Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 6:72-76

In vitro Anti-Candidal effects of Aqueous and Methanolic Extracts of Walnut (*Juglansregia*) Tree Fruit Peel in Comparision with Fluconazole

Ali Naseri[1]*, AbdolmajidFata[1] and Seyyed Ali Akbar Shamsian[2]

[1]*Department of Medical Mycology and Parasitology, Imam Reza Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Department of Medical Mycology and Parasitology, Quaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
Corresponding E-mail: naseria@mums.ac.ir

ABSTRACT

The appearance of fungal species resistant to antifungal drugs among the Candida genus and also their numerous side effects, convince the researchers to work on new therapeutic methods with minimal side effects for humans fungal infections. The aim of this study was to assess of anticandidal activities of aqueous and methanolic extracts of walnut Fruit Peel on common Candida species. In this study, the antifungal effects of aqueous and methanolic extracts of fruit peel of walnut (Juglansregia) and fluconazole against four Candida species were evaluated using broth Microdilution method, based on the Clinical and Laboratory Standards Institute (CSLI) M38-A3 guideline. And then the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of these extracts and antifungal drug were obtained for tested Candida species. Both aqueous and methanolic extracts of fruit peel of walnut showed antifungal effects against Candida species. The MIC of fluconazole, aqueous and methanolic extracts of walnut fruit peel for different Candida species were 0.001- 0.032, 6.25-50, 3.125-25 mg/ml, respectively. The MFC of fluconazole, and methanolic extracts of fruit peel of walnut were 0.001-0.032 and 6.25-25 mg/ml, respectively. The aqueous extracts of walnut fruit peel were without fungicidal effects. The most important result of this study is to show that fruit peel extracts of walnut are effective against named fungal species and it looks promising that in future, we can obtain some effective antifungal agents with minimal side effects from shallot extract.

Keywords: Candida, walnut (*Juglansregia*), aqueous and methanolic extracts, broth microdilution method

INTRODUCTION

The incidence and prevalence of invasive fungal infections have been increasing since the 1980s, especially in immunocompromised patients and hospitalized patients with serious underlying diseases [1, 2]. *Candida* species have isolated from healthy individual's mucosal oral cavity, gastrointestinal tract and vagina as normal flora [3] and are responsible for various clinical manifestations e.g. mucocutaneous infections, bloodstream infections [4]. More than 17 different *Candida* species are isolated from human infections; however, approximately 90% of invasive infections are caused by 5 species including; *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*[5].The factors that contribute to the increase of these infections are immunocompromised patients with intravenous catheters for long times, increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation [6].

Currently, an increase in the number of resistant yeasts to antifungal drugs is identified worldwide [7].Therefore, new antimicrobial agents are needed for treatment of humans and animals infections by drug resistant pathogens [8]. In the recent decades, there has been much interest in natural products as new antimicrobial agents. Antimicrobial effects of different extracts from traditional medicinal plants have been tested and proved [9].Main factors for development of new antimicrobial agents with natural origin are including: the increasing resistance to available drugs, the problems caused by antimicrobial agents added in food and public's pressure on the food industry to produce products without chemical preservatives [10, 11]

The *Juglans* genus (family Juglandaceae) includes several species and is distributed worldwide [12].Walnut (*Juglansregia* L.)Fruit Peel has several properties such as anti-inflammatory, anticancer, blood purify, diuretic, depurative, and laxative activities [13].Some studies have proved the antimicrobial effects of walnut products, particularly of Fruit Peel[14, 15]. The extract of walnut Fruit Peel inhibited the growth of several Gram-positive bacteria and Gram-negative bacteria[16].

The aim of this study is evaluation of anti-candidal activities of aqueous and methanolic extracts of walnut Fruit Peel on common *Candida* species including: *Candida albicans*, *Candida glabrata*, *Candida tropicalis* and *Candida krusei*.

MATERIALS AND METHODS

The Fruit Peel of walnut tree (*Juglansregia*) was collected from Bardaskan city, Khorasan Razavi province, Iran.

