International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 4, Issue 3, 2013, pp 319-323
http://www.bipublication.com

STUDY OF ANTIFUNGAL ACTIVITY OF BORIC ACID ON VAGINAL PATHOGENS

Saindane Dinesh, Pabal Ajit, Purane Madhav, Patil Chetan and Pandit Prachi

Department of Biotechnology, Padmashree Institute of Management and Sciences,
Kommagatta, Kengeri, Banglore-560060, India.
Corresponding author: E-mail: ajpabal@gmail.com, saindane.dinesh60@gmail.com
Tel:09902165724,08197097207

[Received-20/05/2013, Accepted-12/07/2013]

ABSTRACT:

Boric acid is naturally occurring compound containing the elements boron, oxygen, and hydrogen (H3BO3). In nature, the element boron does not exist by itself. Boron is combined with other common elements, such as sodium to make salts like borax and with oxygen to make boric acid. The main cause of vaginal diseases is Yeasts. Yeast is a fungus scientifically referred to as *Candida*. The specific type of fungus most commonly responsible for vaginitis is *Candida albicans*. The Fungal strains used to assess the study *are Candida species, Candida albicans, Candida parapsilosis, Candida tropicalis.* The method used for investigate the boric acid effect is of agar well diffusion. The zone of inhibition was measured and calculated. The zone of inhibition observed for the three strains varies for different concentration. The maximum zone of inhibition for candida albicans was 2.5 cm or 25 mm for 3 mg in 1ml dilution was observed in *candida albicans* compared to the other strains. Supporting to the results obtained we aim to further screen the pathogens study there biochemical and characteristic properties of boric acid and work on the dilutions and its composition to prepare a suitable and effective remedy against the pathogens responsible for vaginal infections because Boric Acid treatment mainly causes cells to form irregular septa and leads to the synthesis of irregular cell wall protuberances that extend far into the cytoplasm.

Keywords: Boric acid, *Candida species, Candida albicans, Candida parapsilosis, Candida tropicalis,* Agar well diffusion, Zone of inhibition,

[I] INTRODUCTION

In the right amounts, boron is an essential nutrient for animals, plants & fungi. However, at high concentration boric acid (BA) become an effective poison that is widely used for the killing of diverse organisms ranging from bacteria to rodents. In medicine, boric acid is used as an alternative treatment for vaginal yeast infection. While the molecular details of boric acid action on yeast remain unclear, it was recently shown that boric acid interferes with morphogenesis, to the effect that it inhibits the transition from yeast to the hyphal from the pathogenic yeast C. Albicans. Because the ability to switch to hyphal growth is an

important virulence factor in C. Albicans, suppression of such elongated growth by boric acid may in part explain its therapeutic effect. The present study was undertaken to assess the effect of boric acid on morphogenesis and cell wall synthesis in yeast, using the well established organism Saccharomyces cerevisiae as a study of subject. In Saccharomyces cerevisiae, morphogenesis and cell wall synthesis depends on the current assembly of cytoskeletal proteins. To guide cell wall synthesis during cytokinesis, a ring of septin filaments form during the G1 phase of the cell cycle and is subsequently completed into a contractile actomysinring (CAR) by the addition of the myosin and actin among other proteins. To complete abscission, the cells first separate mother and daughter cells with a chitin primary septum. The deposition of glucan and mannoprotein-rich cell wall material on the mother and daughter side of the primary septum later completes the trilaminar septum that can be observed under normal culture conditions. A disturbance in the assembly of the septation apparatus, the cohesive functional unit that constructs the primary septum leads to the formation of highly aberrant septa.

The septa formed under these conditions do not allow for the separation of cells after cytokinesis, leading to the formation of chain and clumps of misshaped cells. Based on the observation that Boric Acid causes such clumping and chain formation in S. cerevisiae, the present study was initiated to assess the influence of Boric Acid on the function of the septation apparatus. Boric acid is naturally occurring compound containing the elements boron, oxygen & hydrogen. In nature, the element boron does not exist by itself. Boron is combined with other common elements, such as sodium to make salts like borax & with oxygen to make boric acid. Borates have been used for thousands of years in china and Middle Eastern country. In those areas, borates have been commonly used as a food preservative, cleaning agents, and as an antiseptic. Boric Acid is a mild antiseptic as well as the mild acid that inhibits the growth of microorganisms on the external surface of the body. It is completely used in contact lens solutions, eye disinfectants, vaginal remedies, baby powder, anti-aging preparations and similar external applications.

