ISSN: 1812–1217

The ability of different curcumine solutions on reducing *Candida albicans* biofilm activity on acrylic resin denture base material

Nagham H. Kassab
BDS, MSc (Assist Lect)

Eman A. Mustafa[1]
BDS, MSc (Assist Lect)

Maha T. Al–Saffar[1]
BDS, MSc (Assist Lect)

Department of Prosthetic Dentistry
College of Dentistry, University of Mosul

[1]Department of Basic Sciences
College of Dentistry, University of Mosul

ABSTRACT

Aims: To measure candidal biofilm activity using pH changes of stomastat, to evaluate the effect of different types of curcumine solution on *Candida albicans* biofilm that attached on acrylic resin denture base material (A.R.D.B.M.) and the correlation of initial number of yeasts inoculated with the pH value of stomastat after 24 hours incubation period. **Materials and Methods**: In general, the number of acrylic resin samples that had been used in this study was 20 samples. Curcumine solution had been dissolved in three different solvents, those were ethanol, viscous (glycerine), and sterile distilled water, while cholrhexidine (CHX) alone used as a control negative and distilled water used as a control positive. The diameter of inhibition zone for the different curcumine solution had been measured and compared with control (–ve, and +ve); the biofilm activity of *Candida albicans* on A.R.D.B.M. was measured by a new method using pH change of stomastat. **Results**: Demonstrated that the ability of curcumine solution to decrease fungal biofilm activity on A.R.D.B.M varied depending upon the type of solvent in which the solution had been dissolved, and ethanolic solution (50%) was the most effective in reducing biofilm activity of *C. albicans* on A.R.D.B.M. when compared with CHX solution which is the most commercially used solution as denture cleanser. **Conclusion:** The ethanolic solution of Curcumine can be used in prosthetic dentistry as a new denture cleansing agent.
Keywords: Curcumine solution, *Candida albicans* biofilm, Prosthetic appliances.

Kassab NH, Mustafa EA, Al–Saffar MT. The Ability of Different Curcumine Solutions on Reducing *Candida albicans* Biofilm Activity on Acrylic Resin Denture Base Material. *Al–Rafidain Dent J*. 2007; 7(1): 32–37.

Received: 18/12/2005 ***Sent to Referees:*** 21/12/2005 ***Accepted for Publication:*** 13/2/2006

INTRODUCTION

The formation of *C. albicans* biofilms on prosthetic devices is a significant medical problem and often necessitates removal of the device. [(1)]

It should be noticed that complete denture play two fold role with regards to creating predisposition to candidiasis, first dentures serve as a catalyst to imitation of chronic atrophic candidiasis and second they acts as reservoir for organisms.[(2)]

Denture plaque formed through microorganisms colonization from saliva and oral mucosa and developed by microbial adherence leads to formation of thin biofilm, followed by multilayer and culminates in denture plaque, this biofilm is composed not only of blastospore but also of Candial hyphae [(3,4)], thus denture plaque biofilm means the communities of micro organisms attached to surface [(5)], it represents a protective reservoir for oral microbes [(6)], therefore it is essential to assess the effect of cleansing agents on removing the colonized yeasts particularly fungal biofilm.

Before three years, it was obvious that some of medicinal plants solutions exhibited anti adherent and antifungal effect in removing *C. albicans* cells from acrylic resin denture base material (A.R.D.B.M.) surface and can be used as a mouth rinse or denture soaking solution or boths of

them.[7]

In animals, curcumine was applied topically which exhibited an inhibitory effect on dermophytes and pathogenic fungi affect yeast isolates[8], also it have antifungal activity against *C. albicans*.[9] Curcumine is a yellow pigment contained in the rhizome of the plant, (Curcuma longa) turmeric is the active component, it is an herb of the ginger family that is extensively cultivated in India, it is ideally used in foods for its colour and flavor.[10]

It has long history of safe use in Ayurvedic medicine, and particularly in the treatment of inflammatory disorders. In addition to its anti–inflammatory properties, curcumine is a potent anti–oxidant, stronger than vitamine E in preventing lipid peroxidation in vitro.[11]

Depending on what's previously mentioned; the purposes of this study were to develop a new method to quantify the activity of fungal biofilm and to evaluate the efficacy of curcumine solution as denture cleansing agent comparing it with the most commercially used denture cleanser which is chlorehexidine.

