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ANTIBACTERIAL ACTIVITY AGAINST ORAL PATHOGENS AND HEMOLYTIC PROPERTIES FROM *KAPPAPHYCUS SP* SEAWEED EXTRACT

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ABSTRACT

INTRODUCTION:*Kappaphycus* is a genus of red seaweed that belongs to the family of *Areschougiaceae*. The species within the *Kappaphycus* genus are commonly found in tropical and subtropical regions, particularly in the Indian and Pacific Oceans.The seaweed extract obtained from Kappaphycus species, particularly *Kappaphycus alvarezii*, has gained attention in various fields due to its diverse bioactive compounds and potential applications. Kappaphycus species have been recognized for their rich bioactive compounds, including polysaccharides, peptides, and secondary metabolites, which exhibit diverse biological activities. *Kappaphycus* seaweed extract contains high levels of carrageenan, a hydrocolloid widely used in the food industry as a gelling, thickening, and stabilizing agent.This species has various Biomedical and Pharmaceutical Applications and environmental applications . It also possesses Anti-tumor and Anti-cancer Effects.The antibacterial activity of Kappaphycus seaweed extract against oral pathogens suggests its potential application in oral care products, such as mouthwashes or toothpaste formulations, as a natural alternative or adjunct to conventional antimicrobial agents. The aim of this study is to

analyze the Antibacterial activity against oral pathogens and hemolytic properties from kappaphycus sp seaweed extract.

AIM: To determine the Antibacterial activity against oral pathogens and hemolytic properties from kappaphycus sp seaweed extract.

MATERIALS AND METHODS: The seaweed samples of the plant *kappaphycus alvarezi* was collected and the seaweed extract was prepared.Antibacterial assays and hemolytic assays were done to find the antibacterial and hemolytic properties of the extract.

RESULTS:The production of antimicrobial activity was considered to be an effective indicator of the capability of the seaweeds to synthesize bioactive secondary meta- bolites. Gram positive bacteria were more susceptible to crude extract of seaweed than Gram negative bacteria.The extract showed good hemolytic activity as well .

CONCLUSION: In conclusion, the study on the antibacterial activity against oral pathogens and hemolytic properties of Kappaphycus sp seaweed extract has provided valuable insights into the potential of this natural extract as an antimicrobial agent for oral care applications. The extract has demonstrated significant antibacterial activity against oral pathogens, making it a promising candidate for combating oral infections. Additionally, the extract has shown negligible hemolytic properties, indicating its potential safety for oral use.

KEYWORDS: *kappaphycus* sp , antimicrobial assay , hemolytic assay, oral pathogens ,

Seaweed extract.

INTRODUCTION:

In recent years, the global healthcare community has been grappling with the ever-growing challenge of antibiotic resistance. The excessive and often inappropriate use of antibiotics has led to the emergence of drug-resistant strains of various pathogenic bacteria, rendering conventional treatments less effective. In this context, the search for alternative antimicrobial agents from natural sources has intensified, with marine organisms, including seaweeds, gaining prominence due to their vast biodiversity and potential pharmacological applications.Seaweeds, also known as macroalgae, are abundant and diverse marine organisms that have been utilized for centuries in traditional medicine and culinary practices in various cultures worldwide. (1)Over the years, extensive research has revealed that seaweeds harbor an array of bioactive compounds with remarkable therapeutic properties. Among the many species of seaweeds, *Kappaphycus* sp., a red seaweed commonly found in tropical and subtropical waters, has garnered significant interest for its diverse bioactive constituents.(2) (3)

The oral cavity serves as a habitat for a multitude of microorganisms, including bacteria, fungi, and viruses, forming a complex ecosystem termed the oral microbiome.(4) While many of these

microorganisms are beneficial and contribute to oral health, some are pathogenic and can cause various oral infections and diseases. (5)Dental caries, commonly known as tooth decay, is a prevalent oral disease primarily caused by the cariogenic bacterium Streptococcus mutans, which metabolizes dietary sugars to produce acid that erodes tooth enamel. Moreover, periodontal diseases, such as gingivitis and periodontitis, are primarily caused by bacterial species like Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans, leading to inflammation and damage to the supporting tissues of the teeth.

