

Effect of Olea Extracts on Oral Candida in Patients Wearing Dentures of Different Base Materials

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Abstract

Background: Through the last recent years many researches started to investigate the properties of Aloe vera. Aloe vera proved to have wide ranges of activities in different fields of medicine.

Objective: The study was conducted to evaluate the antifungal activity of Aloe Vera extract in aqueous and alcoholic solvents against *Candida albicans* species which isolated from dentures of patients with denture stomatitis.

Material Method: Twenty six patients with denture stomatitis, *Candida albicans* were isolated and identified by using a routine microbiology method which includes the microscopically examination, culture and biochemical test by using API test. Preparation of aloe Vera extracts (watery and alcoholic) in different concentration was done, then the antifungal activity of aloe Vera for both extracts types were tested by using candida colony count after cultivated on culture media and agar well diffusion method to estimation the diameters of inhibition zone in different concentration.

Results: The results revealed that the Aloe Vera contained substantial antimicrobial efficacy. The alcoholic extracts induce significant inhibition in growth of *candida albicans* in comparison to aqueous one and the inhibitory effect was found to be variable with the applied concentration. The candida species that isolated from nylon dentures was more sensitive than that isolated from acrylic dentures to Olea extract of different forms.

Conclusion: Olea Vera extracts in high concentration have a reliable antifungal activity, alcoholic extract of Olea was more effective than aqueous type and can be used as an alternative to traditional antiseptic solution.

Keywords: Aloe vera; *Candida albicans*; Chloro hexidine; *Candida* species

Introduction

Human use of Aloe Vera is as old where the first record talk about it is use as medical herb discovered engraved on clay tablets during the Mesopotamia civilization circa 2200 BC, in which it is described as a laxative. Use of aloe in ancient times is also documented in Egypt, Greece, and China, Aloe Vera was cultivated in Caribbean islands by Spain and the Netherlands, and was sold in various parts of Europe during the 17th century [1]. The Aloe Vera belongs to the Liliaceous family, of which there are about 360 species. It is a cactus-like plant that grows readily in hot, dry climates and currently because of demand, is cultivated in large quantities [2]. Aloe Vera have more than seventy five nutrients and two hundreds active compounds, including vitamins, enzymes, minerals, sugar, lignin, anthracene quinones, saponins, salicylic acid and amino acids [3]. Aloe Vera has been used to treat various skin conditions such as cuts, burns and eczema. It is alleged that sap from Aloe Vera decreases pain and reduces inflammation [4]. The water content of Aloe Vera ranging from 99% to 99.5% while the solid materials represent 0.5-1.0 only [5]. The physical and chemical constituents of the products derived from Aloe Vera plants differ according to the part of the plant from which it was derived, it is species, the climate conditions, seasonal and grower influences, and processing techniques [6]. The biological inhibitions by different natural substances, such as essential oils and plant extracts have been investigated widely against fungal activities [7]. There are many studies showing that resistance to infection is enhanced by *Aloe* either in humans or in animals, whether the infective agent is a bacterium, virus or fungus [8], response of fungal biomass to the extracts was found to be species specific. The species specificity of phyto toxins has also been demonstrated for other plant species [9].

Candida albicans plays a major role in the pathogenesis of denture stomatitis, oral candidiasis and inflammatory hyperplasia of the palate [10]. This organism has important virulence factors such as proteolytic activity and capacity to adhere and invade the epithelium [11, 12]. The ability of *Candida* species to adhere to oral and plastic surfaces is crucial in pathogenesis. Such adherence enables the microorganism to withstand the mechanical washing action of saliva and it is a prerequisite for successful colonization [13]. According to several studies conducted in universities and hospitals, 65% of denture wearers suffer from problems caused by *Candida albicans* (which is the most adherent *Candida* species). This condition can lead to denture intolerance [10]. Although,

bacteria has a major role in the pathogenesis of periodontal disease, and the yeast *Candida albicans* has also been isolated from periodontal pocket [14]. Nystatin and fluconazole are effective agents in the treatment of various fungal infections. They are the first drugs of choice for the treatment of certain candidiasis. Unfortunately, the toxicities caused by polyene antifungals and resistance to fluconazole limit their use [15,16]. The search for novel antimicrobial agents derived from botanical sources never stopped, where plants provide a source of antimicrobial substances needed to manage some of the emerging resistant microbial species [17].

Antiseptics may play an important adjuvant role to antifungal medication. Chloro hexidine a widely used antimicrobial agent, adversely affects the microbial eukaryotic plasma membrane by nonspecific electrostatic binding to negative protein and phospholipid moieties, causing alteration in the cellular membrane structure and in the cellular osmotic balance [18]. Denture stomatitis, formerly known as denture sore mouth, relates to an inflammatory lesion of the mucosa following the use of complete or partial removable dental prostheses in about 60% of denture wearers [19]. Poor oral hygiene, badly fitting dentures and using denture liners are the most common causes of denture stomatitis so the ability of *C. albicans* to adhere to host mucosal tissues as well as acrylic denture surfaces, the production of proteolytic enzymes that prepare penetration into tissues, phenotype switching of yeast to the hyphal form and several immune modulatory activities are known to be virulent factors for this fungus [7,20]. Donnelly RF, et al. [21], confirmed that the presence of prosthetic device is one of the several reasons for the rise in fungal infection. Salivary flow is also reduced in patients with dentures, which decreases the physiological cleaning properties of the tongue and prepares a suitable environment for microbial survival and colonization in the oral cavity of denture users [22]. *Candida* adherence to oral epithelium, soft denture lining materials and denture base materials has been studied intensively. To date, up to 95% dental prostheses are composed of polymethyl-methacrylate (PMMA). For instance, *Candida* adhesion onto PMMA-based resins is a common source of oral cavity infection and stomatitis [23,24].