Extraction

The Fruit Peel of walnuts were dried and ground into a powder by a blender. The powder was extracted by Maceration method for 48 hours. The extraction was performed using two solvents; water and methanol 70%. The methanolic and aqueous extracts were filtered and evaporated to produce pure extracts by a vacuum rotary evaporator. Afterwards, the extracts were dried and stored at -20°c.

Microorganisms and Inoculum preparation

The *Candida* species, including *Candida albicans* (PTCC 5027), *Candida glabrata*(PTCC 5297), *Candida tropicalis* (PTCC 5028) and *Candida krusei* (PTCC5295) were obtained from the Iranian Research Organization for Sciences and Technology, Tehran, Iran.

Stock inoculum suspensions of the *Candida* species were obtained from 24h cultures on Sabouraud Dextrose Agar (SDA) (Conda, Spanish) at 35°C. A few colonies were suspended in sterile normal saline. The cell densities of the *Candida* suspensions were measured spectrophotometrically at a wavelength of 530 nm, and the transmition was adjusted to 75-77%. The suspensions were diluted to yield final inoculums concentration ranged from 0.5-2.5× 10^3 CFU/ml.

Antifungal susceptibility testing

Antifungal susceptibility test was performed according to the Clinical and Laboratory Standards Institute (CLSI) using micro dilution method as describe previously with minor modification [17, 18] and RPMI 1640 medium (Sigam, USA) supplemented with L- glutamine, without sodium bicarbonate buffered at PH 7.0 with 0.165 M morpholinepropaesulfonic acid (MOPS) (Sigma, Aldrich Chemie).

The all extracts of walnut Fruit Peel were dissolved in dimethylsulphoxide(DMSO). Andfluconazole (Daroupakhsh, Iran)were dissolved in sterile distilled water. Serial dilutions of the extracts and drug were performed in 96-well microplates trays using RPMI 1640. The concentrations of extracts were prepared at a range of 0.02441-50 mg/ml. MIC was determined after incubations of the 96-well microplates at 35°C for 24-48 hours. The growth in each well was compared with control wells. MIC_s were visually determined and defined as the lowest concentration inhibiting growth of Candida species. MFC was determined by culturing 20 µl of the mixed broth culture from the wells with no turbidity on SDA at 35°C for 24 hours on the MIC assay. The MFC was defined as the lowest concentration completely inhibiting the growth of the agents.

RESULTS

The MIC of methanolic and aqueous extracts of walnut Fruit Peel and fluconazole are presented in Table 1. The MIC values of methanolic and aqueous extracts of walnut Fruit Peel ranged from 3.125 to 25 mg/ml and 6.25 to 50 mg/ml, respectively. The least sensitive species to methanolic extract were *Candida albicans* and *Candida glabrata* and the more sensitive species were *Candida tropicalis* and *Candida krusei*. The later species were also more sensitive to aqueous extract. The MFC values of methanolic extracts and fluconazole ranged from 6.25 to 25 mg/ml and 0.001 to 0.032 mg/ml, respectively and the aqueous extracts of walnut fruit peel were without fungicidal effects [Table 2].

Table 1. MIC of methanolic and aqueous extracts of walnut Fruit Peel and fluconazole on *Candida* species

Candida species	MIC (mg/ml)		
	Methanolic extract	Aqueous extract	Fluconazole
Candida albicans	25	25	0.001
Candida glabrata	25	50	0.032
Candida tropicalis	3.125	6.25	0.016
Candida krusei	3.125	12.5	0.002

Table 2. MFC of methanolic and aqueous extracts of walnut Fruit Peel and fluconazole on *Candida* species

Candida species	MFC (mg/ml)		
	Methanolic extract	Aqueous extract	Fluconazole
Candida albicans	25	-	0.001
Candida glabrata	25	-	0.032
Candida tropicalis	6.25	-	0.016
Candida krusei	6.25	-	0.002

