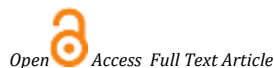


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Research Article

## Multi-Functional Therapeutic Active Extract of Marine *Asterias rubens* against Tooth Decay Pathogen

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### Abstract



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Today, childhood caries is main health related disease it affects many schooling children in many countries especially developed by lactic acid bacteria. So in this present the isolating the 50 lactic acid bacteria from different decay affected children's in Fen Dental Clinic, Tirupur Dt. The emerging bacteria LAB confirmed by phenotype and genotype characterization. After, the pathogen screened for the production of biofilm for to check the resistant mechanism of the *Lactobacillus acidophilus*. In this study the MTLA1-50 isolates produced strong biofilm were confirmed for the *Lactobacillus acidophilus* carrying resistant capability. In particularly, the maximum strong biofilm producing strains MTLA32 and MTLA50 followed by other isolates. Due to pre-dominant resistant activity immediately need innovative drug to eliminate biofilm. Currently star fish extract play a vital role in eliminating the bacterial biofilm for this motive in this study chosen the star fish extract in the concentration of 50, 100, 150µl was performed and executed by well diffusion techniques. The results showed the strain No. MTLA32 and MTLA 50 got the maximum zone of inhibition at 150µl (52mm and 38mm) of extract. Hence this research paper proved the star fish extract has a powerful anti-therapeutic medicine mechanism to treat the dental pathogen.

**Keywords:** Star Fish Extract, Dental Caries, Biofilm Production.

## 1. INTRODUCTION

Human plaque is a prevalent disease in the world. A universal, 36% of the population affected approximately and have decay in their permanent teeth. WHO estimates that virtually all human beings have tooth decay at some point in their lifetimes. Infant teeth it distress about 9% of the residents <sup>1</sup>. In latest year dental disease are most common in human life <sup>2</sup>.

The main reason the formation of tooth decay defines destruction of oral tissues by lactic acid from bacterial fermentation of carbohydrates and in the caries development *Streptococcus* spp. and *Lactobacillus* spp. are major role in establishment of tooth decay <sup>3</sup>. Sugar-added fast has been connected with improved risk of developing tooth decay <sup>4</sup>.

In ancient times, the development of tooth decay mainly exhibited by primary microbe especially *Lactobacillus* spp. <sup>5</sup> and the pathogen appear throughout the foremost years of infant's life involved in root caries and periodontal diseases <sup>6</sup>. Now, resistance of antibiotic drug significantly enhanced in the up to date years and is posturing an ever rising curative problem <sup>7</sup>.

Currently, some anti-drugs prescribed for the treatment of plaque disease. But the distress pathogenic dental microbe fully oppose towards presently updated antibiotic medicine.

One of the techniques to decrease the drug resistant is by using antibiotic resistant inhibitors from starfish *Asterias Rubens* in the way of extract containing therapeutic compound. It is expected that starfish extracts eliminate the target sites of the emerging dental pathogen because of antibacterial potential ability of the phylum Echinodermata - *Asterias rubens* (Star fish) was detected from numerous tissues in the coelomocyte and body wall extracts of star fish <sup>8</sup>. So hence the study highlighted to point out the marine echinoderms especially are prospective sources for innovative types of derivation of new pharmaceutical drug and the research revealed the extraction of therapeutic compound from star fish proved the excellent activity to breakdown the biofilm formation from the childhood cariogenic emerging pathogen in dental caries this will be treatment for the caries infection in future.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plaque Sample

In this study childhood decay samples were collected from 50 patients attending the outdoor patients in Fen Dental Clinic, Tirupur District using sterile forceps. In this research 10% concentration of NaCl saline is used as a transport medium for enumerating the lactic acid bacteria.

## 2.2 Isolation of bacteria

After collection of dental sample, the decay sample serially diluted with peptone broth for minimizing bacterial growth for extracting 0.1ml culture plated over the Man Rogosa Sharpe agar for isolating child major dental pathogen *Lactobacillus acidophilus*.

## 2.3 Identification of Bacterial Isolates

In this research, all plaque pathogenic isolates identified by various biochemical character and 16Sr-RNA gene sequencing method.

## 2.4 Biofilm Assay

The decay isolates were grown on nutrient broth with and without 5% glucose, incubated overnight in microtiter plates at 37°C. End of incubation, the micro titer plate finally treat with crystal violet solution for exactly verify the ring of dental biofilm as visible as purple ring over the microtiter plate. The clinical cariogenic isolate showing maximum biofilm formation was chosen for antibacterial activity.

