J HerbMed Pharmacol. 2015; 4(4): 138-141.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Antifungal effects of *Zataria multiflora* and *Nigella sativa* extracts against *Candida albicans*

Hassan Moghim[1], Simin Taghipoor[1], Najmeh Shahinfard[2], Soleiman Kheiri[3], Ramin Panahi[1]

[1]Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Department of E-learning in Medical Education, Virtual School, Tehran University of Medical Sciences, Tehran, Iran
[3]Social Health Determinants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 12 May 2015
Accepted: 25 September 2015

Keywords:
Candida albicans
Zataria multiflora
Nigella sativa

ABSTRACT

Introduction: Candidiasis is a fungal infection caused by *Candida albicans*. Recent studies suggest that the side effects of herbal drugs with significant therapeutic effects are far less than chemical drugs. This study was therefore, conducted to examine antifungal activities of *Zataria multiflora* and *Nigella sativa* extracts on *C. albicans*.
Methods: Powders of *Z. multiflora* and *N. sativa* were macerated with ethanol 70% and evaporated at 38˚C by rotary evaporator. The suspension of *C. albicans* was prepared according to McFarland at a concentration of approximately 0.5-2.5 × 10^3 CFU/ml. Testing was performed according to microbroth dilution in 96-well micro-dilution plates. Minimum inhibitory concentration (MIC), MIC50%, MIC90% and minimum fungicidal concentration (MFC) were separately evaluated by counting the fungal colonies for *Z. multiflora* and *N. sativa*.
Results: The measured values of MIC, MIC50%, MIC90% and MFC of *Z. multiflora* on the *C. albicans* were 0.13, 0.38, 0.74 and 1.03 mg/ml, and those of *N. sativa* were 10, 27.7, 52.3 and 72.3 mg/ml, respectively.
Conclusion: The results indicate that both *Zataria multiflora* and *Nigella sativa* extracts are effective against *Candida albicans,* but the former species has the highest antifungal activity. If the clinical trials confirm the results of this study, *Z. multiflora*, as a new antifungal agent by replacing chemical drugs can be used to develop antifungal medicinal herbs.

Implication for health policy/practice/research/medical education:
The results indicate that *Zataria multiflora* and *Nigella sativa* extracts are effective against *Candida albicans*, but the former species has the highest antifungal activity. If the clinical trials confirm the results of this study, *Z. multiflora*, as a new antifungal agent by replacing chemical drugs, can be used to develop antifungal medicinal herbs.
Please cite this paper as: Moghim H, Taghipoor S, Shahinfard N, Kheiri S, Panahi R. Antifungal effects of Zataria multifora and Nigella sativa extracts against Candida albicans. J HerbMed Pharmacol. 2015;4(4):138-141.

Introduction

Candidiasis is a primary or secondary infection involving a member of the genus *Candida*. Essentially, this disease is an infection caused by *Candida albicans*. The clinical manifestations of the disease are extremely varied, ranging from acute and/or chronic to episodic involvement probably localized to the mouth, throat, skin, scalp, bronchi, lungs, or the gastrointestinal tract or systemic as in septicemia, endocarditis, and meningitis. Since *C. albicans* is an endogenous species, the disease represents an opportunistic infection (1-3).
Thymus vulgaris (*Zataria multiflora*) is a shrub plant in the family Lamiaceae. It is an aromatic and mountain herb which is commonly used as a spice to flavor food (4,5). Recent studies suggest that the side effects of herbal drugs with significant therapeutic effects are far less than chemical drugs (6). Researches have shown that *Z. multiflora* is effective in treating many diseases, particularly in bacterial and fungal infections and parasitic diseases (7).
Nigella sativa is from the order Ranunculales in the family Ranunculaceae. This aromatic plant with tiny and dark seeds is usually used to flavor food such as bread. *Nigella sativa* is a plant from the buttercup family which grows as self-propelled in various parts of Europe and Asia including Iran (8).
In recent years much research has been done on the ef-

***Corresponding author:** Hassan Moghim, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran. Tel.: +98 381 3346622, Fax: +98 381 3330709, Email: Hassan.moghim@yahoo.com

fectiveness of *N. sativa* extract including thymoquinone, thymohydroquinone, dithymoquinone, thymol and carvacrol, nigellicine, nigellimine-N-oxide, nigellidine and alpha-hedrine compounds (6).