Aims of the Study

To evaluate the antifungal activity of alcoholic and aqueous type of Olea extract upon the growth of *candida*

albicans that isolated from two types of dentures (acrylic and nylon).

Material and Methods

Candida albicans were isolated from twenty six patients with denture stomatitis of both sex and in different age groups who were attended to prosthetic clinic in dentistry college-Al-Mustansiriya University between Jan 2014- Feb 2015. The patients were classified according to type of denture were wearied, nine were nylon type and seventeen were acrylic type. Aqueous and alcoholic acetone aloe Vera extract were prepared in different concentration (15,20,25,30%). *Candida albicans* was isolated from dentures, purified, identification according to gram stain, morphological characteristics on chromogenic agar (CHROM agar *Candida*) and biochemical test results using API *Candida* and counted the candida colony by cultivated on Saouraud Dextrose Agar (SDA), incubation at 37°C aerobically for 48-72 hours and counted at zero time (before using aloe Vera extracts), then the candida inoculum was prepared for each patients by adding 2-3 colonies to normal saline to made suspension using to candida colony count in each concentration of tested extracts by preparation the different concentrations from watery and alcoholic extract in test tube then added 1 ml from candida suspension, incubated at room temperature for 60 minutes then 0.1 ml from each concentration transferred to SDA spreading on surface on agar by using spreader under sterile conditions, incubated for 48 hours at 37°C then colony of candida were counted and compare to

their counted at zero time. The agar well diffusion method was used to determination the antifungal activity of aloe Vera of both extracts by using of single isolates from each subjects to preparation of inoculum, then 0.1 ml from activated candida isolates were spreader separately using glass spreaders, left at room temperature for 10 minutes, then by using cork borer, wells were made on culture media. Then 0.1ml of different concentrations of aloe Vera extracts and chloro hexidine were added to the well. Each well was filled with specific concentration from extracts. Plate left for 15 minutes in room temperature and then incubated aerobically for 24 hours at 37°C. The activity of plant extracts were determined by measuring the diameters of inhibition zone each well by millimeter.

Statistical Analysis

The results were analyzed using SPSS 20.0 version, the results were expressed in mean and SD, independent student T-test, paired sample T-test, ANOV A test and Duncan test were used to assess the significant difference.

Results

Table 1 showed a high statistical significant difference between two types of denture regarding the mean count of candida, where nylon denture reported less contamination than acrylic one at zero time (mean=44.8±7.1SD) also the candida which isolated from nylon denture was more sensitive to alcoholic extract of Olea in all concentrations (15%,20%,25%,30%) as seen in Figure 1.

Conc.	Type of denture	Mean	SD	T test	P value
Zero time	Nylon	44.8	7.1	3.5	0.002
	Acrylic	55.5	7.4		
15%	Nylon	35.3	6.4	3.5	0.002
	Acrylic	46.3	7.9		
20%	Nylon	26.5	6.1	3.1	0.006
	Acrylic	35.8	8.0		
25%	Nylon	19.3	3.2	3.4	0.002
	Acrylic	27.1	6.2		
30%	Nylon	14.1	3.6	3.1	0.006
	Acrylic	20.1	5.2		

Table 1: Mean count of candida at different concentrations of alcoholic extract of Olea for two types of denture.

