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ARTICLE

Antifungal effect of selected European herbs against *Candida albicans* and emerging pathogenic non-albicans *Candida* species

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ABSTRACT The anti-candidal effect of some European medicinal plants *Allium ursinum* (wild garlic or ramson); *Aristolochia clematitis* (birthwort), *Melilotus officinalis* (sweet clover), *Salvia officinalis* (garden sage) and *Viscum album* (mistletoe) was investigated. In general, *C. inconspicua*, *C. zeylanoides* and *C. norvegica* were among the most sensitive species, while *C. albicans* together with C. *orthopsilosis* showed much lower sensitivity. Best results were achieved with wild garlic showing a broad spectrum anti-yeast activity in some cases with fungicidal MIC (3.52 mg/ml). The other plant extracts showed moderate, mainly fungistatic action.
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KEY WORDS

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Candida species are opportunistic pathogens causing candidiasis. Invasive candidiasis is a life-threatening infection in immunocompromised hosts such as bone marrow and organ transplant recipients, in patients receiving intensive chemotherapy regimens and in AIDS patients (Lyles et al. 1999). Development of resistance is common among HIV-positive patients receiving fluconazole for long-term therapy. In some cases, resistance to fluconazole triggers cross-resistance to other azoles or pathogen shift from *Candida albicans* to less sensitive species such as *Candida glabrata* and *Candida krusei* (Bastert 2001). Moreover, systemic *Candida* infections are observed in patients with extensive surgery or burns, intensive antibiotic therapy, indwelling catheters, patients with diabetes mellitus, and in elderly patients (Dean et al. 1996; Wenzel 1995). Nowadays, *Candida* species are important agents of nosocomial bloodstream infections (BSIs) (Pfaller et. al. 2011; Diekema et al. 2012). The incidence of BSIs caused by *Candida* spp. has risen in the past 20 years (Pfaller et al. 1998; Seifert et al. 2007; Diekema et al. 2012). Although *C. albicans* remains the most frequently isolated agent of candidiasis, non-albicans *Candida* (NAC) species now account for a substantial part of clinical isolates collected worldwide in hospitals. In a survey covering 52 hospitals in the USA between 1998 and 2006, *C. albicans* was the most prevalent species, accounting for 50.7% of all isolates, followed by *C. parapsilosis* (17.4%), *Candida glabrata* (16.7%) and *Candida tropicalis* (10.2%); 5.1% BSIs were caused by other *Candida* spp. (Wisplinghoff et al. 2014). NAC species of particular importance include the prominent species *Candida guilliermondii*, *Candida lusitaniae, Candida kefyr, Candida famata* (synonym: *Debaryomyces hansenii), Candida inconspicua, Candida rugosa, Candida dubliniensis* and *Candida norvegensis*.

There is a growing interest to find new, natural compounds with anti-candidal activity because most *Candida* species show reduced sensitivity towards traditional antifungal compounds (Papon et al. 2013). Plant derived antimicrobials are, among others, in the focus of research because of their easy accessibility and wide antimicrobial spectrum. Usually several ingredients with different target sites are responsible for the antimicrobial properties which decreases the possibility of development of microbial resistance. In this study, the anti-candidal effect of European medicinal plants: *Allium ursinum* (wild garlic or ramson); *Aristolochia clematitis* (birthwort), *Melilotus officinalis* (sweet clover), *Salvia officinalis* (garden sage) and *Viscum album* (mistletoe) was investigated.

Allium ursinum is a perennial herbaceous plant, distributed in Europe and Asia. It has been used for centuries in traditional medicine to prevent cardiovascular diseases, to detoxify the body, and to stimulate digestion (Sobolewska et al. 2013). Although bulbs are also edible, leaves are preferred and are consumed mainly fresh. Nowadays, there is a renaissance of wild garlic consumption because of its supposed

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Table 1. Minimum inhibitory concentration (MIC) in mg/ml of the investigated herbs' ethanol (30%v/v) extracts against *Candida* species.

Candida species	*Allium ursinum*	*Aristolochia clematitis*	*Melilotus officinalis*	*Salvia officinalis*	*Viscum album*
C. albicans	3.52	>32.8	19.3	13.2	11.3
C. guillermondii	0.88	32.8	19.3	6.6	11.3
C. glabrata	1.76	>32.8	19.3	13.2	11.3
C. inconspicua	1.76	32.8	9.65	13.2	5.65
C. krusei	1.76	>32.8	19.3	13.2	11.3
C. lusitaniae	0.88	>32.8	19.3	13.2	11.3
C. metapsilosis	1.76	32.8	19.3	6.6	11.3
C. norvegica	0.88	32.8	19.3	6.6	11.3
C. orthopsilosis	3.52	32.8	19.3	13.2	>11.3
C. parapsilosis	1.76	32.8	19.3	6.6	11.3
C. pulcherrima	3.52	32.8	19.3	6.6	11.3
C. zeylanoides	0.88	32.8	9.65	6.6	11.3

health benefits. The main ingredients are sulfur containing compounds: the odorless and non-volatile S-alk(en)yl-L-cysteine-sulfoxides (methiin and alliin) which in crushed leaves hydrolyze to a range of volatile compounds such as thiosulfinates and allicin (Bagiu et al. 2012; Sobolewska et al. 2013). The volatile compounds are thought to be responsible for the wide antimicrobial effect of *A. ursinum* (Bagiu et al. 2012).

