Short Communication

Evaluation of the antifungal activity of olive leaf aqueous extracts against *Candida albicans* PTCC-5027

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Abstract

In this study, antifungal property of olive leaf extracts against *Candida albicans* PTCC-5027 was examined. Fresh olive leaf extracts were prepared using distilled water in a Soxhlet apparatus. The antifungal activity of the extract was analyzed by measuring the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), using the microdilution test and disc diffusion assay. The olive leaf aqueous extracts exhibited antifungal effects against the yeast with an MIC of 24 mg/ml, MFC of 48 mg/ml, and inhibition zone diameter of 21 mm. The results indicated the sensitivity of *Candida albicans* PTCC-5027 to olive leaf aqueous extracts.

Keywords: Antifungal, *Candida albicans*, Olive

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Introduction

Historically, olive leaf extracts have been extensively used in medicine against microbial diseases [1]. Olive leaf com-pounds such as oleuropein and its derivatives including hydroxytyrosol and tyrosol exhibit antimicrobial activities, which can reduce the risk of microbial infections. These compounds can inhibit viruses, retroviruses, bacteria, yeasts, fungi, molds, mycoplasmas, and other parasites, particularly in the gastrointestinal and respi-ratory tracts [2-4].

Candida albicans, which is the most com-monly found yeast on the mucosal membranes of humans, can adhere to epithelial tissues and cause superficial infections. Candidiasis can appear under certain circumstances and has become one of the major causes of morbidity and mortality among immunocompromised individuals worldwide [5].

Considering the increasing number of antimicrobial-resistant strains of microorga-nisms, it is important to employ safe and effective antifungal agents [6]. In this study, the antifungal effect of olive leaf extracts growing in Iran was assessed against a standard *C. albicans* strain (PTCC-5027) through *in vitro* studies.

Material and Methods

Preparation of olive leaf aqueous extracts

In this study, the olive leaves were collected in spring from a northern city in Iran (Rudbar). The leaves (50 g) were homogenized and extracted for 8 h in a Soxhlet apparatus with 250 ml of distilled water. The extracts were concentrated in a rotary evaporator and transferred into sterile vials to be maintained under refrigerated conditions until further use [4]. Sterilization was performed by passing the solution through a filter (pore size of 0.2 μm) [7].

Preparation of the standard strain of fungal cells

The fluconazole-resistant *C. albicans* PTCC-5027 was obtained from the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The strain was cultured on Sabouraud dextrose agar (SDA) and incubated at 37°C for 18 h.

Disc diffusion method

A small number of cultured *C. albicans* colonies were added to sterile normal saline to meet 0.5 McFarland turbidity standards [8]. Afterwards, 200 µl of 0.5 McFarland yeast was applied on the SDA plate, and discs impregnated with 10 µl of olive leaf crude extracts (50 mg/ml) were placed on the plate. A negative control was prepared, using distilled water to dissolve the olive leaf extracts. The plates were incubated at 37°C for 24 h [9, 10]. Antifungal activity was evaluated by measuring the inhibition zone diameter; it should be noted that the assay was repeated three times.

Determination of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

The MIC and MFC values were measured according to the Clinical and Laboratory Standards Institute (CLSI) method by using the microdilution test and RPMI 1640 medium containing L-glutamine (buffered to pH=7.0 and supplemented with 2% glucose). The olive leaf extracts were diluted with distilled water to produce two-fold serial dilutions, ranging from 1 to 144 mg/ml. The test tubes were then incubated at 35°C for 24 h [11].

MIC was defined as the lowest concentration inhibiting the growth of *C. albicans*. MFC was determined by culturing 20 µl of the mixed broth culture from the wells with no visible turbidity on SDA at 35 ºC for 24 h on the MIC assay. The MFC was defined as the lowest concentration completely inhibiting the growth of the yeast [12].

Statistical analysis

Mean comparison was carried out by two-way analysis of variance (ANOVA). The mean comparison was performed at the least significance level of $P<0.05$.

Results

The antifungal effect of olive leaf aqueous extracts on the tested yeast was quantitatively assessed by measuring MIC (µg/ml), MFC (µg/ml), and inhibition zone diameter (mm). MIC, MFC, and inhibition zone diameter of olive leaf extracts against *C. albicans* were 24 µg/ml, 48 µg/ml, and 21 mm, respectively. The results demonstrated the antifungal sensitivity of *C. albicans* PTCC-5027 to olive leaf aqueous extracts.

Discussion

The positive effects of olive tree and its products on health have been recognized for many years [1]. Some phenolic compounds in olive leaf, such as oleuropein and its derivatives, exhibit antimicrobial activities and inhibit the growth of yeasts, fungi, and molds [2-4].

The results of the present study were in accordance with previous research. According to a study by Markin et al., olive leaf aqueous extracts destroyed 15% of *C. albicans* within 24 h [13]. Pereira and colleagues performed a study to analyze the antimicrobial activity of olive leaf extracts and screened the inhibitory effects against *B. cereus*, *B. subtilis*, *S. aureus* (Gram-positive), *E. coli*, *P. aeruginosa*, *K. pneumoniae* (Gram-negative), *C. albicans*, and *C. neoformans* (fungi). The most sensitive microorganisms were *B. cereus* and *C. albicans*, whereas *B. Subtilis* was shown to be the least sensitive [3].

In a previous study, M.G. Soni introduced hydroxytyrosol as an effective polyphenol in olive leaf, with toxic effects against bacteria including *Pseudomonas syringae* (Gram-negative) and *Corynebacterium michiganense* (Gram-positive) (less effective against fungal species) [14, 15].

The observed differences in the antimicrobial effects of olive leaves on different microorganisms are likely to be associated with the crop origin, cultivation conditions, harvesting time, climatic conditions, content of antimicrobial agents in the extracts, sample preparation methods, genus, species, and strain of the yeast, and source of isolation [16]. Further research on olive leaf extracts as a natural alternative to synthetic antimicrobial drugs is required.

Conclusion

The present study focused on the effect of whole olive leaf extract on *C. albicans* (PTCC

5027) and showed its effective antifungal activity. The findings suggest that this herbal extract can be suitable for the prevention and treatment of *Candida* infections such as oral thrush.

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Authors' Contributions

Z.N. designed and supervised the research and M.A. edited the final manuscript**.**

Conflicts of Interest

The authors declare no conflicts of interest.

Financial Disclosure

The authors declare no financial interests related to the materials of the study.

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