Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [4] 2022 : 670-674

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and evaluation Of Crude Extract of *Streptomyces diasticus* in Mouthwash and Tooth Paste against *Candida albicans*

S. Seema*[1],Beema Shafreen[2], Premjanu N[3], R. Ramya[1] and Rita Gupta[4]
1) Department of Biotechnology, Dhanalakshmi Srinivasan College of Arts and Science for Women(A),Perambalur-621212.
2) Department of Biotechnology,Alagappa University, Karaikudi
3)Department of Biochemistry, Dental College and Hospital, Sathyabama University, Chennai
[4]Sr. Lecturer, Babu Banarasi Das University, Lucknow, U.P., INDIA)
Email: seemasiddharthan@gmail.com

ABSTRACT

Formation of dental biofilm is a natural, complex process which involves the association of more than 500 bacterial species. These microorganisms are encased in an extracellular polysaccharide matrix on the surface of the teeth. They are responsible for tooth ache, caries, gingivitis, and periodontal diseases. Hence prevention of various dental disease can be achieved by reducing the formation of dental plaque by regular brushing and usage of mouthwashes. Microorganisms involved in biofilm formation have developed resistance towards synthetic antibiotics and the host immune system. To circumvent this problem secondary metabolite produced by actinomycetes with antibiofilm properties have been utilized. The crude extract which showed the highest activity for inhibition of biofilm formation were extracted from streptomyces diasticus. These extracts were utilized in formulation of mouthwash and toothpaste. Further, they were evaluated for antibiofilm activity against Candida albicans which showed promising result in a dose dependent manner. Thus, the bioactive crude extracts from streptomyces diasticus has a high potential to be used in treating biofilm-related infection and further research is necessary to purify the bioactive compound from the crude extract which has antibiofilm activity against Candida albicans.

Keywords: *Streptomyces diasticus, antibiofilm, Mouthwash, Tooth paste, C.albicans*

Received 04.10.2022 Revised 12.11.2022 Accepted 28.12.2022

INTRODUCTION

Candida albicans(*C. albicans*) is a polymorphic yeast that can exist as normal microbiota of mucosal oral cavity, gastrointestinal and genitourinary tracts in healthy individuals or as an opportunistic pathogens in immunocompromised persons. The virulence of Candida species is associated with ability to form biofilm, dodge host defense, and produce various hydrolytic enzymes such as phospholipases, hemolysin and proteases, which have a tissue damaging effect [1]. Moreover *C. albicans* exhibits dimorphic nature of growth, that is it has the capability of switching from yeast morphology to virulent hyphal morphology. The conversion of yeast morphology to a virulent hyphal morphology is triggered by several factors such as high temperatures (37°C), neutral pH, mammalian serum etc. This virulent hyphal morphology is responsible for biofilm formation and plays a important role during infection [2,3]. These biofilm are formed due to the interaction of various microorganisms with dietary sugar and other food substances in the oral cavity. To maintain healthy mouth it is essential to prevent and remove plaque, which is responsible for various dental diseases. To remove plague various mechanical methods are utilized such as toothbrushes (manual or electric), along with tooth paste, floss, mouthwashes, wood sticks and interdental brushes [4]. A toothpaste is a gel like or a semi-solid substances which is utilized along with toothbrush and plays a significant role in removing plaque. Basically all the tooth paste should contain, abrasive, water, humectants, detergent, sweetener, colour or preservative, thickening, therapeutic and flavouring agent. Various commercial tooth pastes are available which differ in their composition of the basic constituents and the efficacy depends on their chemical composition[5]. Similarly, mouthwash is a non-sterile aqueous alcoholic solution which acts as preservative and a semi-active ingredient. It augments the usage of toothpaste for maintaining oral health. Mainly it is used for the reduction of oral bacteria, removal of food particles, temporarily diminises bad breath and provide a pleasant taste An ideal mouthwash should have these desirable properties. They should not cause physical or chemical harm, decalcification, or interfere with the activity of the saliva. Moreover it should not be toxic or have bad odor or taste. On the other hand it should have the ability to create humid environment and