Aristolochia species are also used in traditional medicine although aristolochic acid with its cytotoxic effect can cause chronic renal failure. Aristolochic acid is supposed to have also antimicrobial properties (Pozdzik 2010; Benzakour 2011).

Melilotus officinalis (L.) Pallas is traditionally used to treat inflammation and infection in the throat and gastrointestinal system. The plant contains melilotin and other coumarin glycosides, and an essential oil, making sweet clover aromatic (Anwer 2008).

Salvia officinalis L. is an aromatic plant and has been used since ancient times in folk medicine. Biologically active compounds are phenol acids, like rosmarinic, caffeic, ferulic acids, and tannins (Then et al. 2004).

Viscum album L. grows as an obligate hemiparasitic plant on the branches of deciduous trees. Mistletoes have been used in traditional medicine in the treatment of many diseases such as diabetes mellitus, stroke, chronic cramps, stomach problems, heart palpitations (Ochocka and Piotrowski 2002). The bioactive compounds are mistletoe lectins and viscotoxins (Urech et al. 2006).

Materials and Methods

Plants

Aristolochia clematitis L., *Melilotus officinalis* (L.) Pallas, *Salvia officinalis* L., *Viscum album* L., were collected in Romania (near Timisoara), and were identified in the University Botanical Garden of Szeged. Leaves of *A. ursinum* were of Hungarian origin and purchased from a local market in Szeged.

Microorganisms

C. norvegica CBS 4239, *C. inconspicua* CBS 180, *C. zeylanoides* CBS 619, *C. pulcherrima* CBS 5833, *C. guilliermondii* CBS 566, *C. albicans* ATCC 10231, *C. krusei* CBS 573, *C. lusitaniae* CBS 6936, *C. glabrata* CBS 138, C. *parapsilosis* CBS 604, *C. metapsilosis* SZMC 8092 and *C. orthopsilosis* SZMC 8116 strains were obtained form the American Type Culture Collection (ATCC, Manassas, VA, USA), from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands), and from the Szeged Microbial Collection (SZMC, Szeged, Hungary). Yeasts were maintained and cultured on YEPD medium (1% (w/v) yeast extract, 2% peptone, 2% glucose, 2% agar) at 30 ºC.

Preparation of herbs extracts

The aerial parts of *A. clematitis, M. officinalis, S. officinalis,* and *V. album* were naturally air dried in shade for 14 days. Once dried, they were milled into a fine powder with an electric grinder. To 10 g powdered plant material, 100 ml 96% (v/v) ethanol was added, and the solution was agitated at room temperature in the dark for 24 hours. The extracts were filtered and concentrated by vacuum drying at 50 ºC. After the dehydration process, dry plant extracts were dissolved in 30% (v/v) ethanol to a final concentration of 50 mg ml^{-1} and were sterilized by filtration through a 0.45 μm membrane filter (Millipore). *Allium ursinum* extract was prepared from fresh material; crushed leaves were macerated in ethanol:water (30:70) solution for 24 h. Thereafter, the crude extract was filtered and the liquid was evaporated at 50 °C. The solid residue was dissolved in 30% (v/v) ethanol and the concentration was adjusted to 50 mg/ml. The solution was sterile filtered in the

Table 2. Minimum fungicidal concentration (MFC) in mg/ml of the investigated herbs' ethanol (30%v/v) extracts against *Candida* species. ND - not detected.

Candida species	*Allium ursinum*	*Aristolochia clematitis*	*Melilotus officinalis*	*Salvia officinalis*	*Viscum album*
C. albicans	3.52	ND	-	ND	ND
C. guillermondii	1.76	ND	19.3	ND	ND
C. glabrata	ND	ND	-	ND	ND
C. inconspicua	0.88	ND	-	ND	ND
C. krusei	1.76	ND	-	ND	ND
C. lusitaniae	0.88	ND	-	ND	ND
C. metapsilosis	14.06	ND	-	ND	ND
C. norvegica	0.88	ND	19.3	ND	ND
C. orthopsilosis	14.06	ND	-	ND	ND
C. parapsilosis	ND	ND	-	ND	ND
C. pulcherrima	1.76	ND	19.3	ND	ND
C. zeylanoides	0.88	ND	9.65	6.6	ND

Table 3. Anti-*Candida* MIC values of the antifungal amphotericin B and different herbs and fruits extracts from the literature. R - resistant; nd - no data.

