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Anticandedal Effect action of Different Concentrations of Aglycon fraction of Anthroquinon (monoanathron) isolated from Aloe vera on Heat cure Acrylic Resins

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Abstract

Aim of study: to investigate the effects of different concentrations of aloe vera , aglycon part (monoanathron) against candida albicance adhere to two types of heat cure acrylic resin denture base (conventional and Hi- impact) on two periods of time (30 minutes and 8 hours).

Materials & methods: A total of 120 specimens were prepared, sixty specimens of each hot cure denture base acrylic resin material, each sixty specimens were farther divided into two subgroups of 30 specimens according to the time of immersion, each 30 specimens were farther subdivided into three 10 specimen groups according to the concentration of aleo vera used, isolation of free aglycon (Monoianthron) from anthroquinon glycoside of aloe vera plant; Candida albicans isolation and identification and preparation of candida albicans suspension; autoclaved specimens of each sub-group for both heat cure acrylic resins were placed into the tubes containing brain heart infusion plus inoculum and remained for 12 hours at 37 ^{0}C in order to favor an initial colonization of the acrylic resin surfaces, the disinfection step was performed over two periods, colony formation was then counted after incubation.

Results: The two concentration of aloe Vera studied in the present investigation exhibited varying degree of inhibitory effect against candida albicans adherent to different type of heat cure denture base acrylic resins. The degree of inhibition varied depending on the concentration of the extract while the period of immersion has no significant effect on the number colony.

Conclusion: with the limitation of this study it can be concluded that 100% and 75% concentrations of monoanthron aglycon part of anthroquinon isolated from aloe vera have anticandidal effect, 100%concentration has higher effect than75%. Immertion period has no effect on the anticandidal activity of the solutions.

Key word: Aloe Vera, denture stomatitis, aglycon, monoanthron, anthroquinon.

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Introduction

The most common mucocutaneous mycosis of the oral cavity is Candidiasis or oral candidiasis which is produced by fungi of the genus *Candida* (*Candida spp.*)[1]. Candida albicans has been shown to be the main Candida strain responsible for inflammatory pathology [2]. Candida albicans is unicellular eukaryotic microorganisms which reproduced by budding .It is a dimorphic fungus, which exists in both blastospore (yeast) and mycelial forms[3]. *Candida* is found n the oral cavity of 53% of the general population as a common commensal organism[1].

Often, when the host defense system suffers because of any changes, like immunodeficiency, candida. albicans become virulent and generates candidiasis, that can be manifested through various clinical forms, involving one or more oral sites, up to affect the whole oral cavity and to disseminate into invasive forms. Candida-associated denture stomatitis is a very common inflammatory process affecting about 60% of the subjects carrier of a prosthesis[4] .Denture induced stomatitis or chronic atrophic candidosis is the commonest form of oral candidosis and is present in 24-60 percent of denture wearers; it may also be associated with orthodontic appliances and obturators. The condition is usually found on the palatal mucosa beneath the fitting surface of the upper denture and both complete and partial denture wearers are affected. It is unusual for the condition to occur on the lower denture bearing mucosa [5].

The treatment of *Candida*-associated denture stomatitis is difficult and complex due to its multifactorial etiology[6], it includes good oral hygiene, denture cleaning procedures, topical or systemic antifungal agents, discontinuation of nocturnal denture wearing habit, and eventually denture replacement [7]. The antifungal treatments more used are antifungal suspensions based on nystatin, amphotericin-B, miconazole and fluconazole [8]. Chlorhexidine in the form of mouth wash (0.2%) and 2% suspension for overnight denture disinfection, can also be used to supplement antifungal drugs [9].

Almost all of the clinically used antifungals encounter from various shortcomings in terms of toxicity, drug-drug interactions, and lack of fungicidal efficacy, high cost, and emergence of resistant strains resulting from frequent usage [10], much recent thoughtfulness has been paid to extracts and biologically active compounds isolated from plants used in herbal medicine[11].

