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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Determination of Antifungal Activity Against Invasive Candidiasis Agents and Trace Element Content of Fig Tree Latex Samples Obtained from Trabzon Province

Trabzon Bölgesinden İzole Edilen İncir Ağacı Latekslerinin İnvazif Kandidiyazis Etkenlerine Karşı Antifungal Aktivitesinin ve Eser Element Düzeylerinin İncelenmes

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Abstract
Candidiasis is a major health concern causing both morbidity and mortality**.** The increasing prevalence of antimicrobial-resistant fungi associated with life-threatening systemic mycoses, led a constant need for new antifungal agents. Herbal medicines have been tried for this purpose for centuries. The antifungal effect of fig tree latex has been reported and some trace elements such as zinc were associated with antifungal effects**.** The aim of this study was to determine the trace element content and in-vitro antifungal activity of fig tree latex sample against *Candida. albicans, C. glabrata, C. tropicalis* and *C. krusei*. Fig tree latex samples were obtained from four different fig tree at Trabzon province in July 2019. The broth microdilution technique was performed to investigate antifungal activity against standard *Candida* strains and trace elements level were detected with Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) analyzer. The most powerful antifungal activity was reached at a concentration of 0.5 for *C. albicans* and *C. tropicalis*, and at a concentration of 0.125 for *C. krusei* and *C. glabrata* in fig tree latex. According to trace element analysis, magnesium had the highest level, followed by calcium and phosphorus. Selenium, aluminium, lead and nickel levels were too low to be measured. As a conclusion, fig tree latex has an antifungal potential against *Candida* species and this may be caused by the high level of magnesium that it contains, however more studies are needed to understand the therapeutic effects of fig tree latex.
Keywords: fig tree latex, *Ficus carica*, *Candida*, antifungal activity, trace elements

Öz
Kandidiyazis, morbidite ve mortaliteye neden olan önemli bir halk sağlığı sorunudur. Hayatı tehdit eden sistemik mikozlara neden olan antimikrobiyal dirençli mantarların sayısındaki artış, yeni antifungal ajanlara olan ihtiyacı doğurmuştur. Bitkisel ilaçlar bu amaçla yüzyıllardır kullanılmaktadır. İncir ağacı lateksinin antifungal etkisi önceki çalışmalarda rapor edilmiş, buna ek olarak çinko gibi bazı eser elementlerin de antifungal etkileri çeşitli çalışmalarla ortaya konmuştur. Çalışmamızda, incir ağacı lateksinin *Candida. albicans, C. glabrata, C. tropicalis* ve *C. krusei*'ye karşı in vitro antifungal aktivitesinin belirlenmesi ve içeriğindeki eser elementlerin ortaya konarak, bu eser elementlerin potansiyel antifungal etkilerinin incelenmesi amaçlandı. İncir ağacı lateks numuneleri, Temmuz 2019'da Trabzon ilindeki dört farklı incir ağacından elde edilmiştir. Latekslerin standart *Candida* kökenlerine karşı antifungal etkisini araştırmak için mikrodilüsyon tekniği uygulandı ve İndüktif Eşleşmiş Plazma Optik Emisyon Spektrofotometresi (ICP-OES) kullanılarak eser element seviyesi tespit edildi. İncir ağacı latekslerinde *C. albicans* ve *C. tropicalis* için 0.5 konsantrasyonda, *C. krusei* ve *C.glabrata* için ise 0.125 konsantrasyonda en güçlü antifungal aktiviteye ulaştığı saptandı. Eser element analizine göre incir ağacı lateksinde en yüksek konsantrasyonda bulunan elementler sırasıyla magnzeyum, kalsiyum ve fosfor olarak saptandı. Selenyum, aluminyum, kurşun ve nikel seviyeleri ise ölçülemeyecek kadar düşük olarak kaydedildi. Çalışmamıza göre incir ağacı lateksinin *Candida* türlerine karşı antifungal potansiyeli olduğu görülmüş ve aktivitenin lateksin içerdiği yüksek magnezyum seviyesinden kaynaklanıyor olabileceği düşünülmüştür. Ancak incir ağacı lateksinin terapötik etkilerinin ortaya konulması için daha fazla çalışmaya ihtiyaç vardır.
Anahtar kelimeler: incir ağacı lateksi, *Ficus carica*, *Candida*, antifungal etki, eser elementler