Candida species	Amphotericin B (μg/ml)	*Rosmarinus officinalis* methanol extract (mg/ml)	*Punica granatum* methanol extract (mg/ml)	*Ribes nigrum* methanol extract (mg/ml)	Xanthorizzol from *Curcuma xanthorriza* (mg/ml)
Reference	Galgóczy et al. 200	Höfling et al. 2010	Höfling et al. 2010	Krisch et al. 2009	Rukayadi et al. 2006
C. albicans	2.0	0.001	0.003	nd	2.5 - 15
C. guillermondii	nd	0.003	0.001	6.13	2.0 – 8.0
C. glabrata	nd	R	0.003	nd	4.0 - 10
C. inconspicua	2.0	nd	nd	4.22	nd
C. krusei	2.0	0.001	0.001	nd	2.5 – 7.5
C. lusitaniae	1.0	0.001	0.001	nd	nd
C. norvegica	0.5	nd	nd	nd	nd
C. parapsilosis	nd	0.003	0.003	4.41	10 - 25
C. zeylanoides	1.0	nd	nd	nd	nd

above mentioned way and the concentration was determined again. All extracts were kept at -20 ºC until use.

Investigation of anti-candidal effect

In vitro anti-candidal activities were evaluated by microtiter plate assay in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA), according to National Committee for Clinical Laboratory Standards (CLSI) recommendations. In each well, 100 µl sterile-filtered (0.45 µm, Millipore, USA) plant extract diluted up to 1/32 of the stock solution was mixed with 100 µl yeast cell suspension (10^5 cells/ml). Each test plate contained an uninoculated control, a positive growth control, a medium-free control and a drug sterile control. Absorbance was measured after 24 h cultivation at 37 ºC. MIC values were determined as the concentration of extracts where the absorbance of the treated culture was ≤10% of the non-treated culture (positive growth control). To determine MFC values, tracking plate method (Jett at al. 1997) was used. MFC was defined as the concentration where no colony growth was observed.

Results and Discussion

The lowest MIC values for *Candida* species were obtained by wild garlic extracts followed by salvia and mistletoe (Table 1). The sensitivity of *Candida* species to the extracts varied substantially. In general, *C. inconspicua*, *C. zeylanoides* and *C. norvegica* were among the most sensitive species, while *C. albicans* together with *C. orthopsilosis* showed much lower sensitivity. In most cases MFC values could not be determined in the investigated concentration ranges, showing that MIC values were mainly fungistatic (Table 2). Best results were achieved with wild garlic showing in some cases fungicidal MICs even at low concentrations (3.52 mg/ml). Lemar and coworkers (2002) have found that fresh garlic and garlic powder extracts had fungicidal effect on *C. albicans* above 10 and 20 mg/ml thus our wild garlic extract showed stronger antifungal activity than garlic. Garlic and wild garlic have common main compounds (alliin, methiin and their derivatives) and these compounds are responsible for the antimicrobial effect. According to literature, these compounds inhibit microorganisms by reacting with the sulfhydryl (SH) groups of cellular proteins (Kyung 2012). It seems that plants with volatile ingredients like allicin or essential oils have good antifungal activity. In our experiments MIC values for *Salvia* were in the range of 6.6-13.2 mg/ml while rosemary extracts investigated by Höfling et al. (2010) showed MIC values close to amphotericin B (Table 3). Extracts rich in flavonoids and anthocyanins as black currant pomace extracts in our previous experiments or a pure phenolic compound, xanthorrizol showed MIC values in the range of 2 - 20 mg/ml (Table 3). Based on MIC values, the effect of most natural antifungals is weaker than that of the usual synthetic agents. Natural antimicrobials may be of importance, however, because they are less likely to induce resistance, and may act synergistically with the synthetic ones.