Natural products express antibacterial and antifungal activities as well as anti-inflammatory and antioxidant effects, and it has been proven that they are the alternative substitution of chemical substances with less adverse effects on humans[3,9]. A wide variety of the plants extracts and essential oils have been traditionally used as antifungal agents against *C. albicans*, therefore their role in treatment of *Candida*-associated denture stomatitis might be of certain importance[12].

Aloe vera is a shrubby or arborescent, perennial, xerophytic, succulent, pea-green color plant. It grows mainly in the dry regions of Africa, Asia, Europe and America[13]. Aloe vera L. is a perennial plant with turgid green leaves joined at the stem in a rosette pattern, it contains major quantities of water and seventy five different ingredients including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic

compounds, lignin, tannic acids, polysaccharide, glycoprotein, saponins, sterols, amino acids and salicylic acid[14]. Aleo vera have therapeutic properties such as immune-stimulation, anti-inflammatory, wound healing, promotion of radiation damage repair, anti-bacterial, anti-viral, anti-fungal and anti-oxidant[15]. *Aloe vera* contains six antiseptic agents: lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur. They all have inhibitory action on fungi, bacteria and viruses[13,16].

The present study was undertaken to investigate the effects of different concentrations of aloevera , aglycon part (monoanathron) against candida albicance adhere to two types of heat cure acrylic resin denture base (conventional and Hi-impact) on two periods of time (30 minutes and 8 hours).

Material and method

1- Specimens grouping:

A total of 120 specimens were prepared, sixty specimens of each heat cure acrylic resin material (conventional heat cure and Hi-impact), each sixty specimens were farther divided into two subgroups of 30 specimens according to the time of immersion (30 minutes and 12 hours (overnight), each 30 specimens were farther subdivided into three 10 specimen groups according to the concentration of aleo vera used as follow, control group (immersed in distil water), 75% aleo vera and 100% aleo vera (figure1).

2- Preparation of Specimens.

To prepare the acrylic samples, a pink modeling wax (Polywax, Bilkim Chemical Company, Izmir/ Turkiye) with dimensions of (10mm × 10 mm× 2 mm)[17] patterns were invested in metallic flask and type III dental stone (Elite model, Zhermack Italy, 117344). One flask contained 6 patterns (figure 2). After the setting of dental stone, the flasks were opened, the wax was eliminated under running hot water and stone surfaces were coated with a thin layer of acrylic separating film. the resins were mixed according to the manufacturers' instructions, powder to liquid ratios were 21.0 g/10.0 ml, for Hi-impact heat polymerized acrylic (Vertex, TUV CERT Netherlands , X4133 P01(powder) , X4111L06(Liquid)), and 23.4 g/10.0 ml, for conventional heat polymerized acrylic (Triplex Hot, Ivoclar Vivadent , Liechtenstein, N74750). The monomer and polymer were mixed at once, allowed to reach a dough stage and placed into the molds For Vertex, the flasks were immersed in water at 73°C for90 min, rising the temperature to 100°C and maintaining the boiling for 30 min. conventional heat polymerized acrylic specimens were polymerized by immersing the flasks in cold water, raising the temperature to 100°C and maintaining this temperature for 45 min (according to the manufacturers instruction for each type). Once the polymerization cycle was completed, the flask was allowed to slow cooling at room temperature followed by complete cooling of the flask with tap water before deflasking. The acrylic specimens were removed from the stone mould trimmed with acrylic bur All specimens were immersed in distilled water at 37ºC for 48±2 hours for elimination of the residual monomer.

3- Method of isolation of free aglycon (Monoanthrone) from anthroquinon glycoside of aloe vera plant

1-156 grams of powdered crude solidified aloe vera juice (which was bought from the local market were placed in a beaker and 1600ml of water were added and

boil gently for 15 minutes, cooled and filtered.