Many reports have been published regarding several pharmacological effects of *N. sativa* extract, some of which have been identified through application of modern techniques, including antimicrobial activity of *N. sativa* oil (extract) and other through medicinal compounds such as thymoquinone and thymohydroquinone, on some bacteria including *Staphylococcus aureus* and particularly recently on *Pseudomonas aeruginosa*, Both *Escherichia coli* and *C. albicans*. Thymoquinone have been reported to be effective on *Aspergillus* (7), and some opportunistic fungi which can cause mycotic infection in patients with AIDS and other immunodeficiency syndromes (7). This study was conducted to examine antifungal activities of *Zataria multiflora* and *Nigella sativa* extracts on *C. albicans.*

Materials and Methods

To prepare extracts, *Z. multiflora* and *N. sativa* were purchased from a reputable grocery and after separation of brushwood with an electric mill, they were pulverized. The resulting powder was passed through a No. 10 sieve and then poured into the flasks and mixed with 300 cc of 70% ethanol. The solution was kept for 72 hours in a dry environment. The solution was passed through the filter paper and then transferred into percolation system for extraction. At a temperature of 38°C of the system, the extract was transferred into the incubator with removing alcohol completely. Finally, various concentrations of the extracts were prepared using distilled water and 0.5 ml of dimethyl sulfoxide (9).

Preparation of *C. albicans* suspensions

The standard fungal strain (code No. PTCC5027) used in this study was provided from Scientific Research Center of Iran. *C. albicans* was cultured on dextrose agar medium at 35°C. After 24 hours, some colonies were transferred to 1 ml normal saline to prepare a solution with a concentration of 0.5 McFarland 1.5×10^6 CFU/ml (neobar slides were used for colony counting). The resulting solution was diluted to a ratio of 1:1000 and suspensions with concentrations of $(0.5\text{-}2.5)\times10^3$ CFU/ml were provided (10).

Preparation of *Z. multiflora* and *N. sativa* extracts

Different dilutions were prepared from *Z. multiflora* and *N. sativa* extracts using microbroth dilution according to the method proposed by the National Committee for Clinical Laboratory Standards for yeasts (NCCLSM27-A). Thus, two-fold dilution of the extract was poured into the first well of 96-well plates, and subsequent dilutions were prepared by serial dilution (10,11).

Different concentrations of the extracts prepared were as follows:

Z. multiflora: 0.093, 0.13, 0.185, 0.38, 0.5, 0.74, 1, 1.03, 1.25, 1.5 mg/ml and *N. sativa :* 5, 10, 20, 30, 40, 50, 60 and 70 mg/ml.

100 µl of each dilution of the extract was added separately to the socket of 96-well plates containing 100 ml of Sabouraud broth liquid medium (Sabouraud broth). Then 100 ml of yeast cell suspension in volumes equivalent to 2500 cells/ml was inoculated into all the sockets, except the control plates. The plates were shaker-incubated at 35°C for 48 hours. After incubation, 10 µl of the contents of each socket was cultured on Sabouraud dextrose agar medium and left for 48 hours at 35°C. After this period, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) (CFU) were separately evaluated based on counting the fungal colonies for *Z. multiflora* and *N. sativa* extracts and compared with the control group (11,12).

Results

Z. multiflora extract

The measured values of MIC, MIC50%, MIC90% and MFC of *Z. multiflora* extract on the *C. albicans* were 0.13, 0.38, 0.74 and 1.03 mg/ml, respectively (Figure 1).

N. sativa extract

The measured values of MIC, MIC50%, MIC90% and MFC of the *N. sativa* were 10, 27.7, 52.3 and 72.3 mg/ml, respectively (Figure 2).

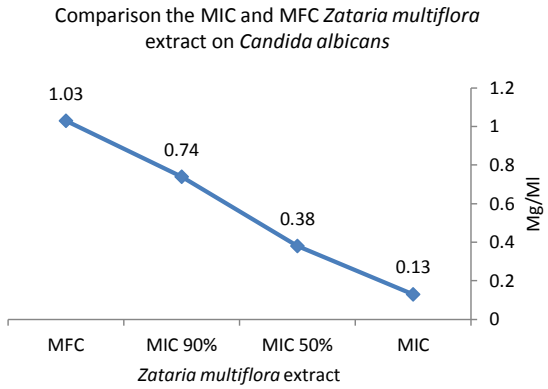

Figure 1. Comparison of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Zataria multiflora* extract on *Candida albicans*.