Boric Acid has been studied in the treatment of VVC. Although it is not commercially available, boric acid is the alternative to the antifungal agents. For each organism, there is an optimal boric acid concentration. Too little boric acid causes symptoms of deficiency while too much boric acid has poorly defined cytotoxic effect. The typical dose of boric acid is 600 mg intravaginally per night for 14 consecutive nights. Boric acid can be used as an antiseptic for minor burns or cuts and is sometimes used in dressing or salves. As an antibacterial compound, boric acid can also be used as an acne treatment. It is also used as prevention of athlete's foot, by inserting powder in socks or stockings.The main cause of vaginal disease is yeasts. Yeast is the fungus scientifically referred as Candida. The specific type fungus most commonly responsible for virginities is Candida Albicans. Yeast is a commonly responsible for virginities is Candida Albicans. The most common form of vaginal infection is called bacterial vaginosis. Women with this infection have large number of organism called Gardnerella Vaginalis, as well as many other organisms, in their vagina. It is considered to be a sexually transmitted infection.

A yeast infection, or Candida virginities, is another common type of vaginal infection. If this is present, there may be larger amounts of thick, white discharge. Or no discharge at all. Other common symptoms include itching, swelling, irritation or redness the vaginal area. The microorganism involved in BV is very diverse, but include Gardnerella Vaginalis, Mobiluncus, Bacteroids & Mycoplasma. A change in normal bacterial flora including the reduction of lactobacillus which may be due to

the use of antibiotics or pH imbalance, allows more resistant bacteria to gain a foot hold and multiply. BV is caused by an imbalance of naturally occurring bacterial flora and is often confused with yeast infection (candidacies), or infection with Trichomonas Vaginalis (trichomoniasis), which are not caused by bacteria. Yeast infection in the vagina, caused by candida albicans, can be treated with medicated suppositories whereas skin yeast infections are treated with medicated ointments. A healthy vagina normally contains many microorganisms; some of the common ones are Lactobacillus crispatus and Lactobacillus jensenii.

[II] MATERIAL AND METHODS:

2.1 Fungal strains:
Candida species
Candida albicans
Candida parapsilosis
Candida tropicalis
2.2 Sub culturing
The cultures were obtained from the National Chemical Laboratory, Pune. The cultures were in the slants, we subcultures the strain in the petri plates by spread plate technique. Media required growing the candida species is Potato Dextrose Agar. Potato Dextrose Agar used only for the growth of fungus. Then the plates placed for incubation for 3-4 days in incubator at 37°C. Generally the fungus takes 48-72 hours to grow.
2.3Control
The control was taken from the market product. Ointments which are used to prevent or control the vaginal disease or vaginal pathogens are taken as control to compare the zone of inhibition or the inhibition activity of the boric acid with it. The product used as Control is of Qupiderm ointment and this product mainly contains the chemical such as Gentamicin, Betamethasone Dipropionate, Tolftate, chlorocresol, chlrohydroxyquinolone respectively.

2.4 **Dilutions:**
The different dilutions were made with boric acid. As per the market product used for the vaginal disease they have some side effects due to their contents concentrations. The boric acid can be used atdifferent concentration for the vaginal disease. If the boric acid can be used in optimum concentration the side effects of boric acid according to other market ointments can be less. To estimate the concentration of Boric Acid to prevent the growth of candida spp. With less or no side effects we had make different dilutions. The dilutions were made in the sodium chloride was taken for the dilution. The dilutions were made by using micropipettes in the 0.5% saline solution.
The following dilutions were made-

Table No.01:

Boric Acid	**Doubled sterilized distilled water**
1 mg	1 ml
2 mg	1 ml
3 mg	1 ml

2.5 **Antifungal Activity**

To carry out the antifungal activity of Boric Acid against the vaginal pathogens (candida spp.) following steps was followed: - The pathogens were isolated, cultured and screened. The pathogen was preserved on the selective media (PDA) and incubates them. Study morphological characteristics and biochemical test was done for the pathogens. A dilution for the sample was made to proceed for the test. The agar well diffusion method was followed for the antifungal activity under aseptic conditions. The zone of inhibition was observed and calculated it in mm.
Procedure: To carry out the zone of inhibition for above experiments following method was followed, Agar well diffusionMaterials: Culture of organism, Solvent (Salt solution), pipette, cork borer, spreader & PDA plate.