Given the significance of oral health in overall well-being, it becomes crucial to identify and develop effective antimicrobial agents to combat these oral pathogens and prevent dental infections. Kappaphycus sp. seaweed, with its rich reservoir of bioactive compounds, has emerged as a promising natural source for potential therapeutic solutions against oral pathogens.(6)Numerous studies have explored the antimicrobial properties of seaweed extracts against various bacterial and fungal strains, revealing their inhibitory effects on the growth and proliferation of these microorganisms. The presence of various secondary metabolites, such as phenolic compounds, polysaccharides, and peptides, has been identified in Kappaphycus sp. (7,8)seaweed, all of which contribute to its bioactivity. These compounds possess the ability to disrupt bacterial cell membranes, interfere with vital cellular processes, and inhibit bacterial adhesion, making them valuable candidates for combating oral infections.(7)

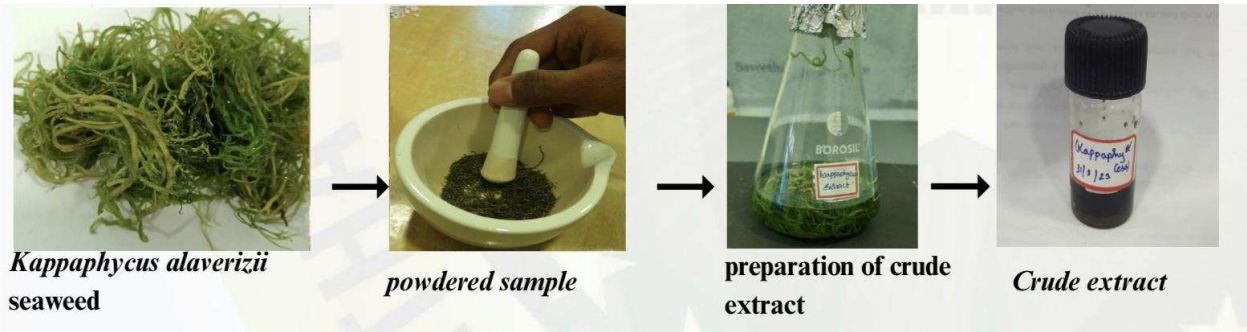
However, before these promising seaweed-derived compounds can be considered for therapeutic applications, it is essential to assess their safety profile thoroughly. Hemolytic activity, thepotential of a substance to cause the lysis or rupture of red blood cells, is a critical factor in determining the safety of any antimicrobial agent. While the destruction of pathogenic bacteria is desirable, it is equally crucial to ensure that the substance does not harm human cells.the exploration of Kappaphycus sp. seaweed extract for its antibacterial activity against oral pathogens and evaluation of its hemolytic properties holds significant promise in the field of oral healthcare. If proven effective and safe, these seaweed-derived compounds could serve as viable alternatives to conventional antibiotics, helping to combat the rising threat of drug-resistant oral pathogens and promote better oral hygiene. Additionally, the findings of this study may also shed light on the broader potential of seaweeds as a valuable and sustainable source of bioactive compounds with diverse applications in medicine and beyond. As we delve into the realm of natural products for healthcare solutions, the untapped potential of the oceans continues to provide us with new and exciting avenues for scientific exploration and innovation.Therefore, alongside evaluating the antibacterial activity of Kappaphycus sp. seaweed extract against common oral pathogens, this study also aims to comprehensively investigate its hemolytic properties. The primary objective is to ascertain whether the extract exhibits selective antibacterial effects while maintaining a safe hemolytic profile.

MATERIALS AND METHODS:

This study was done in blue lab Saveetha dental college and hospital.This research was done for about 3 months .Since this study is in vitro no ethical clearance is required .

Sample collection : kappaphycus alaverizi seaweed samples were collected .Upon collection, the seaweed underwent thorough washing with artificial seawater before being transported to the laboratory. Once completely dried, the seaweed was ground into a fine powder using a mortar and pestle.

To extract the bioactive compounds, 2 grams of the powdered plant material were subjected to extraction using various solvents, namely ethanol, methanol, hexane, butanol, and chloroform (each 20 mL), through the utilization of a Soxhlet apparatus. The resulting supernatant was then filtered using a no. 1 Whatman filter paper with a pore size of 40 µm. This filtration process facilitated the separation of the solvent extracts, which were subsequently stored at 4°C until further analysis.



