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Formulation and Characterisation of Taste Enhancer Mouth Rinse In The Management of Dysgeusia

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ABSTRACT:
AIM: To assess the anti-inflammatory, antioxidant, antimicrobial and cytotoxicity properties of the formulated mouth rinse.
MATERIALS AND METHODS: In this study, the mouth rinse formulation was subjected to anti-inflammatory, anti oxidant, antimicrobial and cytotoxicity testing
RESULTS: Highest anti-inflammatory and antioxidant properties were noted at 50 µl concentration when compared to the control. The antimicrobial activity of the kelp mouth rinse had the highest zone of inhibition seen in 80 µL concentration. The cytotoxic activity was found to be better at all concentrations studied.
CONCLUSION: The formulated mouth rinse exhibited good anti-inflammatory, antioxidant and anti microbial activities and showed low cytotoxicity activity.
KEYWORDS: Dysgeusia, Kelp, Taste perception, Taste sensation, Umami.

INTRODUCTION:

Taste perception is a multifaceted process with many specialized elements. Taste buds, which are situated on raised tongue structures called papillae, are where it all starts. Around 50–100 taste receptor cells are found in each taste bud, and these cells are essential for recognizing particular flavors (AlJulaih & Lasrado, 2023). There are five different kinds of these cells: Type I cells maintain the structure of the taste bud, Type II cells that respond to sweetness, bitterness, and umami, Type III cells react to sourness, Type IV cells that function as stem cells for regeneration, and Type V cells that may help detect fat. When a tastant connects with the appropriate receptor, it causes taste transduction, which results in electrical signals (Hichami et al., 2023). These signals are transmitted to the brain's gustatory cortex via sensory nerve fibers, such as the facial nerve for the front of the tongue. There, the brain combines taste with other sensory inputs to create our whole impression of flavor. The five major tastes sweet, sour, salty, bitter, and umami each play a unique role in our culinary experiences, and heredity and culture also have an impact on how we perceive different flavors (Yang et al., 2023). Understanding these complex dynamics is essential to culinary arts and healthcare. To understand the nuances of taste perception and its broader consequences for human behavior and nutrition, scientists are constantly researching these processes.

The enjoyment of flavor should be one of life's greatest pleasures. However, a decline in taste sensitivity is occasionally linked to aging. Loss of sufficient gustatory function, especially in the elderly, may result in a weak appetite, reduced nutritional intake, and weight loss (Saboowala., n.d.). Dysgeusia is a sensory condition marked by an aberration in taste perception, which results in distorted or changed taste sensations. Disruptions in the complex mechanisms underlying the sensation of taste, a crucial component of human sensory experience, are the cause of this illness. A complicated series of intracellular events known as taste transduction are sparked when a tastant connects with the proper taste receptor. This process results in the production of electrical impulses that represent taste sensations (Chadwick et al., 2008). Dysgeusia can be brought on by a number of things, including illnesses, drugs, dietary deficiencies, and chemical exposures, all of which can interfere with the delicate taste perception systems (Nagata et al., 2023). A variety of taste abnormalities may be a symptom, which has a significant impact on dietary preferences and general wellbeing. Dysgeusia emphasizes the complex nature of taste perception and the ongoing scientific endeavor to comprehend and effectively treat taste abnormalities.

