



EVALUATION OF ANTIMICROBIAL EFFICACY OF FUCOIDAN DERIVATIVES AGAINST ORAL PATHOGENS

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Received 19th May 2021; Revised 18th July 2021; Accepted 10th Aug. 2021; Available online 1st May 2022

<https://doi.org/10.31032/IJBPAS/2022/11.5.6061>

ABSTRACT

Aim: To evaluate the antimicrobial efficacy of fucoidan derivatives against oral pathogens.

Methodology: After characterization of the prepared solutions of ampicillin loaded fucoidan nanoparticles and fucoidan coated metal nanoparticles, the antimicrobial activity of the solutions were assessed against *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans* and *Enterococcus faecalis* by zone inhibition method (agar well diffusion method) and MTT method. MIC of bacterial strains for fucoidan coated metal nanoparticles and fucoidan loaded ampicillin drug was determined according to the broth micro-dilution method.

Results: A statistically significant antimicrobial activity was seen at the MIC of 200 μ L of Fucoidan-chitosan-ampicillin nanoparticles against all the microbial strains tested. Fucoidan-chitosan silver nanoparticles showed antimicrobial activity at the MIC of 100 μ L against *Candida albicans*, *Staphylococcus aureus* and *Streptococcus mutans* and a MIC of 200 μ against *Enterococcus faecalis*.

Conclusion: The study concluded that fucoidan derivatives such as fucoidan-chitosan-ampicillin nanoparticles and fucoidan-chitosan silver nanoparticles can be used as effective antimicrobial agents against oral pathogens.

Keywords: Fucoidan, antimicrobial, oral, pathogens

INTRODUCTION

The human oral cavity is an abode to a variety of micro-organisms. It is comprised of many surfaces, of which each coated with a plethora of bacteria, forming the bacterial biofilm. Some of these bacteria serve as a threat to human beings in the form of oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans.

Several antimicrobial agents have been developed and are available in the market. The focus of interest has been given to marine algae as a new source for bioactive compounds due to their beneficial antimicrobial effects [1, 2]. In terms of antimicrobial agents, various tests have been done globally on marine algae, and various potential candidates, such as algal lectins, bromo-diterpenes, halogenated furanones, phlorotannins, and sesquiterpenes, have been found. Scientists have also proven the antibacterial activity of red, brown, and green algae compounds and their polysaccharides against both Gram positive and Gram negative bacteria [3, 4].

Fucoidan is an anionic sulfated polysaccharide with a rich content of fucose, commonly present in the fibrillar cell walls and intercellular spaces of brown

seaweeds [5]. Though the composition of fucoidan varies among different species of seaweeds, it is basically made up of L-fucose and sulfate with minor quantities of D-galactose, D-mannose, D-xylose and uronic acid [6]. Studies have shown a positive correlation between the sulfates content of fucoidan and its superoxide radical scavenging ability [7].

Nanoparticles (Nanoparticles) have been proposed as valuable carriers for efficiently transporting drugs to the body cells without any phagocytic mechanisms [8, 9]. Nano drug delivery systems can be used for targeted drug delivery at the site of disease or targeting of drugs to a specific site.

These type of drug delivery can improve the drug bioavailability as well as therapeutic efficiency and can also overcome the existing resistance to the drug thereby reducing the harmful effects to the patient [10].

Chitosan (CS) is a cationic polysaccharide derived from chitin by alkaline deacetylation, is widely used as a carrier to improve and control the release of drugs [11]. Chitosan is known for its inherent antimicrobial and anti-biofilm properties. The low cost and ease of chemical

modification makes chitosan suitable for biomedical and pharmaceutical formulations. It has been reported that coupling chitosan with antibiotic increased the efficacy of antibiotics toward bacterial biofilms [12].

Thus incorporating the properties of nanoparticles and chitosan in the capability of drug delivery and taking into account the beneficial properties of fucoidan, chitosan/fucoidan (CS/F) nanoparticles has been developed as carriers for drug delivery systems [13].

MATERIALS AND METHODS

This study was conducted in Yenepoya Research Centre, Mangaluru. Ethical clearance was obtained from the Ethical Review Committee of the Institutional Review Board.

