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Research Article
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In Vitro Antifungal Activities of *Sambucus ebulus* Against Candida Species Using the Standard Protocol CLSI-M27-S3

Arefeh Hosseini[1],Soham Ansari[2*]

[1]School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*Corresponding author:
Soham Ansari,
Department of Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: hosseini.arefeh@yahoo.com

Abstract

Background: The most prevalent fungal infection in the mouth is Candidiasis, which causes various problems for patients. Chemical treatments such as nystatin are the most common methods that are locally used for the mouth that tastes bitter. The repeated use of this method 4 times a day and its preparation during the total consumption lead to patients' dissatisfaction. Herbal treatments due to less medicinal side effects while having equal effects can be a suitable alternative to chemical treatments. Accordingly, this research focused on evaluating the effect of the herbal extract of the antifungal palm on the types of *Candida* and then comparing it with Nystatin.

Methods: To this end, the effect of the herbal extract of antifungal palm on 4 types of *Candida* (i.e., *albicans, glabrata, tropicalis,* and *parapsilosis*) was investigated, and then the results were compared with that of nystatin, which is diagnosed by the broth microdilution method. The result of the study is descriptive, and minimum inhibitory concentration (MIC) in the blue palm herbal extract was studied in comparison with nystatin for 20 isolated *Candida*.

Results: Based on the result, MIC in the palm herbal extract was (mL/mg) 0.25-0.5 while the inhibitory effect of nystatin growth in this type was 0.125-0.5 (mg/L), and the MIC of the remaining types of *Candida*, including *tropicalis*, *glabrata*, and *parapsilosis* was 1-0.5 (mg/mL), 0.5-2, and 0.5-1. Finally, the inhibitory effect of growth (MIC) in nystatin drugs in the mentioned types was 0.5-0.25 (mg/L), 0.5-1, and 0.5-0.25.

Conclusion: Overall, the activity of the antifungal palm herbal extract is suitable against the examined types of *Candida*, and this extract may be used as a drug or mouthwash for candidiasis patients.

Keywords: Mouth fungal infection, Candidiasis, Palm herbal, *Candida albicans*, Oral cavity

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Introduction

The high incidence of oral diseases in the population implies that priority must be given to prevention strategies (1). *Candida* yeast is one of the most important triggers for mouthfungalinfections, belonging to the usual oral flora of many individuals. The three important parameters includethe immune function of the host, the oral condition, and the form of *Candida*, and the number of individuals possessing the infection rises with age (2). Although *Candida albicans*, is the major pathogen, other organisms such as *Candida glabrata* and *Candidia krusei*, which areisolated from the patients, are often considered as the origin of the disease. Given the relative tolerance of some types of strains including *Candida tropicalis* and *C. glabrata*, the value of non-albicans in recent years has increased compared with other antifungal medicines (3). In the meantime, *C. glabrata* is the most prevalent source of infection and resistant to a significant number of azole compounds, particularly fluconazole, after *C. albicans*, which is an oral mucosal, saprophytic microorganism that can be extracted around 31%-55% in normal people's mouth. This fungus may trigger fatal disorders in patients with cancer (4,5). Another characteristic of this pathogenic fungus is the production of biofilm in urinary cathetersor dental appliancesand prostheses (6). Prothesis and oral mucosal surface may provide the best environment for *Candida* to replicate and trigger infection. Any alterations in the oral environment impacting microbial growth variableswill disrupt the natural equilibrium of the microbial community and trigger host opportunistic infections. In comparison with other microorganisms, the over-crowding of opportunistic strainssuch as *Candida* inevitably contributes to mucosal inflammation owing to extracellular enzymes that are responsible for the development of the yeast (7). Local or systemic therapies can be used to manage candidiasis regarding the degree of the lesion and the situation of the patient. Nystatin mouthwash is the local treatment that is applied as the standard. The bitter flavor and frequent usage of nystatin mouthwashfour times per day, along with repetitive preparations contributes to the lack of patient confidence (8). In the last decades,no report has been recorded for the treatment of patients with distinct clinical types of candidiasis and microbial tolerance to antifungal medicines (9). The study will also be beneficial for discovering newcost-effectiveantimicrobial and antifungal ingredients, which have limited complications. The usage of herbs in traditional medicine, including *Sambucus ebulus*, is one of the promising areas. *S. ebulus*, which has many beneficial effects against diseases, is well-known among the typical plants in Balkans and Anatolia. This herb is used for the prevention of gastrointestinal inflammation, influenza, renal and pulmonary disorders, and injuries (10-15). Using the Clinical and Laboratory Standards Institute (CLSI) M-27 A3 procedure, this research was conducted to assess the antifungal activity of the *S. ebulus* extract on the extracted candida strains from the oral cavity.

