Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Impact of apigenin and seashell nano-additives on the antifungal and roughness behavior of a soft denture liner

Nadia Tawfiq Jaffer [1*], Rizgar Mohammed-Ameen Hasan [2], Ghada Abd Alrhman Taqa [3]

[1] Prosthodontics Department-College of Dentistry/ University of Duhok- Iraq
[2] Prosthodontics Department-College of Dentistry/ Hawler Medical University- Iraq
[3] Dental Basic Science Department-College of Dentistry/ Mosul University- Iraq

ARTICLE INFO

Original paper

Article history:
Received: March 24, 2023
Accepted: June 15, 2023
Published: September 30, 2023

Keywords:

Antifungal; Apigenin; Seashell; Soft liner; Roughness

ABSTRACT

Fungal colonization of the soft denture liner is the first step in the development of denture-induced stomatitis. The study aims to assess apigenin and seashell nano-additives for their antifungal efficacy and their impact on the surface roughness of a soft denture liner. The study was accomplished in the Colleges of Dentistry in Duhok, Mosul and Hawler Medical Universities. The Antifungal efficacy against *Candida albicans* was performed by the minimum inhibition concentration (MIC), for apigenin the MIC was determined by agar well diffusion and set at (0.25%, 0.5% and 1%) while for seashells, MIC was determined by broth dilution and set at (1.25%, 2.5% and 5%). Fungal adhesion was conducted on seven groups (unmodified soft liner and six groups of the modified liner with the antifungal concentrations (three for each nanoparticle). A total of forty-nine square-shaped specimens (10*10*2mm) of (GC, Super-soft, heat-cured, USA) soft liner were prepared, the adherent fungal cells were enumerated under a light microscope for each specimen in four fields and the results were expressed as fungal cells/mm^2. For the surface roughness, forty-nine specimens of (20*10*3 mm) of the soft liner were prepared and the average surface roughness was obtained in µm using a profilometer (Talysurf, Taylor Hobson, UK). Apigenin and seashell-modified soft liner observed a significant decrease in both fungal adhesion and surface roughness compared to the unmodified liner and the reduction was related directly to the concentration of both additives. Apigenin and seashell nano-additives were effective as antifungal agents beside improving the surface roughness of the soft liner.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.9.6

Introduction

Soft denture lining materials have been used in the treatment of traumatized denture- bearing mucosa, in sever bony undercuts, in xerostomia, in edentulous arches opposing natural dentition, in oral defects requiring obturations and in advanced resorption of the alveolar ridges where patients cannot tolerate hard denture bases, the rehabilitating effect of these liners on the unhealthy tissues through reducing and evenly distributing masticatory forces on the basal seat mucosa makes the wearing of the denture more comfortable and acceptable (1).

However, during their clinical use soft liners have many drawbacks, the colonization of pathological microorganisms especially *Candida albicans* the most human fungal pathogen responsible for oral mucosa infection, is a crucial first step in the initiation and development of denture-induced stomatitis which is characterized by the inflamed and erythematous mucosa which is covered by the denture (2). Besides the irregularities in the denture fitting surface enhancing the plaque formation and infection potential (3), denture surface roughness has a direct relationship with the microbial retention on the surface making it a reservoir that harbors microorganisms which will adversely affect the liner serviceability and deteriorate it's structure and properties (4). With the emergence of resistant *Candida* strains to conventional antibiotics whether the resistance is built-in or acquired due to prolonged or misuse of the antifungals and the ability of most of the fungi to form biofilms inspite of using the antifungal agents regularly, there is a biological inquiry to use novel and more effective agents in geriatric and immunosuppressed patients (5).

The incorporation of nano-sized particles has been an effective means to get benefits of the superior properties of the nanoparticles (6). Silver, Titanium and Zirconium have been incorporated to reduce the microbial load in the oral cavity and to decrease the chance of developing denture-induced stomatitis (7).

