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POTENTIAL ANTIFUNGAL EFFECT OF ACACIA ARABICA EXTRACT VERSUS STERILE WATER REGARDING SURFACE TOPOGRAPHY OF DENTURE BASE MATERIALS: AN IN-VITRO STUDY

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ABSTRACT

INTRODUCTION: Acacia Arabica (AA) revealed its plant's antibacterial properties. However, there is no information on how it affects the number of Candida albicans (C. *albicans*). Purpose: To ascertain the potential antifungal effect of (AA) gum extract at two time points on different denture resins.

MATERIALS & METHODS: In total, 52 square specimens were fabricated from 4 groups of denture base materials, (n=12) each; Group I: heat cured PMMA; Group II: Polyamide; Group III: milled resin and Group IV: 3D-printed resin. 4 more specimens were prepared for SEM imaging; 1 from each material. For C. *albicans* to colonize and adhere to the specimens, they were incubated with it for 48-hour period. A total of; 6 specimens/group were immersed in sterile water (control group), while 6 additional specimens/group were immersed in 50% (w/v) AA gum extract (test group). CFUs calculated after two time points (3 and 8 hours). ANOVA test was used to evaluate the data, and Fisher's LSD test was then used to perform multiple comparisons between different groups.

RESULTS: After 3 and 8 hours, the amount of C. *albicans* attached to PMMA specimens was significantly lower with AA gum extract ($P \leq 0.05$& $P \leq 0.001$). A reduction of C. *albicans* count was noticed on 3D-printed specimens significantly after 8 hours ($P \leq 0.001$). Conclusions: The use of AA gum extract 50% (w/v) showed a promising antifungal effect by reducing *C. albicans* count on denture base materials, in relation to their methods of manufacturing which affect their surface topography.

RUNNING TITLE: Effect of Acacia Arabica on candida albicans adhered to denture resins.

KEYWORDS: Acacia Arabica, antifungal, CAD/CAM, 3D-printed, milled resins.

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INTRODUCTION

Denture stomatitis (DS) is a common multifactorial mucosal illness affecting complete and partial removable denture wearers. [1] Among those who wear dentures, the incidence of DS varies from 17% to 75%. [2] Oral flora from people wearing dentures are less widely distributed than those who entirely having their teeth remaining, although they may also contain further of Candida fungus. [3] While the etiology of denture stomatitis is not entirely understood, Candida albicans (*C. albicans*) is typically present in conjunction with it. C. *albicans* infection is associated with mucosal damage caused by poor dental hygiene, ill-fitting dentures, xerostomia, and wearing dentures at night. [4] Denture base imperfections that allow yeast cells to colonize them can lead to the development of dental biofilm, or plaque, on the surface of the prosthesis and on mucosal surfaces it comes into contact with. The close fit of the denture creates a protected environment that reduces oxygen and saliva flow to the underlying tissue, which promotes yeast cell entrapment. [5]

Despite the availability of novel antimicrobial drugs, C. *albicans* resistance has emerged as a result of the extensive usage of topical and systemic antifungals. [6] Moreover, systemic candidiasis remains harmful, and recurrence rates for oropharyngeal and vulvovaginal infections are standing rather high. [7] Accordingly, these ramifications dictate further investigation and advancement of naturally occurring antimicrobial substances that target particular oral infections and are safe for the human body.

Plant components known as phytochemicals are non-nutritive substances with anti-disease qualities and offer numerous health benefits. These bioactive components are produced by plants to defend themselves against environmental stresses, but recent studies show that many phytochemicals can also shield humans from

illness. [8] As a matter of fact, a large proportion of pharmaceuticals that is currently in use in healthcare facilities are either products of plant origin or have been inspired by plant-based sources. [9] Acacia Arabica has been used since ancient times. Their phytochemicals have a chemical contribution to several classes, including phenols and phenolic glycosides, alkaloids, volatile essential oils, resins, oleosins, steroids, tannins, and terpenes. [10] Gum extract has tonic, styptic, and astringent properties. [11] It is used to treat oral cavity lesions, amoebic dysentery, dry cough symptoms and as tonic, analgesic, and anti-asthmatic. [12] It is a naturally occurring, non-toxic excipient that is utilized to supply the bioactive formula in medications for prolonged release. [13]