Materials and Methods

Fungal Strains

In general, 20 Candida strains were extracted from dental stomatitis patients aged around 40-60 years. Sterile swabs were employed to collect these samples, which werecultivated in Sabouraud dextrose agar (SDA) and incubated at35˚C.

Extraction

The samples of *S. ebulus* leaves were prepared from the grocery shop by a botanist at the Shahid Beheshti School of Pharmacy. The maceration process was used for extraction. To this end, 100 g of *S. ebulus* powder was added since grinding, to twoliters of distilled water seethedand immediately removedfrom warmth. The dish was then wrapped with foil, and its components were filtered inthe laboratory with filter paper (Whatman 92 paper). Next, the dish was put on a water bath for 2 hours with dry extracts prepared following solvent evaporation (16).

Drug Sensitivity Evaluation

To evaluate the minimum inhibitory concentration (MIC) of the *S. ebulus* aquatic extract in four *Candida* strains including *albicans, glabrata*, *tropicalis*, and *parapsilosis*, eleven successiveextract concentrationswith 1/2 dilution have been applied,and the maximumand minimum concentrations were 20 mg/mL and 0.02 mg/mL, respectively. Nystatin was also included in this analysis as the monitor, and the maximum and minimum concentrations at 11 consecutive doses were 32 mg/L and0.032 mg/L, respectively. All strains were cultivated in SDA media and put at 35˚C for 72 hours to measure the anti-fungal effect of the *S. ebulus* extract. Then, 96-well plateswith a culturemedium of 1640 (Roswell Park Memorial Institute-1640)-RPMI and –MOPS (3-(N morpholino) propanesulfonic acid)and separate dilutions of the required extract were prepared as well. Next, the formulated fungal suspension was added to the plates, which were incubated at 35˚C for 24-48 hours. The extent of the induced inhibition by the extract was then calculated according to the parameters of the CLSI protocol. The concentrations of antifungals hindering the growth of at least 90% and 50% of the strains and the candidastrains were identified as MIC90 and MIC50, respectively. To acquire MIC90 and MIC50, there should be at least 10 strains for each fungus. The geometric mean was also computed from the Excel-2013 software using the Geomean model, and each examination was performed twice in the present analysis.

Results

Based on the findings (Table 1), the geometric mean for *S. ebulus* and nystatin was 0.82 mg/mL and 0.36 mg/L for the 20 included *Candida* strains in this analysis. The development of the 20 isolated *Candida* strains from theoral cavitywas inhibited by nystatin and *S. ebulus* in the concentration rangeof0.125-1 mg/Land 2-5.0 mg/mL, respectively.

Candida albicans

The obtained data (Table 2) reveal that the *S. ebulus* extract within the range of 0.5-2 mg/mL inhibits the growth of *C. albicans* in nine different studied strainswhereas nystatin at the concentration of 3-10 mg/mL (0.25, 0.5, and 0.125) applieda growth inhibitory impact on *C. albicans*.

Candida glabrata

The findings (Table 3) suggested that the extract of *S. ebulus* in four strains of *C. glabrata* yeast at a concentration of 0.5-2 mg/mL suppressesthe proliferation of this fungus. Nystatinhasa growth inhibitory impact on *Candida* at concentrations of 3-10 mg/mL (0.25, 0.5, and 1).

Candida tropicalis

Based on the results (Table 4), the *S. ebulus*extract in four strains of*C. tropicalis*within a range of 3-10 (0.25-0.5) mg/mL and nystatin within a range of 0.125 to 1 mg/mL might

Table 1. Correlation of Geometric Mean, Minimum and Maximum Values of MIC50, MIC, and MIC90 for 20 *Candida* Strains

	Nystatin (mg/L)	*Sambucus Ebulus* (mg/mL)
Geometric mean	0.36	0.82
Minimum range	0.125	0.5
Maximum range	1	2
MIC50	0.5	1
MIC90	1	2

Note. MIC: Minimum inhibitory concentration.