Nano-particle incorporation of a natural origin into soft liners is so practical. Apigenin a natural trihydroxy flavone, mostly presents in fruits and vegetables like parsley, oranges, onion and tea (8) has gained attention due to its multiple bioactivities, the revealed biological functions of apigenin include anti-oxidant, antimutagenic, anticarcinogenic, anti-inflammatory, in addition to antibacterial, antiviral, antifungal, and antiparasitic effectiveness (9). Medically, apigenin possessed oxidative damage of the free radicals, a decrease in the glucose level as well as an acceleration in the wound healing rate (10-12).

Seashell, a protective structure of mollusk's, such as oyster's or a clam's (13), is mostly composed of calcium and phosphate and a source of hydroxyapatite (HA) (14). Chemical analysis of different shell species revealed many oxides, mainly calcium oxide followed by magnesium

* Corresponding author. Email: nadiya.tawfiq@uod.ac

oxide and trace amount of iron oxide. Seashell had been used in the synthesis of (HA) and the preparation of calcium-based dental cements, and proved to demonstrate antibacterial activities against *Streptococcus mutans*, oral *Lactobacilli* and *Enterococcus faecalis* (15,16).

The present study aims to assess apigenin and seashell nano-additives for their antifungal efficacy and their impact on the surface roughness of a soft denture liner.

Materials and Methods

Isolation of *Candida albicans*

Candida albicans had been selected as a model fungal organism in this study as it is the main cause of candidiasis, culture was obtained from the microbiology laboratory center- dental basic science department in the College of Dentistry/Duhok University. To ensure its purity, *Candida albicans* was cultivated on Sabouraud Dextrose agar and germ tube test by a special microbiologist (17). The culture was then subcultured on Sabouraud dextrose agar (SDA); (Oxoid, UK) plates and incubated for 24 h at 37 °C. Cells were harvested by refrigerated centrifuge (6000 rpm/4°C/15 min) and then were washed twice in phosphate–buffer saline PBS, *Candida albicans* was diluted to a suspension of (0.5 McFarland standards) which is about 10^7 colony forming unit (CFU/ml), 2 ml of the suspension in PBS was added to a petri dish that contained the test samples and incubated for 1hr at 37°C (18).

Determination of antifungal effect

The Minimum Inhibitory Concentration (MIC) is the lowest antimicrobial agent concentration that prevents the visible growth of a microorganism. These evaluations are quite useful to determine the most appropriate concentrations required in the final antimicrobial agent that will be dependent on the adhesion assay.

Determination of MIC for apigenin nanoparticles (Agar well diffusion method)

A volume of *Candida albicans* inoculum had been spread on the entire plate surface of Sabouraud Dextrose agar. Then, four holes with a diameter of 6 mm and 1 mm depth were punched aseptically with a sterile cork-borer on each plate, so that the wells were lying within the lawn culture, Nystatin was used as a control antifungal agent and a volume of the apigenin solution at concentrations of (0.25%, 0.5%, 1%, 2%, 4%, 6%, 8% and 16%) were introduced into the wells. Then, the plates were incubated at 37°C in a shaking incubator. The lowest concentrations that exhibited clear zones around the wells (minimum inhibition zone) representing the diffusion of the apigenin in the agar medium and inhibited the growth of the microbial strain was determined visually and considered as the MIC (19). The mean inhibition diameter was measured by the average diameter around the well using a metallic scale (20). Five independent experiments (n=5) were performed.

Determination of MIC for seashell nanoparticles (Broth dilution method)

The procedure involved the preparation of two-fold serial dilutions of the antimicrobial agent in brain heart infusion broth (21), six times the serial dilution was carried out to obtain suspension with 0.312%, 0.625%, 1.25%, 2.5%, 5% and 10% of the seashell nanoparticle concentration. Besides, two tubes were prepared, the negative control (the seashell nanoparticles with the broth without C. albicans inoculum) and the positive control (the broth inoculated with *Candida albicans* without nanoparticle). A volume of 0.1 ml of *Candida albicans*-containing broth was dispensed in all tubes containing 2 ml (macrodilution of nanoparticle concentrations) Then, after well-mixing, the inoculated tubes were incubated for 24 h at 37°C. The MIC was determined and confirmed after culturing 0.1 ml of each tube solution in a previously prepared plate containing Sabouraud Dextrose agar and after incubation in a shaking incubator at 37°C for 24 h.