There are several different types of polymers used in clinical dentistry for different purposes. [14,15] The most widely utilized polymer among them is polymethyl methacrylate (PMMA). [16] Another polymer is thermoformed acrylic resin; it is a versatile, injectable, heat-sensitive material free of monomers. [17,18] Thanks to recent technological advances computer-aided design/computer-aided manufacture (CAD/CAM) milled and three-dimensional (3D) printing are now available for denture base fabrication. Because the CAD/CAM milled denture bases are made from industrially pre-polymerized resin pucks, they have a higher degree of polymerization and are less likely to be porous. It is important to note that the amount of pores on the denture surface and, consequently, its vulnerability to microbial colonization and biofilm formation, can be affected by the curing process. [19]

Due to the financial strain and harmful effects of DS on denture wearers, there is an urgent need to find new treatments and preventative measures to counteract the rise in Candida colonization, the most common sign of oral candidiasis that causes denture wearers to develop Candida-associated denture stomatitis. A previous study [20] showed that Acacia Arabica possesses antimicrobial activities against dental pathogens. However, there is no data on its effectiveness on reducing *C. albicans*. Consequently, the purpose of this study was to ascertain how Acacia Arabica gum extract affected candida that was adherent to various denture base resins. The null hypothesis states that there is no difference in the number of C. *albicans* adhered to PMMA, Polyamide, milled or 3D-printed denture resins after immersion in Acacia Arabica gum extract after 3 or 8 hours' time points.

MATERIALS AND METHODS

This research study was approved by the Research Ethics Committee of Alexandria University, Egypt, Faculty of Dentistry (IRB No. 00010556 –IORG 0008839) prior to any research-related activities.

The estimated sample size was based on a 5% level of significance and an 80% study power (alpha error accepted= 0.05). The minimum sample size was calculated to be 12 per group (n=12). The total required sample size for testing = 48 specimens. [21] In addition to; 4 specimens were used; 1 from each material for SEM imaging. To keep the sample size constant, any specimens lost from the study sample as a result of processing errors were replaced. [22] Sample size was estimated by software: G*Power Version 3.1.9.7. [23]

Specimens' Fabrication:

Square-shaped specimens were fabricated in dimensions 12x12x3 mm [24] following the manufacturer's instructions. Group I and II specimens were assembled in molds created by inserting stainless steel plates into type III dental stone-filled metal dental flasks. When the stone was completely set, the stainless steel plates were taken out, and the mold was prepared for making test specimens. Group 1: Heat-polymerized polymethyl methacrylate (PMMA) specimens (Acrostone heat-cured denture base (© 2023 Acrostone Dental & Medical Supplies. Egypt) were polymerized by conventional technique for eight hours, at 74°C water bath. [25] Group II: Polyamide: (Flexi fast – Flexi Ultra Flexafil S.A.C.I. leopoldo marechal, Argentina "orange pink 78"). In compliance with the manufacturer's instructions, specimens were plasticized at 280° C for 15 minutes under 7.5 bars of pressure. [26] Group III: CAD/CAM subtractive milled resins (AvaDent denture base Puck, AvaDent, Global Dental Science Europe, Tilburg, The Netherlands. Shade: light pink). Using the subtractive method, the denture base is milled from a block of pre-polymerized resin to create the specimens [27], by a diamond saw in a wet environment attached to the cutting machine (IsoMet 5000 Linear Precision Saw, Buehler, USA). [28] Group IV: CAD/CAM 3D-printed resins (Formlabs Inc., Somerville, MA, USA .Shade: Light pink). Open software (123D design, Autodesk, version 2.2.14, CA, USA) was used to construct the specimens. It designed the 3D-printed specimens and generated the necessary STL file. The printer received the printing request (Form 2; Formlabs). The printer's specifications were set in accordance with the guidelines provided by the manufacturer. [29] The process of fabrication involves photo-polymerization, wherein layers are produced with a thickness of 50 µm and 90-degree printing orientations. Then specimens were post cured in the corresponding unit according to manufacturer's instructions as well as finishing of all specimens which were finished by one trained investigator. Specimens were kept unpolished to simulate the fitting surface of the denture. Every specimen was kept for a minute in a flask filled with 90% ethanol, then cleaned in distilled water

and immersed in sterile water for 24 hours at 37°C. 30

Scanning Electron Microscopy imaging (SEM):