ANTIMICROBIAL ACTIVITY:

According to the protocols from the Clinical and Laboratory Standards Institute (CLSI, http://www.clsi.org), minimal inhibitory concentrations (MIC) and growth inhibition curves were obtained using pure peptides in the presence of bacteria using two different methods, agar diffusion susceptibility assays and broth microdilution assays.

A 10 mL Mueller-Hinton agar (MHA) underlay was used for the agar diffusion susceptibility assay on a Petri dish plate, and a 0.1 mL aliquot of a mid-logarithmic-phase (16108 CFU/mL in MHB with A625nm = 0.5) culture was added to a sterile tube containing 9.9 mL of non-solidified MHA and mixed. The previously poured MHA Petri plate was then covered with the contents of the tube. then 5 mL aliquots at 300, 100, and 50 of a diluted antimicrobial peptide.

HEMOLYTIC ACTIVITY:

Hemolytic activity was determined by incubating suspensions of human red blood cells with serial dilutions of each selected peptides. Red blood cells were rinsed several times in PBS by centrifugation for 3 min at 3,000 g until the OD of the supernatant reached the OD of the control (PBS only). Red blood cells were counted by a hemocytometer and adjusted to 7.7610660.36106

cells/mL. Red blood cells were then incubated at room temperature for 1 h in 10% Triton X-100 (positive control), in PBS (blank), or with amphipathic peptides at concentrations of 0.4, 0.8, 1.6, 3.1, 6.2, 12.5 and 25 mM, only for Pin2 [14] and Pin2 [17] the 50 and 100 mM concentrations were evaluated. The samples were then centrifuged at 10,000 g for 5 min, the supernatant was separated from the pellet, and its absorbance measured at 570 nm. The relative optical density compared to that of the suspension treated with 10% Triton X-100 was defined as the percentage of hemolysis.

RESULTS:

Zone of inibition	Control	150µg/ml	200µg/ml
Streptococcus mutans	29mm	16mm	17mm
Salmonella typhii	21mm	17mm	20mm
Klebsiella pnumoniae	24mm	17mm	18mm
Escherichiea coli	16mm	12mm	14mm
Staphylococcus aureus	23mm	18mm	19mm

FIG:1-ANTIMICROBIAL ACTIVITY- The results indicate the zone of inhibition, which represents the area around a paper disc containing different concentrations (in this case, 150 µg/ml and 200 µg/ml) of a tested substance (possibly an antibacterial agent). This area where bacterial growth is inhibited can provide insights into the substance's effectiveness against various bacterial strains.