The umami flavor was suggested as a basic taste by the Japan Chemist Kikunae Ikeda in 1908, but to the western world the advent of the umami flavor was slow. The discovery of the first umami receptor, the metabotropic glutamate receptor taste-mGluR4, a unique dimeric G protein-coupled receptor found in the membranes of the taste cells of the taste buds, marked a significant development in the year 2000 (Kawamura & Kare, 1987). Taste-mGluR4 is a shortened variant of the well-known glutamate receptor mGluR4 in the brain, and it is responsive to L-glutamate. Umami flavor can potentially be helpful for individuals with dysgeusia in several ways. Umami flavor can impart an enhanced palatability by providing a more pleasant and appealing flavor profile. It can serve as a way to make food more palatable, and encourage those inflicted with dysgeusia to eat even when taste distortions are present (Kawakami et al., 2023). The presence of dysgeusia can often result in metallic, bitter and other off-putting tastes. Umami's strong and savory quality has the potential to mask or balance out these unpleasant tastes, making the overall eating experience more tolerable. Dysgeusia also leads to reduced appetite due to the unpleasant taste sensations. Umami, with its appetizing and satisfying qualities, may stimulate appetite and encourage individuals to eat more, ensuring they receive adequate nutrition.There are a number of ways umami flavor may be beneficial for people who have dysgeusia (Thomas et al., 2022). Umami flavor contributes to a more pleasant and appealing flavor profile, which can increase palatability. Making food more appealing may help persons who suffer from dysgeusia continue to eat despite taste distortions. Dysgeusia is frequently accompanied by metallic, bitter, and other unpleasant tastes (Risso et al., 2020). These disagreeable tastes may be concealed or balanced by the powerful and delicious property of umami, improving the entire eating experience. Due to the unpleasant taste sensations, dysgeusia also causes a decrease in appetite. Umami's savory and gratifying properties may pique appetites and encourage people to eat more, ensuring they get the nutrition they need (Nakagawa et al., 2009). Different civilizations have long used kelp, a form of seaweed that is rich in vital nutrients including iodine, vitamins, and minerals, for therapeutic purposes. Due to its possible medicinal benefits, kelp has recently attracted more scientific attention. According to studies, kelp extracts contain bioactive substances like minerals, fucoidans, and polyphenols that have antioxidant, anti-inflammatory, and taste-modifying properties (Kim & Bhatnagar, 2011). These substances might be quite important in reducing dysgeusia-related taste abnormalities. The aim of this study is to assess the antioxidant, anti inflammatory and antimicrobial potentials of a kelp based mouth rinse.

MATERIALS AND METHODS:

Preparation of the Mouth rinse:

2 grams of powdered Kelp was sourced from the local market of Japan which was mixed in 100 ml of water. 0.02 grams of cetylpyridinium chloride and 0.1 grams of sodium carbonate were added. It was boiled at 60 degrees and stirred using the automatic stirrer at 5000 rpm for 3 hours and filtered.

1. **Antioxidant test**

DPPH ASSAY

The DPPH free radical is a long-lived organic nitrogen radical with a deep purple color. When a DPPH solution is mixed with an antioxidant, its color turns from purple to yellow of the corresponding hydrazine. The reducing ability of antioxidants toward DPPH can be evaluated by monitoring the decrease of its absorbance at 515–528 nm. The results are expressed as IC50 or as % scavenging of DPPH at a fixed antioxidant concentration for all the samples.

Preparation of DPPH solution

DPPH solution was prepared by taking 7.89 mg of DPPH using a chemical balance, dissolving with 100 ml 99.5% ethanol, and finally kept in dark for 2 hr.

DPPH assay procedure

1,000 µl DPPH solution was combined with 800 µl of Tris-HCl buffer (pH 7.4) in a test tube. Subsequently, 200 µl of the sample solution was added and mixed rapidly. The mixture was then allowed to sit at room temperature for 30 minutes. The absorbance at 517 nm was measured. A blank solution, consisting of 1,200 µl of ethanol and 800 µl of Tris-HCl buffer (pH 7.4), was prepared for comparison. The inhibition ratio (%) was calculated using the following equation:

Inhibition ratio (%) = (A1 − A2) × 100 / A1,

where A1 is the absorbance of the addition of ethanol instead of testing sample and A2 is the absorbance of testing sample solution.