DISCUSSION

The emergence of drug-resistant strain of various fungal species such as *Candida*, *Dermatophytes* and *Cryptococcus neoformans* has prompted into developing new strategies for treatment of fungal infections which may be less toxic to human [19].Some studies have demonstrated the antibacterial and antifungal effects of walnut extract. Noumi et al. evaluated anticandidal effect of three type extracts (methanol, acetone and ethyl acetate) of *Salvadorapersica* and *Juglansregia* on different *Candida* species at 2009. In this study, it is indicated that ethyl acetate extract of walnut Fruit Peel was more effective than other extracts. *Candidaalbicans* was the most sensitive species to ethyl acetate extract of *juglansregia* Fruit Peel and *Candida glabrata* was the most resistant species to this extract[12]. In other study, Citoglu et al. investigated antibacterial and antifungal effects of methanolic extract of walnut leaves. The results of this study showed that *Candida albicans* in comparison with *Candida glabrata* and *Candida krusei* was more sensitive to walnut extract[19].In a study by Yigt et al., antifungal and antibacterial activities of aqueous and methanolic extracts of leaves and husk of walnut were proved. The results showed that the MIC values of aqueous extracts of leaves and husk on three *Candida* species were 2.5 mg/ml and the MIC of alcoholic extracts on all *Candida* species were 1.5 mg/ml[20].In a study at 2010, Coban et al. assessed antibacterial and antifungal properties of hydro-alcoholic extract of walnut leaves. In this study, the most sensitive species was *Candida utilis* and *Candida tropicalis* was the most resistant species in comparison with other tested *Candida* species[21].

The results of present study are in agreement with the results of some researchers, but differ from the other studies [20, 21].It seems that the main effective factors in differences between the results of these studies might be include extraction method, fungal species differences, soil ingredients, climatic conditions of the regions, age of plants, and time of collection of plant materials [22, 23].

The results indicated that anticandidal activity of methanolic extract of walnut Fruit Peel on tested *Candida* species is more than aqueous extract. It shows that effective ingredients of walnut Fruit Peel with antifungal effects dissolve in methanol better than water, therefore; it is recommended that alcoholic extracts are used for prospective complementary researches.

Comparison of the MIC of methanolic and aqueous extracts of walnut with the MIC values of fluconazole indicated that the effective concentrations of extracts on tested *Candida* species are higher than fluconazole. According to the

fact that the methanolic and aqueous extracts of walnut were crude extracts and contain ineffective ingredients, it seems that the differences between the MIC of extracts and fluconazole are reasonable.

Interestingly, the MFC results show that the aqueous extract of walnuts has no fungicidal effects and only stops *Candida* species growth, whereas the alcoholic extract kill the *Candida* cells and even inhibit growth of fungi by killing the *Candida albicans* and *Candida glabrata* cells.

CON**CLUSION**

The most important result of this study is to demonstrate the effectiveness of walnut extract against *Candida* species and it looks promising that in future, we can achieve an appropriate antifungal drug with minimal side effects from walnut extract for treatment of candidiasis, but *in vivo* studies are required for evaluation of pharmacokinetic effects of walnut.

Acknowledgments
This work was financially supported by Grant Number 901074 from Vice-Chancellor for research Affairs of Mashhad University of Medical Sciences.

