**Antifungal Evaluation of Different Concentrations of Aglycon fraction of Anthroquinon (dianathron)** **isolated from Aloe vera on Acrylic Resins**

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**Abstract**

**Background:** Denture-related stomatitis is a common condition where mild inflammation and redness of the oral [mucous membrane](https://en.wikipedia.org/wiki/Mucous_membrane) occurs beneath a [denture](https://en.wikipedia.org/wiki/Denture). It is the most common form of [oral candidiasis](https://en.wikipedia.org/wiki/Oral_candidiasis) (a yeast infection of the mouth). Aloe Vera is a species of Aloe, native to northern Africa. It is a stemless or very short-stemmed succulent plant growing to 80-100 cm tall, spreading by offsets and root sprouts. The leaves are lanceolate, thick and fleshy, green to grey-green, with a serrated margin.

**Aim of study:** to investigate the effects of different concentrations of aloe.vera , aglycon part (dianathron ) against candida albicance adhere to heat cure and cold cure acrylic resin denture base on two periods of time (30 minutes and 12 hours).

**Materials & methods:** A total of 120 specimens were prepared, sixty specimens of each denture base acrylic resin material (heat cure and cold cure), 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 (Dianthron) from anthroquinon glycoside of aloe vera plant;C. albicans isolation and identification and preparation of candida albicans suspension; autoclaved specimens of each sub-group for both heat cure and cold cure acrylic resins were placed into the tubes containing BHI plus inoculum and remained for 11 h at 37oC 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 denture base acrylic resins (hot and cold cure acrylic resins). The degree of inhibition varied depending on the concentration of the extract. Highest concentration of extracts of *A. vera* displayed

**Conclusion:** with the limitation of this study it can be concluded that 100% and 75% concentrations of dianthron 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, dianthron, anthroquinon.

**Introduction**

Denture stomatitis is defined as inflammation of the oral mucosa in contact with a denture fitting surface(1) . Denture stomatitis is recognized as having a multifactorial etiology including such causative factors as: *Candida* yeast infections, bacterial infections, poor oral and denture hygiene, unrelieved denture use, denture trauma, allergic reactions to denture materials, immunological factors, dietary factors, various medications and predisposing systemic pathologies(2).

The presence of the denture on the oral mucosa alone serves as a catalyst for the initiation of denture stomatitis by altering the local microenvironment by decreasing pH, saliva flow and mechanical cleansing, serving as a reservoir for harbouring microorganisms. Of these microorganisms, it is generally regarded that *Candida* species, particularly *Candida albicans*, is one of the most common causative agents of denture stomatitis(3) .

Candida spp. are oral commensals present in up to 90% of healthy persons and Candida albicans (C. albicans) is the most frequent colonizer fungi(4-6). Denture stomatitis is clearly associated with oral infection by yeasts, mainly C. albicans(7). Candida-associated denture stomatitis has been found in 60-65% of the subjects carriers of prosthesis(8).

Treatments currently available and indicated include: denture cleaning procedures, discontinuation of nocturnal denture wearing, denture replacement or realignment, topical or systemic antifungal agents, microwave irradiation, disinfecting solutions - such as chlorhexidine and sodium hypochlorite - and diode laser irradiation(2).

Considering the increasing resistance of the fungi against antifungal agents, formation of *Candida* biofilm, and generally positive attitude of patients for herbal treatment, natural products could be the alternative substitutions of chemical substances with less adverse effects on humans, which is relevant for effective treatment of *Candida* infections aloe vera plant was used in this study.

Aloe vera is native to southern and eastern Africa along the upper Nile in the Sudan, and it was subsequently introduced into northern Africa and naturalized in the Mediterranean region and other countries across the globe(9). The botanical name of Aloe vera is Aloe barbadensis miller. It belongs to Asphodelaceae (Liliaceae) family, and is a shrubby or arborescent, perennial, xerophytic, succulent, pea-green color plant. The aloe plant has long (up to 20 inches long and 5 inches wide), triangular, fleshy leaves that have spikes along the edges. The fresh parenchymal gel from the center of the leaf is clear; this part is sometimes dried to form aloe vera concentrate or diluted with water to create aloe juice products. The sticky latex liquid is derived from the yellowish green pericyclic tubules that line the leaf (rind); this is the part that yields laxative anthraquinones(10). Aloe vera (Aloe barbadensis Miller) is most biologically active among 400 species(11).

