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Abstract

Objectives: was to evaluate antimicrobial effect of Coriander essential oil as denture disinfectant on the microorganism related to the fitting surface of the dentures and its effect on the color of denture.

Materials and Methods

Microbiological test: Forty complete denture wearers were selected. swabs were taken from the fitting surface of the dentures, culturing and differentiation and counting of microorganism were done.

Participants were instructed to apply Coriander essential oil to the fitting surface of the dentures for ten days. Swabs from the fitting surface of the dentures after application of materials for ten days were taken, culturing and differentiation and counting of microorganism were done.

Color stability test: 12 clear and 12 Pink Specimens were received Coriander oil and 2 clear Specimens and 2 pink Specimens as control (without storage in any solution). Test specimens were immersed in coriander essential oil for 30 days. Spectrophotometer were used to measure color change.

Results

Microbiological results: There was statistically significant decrease with *Streptococcus, Coliforms* and decrease with *Staph epidermis, Staph aureus* and *Candid*a but statistically non-significant.

Color change results: There was significant change in color of clear and pink heat cured acrylic resin after imme.

Conclusion: Coriander essential oil had remarkable antiseptic effect on oral microorganisms related to the fitting surface of dentures.

Coriander essential oil as denture disinfectant can affect color stability of heat cured acrylic resin denture base but in comparing with 6 years simulated period of daily immersing for 20 minutes, it considered very little change.

Keywords: Denture Hygiene; Denture Cleanser; Essential Oil; Disinfectant; Complete Denture

Abbreviations

hrs: Hours; CFU: Number of Colony-Forming Units; nm: Nanometer; cm²: Square Centimeter(s)

Introduction

Most developed world countries have accepted the chronological age of 65 years as a definition of 'elders' or older person While this definition is somewhat arbitrary, it is many times associated with the age at which one can begin to receive pension benefits. The portion of older people is growing faster than of any other age group [1]. Approximately 600 million are aged 60 years and over, and this number will double by 2025 [2]. By 2050, it will be 2 billion, 80% living in developing countries [1,2].

Not only chronic systemic diseases are prevalent in old age [3], but also poor oral health and high prevalence rate of oral diseases has been seen [4].

Removable dentures especially complete dentures are frequent among elder people.

Wearing dentures alters the oral flora. Oral flora are micro-organisms that are present in a healthy mouth as commensals and normal inhabitant without causing any disease. It includes numerous micro- organisms as bacteria, fungi, mycoplasma and viruses and on occasion's even protozoa [5].

Denture materials when exposed to oral cavity it formed dental biofilm by Formation of pellicle, Bacteria attach to the pellicle, Bacterial multiplication and colonization, Biofilm growth and maturation and Matrix formation.

The cultivable flora of the denture showed a complex bacterial community 56.8% of these were aerobic bacteria, 36.9% were anaerobic bacteria and 13% were yeasts [6].

Different studies have suggested that oral bacteria may be risk factors for a number of prevalent systemic diseases. Oral bacteria have been implicated in bacterial endocarditis, aspiration pneumonia, gastrointestinal infection and chronic obstructive pulmonary disease, among others, and dentures offer a reservoir for microorganisms associated with these infections [7,8].

Wearing removable dentures induces local environmental changes that may result in denture stomatitis which is a common condition characterized by an inflammatory response of the denture- bearing mucosa. The prevalence rate of denture stomatitis is reported within the range of 11 to 67% in complete denture wearers. [9-11].

Many modalities were used to keep denture hygiene either mechanical and chemical (soap, alkaline peroxides, alkaline hypochlorite, acids, disinfectants, enzymes [12-17] and mouth washes.

Return to the nature in medical field has a great concern nowadays to be away from the side effect of chemicals and synthetic medication. In ancient Egyptian, Indian and Buddhist culture many plants were used to treat diseases.

Dioscorides, "the father of pharmacognosy," who, as a military physician and pharmacognosist of Nero's Army, studied medicinal plants wherever he travelled with the Roman Army.