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I. INTRODUCTION

Candidiasis is a major health concern increasing both morbidity and mortality. It is known that there are at least 15 different *Candida* species that cause infections in humans. However, the four most common species detected in more than 90% of invasive diseases related to *Candida* species are *Candida albicans, C. glabrata, C. tropicalis* and *C. krusei*. Each of these organisms has unique virulence potential, antifungal sensitivity, and epidemiology [1]. As a result of the increasing prevalence of antimicrobial-resistant fungi associated with life-threatening systemic mycoses, there is a constant need for new antifungal agents. Because fungi have eukaryotic cell structure, their similarity with host cells creates additional problems in the design of drugs with selective toxicity to fungal cells in the treatment of these infections [2]. Herbal medicines have played an important role in the protection of individuals' health for thousands of years all over the world [3]. When the frequency of the usage of complementary and alternative drugs in general populations is examined, it has been understood that herbal drugs are quite popular in recent years [4]. The fig tree is one of the oldest trees belonging to the *Moraceae* family. Its product, *Ficus carica* L., is one of the earliest cultivated crops in the world due to its nutritional and medicinal benefits. The fruit can be consumed both in dry and fresh form. Mediterranean countries such as Turkey are the leading manufacturers of this product due to suitable climate [5]. In addition, fig tree latex ("ficin", a common milky secretion of fig tree leaves and fruits) has different therapeutic effects [6]. The antifungal effect of fig tree latex has been reported in previous studies, however, the cause of this antifungal effect was not examined in these studies [7, 8].

Trace elements are essential inorganic elements for life and are cofactors or catalysts in enzyme activity. When taken insufficiently, it causes dysfunction by affecting biological functions and only physiological doses are needed for the continuation of normal tissue functions. Trace elements such as iron (Fe), copper (Cu), zinc (Zn), selenium (Se), etc. play crucial roles in many biological systems [9, 10]. For instance, in *C. albicans*, these ions can participate in the provision of membrane potential, regulation of cell volume, cell proliferation and apoptosis mechanisms [10]. In addition to this, some trace elements such as Zn and Se compounds were associated with antifungal agents [11, 12]. When the trace elements found in figs were examined, the presence of different trace elements was reported [13].

In our study, it was aimed to reveal the in-vitro antifungal activity of fig tree latex against *C. albicans, C. glabrata, C. tropicalis* and *C. krusei* which are the species responsible for over 90% of invasive candidiasis, also to determine the levels of the trace elements in the fig tree latex and to evaluate the trace elements that may contribute to the antifungal activity.

II. MATERIALS AND METHODS

2.1.Antifungal Activity Assays

In this study, the broth microdilution technique was utilized to investigate the effect of fig tree latex on the growth of *Candida* species according to Clinical Laboratory Standards Institute (CLSI) criteria. In order to achieve this objective, *C. albicans* American Type Culture Collection (ATCC) 10231, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 ve *C. glabrata* ATCC 2001 strains were used. Fig tree latex samples were obtained from four different fig tree from Trabzon region (Turkey) in July 2019 and latexes were transferred to the laboratory under sterile conditions in microcentrifuge tubes. Each fig tree latex was individually tested against four different *Candida* species.10 µL fig tree latex sample was added directly to the first well of the 96 well plate which contains 80 µL Brain Heart Infusion (BHI) broth in each well. Then, latex diluted 0.5, 0.25, 0.125 ve 0.0625(dilution coefficient) fold with BHI broth. Also, the same volume of BHI was used as the negative control. Broth cultures of *Candida* species were adjusted to 0.5 McFarland turbidity standard individually and were inoculated into each well. The absorbances of the time T0 were measured spectrophotometrically at optical density at 600 nm (OD 600) in the Epoch System (Biotek Instruments, USA) according to the manufacturer's instructions. After incubation at 37ºC for 24 hours, the absorbance values of T24 time were measured and values were compared with T0 absorbances in order to evaluate fungal growth. All experiments were performed in triplicate [14].

2.2. Trace Element Analysis

Fig tree latex samples were prepared for element measurements by diluting 1:10 with deionized water. The analysis of chromium (Cr), Cu, Fe, magnesium (Mg), manganese (Mn), Se, Zn, aluminium (Al), calcium (Ca), phosphorus (P), cobalt (Co), cadmium (Cd), lead (Pb), arsenic (As), boron (B), nickel (Ni) and silicium (Si) levels were performed with ICP-OES Thermo iCAP 6000 series at Trace Element Analysis Laboratory of Biophysics Department of Cerrahpasa Medical Faculty at Istanbul University-Cerrahpasa.

