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Questioning: Is *Cocos nucifera* oil antifungal effective for dental and oral health or not?

Sorgulama: *Cocos nucifera* yağı diş ve ağız sağlığı için antifungal etkili midir, değil midir?

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ABSTRACT

Objective: Aim of this study was to investigate the antifungal properties of different acrylic resins coated with *Cocos nucifera* oil. The escalating threat to human health posed by serious *Candida* spp. infections and the growing challenge of antimicrobial resistance underscores the pressing need for novel antifungal compounds. In response to this, our study explored the antifungal properties arising from the application of *Cocos nucifera* oil coating on different acrylic resins. Since there are no previous studies in the literature investigating the relationship between *Candida albicans*, *Cocos nucifera* oil, and commonly used 3D-printed acrylic resins, this topic deserves further investigation.

Methods: In our study, six discs were fabricated using the heat-cured method, and the other six discs were fabricated using the 3D-printing method. The surfaces of the experimental acrylic discs were coated with *Cocos nucifera* oil, but the surfaces of the acrylic discs in the control group were not coated with *Cocos nucifera* oil. The antifungal activities of acrylic resins

ÖZET

Amaç: Bu çalışmanın amacı *Cocos nucifera* yağı ile kaplanmış farklı akrilik reçinelerin antifungal özelliklerinin araştırılmasıdır. Ciddi *Candida* spp. enfeksiyonlarının insan sağlığına yönelik tehditini arttırması ve antimikrobiyal direncin giderek artması, yeni antifungal bileşiklere olan acil ihtiyacın altını çizmektedir. Bu sorunlara cevap olarak, çalışmamızda *Cocos nucifera* yağ kaplamasının farklı akrilik reçineler üzerine uygulanmasından kaynaklanan antifungal özellikleri araştırılmıştır. Literatürde *Candida albicans*, *Cocos nucifera* yağı ve yaygın olarak kullanılan 3D baskılı akrilik reçineler arasındaki ilişkinin araştırılması konusunda daha önce yapılmış herhangi bir çalışma olmadığından dolayı bu konunun araştırılmasına ihtiyaç vardır.

Yöntem: Çalışmamızda, altı adet disk, ısıyla sertleştirme yöntemi kullanılarak, diğer altı adet disk ise 3D baskı yöntemi kullanılarak üretilmiştir. Deneysel akrilik disklerin yüzeyleri *Cocos nucifera* yağı ile kaplanmıştır. Kontrol grubundaki akrilik disklerin yüzeyleri ise *Cocos nucifera* yağı ile kaplanmamıştır. *Cocos nucifera* yağı ile kaplanmış akrilik reçinelerin *C.*

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coated with *Cocos nucifera* oil against *C. albicans* ATCC 90028 strains were determined using the plate counting method.

Results: Contrary to expectations, *Cocos nucifera* oil did not exhibit any antifungal effects; it was determined that *C. albicans* promoted growth increase on acrylic discs coated with *Cocos nucifera* oil than on the control acrylic discs. Thus *Cocos nucifera* oil was found to promote *C. albicans* growth.

Conclusion: This study demonstrated that both heat-cured and 3D-printed acrylic resins coated with *Cocos nucifera* oil did not have any antifungal effects against *C. albicans*, the contrary, the acrylic resins coated with *Cocos nucifera* oil promoted *C. albicans* growth.

Key Words: *Candida albicans*, antifungal, coconut oil, 3D-printed acrylic resins, heat-cured acrylic resin

albicans ATCC 90028 suşlarına karşı antifungal aktiviteleri plate counting yöntemi kullanılarak belirlenmiştir.

Bulgular: Beklenenin aksine, *Cocos nucifera* yağının antifungal etki göstermediği; aksine, *Cocos nucifera* ile kaplanmış akrilik diskler üzerinde, kontrol akrilik disklere göre daha fazla *C. albicans* üremesi olduğu görülmüştür. *Cocos nucifera* yağının *C. albicans* üremesini teşvik ettiği belirlenmiştir.