2. **Anti inflammatory test:**

Inhibition of protein denaturation

Mizushima et al.'s method was slightly modified and the reaction mixture included test extracts at varying concentrations and a 1% aqueous solution of bovine albumin fraction. The pH of the mixture was adjusted with a small amount of 1N HCl. Diclofenac sodium was used as the standard drug. Samples were incubated at 37°C for 20 minutes, then heated to 57°C for 30 minutes. After cooling, turbidity was measured spectrophotometrically at 660 nm. The experiment was conducted in triplicate, and the percent inhibition of protein denaturation was calculated as follows:

Percentage Inhibition = (A of Control – A of Sample)/A of Control x100

3. **Antimicrobial test**

Antimicrobial activity-Minimal Inhibitory Concentration (MIC)

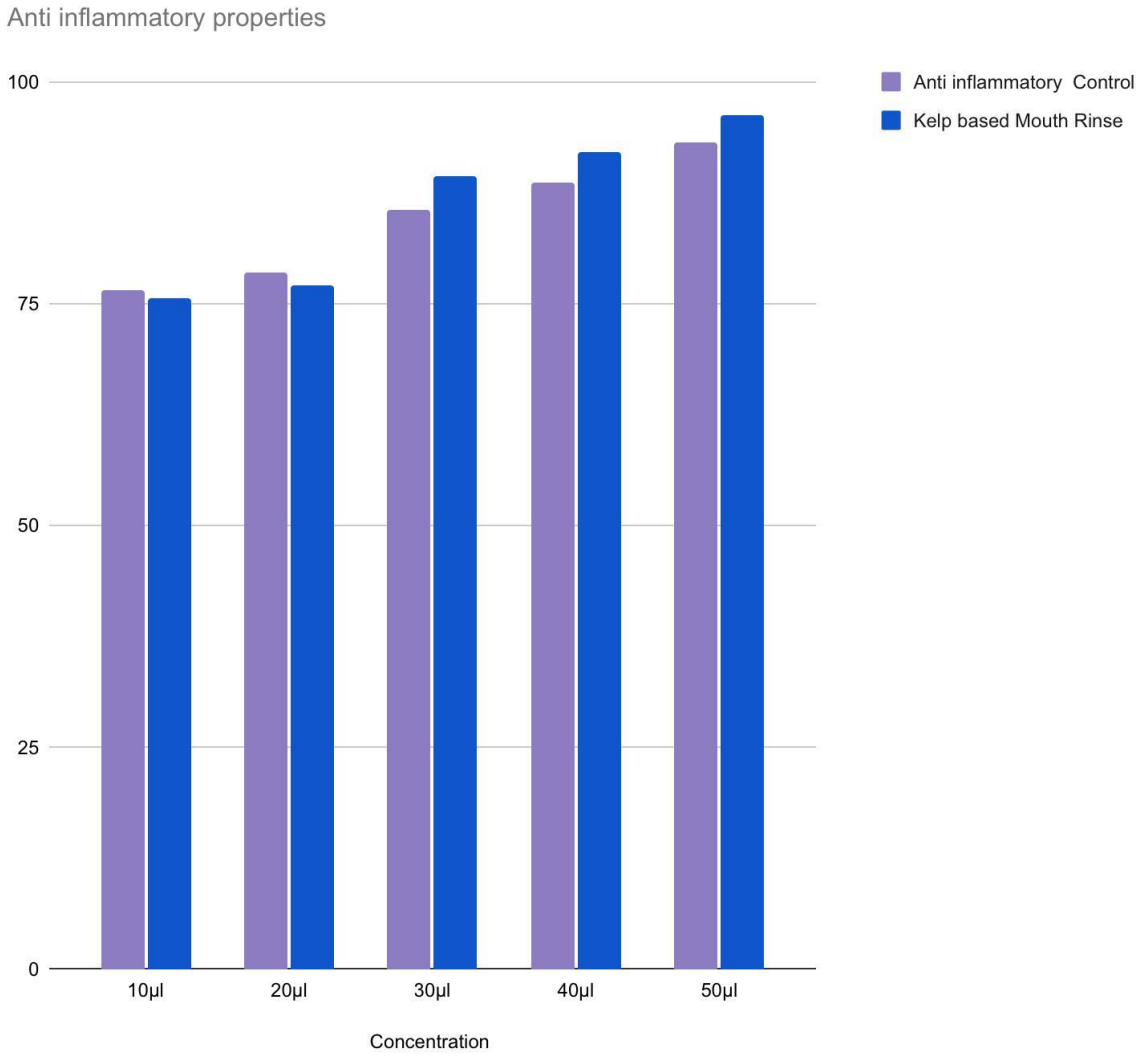
The minimal inhibitory concentration (MIC) test is performed using Mueller Hinton Agar (MHA), which is the best medium for routine susceptibility tests because it has good reproducibility, low in sulfonamide, trimethoprim, and tetracycline inhibitors, and gives satisfactory growth of most bacterial pathogens.