## 2.5 Collection of Star Fish

Sea star - *Asterias Rubens* specimen were collected from Mandapam Coast, Chinnapalam - Thoppukadu Theevu, Rameshwaram Dt. The specimen were cleaned with sea water at the sampling site of Thoppukadu to remove sediments and contamination, then it was put in sterile polythene bags and stored in ice box suddenly transported to the laboratory for isolation of bio-medically active extract for treatment of dental pathogen.

## 2.6 Preparation of the crude extracts:

The samples of star fish *Asterias Rubens* were grinded by mortar and pestle instrument. Then, the extracts were filtered using Whatman No. 1 filter paper and it was mixed with dimethyl sulphoxide solution (10ml). In this study the antagonistic activity of starfish with DMSO extract was screened against dental pathogen.

## 2.7 Antibacterial bioassay

Pharmaceutical activity of 50, 100 and 150µl concentration of starfish extract was performed by well assay against lactic acid

bacteria and the results were detected by measuring the diameter of inhibition zone around the dental bacterial colony and the measurement expressed in mm in diameter.

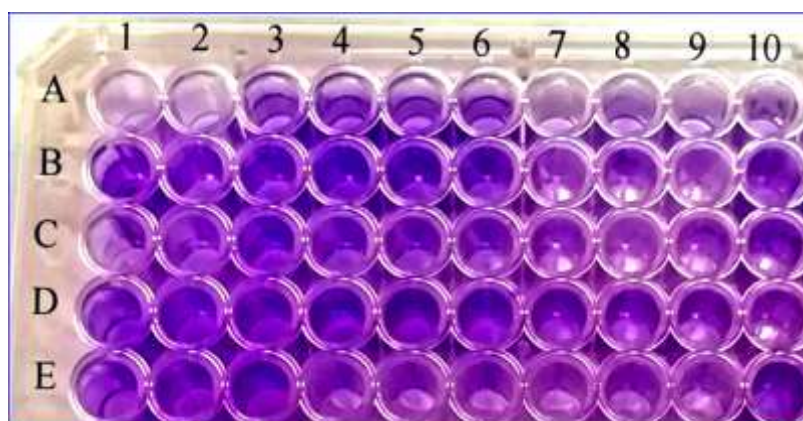
## RESULTS AND DISCUSSION

Totally, 50 *L. acidophilus* lactic acid acidic strains isolated from 50 dental samples identified by biochemical, biofilm production and 16SrRNA Gene Sequencing.

In this study screening of cariogenic dental biofilm was analyzed with 5% concentration of glucose in the nutrient medium at 37°C. The micro titer plates filed with 5% concentration of glucose concentrated medium with cariogenic grown culture inoculated in each well. After incubation the plates analyzed for the production of biofilm around the well of micro titer plate. In this present confirmed all microbial colony ability to produce the biofilm in 5% concentration glucose. Among 50 isolates the utmost strong effect of biofilm was observed in the strain no. MTLA32, MTLA50 followed by the strain no. MTLA2, MTLA3, MTLA4, MTLA5, MTLA6, MTLA7, MTLA8, MTLA9, MTLA10, MTLA11, MTLA12, MTLA13, MTLA14, MTLA15, MTLA16, MTLA17, MTLA18, MTLA19, MTLA20, MTLA21, MTLA22, MTLA23, MTLA24, MTLA25, MTLA26, MTLA27, MTLA28, MTLA29, MTLA30, MTLA31, MTLA33, MTLA34, MTLA35, MTLA36, MTLA37, MTLA38, MTLA39, MTLA40, MTLA41, MTLA42, MTLA43, MTLA44, MTLA45, MTLA46, MTLA47, MTLA48 and MTLA49 (Fig. 1).

In the present study marine star fish (Fig. 2) collected from Thoppu Kaadu (Fig. 3), Rameshwaram, Mandapam Coastal area with the help of sea shore community peoples and the marine star fish identified at CMFRI.

In this research anti-cariogenic activity of marine star fish *Asterias Rubens* extract was performed by well assay against dental acidic pathogen. Result indicated that this study the utmost zone of inhibition 32mm, 48mm and 52mm was observed in strain no. MTLA32 followed by the zone of inhibition 25mm, 28mm and 38mm was observed in strain no. MTLA50 at the 50, 100 and 150µl concentration of novel star fish extracts (Table: 1; Fig.4).