MATERIALS AND METHODS

Heat polymerized denture acrylic resin sheets (10x10x1.5mm $\pm$ 0.5) were fabricated in which polymer and monomer were mixed according to manufacturer's instruction; A smooth surface was obtained by pressing the mixture between two glass slides surfaces. The mixture was flasked and processed in water tank at 70 ◦C for 90 minutes. and then at 100 ◦C for 30 minutes, then the processed acrylic resin sheets were cut into (10x10x1± 0.5) mm, the number of total samples were processed and used in this study were 20 samples.[3,7]

Brain heart infusion broth was used in this assay for culturing *Candida albicans* which was isolated on sabourauds dextrose agar from the inner surface of upper complete denture.[7]

The rhizome of the *Curcumine longa* plant is the portion that used medically it is usually cleaned boiled, and dried yielding a yellow powder (Curcumine).[12] In the this study three different solution of Curcumine were used and in two concentration. For each one 50%, and 100% the first one was ethanolic solution of Curcumine (ESC), the second one was viscous soluteion of Curcumine (VSC) and the third one was watery solution of Curcumine (WSC).

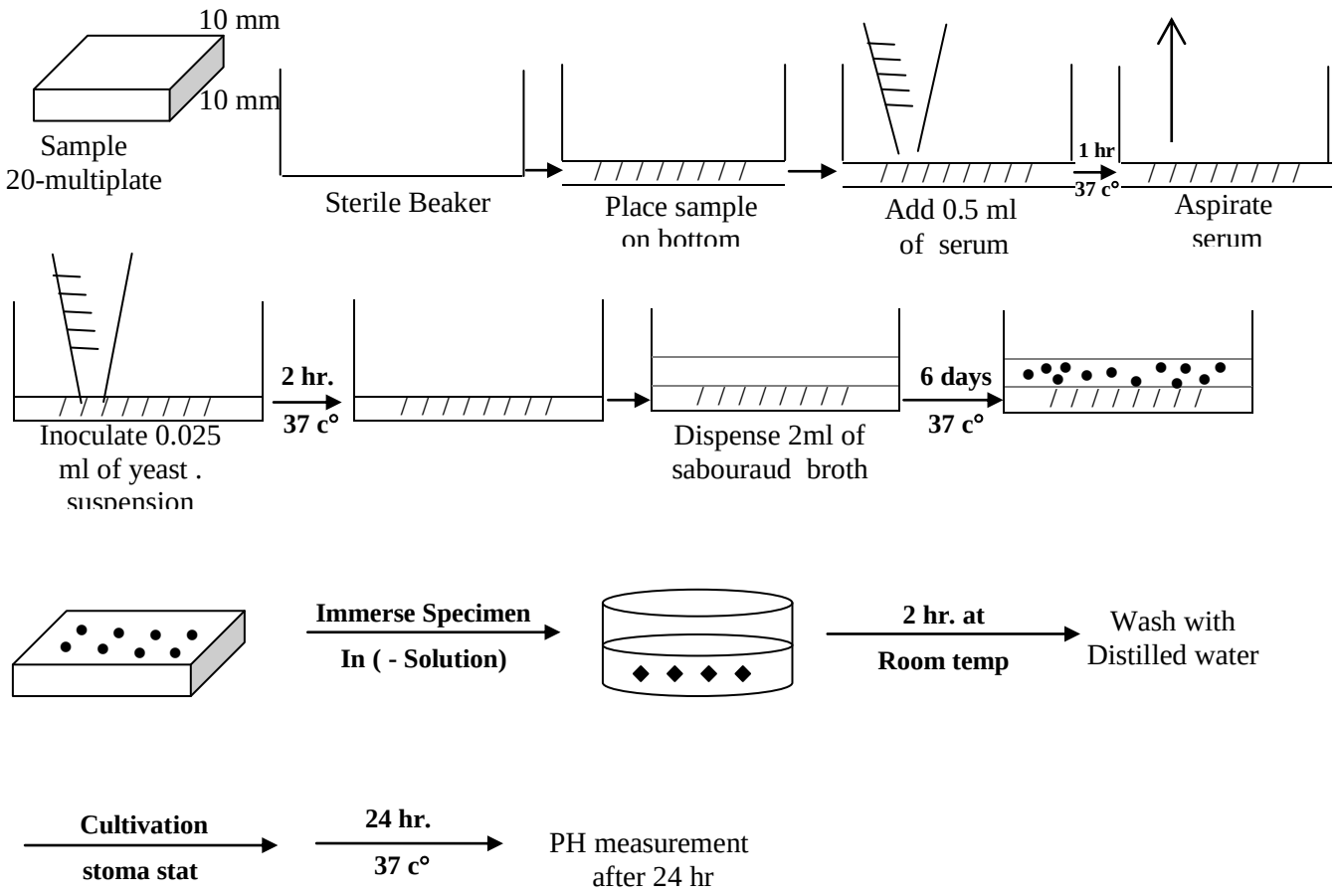
The ethanolic solution of curcumine (ESC) 50% was prepared by resuspending 50 gm of Curcumine in 100 ml of ethanol (95%), while 100% solution of ESC was prepared by resuspending 100 gm of Curcumine in 100 ml of ethanol 95%.