There are more than 200 compounds found in Aloe barbadensis, about 75 of which have biological activity(12) , including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid(13-14). The plant contains many vitamins, including Vitamins A, C and E, which are antioxidants. It also contains thiamine, niacin, riboflavin, vitamin B12, choline and folic acid, Enzymes such as acid phosphatase, alkaline phosphatise, amylase, lactic dehydrogenase and lipase. When taken orally, these biochemical catalysts, amylase and lipase aid in digestion by breaking down fats and sugars(15). Minerals: Sodium, potassium, calcium, magnesium, selenium, manganese, copper, zinc, chromium and iron are all found in the aloe plant(16). Sugars are located in the mucilaginous layer of the plant under the rind of the leaf. It includes monosaccharides (glucose and fructose) and polysaccharides (glucomannose and polymannose)(17). Anthraquinones: The bitter reddish yellow exudates, located beneath the outer green rind, contains anthraquinones and their derivatives, Barbaloin, aloeemodin- 9-anthrone, lsobarbaloin, Anthrone-C-glycosides and chromones. These are phenolic compounds, traditionally known as laxatives. These compounds exert a powerful purgative effect, when in large amount, but when smaller they appear to aid absorption from the gut and are potent antimicrobial agents and possess powerful analgesic effects(18). Sterols: These include cholesterol, Campesterol, β- Sitosterol and Lupeol. All these have anti- inflammatory action and lupeol also possesses antiseptic and analgesic properties(16). Salicylic acid: This is an aspirin-like compound possessing anti inflammatory and antibacterial properties Amino acids: Aloe vera gel provides the amino acids required for repair and growth. It includes 20 of 22 nonessential amino acids and 7 of 8 essential ones(18). Lignin: It is an inert substance which when included in topical preparations, enhances penetrative effect of the other ingredients into the skin. Saponins: These are the soapy substances that have cleansing and antiseptic properties(16).

The pharmacological actions of Aloe vera gel as studied in in vitro and in vivo include: Burn and wound healing property, moisturizing and anti-aging effect, immune system restoration. immunomodulatory effect, anti-inflammatory action, anti-diabetic effects, antimutagenic effects, anti-oxidant effects, anti-bacterial, anti-fungal and anti-viral actions.

Aloe vera is best known for its soothing and healing effects on burn and other wounds. A glycoprotein, isolated from A. vera showed an increase in epithelial cell migration and enhanced wound healing process(19). The enhancement in content of hyaluronic acid and dermatan sulphate in the granulating tissue of healing wound(16), increases the collagen content and extent of collagen cross linking of the wound, resulting in enhanced wound contraction and breakage of scar tissue(20). It does so by accelerating the flow of blood towards the wounded area. Aloe is the best wound dressing ever discovered(21).

Aloe vera possesses implausible moisturizing properties, by producing collagen and elastin fibers, making the skin more elastic and less wrinkled, by its cohesive action on superficial flaking epidermal cells and also by the action of amino acids(16,21). For such incredible characteristics, Aloe vera is an ideal ingredient in cosmetics and dermatological procedures.

Aloe vera has been reported to protect the skin against damage caused by radiation(22). The administration of Aloe vera gel results in generation of an antioxidant protein metallothionein, which act as a scavenger for hydroxy radicals, hence protecting the skin from oxidative damage(23).

Jyotsana et al.(24) showed a significant increase in total white blood cell and macrophage count upon administration of Aloe vera extract. Some immunomodulatory effects were shown to be associated with glycoproteins, namely lectins, found in aloe gel(25).

The effects observed for acetylated mannan in aloe gel resembles the anti inflammatory action of mannose-6-phosphate(25). Aloe vera also inhibits the cyclooxygenase pathway, reducing the production of prostaglandins, thereby reducing the inflammation(16,26).

Aloe vera gel is well known for reducing the blood sugar level(26). It also reduces hepatic transaminases, plasma and tissue cholesterol, triglycerides, free fatty acids and phospholipids(25). Aloe vera gel significantly lowered the triglycerides level(27). This explains its hypoglycaemic and hypolipidemic effects(18).