Soft liner specimens' preparation

A total of forty-nine square- shaped specimens (10mm×10mm×2mm) of (GC, heat cured Super- soft, USA) acrylic soft denture lining material were prepared for testing the fungal adherence ability and additional forty-nine specimens of (10mm×20mm×3mm) dimensions were prepared for roughness measurement and distributed into seven groups of the study (control, apigenin modified with 0.25, 0.5 and 1%, seashell modified with 1.25, 2.5 and 5%) with a mixing ratio of 5 gm powder to 4 ml liquid according to the manufacturers' instructions. The additives were added to the soft liner's liquid, and the weight of the additive powder was subtracted from the soft liner powder's weight. The additive powder with the liner's liquid were mixed together and put inside a sonicator in order to uniformly distribute the nano-powder, the liquid and powder of the soft liner were mixed together and covered till reached the dough stage then the mixture was packed into stone molds previously prepared in the required specimens' dimensions. The material was cured in hot water at 165°F for approximately 30 minutes and then boiled for ten minutes according to the instructions of manufacturers'.

Adhesion assay

The MIC results of the apigenin and seashell nanoparticles had been determined to use the most effective concentrations for each nanoparticle in the adhesion assay. Apigenin was recorded sharply by agar well diffusion against *Candida albicans* at three concentrations (0.25%, 0.5% and 1%), while seashell registered its most powerful antifungal concentrations by broth diffusion method at (1.25%, 2.5% and 5%), Figure (1).

Candida albicans adherence ability

A suspension of 0.5 McFarland standards *Candida albicans* was prepared. The sterile specimens of the soft liner were deposited in sterile plates containing 20ml of the prepared suspension and incubated for 1hour at room temperature. Then, the specimens were removed from the

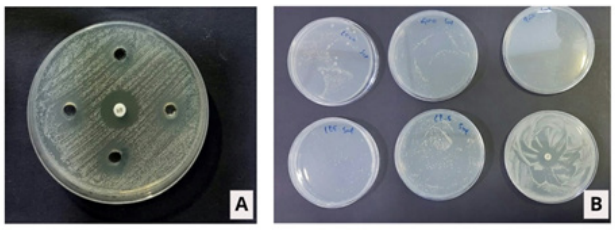

Figure 1. MIC against *C. albicans*, A: apigenin antifungal activity by agar well diffusion, B: seashell antifungal activity by broth dilution.

suspension and rinsed twice for one minute by phosphate buffered saline solution (PBS) to eliminate the non-adhered cells, and with sonication for 15min, the specimens were dried and fixed with 1 ml of methanol (7,22). The staining was carried out using crystal violet for 30 seconds, specimens were washed with (PBS) solution again for 30 seconds, and were dried with filter paper to be inspected under a light microscope 10X (Carl Zeiss, Germany). The adhered cells were enumerated for each specimen in four fields and the results were expressed as fungal cells/mm^2.

Surface roughness measurement

The soft lining specimens were inspected for surface roughness using a profilometer (Talysurf, Taylor Hobson, UK) with a 0.25µm Diamond stylus head at 0.5 mm/sec speed and a cut-off length of 2.5 mm. Three readings were obtained at three different sites of each specimen surface and the mean of the readings was calculated and considered as the average roughness (Ra µm).

Statistical Analysis

The data were expressed as mean ± SD, for multiple comparisons, and the study groups were analyzed using One-way ANOVA analysis of variance and LSD post-hoc test. The threshold significance was set at $P \leq 0.05$.

Results

Adhesion assay, fungal cell/unit area

Table (1) ANOVA showed that there was a highly significant difference among the groups ($p < 0.05$). Figure (2) showed the results of the Post- hoc LSD (Duncan's) test, the highest mean of the adherent fungal cells was obtained for the control group (unmodified soft liner), The liner-modified groups observed a decrease in the adhesion of fungal cells in both apigenin and seashell and the adhesion decreased significantly with the increase in the concentration of the additive material, the lowest means were recorded for seashell 5% and apigenin 1% modified liner.