Sonuç: Bu çalışma ile *Cocos nucifera* yağı ile kaplanmış hem ısıyla sertleştirilmiş hem de 3D baskılı akrilik reçinelerin *C. albicans*'a karşı herhangi bir antifungal etkiye sahip olmadığı, aksine *Cocos nucifera* yağı ile kaplanmış akrilik reçinelerin *C. albicans* üremesini arttırdığı sonucuna varılmıştır.

Anahtar Kelimeler: *Candida albicans*, antifungal, hindistan cevizi yağı, 3D-baskılı akrilik reçine, ısıyla sertleştirilmiş akrilik reçine

INTRODUCTION

Yeasts of the genus *Candida* are inherent components of the typical microbiota found in the skin, oral cavity, gastrointestinal tract, and genitourinary tract (1). *Candida albicans* is the most prevalent strain in oral candidiasis and denture stomatitis (2), a common inflammatory condition of oral mucosa in individuals wearing dentures (3). Although other *Candida* species can cause infections, they occur less frequently (4). In addition to infections, several other factors affect the development of denture stomatitis, including the nocturnal use of oral cavity prostheses and poor oral hygiene (5). The irregularities and pores that develop on the surface of acrylic resin denture base materials can transform the inner surface of dentures into a reservoir of microorganism (6).

Complete and partial edentulism lead to an imbalance in all components of the stomatognathic system. Consequently, practitioners and researchers aim to restore all affected functions (7). In the past decade, digital technologies have emerged as alternatives to conventional (heat-cured) fabrication of acrylic removable dentures (8). Computer-aided design and computer-aided manufacturing (CAD-CAM), incorporating additive techniques such as 3D-printing, has facilitated the manufacture of removable dentures (9). The advantages of 3D-printing include the ability to build intricate geometries by adding materials layer-by-layer, thereby minimizing waste (10). Therefore, the use of 3D-printing to create completely removable denture bases has become increasingly popular in both research and clinical practice. Although it is equal to or surpasses the conventional method in terms of the overall accuracy of the produced dentures (11), this method still has some limitations concerning its mechanical and physical properties (10, 12). Research has indicated that 3D-printed acrylic resins exhibit higher surface roughness and inferior surface characteristics than heat-cured acrylic resins (7, 13, 14).

The widespread use of acrylic resin for edentulism has led to growing emphasis on maintaining optimal denture hygiene. Mechanical and chemical methods are commonly recommended to prevent the buildup of microorganisms and subsequent oral mucosal inflammation during denture hygiene (6). Efforts have been made to produce an antimicrobial acrylic resin. Nonetheless, the inclusion of such agents may alter the characteristics of acrylic resins, ultimately affecting their appearance and strength (15). Additionally, a variety of antifungal agents are readily available for treating oral *Candida* infections. Despite their proven efficacy, these drugs are associated with undesirable side effects such as bitter taste, allergic reactions, and potential drug interactions. Investigating natural products obtained from plants that are traditionally used in medicine can offer valuable substitutes for antifungal agents (5). Studies have indicated that natural substances, particularly plant essential oils, have the potential to combat various strains of *Candida* spp., including the highly prevalent *C. albicans* (16). Among these, coconut oil, scientifically known as *Cocos nucifera*, has garnered attention because of its efficacy against fungal infections. Given the accessibility, unique composition, and affordability of coconut oil, it is crucial to investigate its efficacy as an antifungal agent (17). Although they are widely used in clinical practice, the effects of natural products against fungal pathogens remains controversial (5, 18). Furthermore, there is a notable gap in studies investigating the effects of coconut oil on different types of denture resins against *C. albicans*. Thus, this study aimed to assess the antifungal activity of *Cocos nucifera* oil on both heat-cured and 3D-printed acrylic resin surfaces and compare their effectiveness.

The hypotheses tested were as follows:

1) The application of *Cocos nucifera* oil would have antifungal activity against *C. albicans* growth

2) 3D-printed resin discs would provide an equal surface topology against *C. albicans* compared to heat-cured acrylic resin.

