

Study of the Effect of Betel Leaf and Lemon Juice on *Candida Albicans*

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Abstract: *Candida albicans* is a virulent human pathogen and can cause a variety of superficial and deep infections in man like oral thrush, cystitis and liver abscess. Biofilm formation and resultant antifungal resistance is an upcoming problem in this pathogen. Hence we evaluated the effect of extract of betel juice and lemon juice at different concentrations on this pathogen. It was found that at concentration of 4 gram%, betel leaf extract significantly reduced the growth, lipase expression and biofilm of *Candida albicans* in vitro. This study can pave the way for future studies.

Keywords: *Candida albicans*, pathogen, cystitis, oral thrush, liver abscess.

INTRODUCTION

Candida albicans is a fungal pathogen causing a variety of infections in man, and is particularly worrisome due to the high mortality of systemic infection [1]. Drug resistance is also an upcoming problem in *Candida albicans*, especially in oropharyngeal candidiasis caused by *Candida albicans* and also to a lesser degree in Candidemia [2]. Hence it should be interesting to note and observe the effect of natural compounds on growth and biofilm formation and also virulence factors of *Candida albicans*. It is important to mention here that curcumin, a natural compound, has shown anti-adherence effect on *Candida albicans* on denture materials [3]. Ethyl acetate extract of Neem leaf (*Azadirachta indica*) also has the power of inhibiting the growth of *Candida albicans* [4]. Keeping all these things in mind, we wanted to evaluate the effect of betel leaf extract and lemon, two commonly used food items and additives, on the growth and virulence traits of *Candida albicans*.

MATERIALS AND METHODS

This was a lab based observational study, carried out in the department of Microbiology of the institute from June to November 2017 as part of short term project. Five (5) randomly selected clinical isolates of *Candida albicans* were chosen for the study. *Candida albicans* strains were identified by phenotypic tests done from colonies on Sabouraud's dextrose broth, like positive Germ tube test, fermentation of glucose and maltose but not lactose, positive growth at 44 °C and production of terminal chlamyospores at room temperature after 48 hours. Betel leaf (sweet variant) and lemon were procured from the market, each time from different vendors. Leaves of betel (*Piper betle*) were washed, dried, torn into small pieces and added to 100 ml peptone water in sets of different concentrations, like 1 gram%, 2 gram% and 4 gram%. Similarly lemon juice was extracted from lemon and added and volume made up to 100 ml peptone water in the same concentrations. *Candida albicans* colonies were inoculated in: (a) Peptone water, (b) Peptone water with betel extract at different concentrations, and (c) Peptone water with lemon extract at different concentrations. In each case turbidity was adjusted to

0.5 Mac Farland standards. After incubation at 37 °C for 1 hour, 12 hours and 24 hours, 1 loopful from each tube was inoculated on Mueller Hinton agar and Egg yolk agar prepared in-house by adding 10 ml beaten egg yolk to 90 ml sterile molten Nutrient agar (time kill assay). In egg yolk agar, alterations in virulence factors by the extracts were studied. Lecithinase was denoted by presence of halo around the colonies while lipase and protease were denoted by appearance of pearly shine over surface of colonies and appearance of zone of clearing around colonies. After subculture from peptone water and extracts, liquid content of the tubes were decanted and biofilm formation was assessed by the Test tube method, in which 1 ml aqueous Safranin (1%) was added to the tubes and kept for 1 minute. After that dye was poured off and tubes washed thrice with normal saline. Then tubes were dried in inverted position and observed visually for biofilm formation. Also, the toxicity of the extracts were studied by mixing 1 drop of extracts with 1 drop of buffy coat from leftover human plasma samples (received in the lab for other purposes) and observing microscopically for WBC and RBC cell lysis. All experiments were done thrice with each isolate.

RESULTS

At concentrations of 1 gram and 2 gram%, there was little or no effect on growth of *Candida albicans* shown by the extracts. However, at the concentration of 4 gram %, betel leaf extract markedly inhibited the growth, biofilm and lipase (principal virulence factor) of *Candida albicans*. None of the *C. albicans* isolates showed protease activity. Lemon

extract at 4 gram% showed no inhibition of growth, biofilms or lipase. In the time kill assay, it was found that the maximum inhibition was shown after a time of 12 hours. Incubation upto 24 hours did not add to the growth inhibition and lipase expression. Extracts were non toxic to human RBC and WBC since there was no cell lysis. Results have also been shown below in tabular form. Images also highlight the findings.

Table-1: Effect of 2% paan extract on *Candida albicans*

	Total number of exp.	Normal Peptone water	2% PAAN Extract with peptone water		2% Lemon Extract with peptone water	
			Positive	Negative	Positive	Negative
Biofilm	15	15	11	04	12	03
Growth	15	15	10	05	12	03
Lipase	15	12	10	05	12	03
Protease	15	00	00	00	00	00
Lecithinase	15	00	00	00	00	00

Positive: Organisms growth have seen in different % of PAAN and Lemon extract

Negative: Organisms growth have inhibited in different % of PAAN and Lemon extract

Table-2: Effect of 4% paan extract on *Candida albicans*

	Total number of exp.	Normal Peptone water	4% PAAN Extract with peptone water		4% Lemon Extract with peptone water	
			Positive	Negative	Positive	Negative
Bio film	15	15	03	12	10	05
Growth	15	15	03	12	11	04
Lipase	15	12	03	12	10	05
Protease	00	00	00	00	00	00
Lecithinase	00	00	00	00	00	00

Positive: Organisms growth have seen in different % of PAAN and Lemon extract

Negative: Organisms growth have inhibited in different % of PAAN and Lemon extract



Fig-1: Growth inhibition of *Candida albicans* by 4% paan extract



Fig-2: Inhibition of biofilm of *Candida albicans* by 4% paan extract

DISCUSSION

In the modern era, with the prevalence of malignancies and HIV infection, there has also been a sharp increase in opportunistic infections like *Candida albicans*; in fact, *Candida albicans* now causes 5-10% of all bloodstream infections [5]. Hydrolytic enzymes like lipases, and secreted aspartic proteinases (SAP) and also lipase play a major role in the virulence of *Candida albicans* since they also help in destroying cell membrane of host system [6]. This inhibition of virulence traits can be helpful in treating Candidiasis, since anti-virulence therapy is also sometimes helpful in eradicating or mitigating infections in the face of high drug resistance. Our studies show that the extracts showed inhibitory property even after preparing by autoclaving. So they can be helpful in febrile states. Also they were safe to human cells. A study from Malaysia shows the inhibitory effect of Piper betel (paan) aqueous extract on *Candida albicans* biofilm, and also proves that it possibly does so by inhibiting the expression of Hyphal wall protein (HWP) which is needed for optimum biofilm formation by this pathogen [7]. Paan (betel) is frequently consumed in our country and it should be interesting to see the effect of paan extract on oral candidiasis, which is mainly caused by *Candida albicans*, since studies suggest that chewing betel leaf can have deleterious effect on normal oral microbiota also [8]. One drawback of our study was that it was done with 5 isolates only. More studies with more clinical isolates are needed. Such studies have not been carried out earlier in our country with simple aqueous extracts of these isolates, and should also pave the way for further research in this area.

CONCLUSION

Betel leaf extract inhibits the growth and biofilm formation in *Candida albicans* effectively. Same thing is not observed with Lemon extract. This can be the topic of further studies.

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