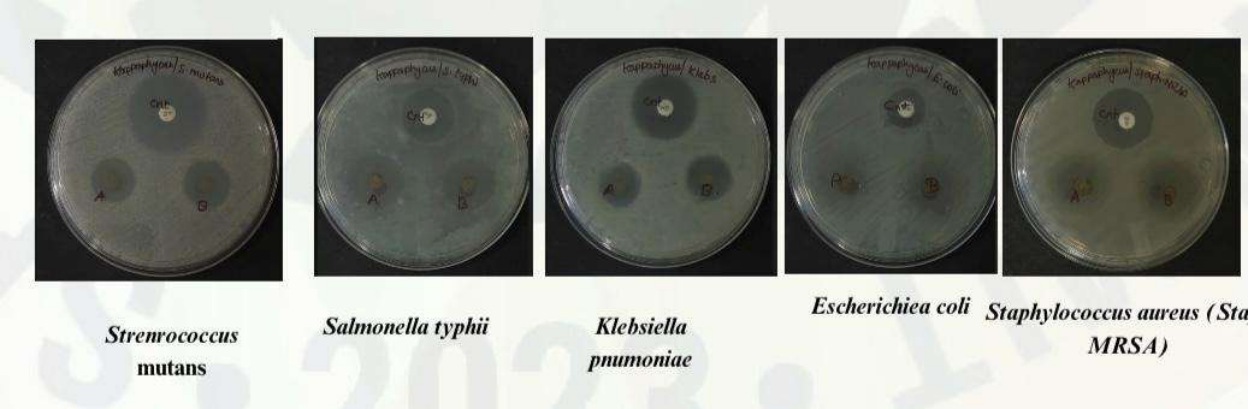
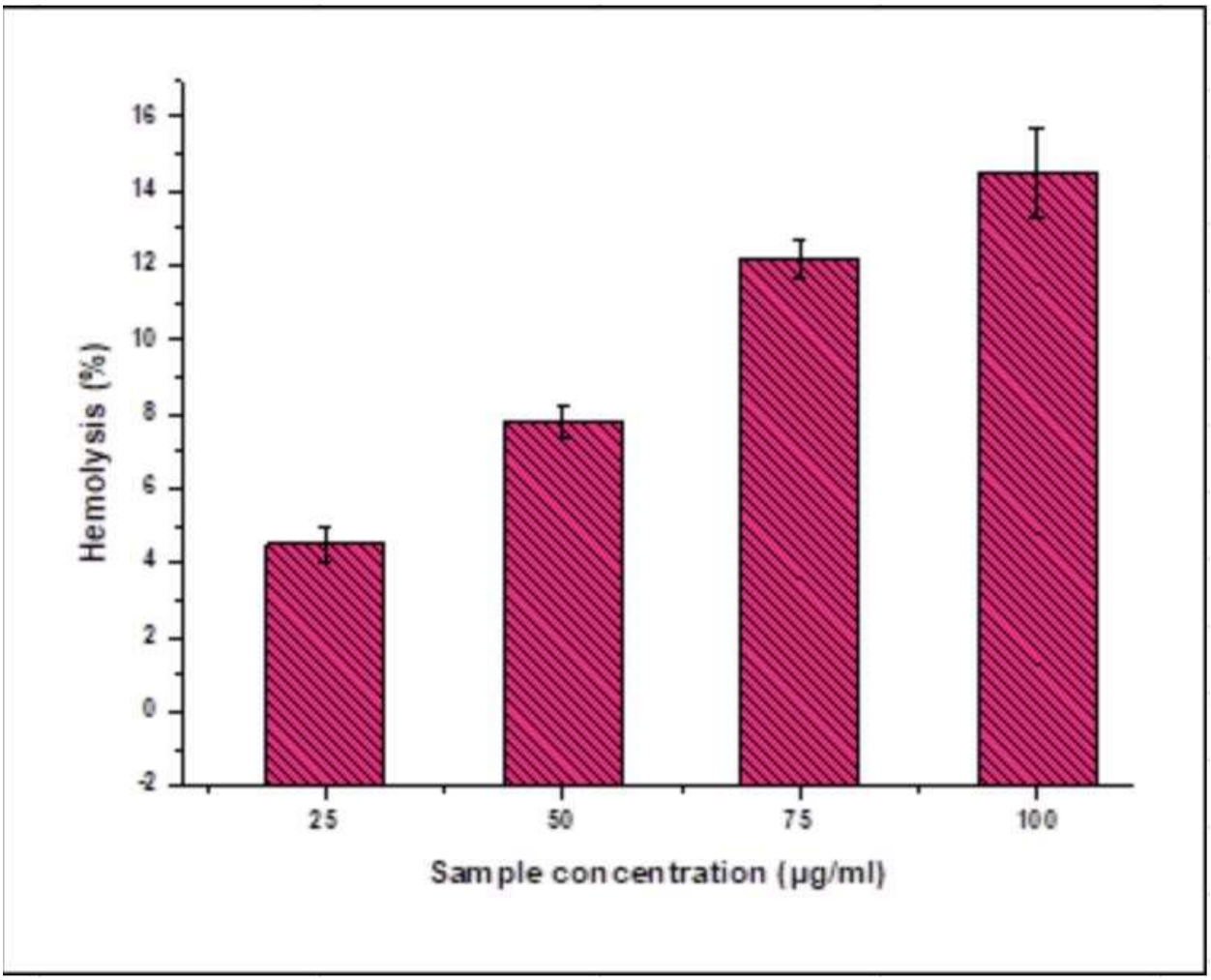


FIG:2-HEMOLYTIC ACTIVITY- demonstrate a clear correlation between the concentration of the tested substance and its ability to induce RBC lysis. As the concentration increases, so does the extent of RBC lysis, highlighting the dose-dependent nature of the substance's impact on these cells. This information is crucial for understanding how the substance interacts with RBCs and

may have implications for various medical or research contexts where RBC lysis is a relevant factor.