This study has the potential to shed light on the role of alternative medicine in digital and conventional dentistry. Previous literature lacks evidence of the effects of *Cocos nucifera* oil on both conventional and 3D-printed denture resins against *C. albicans*. This study was prompted by the urgent need to control the growth of *C. albicans*, a major cause of denture stomatitis.

MATERIAL and METHOD

The sample designs

In this study, a total of 12 acrylic discs were fabricated: heat-cured and 3D-printed. Six discs were fabricated by heat-cured method and other six discs were fabricated by 3D-printing method. The fabricated rectangular samples had a width of 2 cm, length of 5 cm, and thickness of 1 cm.

Heat-cured acrylic resin

The heat-cured acrylic resin discs were fabricated according to the manufacturer's instructions, as described in a previous study (7). Wax patterns were cut using modelling wax (Hindustan Modelling Wax No. 2, The Hindustan Dental Products, Hyderabad, India) according to predetermined dimensions and invested in Type 3 dental stones (Moldano, Kulzer GmbH, Hanau, Germany) using a mixing ratio of 100 g powder to 30 mL water under vacuum for 30 min, following the manufacturer's instructions. Subsequently, the flask was immersed in water at 100°C for five minutes. Once the flask was separated, any remaining wax remnants were removed. A gypsum separator (Model Sep, Harvest Dental Inc., California, United States) was used to isolate the mold. The resin was mixed in a beaker at a ratio of 35 g powder to 14 mL liquid and stirred with a spatula for one minute.

The acrylic resin (Meliodent Heat Cure, Kulzer GmbH, Hanau, Germany) reached packable consistency within 10 min and was poured into the mold. The flask underwent a 15-minute immersion in boiling water, after which the heat source was deactivated. The polymerization process utilized a short cycle method

according to the manufacturer's guidelines. After a 20-minute boiling period, the flask was cooled gradually in a water bath. Following the completion of the polymerization phase, the specimens were removed from the mold. Subsequently, the printed specimens were trimmed, ground, and buffed using a polishing machine (Interlab, Hull, UK) with the application of pumice powder, emery paper, and tungsten carbide bur.

3D-printed acrylic resin

Wax patterns were digitized to obtain a high-resolution STL format using a chairside software (CEREC 5.0.0, Dentsply-Sirona, NY, USA). The resulting STL file was processed using a software (Blender for Dental 3.3.1, Blender, New York, USA). Subsequently, resin discs were produced through 3D printing using an LCD printer (FreeShape 120, Ackuretta, Taipei, Taiwan), and resin material (Optiprint Laviva, Dentona AG, Dortmund, Germany) in a light pink hue (LOT:48230) was formulated for disc fabrication. The printing was completed with a wavelength of 405 nm, maximum laser speed of 5000 mm/s, layer thickness of 100 µm, and build angle of 90°, as recommended to achieve optimum material properties (19, 20). After printing, the specimens were cleaned using a dual-tank cleaning bath treatment for 3-minute each, containing 99.8% isopropanol (CLEANI; Ackuretta, Taipei, Taiwan), followed by a 3-minute post-curing phase using an ultraviolet curing unit (Tray-LED; Ackuretta, Taipei, Taiwan) with a 405 nm LED wavelength and 39 W power at 60 °C, following the manufacturer's instructions. The printed specimens were ground using water lubricant, followed by polishing using silicon carbide papers with grain sizes of 400 (P600), 600 (P1200), and 1200 (P2500). Subsequently, they were polished with 0.05 µm MasterPrep Alumina discs.

Test microorganism

The test microorganism, *C. albicans* ATCC 90028, was obtained from the culture collection of the School of Pharmacy, Department of Pharmaceutical Microbiology Research Laboratory at Istanbul Medipol University.

Inoculum preparation

C. albicans suspension was prepared from 3 days culture and the yeast density was adjusted to $4.6x10^3$ CFU/mL by using a densitometer and diluting (21).