Glycoprotein and polysaccharide (acemannan) fractions of Aloe possess anti tumour activity(27). The polysaccharide fraction of Aloe gel showed chemo preventative and anti-genotoxic effects by preventing the ormation of benzo[α]pyrene- DNA adducts(28).

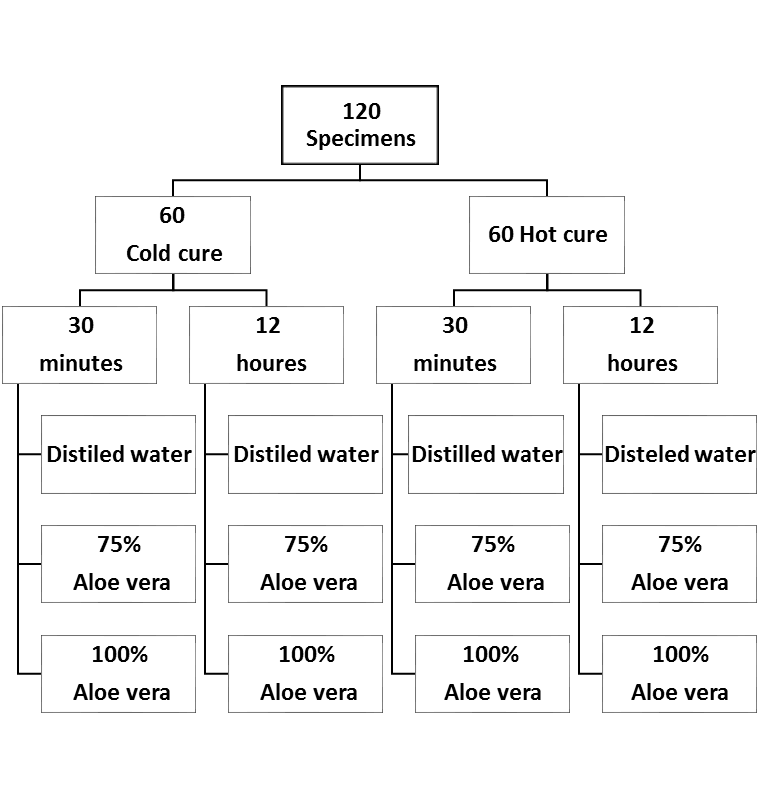
Aloe vera possesses enormous antioxidant effect. Glutathione peroxidise activity, superoxide dismutase enzymes and a phenolic anti-oxidant were found to be present in aloe vera gel, which may be responsible for these anti-oxidant effects(26,29).

Aloe gel acts against both gram positive and gram negative bacteria(30). Aloe gel preparation is also inhibitory to Candida albicans(31). The anthraquinone derivatives of Aloe leaf have shown virucidal effects on enveloped viruses(32-33). Aloe emodin inactivates most of the viruses, including Varicella zoster, influenza and pseudorabies virus and herpes simplex viruses(30,32).

The present study was undertaken to investigate the effects of different concentrations of aloe.vera , aglycon part (dianathron ) against candida albicance adhere to heat cure and cold cure acrylic resin denture base on two periods of time (30 minutes and 12 hours).

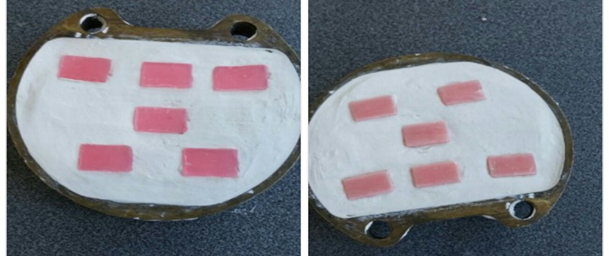
**Materials and methods**

**1- Specimens grouping:** A total of 120 specimens were prepared, sixty specimens of each denture base acrylic resin material (heat cure and cold cure), 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).



**Figure 1:** Specimens groups.

**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)(34) 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 heat-cured acrylic denture-based resin (Triplex Hot, Ivoclar Vivadent Liechtenstein, and N 74750) was mixed for with a ratio of 23g/10 ml powder/liquid according to manufacturer’s recommendations. The acrylic resin was packed into dental stone mold at the dough stage 12-15minutes after mixing and the polymerization process was carried out in a water bath at 70∘C for 1 h followed by boiling for 30 min. Autopolymerised acrylic resin (Triplex Cold, Ivoclar Vivadent Liechtenstein, N 69982) was packed in the molds and the polymerization process was carried out at 25∘C for 10 minutes. No finishing and polishing procedures were done in order to simulate the inner surface of a complete denture. Only remaining excesses were removed with the aid of acrylic burs. All specimens were and immersed in distilled water at 37ºC for 48±2 hours for elimination of the residual monomer.