Method:

The inoculums were prepared in aseptic conditions.4mm holes were punched in the agar using sterilized cork borer on the PDA (Potato Dextrose Agar) plate aseptically. The Petri plates were marked to label the plate by using wax. The organism were sprayed aseptically onto the PDA plate & the plates were stand for 5 minutes. 1, 2, 3 drops of filtered growth was poured in appropriate wells of medium. The plates were incubated at 35°C for 24 hours. The zone of inhibition in mm was measured using the ruler on undesirable part of the plate. The zone of inhibition size was recorded and all cultures were discarded in the autoclaved.

2.6 Observation:

To obtain the perfect zone of inhibition on petri plate we had done following steps:-First we made a 0.5% saline solution in the test tubes. Then we have taken a loop full of candida strain and put it in the saline solution. And then mix it properly in the saline solution by vortexing. Then the petri plates were made of having P D Agar media for the growth of fungus. Then with the help of micropipette pour the 0.5% saline solution (containing the candida strain) and spread with the spreader. Then with the help of cork borer wells were made in the middle of petri plates. Then in each plate the different boric acid dilutions are poured with the help of micropipette. The experiment was performed in triplicates. But to check the effects of boric acid against the candida spp. we made additional petri plates (2 petri plates for each strain), in that we directly put a pinch of boric acid in the well. Then the both plates were kept for incubation at 37 c in the incubator for 3-4 days. Zone of inhibition was observed in agar plates. Zone of inhibition of different strains varies in each concentration of boric acid. For the candida albicans the zone of inhibition is larger than the other strains because different strains of candida species show different response to the boric acid. The zone of inhibition of control was also observed to check and compare the zone of inhibition of control and sample i.e. Boric Acid.

[III] RESULTS

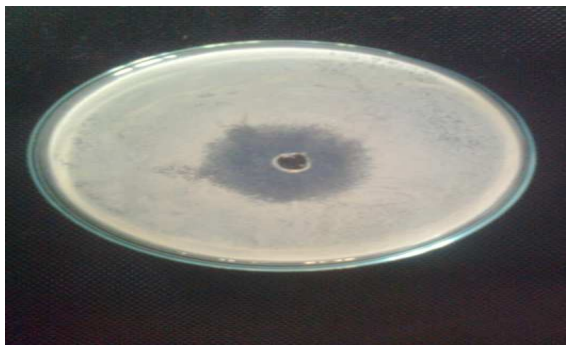

Fig.No.1. Zone of inhibition observed in candida albicans of 1 mg in 1ml concentration.

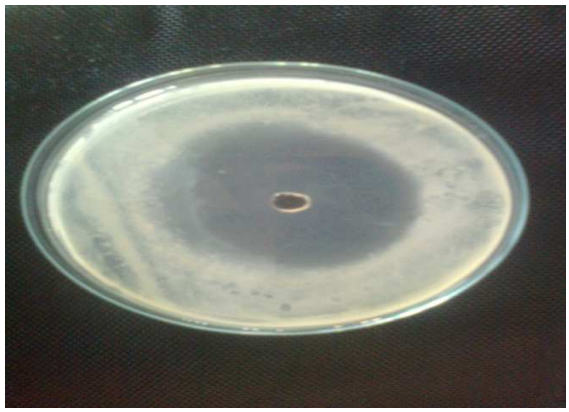

Fig.No.2. Zone of inhibition observed in candida albicans of 3 mg in 1 ml concentration

The zone of inhibition observed for the three strains varies for different concentration. The maximum zone of inhibition for candida albicans was 2.5 cm or 25 mm for 3 mg in 1ml dilution was observed in *candida albicans* compared to the other strains.