Determination of the *Candida* growth on acrylic resin discs

The antifungal activities of acrylic resins coated with *Cocos nucifera* oil against *C. albicans* ATCC 90028 strains were determined using the plate counting method described by Hoş et al. (21).

Each the acrylic disc surfaces (2 cm x 5 cm) were sterilized for 10 min using UV light (Philips TUV 30 W, Ultraviolet) (21). The test (experimental) group was flooded with 1.5 mL of warm (37 °C) *Cocos nucifera* oil (Bio Planete, Bram, France), and the control group was kept without the *Cocos nucifera* oil coating. A 100 µL of a 24-hour yeast culture (4.6×10^3 CFU/mL) was transferred onto the test and control groups, and the surfaces were covered with polyethylene (PE) film (1.5 cm × 4 cm). Three discs of 3D-printed acrylic resin were coated with *Cocos nucifera* oil and other three discs of 3D-printed acrylic resin were not coated with *Cocos nucifera* oil, as those were controls. Three discs of heat-cured acrylic resin were coated with *Cocos nucifera* oil and other three discs of heat-cured acrylic resin were not coated with *Cocos nucifera* oil, as those were controls. Hence, the tests were performed with three replicates. The discs were incubated for 24 h at 37 ±1°C with relative humidity (RH) exceeding 90%. Following incubation, the discs were washed with 20 mL of a 0.87% NaCl solution. A 100 µL of this solution was spread-plated onto Potato Dextrose Agar. After the 72-hour incubation at 37 ±1°C, the colonies were counted.

Statistical analysis

Statistical analyses were performed using SPSS version 26 (SPSS Inc., Chicago, IL, USA). The

distribution and homogeneity of the data were assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. The findings revealed that the data did not display a homogenous distribution, and non-parametric tests were employed as a result of the limited sample size. Mann-Whitney U test was used for statistical analysis, and a significance level of $p < 0.05$ was used for the analysis.

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Date: 22.04.2022 and Number: 363).

RESULTS

Cocos nucifera oil

Table 1 presents the number of *C. albicans* colonies for control without *Cocos nucifera* oil and experimental resins coated with *Cocos nucifera* oil. The findings revealed that *C. albicans* demonstrated growth increase on *Cocos nucifera* oil-coated acrylic discs than on the control acrylic discs. Notably, there was a statistically significant difference between the control group and the *Cocos nucifera* oil-coated acrylic discs for the 3D-printed acrylic resins (p:0.022). In the control group of 3D-printed acrylic resin discs, the median value of *C. albicans* count was 266 (172-465) CFU/sample whereas the median value was 1817 (882-1907) CFU/sample for 3D-printed acrylic resin discs coated with *Cocos nucifera* oil.

Additionally, heat-cured acrylic resins revealed a statistically significant difference between the control group and the *Cocos nucifera* oil-coated discs (p =0.001). Although the control group did not show *C. albicans* growth, the *Cocos nucifera* oil-coated discs had a median value of 315 (302-324) CFU/sample. The findings are shown in Figure 1.

Table 1. Comparison of the numbers of *C. albicans colonies on acrylic resin surfaces (3D-printed / Heat-Cured) coated with/without Cocos nucifera oil*

	3D- Printed				Heat Cured				
	Mean	S.D.	Med.	Min-Max	Mean	S.D.	Med.	Min-Max	*p*
Control	301	149.6	266	172-465	0	0	0	0	0.037
Experimental	1535.3	567.6	1817	882-1907	313.7	11.1	315	302-324	0.050
p	0.022				0.001				

Mean= mean, S.D.= standard deviation, Med= Median, Min-Max= Minimum-Maximum, *p*= p-value