lubricating effect apart from aiding in the degradation of the food debris[6] Hence researchers have inclined towards nature for obtaining natural compounds either from plants or microorganism as they produce them as secondary metabolite. These secondary metabolites have, antifungal, antibacterial, antiviral, anti-inflammatory and anti-biofilm properties. Actinomycetes are a microorganism which has characteristic features in between fungi and bacteria. About 70% of the antibiotic available commercially is contributed by actinomycetes. Out of which Streptomyces sp is the major contributer. In this context as a search for new therapeutic mouthwash and tooth paste actinomycetes namely *Streptomyces diasticus* is used for this study. The aim of this study was to formulate and evaluate the efficacy of *Streptomyces diasticus* extract in the reduction of dental biofilm. This in turn may lead to possibility of finding a new type of formulation for use in oral hygiene that may contribute to prevent dental plaque and biofilm formation.

MATERIAL AND METHODS

Tooth Paste and Mouthwash solutions:

Mouthwash solution and tooth paste were formulated. Table 1 shows the ingredients, and pH of the mouthwash solution and tooth paste.

S.N0	Categories	Ingredients	pH
1	**Mouth wash**	Sodium chloride, Distilled water, Crude extract	7.0
2	**Tooth paste**	Calcium carbonate, saccharin sodium, camphor, hard soap, glycerin, crude extract	7.0

Culture Inoculum:

Clinical isolate of *C. albicans* which forms substantial biofilm was selected for this study. The strain was procured from the Sathyabama Dental College and Hospital, Chennai. The strain was maintained in Potato Dextrose (PD) agar (HiMedia) plates. The yeast cells suspension containing 10^{-7} cells mL^{-1} in 0.1 M phosphate buffer saline were prepared according to the previous methods [7] and 10 µl of yeast cells suspension was used for further assays.

Biofilm Inhibition assay:

Streptomyces diasticus extract of varying concentration ranging from 10 µg to 30 µg were added to the PD broth containing the 10^{-7} cells mL^{-1} of *C. albicans* and their biofilm inhibitory concentration (BIC) were determined. The microtitre plates (24 well) were incubated for 24 h at 37°C. After incubation, the yeast biofilm was stained with 0.4% crystal violet (CV). The wells contacting the crystal violet solution were discarded, washed with MilliQ and dried. Later 1 ml of ethanol was added to the wells and recorded the optical density (OD) using multi-label reader (PerkinElmer, EnSpire, USA) at 570 nm.

Light microscopic observation:

To observe the antibiofilm activity of *Streptomyces diasticus*, *C. albicans* were grown on glass slides (1 X 1 cm). The slides were kept inside the wells of the microtitre plates containing PD broth supplemented with BIC of mouthwash .The wells without mouthwash served as the control and subsequently the microtitre plates were incubated for 72 h without shaking at 37°C. After incubation the slides were removed and rinsed with MilliQ and used for light microscopic observations [8]. Antibiofilm and ability to inhibit the yeast to hyphal formation by *S. diasticus* extract was revealed through the light microscopy. The control and treated slides were stained with 0.4% crystal violet. The slides were rinsed with deionised water and air dried. The biofilm slides were observed under 400× magnification and the images were captured and processed using light microscope (Lieca, DM2000 software LAS 4.9).

Fluorescence microscopy:

For fluorescence microscopic analysis, working solution of Acridine Orange: Ethidium Bromide (AO:EtBr) was prepared by dissolving 10 µl each of Acridine Orange (AO) 5 mg/ml in 95% ethanol and ethidium bromide (EtBr) 3 mg/ml in absolute ethanol in 10ml of phosphate buffered saline (PBS). After 24h of incubation at 37°C, cells were rinsed thrice with PBS and stained with equivalent mixture of AO:EtBr mixture (1:1 ratio, 10µl). The cells were then visualized under fluorescence microscope.