Scanning electron microscopy imaging was conducted by SEM (JEOL, JSM-5510LV, Peabody, MA, USA) at (Tissue Engineering Laboratory, Scanning Electron Microscope Unit, Faculty of dentistry, Alexandria University, Egypt) to analyze the surface topography of the different denture base materials. One specimen from each denture base materials was randomly selected for qualitative assessment via SEM using magnification of x50 with 10 KV. Because the polymeric resins are nonconductive, specimens were gold-coated to decrease the surface charging effect, which would otherwise occur because of the accumulation of static electric fields. [30]

Acacia Arabica gum extract preparation:

Commercial Acacia Arabica gum (Figure 1: a) was prepared at (Microbiology and Immunology Department, Faculty of pharmacy, Alexandria University, Egypt) to obtain the extract. After removing any contaminants with a sharp object, it was thoroughly cleaned three times for 10 minutes using sterile water free of lipopolysaccharides (LPS), [31] in order remove any possible contaminants. The extract was dissolved in sterile LPS-free water at a concentration of 50% (w/v), and in order to maintain the high quality of the extract by preventing deterioration, it was agitated with a magnetic stirrer until it was entirely dissolved. It was just before usage, freshly made.

Microbiological Evaluation:

To get rid of any microbiological contamination, specimens were immersed in 70% ethanol for 30 minutes. Any remaining ethanol was then rinsed with saline. Following that, the specimens were placed at the bottom of a sterile 24-well cell culture plate (Figure 1: b), and incubated with a 2 ml of standard strain of *C. albicans* (ATCC 10231) suspension (1×10^6 CFU/ml to permit the growth and adhesion of C. *albicans* on the surface of denture base materials. Then specimens were rinsed three times with saline after a 48-hour incubation period in order to eliminate any non-adherent cells. For two different time points (3 hours and 8 hours), A total of; 6 specimens/group were immersed in sterile water (control group), while 6 additional specimens/group were immersed in 50% (w/v) AA gum extract (test group). The amount of C. *albicans* still adhered to the specimens' surface was counted after the specimens underwent another saline wash to get rid of any remaining GA extract. [32] Using cell scrappers, the adhered C. *albicans* cells were scraped off the specimen surfaces in 2 ml of saline. Each scraped extract was placed in a 15 ml polypropylene tube together with its specimen (Figure 2: a) then homogenized for three minutes with a homogenizer to enable any remaining C. *albicans* cells on the specimens' surface to be removed. After that, the extract was serially diluted in saline and 20 µl of each dilution were plated on Sabouraud dextrose agar (SDA) plates (Muller Hinton Agar plates, Oxoid, Basingstoke, UK) (Figure 2: b) for selective fungal growth. Viable cells were counted following a 48-hour incubation period, and the total number of candida cells attached to each specimen for each experimental condition was calculated and expressed as colony-forming units per plate (CFUs/plate).

Statistical analysis:

Data were collected and statistical analyses were carried out using GraphPad Prism and one-way analysis of variance (ANOVA), with multiple comparisons between various groups being tested using Fisher's LSD test.

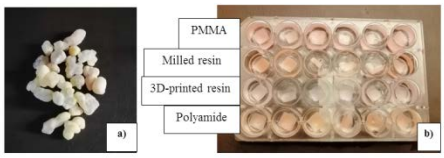

Figure 1: a): Acacia Arabica gum, b): 24-well cell culture plate with different denture base specimens.

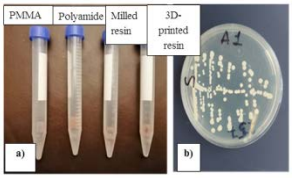

Figure 2: a): Each scrapped extract and its specimen were transferred to a 15 ml polypropylene tube. b): Sabouraud dextrose agar (SDA) plate with candida albicans.