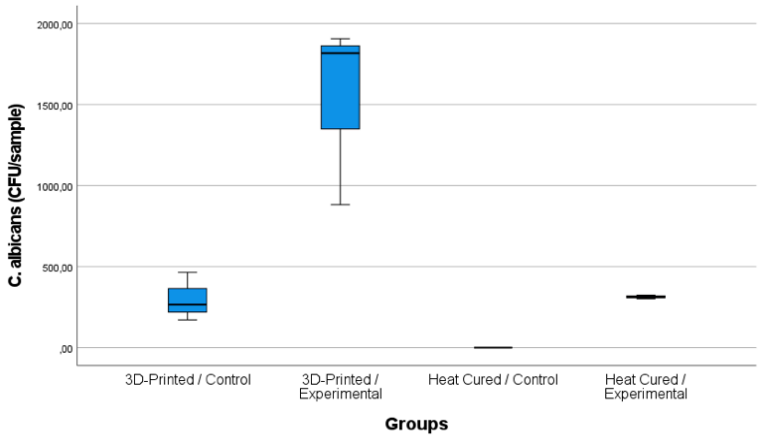

Figure 1. Effects of *Cocos nucifera* oil on *C. albicans* growth

Manufacturing techniques

The number of *C. albicans* colonies for 3D-printed acrylic resins and heat-cured acrylic resins coated with *Cocos nucifera* oil are shown in Table 2. It was found that *C. albicans* exhibited growth increase on 3D-printed acrylic resin discs than on heat-cured acrylic resins. The number of *C. albicans* colonies was statistically significant in control groups (p :0.037), with no growth observed in heat-cured acrylic resin discs, but a median value of 266 (172-465) CFU/sample was found in 3D-printed acrylic resin discs. The effects of different manufacturing techniques on *C. albicans* growth were highlighted by these findings. Statistically significant differences were found between the manufacturing techniques (p :0.050) in the groups coated with *Cocos nucifera* oil. The median values of *C. albicans* count in 3D-printed acrylic resin discs coated with *Cocos nucifera* oil was 1817 (882-1907) CFU/sample, while in heat-cured acrylic resin discs coated with *Cocos nucifera* oil, it was 315 (302-324) CFU/sample. The schematic representation is given in Figure 2.

Table 2. Comparison of the number of *C. albicans colonies in terms of the control and experimental groups*

		n	M	S.D.	p
3D -Printed	Control	3	301.0	149.6	0.022*
	Experimental	3	1535.3	567.6	
Heat-cured	Control	3	0.0	0.0	0.001*
	Experimental	3	313.7	11.1	

n= sample size, M= mean, S.D.= standard deviation, p= p-value

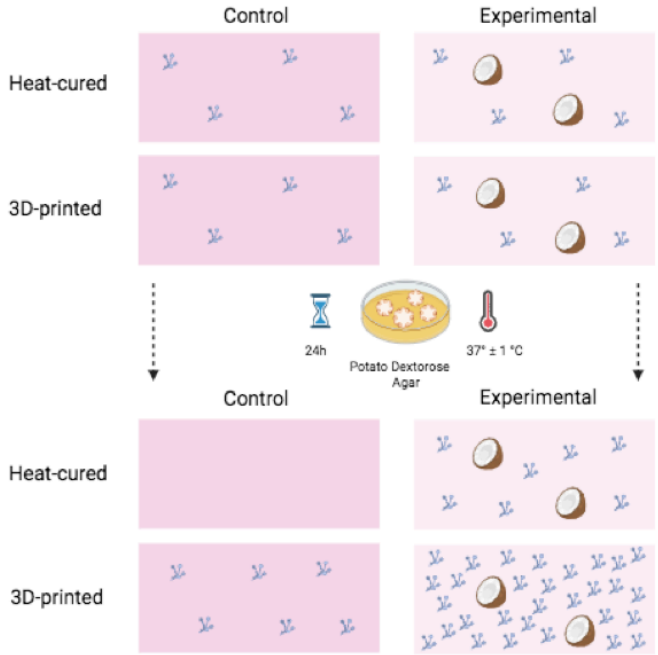

Figure 2. Graphical abstract
Schematic representation of the effects about differently manufactured acrylic resin discs coated with *Cocos nucifera* oil against to *C. albicans*.
: *Cocos nucifera* : *C. albicans*