Surface roughness measurement

There was a significant difference among all groups in surface roughness mean values according to the ANOVA test, Table (2). Post hoc LSD test, Figure (3) showed that the highest mean value was obtained for the control group (unmodified soft liner), while a high decrease was obtained for the groups of apigenin and seashell modified liner and the decrease was related directly to the increase in the additives' concentration, the lowest mean was recorded for seashell 5% modified liner.

Discussion

It is interesting to test the antifungal efficacy of pharmaceutical materials from natural origin at nano scale size impregnated in a soft liner against *Candida albicans* the commensal and most prevalent opportunistic human fungal pathogen, when there is impairment in the host immunity to reduce as much as possible the development of the denture-induced stomatitis.

Apigenin, a naturally, organic, and small occurring biomolecules has been approved to be antimicrobial, antiviral

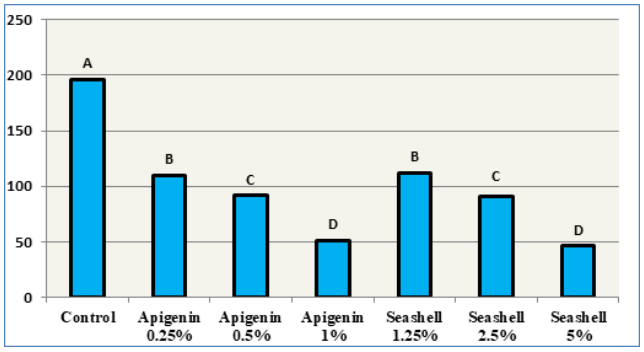

Figure 2. Multiple comparison Duncan-LSD test of the mean adherent *C. albicans* colonies for the soft liner modified with apigenin and seashell nanoparticles, different letters mean significance.

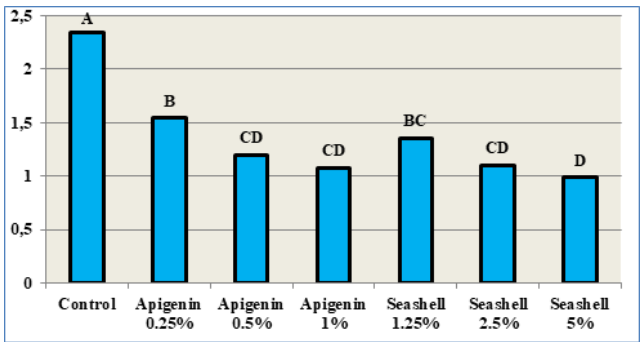

Figure 3. Multiple comparison Duncan-LSD test of the mean surface roughness (µm) for the soft liner modified with apigenin and seashell nanoparticles, different letters mean significance.

Table 1. One-way ANOVA test for *Candida albicans* adherent colonies.

Group	N	Mean ± SD			Sum of squares	df	Mean square	F	Sig-P value
Control	7	195.857(25.47)	Variance Analysis	between groups	104220.7	6	17370.12	144.374	.000
Apigenin 0.25%	7	109.857(7.44)							
Apigenin 0.5%	7	91.42(6.32)							
Apigenin 1%	7	51.142 (3.84)							
Seashell 1.25%	7	112.00(6.48)		within groups	5053.143	42	120.313		
Seashell 2.5%	7	90.857 (5.52)							
Seashell 5%	7	46.00(3.2)							
Total	49	99.59(47.71)		Total	109273.8	48			

Table 2. One-way ANOVA test for surface roughness.

Group	N	Mean ± SD			Sum of squares	df	Mean square	F	Sig-P value
Control	7	2.34 ± 0.30	Variance Analysis	between groups	9.147	6	1.524	19.955	.000
Apigenin 0.25%	7	1.54 ± 0.58							
Apigenin 0.5%	7	1.20 ± 0.17							
Apigenin 1%	7	1.08 ± 0.18							
Seashell 1.25%	7	1.357 ± 0.139		within groups	3.209	42	.076		
Seashell 2.5%	7	1.10 ± 0.081							
Seashell 5%	7	0.985 ± 0.069							
Total	49	1.373(0.507)		Total	12.356	48			

and antiparasitic flavone (23) and had fulfilled the required conditions of periodontal pathogens species drugs (24).