Row A: MTLA1,2,3,4,5,6,7,8,9,10  
 Row B: MTLA11,12,13,14,15, 16,17,18,19,20  
 Row C: MTLA21,22,23,24,25,26,27,28,29,30  
 Row D: MTLA31,32,33,34,35,36,37,38,39,40  
 Row E: MTLA41,42,43,44,45,46,47,48,49,50

Figure1: Biofilm Production from Dental Pathogen *Lactobacillus acidophilus*



MTLA – MICRO TECH LACTOBACILLUS ACIDOPHILUS

Figure 2: Marine Star Fish



Figure 3: Sample Collection Site with Sea Shore Community People (Thoppu Kaadu Theevu, Rameshwaram)

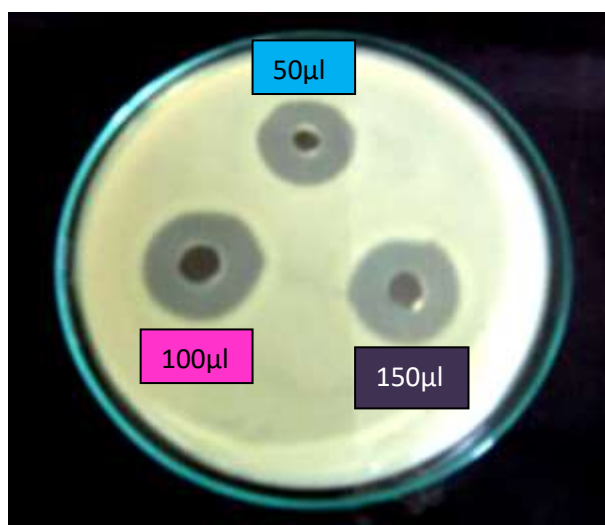


Figure 4: Antibacterial activity of the starfish against biofilm producing *Lactobacillus acidophilus*

**Table 1: Antibacterial activity of the starfish against biofilm producing *Lactobacillus acidophilus***

| S. No | Strain. No | Starfish concentration |       |       |
|-------|------------|------------------------|-------|-------|
|       |            | 50µl                   | 100µl | 150µl |
| 1     | MTLA32     | 32                     | 48    | 52    |
| 2     | MTLA50     | 25                     | 28    | 38    |

In this study examination of cariogenic strain *Lactobacilli* in 50 deep caries samples the strain No. MTLA32 and MTLA50 is predominantly exhibit strong the biofilm by tooth decay isolates. This is due to the presence of acidogenic microbe *Lactobacillus* in the oral cavity it depends on various factors such as the existence of biological niches <sup>9</sup>.

In present research prove the, utmost biofilm creation as induced in the presence of 5% concentration of glucose by the dental pathogen *L. acidophilus*. This result is in agreement by the novelist found that the glycocalyx development and bacterial observance both are improve the strong biofilm creation due to the presence of glucose absorption in bacterial growth media <sup>10</sup>.

Remarkably, Phylum Echinodermata - Marine starfish, have a huge number of therapeutic drug in their body, therefore, our research was to focus on the therapeutic properties of bioactive substances in the form of crude extracts of star fish collected from the Rameshwaram Sea, Thoppukadu Theevu. The medical activity of the extract was done by well assay techniques. In the present investigation, a well-defined antimicrobial activity of sea star crude extracts has been observed beside 2 strong biofilm producing dental strains MTLA32 and MTLA50. However, the values of the antibacterial activity ranged between 25 mm to 52 mm as inhibition zones. The potential ability of the therapeutic activity agreed with the scientist extracted the pharmaceutically active drug from marine sea star *Linckia laevigata* and *Oreaster nodosus* exhibit biomedical activity beside the pathogenic *Escherichia coli* <sup>11</sup>.

## CONCLUSION

The present research concluded that the marine *Asterias Rubens* – star fish extract is drastically effective against tooth decay organism *Lactobacillus acidophilus* at 50, 100 and 150µl. Hence these therapeutic extracts act as gold standard multi functional broad spectrum activity to kill the dental pathogen around the oral surroundings of infants and this paper assure that there is no any side effect at the time of treatment of dental caries in future. So this research paper proved to exhibit various kind of biomedical applications of the phylum Echinodermata make it a viable option for use in all dental emergencies in childhood caries in future.

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## AUTHORS CONTRIBUTIONS

Each author has given considerable and equal contributions to this research

## CONFLICTS OF INTEREST

The authors have no conflict of interest

## AUTHORS FUNDING

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