MATERIALS

1. Chemicals:

- a) Fucoidan
- b) Silver nitrate
- c) Chitosan
- d) Sodium triphosphate
- e) Ampicillin
- f) Luria broth

2. **Microbes:** four commonly found oral pathogens- *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* were tested.

METHODS

a. Preparation of chitosan solution :

300 mg of chitosan was added in 100 ml of 1% acetic acid solution.

The mixture was kept for stirring on a magnetic stirrer for an hour to dissolve chitosan completely. Further, the pH of the solution was adjusted to 5.5 by using 1 M sodium hydroxide.

b. Preparation of tetraphenylporphyrin (TPP) solution:

100 mg of TPP reagent was added in 100 ml of distilled water. This mixture was kept on a magnetic stirrer for half an hour and solution obtained was used for further experiments.

c. Preparation of ampicillin loaded fucoidan nanoparticles and silver coated fucoidan nanoparticles:

1 ml of fucoidan (1mg/ml) solution was added with constant stirring to 1 ml of chitosan (3mg/ml) solution in a 100 ml beaker. To this, 0.5 ml of TPP solution was added and further stirring was continued up to 4 hours (**Figure 1**). To develop derivatives of chitosan-fucoidan nanoparticles, the procedure followed as mentioned above and 5 mg of ampicillin drug were added. Next, stirring was continued up to 4 hours. To develop silver nanoparticles containing derivatives of chitosan-fucoidan nanoparticles, the procedure followed as mentioned above, and 5 ml of bramhakamal coated silver

nanoparticles (Silver nanoparticles) were added. Next, stirring was continued up to 4 hours. The developed fucoidan coated silver

nanoparticles and ampicillin loaded nanoparticles were separated through centrifugation and lyophilisation (**Figure 2**).

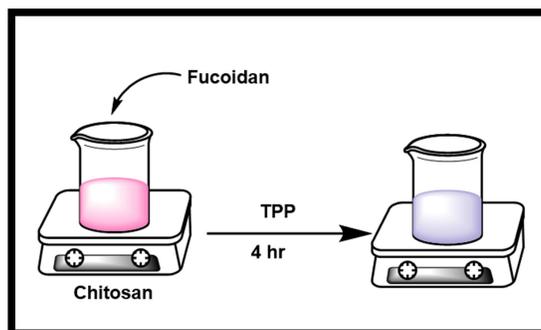


Figure 1: Preparation of fucoidan-chitosan-ampicillin nanoparticles

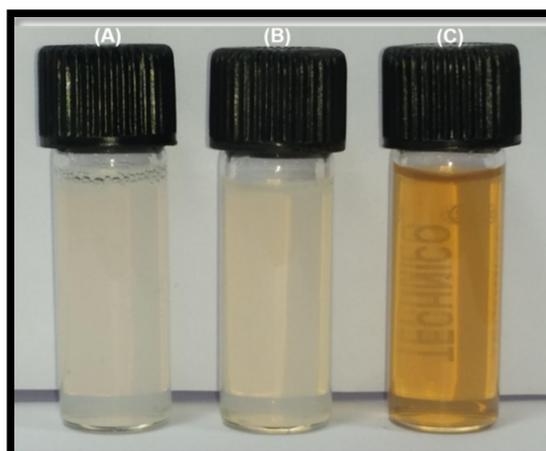


Figure 2: Prepared samples of (a) fucoidan-chitosan nanoparticles, (b) fucoidan-chitosan-ampicillin nanoparticles, (c) fucoidan-chitosan-silver nanoparticles

Characterization of fucoidan coated metal nanoparticles and drug loaded nanoparticles was performed. The following characterizations method was performed to ensure the formation of nanoparticles:-

- UV-Visible spectroscopy
- Fourier Transform Infrared Spectroscopy (FT-IR)
- Thermal gravimetric analysis (TGA)
- X-Ray diffraction (XRD)
- Field emission scanning electron microscopy (FE-SEM)
- Transmission electron microscopy analysis (TEM)
- Electron Dispersion X-ray diffraction (EDX)

Evaluation of antimicrobial activity

Candida albicans, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus mutans* microbial strains were used in the study. Luria broth culture was taken for culturing *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus mutans* microbial strains. Potato dextrose was the media used for culturing *Candida albicans*. The antimicrobial activity of fucoidan coated metal nanoparticles and ampicillin loaded fucoidan nanoparticles was measured by Zone inhibition method (agar well diffusion method) and MTT method. MIC of bacterial strains for fucoidan coated metal nanoparticles and fucoidan loaded ampicillin drug was determined according to the broth micro-dilution method.