2-The filtrate was placed in a separatory funnel and extracted by shaking with two quantities of (2×30 ml) chloroform. The chloroform layer will contain the free aglycone. While the upper aqueous layer contain the whole glycoside which is placed in a 250-rouned buttomed flask and adding to it 3.5 ml ferric chloride solution (60% w/v) . Reflux for20 minutes and add 2ml of concentrated Hcl acid. Continue heating for further 20 minutes, shaking the flask occasionally to dissolve the precipitate, and allow cooling.

3- Place the hydrolysate in a separatory funnel and extract with two quantities of (2× 30 ml) chloroform.

4-concentrate the bulked chloroform extract which is the pharmacologically active aglycon part (monoanthrone).

5-The prepared monoanthrone was used as disinfectant solution and referred to as 100% aloe vera solution.

6-A 75% concentration was prepared from 100% aloe vera solution and referred to it as 75% aloe vera solution and used as disinfectant solution.

4- C. albicans Isolation and Identification:

C. albicans was obtained by taking swabs from seven volunteers wearing old dentures attending Department of Prosthodonotics at College of Dentistry / Al-Mustansiriya University. The collected swabs cultured on Sabouraud's dextrose agar and incubated at 37°C for 24 hours.

To identify and select C. albicans after incubation the following tests were done:

1. Culture morphological features assessment for *C. albicans* colonies, it should be creamy to white, flat or domed, and have a dry glistering or waxy surface (figure 2)
2. Candida albicans takes gram positive stain. It appears under light microscope as spherical to oval budding cells (3-6 nm) in the yeast or the blastospore form (figure 2)
3. The isolated fungus was also identified by *C. albicans* API kit which is a standardized system for Candida species identification. ID No, 7102.

Api Candida System:

Inoculation of the tubes was performed by adding suspension of inoculum in saline (McFrland standard of 3). After 18-24 hr. incubation at 37 ^{0} C, the reactions were read visually without addition of reagents. The results were transfered into numerical profile which was compared with the profile index.

5- preparation of candida albicans suspention

Candida albicans were grown on Sabouraud Dextrose Agar (LAB 009) plates (containing 500 mMol/L of sucrose) at 25^{0} C. For 24 h., the colonies were suspended in tubes containing 5 mL of brain heart infusion (BHI) broth (Salucea Lot 1202)) were prepared according to its manufacter's instruction and autoclaved at 121^{0} C for 15 minutes.

The cell suspension in each tube was adjusted spectrophotometrically to 0.5 McFarland.

6- Contamination

Next, autoclaved specimens of each sub-group for both conventional and Hi-impact heat cure acrylic resins were placed into the tubes containing BHI plus inoculum and remained for 12 h at

37°C in order to favor an initial colonization of the acrylic resin surfaces.

Each specimen was first washed with saline after immersion in the contaminated culture broth. Saline excess was removed with a gentle compression of sterile gauze, and they were used as the *C. albicans*-adherent specimens.

7- Disinfection

Then, the disinfection step was performed over two periods: 30 minutes and 8 hours as follows:

a) Control Group – 10 contaminated specimens, exposed to distilled water.

b) 10 contaminated specimens exposed to 100% aleo vera solution.

c) 10 contaminated exposed to 75% aleo vera solution.

8- Colony Counting:

Each specimen was then washed again with saline to remove the loosely adherent *C. albicans.* And the excess was removed with sterile gauze. It was then transferred to individual tubes containing 5 mL of BHI broth. After 24 h of incubation, solutions were serially diluted in nutrient broth, and then 100µL of each diluted supernatant was placed by using glass spreader on Petri dish plates that contained Sabouraud's dextrose agar. The plates were returned to the incubator at 37°C for 24 hours. Colony formation was then counted after incubation.

The mean values and standard deviations, of the obtained data were calculated with descriptive statistics. The data were statistically analyzed with factorial ANOVA Statistical analysis was performed with the SPSS software for Windows (v. 19.0)

The culture preparation , the growth of C. albicans on the specimens prepared, disinfection and colony counting were conducted in the Central health laboratories-ministry of health.