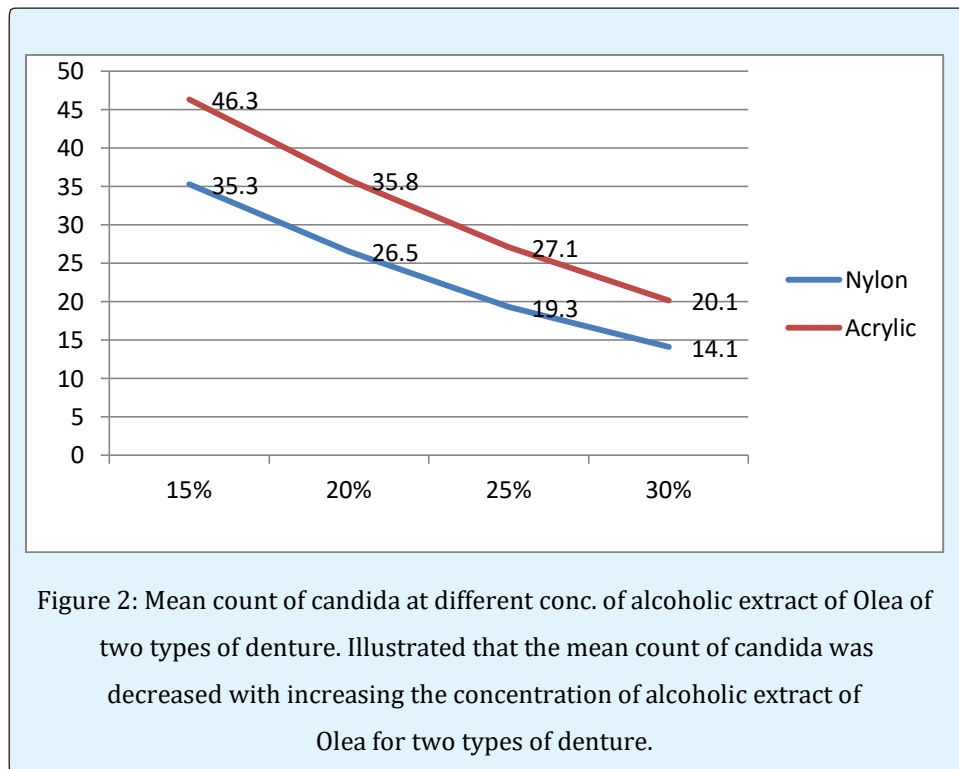
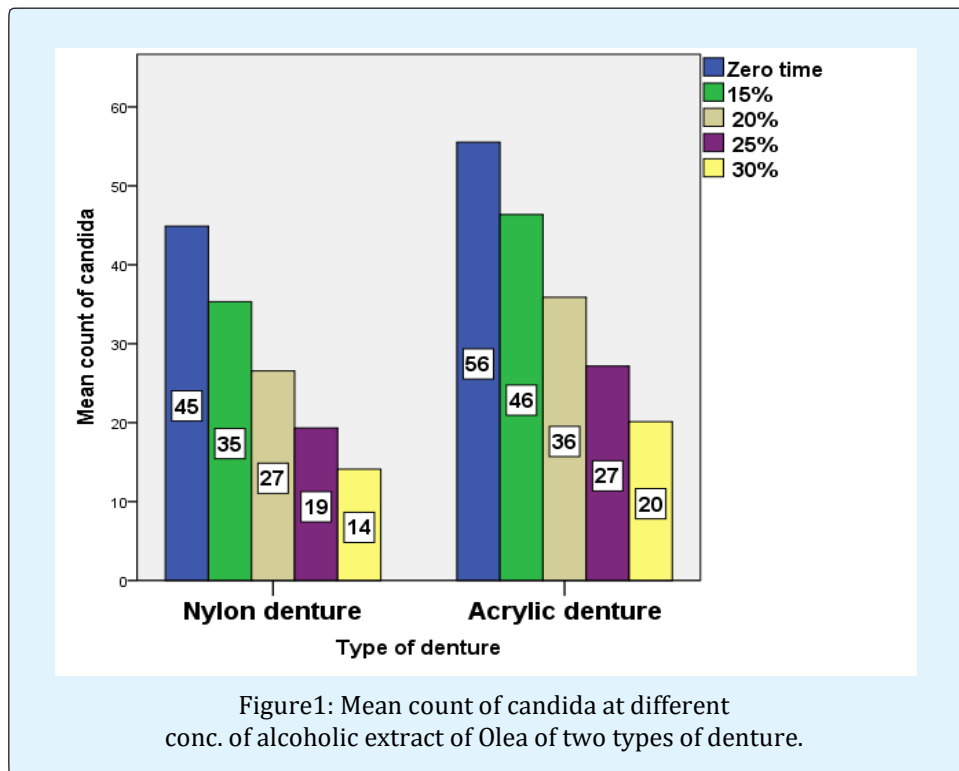


Figure 2 Illustrated that the mean count of candida was decreased with increasing the concentration of alcoholic extract of Olea for two types of denture.

Table 2 and Figure 3 demonstrated that the candida which isolated from nylon denture was more sensitive to watery extract of Olea in all concentrations (15%,20%,25%,30%).

	Type of Denture	Mean	SD	T test	P value
Zero time	Nylon	44.8	7.1	3.506	0.002
	Acrylic	55.5	7.4		
15%	Nylon	43.0	8.2	2.119	0.04
	Acrylic	50.1	8.0		
20%	Nylon	37.4	8.0	2.324	0.02
	Acrylic	44.3	6.7		
25%	Nylon	30.3	9.0	2.445	0.02
	Acrylic	37.9	6.6		
30%	Nylon	25.4	7.5	2.052	0.05
	Acrylic	30.9	5.9		

Table 2: Mean count of candida at different concentrations of watery extract of Olea of two types of denture.

No: Acrylic=17, Nylon=9.SD=standard deviation.

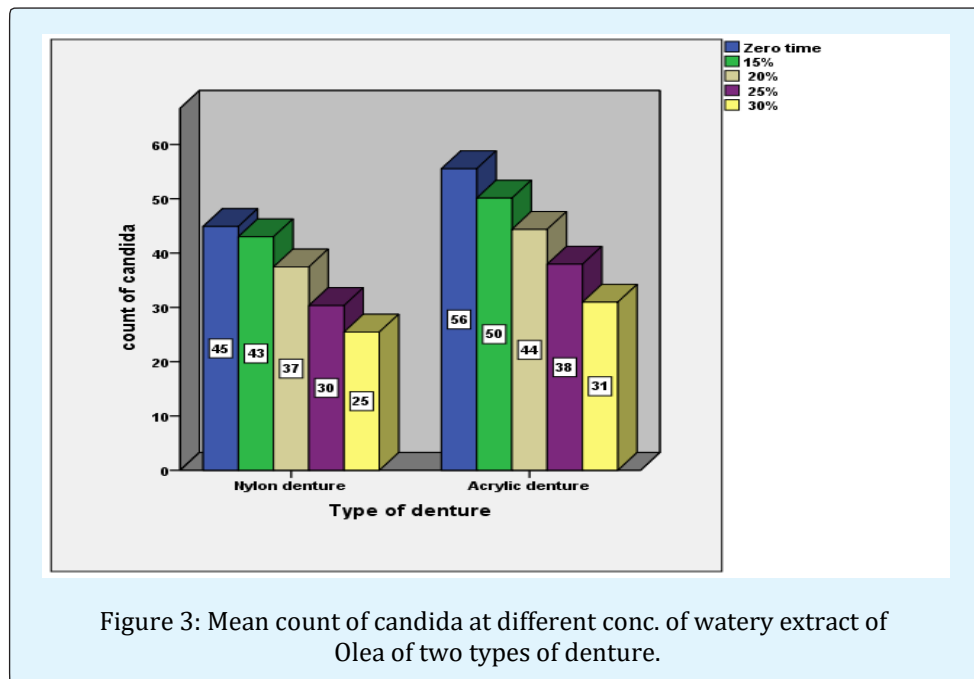


Figure 3: Mean count of candida at different conc. of watery extract of Olea of two types of denture.