4. **Cytotoxicity test using Brine Shrimp:**

10 newly hatched Brine Shrimp (Artemiasalina) larvae (Nauplii) were taken and transferred into 6 wells. The synthesized mouthwash was introduced into each of the wells of varying concentrations of 10, 20, 40 and 80 µl. One well with live nauplii was without the mouthwash and it served as the standard sample and was left undisturbed for 48 hours. The number of live nauplii after 24 and 48 hours were noted and the data was plotted in the form of a graph.

RESULTS:

Kelp mouth rinse had better antioxidant property at 50µl concentration and was also found almost equal to the standard Vitamin E. (Graph 1, Table 1).



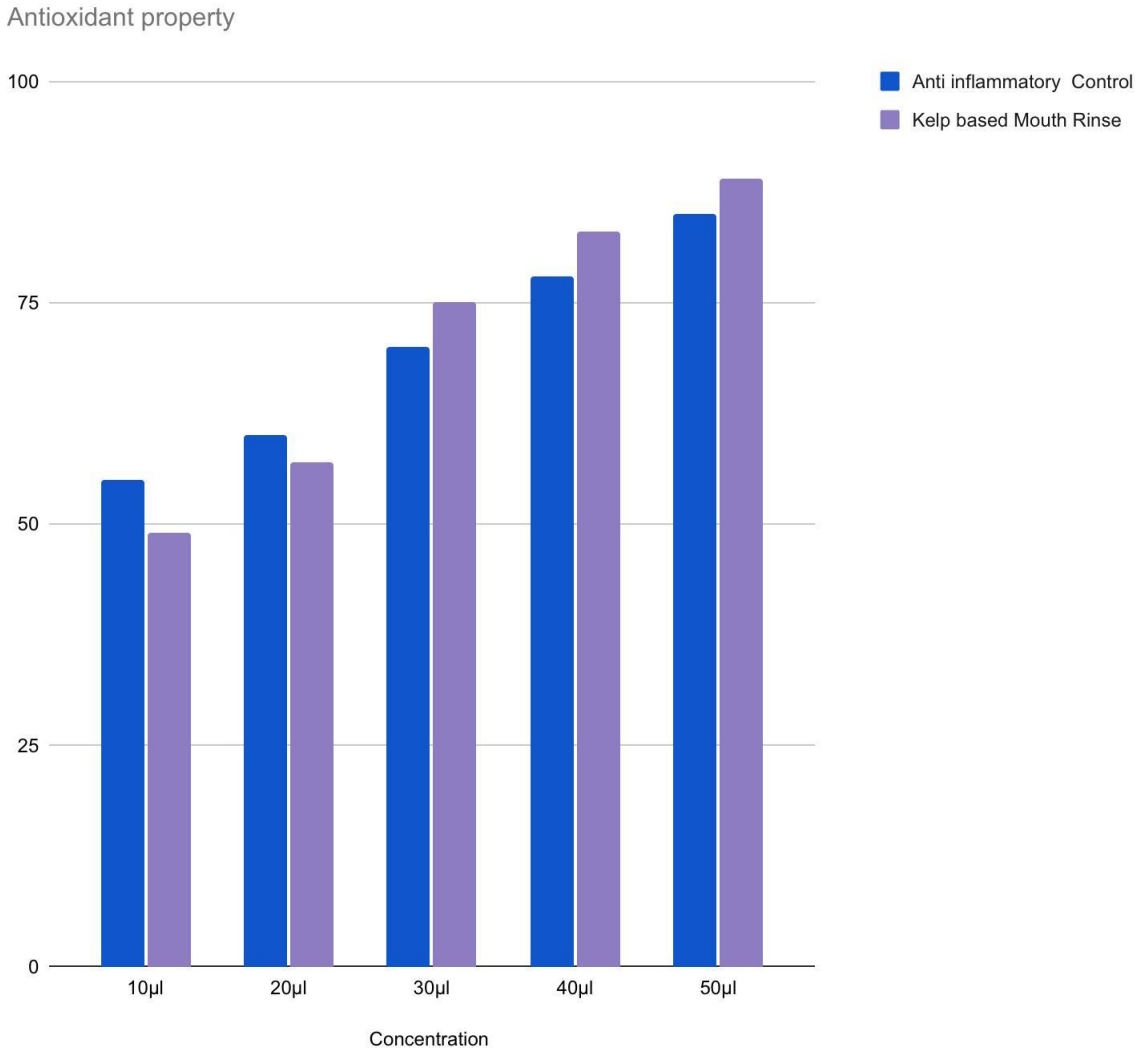
Graph 1: ANTI INFLAMMATORY PROPERTIES OF KELP BASED MOUTHRINSE