RESULTS

The surface topography of the denture bases are shown by the qualitative SEM assessment, which revealed that the surface of CAD/CAM milled specimens was the smoothest, followed by polyamide specimens unlike those of heat cured PMMA and 3D-printed which showed surface irregularities. (Figure 3)

At the two time intervals studied (3 and 8 hours), PMMA denture base specimens demonstrated a significant reduction in the count of candida cells adherent to the denture base specimens following immersion in GA extract compared to sterile water ($P \leq 0.05$ and $P \leq 0.001$), respectively. Remarkably, after leaving the denture in sterile water for 8 hours as opposed to 3 hours, there was a significant increase in the candida count ($P < 0.01$). However, after 3 hours, the candida count was significantly lower in the GA extract group compared to the 8 hours' sterile water group. (Figure 4: a)

Polyamide denture base specimens revealed a reducing tendency with no significant difference in the candida count following a 3 hours' immersion in GA extract as opposed to sterile water at the same time. Compared to the 3 hours' time point, the candida count was significantly lower after 8 hours of immersion in either sterile water or GA extract. That reduction in count of candida adhered to the polyamide dentures after 8 hours was with no significant difference (*P*=0.09). (Figure 4: b)

Milled denture base specimens also showed a reducing trend in the candida count when immersed in GA extract for 3 hours as opposed to sterile water throughout the same time point. However, that difference was non-significant (P=0.09). There was non-significant increase in the candida count when immersed in GA extract for 8 hours compared to 3 hours' immersion in sterile water. (Figure 4: c)

3D-printed denture base specimens revealed that there was no significant difference in the number of candida between denture bases immersed in sterile water or GA extract after 3 hours' time point. Nevertheless, compared to the 3 hours' time point, the number of adherent candida cells to 3D-printed denture base specimens was significantly lower than after 8 hours' time point in both sterile water and GA extract ($P \leq 0.05$). There was a greater reduction in candida count in denture base specimens immersed in sterile water at 8 hours compared to 3 hours' time point ($P \leq 0.05$). Moreover, the count was significantly less in denture base specimens immersed in GA extract at 8 hours' time point than those immersed in sterile water at the same time point ($P \leq 0.05$). (Figure 4: d)

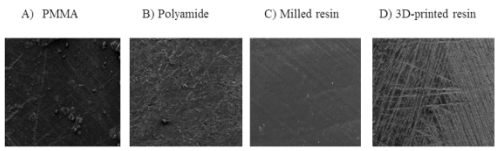

Figure 3: SEM by magnification x50 showing A) PMMA. B) Polyamide. C) Milled resin. D) 3D-printed resin.

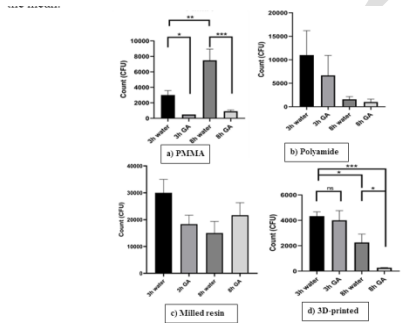

Figure 4: Count of *C. albicans* (CFU/ml) adhered cells to denture base material after immersion of candida coated a) PMMA, b) polyamide, c) Milled resin, d) 3D-printed specimens in sterile water or AA gum extract test for 3 hours and 8 hours. (*p < 0.05, ** p < 0.01, *** p < 0.001). Data are expressed as mean; error bars represent standard error of the mean.

DISCUSSION

This study aimed to evaluate the potential antifungal effect of GA extract on different denture bases at two time points. The methodologies used in this research were derived from previous investigations. Colony forming units (CFUs) method was used in the current study to evaluate the *C. albicans* count as it is the gold standard method for biofilm growth assessment of this organism. [33]