Table 2. Minimum Inhibitory Concentration of the Aqueous Leaf Extract of *Sambucus ebulus* Relative to Nystatin for the Proliferation of *Candida albicans*

Strains	Nystatin (mg/L)	*Sambucus ebulus* (mg/mL)
C1	0.5	0.5
C2	0.25	0.5
C3	0.125	1
C4	0.125	0.5
C9	0.125	0.5
C10	0.25	1
C12	0.5	1
C19	0.5	2
C20	0.25	1

Table 3. MinimalInhibitory Concentration of the *Sambucus ebulus* Aqueous Extract Relativeto Nystatin on the Development ofStrains

Strains	Nystatin (mg/L)	*Sambucus ebulus* (mg/mL)
C5	0.5	0.5
C6	0.25	1
C14	1	1
C15	1	2

Table 4. Minimum Inhibitory Concentrationof the *Sambucus ebulus* Aqueous Extract Relative to Nystatin on *Candida tropicalis* Development

Strains	Nystatin (mg/L)	*Sambucus ebulus* (mg/mL)
C8	0.25	1
C11	0.5	0.5
C16	0.25	1
C17	0.5	0.5

inhibit fungal growth.

Candida parapsilosis

The *Sambucus ebulus* extract with the concentration of 3-10 (0.25-0.5) mg/mL and nystatin with the concentration range of 0.5mg/mL prevented the development of this fungus in 3 strains of *C. parapsilosis* (Table 5).

Discussion and Conclusion

Denture stomatitis is a fungal infection that is triggered by various Candida types and happens when dentures are used in patients and the quality of life represents a decrease. This lesion is typically controlled with nystatin (11). In recent years, several instances of drug tolerance have been observed in a range of bacteria and fungi, probably rendering that alternative treatments must be sought in this regard. In addition, the finding of new plant substitutes for chemical therapies was granted greater consideration in view of the appropriate results and lower complications of herbal medicines versus chemical medicines. Considering that no herbal remedy has been established as a chemical solution for the treatment of denture stomatitis, this study focused on identifying this alternative. In this study, *S. ebulus* was evaluated, which is a plant that grows in numerous countries including Iran. It has been recognized as a plant of the culture of Iranian traditional medicine and is typically applied for treating different inflammations including gastrointestinal, flu, and renal and pulmonary diseases and various injuries such as wounds. It is further a popular plant in the Balkans and Anatolia. Several recent trials have confirmed that this plant can be used as an immune stimulant for managing respiratory disorders and gastrointestinal inflammation, and as an anti-tumor agent (15-17). Different groupings have already examined *S. ebulus*. Although numerous experiments have been performed in this area to examine different effects of this plant, this is the first study to specifically evaluate antifungal characteristics in theoral cavity. Unfortunately, a limited number of studies have investigated the antimicrobial properties of this herb. Asgarpanah et al (17) performed an analysis through the agar diffusion process in one of the most current studies on the antifungal properties of four plants such as *S. ebulus*. The researchfocusedon the impactof the extract treatment of these four plantson eight fungal strains including *C. albicans* although no obvious substantial findings were obtained from the *S. ebulus*. In this analysis, the agar diffusion approach, which is a qualitative tool, was used in comparison to our research. Further studies are recommended in this area. According to the universal standard protocol CLSI M27-A3, the microdilution approach was employed in our experiment and the findings were quantitative. In their research, Salehzadeh et al investigated the bactericidal effect of the *S. ebulus* extract throughdisk agar diffusionand found that all the examined bacteria were susceptible to the *S. ebulus* extract. Therefore, the plantextract can be regarded as a natural antibiotic candidate (18). Similarly, Schwaiger et al studied the anti-inflammatory activity of this plant and demonstrated that the *S. ebulus* leaf extract may suppresstumor necrosis factor,Vcam-11,and ICAM-12 in HUVEC 3. The anti-inflammatory function of this plant was documented and the origin of this impact was established to be due to abundant reserves of ursolic acid (8).