DISCUSSION

In the present study, we investigated the antifungal activity of coconut oil on heat-cured and 3D-printed acrylic resin discs. As modern medicine comes with the risk of side effects, individuals are increasingly turning towards traditional practices as complementary therapies to improve their overall health. This quest for a solution for denture stomatitis has unveiled a potential natural remedy. According to Kumar et al. (17) and Krishnamoorthy et al. (16), in vitro studies have demonstrated that when *Cocos nucifera* oil is incorporated into tissue conditioner, it was showed a significant reduction in the colonization of *C. albicans*. However, our study yielded contrasting results, with *C. albicans* growth in the presence of *Cocos nucifera* oil. Shino et al. (22) conducted a study on the impact of *Cocos nucifera* oil on early childhood caries. They concluded that *Cocos nucifera* oil showed antifungal activity. Our findings challenge the literature on the potential benefits of *Cocos nucifera* oil for inhibiting *C. albicans* growth.

The initial step in the colonization and invasion of host tissues by *C. albicans* is its ability to adhere to acrylic resin surfaces. In addition, rough surfaces can increase the accumulation of organisms in prostheses even after cleaning. Yeast growth is aided by rough surfaces. The topology of improperly polished prostheses also favors yeast growth (15). Baciu et al. (7) revealed that among the tested denture base resins (heat-cured, CAD/CAM milled, and 3D-printed), 3D-printed acrylic resin exhibited the highest surface roughness. These results are supported by those of Helal et al. (13) and Zeidan et al. (14) In this study, *C. albicans* showed more growth increase on the 3D-printed acrylic resin surface coated with *Cocos nucifera* oil than on the heat-cured acrylic resin surface coated with *Cocos nucifera* oil.

A study conducted by da Silva et al. (19) showed that, despite the lower surface roughness compared to heat-cured acrylic resins, *C. albicans* exhibited growth increase on 3D-printed acrylic resins. They indicated that surface free energy of 3D-printed acrylic resins may be more likely associated with that colonization than their surface roughness. As previously described by Gad et al. (23), the surface roughness, the surface free energy, and the contact angle of the resin are also responsible for *C. albicans* growth. Moreover, the inferior surface characteristics of 3D-printed acrylic resin can be linked to the processing techniques employed, including light polymerization and printing methodologies, such as the layering technique, printing orientation, and associated parameters (7).

In conclusion; To sum up, the first hypothesis was rejected, as *Cocos nucifera* oil did not have antifungal activity against *C. albicans*. Furthermore, the second null hypothesis was also rejected, as the 3D-printed acrylic resin discs coated with *Cocos nucifera* oil showed more *C. albicans* growth increase compared to the heat-cured acrylic resin discs coated with *Cocos nucifera* oil.

In conclusion, this pioneer study proved that both heat-cured and 3D-printed acrylic resin discs coated with *Cocos nucifera* oil do not have any antifungal effects against *C. albicans*. This is the first report proving that both heat-cured and 3D-printed acrylic resins coated with *Cocos nucifera* oil do not have antifungal effects against *C. albicans*. Contrary to expectations, *Cocos nucifera* oil did not exhibit antifungal effects; instead, it unexpectedly promoted a favorable environment for *C. albicans* growth, particularly on 3D-printed acrylic resin discs. These findings challenge the initial hypotheses and underscore the interactions between natural products, material surfaces, and microbial behavior. Additionally, 3D-printed acrylic resin promoted more *C. albicans* growth increase compared to conventional acrylic resin. It is needed further investigation to determine its efficacy against other fungal species. To expand on these findings, it is imperative to thoroughly investigate relationship among *C. albicans*, *Cocos nucifera* oil and widely utilized 3D-printed resins. Future research should

focus on elucidating the underlying mechanisms that contribute to the observed discrepancies and investigating ways to reduce the enhanced growth of *C. albicans* in 3D-printed acrylic resin.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Date: 22.04.2022 and Number: 363).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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