RESULT AND DISCUSSION:

Biofilm inhibition assay

Biofilm formation was observed in 24 h incubated culture of *C. albicans* in spider medium upon staining with crystal violet. A dose dependent reduction in biofilm formation was observed around the walls of the test tube at (Fig 1A & 1B).This shows that *S. diasticus* crude extract present in toothpaste and mouthwash

exhibited significant antibiofilm activity against *C. albicans* biofilm. The BIC was performed to quantitate the amount of biofilm inhibition by various concentration ranging from 25 to 100 µg/ml and 25µl to 100 µl /ml in the case of tooth paste and mouthwash respectively as shown in(fig 2A and 2B) .The switching of yeast morphology to hyphal morphology is responsible for infection,virulence and biofilm formation [9]. Recently many researchers have reported about the ability to suppress biofilm metabolic activity by various natural and synthetic compounds [10, 11]. Interestingly mouthwash showed better result compared to toothpaste contradictory to the findings reported by Majed M*et al.,* 2013 that biofilm bacteria are less state susceptible to mouth wash solutions [12] .This may be due to the increased solubility, permeability and penetrative capacity of the chemical components present in the extract.

Light Microscopic observation

Light microscopic analysis was performed to visualize the antibiofilm activity of *S. diasticus* extract against *C. albicans* biofilm which were grown on SDA medium. The SDA medium enhances the biofilm formation by *C. albicans*. The mouthwash containing *S. diasticus* extract at 100 µl/ml concentration exhibited good antibiofilm activity as there was less aggregation of cells when compared to control and tooth paste containing the same extract (Fig 2). Tooth paste containing the extract also showed significant reduction in aggregation of cells at the concentration of 100µg/ml but showed less antibiofilm activity compared to mouthwash which is further proved by anti biofilm inhibition assay. The probable reason may be due to the presence of calcium carbonate and glycerol in the tooth paste which might inhibit the solubility of the extract. The results have show that the extracts of *S. diasticus* augurs well as an effective anti-bio film agent since it inhibited hyphal development effectively. Our results were similar to the findings where usnic acid showed inhibition of hyphal development [13].

Fluorescence Microscopic observation:

To investigate the live and dead cells of *C. albicans* biofilm was visualized under Fluorescence Microscopic.*C. albicans* biofilm cells were stained with AO/EtBr, which give green fluorescence in the nuclei of live cells and orange to red fluorescence in the dead cells. Cells exposed to a BIC of mouth wash and tooth paste showed enormous numbers of dead cells compare to control (Fig 3). The above result findings were similar to *In vitro* antifungal activity of equol against *Candida albicans*[14].

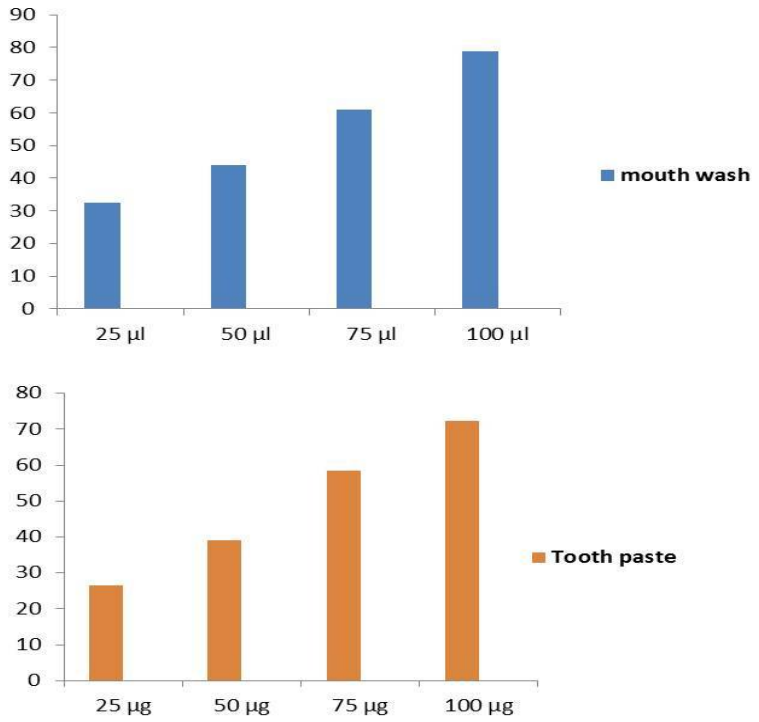

Fig 1: Biofilm inhibition by crystal violet assay (OD 570nm). A) Biofilm inhibition was performed using Mouth wash. B) Biofilm inhibition was performed using Tooth paste.