Zone of Inhibitions

Table No.02

SR NO.	STRAINS	ZONE OF INHIBITION					
		1mg		2mg		3mg	
		cm	mm	cm	mm	cm	mm
1.	Candida Albicans	1.2	12	2.1	21	2.5	25
2.	Candida Parapsilosis	0.8	8	2.2	22	1.8	18
3.	Candida Tropicalis	0.6	6	1.8	18	1.9	19
4.	Control (Qupiderm)	1.1	11	1.9	19	2.1	21

Graphical representation:

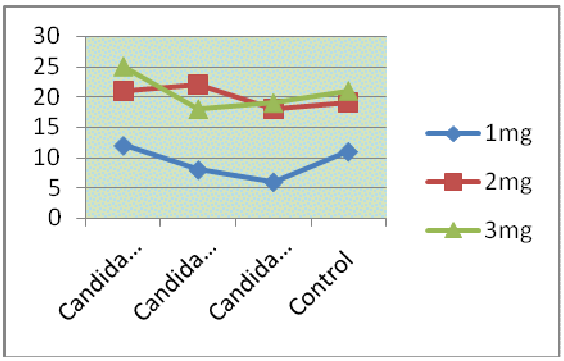

Fig.No.03 Comparison between zone of inhibition.

According to the observation of zone of inhibition in plates of each strain for each concentration the graph is plotted. After studying the graph we can say that the candida albicans shows the maximum zone of inhibition for 3 mg concentration. Compare to the other strains and control the candida albicans plate shows highest growth of inhibition.

[IV] CONCLUSION

As per the review done on this topic and the results obtained in the present study it gives an idea about the activity of Boric acid against the pathogen candida albicans. It can be concluded that natural components like Boric acid can be beneficial against vaginal diseases.

[V] FUTURE ASPECT

Supporting to the results obtained I aim to further screen the pathogens study there biochemical and characteristic property further study the properties of boric acid and work on the dilutions and its composition to prepare a suitable and effective remedy against the pathogens

[VI] ACKNOWLEDGEMENTS

The authors are sincerely grateful to the management of Padmashree Institute of management and science, Bangalore-60 for their valuable support and encouragement in this study.

[VII] REFERNCES

[1] A. Fleischer, C. Titel, and R. Ehwald, “The boron requirement and cell wall properties of growing and stationary suspension-cultured *Chenopodium album L* cells,” *Plant Physiology*, vol. 117, no. 4, pp. 1401–1410, 1998.

[2] A. Bennett, R. I. Rowe, N. Soch, and C. D. Eckhert, “Boron stimulates yeast (*Saccharomyces cerevisiae*) growth,” *Journal of Nutrition*, vol. 129, no. 12, pp. 2236–2238, 1999.

[3] E. Siegel and S. Wason, “Boric acid toxicity,” *Pediatric Clinics of North America*, vol. 33, no. 2, pp. 363–367, 1986.

[4] F. De Seta, M. Schmidt, B. Vu, M. Essmann, and B. Larsen, “Antifungal mechanisms supporting boric acid therapy of Candida vaginitis” *Journal of Antimicrobial Chemotherapy*, vol. 63, no. 2, pp. 325–336, 2009.

[5] G. Lesage and H. Bussey, “Cell wall assembly in *Saccharomyces cerevisiae*” *Microbiology and Molecular Biology Reviews*, vol. 70, no. 2, pp. 317–343, 2006.

[6] J. B. Moseley and B. L. Goode, “The yeast actin cytoskeleton: from cellular function to biochemical mechanism,” *Microbiology and Molecular Biology Reviews*, vol. 70, no. 3, pp. 605–645, 2006.

[7]J. D. Sobel, G. Muller, and H. R. Buckley, “Critical role of germ tube formation in the pathogenesis of candidal vaginitis,” *Infection and Immunity*, vol. 44, no. 3, pp. 576–580, 1984.

[8] M. Benderdour, T. Bui-Van, A. Dicko, and F. Belleville, “In vivo and in vitro effects of boron and boronated compounds,” *Journal of Trace Elements in Medicine and Biology*, vol. 12, no. 1, pp. 2–7, 1998.

[9] M. Schmidt, B. Bowers, A. Varma, D. H. Roh, and E. Cabib, “In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other,” *Journal of Cell Science*, vol. 115, no. 2, pp. 293–302, 2002.

[10] S. M. Prutting and J. D. Cerveny, “Boric acid vaginal suppositories: a brief review,” *Infectious Diseases in Obstetrics and Gynecology*, vol. 6, no. 4, pp. 191–194, 1998.