Sample concentration(µ/ml)	RBC cell lysis(%)	St.Er
25	4.5	0.5
50	7.8	0.4
75	12.2	0.5
100	14.5	1.2

DISCUSSION:

The antimicrobial activity was assessed. The zone of inhibition was assessed in two different concentrations 150 µg/ml and 200 µg/ml.Five microorganisms were tested for zone of inhibition. Amongst the five microorganisms staphylococcus aureus showed the maximum zone of inhibition in both the concentrations. Gram positive bacteria were more susceptible to crude extract of seaweed than gram negative bacterias . The production of antimicrobial activity was considered to be an effective indicator of the capability of the seaweed extract to synthesize bioactive secondary metabolites. The hemolytic activity was also assessed and the extract showed high hemolytic activity at 100 µg/ml concentration.It also demonstrate a clear correlation between the concentration of the tested substance and its ability to induce RBC lysis. As the concentration increases, so does the extent of RBC lysis, highlighting the dose-dependent nature of the substance's impact on these cells. This information is crucial for understanding how the substance interacts with RBCs and may have implications for various medical or research contexts where RBC lysis is a relevant factor.

In other studies they concluded that the Gram-negative bacterium E. coli is prevalent in many gastrointestinal infections and other infection conditions, the Gram-positive bacterium S. aureus is one of the key concerns in various topical infections.Both S. aureus and E. coli grew in broth culture conditions in the presence of repeated concentrations of all Pin2 variations ranging from 25 to 0.4 mM. S. aureus and E. coli both grew in broth culture conditions in the presence of serial concentrations of all Pin2 variations ranging from 25 to 0.4 mM.(9) The hemolytic activity was also detected in order to relate the variations in the amphipathicity of the various variants to the observed hemolytic activity discrepancies. (10)The decrease in the hemolytic profile of the short variants may be connected to the reduction of hydrophobic residues since the helical wheel projection of the short variants shows the amphipathic distribution as it was originally intended. These findings might be connected to the assays for hemolytic activity.(11)

Many researchers have investigated the antimicrobial capabilities of seaweed extracts against different bacterial and fungal strains, indicating their inhibitory effects on the growth and multiplication of these pathogens. Kappaphycus sp. seaweed has been found to have a number of secondary metabolites that all contribute to its bioactivity, including phenolic compounds, polysaccharides, and peptides. These chemicals make excellent candidates for treating oral infections since they have the power to damage bacterial cell membranes, obstruct crucial cellular functions, and limit bacterial adherence.Previous studies presents data on the antimicrobial activity of different peptides and antibiotics against specific microorganisms. The MIC values vary significantly, suggesting differences in the potency of these substances against the tested pathogens. This information is valuable for evaluating the potential effectiveness of these peptides and antibiotics in treating microbial infections, with lower MIC values indicating stronger antimicrobial activity.our study showed that the production of antimicrobial activity was considered to be an effective indicator of the capability of the seaweed extract to synthesize bioactive secondary metabolites.Further research is needed to explore the extract's efficacy, safety,

optimal dosage, and potential mechanisms of action in more detail. These findings contribute to the growing body of knowledge on natural antimicrobial agents and support the development of sustainable and eco-friendly approaches to oral health maintenance.

CONCLUSION:In conclusion, the study on the antibacterial activity against oral pathogens and hemolytic properties of Kappaphycus sp seaweed extract has provided valuable insights into the potential of this natural extract as an antimicrobial agent for oral care applications. The extract has demonstrated significant antibacterial activity against oral pathogens, making it a promising candidate for combating oral infections. Additionally, the extract has shown negligible hemolytic properties, indicating its potential safety for oral use.

CONFLICT OF INTEREST : The author has no conflict of interest regarding the study.

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ETHICAL CLEARANCE: This study requires no ethical clearance since it is a in vitro study

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