Anti inflammatory properties		
Control	**Kelp mouth rinse**	**Sig.**
10 µl	10 µl	0.045
20 µl	20 µl	0.001
30 µl	30 µl	0.007

40 µl	40 µl	0.001
50 µl	50 µl	0.009

Table 1: Paired t test anti inflammatory properties

Anti-inflammatory activity was also found to be good when the solution was formulated at 50µl concentration and showed properties equal to the gold standard drug of choice diclofenac sodium. (Graph 2, Table 2).



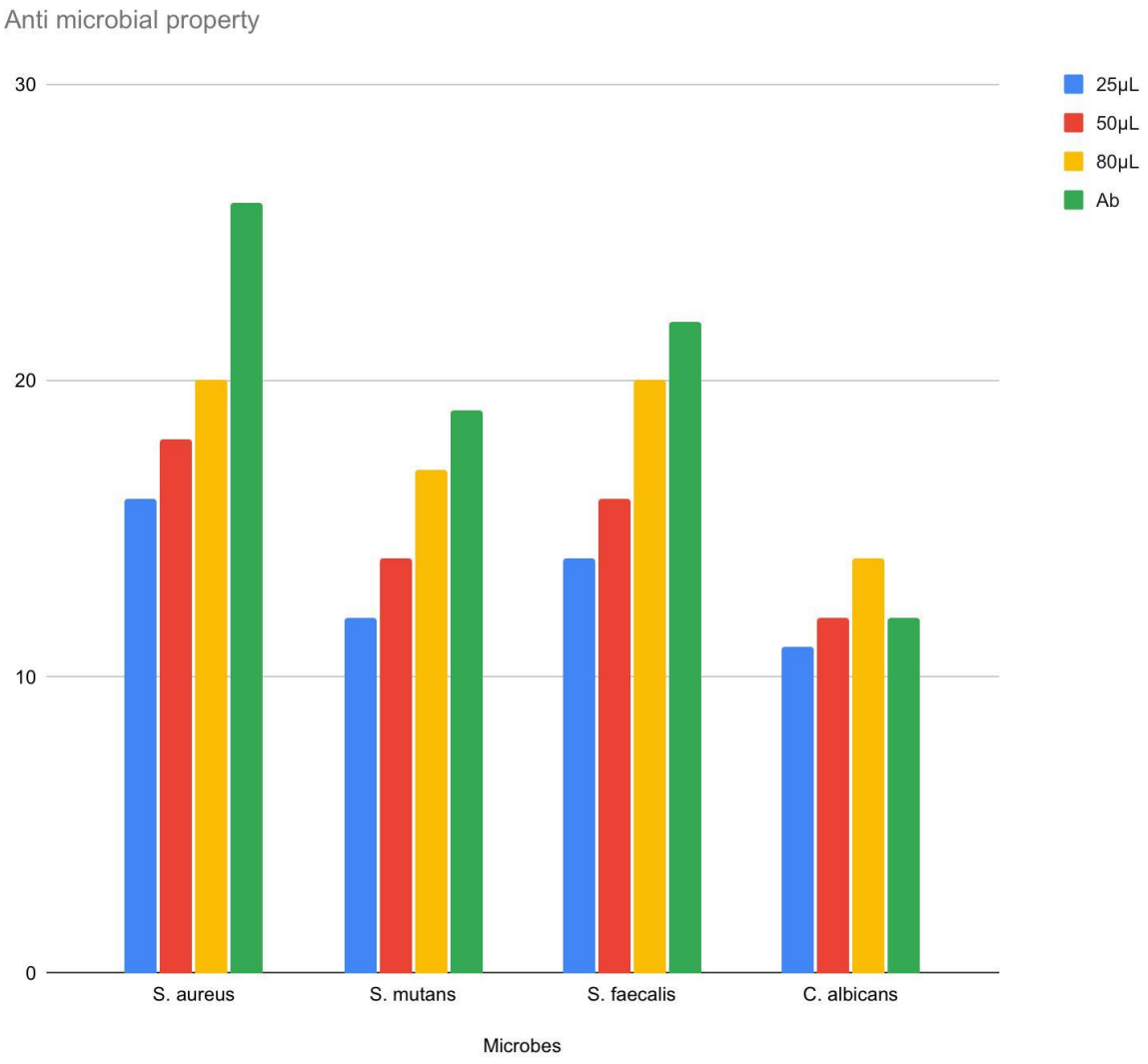
Graph 2: ANTIOXIDANT PROPERTIES OF KELP BASED MOUTHRINSE

Antioxidant properties		
Control	**Kelp mouth rinse**	**Sig.**

10 µl	10 µl	0.050
20 µl	20 µl	0.008
30 µl	30 µl	0.049
40 µl	40 µl	0.003
50 µl	50 µl	0.021

Table 2: Paired t test antioxidant properties

The antimicrobial activity of the kelp mouth rinse had the highest zone of inhibition seen in 80 µL concentration for S. aureus and S.mutans, but the mouth rinse had reduced antimicrobial activity when compared to the control. (Graph 3, Table 3).