Results

Table 1 and Figure 3 show the means of candida albicans numbers on two type of heat cure acrylic resins (Conventional and Hi-impact) after immersion in distilled water (control) and two different concentrations of aleo vera (75%and 100%) for two periods (30 minutes and 12 hours). 3 way ANOVA indicates that the main effect of denture base type was significant, F (1, 108) = 18.563, p = .000 and the main effect of immersion material on candida number was significant F(2, 108) = 284.970, p = .000, while the immersion time have no significant effect on the number of candida albicans (Table2) .the significantly lowest number of candida albicance was found in Aleo vera 100% (Table 3).

Discussions

One outcome of denture wearing among others is an increase of the rate of *Candida* presence and infection by this yeast, an observation which has been confirmed for many years. Placement of a prosthesis in the oral cavity results in thoughtful alterations of the environmental conditions as the prosthesis becomes colonized with oral microorganisms and cuts off the underlying mucosa from the mechanical cleansing effect of the tongue and the free flow of saliva with its antimicrobial substances[18] .

Candida-associated denture stomatitis is a very common inflammatory process affecting about 60% of the carriers of a prosthesis. The major etiologic agent is Candida. albicans .These microorganisms can stick and proliferate through the hard and soft tissues of the oral cavity, and

colonize the inner denture surfaces forming a biofilm with different kinds of bacteria[١٩] .Thus, dentures act as a container for microorganisms, enabling Candida to re-infect the mucosal surface continually, contributing to the development of denture stomatitis[20].

The tissue surface of the dentures usually shows micropits and microporosities .microorganisms harboring booking in these areas are difficult to remove mechanically or by chemical cleansing. According to several in vitro studies, the microbial contamination of denture acrylic resin occurs very quickly, and yeasts seem to adhere well to denture base materials[21] . Acrylic resin has liquid sorption capacity and when it comes into contact with the oral cavity, it is capable of absorbing and adsorbing saliva and blood, consequently becoming a vehicle of cross contamination [22].

Irrespective of the existence of potent antibiotic and anti-fungal agents, resistant or multi-resistant strains are continuously appearing, striking the need for a permanent search and development of new drugs. Plants are the cheapest safer and time-tested alternative sources of antimicrobials [23] , accordingly re-searchers are interested in herbal remedies for medication and aim to substitute herbal material instead of chemical formula with limited side effects for human beings [24]. Medicinal plants express a productive source of antimicrobial agents[25] . Plant products still remain the fundamental source of pharmaceutical agents used in traditional medicine[26] . plant utilization as traditional health remedies is most popular for 80% of world population in Asia and all over the world and is reported to have minimal side effects[27] .

There are many researches in the literature about medical plants as antifungal. A study conducted by Kassab-Bashi et al[28] to evaluate the antifungal anti-adherent effect of medicinal plant extracts (peppermint,rue, pomegranate and garlic) on the colonization of *C. albicans* on acrylic *resin* denture base surface . Another study carried on by Marcos-Arias et al[29] to investigate the in vitro activity of eight terpenic derivatives (carvacrol, farnesol,geraniol, linalool, menthol, menthone, terpinen-4- ol, and a-terpineol), a phenylpropanoid (eugenol) and a phenethyl alcohol (tyrosol) against oral Candida isolates from patients suffering from denture stomatitis. A pretreatment of acrylic surface with ethanolic extract of *Boesenbergia pandurata* (Roxb.) Schltr. rhizome resulted in significantly reduced adhesion of *C. albicans* to denture acrylic surface[30]. Sookto et al.[31] found that essential oil of *Salvia officinalis* L. manifested strong anti-candidal activity and expressed inhibitory effects on the adhesion of the *C. albicans* to polymethyl methacrylate resin surface.. *Zataria multiflora* Boiss. has shown the beneficial effect to the treatment of CADS.[32] . Much work has been performed to monitor the antimicrobial effect of *Satureja hortensis* L. [33], *Pelargonium graveolens* L.[34], and *Streblus asper* Lour[35] . Barani, et al. investigated the anti-fungal activity of Morinda citrifolia fruit extract on Candida albicans[36]