The viscous solution of Curcumine (VSC) was prepared by resuspending 50 gm of Curcumine in 100 ml of glycerine solution (50% solution), while 100% solution was prepared by resuspending 100 gm of Curcuminme in 100 ml of glycereine solution.[13] Watery solution of Curcumine (WSC) 50% was prepared by resupending 50 gm of Curcumine in 100 ml of sterile distilled water, while (100%) solution was prepared by resuspending 100 gm of Curcumine in 100 ml of sterile distilled water.

Discs were prepared by adding 1.0 ml of ESC, VSC and WSC for each concentration (50, 100%) to 10 discs. Chlorhexidine gluconate solution 0.2% was used as control. Colonies were streaked on sabouraud agar medium within 15 min[14] after plates were inoculated with the previously prepared discs of both concentration, 50% and 100% for each solution (ESC, VSC, and WSC) applied to the inoculated plates by sterile forceps. Then the plates were placed in an incubator at 37 C for 24 hours.[15–17] After incubation the plates were examined and the zones of inhibition of growth were noticed and measured in mm. Results were interpreted by comparing the zone with chlorhexidine gluconate 0.2%.

Since *Candida albicans* biofilm are multi layered with aggregated blastospore and hyphae[18], It's difficult to quantify the cell number by conventional vital counting or direct counting under light or sca-nning electron microscopy, therefore, the number of vital cell of colonized yeasts (biofilm activity) was studied by the following new method(Figure 1).[19]

Each sample of denture base has been placed on the bottom of sterile beaker. Add 0.5 ml of serum then incubated for 1 hour, at 37 ◦C after that serum was aspirated then 0.025 ml of yeast suspension 10^6 cell/ml was inoculated.



Figure(1): Schemetic diagram of biofilm assay procedure

Yeast suspension was determined by total cell count using haemocytometer [20], then incubated for 2 hours, at 37 ◦C after that 2 ml of sabouraud broth was added to each beaker, incubated for 6 days at 37◦C. To assess the ability of Curcumine solutions to remove Candidal biofilm, yeast colonized suspension were immersed in to 50 ml of each of the following:

- ESC 50% (Ethanolic solution of Curcumine)
- VSC 50% (viscous solution of Curcumine)
- WSC 50% (watery solution of Curcumine)
- Chlorhexidine gluconate solution 0.2% as control positive.
- Sterile distilled water as control negative.

After that each beaker was incubated for 2 hours washed with sterile distilled water and then 2 ml of stomastat (modified sabouraud broth containing 0.01% wt/vol chloramphenicol)[21–22] were added to each beaker. The initial number of yeasts inoculated was determined by total cell count using haemocetometer, than after 24 hr of incubation the pH of medium with each specimen was measured using a pH meter instrument (HNNA, CE, Italy).