STATISTICAL ANALYSIS

All the experiments were performed in triplicate. Data was analysed using Student's t-test at a significance level of $p < 0.05$ and presented as mean \pm standard deviation.

RESULTS

Results of broth-microdilution assay against *Candida albicans*

A concentration-dependent antimicrobial activity was seen in fucoidan-chitosan nanoparticles, fucoidan-chitosan-ampicillin nanoparticles, and fucoidan-chitosan-silver nanoparticles against *Candida albicans* (Graph 1).

The highest growth inhibition was observed at a concentration of 500 μ L. It was seen that fucoidan-chitosan-silver nanoparticles (75.24 \pm 2.91%) showed more growth inhibition as compared to fucoidan-chitosan nanoparticles (21.01 \pm 6.58%) and fucoidan-chitosan-ampicillin nanoparticles (57.76 \pm 1.45%) at the concentration of 500 μ L.

Results of broth-microdilution assay against *Staphylococcus aureus*

A concentration-dependent antimicrobial activity was seen in fucoidan-chitosan nanoparticles, fucoidan-chitosan-ampicillin nanoparticles, and fucoidan chitosan-silver nanoparticles against *Staphylococcus aureus* (Graph 2).

The highest growth inhibition was observed at a concentration of 500 μ L. It was seen that fucoidan chitosan-silver nanoparticles (77.30 \pm 0.34%) showed more growth inhibition as compared to fucoidan-chitosan nanoparticles (38.75 \pm 4.60%) and fucoidan-chitosan-ampicillin nanoparticles (70.98 \pm 1.65%) at the concentration of 500 μ L.

Results of broth-microdilution assay against *Streptococcus mutans*

A concentration-dependent antimicrobial activity was seen in fucoidan-chitosan nanoparticles, fucoidan-chitosan-ampicillin nanoparticles, and Fucoidan chitosan - silver nanoparticles against *Streptococcus mutans* (Graph 3).

The highest growth inhibition was observed at a concentration of 500 μ L. It was seen

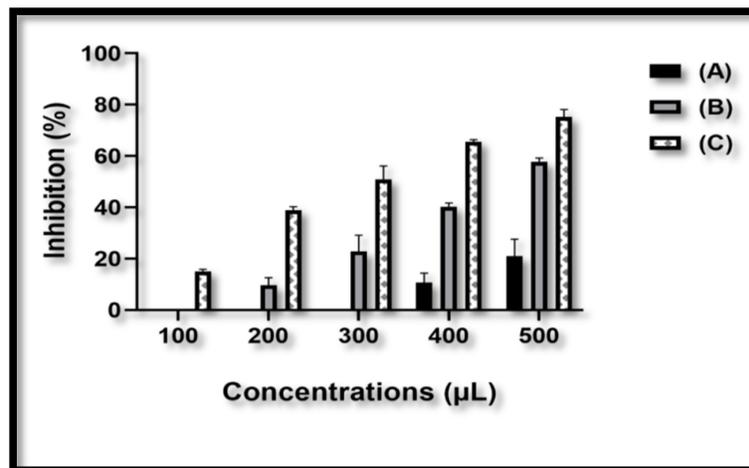
that fucoidan chitosan-silver nanoparticles ($81.49 \pm 1.63\%$) showed more growth inhibition as compared to fucoidan-chitosan nanoparticles ($44.48 \pm 1.06\%$) and fucoidan-chitosan-ampicillin nanoparticles ($65.83 \pm 1.06\%$) at the concentration of 500 μL .