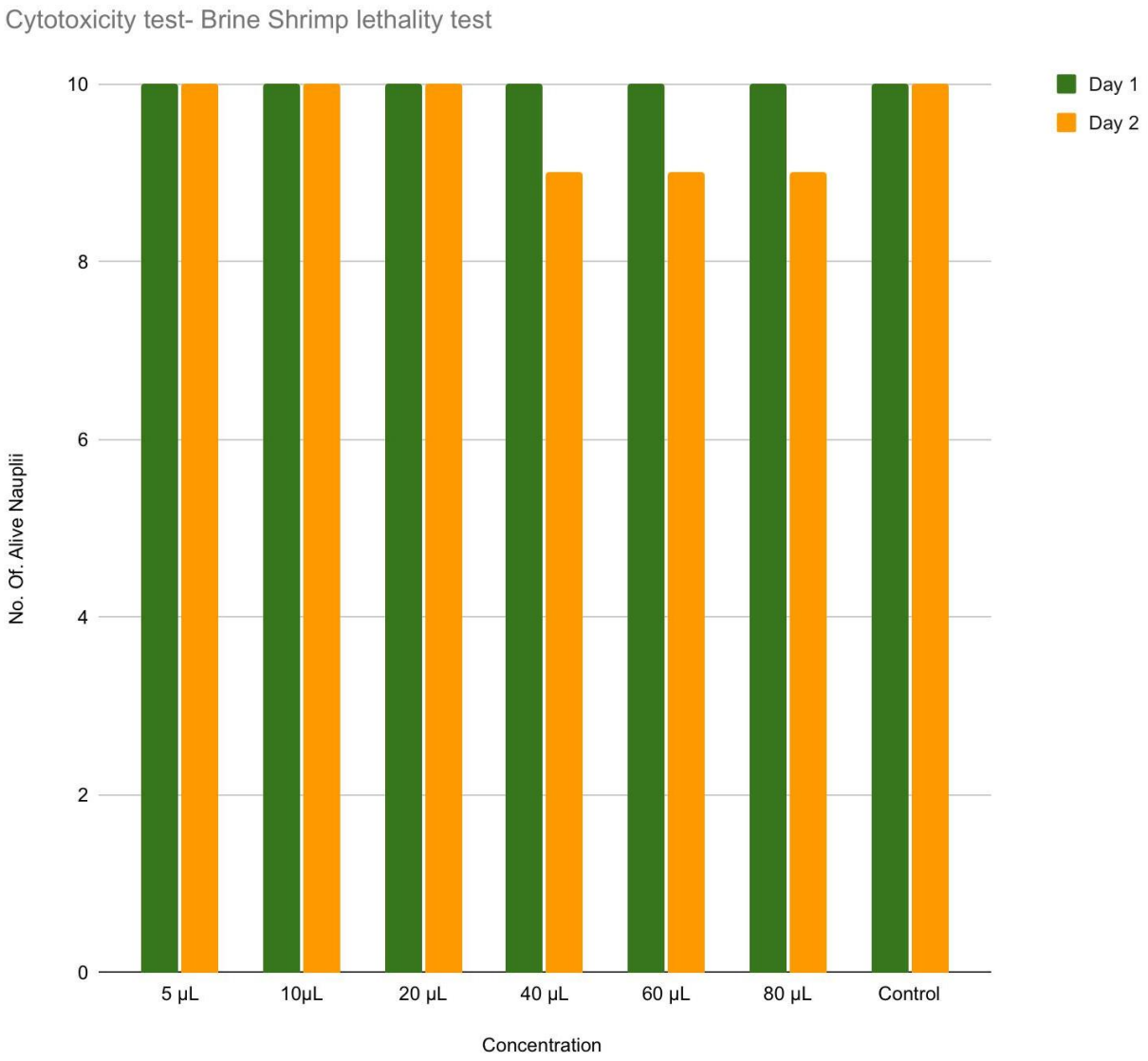
Graph 3: ANTIMICROBIAL ACTIVITY OF KELP BASED MOUTH RINSE

Anti inflammatory properties			
Microbe	**Control**	**Kelp Mouth rinse**	**Sig.**
S. aureus	80 µl	25µl	0.032
	80 µl	50 µl	0.005
	80 µl	80 µl	0.012
S. mutans	80 µl	25µl	0.006

	80 µl	50 µl	0.024
	80 µl	80 µl	0.016
E. faecalis	80 µl	25µl	0.056
	80 µl	50 µl	0.004
	80 µl	80 µl	0.009
C. albicans	80 µl	25µl	0.04
	80 µl	50 µl	0.059
	80 µl	80 µl	0.001

Table 3: ONE WAY ANOVA OF ANTIMICROBIAL PROPERTIES OF KELP BASED MOUTH RINSE

The cytotoxic activity was found to be better at all concentrations studied. 10 nauplii survived at 5µL, 10µL and 20µL and 9 nauplii survived at 40 µL, 60 µL and 80µL after 48 hours implicating the low cytotoxicity associated with the kelp formulation. (Graph 4).