Apigenin has observed in this study a potent antifungal effect at different concentrations especially at (0.25%, 0.5% and 1%). The antifungal mechanism of apigenin is related to its ability to cause perturbations of the cell membrane, causing cell shrinkage and leakage of the intracellular components and a reduction in the ability of the fungal membrane to maintain its osmotic balance. Additionally, the inhibition of *Candida albicans* biofilm formation by apigenin will result in an alteration of the membrane and a physical damage of the fungal pathogen by inducing the mitochondrial-mediated apoptotic pathway, and mitochondria calcium signaling is the main factor in its pathway in *C. albicans.* These activities render apigenin to be effective as a therapeutic antifungal agent and participate in its defense against infection by pathogenic microorganism (25, 26).

Seashells antifungal effect was more potent at concentrations (1.5%, 2.5% and 5%) composed mainly of calcium carbonate and via heat treatments to produce nanoparticles converted into CaO. Subsequently CaO hydrate to produce Ca(OH)2. These seashell-derived components have observed excellent microbicidal activities related to their alkalinity, the primary mechanism of antimicrobial activity (27). In addition the HA hydroxyapatite content in seashell has antibiofilm activity, influencing the production of extracellular matrix, altering the morphological characteristics of cells wall, and decrease in the hyphal cells density, a mechanism that justifies the antifungal activity (28). The secondary mechanism of antimicrobial activity is by the dissociation of CaO in the slurry phase and generation of the high content of Ca2+ and ROS (Reactive Oxygen Species), a cationic environment that bonds with the cardiolipin (negative surface charge) the main lipid in the cell membrane of the pathogen. The binding of Ca2+ and cardiolipin results in changing the cell metabolism, starvation and cell wall rupture of the fungal cell (29,30).

Low roughness values are important to prevent the adhesion of microorganisms. The surface energy is effective in initial adhesion, while surface roughness provides a large surface area enabling the attachment as well as protecting the environment until completing the firm attachment (31).

Both apigenin and seashell nano-additives reported enhancements in the surface roughness of the soft liner when compared to the untreated liner and the effect positively appeared as the concentration of the additives increased. The results are attributed to the small size of the reinforcement materials and the uniform dispersing of the nano additives preventing agglomeration within the soft liner and the small size of the reinforcement materials, besides the higher the concentration of the nanoparticles the greater will be the spaces being filled and the more contact points between the liner and nano additives, thereby providing mechanical interlocking and resulting in a decrease in the plasticizers leakage and surface porosities (32).

Conclusion

The modification of the soft denture liner with apigenin and seashell nano-materials had greatly enhanced its antifungal activity and reduced the fungal adhesion to the liner material. In addition, an improvement in the surface roughness was observed. This modification of the soft liner can be considered as a promising in the prevention of denture-induced stomatitis and providing the patient a more healthier and comfortable denture.

Conflict of Interest

There are no conflicts of interest.

Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data that were generated during this study are included in this published article

Authors' Contribution

All authors had equal role in study design, work, statistical analysis and manuscript writing.

Funding

This research was not funded in the commercial and public.

References

1. Borg HS. One-year clinical investigation of the inclusion of

three nanoparticles in the heat-cured acrylic resin denture base on the adhesion with the silicone soft liner. Egypt Dent J 2021; 67(2):1527-1536. DOI: 10.21608/edj.2021.59178.1463