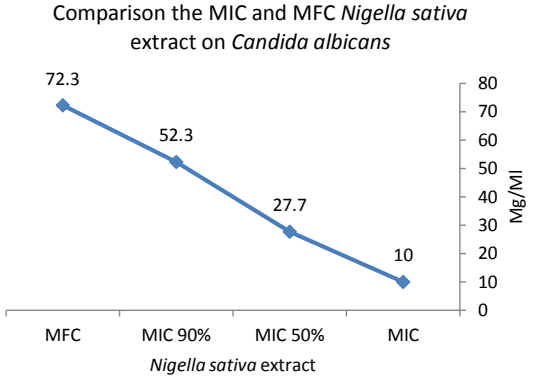

Figure 2. Comparison of the minimum inhibitory concentration and minimum fungicidal concentration of *Nigella sativa* extract on *Candida albicans*

Comparison of inhibitory and fungicidal effects of extracts

The logistic regression test showed that there were significant differences between the inhibitory and fungicidal effects of each of the plants extracts ($P<0.05$). *Z. multiflora* had the highest inhibitory and fungicidal effects on *C. albicans* but *N. sativa* had the lowest antifungal effect (Figure 3). The measured values of MIC, MIC50%, MIC90% and MFC of *Z. multiflora* and *N. sativa* extracts are compared in Table 1.

Discussion

Candidiasis is a primary or secondary infection involving a member of the genus *Candida.* Essentially, this disease is an infection caused by *C. albicans.* The clinical manifestations of the disease are varied, including acute, chronic and episodic involvement which is occasionally localized to the mouth, throat, skin, scalp, bronchi, lungs, or the gastrointestinal tract or systemic as in septicemia, endocarditis, and meningitis. Since *C. albicans* is an endogenous species, the infection is opportunistic (1-3).

Various studies have shown that many species of *Candida* have developed resistance to antifungal drugs. Nowadays traditional medicine and the use of herbal medicines to treat fungal infections are important because herbal medicines have fewer side effects and are less likely to develop drug resistance compared with chemical ones (1-3).

Due to difficulties in treatment of candidiasis, it is necessary to find new antifungal drugs. This increases the spectrum of antifungal drugs and eliminates the drug resistant strains. Herb plants can be beneficial for their therapeutic effects.

Akbari evaluated antifungal activities of *Thymus vulgaris* L. and *Origanum vulgare* L against fluconazol-resistant susceptible *C. albicans* isolates and found that the essence and extract of both plants were able to inhibit the growth of the isolates susceptible to fluconazole in a predictably concentration-dependent manner. The methanol extracts of *Origanum* had the highest antifungal effect with 0.49 mg/ml MIC (13). In our study, the obtained MIC of Z. *multiflora* extract was 0.13 mg/ml for *C. albicans*, which is greater than the effect on *C. albicans* obtained for this extract in Akbari's study. The contrasting results could be due to the different species of *Z. multiflora*, plant chemical compounds and/or methodologies.

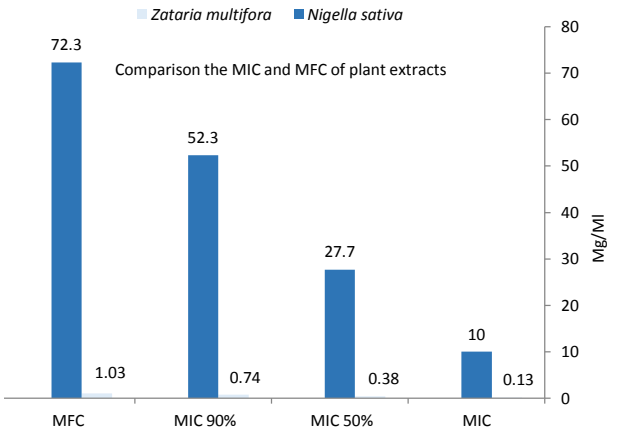

Figure 3. Comparison of minimum inhibitory concentration (MIC), MIC50%, MIC90%, and minimum fungicidal concentration of *Zataria multifora* and *Nigella sativa* extracts on *Candida albicans.*

Table 1. MIC, MIC50%, MIC90% and MFC of *Zataria multifora* and *Nigella sativa* extracts on *Candida albican* (mg/ml)

Plant	MIC	MIC50%	MIC90%	MFC
Zataria multiflora	0.13*	0.38	0.74	1.03
Nigella sativa	10	27.7	52.3	72.3

Abbreviations: MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration.