The favorable wavelengths, 267.716, 324.754, 259.940, 285.213, 257.610, 196.090, 206.200, 167.090, 317.933, 177.495, 228.616, 249.773, 189.042, 249.773, 221.647 and 251.611 nm, were used for the determinations of Cr, Cu, Fe, Mg, Mn, Se, Zn, Al, Ca, P, Co, Cd, Pb, As, B, Ni and Si levels, respectively, in the ICP-OES device.

2.3. Reagents

ICP-OES labor standards were prepared from appropriate standard solutions including 1.000 ppm for each tested element obtained from Chem Lab NV located in Zedelgem/Belgium. Reagents with analytical reagent grade and deionized water were used. Stock solutions of Cr, Cu, Fe, Mg, Mn, Se, Zn, Al, Ca, P, Co,

Cd, Pb, As, B, Ni and Si were prepared by taking the appropriate standard in deionized water. Solutions were prepared freshly before using doubly deionized water was used in this study. To reduce the risk of contamination from ambient air and dust, all process was performed on a clean bench. All the volumetric flasks used were cleaned soaking in with 10% (v/v) nitric acid (HNO_3) solution during the day before use. These were rinsed with deionized water thoroughly and dried in an oven overnight at 100 ^{0}C [15]. *F. carica* L. levels of analyzed elements were expressed in micrograms per milliliter (µg/mL) [16]. Measurement of each trace element level was carried out three times and averaged.

III. RESULTS AND DISCUSSION

Antifungal activity assays revealed the most powerful antifungal activity was reached at a concentration of 0.5 for *C. albicans* and *C. tropicalis*, and at a concentration of 0.125 for *C. krusei* and *C. glabrata* in fig tree latex taken at different times. The fungal growth suppression rates of *Candida* strains according to the fig tree latex dilution rates are given in Table 1 and Figure 1, and the relative growth rates of *Candida* strains as colony-forming unit (CFU) are given in Table 2.

Minerals, trace and toxic element levels of *F. carica* L samples were given in Table 3. According to trace element analysis, Mg has the highest level, followed by Ca and P. Se, Al, Pb and Ni levels were too low to be measured. All element levels are given in Table 3.

Table 1. Growth suppression rates of *Candida* strains according to the concentrations of fig tree latexes (%)

	1	**0.5**	**0.25**	**0.125**	**0.0625**
C. albicans	55.19%	90.74%	86.44%	87.04%	69.99%
C. tropicalis	67.36%	68.06%	63.92%	65.98%	51.23%
C. krusei	75.20%	75.35%	72.03%	79.73%	63.25%
C. glabrata	64.59%	51.99%	84.98%	87.47%	73.36%

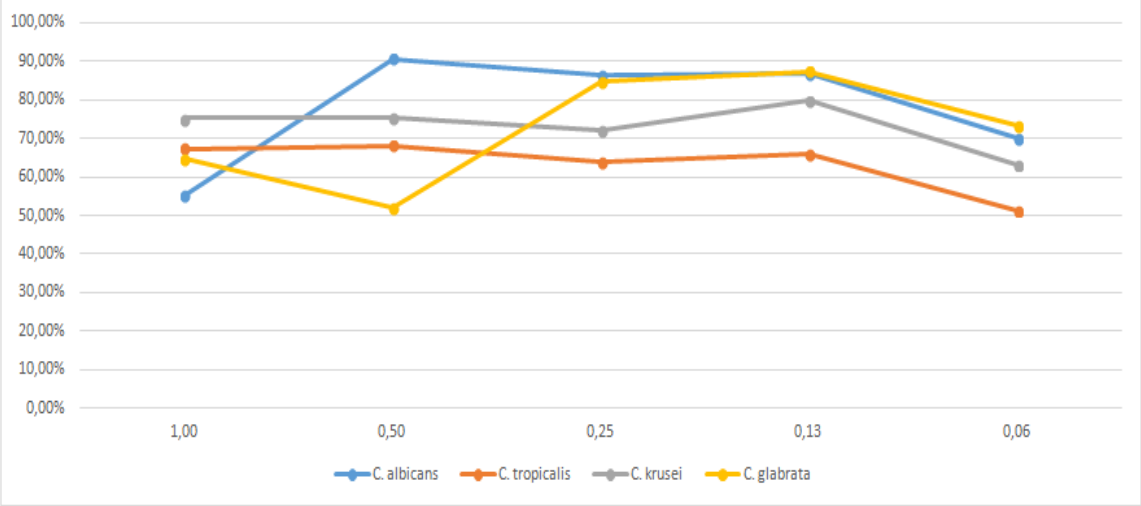

Figure 1. Growth suppression rates of *Candida* species by fig tree latex (%)