Dioscorides listed coriander within most appreciated domestic plants, Coriander is a summer plant, originating from Southern Europe spanning to North Africa and southwestern Asia. It has therapeutic properties as Antioxidant, Hypoglycemic, Hypolipidimic, Antibacterial, anti-inflammatory and Antimutagenic Potential and it was used by several cultures [18].

Recent studies discovered that Coriander essential oil has anti-bacterial and anti-fungal effect. Oil of coriander is also preferably used for prophylaxis and/or therapy of microbial diseases of the mucous membranes, particularly for diseases of the mucous membranes caused by *Streptococci, Staphylococci* (including methicillin-resistant strains of *Staphylococcus aureus*), *Pseudomonades, E. coli*, but also yeast-like fungi and molds [19].

Plymethyl methacrylate resin has been used for a long time as the best choice to fabricate full or partial dentures because of its esthetic qualities and ease of manipulation. Discoloration of acrylic resins may be caused by several factors. Intrinsic factors and extrinsic factors. It has been reported that certain beverages, such as tea, coffee, and wine, cause discoloration of acrylic resins [20]. Some of the extrinsic factors are: the effect of cleaning solutions, tobacco, composition of saliva and denture hygiene habits [21].

Aim of the work was to evaluate antimicrobial the effect of Coriander essential oil as denture disinfectant on the microorganism related to the fitting surface of the denture and its effect of color stability of denture base.

Materials and Methods

Microbiological test

Material used: Coriander essential oil (Asala., Natural Product, Alexandria, Egypt) available in the market.

Participants: Forty complete denture wearers were selected (22 males and 18 females) and age were ranges between 60 - 70 year. After detailed clinical interview and oral examination to be sure of all participants are free from any signs of inflammation, swabs were taken from the fitting surface of the dentures, culturing and differentiation and counting of microorganism were done.

After that the participants were instructed to clean the fitting surface of the denture with brush and running water then apply Coriander essential oil with fine brush or cotton pellet and put the denture in glass of water over night and at morning participants were instructed to rinse the denture with running water before using and this were repeated for ten days. Swabs from the fitting surface of the dentures after application of materials for ten days were taken, culturing and differentiation and counting of microorganism were done.

Microbiological test

Routinely method to isolate bacteria, by spreading dental swab on agar plate (blood, MacConkey) as primary inoculum and the distributed thinly over the plate by streaking it with loop in a series of parallel lines in different segments of the plate. Loop was flamed and cooled between different sets of streaks. The plate was incubated for 48 hrs at 37°C aerobically. On incubation growth may be confluent at the site of original inoculation but it become progressively thinner and well separated colonies were obtained over the final series of the streaks (Figure 1). To differentiate between gram negative and gram positive bacteria Macconkey's agar was used. The number of colonyforming units (CFU) per cm² were calculated.

This study was performed with the approval of the Ethics Committee of Pharos University, Faculty of Dentistry, and written informed consent was obtained from the participants.

Color stability test

28 specimens 14 clear and 14 pink heat cured acrylic denture base (Acrostone, acrostone Dental and Medical Supplies, Egypt), (25 x 15 x 2.5 mm) were prepared. These specimens were wet-polished with up to 1200 grit abrasive paper. After immersion in distilled water at 37°C for 48 hours, the specimens were dried.

Application of coriander oil

12 clear and 12 Pink Specimens were received Coriander oil and 2 clear Specimens and 2 pink Specimens as control were measured at baseline (day 0) without storage in any solution.

Specimens were immersed in coriander essential oil and the all surfaces were covered with the oil and every day the samples were washed with running water and the oil applied to the Specimens as the same manner for 30 days. one hour represented 3 immersions of 20 min and each 24 h (one day) corresponded to 72 immersions of 20 min per day. 72 immersions of 20 min per day for 30 days simulating about six years of using.

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Color measurements

After 30 days, Specimens were washed and dried and grinding were done by using of acrylic stone and the powder collected in zipper bag and the number of every sample was written.