2. Baygar T, Ugur A, Sarac N, Balci U, Ergun G. Functional denture soft liner with antimicrobial and antibiofilm properties. J Dent Sci 2018; 13(3): 213-219. doi: 10.1016/j.jds.2017.10.002.
3. Urban VM, Lima, TF, Bueno MG, Giannini M., Arioli Filho JN, de Almeida AL, Neppelenbroek KH. Effect of the addition of antimicrobial agents on Shore A hardness and roughness of soft lining materials. J Prosthodont 2014; 24(3):207-214. doi: 10.1111/jopr.12205.
4. Elawady, AF, Mohamed SE, Nassif MR. A study to compare adhered oral flora to soft liner and conventional denture base surface in complete denture patients. Egypt Dent J 2019; 65(4): 3777-3786.
5. Hossain CM, Ryan LK, Gera M, Choudhuri S, Lyle N, Ali KA, Diamond G. Antifungals and Drug Resistance. Encyclopedia. 2022; 2(4):1722-1737. https://doi.org/10.3390/encyclopedia2040118
6. Akay C, Avukat EN. Effect of Nanoparticle Addition On Polymethylmethacrylate Resins. Acta Sci Dent Sci 2019; 3(7): 91-97. DOI: 10.31080/ASDS.2019.03.0577
7. Ahmed AQ, Ali MM. The influence of Titanium Dioxide nanoparticles incorporation into soft denture lining material on *Candida albicans* adherence and some mechanical and physical properties. J Pure Appl Microbiol 2018; vol 12(2): 783-791. DOI: https://dx.doi.org/10.22207/JPAM.12.2.39
8. Wang M, Firrman J, Liu L, Yam K. A review on flavonoid apigenin: dietary intake, ADME, antimicrobial effects, and interactions with human gut microbiota. Biomed Rese Int 2019; 9: 1-18. DOI: 10.1155/2019/7010467
9. Morimoto Y, Baba T, Sasaki T, and Hiramatsu K. Apigenin as an anti-quinolone-resistance antibiotic. Int J Antimicrob Agents 2015; 46(6): 666–673. doi: 10.1016/j.ijantimicag.2015.09.006.
10. Ibrahim RT, Mahmmod AA, Taqa, GA. Synthesis of Silver Nano particles using Catechin and Apigenin and study their anti-oxidant effect on the laboratory Rats. College Of Basic Education Researches Journal 2020a; 16(3), 919-943. DOI:10.33899/BERJ.2020.166004
11. Ibrahim RT, Mahmmod AA, Taqa GA. The protective and Antioxidant Effect of Catechin and Apigenin on Some Biochemical Parameters in Blood Serum of Rats Exposed to Oxidative Stress Induced by H2O2. College Of Basic Education Researches Journal 2020b, 16(3):813-852. DOI:10.33899/BERJ.2020.166002
12. Rajab AM, Al-Wattar WT and Taqa GA. The roles of apigenin cream on wound healing in rabbits model. J Appl Vet Sci 2022, 7 (1): 1-5. DOI: 10.21608/JAVS.2021.97151.1104
13. Moss AS, Ishikawa M, Koshio S, Yokoyama S, Dawood MAO. Effects of Different Levels of Marine Snail Shells in the Diets of Juvenile Kuru mps *Marsupenaeus japonicus* as a Source of Calcium. North American Journal of Aquaculture 2019, 81 (1): 55-66. https://doi.org/10.1002/naaq.10066.
14. Orman Z, Yucel S, Sahin YM, Gunduz O, Oktar FN. Bioactivity of Hydroxyapatite Produced from Sea Snail Turritella Terebra. Acta Physica Polonica A 2019;135(5): 1089-1092. DOI: 10.12693/APhysPolA.135.1089
15. Al-Yousifany NN, Chakmakchi M and Taqa AA. Preparation and evaluation of antimicrobial activity of novel calcium based dental cement. International Journal of Enhanced Research in Science Technology & Engineering 2015; Vol. 4 (3): 99-105.
16. Banan N. Alhussary, Ghada A. Taqa, Amer A. Taqa. Preparation and characterization of natural nano hydroxyapatite from eggshell and seashell and its effect on bone healing. J. Appl. Vet Sci. 2020; 5 (2): 25 -32. DOI: 10.21608/javs.2020.85567
17. Abdulla H, Mustafa EA. Rapid Detection of Candida species Isolated from Denture Stomatitis Patients using Phenotypic methods and Chromogenic agar media. Al-Rafidain Dent J. 2020; 20: 125-133. DOI:10.33899/rden.2020.126821.1029