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**Figure 2: Specimens preparation**

**3- Method of isolation of free aglycon (Dianthron) from anthroquinon glycoside of aloe vera plant:**

**1-** 156 gm of powdered crude solidified aloe vera (which was bought from the local market figure 3) 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 extracts were combined, and then concentrated. The chloroform layer will contain the free aglycone.

**3-** The prepared chloroform extract was used as disinfectant solution and referred to as 100% aloe vera solution.

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



**Figure 3:** Solidified aloe vera

**4- Candida 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.

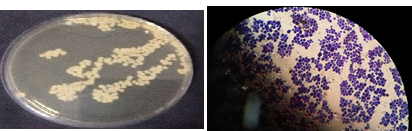
To identify and select C. albicans after incubation the follow­ing tests were done:

**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 4).

**2-** C. 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 4).

**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 C , the reactions were read visually without addition of reagents. The results were transfered into numerical profile which was compared with the profile index.



**Figure 4:** Candida albicans

**5- Preparation of candida albicans suspention:** C. albicans were grown on Sabouraud Dextrose Agar (LAB 009) plates (containing 500 mMol/L of sucrose) at 25 C°. For 24 h., the colonies were suspended in tubes containing 5 mL of brain heart infusion (BHI) broth (Salucea Lot 1202) were preparedaccording to its manufacter's instruction and autoclaved at 121C° 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 heat cure and cold cure acrylic resins were placed into the tubes containing BHI plus inoculum and remained for 11 h at 37oC in order to favor an initial colonization of the acrylic resin surfaces (figure5). 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.

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**Figure 5:** Autoclaved specimenswere placed into the tubes containing BHI plus inoculum

**7- Disinfection:** Then, the disinfection step was performed over two periods: 30 minutes and 12 hours as follows:

**a)** Control Group – 10 contaminated specimens, exposed to saline solution;

**b)** 10 contaminated specimens exposed to 100% aleo vera solution; and

**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 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.

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).

**Results**

Table 1 represents descriptive statistics of study's groups. Factorial ANOVA (Test of Between subjects Effects) indicates that the main effects of denture base type and immersion material on colony number were significant, while the immersion time have no significant effect on the number of colony forming units (Table2). Immersing in distilled water had higher number of candida albicans than immersing in 75% and 100% aloe vera and the difference is highly significant (Table3).

**Table 1 Descriptive Statistics of candida albicans adherence to the surface of heat cure and cold cure acrylic denture base groups measured in cell count ×105 /ml**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **N** | **Std. Deviation** | **Mean** | **Immersion material** | **Immersion time** | **Denture base type** |
| 10 | 3.24 | 11.5 | Control (distilled water) | **30 minutes** | **Hot cure acrylic** |
| 10 | 1.51 | 2.5 | 75% Aloe vera |
| 10 | .97 | 1.6 | 100% Aloe vera |
| 10 | 3.36 | 12.2 | Control (distilled water) | **12 hours** |
| 10 | .97 | 2.6 | 75% Aloe vera |
| 10 | .84 | 1.4 | 100% Aloe vera |
| 60 | 2.11 | 5.3 | **Total** | | |
| 10 | 2.99 | 14.9 | Control (distilled water) | **30 Minutes** | **Cold cure** |
| 10 | 2.17 | 4.5 | 75% Aloe vera |
| 10 | 1.72 | 3.1 | 100% Aloe vera |
| 10 | 2.95 | 15.5 | Control (distilled water) | **12 hours** |
| 10 | 2.17 | 5.4 | 75% Aloe vera |
| 10 | 1.71 | 4.6 | 100% Aloe vera |
| 60 | 5.53 | 8.50 | Total | | |