The finely crushed sample was mixed with Nujol (which are mulling agents) in a marble mortar, with a pestle to make a thick paste. 0.1 gm of the paste was weighted by sensitive balance, dissolved in 1 ml distilled water, Incubated at 37°C for 60 minutes, Centrifuged to separate the color from the mixture and cuvette was loaded Finally the intensity of the color is measured using Spectrophotometer 360 nm by measuring the intensity of wavelengths in a spectrum of light compared with the intensity of light from a standard source. Three readings of each sample were taken and the average of the three readings was recorded.

Results and Discussion

Microbiological Results

From the results of present study (Table 1 and Figure 1) it was found that the main micro-organism related to the fitting surface of removable denture was *Streptococcus* followed by *Coliforms* followed by *Candida*.

	Before using of coriander	After using of coriander	р
Staph epidermis			
Min. – Max.	2.0 - 20.0	1.0 - 14.0	0.245
Mean ± SD.	13.0 ± 7.21	7.80 ± 5.40]
Median	15.0	8.0]
мwp ₁		0.175	
Staph aureus			
Min. – Max.	3.0 - 50.0	3.0 - 35.0	0.580
Mean ± SD.	22.0 ± 18.43	16.40 ± 12.10]
Median	15.0	14.0]
мwp1		0.675	
Streptococcus			
Min. – Max.	56.0 - 200.0	60.0 - 85.0	0.019*
Mean ± SD.	112.20 ± 55.83	73.0 ± 10.37]
Median	105.0	75.0]
мwp ₁		0.295	
Coliformes			
Min. – Max.	55.0 - 130.0	40.0 - 80.0	0.038*
Mean ± SD.	87.0 ± 29.07	59.0 ± 16.73	
Median	80.0	60.0	
^{MW} p ₁		0.115	
Candida			
Min. – Max.	15.0 - 65.0	12.0 - 49.0	0.632
Mean ± SD.	41.0 ± 19.81	33.0 ± 15.95]
Median	40.0	35.0	
^{MW} p ₁		0.465	

Table 1: Comparison between control group and post coriander group regarding types of isolated micro-organism.

 p_1 : p value for Mann Whitney test for comparing between control and test group *: Statistically significant at $p \le 0.05$



Figure 1: Comparison between mean of control and post coriander groups regarding types of micro-organisms.

The minimal amount of micro-organism related to the fitting surface of the denture was Staph aureus followed by Staph epidermis.

It was found that coriander essential oil was effective in decreasing the amount of microorganism related to the fitting surface of the denture regarding the five types of microorganism that were isolated and differentiated with significant decrease related to *Streptococcus* and *Coliforms*.

It was found that (Table 2), after using of coriander essential oil as disinfectant for fitting surface of denture *Staph epidermis* reduced by40%, *Streptococcus* by 35%, *Coliforms* by 32%, *Staph aureus* by 25.5% and *Candida* by 20%.

	Before using of coriander	After using of coriander
Staph epidermis	15 (2.0 – 20.0)	8.0 (1.0 - 14.0)
Mean ± SD.	13.0 ± 7.21	7.80 ± 5.40
% of reduction from control		40.0
Staph aureus	15 (3.0 – 50.0)	14 (3.0 – 35.0)
Mean ± SD.	22.0 ± 18.43	16.40 ± 12.10
% of reduction		25.5
Streptococcus	105 (56 – 200)	75 (60.0 – 85.0)
Mean ± SD.	112.20 ± 55.83	73.0 ± 10.37
% of reduction		34.9
Coliforms	80 (55.0 – 130.0)	60 (40.0 - 80.0)
Mean ± SD.	87.0 ± 29.07	59.0 ± 16.73
% of change		32.2
Candida	40 (15.0 – 65.0)	35 (12.0 – 49.0)
Mean ± SD.	41.0 ± 19.81	33.0 ± 15.95
% of change		19.5

Table 2: % of reduction from control to post coriander group regarding types of isolated micro-organism.

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Color stability Results

From the results of present study (Table 3, 4 and Figure 2) it was found that significant increase in colour change of pink and clear acrylic denture base and significant increase in colour change of clear denture base with Coriander in comparison with Control after 30 days of application of coriander essential oil.