18. Mohammed M Sadoon, Ammar K Al-Noori. Effect of Different Nanoparticles Incorporation in Acrylic Based Soft Liner on Candida albicans Adhesion. Al-Rafidain Dent J 2022; 22(1):158-169. DOI: 10.33899/rdenj.2022.173032
19. Godil AZ, Bhagat D, Das P, Kazi AI Dugal R, Surekha Satpute S. Incorporation of Fluconazole and *Ocimum Sanctum* Oil in Soft Denture Liners to Treat Biofilms of *Candida albicans* Associated with Denture Stomatitis. Dentistry 3000 2021; Vol 9 (1). DOI 10.5195/d3000.2021.120
20. Rawat P, Agarwal S and Tripathi S. Effect of Addition of Antifungal Agents on Physical and Biological Properties of a Tissue Conditioner: An In-Vitro Study. Adv Pharm Bull 2017; 7(3), 485-490 doi: 10.15171/apb.2017.059 http://apb.tbzmed.ac.ir
21. Parvekar P, Palaskar J, Metgud S, Maria R and Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus.* Biomater Investg Dent. 2020; 23;7(1):105-109. doi: 10.1080/26415275.2020.1796674
22. Gad MM, Bahgat HA, Edrees MF, Alhumaidan A, Khan SQ, Ayad NM. Antifungal activities and some surface characteristics of denture soft liners containing silicon dioxide nanoparticles. J Int Soc Prev Community Dent 2022; 12(1):109-116. doi: 10.4103/jispcd.JISPCD_286_21.
23. Younes KA, Taqa GA, Nayef NS. Effect of some materials and drugs on Entamoeba gingivalis culture. Rafidain Journal of Science 2019*;* Volume 28(4):40-49 10.33899/rjs.2019.163300
24. Cha S, Kim G, Cha J. Synergistic antimicrobial activity of apigenin against oral Pathogens. International Journal of Engineering Research & Science (IJOER) 2016; 2(1):27-37.
25. Lee H, Woo E, Lee DG. Apigenin induces cell shrinkage in Candida albicans by membrane perturbation. FEMS Yeast Res 2018; 18(1): 1-9. doi: 10.1093/femsyr/foy003
26. Lee W, Woo E, Lee D. Effect of apigenin isolated from *Aster yomena* against *Candida albicans*: apigenin-triggered apoptotic pathway regulated by mitochondrial calcium signaling. J Ethnopharmacol 2019; 231(1):19-28. doi: 10.1016/j.jep.2018.11.005
27. Gultekin N and Kucukates E. Evaluation of antimicrobial activity of scallop shell powder against Staphylococci species and gram negative bacteria isolated from patients intensive care units. Biomed J Sci & Tech Res 2019; 13(4):10176-10180. DOI: 10.26717/BJSTR.2019.13.002445
28. Zamperini CA, André RS, Longo VM, Mima EG, Vergani CE, Machado AL, Varela JA and E. Longo E. Antifungal Applications of Ag-Decorated Hydroxyapatite Nanoparticles. Journal of Nanomaterials Volume 2013; Article ID 174398, 9 pages http://dx.doi.org/10.1155/2013/174398
29. Roy A, Gauri SS, Bhattacharya M and Bhattacharya J. Antimicrobial activity of CaO nanoparticles. J. Biomed. Nanotechnol 2013; 9, 1570-1578. doi:10.1166/jbn.2013.1681
30. Hata Y, Bouda y, Hiruma S, Miyazaki H and Shingo Nakamura S. Biofilm Degradation by Seashell-Derived Calcium Hydroxide and Hydrogen Peroxide. Nanomaterials 2022; 12, 3681. https://doi.org/10.3390/nano12203681
31. Kreve S, Oliveira VC, Bachmann L, Alves OL and Dos Reis AC. Infuence of AgVO3 incorporation on antimicrobial properties, hardness, roughness and adhesion of a soft denture liner. Scientific Reports 2019; 9:11889 https://doi.org/10.1038/s41598-019-48228-8.
32. Gad MM, Abualsaud R, Rahoma A, Al-Thobity AM, Al-Abidi KS, and Akhtar S. Effect of zirconium oxide nanoparticles addi-

tion on the optical and tensile properties of polymethyl methacrylate denture base materials. Int J Nanomedicine 2018; 13: 283-292. DOI: 10.2147/IJN.S152571