Table 2. Relative growth rates of *Candida* strains as CFU in medium supplemented with different concentrations of fig tree latex samples (Means + SD.) (CFU/mL*10^5)

	1	**0.5**	**0.25**	**0.125**	**0.0625**
C. albicans	8.96±6.49	1.85±2.22	2.71±4.14	2.59±2.97	6.00±3.45
C. tropicalis	6.53±3.95	6.39±3.12	7.22±7.28	6.80±4.40	9.75±6.34
C. krusei	4.96±3.92	4.93±4.47	5.59±6.18	4.05±5.20	7.35±6.22
C. glabrata	7.08±7.64	9.60±6.92	3.00±5.20	2.51±2.72	5.33±4.99

CFU: Colony-forming unit

Table 3. Trace element levels of *F. carica* L. samples

Element	Results (µg/mL)	SD
Cr	0.06	0.03
Cu	0.27	0.13
Fe	0.37	0.21
Mg	2688.93	610.30
Mn	0.16	0.07
Se	**ND**	**ND**
Zn	0.35	0.22
Al	**ND**	**ND**
Ca	70.98	10.49
P	29.42	9.18
Co	0.04	0.03
Cd	0.03	0.01
Pb	**ND**	**ND**
As	0.51	0.21
B	0.68	0.43
Ni	**ND**	**ND**
Si	0.96	0.25

Cr, chromium; Cu, copper; Fe, iron; Mg, magnesium; Mn, manganese; Se, selenium; Zn, zinc; Al, aluminium; Ca, calcium; P, phosphorus; Co, cobalt; Cd, cadmium; Pb, lead; As, arsenic; B, boron; Ni, nickel; Si, silicium. ND: non-detectable. Data are shown as the Means ± SD.

Candida species are the most important pathogens of opportunistic mycoses. In addition to superficial and mucosal infections such as oral candidiasis and vulvovaginal candidiasis, which are common in healthy hosts, they also have the potential to cause systemic infection in the immunosuppressive host. Although more than fifteen *Candida* species that can infect humans have been identified, it is known *that C. albicans, C. glabrata, C. tropicalis* and *C. krusei* are responsible for 90% of human infections [1]. Antifungal resistance emerging in *Candida* is an important public health problem that causes failure in the treatment of candidiasis. The development of new antifungals against increased antifungal resistance and the discovery of substances with antifungal activity are crucial [2]. Herbal treatment methods have been used in the treatment of superficial infections for many years, and especially in recent years, interest in these traditional treatment approaches has increased [3, 4]. One of the plants that have been used for many years for this purpose is fig, in which Turkey is among the leading producers. Fig (*Ficus carica* L.) is a nutritious plant belonging to the *Moraceae* family, whose therapeutic effects have been revealed in many studies [5-8]. One of the most important therapeutic effects of *F. carica* L. is the wound healing effect, which is claimed to be caused by the latex of the fig tree. Fig tree latex is a milky substance that is highly secreted from many parts of the fig tree such as young shoots, pedicles and leaves. Fig tree latex, which is secreted especially from the injured area of the fig tree, covers the area and protects it against to invasion of pathogens [8]. Probably with the observation of this effect, fig tree latex has begun to be used in traditional treatment of human wounds. In addition to its wound healing effect, there are many studies investigating the antifungal effect of fig tree latex [7, 8]. Despite its proven antifungal effect, there are no studies examining which active ingredient of the fig tree latex causing this antifungal effect. Therefore, in our study, it was aimed to investigate the antifungal effect of fig tree latex on *Candida* species, and to reveal the trace elements in the fig tree latex collected from our country and to examine the potential antifungal effects of these trace elements.

Aref et al. examined the antifungal effects of the extracts prepared from fig tree latex in their studies and they demonstrated that different extracts exerted different antifungal effects on different fungal species such as *C. albicans, Aspergillus fumigatus, Microsporum canis, Cryptococcus neoformans Trichophyton rubrum, T. soudanense* and *Scopulariopsis brevicans*. The most resistant species were determined as *T. rubrum, T. soudanense* and *S. brevicans*, and it was observed that fig tree latex inhibit the related species at rates varying between 0% and 60% [7].

Raskovic et al. investigated the antifungal effect of fig tree latex collected between May and August from Bar Region of Montenegro on *Saccaromyces cerevisiae*. According to the results of their study, that fig milk collected between May and June had a higher antifungal effect than the latex collected between July

and August due to the high chitinolytic activity in the fig tree in the relevant months [8].