In the current study, it is found that the effect of GA extract on these denture bases may be varied according to: the manufacturing method of the denture bases itself; the concentration of AA and the immersion time. The different manufacturing methods of denture bases may clarify its behavior to the attachment of *C. albicans.* This may be elucidated by the theory that the surface characteristics of denture base affects C. *albicans* adherence, also among other factors; is the chemical composition of the denture base material which significantly influences the pathogenic yeast cells' capacity to adhere and form biofilms, in addition to the microorganism strain is another important factor. [34] This is confirmed by the results of SEM images of this study. In the present study, dentures were immersed for 8 hours in order to replicate their removal and overnight soaking. [35,36] There was a significant reduction in the count of candida cells adhered to (Acrostone) PMMA denture base materials. That indicates the efficiency of GA extract in reducing the count of candida cells from the PMMA denture. Remarkably, When PMMA specimens were immersed in water for 8 hours as opposed to 3; there was a significant increase in the candida count. That is consistent with the findings of Verhaeghe TV et al 2019 study [37] which uncovered an unexpected fact: leaving the dentures dry overnight is preferable to storing them in water without cleaning them, as the latter may promote the colonization of C. *albicans.* The adherence of C. *albicans* to PMMA; can be explained by incomplete mixing of the polymer and monomer, air trapped during mixing, and inadequate flask compression, all of which might affect the adherence of microorganisms and encourage their colonization on the surface of the denture. [38] After being immersed in GA extract, (Flexi Ultra) polyamide specimens showed a decreasing trend with non-significant change in the adherent candida count. Considering the data acquired from Aslanimehr M et al in 2017 [34] who stated that; compared to conventional pressure-coated surfaces,

injection-molded acrylic materials have fewer C. *albicans* adhered to them, and the results were statistically significant (p=0.001) when using acrylic resins. The distinct preparation techniques applied to the two materials may have led to this result. Injection molding resins are available in cartridges that have already been pre-mixed, creating a smooth surface that reduces the adhesion of C. *albicans* to the material. [34, 39] In regards to CAD/CAM specimens; (AvaDent) milled specimens, there was a non-significant reduction in *C. albicans* count. The reduction was possibly due to the reduced initial adherence to the smooth surface. This is consistent with the results of studies which reported that computerized milling significantly lowered microbial adhesion. [40,41] As in comparison to conventional groups, CAD/CAM demonstrated more desirable surface qualities, such as a reduced error rate, more precision, better consistency, greater adaptability and less porous. [42] The high pressure, the manufacturer utilized during polymerization causes the industrially polymerized resin pucks from which the denture bases are milled, to have highly condensed resin. [43] Fouda et al [44] discovered that whereas C. *albicans* adhered to the surfaces of milled, 3D-printed and heat polymerized denture base resins similarly, milled resins had the lowest count. However, the non-significant increase in candida after immersion in GA extract for 8 hours maybe consistent with, Schubert et al [45] who discovered more candida adherence on CAD/CAM 3D-printed and milled oral splints compared to conventionally manufactured ones, confronting all prior findings..

The number of attached candida cells was significantly reduced in (Formlabs) 3D-printed specimens. Studies [40,41] reported that 3D printing greatly increased *C. albicans* adhesion to denture foundation acrylic resin discs, during 4-hour adhesion period of C. *albicans*. This could be as a result of the 3D-printed resins' layer-by-layer manufacturing, which leaves pores and grooves on their surface that promote the attachment of microorganisms. Furthermore, compared to conventional heat-polymerized unpolished specimens and even after polishing the specimens, Osman RB et al [46] found increased Candida adhesion on printed resins following a 24-hour incubation period. Nevertheless, Fiore AD et al [47] found that C. *albicans* adhered more to the heat-polymerized PMMA resin in 90 minutes than to the milled denture base resins and 3D-printed using the SLA technique. After 16 hours of incubation, the microbial adhesion of all resins was similar.

Although information about the antifungal effect of AA extract in relation to time is scarce, however, in this study; the count of C. *albicans* was reduced after immersion of different denture base material in GA extract which rejected the null hypothesis.

In attention to the limitation of this study, further in-vitro studies with larger sample size and clinical studies are required and hence support the findings of this study to use GA extract as an antifungal agent. Moreover further studies comparing Acacia Arabica extract to other antifungal agents is recommended to be used in denture care products (cleansers) in near future.

CONCLUSIONS

The use of GA extract significantly reduced the number of C. *albicans* attached to PMMA and 3D-printed specimens (irregular surface topography); and non-significantly in polyamide and milled specimens (smooth surface topography) after overnight soaking.

C. *albicans* count increased significantly when PMMA specimens immersed in water overnight.

The concentration of 50% (w/v) GA extract showed a potential antifungal activity in reducing C. *albicans* count adhered to different denture bases.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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