The result were tested for their significant by Student's t- test, F -test and Duncan multiple range test ($P<0.05$ probability).

RESULT AND DISCUSSION

According to the result that obtained from measuring (the means of the inhibition zone diameter for all solutions of Curcumine) the ethanolic solution of 50% concentration have the greatest diameter zone of inhibition (13 mm) comparing it with the control solution (CHX) which has the diameter zone of inhibition 15.5 mm, in which the effect was not significantly different between them while 100% concentration of E.S.C and the other types of Curcumin solution in their different concentration, 50%, and 100% had antifungal effect

which were significantly different from CHX and ESC (50%). this mean those solutions had the least antifungal effect as shown in Figure (2), for this reason the second concentration of 100% for all soluteons were excluded, and used only the first concentration (50%) for all curcumine solution in the second new method which are used for demonstrating the reduction *Candida albicans*, biofilm activity so the result of present study demonstrated the antifungal activity of curcumine solution against *C. albicains* cells. This agreed with different studies[8,9], this is because turmeric solution and the essential oil of *Curcuma longa* inhibit the growth of a variety of bacteria, parasites and pathogenic fungi.[23]

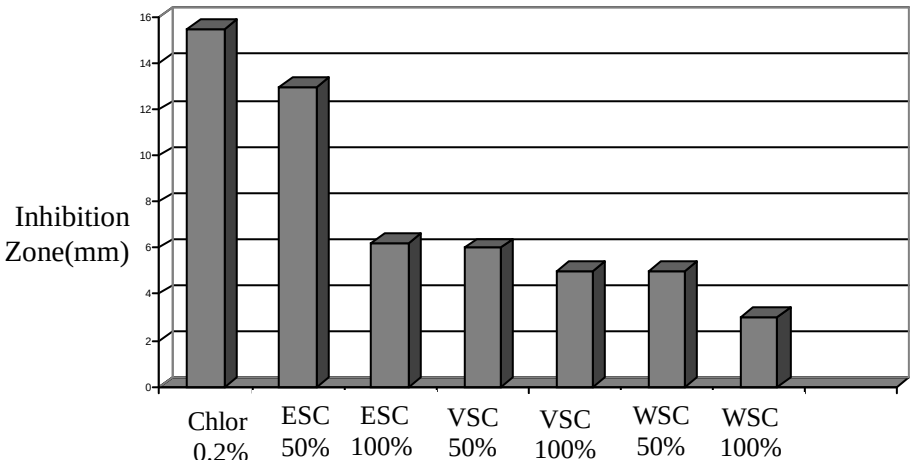


Figure (2): Antifungal effect of different Curcumine solutions compared with control treatment. ESC: Ethanolic solution of Curcumine; VSC: viscous solution of Curcumine; WSC: watery solution of Curcumine

From Figure (3) and Table (1) demonstrated the effect of E.S.C in 50% concentration, with CHX in 0.2% concentration, and D.W on initial number of *C. albicans* cells inoculated in stomastat and the result showed that both of CHX and E.S.C had the highest effect on reducing the number of *C. albicans* cells in which the effect between them was not significant while the effect was significant when compared with others. The same table showed that the most effective solutions in reducing the initial number of *C. albicans* were CHX and E.S.C, both of them had their effect in changing the pH of stomastat toward neutral pH.

Table (1) : Relationship between the number of yeasts initially inoculated and the pH value of Stomastat after 24 hr.

Treatments	pH of stomastat after 24 hr	Initial number of yeasts inoculated
	Mean ±SD	Mean ±SD
Chlorhexidine gluconate 0.2%(control +)	6.23 ± 0.017	4.38 ± 0.75
Distilled water(control –)	4.80 ± 0.01*	26.7 ± 3.8*
ESC 50%	6.19 ± 0.03	4.45 ± 0.54
VSC 50%	5.89 ± 0.007*	8.75 ± 1.1*
WSC 50%	5.13 ± 0.009*	16.50 ± 1.6*

SD: Standard deviation; ESC: Ethanolic solution of Curcumine; VSC: viscous solution of Curcumine; WSC: watery solution of Curcumine.
Significantly different from Chlorhexidine at P>0.05