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References

Anwer M, Suhail M, Mohtasheem IA, Ahmed SW, Bano H (2008) Chemical constituents from *Melilotus officinalis*. J Basic Appl Sci 4(2):89-94.
Bagiu RV, Vlaicu B, Butnariu M (2012) Chemical composition and in vitro antifungal activity screening of the *Allium ursinum* L. (Liliaceae). Int J Mol Sci 13:1426-1436.
Bastert J (2001) Current and future approaches to antimycotic treatment in the era of resistant fungi and immunocompromised hosts. Int J Antimicrob Agents 17:81-91.
Benzakour G, Benkirane N, Amrani M, Oudghiri M (2011) Immunostimulatory potential of *Aristolochia longa* L. induced toxicity on liver, intestine and kidney in mice. J Toxicol Environ Health Sci 3(8):214-222.
Clinical and Laboratory Standards Institute (CLSI), Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard Second Edition (M27-A2), 2002
Dean DA, Burchard KW (1996) Fungal infection in surgical patients. Am J Surg 171:374-382.
Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M (2012) The changing epidemiology of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis 73:45-48.
Galgóczy L, Bácsi A, Homa M, Virágh M, Papp T, Vágvölgyi Cs (2011) In vitro antifungal activity of phenothiazines and their combination with amphotericin B against different *Candida* species. Mycoses 54:737-743.
Höfling JF, Anibal PC, Obando-Pereda GA, Peixoto IAT, Furletti VF, Foglio MA, Gonçalves RB (2010) Antimicrobial potential of some plant extracts against *Candida* species. Braz J Biol 70(4):1065-1068.
Jett BD, Hatter, KL, Huycke MM, Gilmore MS (1997) Simplified agar plate method for quantifying viable bacteria. BioTechniques 23:648-650.
Krisch J, Ördögh L, Galgóczy L, Papp T, Vágvölgyi Cs (2009) Anticandidal effect of berry juices and extracts from *Ribes* species. Cent Eur J Biol 4:86-89.
Kyung KH, Lee YC (2001) Antimicrobial activities of sulfur compounds derived from S-alk(en)yl-L-cysteine sulfoxides in *Allium* and *Brassic*a. Food Rev Int 17(2):183-198.
Lemar KM, Turner MP, Lloyd D (2002) Garlic (*Allium sativum*) as an anti-*Candida* agent: a comparison of the efficacy of fresh garlic and freeze-dried extracts. J Appl Microbiol 93: 398–405.
Lyles RH, Chu C, Mellors JW, Margolick JB, Detels R, Giorgi JV, Al-Shboull Q, Phair JP (1999) Prognostic value of plasma HIV RNA in the natural history of *Pneumocystis cariniipneumonia*, cytomegalovirus and *Mycobacterium avium* complex. Multicenter AIDS cohort study. AIDS 13:341-349.
Ochocka JR, Piotrowski A (2002) Biologically active compounds from European mistletoe (*Viscum album* L.). Can J Plant Pathol 24:21-28.
Papon N, Courdavault V, Clastre1 M, Bennett RJ (2013) Emerging and emerged pathogenic *Candida* species: beyond the *Candida albicans* paradigm. PLOS Pathogens 9(9):1003550. doi:10.1371/journal.ppat.1003550.
Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP (1998) National surveillance of nosocomial blood stream infection due to species of *Candida* other than *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE Program. Diagn Microbiol Infect Dis 30:121-129.
Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M (2011) *Candida* bloodstream infections: comparison of species distributions and antifungal resistance patterns in community-onset and nosocomial isolates in the SENTRY Antimicrobial Surveillance Program, 2008-2009. Antimicrob Agents Chemother 55:561-566.
Pozdzik AA, Berton A, Schmeiser HH, Missoum W, Decaestecker C, Salmon IJ, Vanherweghem JL, Nortier JL (2010) Aristolochic acid nephropathy revisited: A place for innate and adaptive immunity? Histopathol 56(4):449-463.
Rukayadi1Y, Yong D, Hwang J-K (2006) In vitro anticandidal activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. J Antimicrob Chemother 57:1231-1234.
Seifert H, Aurbach U, Stefanik D, Cornely O (2007) In vitro activities of isavuconazole and other antifungal agents against *Candida* bloodstream isolates. Antimicrob Agents Chemother 51:1818-1821.
Sobolewska D, Podolak I, Makowska-Was J (2013) *Allium ursinum*: botanical, phytochemical and pharmacological overview. Phytochem Rev. DOI 10.1007/s11101-013-9334-0.
Stajner D, Milic N, Canadanovic-Brunet J, Kapor A, Stajner M, Popovic BM (2006) Exploring *Allium* species as a source of potential medicinal agents. Phytother Res 20:581-584.
Then M, Vásárhelyi-Perédi K, Szöllősy R, Szentmihályi K (2004) Polyphenol-, mineral element content and total antioxidant power of sage (*Salvia officinalis* L.) extracts. Acta Hort 629:123-129.
Urech K, Schaller G, Jäggy C (2006) Viscotoxins, mistletoe lectins and their isoforms in mistletoe (*Viscum album* L.) extracts in vitro. Drug Res 56(6):428-434.
Wisplinghoff H, Ebbers J, Geurtz L, Stefanik D, Major Y, Edmond MB, Wenzel RP, Seifert H (2014) Nosocomial bloodstream infections due to *Candida* spp. in the USA: species distribution, clinical features and antifungal susceptibilities. Int J Antimicrob Ag 43:78- 81.