In the study conducted by Ahmed in 2016, the antimicrobial effect of latexes obtained from *Ficus carica* and *Ficus elastica* species was examined on many bacteria and fungal species, and it was reported that the antifungal activity of fig tree latex was high against all the yeast strains examined. It was stated that the fig latex obtained from *F. carica* was more effective compared to *F. elastica* latex and according to agar well diffusion results, inhibition zone diameters varied between 30 mm and 12 mm against different yeast species. The highest inhibition zone was observed against *C. tropicalis* (30 mm), followed by *C. albicans* (25 mm), *C. sojae* (22 mm), *C. kefyr* (19 mm) species, and the lowest antifungal effect was observed against *C. krusei* (14mm) and *C. neoformans* (12mm) species [17].

In our study, it was observed that fig tree latex in different concentrations suppressed the growth of different *Candida* species at different rates. It was observed that fig tree latex showed the highest antifungal activity against *C. albicans* and *C. tropicalis* at the concentration of 0.5, and the concentration of 0.125 against *C. krusei* and *C. glabrata*. Growth of *C. albicans* was suppressed 91% at 0.5 concentration, while *C. tropicalis* growth was suppressed by 68%. The growth of *C. krusei* was inhibited by 80% at the concentration of 0.125 and the growth of *C. glabrata* was suppressed by 87%. It is noteworthy that fig tree latex suppresses the growth of different *Candida* species at different dilution rates. It is also striking that, contrary to expectations, higher antifungal activity was not obtained when fig tree latexes were applied to *Candida* species at maximum concentration without dilution.

Trace elements such as Mg, Ca, Fe, Cu, Zn, Se, etc. plays vital roles in biological systems such as, providing membrane potential, regulation of cell volume, cell proliferation or participation in apoptosis mechanisms [9-12]. In addition to this, the antimicrobial activities of some trace elements are known [11, 12]. Although the antifungal effectiveness of fig tree latex has been known for years, there are no studies on active compounds that cause this activity. In our study, we aimed to examine the trace elements found in fig tree latex and reveal the elements that may cause or support this antifungal activity.

In our study, the minerals, trace and toxic element levels in fig tree latex were examined, and the elements with the highest concentrations were found as Mg, Ca and P, respectively. There are studies in the literature regarding the antifungal effectiveness of some trace elements, particularly magnesium oxide. Karimiyan et al. investigated the antifungal activity of magnesium oxide, zinc oxide, silicon oxide and copper oxide nanoparticles on *C. albicans* in their study and reported that the minimum inhibitory concentration (MIC) value of magnesium oxide nanoparticles against *C. albicans* was higher than 3200 µg/mL. In our study, the magnesium ratio in fig tree latex was found to be 2989 µg/mL, suggesting that the magnesium concentration may have contributed to the antifungal effect of fig tree latex. In the same study, the MIC values of zinc oxide and copper oxide nanoparticles against *C.albicans* were found to be 200 and 400 µg/mL, respectively, and their antifungal activities were evaluated as higher than magnesium oxide nanoparticles. However, in our study, because the zinc and copper ratios in fig tree latex were detected as very low, it cannot be said that the antifungal effect of fig tree latex we observed in our study was caused by the relevant elements [18].

Kong et al. investigated the antifungal effectiveness of magnesium oxide nanoparticles against *C. albicans* in 2020 and they found the MIC value as 391 µg/mL. Besides, it has been demonstrated that magnesium oxide nanoparticles significantly inhibited the adhesion of *C. albicans* by adhesion experiments ($p < 0.001$). As a result, they reported that magnesium oxide nanoparticles effectively inhibited the growth, adhesion, morphological transformation and biofilm formation of *C. albicans* and mentioned that magnesium oxide nanoparticles may be an effective antifungal candidate [19].

IV. CONCLUSION

In our study, the most common elements found in fig tree latex after Mg were Ca and P, respectively. However, there have been no studies investigating the antifungal efficacy of these two elements in the literature, so it is not possible to compare the antifungal efficacy of the related elements at this stage. More studies are needed in this field in order to evaluate the antifungal potential of other minerals, trace and toxic elements measured in fig tree latex.

In conclusion, fig tree latex has an antifungal potential on *Candida* species and this may be caused by the high level of magnesium that it contains however more studies are needed on the therapeutic use of fig tree latex. Also, according to the results of our in vitro study, before the usage of fig tree latex for antifungal purposes, it is necessary to know which fungal agent it will be used against and to use it at the appropriate concentration for that agent.

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