Figure 4 Illustrated that the mean count of candida was decreased with increasing the concentration of watery extract of Olea for two types of denture.

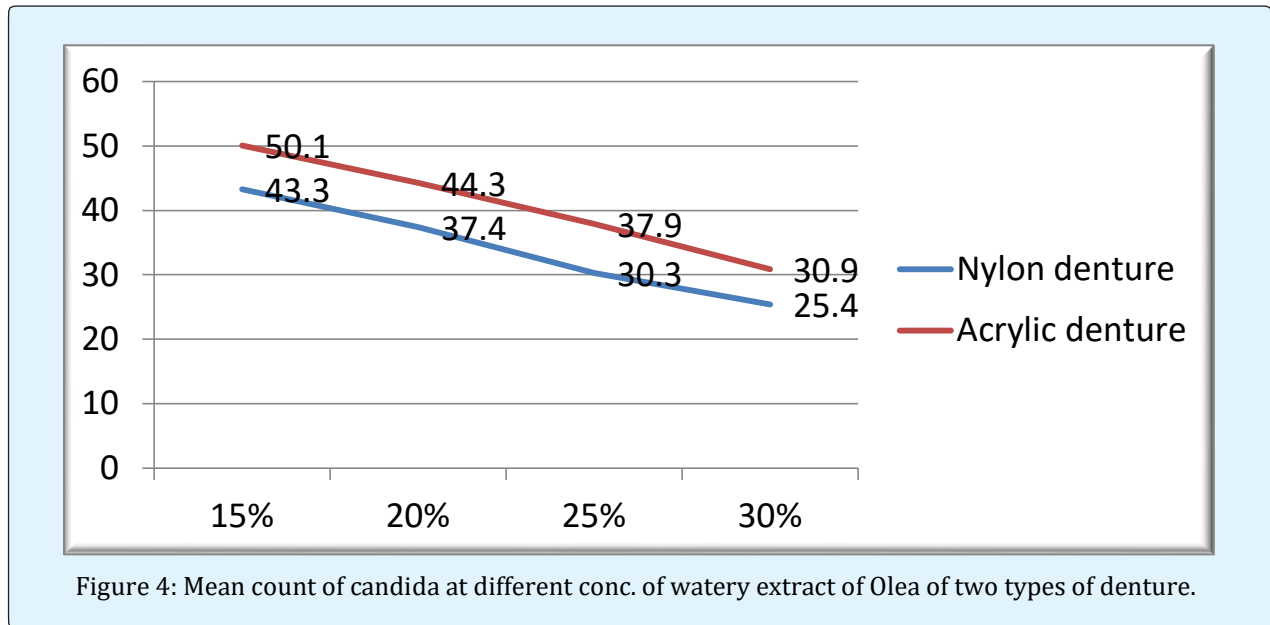


Figure 4: Mean count of candida at different conc. of watery extract of Olea of two types of denture.

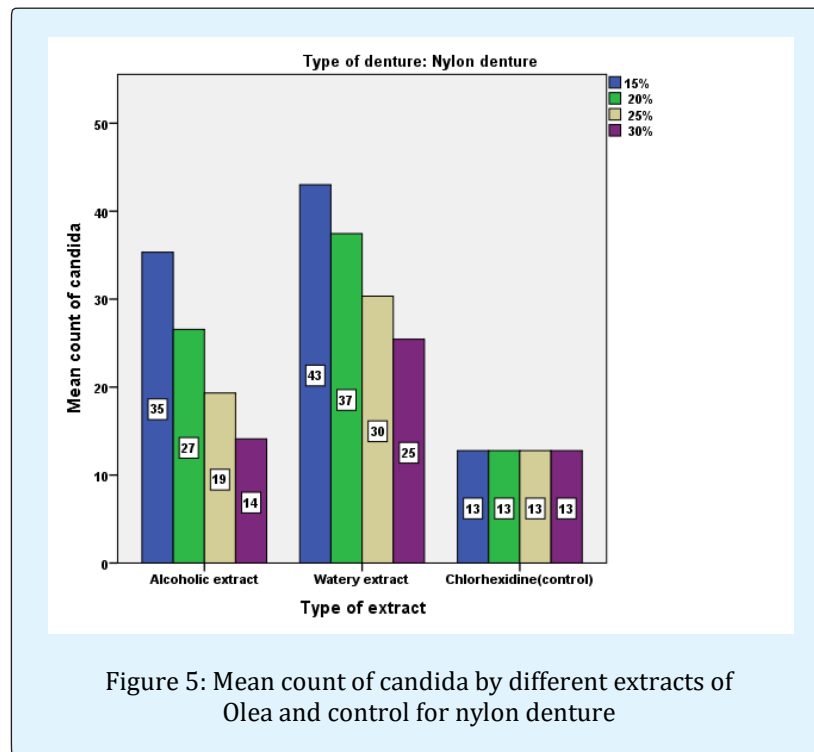
Table 3 and Figure 5 illustrated that the alcoholic extract of Olea was more effective on count of candida isolated from nylon denture than watery one but less than control (chlorhexidine2%) in all concentrations with a high

significant statistical difference (p value ≤ 0.05). On multiple comparison no statistical significant were found between alcoholic extract and control (chlorhexidine2%) at 25 and 30% concentrations. As seen in Table 4.