Pink				
	Post coriander	Control		
Min. – Max	0.04 - 0.09	0.026		
Mean ± SD.	0.05 ± 0.01			
р	< 0.001*			

Table 3: Comparison between control and post coriander color change with pink heat cured acrylic resin.

 p: p value for Student t-test for comparing between

Control with post Coriander

*: Statistically significant at $p \le 0.05$

Clear					
	Post coriander	Control			
Min. – Max	0.04 - 0.18	0.035			
Mean ± SD.	0.13 ± 0.05				
р	< 0.001*				

 Table 4: Comparison between control and post coriander
 color change with clear heat cured acrylic resin.

p: p value for Student t-test for comparing between Control with post Coriander

*: Statistically significant at $p \le 0.05$





Removable dentures are particularly frequent among older people in the industrialized world [22]. Some countries report that onethird to half of the older people wear full dentures while up to three-quarters wear removable full and/or partial dentures. Again, the prevalence of removable dentures shows considerable variation by socio-economic status the rates are high among the socio-economic cally disadvantaged [23,24].

Oral flora are micro-organisms that are present in a healthy mouth as commensals and normal inhabitant without causing any disease. It includes numerous micro- organisms as bacteria, fungi, mycoplasma and viruses and on occasion's even protozoa [5].

The presence of a denture on the oral mucosa alters the local environmental conditions due to the inaccessibility of saliva and lack of mechanical cleaning by the tongue. Hence, dentures act as reservoirs that harbor a mixed species of bacterial biofilm [25].

Dental biofilm is a matrix of enclosed bacterial population which is adherent to each other and /or to surfaces and interfaces. It adheres firmly to the acquired pellicle and hence to the teeth, calculus, and fixed and removable restorations. Dental biofilm may contain microorganisms other than bacteria; the organisms may include yeast, protozoa and viruses [5].

From the results of present study, the most predominant microorganism related to fitting surface of removable denture was *Streptococcus*, many studies also reported that streptococci are associated with denture plaque from those with healthy [26,27], and diseased mouths [28,29].

Streptococcus bacteria are two types: group A and group B. Group A cause throat infection, group B cause blood infections and pneumonia in elderly or already have health problems.

In present study, *Candida albicans* was isolated from the fitting surface of the denture, which responsible for denture stomatitis, in 1936, Cahn first proposed that infection by *Candida albicans* was responsible for denture stomatitis [30].

Denture stomatitis was first classified by Newton [31], according to its clinical appearance as type 1: A localized simple inflammation, Type 2: An erythematous or generalized simple type seen as more diffuse erythema involving a part or the entire denture covered mucosa and Type 3: Agranular type (inflammatory papillary hyperplasia involves epithelial response to chronic inflammatory stimulation secondary to yeast colonization).

Other studies have shown *Candida* incorporation into biofilms covering different biomaterials such as dentures: these biofilms may be an increased risk factor for invasive candidiasis when the host immune system is compromised [27,32].

In present study, *Staphylococcus* spp. Was isolated from fitting surface of removable denture. This can be responsible for aspiration pneumonia, a life threatening infection, especially in geriatric patients [33].

So keep denture hygiene is very important procedure, and there are many modalities [12-17], either mechanical by brushing or chemical by using sodium hypochlorite. Soap is one of the auxiliary agents that can be used. Mouthwashes as chlorhexidine and Listerine have been used for cleansing dentures.

Authors also recommended that treatment should include antimicrobial therapy of denture and removal of denture for a period of time in every 24 hours.

Also there are many evidences showing that the use of microwave irradiation at specified setting and exposure time are bactericidal and fungicidal [14].

Oil of coriander is particularly preferred for prophylaxis and/or therapy of stomatitis diseases, periodontitis diseases, dental caries plaque generation, and halitosis. Oil of coriander may also be used against certain diseases (assumedly caused by viruses), as for example aphthae and Herpes labialis [19].

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In present study, coriander essential oil was used as disinfectant for denture by applying it by soft brush or cotton pellet after brushing with water and the result showed that it had effective anti-microbial and anti-fungal effect.

From the results of present study, it was found that coriander essential oil had effective bactericidal activity with gram positive (*Staph epidermis, Staph aureus* and *Streptococcus*) and gram-negative (*Coliforms*) bacteria, with more action with gram negative than gram positive bacteria.