Results of broth-microdilution assay against *enterococcus faecalis*

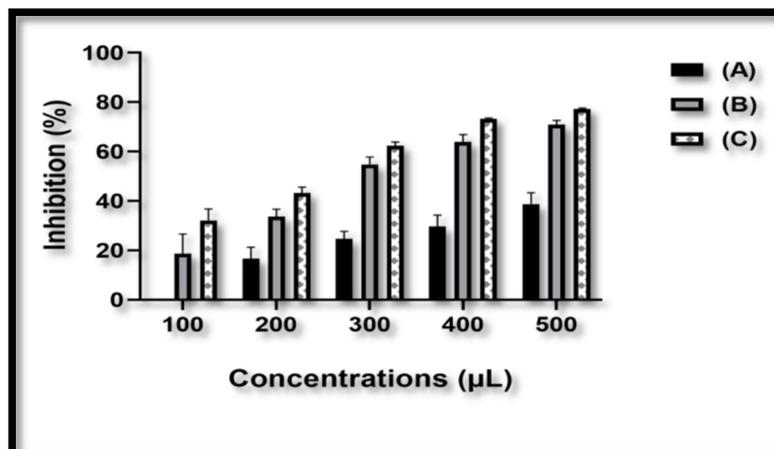
A concentration-dependent antimicrobial activity was seen in Fucoidan-chitosan nanoparticles, Fucoidan-chitosan-ampicillin nanoparticles, and Fucoidan-chitosan silver

nanoparticles against *Streptococcus mutans* (Graph 4).

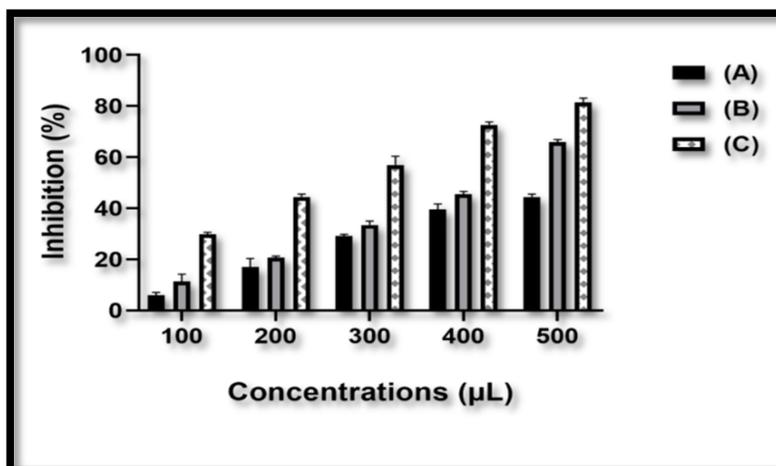
The highest growth inhibition was observed at a concentration of 500 μL . It was seen that Fucoidan-chitosan-silver nanoparticles (92.76 ± 1.63) showed more growth inhibition as compared to Fucoidan-chitosan nanoparticles (61.11 ± 3.11) and Fucoidan-chitosan-ampicillin nanoparticles (91.71 ± 0.14) at the concentration of 500 μL (Table 1).



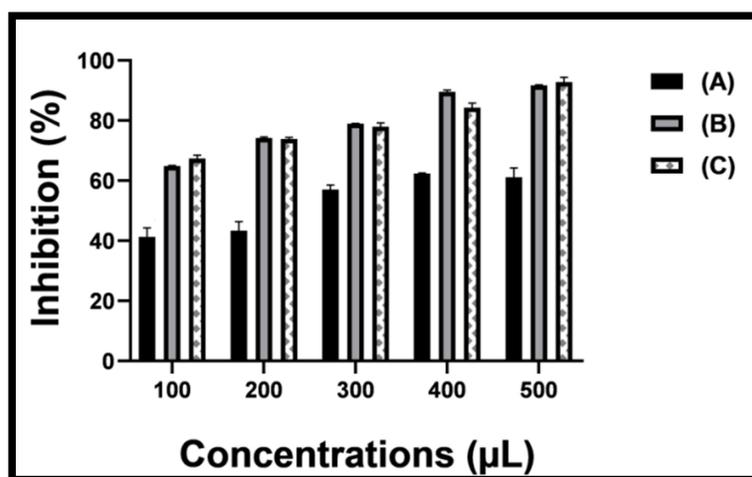
Graph 1: Results of broth-microdilution assay against *Candida albicans* (a) fucoidan-chitosan nanoparticles, (b) fucoidan-chitosan-ampicillin nanoparticles, (c) fucoidan-chitosan-silver nanoparticles