		Mean	SD	95% C.I		F test	P-value
				Lower Bound	Upper Bound		
15%	Alcoholic	35.3	6.4	30.3	40.2	50.1	0.001
	Watery	43.0	8.2	36.6	49.3		
20%	Alcoholic	26.5	6.1	21.8	31.2	33.1	0.001
	Watery	37.4	8.0	31.2	43.6		
25%	Alcoholic	19.3	3.2	16.8	21.7	18.5	0.001
	Watery	30.3	9.0	23.3	37.2		
30%	Alcoholic	14.1	3.6	11.2	16.9	13.9	0.001
	Watery	25.4	7.5	19.6	31.2		
	Chx.(control)	12.7	4.7	9.1	16.4		

No=9, CHX=ChlorHexidine

Table 3: Mean count of candida by different extracts of Olea and control at different concentrations for nylon denture.



Conc.	(I) Type of Extract	(J) Type of Extract	Mean Difference (I-J)	Sig.	95% C.I	
					Lower Bound	Upper Bound
15%	Alcoholic	Chx.(control)	22.5	0.001	15.1	29.9
	Watery	Chx.(control)	30.2	0.001	22.8	37.6
20%	Alcoholic	Chx(control)	13.7	0.001	6.6	20.9
	Watery	Chx(control)	24.6	0.001	17.5	31.8
25%	Alcoholic	Chx(control)	6.5	0.062	5.3	13.4
	Watery	Chx(control)	17.5	0.000	10.7	24.4
30%	Alcoholic	Chx(control)	1.3	0.832	4.8	7.5
	Watery	Chx(control)	12.6	0.000	6.4	18.8

CHX=ChlorHexidine

Table 4: Multiple comparisons of extracts and control group.

Table 5 and Figure 6 illustrated that the alcoholic extract of Olea was more effective on count of candida isolated from acrylic denture than watery extract but

less than control (chlorhexidine2%) in all concentrations with a high significant statistical difference (p value ≤ 0.05).

Conc.	Extract Types and Control	Mean	SD	95% C.I		F test	P value
				Lower bound	Upper bound		
15%	Alcoholic	46.3	7.9	42.2	50.4	142.9	0.001
	Watery	50.1	8.0	45.9	54.2		
20%	Alcoholic	35.8	8.0	31.7	40.0	102.3	0.001
	Watery	44.3	6.7	40.8	47.8		
25%	Alcoholic	27.1	6.2	23.9	30.4	76.4	0.001
	Watery	37.9	6.6	34.5	41.3		
30%	Alcoholic	20.1	5.2	17.4	22.8	50.2	0.001
	Watery	30.9	5.9	27.9	33.9		
	Chx(control)	12.1	5.2	9.4	14.8		

No=17, CHX=ChlorHexidine

Table 5: Mean count of candida by different extracts of Olea and control at different concentrations for acrylic denture.

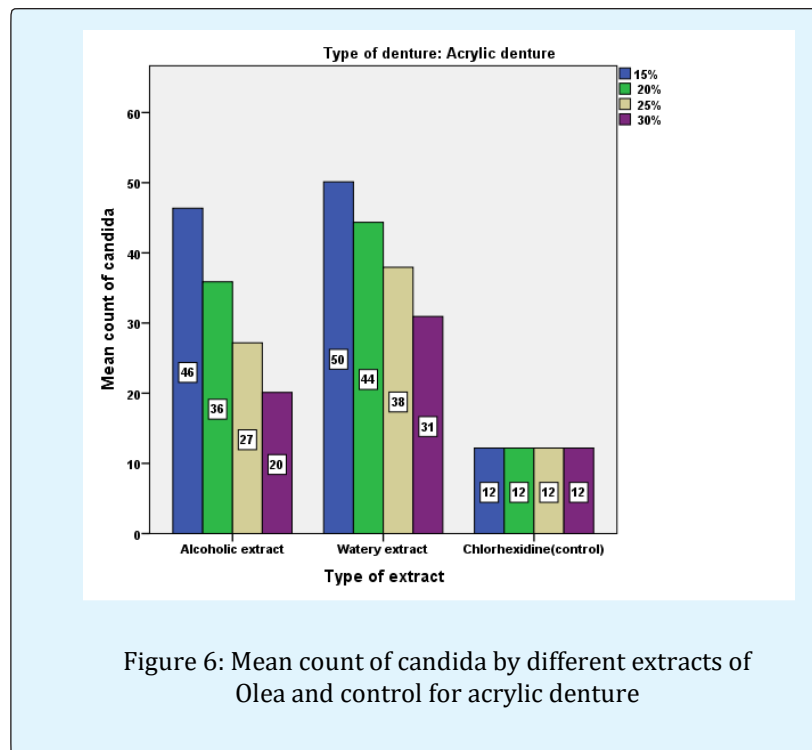


Figure 7 and 8 demonstrated strong positive correlation between count of candida at zero time and wearing time in months for nylon and acrylic dentures ($R=0.8, 0.6$)

accordingly, where the mean count of candida increased with increasing wearing time.