The current study was conducted to assess aloe vera antifungal properties against Candida albicans. Aloe vera is used from more than 2000 years ago, it is well known for its medical properties1[37]. Aloe Vera possess a number of therapeutic uses viz: antiinflamatory, immunostimulatory, antibacterial, antifungal and cell growth stimulatory activity[38]. it has been found to be beneficial in oral

problems like apthous ulcers[39]. gingivitis[40] ,periodontitis[41] , oral Lichen Planus[42] , halitosis[43], denture adhesives and denture cleanser[44] .

There are many studies about the antifungal activity of crude extractive of aleo vera, Nebedum et al [45], Rosca-Casian et al.[46] , Subramanian et al[47], Shamim et al[48], Ali et al [49] Saks and Barkai-Golan[50], Nidiry et al[51].

The present study shows that aleo vera has anti-candidal activity. This is in agreement with studies done by George et al.[52] , Shireen et al[53], Saran et al[54] and Heggers et al[55]. The activity increases with the increase in the dose. This is also in agreement with studies done by Heggers et al[55], Shireen et al and Saran et al.[54].

The anti-microbial effect of aleo vera is attributed to the component called as plant's natural anthraquinones. Anthraquinones are naturally occurring aromatic compound that are found in plants that are applicable in the field of medicine and the dye industry[56]. Aloe vera provides 12 Anthraquinones, including aloe emodin, aloetic acid, aloin, anthracine, anthranon, barbaloin, chrysophanic acid, emodin, ethereal oil, ester of cinnemonic acid, isobarbaloin, and resistannol. In small quantities, anthraquinones act as potent antimicrobial [57]. This research was conducted by using monoantheron aglycol solution as anticandidal agent.

Although denture induced candidosis could be treated by methods pointed towards the oral mucosa, other treatment modalities are directed toward the denture base.

Several researches investigated the possibility of using drug delivery system by incorporation of antifungal or antimicrobial agents, with denture acrylic resin[58,59]

or with soft liners[60, 61]. The idea suggested the use of polymerized acrylic as carriers for drugs orally. Similarly, soft liners placed indentures have been used as carriers for antifungal drugs in treating denture stomatitis. It has been found that the release of the drug out of the polymeric carriers continued for more than 100 days[62]. However, the physical properties of the resin were affected due to the presence of the drug particles, which may dissolve and result in porosity in the acrylic base [63].

The current study was carried out by immersion heat cure acrylic specimens (conventional and Hi-impact) on aleo vera monoanthron solution for either 30 minutes or 8 hours, . Further studies are required to determine the physical properties of different types of heat cure acrylic resins after immersion in aleo vera solutions.

This study revealed that monoanthron isolated from aloe vera can be added as anticandidal agent.

Conclusion

With the limitation of this study it can be concluded that 100% and 75% concentrations of monoanthron aglycon part of anthroquinon isolated from aloe vera anticandidal effect, 100%concentration has higher effect than 75%. Immertion period has no effect on the anticandidal activityof the solutions.

It is desired that this research would lead to the creation of some compounds that could be used to formulate new and more potent anticandidal drugs of natural source to treat denture stomatitis.

Further investigations should be made to investigate anti-candida effect of other fraction of aloe vera.

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Table 1 descriptive statistics of study's groups

N	Std. Deviation	Mean	Immersion material	Immersion time	Heat cure acrylic type
10	1.4	9.1	Distilled water	30 Minutes	**conventional heat cure acrylic**
10	1.6	4.3	Aleo vera 75%		
10	1.6	2.9	Aleo vera 100%		
10	1.7	9.5	Distilled water	8 Hours	
10	1.2	3.8	Aleo vera 75%		
10	1.3	2.2	Aleo vera 100%		
60					**Total**
10	1.8	8.4	Distiledl water	30 Minutes	**Hi-impact heat cure acrylic**
10	.73	3.1	Aleo vera 75%		
10	.87	1.1	Aleo vera 100%		
10	1.8	8.9	Distiledl water	8 Hours	
10	.67	2.3	Aleo vera 75%		
10	.84	1.4	Aleo vera 100%		
60					**Total**

Table 2 3 Way ANOVA, Test of Between subjects Effects .Dependent variable; Candida albicans number.