**Table 2**  **Test of Between subjects Effects .Dependent variable; colony forming unit**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source of error** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| **Denture base type** | 218.700 | 1 | 218.700 | 43.952 | .000\* |
| **Immertion time** | 10.800 | 1 | 10.800 | 2.170 | .144 |
| **Immertion Material** | 2859.050 | 2 | 1429.525 | 287.288 | .000\* |
| **Dentbastype \* ImmTime** | 4.800 | 1 | 4.800 | .965 | .328 |
| **Dentbastypype \* immMat** | 6.350 | 2 | 3.175 | .638 | .530 |
| **ImmTime \* immMat** | .150 | 2 | .075 | .015 | .985 |
| **Dentbastyp \* ImmTime \* immMat** | 4.050 | 2 | 2.025 | .407 | .667 |
| **Error** | 537.400 | 108 | 4.976 |  |  |
| **Total** | 8948.000 | 120 |  |  |  |

\*Highly significant P<0.01

**Table3** Multiple **Comparisons. LSD test. Immersion materials**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **(I) immersion materials** | **(J) immersion materials** | **Mean Difference (I-J)** | **Std. Error** | **Sig.** |
| **control (distilled water)** | 75% aloe vera | 9.7750\* | .49879 | .000\* |
| 100% aloe vera | 10.8500 | .49879 | .000\* |
| **100% aloe vera** | 75% aloe vera | -1.075 | .49879 | .033\*\* |

\* Highly significant P<0.01

\*\* Significant P<0.05

**Discussion:**

The Aloe vera plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. Aloe vera has been used for medicinal purposes in several cultures for millennia: Greece, Egypt, India, Mexico, Japan and China(35).

The pharmacological actions of AV include antiinflammatory, antibacterial, antiviral and antifungal properties, and hypoglycaemic effects (36-38).

There are eight main uses of aloe vera in dental practice(39):

**1.** Periodontal surgery.

**2.** Applications to the gum tissues when they have been traumatized or scratched by toothbrush-dentifrice abrasion, sharp foods, dental floss, and toothpick injuries.

**3.** Chemical burns from accidents with aspirin.

**4.** Extraction sockets**.**

**5.** Acute mouth lesions such as herpetic viral lesions, aphthous ulcers, canker sores & cracks occurring at the corners of our lips. Gum abscesses are soothed by the applications as well.

**6.** Chronic oral diseases Lichen Planus and Benign Pemphigus, gum problems associated with AIDS and Leukemia. Migratory glossititis, geographic tongue and Burning Mouth Syndrome.

**7.** Denture patients with sore ridges and ill-fitting dentures & partials.

**8.** Dental implants.

In the present investigation, anti-fungal activity of different concentrations of aglycon fraction of anthroquinon (dianathron) of aloe vera was evaluated. 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 denture base acrylic resins (hot and cold cure acrylic resins). The degree of inhibition varied depending on the concentration of the extract. Highest concentration of extracts of *A. vera* displayed higher effect.

Aloe vera juice has antimicrobial activity against *M. smegmatis*, *K.pneumoniae*, *E. faecalis*, *M. luteus*, *C.albicans* and *B. sphericus* as determined by a study conducted by Alemdar and Agaoglu(40). Aloe vera has found by - Vogler and Ernst to inhibit the growth of Candida albicanas (41). Agarry et al(42). Antimicrobial susceptibility test showed that aloe vera leaf possesses inhibitory effects on both *P.aeruginosa* and *C. albicans*. This antifungal activity meight be due to different conistitues of aloe vera.

*Aloe vera* contains 6 antiseptic agents: Lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur. They all have inhibitory action on fungi, bacteria and viruses (43).

Anthraquinones are the phenolic compounds that found in the Aloe vera. The Aloes consist of free anthraquinones and their derivatives: barbaloin, isobarbaloin, anthrone-c-glycosides and cromones. In large amounts, these compounds exert a strong purgative effect, but in smaller amounts they appear to aid in absorption from the gut and considered as potent antimicrobial agents and possess powerful analgesic effects(44).

Saponins are soapy substances form 3 percent of the gel and are general cleanser, having antiseptic properties. These act powerfully as anti-microbials against bacteria, viruses, fungi and yeasts(45).

The Aloe protein of 14 kDa from the A. vera leaf gel was isolated and thepurified Aloe protein exhibited a potent antifungal activity against Candida paraprilosis, Candida krusei, and Candida albicans(46).

This study revealed that Dianthron 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 dianthron 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.

It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent anticandidal drugs of natural origin to treat denture stomatitis.

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

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