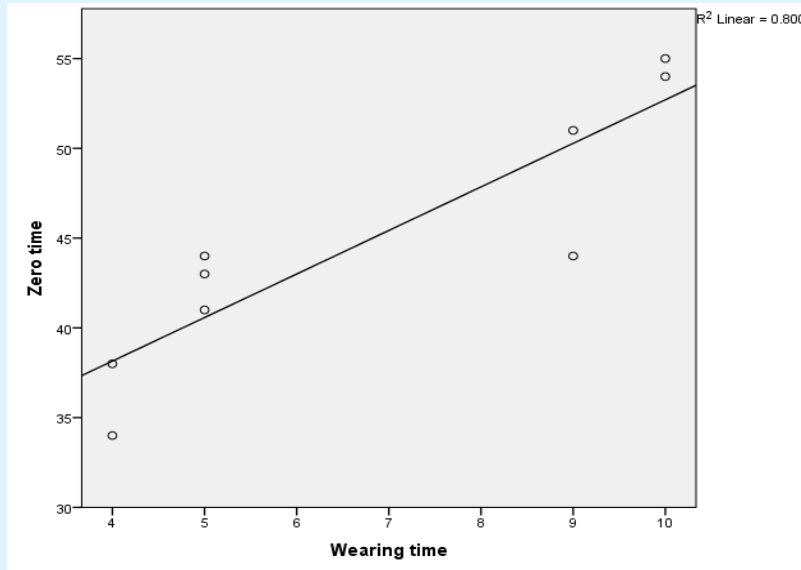


Figure 7: Correlation of mean count of candida at zero time with wearing time for nylon denture.

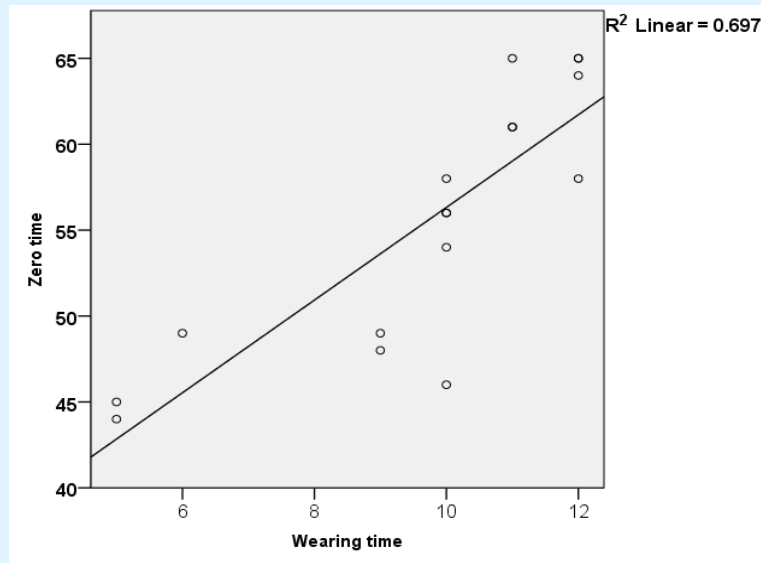


Figure 8: Correlation of mean count of candida at zero time with wearing time for acrylic denture.

Table 6 and Figure 9 demonstrated that the inhibition zone induced by alcoholic extracts of Olea was more for candida isolated from nylon denture than acrylic denture

with a high statistical significant difference in all concentrations ($p \leq 0.05$).

Conc.	Type of denture	Mean	SD	T test	P value
15%	Nylon	5.2	0.8	5.897	0.001
	Acrylic	3.6	0.5		
20%	Nylon	5.9	1.0	6.474	0.001
	Acrylic	4.0	0.5		
25%	Nylon	6.4	1.3	5.744	0.001
	Acrylic	4.2	0.6		
30%	Nylon	6.9	1.0	7.617	0.001
	Acrylic	4.6	0.4		

No: Acrylic=17, Nylon=9

Table 6: Mean of inhibition zone by different concentration of alcoholic extract of Olea for two types of denture.

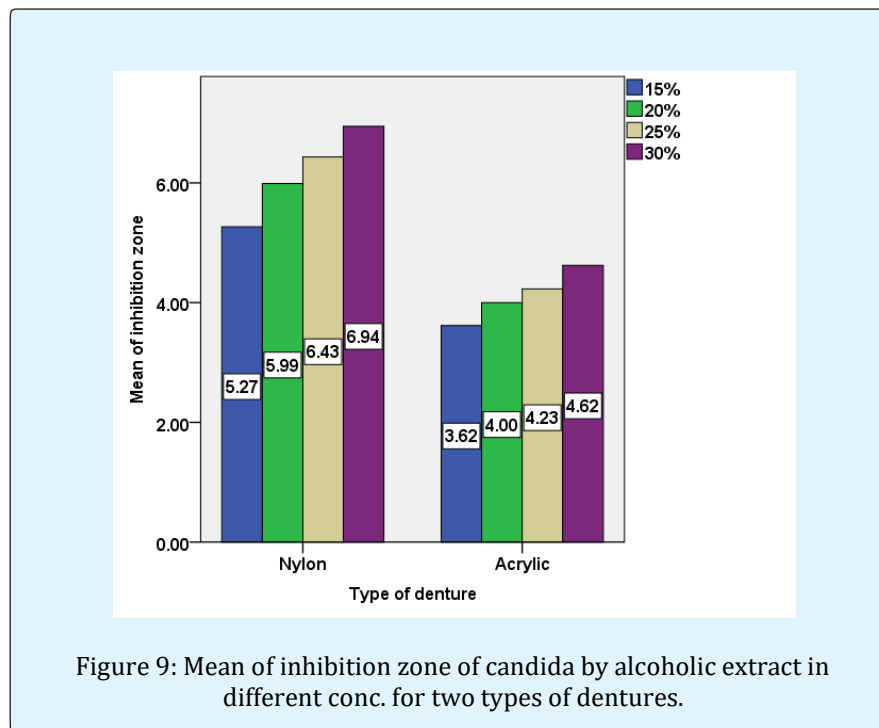


Figure 9: Mean of inhibition zone of candida by alcoholic extract in different conc. for two types of dentures.