REFERENCES

[1]Arendrup MC, Fuursted K, Gahrn-Hansen B, Jensen IM, Knudsen JD, Lundgren B, et al. Seminational surveillance of fungemia in Denmark: notably high rates of fungemia and numbers of isolates with reduced azole susceptibility. J ClinMicrobiol 2005; 43: 4434–4440.
[2]Espinel-Ingroff A, Canton E, Peman J, Rinaldi MG, Fothergill AW. Comparison of 24-hour and 48-hour voriconazole MICs as determined by the Clinical and Laboratory Standards Institute broth microdilution method (M27–A3 document) in three laboratories: results obtained with 2,162 clinical isolates of *Candida* spp. and other yeasts. J ClinMicrobiol 2009; 47: 2766–2771.
[3]Shao LC, Sheng CQ, Zhang WN. Recent advances in the study of antifungal lead compounds with new chemical scaffolds. Yao XueXueBao 2007; 42: 1129–1136.
[4]Eggimann P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. Lancet Infect Dis 2003; 3: 685–702.
[5]Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the VITEK 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against *Candida* spp. J ClinMicrobiol 2007; 45: 3522–3528.
[6]Ortega M, Marco F, Soriano A, Almela M, Martinez JA, LopezJ, et al. *Candida* species bloodstream infection: epidemiology and outcome in a single institution from 1991 to 2008. J Hosp Infect 2011; 77: 157–161.
[7]Ingham CJ, Boonstra S, Levels S, de Lange M, Meis JF, Schneeberger PM. Rapid susceptibility testing and microcolony analysis of *Candida* spp. cultured and imaged on porous aluminum oxide. PLoS ONE 2012; 7:318-338.
[8]Wijesekera RB. Plant derived medicines and their role in global health. The Medicinal Plant Industry, Boca Raton: CRC Press; 1991.
[9]Evans CE, Banso A, Samuel OA. Efficacy of some nupe medicinal plants against Salmonella typhi: an in vitro study. J of Ethnopharmacology 2002; 80: 21-24.
[10]Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. IntJ Food Microbiol 2000; 56: 3-12.
[11]Proestos C, Chorianopoulos N, Nychas GJE, Komaitis M. RPHPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity.J of Agricultural and Food Chem 2005; 53: 1190-1195.
[12]NoumiE,SnoussiM, HajlaouiH, Valentin E, Bakhrouf A. Antifungal properties of *Salvadorapersica* and *Juglansregia* L. extracts against oral *Candida* strains. Eur J ClinMicrobiol Infect Dis 2010; 29: 81–88.
[13] Bhatia K, Rahman S, Ali M, Raisuddin S. In vitro antioxidant activity of *Juglansregia* L. bark extract and its protective effect on cyclophosphamide-induced urotoxicity in mice, Redox Report 2006; 11 (6): 273–279.
[14]Alkhawajah AM.Studies on the antimicrobial activity of *Juglansregia*.Am J Chin Med 1997; 25:175–180.
[15]Falahati M, Fateh R, Sharifinia S. Anti-candidal effect of shallot against chronic candidiasis. Iranian J of pharmacol& therapeutics. 2011; 10: 49-51.

[16]Zakavi F, GolpasandHagh L, Daraeighadikolaei A, Farajzadeh Sheikh A, Daraeighadikolaei A, LeilaviShooshtari Z. Antibacterial effect of *Juglansregia*bark against oral pathologic bacteria. Int J of Dentistry 2013; 5-10.
[17] Arji P, Naseri A, Rakhshandeh H, Najafzadeh M. Investigation of antifungal activity of methanol and aqueousextracts of walnut *(Juglansregia)* leaves and peel against candida species.Journal of Birjand University of Medical Sciences 2015; 22(2): 114-124.
[18]Johan HR, Barbara DA, Andes D, Arthington-Skaggs B, Brown SD, Chaturvedi V, et al. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standards-Third edition. Clin Lab Stand Inst. 2008; 28(14):13-25.
[19]Citoglu GS, Altanlar N. Antimicrobial activity of some plants used in folk medicine. J Fac Pharm Ankara 2003; 32: 159-163.
[20]Yigit D, Yigit N, Aktas E, Ozgen U. Antimicrobial activity of walnut (*Juglansregia* L.). J Turkish Society Microbiol 2009; 39: 7-11.
[21]Coban EP, Biyik H. Antimicrobial activity of the ethanol extracts of some plants natural growing in Aydin, Turkey. African J Microbial Res 2010; 4: 2318-2323.
[22]Fateh R, NasiriKashani MJ, Motevallian M, Falahati M, Yazdanparast A. In vitro antifungal activity of *Allium hirtifolium* in comparison with the miconazole. Med J the Islamic Republic Iran 2010; 24: 17-22.
[23]Aiyegoro OA, Okoh AI. Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysumpedunculatum.* Int. J. Mol. Sci. 2009; 10(11): 4990-5001.