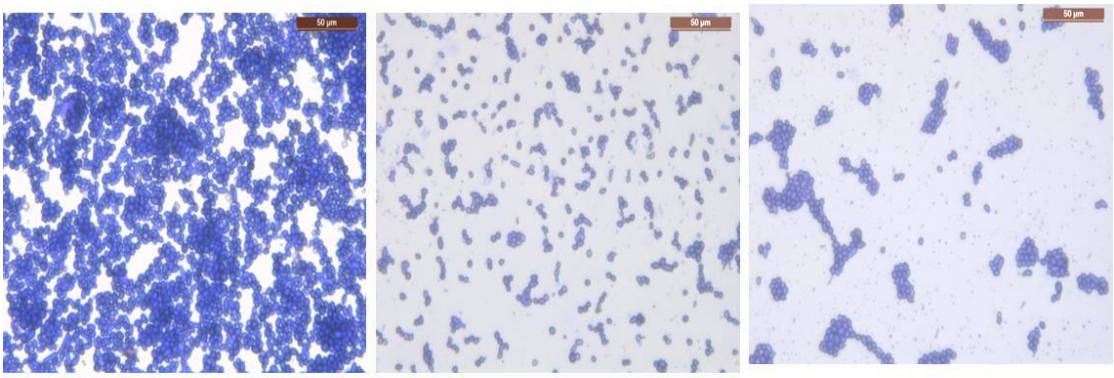

Fig 2: Light Microscopic observation of *C. albicans* biofilm showed the morphology of biofilm

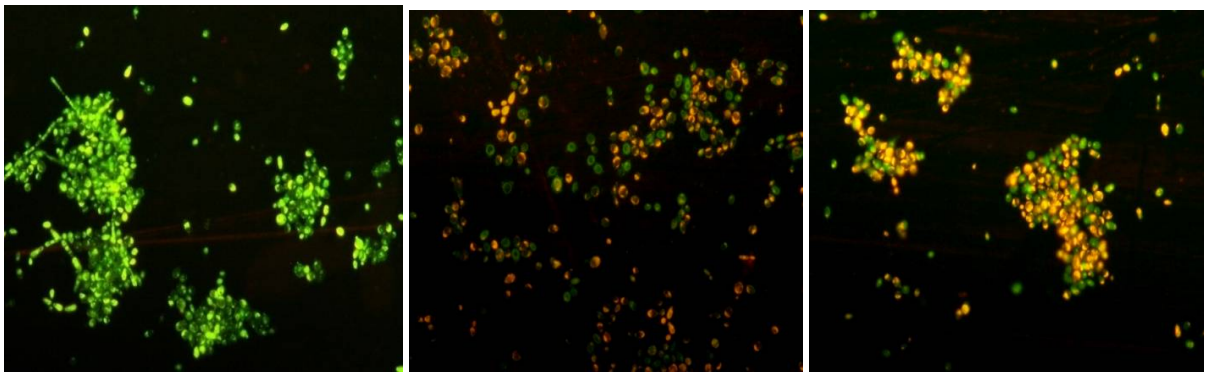

Fig 3: Fluorescence microscopic observation of *C. albicans* cells stained with Acridine orange (AO)/ Ethidium bromide (EtBr). Control cells showed green color indicate live cells, Mouth wash and Toothpaste Treated cells at BIC showed orange red color indicate dead cells.

CONCLUSION

In conclusion the bioactive crude extracts from actinomycetes S*treptomyces diasticus* extract has shown a significant potential in inhibition of *C. albicans* biofilm formation. Hence it may be used in treating various biofilm-related infection. The further research should include identification and purification of bioactive compounds having antibiofilm activity and also the ADMET property of the compound and long term effect on humans.