Table 7 and Figure 10 demonstrated that the inhibition zone induced by watery extract of *Olea* was more for candida isolated from nylon denture than acrylic denture with a high statistical significant difference in the

concentrations (20, 25, and 30%) ($p \leq 0.05$), no statistical significant difference was noted at conc. of 15% (p value ≥ 0.05).

Conc.	Type of denture	Mean	SD	T test	P value
15%	Nylon	6.8	1.1	1.937	0.06
	Acrylic	5.9	1.2		
20%	Nylon	7.8	1.1	2.296	0.03
	Acrylic	6.8	1.1		
25%	Nylon	8.9	1.5	2.114	0.04
	Acrylic	7.7	1.2		
30%	Nylon	9.9	1.7	2.151	0.04
	Acrylic	8.6	1.2		

No=Acrylic=17, Nylon=9

Table 7: Mean of inhibition zone by different concentration of watery extract of *Olea* for two types of denture.

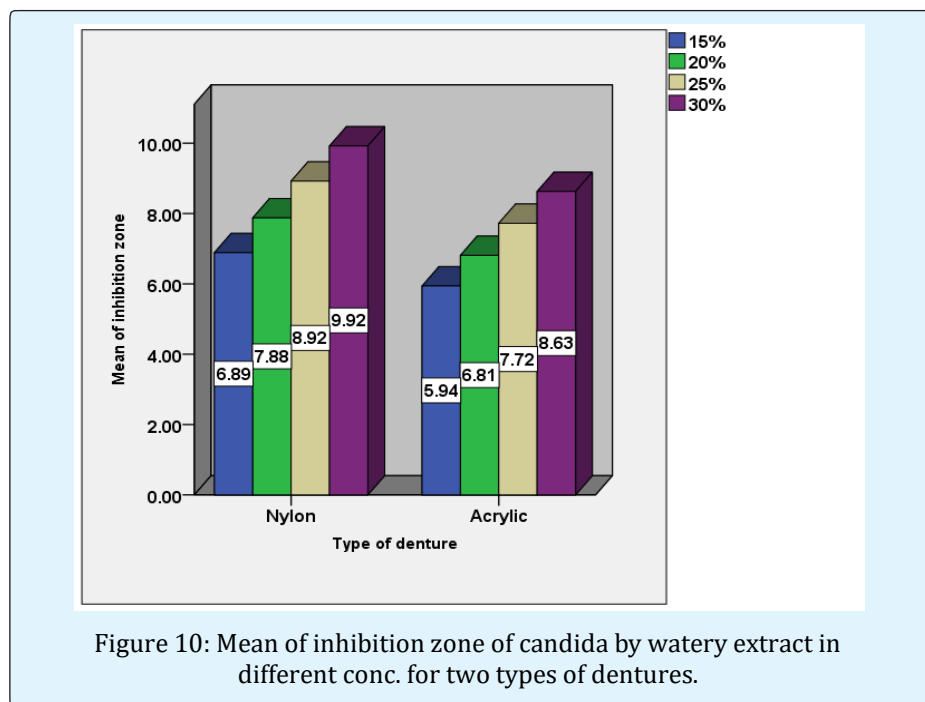


Figure 10: Mean of inhibition zone of candida by watery extract in different conc. for two types of dentures.

		Mean	SD	95% C.I		F test	P value
				Lower Bound	Upper Bound		
15%	Watery	5.2	0.8	4.5	5.9	100.2	0.001
	Alcoholic	6.8	1.1	6.0	7.7		
20%	Watery	5.9	1.0	5.1	6.7	77.5	0.001
	Alcoholic	7.8	1.1	7.0	8.7		
25%	Watery	6.4	1.3	5.4	7.4	50.4	0.001
	Alcoholic	8.9	1.5	7.7	10.1		
30%	Watery	6.9	1.0	6.0	7.7	41.2	0.001
	Alcoholic	9.9	1.7	8.5	11.2		
	Chx	13.8	1.8	12.4	15.3		

No=9, CHX=ChlorHexidine

Table 8: Mean of inhibition zone of candida by different extracts of Olea and control at different concentrations for nylon denture.

Table 8 illustrated that the alcoholic extract of Olea was induce more inhibition zone than watery on candida isolated from nylon denture than watery extract but less than control (chlorhexidine 2%) in all concentrations with

a high significant statistical difference ($p \text{ value} \leq 0.05$). On multiple comparisons a statistical significant difference was noted as seen in Table 9.

		Mean	SD	95% C.I		F test	P value
				Lower Bound	Upper Bound		
15%	Watery	3.6	0.5	3.3	3.9	127.1	0.001
	Alcoholic	5.9	1.2	5.3	6.5		
20%	Watery	4.0	0.5	3.7	4.2	116.1	0.001
	Alcoholic	6.8	1.1	6.2	7.3		
25%	Watery	4.2	0.6	3.8	4.5	103.1	0.001
	Alcoholic	7.7	1.2	7.0	8.3		
30%	Watery	4.6	0.4	4.3	4.8	95.1	0.001
	Alcoholic	8.6	1.2	7.9	9.2		
	Chx.	14.5	3.3	12.7	16.2		

No=17, CHX=ChlorHexidine

Table 9: Mean of inhibition zone of candida by different extracts of Olea and control at different concentrations for acrylic denture.