Graph 2: Results of broth-microdilution assay against *Staphylococcus aureus* (a) fucoidan-chitosan nanoparticles, (b) fucoidan-chitosan-ampicillin nanoparticles, (c) fucoidan-chitosan-silver nanoparticles



Graph 3: Results of broth-microdilution assay against streptococcus mutans (a) fucoidan-chitosan nanoparticles, (b) fucoidan-chitosan-ampicillin nanoparticles, (c) fucoidan-chitosan-silver nanoparticles



Graph 4: Results of broth-microdilution assay against enterococcus faecalis (a) fucoidan-chitosan nanoparticles, (b) fucoidan-chitosan-ampicillin nanoparticles, (c) fucoidan-chitosan-silver nanoparticles

Table 1: Minimum inhibitory concentration of fucoidan-chitosan-ampicillin nanoparticles and fucoidan-chitosan-silver nanoparticles

Microbial strains	Minimum Inhibitory Concentration (µL)	
	Fucoidan-chitosan-ampicillin nanoparticles	Fucoidan-chitosan-silver nanoparticles
<i>Candida albicans</i>	200	100
<i>Staphylococcus aureus</i>	200	100
<i>Streptococcus mutans</i>	200	100
<i>Enterococcus faecalis</i>	200	200

DISCUSSION

Fucoidan is a complex sulfated polysaccharide that is found in the cell walls of several edible brown algae such as *Fucus vesiculosus*. Fucoidan is a group of certain fucose-containing sulfated

polysaccharides (FCSPs) that have a backbone built of (1→3)-linked α -L-fucopyranosyl or of alternating (1→3)- and (1→4)-linked α -L-fucopyranosyl residues. Fucoidan can hamper oral inflammation by neutralizing endotoxins released from oral

biofilm and inhibit oral biofilm formation by anti-adhesion activity to tooth surfaces [14].

Lee *et al* has proven the considerable antimicrobial activity of fucoidan against *C. albicans*, *S. mutans*, *P. Gingivalis* and *S.aureus*. Study by Tsubura *et al* showed markedly improvement of recurrent oral herpes labialis on application of fucoidan cream in terms of healing time and time for loss of discomfort [16]. The antimicrobial activity of most of the marine algae has been related to their phenolic compounds and sulphated polysaccharides. These phenolic compounds can lead to the widening of the pores of bacterial membrane, resulting in the loss of intracellular macromolecules such as nucleotides and proteins [17].

Chitosan, a polycationic macromolecule is the N-deacetylated derivative of chitin. It has been confirmed to have inherent antimicrobial properties and also anti-biofilm activities [18]. Zhang *et al* has demonstrated an innovative approach in which chitosan (covalent carrier) was linked with streptomycin (antibiotic) against biofilm bacteria. Conjugation of streptomycin with chitosan has resulted in increased access of the antibiotic into the biofilm and showed that antibiotic covalently linked to carbohydrate carriers can overcome antibiotic resistance of microbial biofilms [12].

In the present study, antimicrobial activity of fucoidan derivatives were tested against the commonly found oral pathogens - *E.faecalis*, *S.mutans*, *S.aureus* and *Candida albicans*. The derivatives tested against the pathogens were ampicillin loaded fucoidan nanoparticles and fucoidan coated metal nanoparticles.

