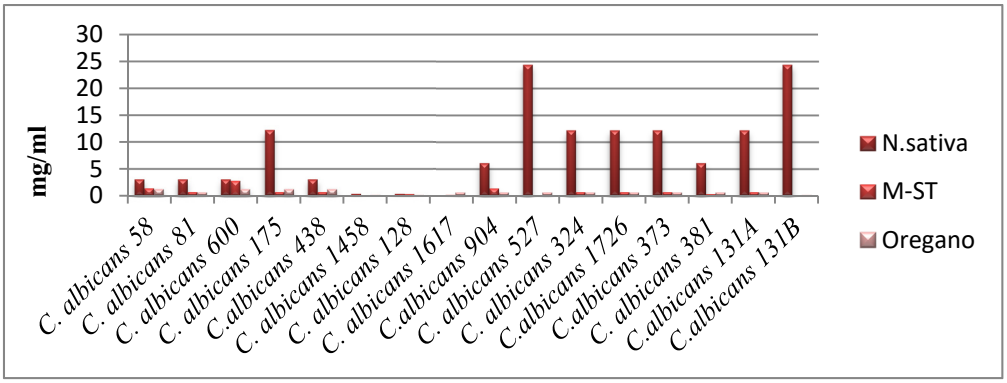

Figure 2. MBEC values obtained against *C. albicans* strains for *Nigella sativa*, *Thymus vulgaris* - *Eugenia aromaticum* mixture (M-TS) and *Origanum vulgare* EOs.

4. Conclusions

The obtained results demonstrated that *Thymus vulgaris* - *Syzygium aromaticum* mixture and *Origanum vulgare* essential oils exhibited very good antifungal activity against *C. albicans* nosocomial strains and also antibiofilm activity at subinhibitory concentrations against all tested *C. albicans* isolates. Future assays will be made for the comparative analysis of the

antifungal activity exhibited by commercial *versus* freshly extracted fatty and essential oils from the studies plant species.

Acknowledgement
The financial support of PN-III-P2-2.1-BG-2016-0369, granted by the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI) is gratefully acknowledged.

References

1. MANOLAKAKI D, VELMAHOS G, KOURKOUMPETIS T, CHANG Y, ALAM HB, DE MOYA MM, & MYLONAKIS E. Candida infection and colonization among trauma patients. *Virulence*, 1(5): 367-375, (2010).
2. FRANÇOIS LM, DUNCAN W, BERNHARD H. *Candida albicans* pathogenicity mechanisms. *Virulence*, 4 (2):119-128, (2013).
3. SCHALLER M, BORELLI C, KORTING HC, HUBE B SCHALLER M, BORELLI C, KORTING HC, HUBE B. Hydrolytic enzymes as virulence factors of *Candida albicans*. *Mycoses*, 48:365-377, (2005).
4. SARDI JC, DUQUE C, CAMARGO GA, HOFL ING JF, GON Ç ALVES RB. Periodontal conditions and prevalence of putative periodontopathogens and *Candida* spp. in insulin-dependent type 2 diabetic and non-diabetic patients with chronic periodontitis. A pilot study. *Arch Oral Biol* 56 : 1098 – 1105,(2011).
5. INGHAM, C. J., BOONSTRA, S., LEVELS, S., DE LANGE, M., MEIS, J. F. & SCHNEEBERGER, P. M. Rapid susceptibility testing and microcolony analysis of *Candida* spp. cultured and imaged on porous aluminum oxide. *PLoS ONE* 7, e33818, (2012).
6. ABDURAHMAN HN, RANITHA M, AZHARI HN. Extraction and characterization of essential oil from Ginger (*Zingiberofficinale roscoe*) and Lemongrass (*Cymbopogon citratus*) by microwave-assisted hydrodistillation. *Int J Chem Environ Engin*.4:221–226, (2013).
7. RAHMAN, M.M. AND HOSSAIN, M.N. Antibiotic and herbal sensitivity of some *Aeromonas* sp. isolates collected from diseased carp fishes. *Progress.Agric*, 21(1&2): 117-129, (2010).
8. PAPARELLA A., L. TACCOGNA, I. AGUZZI. Flow cytometric assessment of the antimicrobial activity essential oils against *Listeria monocytogenes Food Control*.,pp. 1174–1182, (2008).
9. SAVIUC C, GRUMEZESCU AM, OPREA E. Antifungal activity of some vegetal extracts on *Candida* biofilms developed on inert substratum. *Biointerface Research in Applied Chemistry*.1:15–23., (2011).
10. SARDI J. C. O., SCORZONI L., BERNARDI T., FUSCO-ALMEIDA A. M. AND MENDES GIANNINI M. J. S. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology* .62, 10–24, (2013).
11. CHIFIRIUC C, GRUMEZESCU V, GRUMEZESCU AM, SAVIUC C, LAZĂR V, ANDRONESCU E. Hybrid magnetite nanoparticles/*Rosmarinus officinalis* essential oil nanobiosystem with antibiofilmactivity.*Nanoscale Res Lett*. Apr 10;7:209. doi: 10.1186/1556-276X-7-209, (2012).
12. SINGH S, DAS SS, SINGH G, SCHUFF C, DE LAMPASONA MP, CATALÁN CA. Composition in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (Nigella sativa L.) *Biomed Res Int*.2014:918209. doi:10.1155/2014/918209, (2014).
13. SHAABAN HA, SADEK Z, EDRIS AE, SAAD-HUSSEIN A. Analysis and antibacterial activity of Nigella sativa essential oil formulated in microemulsion system. *J Oleo Sci*.;64:223–32, (2015).
14. HALAWANI, E. Antibacterial activity of thymoquinone and thymohydroquinone of Nigella sativa L. and their interaction with some antibiotics. *Advances in Biological Research*, 3, 148-152, (2009).
15. BAKKALI, F., AVERBECK S, AVERBECK D, IDAOMAR M. Biological effects of essential oils: a review. *Food and Chemical Toxicology*, v. 46, p. 446-475, (2008).
16. SOKOVIĆ M., GLAMOČLIJA J., MARIN P.D., BRKIĆ D., VAN GRIENSVEN L.J.L. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules*, 15: 7532–7546, (2010).
17. ZEGHAD N AND MERGHEM R. Antioxidant and antibacterial activities of *Thymus vulgaris* L., *Medicinal and Aromatic Plant Research Journal*; 1: 5-11, (2013).
18. NIKOLIC M, GLAMOCLIJA J, FERREIRA IC, CALHELHA RC, FERNANDES Â, MARKOVIC T, MARKOVIC D, GIWELI A, SOKOVIC M. Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. andReut and *Thymus vulgaris* L. essential oils. *Ind Crop Prod*;52:183-90, (2014).
19. EL-ABED, S.; HOUARI, A.; LATRACHE, H.; REMMAL, A.; KORAICHI, S.I. In vitro activity of four common essential oil components against biofilm-producing *Pseudomonas aeruginosa*. *Res. J. Microbiol*., 6, 394–401, (2011).