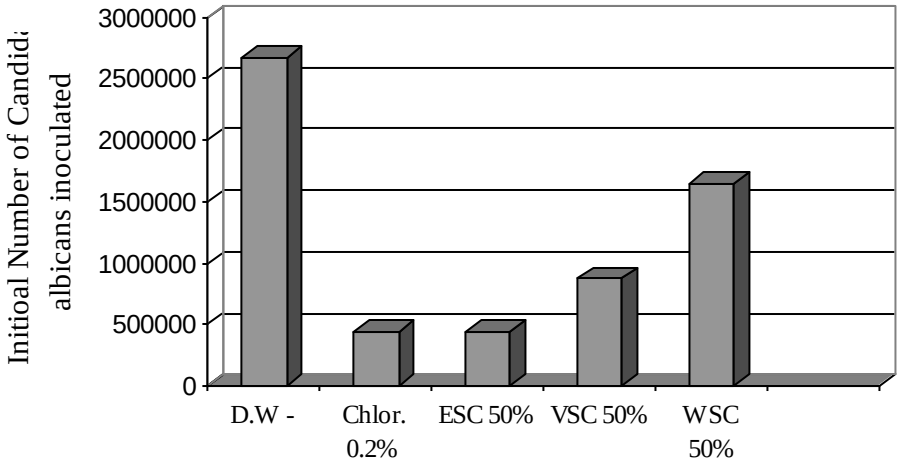


Figure (3): Efficacy of ESC, VSC and WSC to reduced the fungal biofilm activity compared with the control treatments. DW: Distilled water; Chlor: Chlorhexidine; ESC: Ethanolic solution of Curcumine; VSC: viscous solution of Curcumine; WSC: Watery solution of Curcumine

This result is very important because an increase in *C. albicans* cells growth was noticed and their activity in acidic medium and vasa versa [24,25] the data obtained by this method are directly implicated in the etiology of denture induced stomatatis, since the pH reduction caused by acid production of fungi in the residual biofilm was directly measured.

The commonly used method for examining the ability of denture cleansing agents in reducing or removing the colonized yeast and fungal biofilm from acrylic resin surface is the conve-ntial vital counting which has short comings particularly for the hyphal emergence which was aggregation of yeasts in both blastospore and hyphal phases which commonly take place in the colonization process, therefore this method may give misleading vital count result[3], for this reason a more sensitive and accurate method to measure biofi-lm activity of *C. albicans* was used in this study.

CONCLUSION

The method of using pH change of stomastat was sufficiently sensitive and quantitative to assess fungal biofilm activity.

The ability of Curcumine solution to decrease fungal biofilm activity were essentially similar to the result of antifungal effect by measuring the diameter zone inhibition.

The method of using, pH change of stomastat was used for the first time in this study for studding the biofilm activity (number of colonized *C. albicans* cells that adhered to acrylic resin denture base material.

The ethanolic solution solution of Curcumine was the most effective one in reducing *C. albicans* biofilm activity on A.R.D.B.M and can be used as a new denture cleansing solution.

REFERENCES

1. Hoyer LL, Rfundyga JE, Heeht JC, Kapteyn FM , Klis JA. Characterization of ALS genes from non– *albicans Candida* and Phylogenetic analysis of the ALS family. *Genetics*. 2001; 157: 1555–1567.
2. Budtz–Jorgensen E. Etiology, pathogenesis therapy, and prophylaxis of oral yeast infections. *Octa Odontal Scand*. 1990; 48: 61–69.
3. Nikawa Hayashi S, Nikawa Y, Hamada T, Samaranayake LP. Interaction between denture lining material, protein pellicles and *Candida albicans*. *Archs Oral Biol*. 1993; 38: 631–634.
4. Nikawa H, Nishimura H, Makihira S, Hamada T, Sadamori S. Samaranyake LP. Effects of serum concentration on Canida biofilm formation on acrylic surfaces. *Mycoses*. 2000; 43(3–4): 139–143.