Likewise, Jabbari et al evaluated the impact of the topical treatment of this plant gel on joint pain, particularly knee osteoarthritis. This randomizeddouble-blind, active-controlled clinical trial focused on examining 106 samples. In all cases, the consequencesof four weeks of gel usage were favorable (including relief from pain). This plant

Table 5. Minimal Inhibitory Concentration for the *Sambucus ebulus* Aqueous Extract Relative to Nystatin Aboutthe Development of *Candida parapsilosis* Strains

Strains	Nystatin (mg/L)	*Sambucus ebulus* (mg/mL)
C7	0.5	1
C18	0.25	0.5
C16	0.25	0.5

possessesflavonoid and lectin, which have recently been used in cancer therapy and have resulted in promising outcomes (20). In another study, Ivanova et al investigated the lipid profile enhancement and serum antioxidant potential of normalvolunteers between the ages of 20 and 58 after soaking the fruit of *S. ebulus*. In this analysis, significant quantities of TAC, TPC, and AC were contained in the soaked fruit of this plant, and 200 mL of the *S. ebulus* soaked fruit was eaten by a community of human volunteers every day for onemonth. The blood samples were obtained before and afterthe experiment, and there was a substantial drop in triglycerides, total cholesterol, and LDL-C at the end of the analysis. However, the HDL-C/LDL-C proportion represented an enhancement. The antioxidant potential of serum and total thiol concentrations increased as well. The findings showed how the lipid profile and the antioxidant capacity in humans can be improved usingthis plant (21). Ferrares et al fromthe University of Valladolid (Spain) also examined the effects of *Sambucus nigra* (a strainthat is highly similar to *S. ebulus*) and *S. ebulus* itself on cancer therapyand reportedpositive results in this regard. This paper provides evidence that ahemoglobin targeting cancer cell isproduced using both *S. ebulus* and *S. nigra* (22). The broth microdilution process, as previously explained in detail, was used for the analysis in order to improve final precision. To this end, 20 individuals with denturestomatitis were examined in this research. These samples were then defined, followed by precisely specifying what sort of candidawas hosted by each individual. It is worth remembering that the clinical studies on a specific fungus (e.g., *C. albicans*)have produced contradictory effects in multiple individuals, but this is also the case in the licensed medication,nystatin. The origin of this variation is the discrepancy between the host setting and the difference between the usual floras of each checked individual. Candida strains in this analysis comprised*C. albicans* (n= 9), *C. tropicalis* (n= 4), *C. glabrata* (n= 4), *C. parapsilosis* (3= n). Eventually, the impact of the *S. ebulus* extract was evaluated in comparison with nystatin. Promising results were obtained, and the anti-candidal characteristics of this plant were demonstrated as well.