ACKNOWLEDGEMENT

The authors acknowledge Chancellor Shri A.Srinivasan, Dhanalakshmi Srinivasan Group of Institutions, for the financial support of this work .

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES:

1. Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W., & Azeredo, J. (2011). Adherence and biofilm formation of non-Candida albicans Candida species. Trends in microbiology, 19(5), 241-247.
2. Ramage, G., Saville, S. P., Thomas, D. P., & Lopez-Ribot, J. L. (2005). Candida biofilms: an update. Eukaryotic cell, 4(4), 633-638.
3. Vediyappan, G., Dumontet, V., Pelissier, F., & d'Enfert, C. (2013). Gymnemic acids inhibit hyphal growth and virulence in Candida albicans. PloS one, 8(9), e74189.
4. Ahuja, S., Dodwad, V., Kukreja, B. J., Mehra, P., & Kukreja, P. (2011). A comparative evaluation of efficacy of Punica granatum and chlorhexidine on plaque and gingivitis. Journal of the International Clinical Dental Research Organization, 3(1), 29.

5. Vranic, E., Lacevic, A., Mehmedagic, A., & Uzunovic, A. (2004). Formulation ingredients for toothpastes and mouthwashes. Bosnian journal of basic medical sciences, 4(4), 51.
6. Shin, A. R., & Nam, S. H. (2018). The effects of various mouthwashes on the oral environment change for oral health care. Biomed Res, 29(8), 1724-9.
7. Beema Shafreen, R. M., Selvaraj, C., Singh, S. K., & Karutha Pandian, S. (2014). In silico and in vitro studies of cinnamaldehyde and their derivatives against LuxS in Streptococcus pyogenes: effects on biofilm and virulence genes. Journal of Molecular Recognition, 27(2), 106-116.
8. Nithyanand, P., Shafreen, R. M. B., Muthamil, S., & Pandian, S. K. (2015). Usnic acid inhibits biofilm formation and virulent morphological traits of Candida albicans. Microbiological research, 179, 20-28.
9. Kavanaugh, N. L., Zhang, A. Q., Nobile, C. J., Johnson, A. D., & Ribbeck, K. (2014). Mucins suppress virulence traits of Candida albicans. MBio, 5(6), e01911-14.
10. Tsang, P. W. K., Bandara, H. M. H. N., & Fong, W. P. (2012). Purpurin suppresses Candida albicans biofilm formation and hyphal development. PLoS One, 7(11), e50866.
11. Almangush, A., Bello, I. O., Keski–Säntti, H., Mäkinen, L. K., Kauppila, J. H., Pukkila, M., ... & Salo, T. (2014). Depth of invasion, tumor budding, and worst pattern of invasion: prognostic indicators in early-stage oral tongue cancer. Head & neck, 36(6), 811-818.
12. Masadeh, M. M., Gharaibeh, S. F., Alzoubi, K. H., Al-Azzam, S. I., & Obeidat, W. M. (2013). Antimicrobial activity of common mouthwash solutions on multidrug-resistance bacterial biofilms. Journal of Clinical Medicine Research, 5(5), 389.
13. Nithyanand, P., Beema Shafreen, R. M., Muthamil, S., & Karutha Pandian, S. (2015). Usnic acid, a lichen secondary metabolite inhibits Group A Streptococcus biofilms. Antonie Van Leeuwenhoek, 107(1), 263-272.
14. Naik, L. K., Shetty, P., Prasad, M. K., Karnaker, V. K., Shroff, S. E., & Madathil, L. P. (2016). Fluorescence of Candida in diagnosis of oral candidiasis. Indian Journal of Dental Research, 27(6), 618.

CITATION OF THIS ARTICLE

S. Seema,Beema Shafreen, Premjanu N, R. Ramya and Rita Gupta: Formulation and evaluation Of Crude Extract Of *Streptomyces diasticus* In Mouthwash And Tooth Paste Against *Candida albicans.* Bull. Env. Pharmacol. Life Sci., Spl Issue [5]: 2022: 670-674.