5. Toole G, Kaplan H, Kotter R. Biofilm formation as microbial development. *Ann Re Microbiol.* 2000; 54: 49–79.
6. Chandra J, Mukherjee P K, Leidich SD, Faddoul FF, Hoyer LL, Donhlas LJ, Channoum MA. Antifungal resistance of candidal biofilms formed on denture acrylic in vitro**.** *J Dent Res*. 2001; 80(3): 903–908.
7. Kassab, N.H. Antifungal effect of some agents on *Candida albicans* growth on acrylic resin denture base surface (In vitro study). MSc Thesis. College of Dentistry. University of Mosul. 2002.
8. Appisaviyakula A, VaniHanakom N. Antifungal of Curcumine. *J Ethano Pharma*. 1995; 49: 63–69.
9. Roth G N, Chandra A, Nair MG. Novel Biological activities of Curcuma longa Constituents. *J Nat Prod*. 1998; 61(4): 542–555.
10. Ammon HPT, Safayhi H, Mack T, Sabieraj J. Mechanism of anti inflammatory actions of Curcumine and boswellic acids. *J Ethnopharamacol*. 1993; 38: 113–119.
11. Wallace JM. Nutritional and botancial modulation of inflammatory cascade eicosanoids, cyclooxygenases and lipoxygenease As an adjunct in cancer therapy. *Integr cancer Ther*. 2002; 1(1):7–37.
12. Leung A. Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. John Wiley and Sons Inc. New York. USA. 1980; Pp: 313–314.
13. Al–Saffar MT. The Therapuetic effect of viscous solution of Curcumine in the treatment of recurrent apthous stomatitis (RAS). Al–Rafidian Dent J. 2006; 6: 48–52
14. Waage SK, Hedin PA. Quercetin 3-O-galactosyl-(1 → 6)-glucoside, a compound from narrowleaf vetch with antibacterial activity. *Phytochemistry*. 1985; 24: 243–245.
15. Prescott LM, Hareley PO, Klein DA. Microiology. 3rd ed. Brown a film activity London. 1996.
16. Bauer AW, Kiraby WA, Sherris JS, Tark M. Antibiotic susceptibility testing by tenderized; Single disc method. *Am J clin pathol*. 1996; 45: 493–496.
17. Nawas TF, Mawahdeh, Dabneh A, Al–Omari A. In vitro activity of Antimicrobial agents against proterus spp from clinical specimens. *Br J BioMed Sc*. 1994; 51(2): 95–99.
18. Nikawa H, Nishimura H, Yamamoto T, Hamada T, Samaranayake LP. A novel method to study the hyphal phase of C. albicans and evailation of its hydrophobicty. *Oral Microbial Imunol*. 1995; 10: 110–114.
19. Nikawa H, Yamamoto T, Hamada T, Samori S, Agrawal S. Cleansing efficacy of commercial denture cleanser: Ability to reduce *Candida albicans* (biofilm activeity). *J Prosthet Dent*. 1995; 8:527–534.
20. Aneja KR. Counting of Cells/Spores of Microorganisms in: Experiments in Micrrobiology plant pathology and Biotechnology. 4th ed. New Age International Ltd. New Delhi. India. 2004. Pp: 66–69.
21. Hamada T, Yuhda S, Shieto N, Tamamoto M, Nahara Y,Sadamori S, A simplified culture for the diagnosis of denture stomatatis. *HiroShima J med SciI.* 1987; 36: 284–289.
22. Nikawa H, Kotani H, Sadamori S, Hamada T. denture stomatitis and ABO blood types. *J Prosthet Dent.*1991; 66: 391–394.
23. Allen PC, Danforth HD, Augustine PC. Dietary modulation of avian Coccidiosis. *Int J Parasitol*. 1998; 28:1131–1140.
24. Holmes AR, Cannon RD, Jenkinson H. Interactions of *Candida lbicans* with bacteria and salivary molecules in oral biofilm. *J Indust Microbiol*. 1995; 15: 208–213.
25. Radford DR, Challacombe ST, WalterJD. Denture plaque and adherence of *Candida albicans* to denture base materials (in vitro and vivo). *Crit Rev Oral Biol Med*. 1999; 10(1) :99–116.