Table 9 illustrated that the alcoholic extract of *Olea* was induce more inhibition zone than watery on candida isolated from acrylic denture than watery extract but less than control (chlorhexidine 2%) in all concentrations with a high significant statistical difference (p value ≤ 0.05). On multiple comparison a statistical significant difference was noted.

Discussion

Antifungal activity of Aloe Vera extract was determined first against candida albicans where our results shows it is has an inhibitory effect on growth of *candida albicans* in comparison to zero time with two types of extracts but it is ability of inhibition was less than that of control with all concentrations were used rather significant difference from control was reported with low concentration of *Olea* extract (15%, 20%) only, this finding indicate that the inhibitory effect of *Olea* in high concentration not differed significantly from control and we can use it as alternative to traditional antiseptic, our finding in consistent with authors Vidhya & Udayakumar [25], where they confirmed the inhibitory activity of *Olea* extract and they link this activity to its ingredient, where they reported it contains about twelve active ingredient among these, the four compounds such as octadecanoic acid ethyl ester (1.19%), phytol (4.76%), 6,9,12-octadecatrienoic acid, phenyl methyl ester (25%) and octanal, 7-methoxy-3,7-dimethyl (14.29%) and these ingredients possess insecticidal, anti candidal and antifungal activities. The antifungal activity of aloe extract also confirmed by authors Mc Guffin, et al. [26] where they reported that the *Olea* extract has bactericidal and antifungal activity. The authors Boudreau M, et al. [27] reported that aloe Vera leaves contain many biologically active compounds such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones and various lectins which have antifungal activity. Our finding of positive effect of *Olea* extract was similar to finding of authors Agarry O, et al. [28] where they reported that the inhibitory activity of aloe Vera induced by their leaf extract and this positive effect produced by anthraquinone glycoside that present in the leaf extract only.

Our results confirmed that the *Candida albicans* which isolated from nylon dentures was more sensitive than that isolated from acrylic one, the most accepted explanation for this difference, is that the candida is more adherent to acrylic base material dentures than nylon base materials therefore it is less sensitive to *Olea* extract. It is well recognized that inert surfaces which are either

implanted or in superficial contact with the host mucosa frequently act either as conduits of infection transmission or as reservoirs of infection, for this reason a number of researchers have investigated the adherence of *Candida* to a variety of materials found in medical devices such as oral prosthesis, Schaller M, et al. 2002 [7] and Radford DR, et al. [20], reported that the *candida albicans* has ability to adhere to acrylic dentures. Authors Luo & Samaranayake 2002 [29] reported that the adherence ability of candida to acrylic is species specific and depend on Cell Surface Hydrophobicity (CSH). The process of candidal adhesion is rather complex and involves both biological and non-biological factors. The relative CSH of *Candida*, which is a property of the cell wall, is widely considered as a non-biological factor of critical importance pertaining to candidal adhesion. A statistically significant positive correlation between CSH and candidal adhesion to buccal epithelial cells and denture acrylic surfaces has also been reported by a number of workers [30]. Ozcan M, Nevzalolu EU, et al. [31] reported that materials with the roughest surface may serve as reservoir, with surface irregularities providing an increase microorganism retention and protection from shear forces. Kawai K & Uranom Ebisus [32] recorded that the reason for this adhesion, that rough surface has irregularities inducing adhesion of *Candida* and bacteria, these superficial defect such as voids and micro cracks on surface were possible sites for Candidal adhesion. Radford DR, Sweet SP, et al. [33] reported that the presence of saliva decrease Candidal attachment this may be explained that, also the pellicle of the Saliva could act as blocker of a nonspecific adhesion of the yeast to the surface of the acrylic, so Saliva significantly reduce the adherence of *C. albicans* to the surface in vitro, another explanation is that thin bio film of the acquired salivary pellicle can significantly reduce free energy on hard intraoral surfaces that may affect Candidal adhesion.

In this study we compare the effect of two types of *Olea* extracts:- aqueous and alcoholic extract in different concentrations on oral candida isolated from dentures with different base materials:- nylon and acrylic based (PMMA-based resin and our finding revealed that there is significant inhibitory effect with alcoholic base extract in comparison to aqueous one and the inhibitory action of *Olea* of aqueous and alcoholic extract was increased with increasing their concentration, these result enclose with the finding of Arun kumar & Muthuselvam [34], where they reported that the maximum antifungal activity of Aloe Vera was observed in acetone extract against candida albicans, but conflict with the finding of Bruneton [35], where he reported that the highest inhibitory effect

of fungal biomass was achieved with aqueous extract of the tested plant species that may be attributed to the presence of main active constituent of A. Vera plant extract in aqueous one.

Regarding the inhibition zone induced by Olea extracts, our finding in line with Shamim, et al. [36] where he also noted high zone of inhibition with ethanol extracted from Aloe Vera against *Candida* species. Ibrahim, et al. [37] investigated the phyto constituents and antimicrobial activity of aqueous, ethanol and acetone extracts of the Aloe Vera against some human and plant pathogens by disc diffusion method and he reported, among the three extracts, ethanol and acetone extracts recorded significant antimicrobial activity against all test pathogens.

Conclusion

AloeVera extracts are suitable alternative of herb origin to traditional antiseptic as have an antifungal activity and that of alcoholic base in high concentration is the best alternative one, in addition the candida species that isolated from nylon denture was more sensitive to Olea extracts than that isolated from acrylic one.

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