References

1. Kandil O, Radwan NM, Hassan AB, Amer AMM, El-Banna HA, Amer WMM. Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. J Ethnopharmacol. 1994;44(1):19-24. doi: 10.1016/0378-8741(94)90094-9.
2. Neville BW, Damm DD, Allen C, Chi AC. Oral and Maxillofacial Pathology. Elsevier Health Sciences; 2015.
3. Little JW, Miller C, Rhodus NL. Dental Management of the Medically Compromised Patient-E-Book. Elsevier Health Sciences; 2017.
4. Ruddock PS, Liao M, Foster BC, Lawson L, Arnason JT, Dillon JA. Garlic natural health products exhibit variable constituent levels and antimicrobial activity against *Neisseria gonorrhoeae*, *Staphylococcus aureus* and *Enterococcus faecalis*. Phytother Res. 2005;19(4):327-34. doi: 10.1002/ptr.1667.
5. Saeki Y, Ito Y, Shibata M, Sato Y, Okuda K, Takazoe I. Antimicrobial action of natural substances on oral bacteria. Bull Tokyo Dent Coll. 1989;30(3):129-35.
6. Jiménez P, Tejero J, Cordoba-Diaz D, Quinto EJ, Garrosa M, Gayoso MJ, et al. Ebulin from dwarf elder (*Sambucus ebulus* L.): a mini-review. Toxins. 2015;7(3):648-58. doi: 10.3390/toxins7030648.
7. Rahimi-Esboei B, Ebrahimzadeh MA, Gholami S, Falah-Omrani V. Anti-giardial activity of *Sambucus ebulus*. Eur Rev Med Pharmacol Sci. 2013;17(15):2047-50.
8. Schwaiger S, Zeller I, Pölzelbauer P, Frotschnig S, Laufer G, Messner B, et al. Identification and pharmacological characterization of the anti-inflammatory principal of the leaves of dwarf elder (*Sambucus ebulus* L.). J Ethnopharmacol. 2011;133(2):704-9. doi: 10.1016/j.jep.2010.10.049 .
9. Süntar IP, Akkol EK, Yalçin FN, Koca U, Keleş H, Yesilada E. Wound healing potential of *Sambucus ebulus* L. leaves and isolation of an active component, quercetin 3-O-glucoside. J Ethnopharmacol. 2010;129(1):106-14. doi: 10.1016/j.jep.2010.01.051.
10. Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B, Yankova T. Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytother Res. 2006;20(11):961-5. doi: 10.1002/ptr.1985.
11. Ebrahimzadeh MA, Enayatifard R, Khalili M, Ghaffarloo M, Saeedi M, Yazdani Charati J. Correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents of some medicinal plants. Iran J Pharm Res. 2014;13(3):1041-7.
12. Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Todorovic B, Veberic R, Stampar F, et al. Investigation of anthocyanin profile of four elderberry species and interspecific hybrids. J Agric Food Chem. 2014;62(24):5573-80. doi: 10.1021/jf5011947.
13. Ding M, Feng R, Wang SY, Bowman L, Lu Y, Qian Y, et al. Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. J Biol Chem. 2006;281(25):17359-68. doi: 10.1074/jbc.M600861200.
14. Girbés T, Ferreras JM, Arias FJ, Stirpe F. Description, distribution, activity and phylogenetic relationship of ribosome-inactivating proteins in plants, fungi and bacteria. Mini Rev Med Chem. 2004;4(5):461-76. doi: 10.2174/1389557043403891.
15. Stirpe F. Ribosome-inactivating proteins. Toxicon. 2004;44(4):371-83. doi: 10.1016/j.toxicon.2004.05.004.
16. Lapadula WJ, Sánchez Puerta MV, Juri Ayub M. Revising the taxonomic distribution, origin and evolution of ribosome inactivating protein genes. PLoS One. 2013;8(9):e72825. doi: 10.1371/journal.pone.0072825.
17. Asgarpanah J, Hashemi SJ, Hashemi E, Askari K. In vitro antifungal activity of some traditional Persian medicinal plants on pathogenic fungi. Chin J Integr Med. 2017;23(6):433-7. doi: 10.1007/s11655-015-2181-7.
18. Salehzadeh A, Asadpour L, Naeemi AS, Houshmand E. Antimicrobial activity of methanolic extracts of *Sambucus ebulus* and *Urtica dioica* against clinical isolates of

methicillin resistant Staphylococcus aureus. Afr J Tradit Complement Altern Med. 2014;11(5):38-40. doi: 10.4314/ajtcam.v11i5.6.

19. Jabbari M, Hashempur MH, Emami Razavi SZ, Raeisi Shahraki H, Kamalinejad M, Emtiazy M. Efficacy and short-term safety of topical dwarf elder (*Sambucus ebulus* L.) versus diclofenac for knee osteoarthritis: a randomized, double-blind, active-controlled trial. J Ethnopharmacol. 2016;188:80-6. doi: 10.1016/j.jep.2016.04.035.
20. Ivanova D, Tasinov O, Kiselova-Kaneva Y. Improved lipid profile and increased serum antioxidant capacity in healthy volunteers after *Sambucus ebulus* L. fruit infusion consumption. Int J Food Sci Nutr. 2014;65(6):740-4. doi: 10.3109/09637486.2014.898256.
21. Shariateh M. Extraction of Medicinal Plants and Methods of Their Identification and Evaluation. Tehran: Mani Publications; 1991:6-14. [Persian].
22. Ferreras JM, Citores L, Iglesias R, Jimenez P, Girbes T. Sambucus ribosome- inactivating proteins and lectins. Toxic plant Proteins.2010; 18: 107-131.