In another study by Fuladi et al (14), the inhibitory effects of clotrimazole cream and herbal vaginal thyme cream were compared and the obtained MIC of *Z. multiflora* methanol and ethanol extracts were estimated to be 0.079 and 0.125 mg/ml, respectively.

Katiraei et al (15) compared the MIC of essential oils of *Z. multiflora*, Geranium and Artemisia with regard to the growth of the *C. albicans* isolates resistant to azole drugs. They showed that the MIC levels of essential oils of the plants were statistically significant from those of azoles. In that study, the obtained MIC of *Z. multiflora* for *C. albicans* was 0.18 mg/ml.

The effect of alcoholic extract of thyme on the growth of *Aspergillus* was investigated and found that the use of plant extracts in food was useful for control of fungal growth without production of toxins in foods (16).

In another study performed by Gandomi Nasr Abadi et al (17), the effect of essential oils of *Z. multiflora* on *Aspergillus flavus* was evaluated . It was found that all concentrations of oils had a significant effect on the growth and sporulation of *A. flavus*. Also, they reported the levels of MIC and MFC 400 and 1000 PPM, respectively. This extract has inhibitory effects on the mold and hence, it is recommended as alternatives to chemical preservatives in the food industry (17).

In a study by AL-Quarashi et al (18) on the antifungal activity of *N. sativa* extract (thymoquinone) against *Aspergillus niger* and amphotericin b was investigated. It was found that the oil extract of *N. sativa* affected the growth of *A. niger* by 100% and could inhibit the growth of *A. niger* by 93.8%. The antifungal activity of *N. sativa* could be attributed to the thymoquinone.

To the best of our knowledge, No study has yet been conducted to assess the MIC and minimum bactericidal concentration of *N. sativa* on *C. albicans*. Further studies will be needed to investigate antifungal activities of *N. sativa*.

In our study, the obtained values of MIC, MIC50%, MIC90% and MFC of *Z. multiflora* extract on the *C. albicans* were respectively 0.13, 0.38, 0.74 and 1.03 mg/ml, which are consistent with other studies. Therefore Z. *multiflora* extract has antifungal effect against *C. albicans* and can be used as an antifungal agent to treat fungal infections such as candidiasis.

In the present study, the obtained values of MIC, MIC50%, MIC90% and MFC of *N. sativa* were 10, 27.7, 52.3 and 72.3 mg/ml, respectively. Both *Z. multiflora* and *N. sativa* ex-

tracts are able to inhibit the growth of *C. albicans*, but the former species has a greater impact on fungal infections.
The major constituents of *Z. multiflora* are carvacrol, thymol, and phenolic compounds with antifungal activities (5). In recent years much research has been done on the effectiveness of *N. sativa* extract including thymoquinone, thymohydroquinone, dithymoquinone, thymol and carvacrol compounds. It has been shown that the antifungal activity of *N. sativa* could be attributed to thymol and carvacrol contents (6).

Conclusion

In this study, antifungal effects of *Z. multiflora* and *N. sativa* extracts were determined for *C. albicans*. It was shown that, an increase in the concentration of plant extracts caused fungal growth inhibition. Antifungal drugs are expensive with side effects and harmful for patients which frequently lead to drug resistance. As herbal medicines have fewer side effects and are less likely to develop drug resistance compared with chemical ones, they could be useful for treatment.
The results of the current study show that *Z. multiflora* extract plays a significant role in preventing of *C. albicans* growth. If the clinical trials confirm the results of this study *Z. multiflora*, as a new antifungal agent by replacing chemical drugs can be used to develop antifungal medicinal herbs.

Acknowledgements

This paper was derived from MD thesis of Ramin Panahi. The authors gratefully thank the Medical Plants Research Center staff for their assistance.