Sig.	F	Mean Square	df	Type III Sum of Squares	Source
.000	18.563	36.300	1	36.300	**Heat cure acrylic type**
.603	.273	.533	1	.533	**Immersion time**
.000	284.970	557.275	2	1114.550	**Immersion material**
.603	.273	.533	1	.533	**Heat cure acrylic type * Immersion time**
.461	.780	1.525	2	3.050	**Heat cure acrylic type * Immersion material**
.214	1.564	3.058	2	6.117	**Immersion time * Immersion material**
.569	.567	1.108	2	2.217	**Heat cure acrylic type * Immersion time * Immersion material**
		1.956	108	211.200	**Error**
			120	4082.000	**Total**

Table3 Multiple Comparisons. LSD test. Immersion materials

Sig.	Std. Error	Mean Difference (I-J)	(J) Immersion material	(I) Immersion material
.000	.31269	5.6000*	Aleo vera 75%	**Distilled water**
.000	.31269	7.0750*	Aleo vera 100%	
.000	.31269	1.4750*	Aleo vera 100%	**Aleo vera 75%**

* Highly significant p<0.01

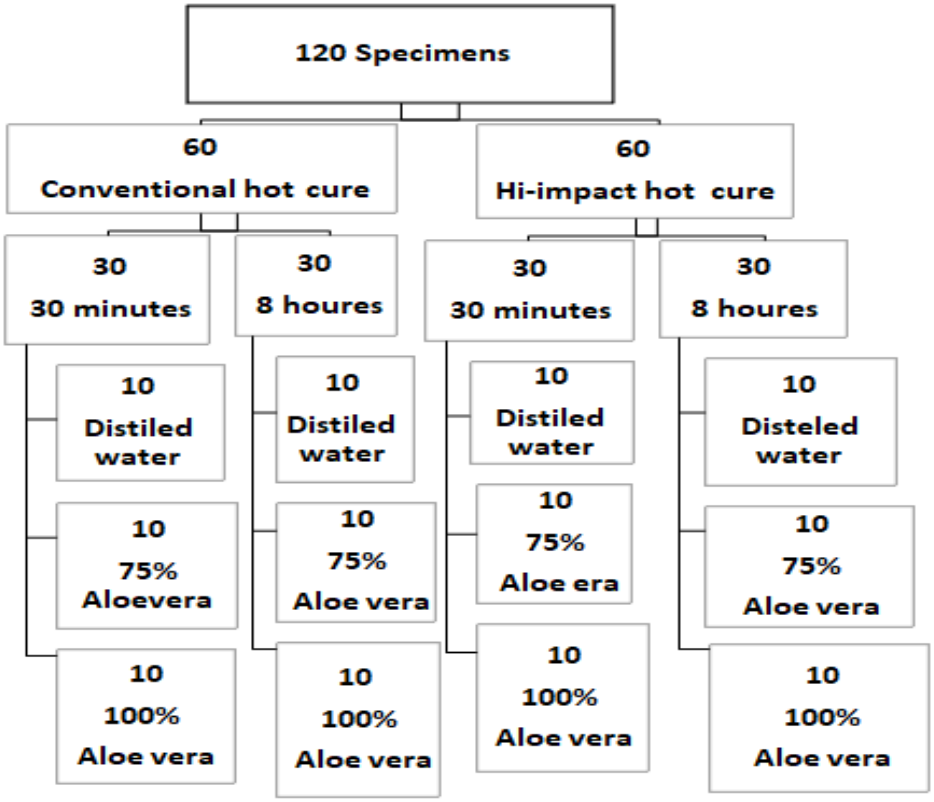

Figure (1) Specimens groups

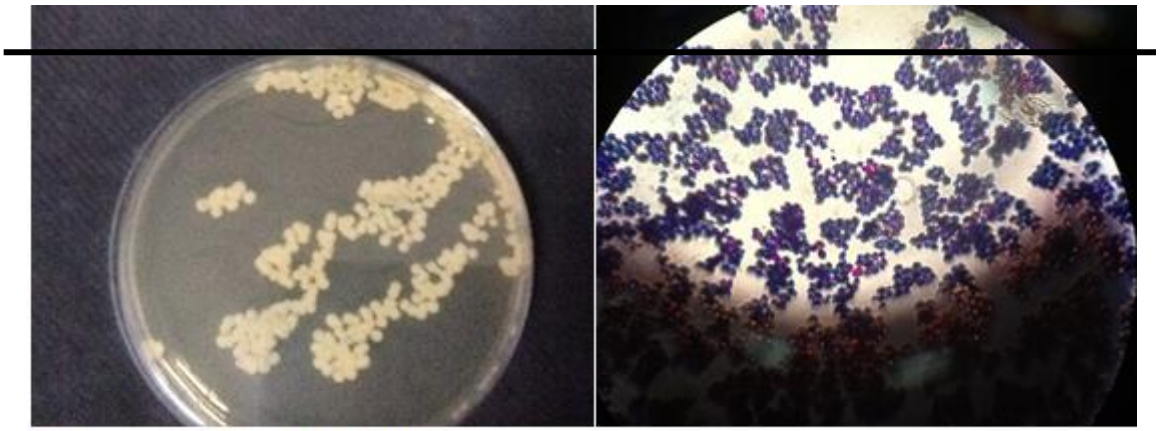

Figure (2) Candida albicans.

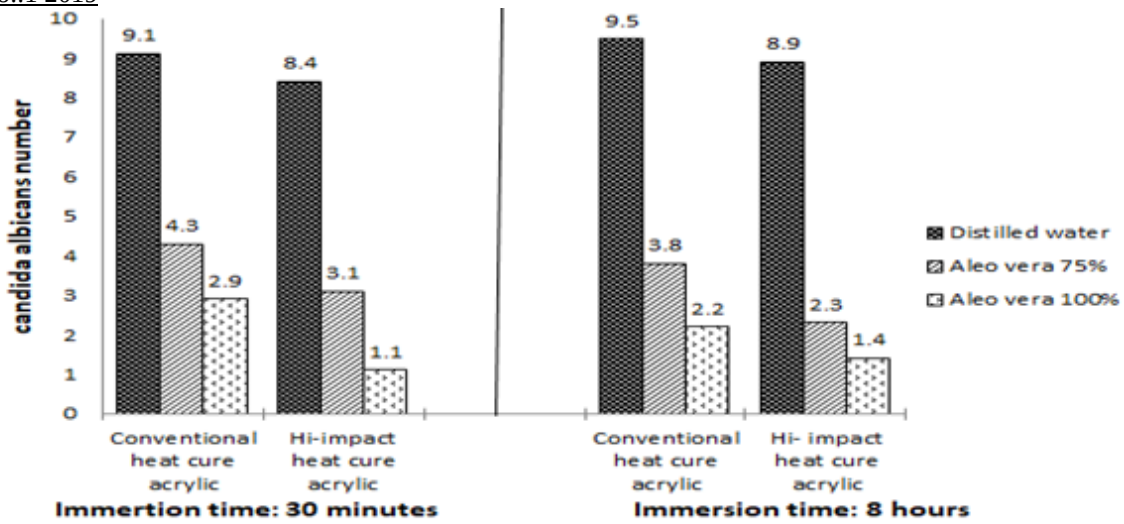

Figure (3) Candida albicans distributions