Graph 4: CYTOTOXICITY TEST

DISCUSSION:

Kelp (*Laminaria japonica*), is a fast-growing marine plant that does not require freshwater or fertilizers to grow. It absorbs excess nutrients and carbon dioxide from the surrounding water, making it an environmentally friendly resource. (Li et al., 2023). Utilizing kelp in oral care products could contribute to reducing the ecological footprint associated with traditional mouthwashes. Kelp-based mouthwash may potentially offer anticavity effects due to its mineral content, including calcium and fluoride. These minerals are known to promote enamel remineralisation and strengthen teeth, thus reducing the risk of cavities. It has proven antibacterial properties that helps combat common oral pathogens, thereby reducing the risk of dental caries. (Barba et al., 2019)The anti-inflammatory and antioxidant properties of kelp based mouthrinse may have soothing properties for oral tissues.

The presence of bioactive compounds, such as polysaccharides and fucoidans, in kelp extracts may help alleviate oral inflammation and promote the healing of oral wounds or ulcers. In a study by D. Yin et al, the kelp was extracted using various methods such as hot water extraction

(HWE), ultrasound extraction (UAE), enzyme assisted extraction (EAE) and acid assisted extraction (AAE). It was found that the polysaccharide extracted using the ultrasound extraction and enzyme assisted methods showed higher antioxidant activities. The study also showed that there was a significant positive correlation between the presence of sulfate and mannose content present in kelp. (Lu Bai et al., 2021). The results of the previous studies were consistent with the results of the present study.

Previous studies investigated the anticariogenic potential of different seaweed extracts, including kelp, against cariogenic bacteria. The results indicated that kelp extracts exhibited inhibitory effects on bacterial growth, suggesting its potential as a natural ingredient in preventing dental caries. Bioactive compounds found in kelp, such as fucoidans and phlorotannins, have demonstrated potential anticancer properties in various studies. RE Cian et al, found that peptides extracted from kelp (*P. Columbiana*) promoted iron absorption and showed good anticariogenic activity. (Cian et al., 2016)

According to the study by T. Sassano et al, the use of umami taste stimulation increases the flow rate of saliva due to the triggering of the gustatory-salivary reflex. Japanese Konbucha was used to stimulate reflexive salivation by stimulating the umami taste, which resulted in significant improvements in the taste function, appetite, weight and overall health of the test subjects. (Sasano T et al.,2015).

Dysgeusia is more common in the elderly population, due to the altered sensation of taste, most individuals fail to receive proper nutrition. Herbal mouth rinses can help combat it while having minimal cytotoxicity, which have been proven in various previous studies, wherein herbal mouth rinses had comparable antioxidant (Chatterjee et al., 2023; G et al., 2023; Shanmugam et al., 2023; V et al., 2023) , anti inflammatory (Swarna Meenakshi et al., 2023), anti microbial properties(Munusamy & Shanmugam, 2023)(Begum et al., 2023)(Bl et al., 2024)along with reduced cytotoxic effects (Singh et al., 2023; Varghese et al., 2024). The limitation of the present study is that it was conducted in an invitro set up and the results can not be generalized to clinical setting, and further research is required to assess the effectiveness of kelp based mouth rinse to combat dysgeusia and also to assess patient acceptability. A study is being carried out to assess the acceptability and clinical translation of the formulated mouth rinse.

CONCLUSION:

The novel formulation of kelp based mouth rinse showed enhanced anti-inflammatory, antioxidant and antimicrobial activity, as well as reduced cytotoxic effects, which are comparable to the traditional mouthwashes and mouth rinses. In the future clinical trials can be conducted using the formulated mouth rinse, to assess its clinical efficacy in combating dysgeusia along with patient tolerance, to assess the perception, taste and overall satisfaction of users through surveys or focus groups for further development and research.

CONFLICT OF INTEREST: None Declared

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