Authors' contributions

HM prepared the proposal and the manuscript draft as well as conducting the study. All authors contributed to the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

Conflict of interests

The authors declare no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

The research was funded by Deputy of Shahrekord University of Medical Sciences, Iran (code number: 290-75-01-1389).

References

1. Moghim H, Taghipour S, Shahinfard N, Kheiri S, Heydari S, Rafieian S. Antifungal effects of Allium ascalonicum, Marticaria chamomilla and Stachys lavandulifolia extracts on Candida albicans. J HerbMed Pharmacol. 2014;3(1):9-14.
2. Moghim H. Role of cellular immunity in prevention of Candidiasis in Leukemia and Lymphomatic patients using flowcytometry method. J Shahrekord Univ Med Sci. 2001;3(3):42-48.
3. Moghim H. Detection of CH50 and role of C3, C4 complement in patients with Candidiasis. J Shahrekord Univ Med Sci. 2000;2(1):62-65.
4. Hematkhah F. Medicinal Plants. 2nd ed. Iran: Asreketab Publisher; 2004: 48-118.
5. Velag G. Medicinalplans. 2nd ed. Iran: Ghoghnoos publisher; 2007:80-87.
6. Saad B, Azaizeh H, Said O. Tradition and perspectives of Arab herbal medicine: a review. Evid Based Complement Alternat Med. 2005;2:475-479.
7. Samsam SH. Breedingand propagation of medicinal plants. Iran: Mani Publisher; 2005:125-7.
8. Shabooee E. Medicinal Plants and Fruits. Iran: Barbod Publisher; 2001.
9. Sharafati R, Sherafati F, Rafieian-Kopaei M. Biological characterization of Iranian walnut (Juglans regia) leaves. Turk J Biol. 2011;35:635-639.
10. Willinger B, Apfalter P, Hirschl AM, Makristathis A, Rotter M, Seibold M. Susceptibility testing of Candida species: comparison of NCCLS microdilution method with Fungitest. Diagn Microbiol Infect Dis. 2000;38(1):11-15.
11. Moghim H, Taghipour S, Shahinfard N, Kheiri S, Khabbazi1 H. Comparative study on the antifungal activity of hydroalcoholic extract of Iranian Propolis and Royal jelly against Rhizopus oryzae. J HerbMed Pharmacol. 2015;4(3):89-92.
12. Razzaghparast A, Shams Ghahfarokhi M, Yadegari MH, Razzaghi Abyaneh M. Antifungal effects of Allium cepa and some azoles in intact forms and in combinations to each other against pathogenic yeasts. Kowsar Med J. 2008;13(2):103-113.
13. Akbari S. Antifungal activity of Thymus vulgaris L. and Origanum vulgare L. against fluconazol-resistant and susceptible Candida albicans isolates. J Med Plants. 2007;6(1):53-62.
14. Fuladi Z, Afshari P, Gharibi T, Dabbagh MA. The comparison oF Zataria multiflora boiss (Avishan Shirazi) and Clotrimazol vaginal cream in the treatment of candidiasis vaginitis. Iranian South Medical of Journal. 2009;12(3):214-224.
15. Katiraei F, Eydi S, Bahonar Ar, Zarinfar H, Khosravi A. Comparision of MICS of some Iranian herbal essences against azole resistance and azole susceptible of Candida albicans. Journal of Medicinal Plants. 2008;7(27):37-44.
16. Javanmard M, Ramazan Y. Application of edible coatings incorporated Avishan-E Shirazi (Zalaria multiflora) alcoholic extract for inhibition of Aspergillus flavus growth on the pistachio kernel. J Med Plants. 2009;8(30):61-65.
17. Gandomi Nasr Abadi H, Misaghi A, Akhoundzadeh Basti A, Khosravi AR. Effects of Zataria multiflora Boiss essential oil on Aspergillus flavus. Journal of Medicinal Plants. 2008;7(27):45-51.
18. AL-Quarashi AR, Akhtar N, Al-Jabre S, AL-Akloby O, Randhawa MA. Anti-fungal activity of Thymoquinone and Amphotericin B against Aspergillus niger. Scientific Journal of King Faisal University. 2007;8(1):143-8.