This result is the same with the result of previous study [34] which found that Gram-negative strains and S. *aureus* strains inhibited by coriander oil, were found not to be able to regrow in fresh culturing medium after transfer and incubation for 24 h.

This results explained by using flow cyto-metric experiments conclude that its primary mode of action seems to be bacterial cell permeabilization, and the mode of action of coriander oil is similar in both Gram-positive and -negative bacteria. As a result of membrane permeabilization, all other cellular functions such as membrane potential, respiratory activity or efflux pump activity are also compromised [34].

From the results of present study, it was found that coriander essential oil as disinfectant for denture had antifungal activity. The antifungal activity may be inhibition visible fungal growth or allowed no visible growth on the solid medium and this expressed as fungicidal [35].

The antifungal activity of essential oil depends fundamentally on their ability to pass through the cell wall and penetrate between fatty acid chains of the lipid bilayer, altering membrane fluidity and permeability and damaging membrane proteins, leading to degradation of the cytoplasmic membrane and to cell death [36,37].

The initial stages of biofilm formation and adherence to a substrate material are mediated by both abiotic factors, such as surface hydrophobicity, and biotic factors, such as increased expression of adhesions and other cell-surface proteins [38]. So the inhibition of adherence of yeast cells may constitute a main target for disrupting the initial stages of biofilm formation of *Candida* [35].

Plymethyl methacrylate resin has been used for a long time as the best choice to fabricate full or partial dentures because of its esthetic qualities and ease of manipulation [39].

Many factors contribute towards discoloration of acrylic resin. They include dissolution of ingredients, stain accumulation, surface roughness, water sorption and degradation of intrinsic pigments. Intrinsic factors are those factors that are involved in changes at the interface of matrix and fillers as well as within the resin matrix. In general, internal color changes occur due to the physical-chemical conditions such as variations in temperature and moisture. Extrinsic factors involved adhesion of ions or molecules by the process of adsorption on the surface of the dental resins [40].

It was found that the color stability of denture base resins can be significantly affected by disinfectant solutions such as glutaraldehyde, chlorhexidine, phenolic-based, alcohol-based and hypochlorite disinfectants [41]. Exposure of denture base resin to the commonly consumed beverages, oral fluids and denture cleansers has been demonstrated in the color changes of these materials. This is because of entry of colored mixtures into resin matrix causing oxidation of amine accelerator [42]. Also Absorption and adsorption of pigments present in the oral environment accelerate the discoloration and have a greater effect on chromatic changes of material compared with intrinsic factors [43].

From the result of present study it was found that coriander oil as disinfectant caused significant color change in pink heat cured acrylic resin denture base with significant increase with clear denture base that is in agree with results of all previous studies [44-46].

It was reported that the color change of denture base heat cured acrylic resin increased as the immersion time increased [47]. The literature has shown that sodium hypochlorite and alkaline peroxides are the two main classes of immersion denture cleansers the im-

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mersion procedures can be performed for 3 to 20 min, or for 8 h [48]. In present study we immersed specimens in coriander essential oil 24h for 30 days (almost 6 years immersion simulation (as one hour represented 3 immersions of 20 min and each 24h (one day) corresponded to 72 immersions of 20 min per day.

In present study, there is statistically significant color change after immersing in coriander oil, with pink heat cured acrylic material (control was 0.026 and test group was (0.05 ± 0.01) while with clear heat cured acrylic material (control was 0.036 and test group was (0.13 ± 0.05) , but the rang of difference after simulating almost 6 years of immersion is considered very little change.

Conclusion

The microbiological results obtained from present study proved that, coriander essential oil has a remarkable antiseptic effect on oral micro-organisms related to fitting surface of removable dentures.

Coriander essential oil as denture disinfectant can affect color stability of heat cured acrylic resin denture base but in comparing with 6 years simulating period of use, it considered very little change.

Coriander essential oil as denture disinfectant is natural remedy so the denture patient is away from adverse reactions of chemical ingredients.

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Conflict of Interest

There is no conflict of interest with this research.

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