The fucoidan-chitosan nanoparticles group was taken as a control group. It was observed that Fucoidan-chitosan nanoparticles showed a concentration-dependent antimicrobial activity against all the four bacterial strains. The results showed that a concentration of 100 μ L of fucoidan chitosan nanoparticles did not cause any inhibition against the growth of *Candida albicans* and *Staphylococcus aureus*. But the same concentration showed inhibition in both *Streptococcus mutans* (6.04 \pm 1.06) and *Enterococcus faecalis* (41.29 \pm 2.96). At the concentration of 500 μ L, fucoidan chitosan nanoparticles showed the highest growth inhibition against *Enterococcus faecalis* (61.11 \pm 3.11).

Chotigeal *et al* demonstrated the antimicrobial activity of crude fucoidan from *Sargassum polycystum* against *Staphylococcus aureus* at a concentration of 12 mg/ml [19]. Marudhupandi *et al* showed that fucoidan from *Sargassum wightii* exhibited antibacterial activity against the human pathogens such as

Escherichia coli, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Proteus proteus*, *Shigella sonnie*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella sp.* [20] Pierre *et al.*, reported that the sulfated galactan from *Chaetomorpha aerea* has been found to have antimicrobial activity against *Staphylococcus aureus*, *Salmonella enteritidis*, *P. aeruginosa*, *Enterococcus faecalis*, *Bacillus subtilis*, *Micrococcus luteus* and *Candida glabrata* [21].

In the present study, ampicillin loaded fucoidan nanoparticles also exhibited a concentration-dependent antimicrobial activity against all the four microbial strains. The minimum inhibitory concentration of 200 μ L of ampicillin loaded fucoidan nanoparticles was observed to be effective against all the microbial strains. At this concentration of 200 μ L, a growth inhibition of 9.70 \pm 2.91 was observed against *C. albicans*, 33.73 \pm 3.01 against *S. aureus*, 20.64 \pm 0.61 against *S. mutans* and 74.21 \pm 0.29 against *E. faecalis*. However, the highest growth inhibition of all the microbial strains was seen at the concentration of 500 μ L and among the strains it was observed against *E. faecalis* (91.71 \pm 0.14). Lee *et al* has investigated the effects of fucoidan alone and fucoidan with antibiotics against specific cariogenic and periopathogenic bacteria. The MIC values were observed to have reduced 4 folds when fucoidan was

used in combination with antibiotics. It was concluded that there exist considerable synergistic effects of fucoidan with antibiotics against cariogenic and periopathogenic bacteria [15]. Choi *et al* has confirmed the synergistic effects of fucoidan and antibiotics such as ampicillin and oxacillin against clinic isolated methicillin-resistant *Staphylococcus aureus* (MRSA) [22].

A concentration-dependent antimicrobial activity was seen by fucoidan coated metal nanoparticles against all the four microbial strains. The minimum inhibitory concentration of 100 μ L was found to be effective against all the microbial strains. At this concentration, growth inhibition of 15.04 \pm 0.84 was observed against *C. albicans*, 32.12 \pm 4.68 against *S. aureus*, 29.89 \pm 0.61 against *S. mutans* and 67.40 \pm 1.03 against *E. faecalis*. However, the highest growth inhibition of all the microbial strains was seen at the concentration of 500 μ L and among the strains it was observed against *E. faecalis* (92.76 \pm 1.63).

The increasing resistance to the existing antimicrobial agents have led to the need for developing new therapeutic agents. Silver nanoparticles exhibit good antibacterial properties. Rajawat *et al* has obtained an enhanced action of antibiotics against pathogenic bacteria when conjugated with silver nanoparticles [23].

Rajeshkumar *et al* has synthesized fucoidan-mediated silver nanoparticles and showed remarkable changes in the antiantibacterial activity of antibiotics when combined with silver nanoparticles [24].

In the light of the findings of this study, fucoidan coated metal nanoparticles and ampicillin loaded fucoidan nanoparticles can be used as an effective antimicrobial agent against oral pathogens. However, there is a need for further research to thoroughly understand the mechanism of antimicrobial activities of these fucoidan derivatives.

CONCLUSION

This study suggests that fucoidan derivatives such as ampicillin loaded fucoidan nanoparticles and fucoidan coated metal nanoparticles possess antimicrobial activity and can be used as effective antimicrobial agents against oral pathogens.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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