ORIGINAL RESEARCH

Sargassum polycystum and *Turbinaria conoides* Seaweed-based Novel Denture Cleanser: An *In Vitro* Study

Sree Roopa Gogula[1], Shivasakthy Manivasakan[2], David Livingstone[3], Jahnavi Madaan[4]

Abstract

Aim: The study ventures into evaluating the antifungal and antibacterial efficacy of commercially available denture cleanser with *Sargassum polycystum*, *Turbinaria conoides* seaweeds, and the combination of seaweeds.

Materials and methods: Poly(methyl methacrylate) disks measuring 10 x 2 mm were fabricated. The samples are divided into four groups of 21 samples each. The denture base was coated with *Candida albicans* and *Streptococcus mutans* individually. Group I was treated with Fittydent, group II (*S. polycystum* and *T. conoides* seaweeds combination), group III (*S. polycystum*), and group IV (*T. conoides*). The colony-formation units present on the surface of the denture were evaluated before and after treatment with different denture cleansers using the serial dilution method. Statistical analysis was done using descriptive statistics, analysis of variance, and *post hoc* Bonferroni analysis.

Results: At 10^{-5} dilution, *T. conoides* (group IV) was statistically significant in reducing both *C. albicans* and *S. mutans*. At 10^{-10} dilution, *T. conoides* (group IV) and *S. polycystum* and *T. conoides combination* (group II) had high antibacterial efficacy and were statistically significant. Fittydent (group I) had higher antifungal efficacy and was statistically significant in comparison to *S. polycystum* (group III) alone. At 10^{-10} dilution, the *T. conoides* (group IV), *S. polycystum*, and *T. conoides combination* (group II) showed no evidence of a significant difference in comparison to Fittydent (group I). Fittydent had higher antibacterial efficacy and was statistically significant in comparison to *S. polycystum* (group III) alone.

Conclusion: *Sargassum polycystum* and *T. conoides* combination and *T. conoides* were found to have higher antibacterial efficacy in comparison to commercially available denture cleanser and also were found to have equal antifungal efficacy in comparison to commercially available denture cleanser.

Keywords: *Candida albicans*, Denture cleanser, *Sargassum polycystum*, *Seaweed*, *Streptococcus mutans*, *Turbinaria conoides*.

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Introduction

Older age-groups are most commonly affected with edentulism.[1] A survey was done in 2014 among completely edentulous adults of 50 years and older and they found that the prevalence of edentulism in India was 16.3%. A removable complete denture is the first and foremost option considered for rehabilitation.[2] In the oral cavity, microorganisms form complex structures and microbial communities known as biofilm. They produce polysaccharides and extracellular matrix that aid in the attachment of various other microorganisms, like *Candida albicans*.[3] The proliferation of fungi and bacteria leads to chronic atrophic candidiasis or denture stomatitis, which was found in about 11 to 67% of patients using dentures.[3] *Candida* spp. interact with other microorganisms in the oral cavity, particularly *Streptococcus* spp. and *Staphylococcus* spp., thus resulting in a complex biofilm formation with an organized structure that is difficult to remove.[4,5]

Denture maintenance plays an important role in preventing oral lesions.[6] Denture cleansing is done by combining mechanical cleaning with a chemical cleanser, which is an efficient way to remove biofilm in older people with less manual dexterity. Herbal products like Triphala, turmeric, cloves, miswak, cashew and guava leaves, aloe vera, grape seed oil, and origanum oil[6] have recently become popular[7] and are the most cost-effective alternatives and lack side effects. Seaweed algae are one of the naturally available products in the environment. They have been broadly classified into three groups: brown, red, and green.[8] In dentistry, seaweeds have been used in impression material (polysaccharides derived from algae like alginate and agar),[9] toothpastes,[10,11] and mouthwashes.[12]

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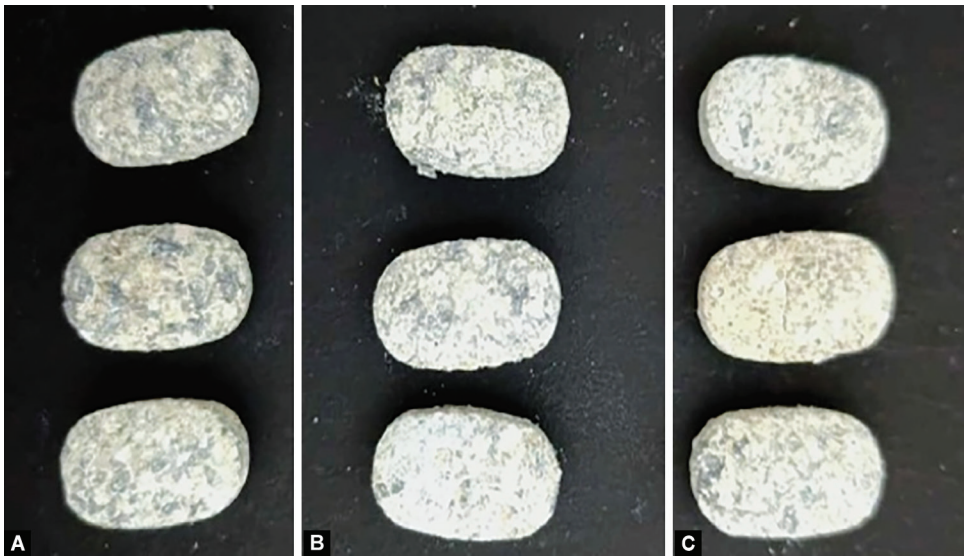
Turbinaria conoides and *Sargassum polycystum* are seaweeds that are available in the coastal sea. These seaweeds have extraordinarily promising antiproliferative, anti-inflammatory, antiviral, and antimicrobial properties because of the bioactive compound.[13]

Gulfweed (*Sargassum*) is common as free-floating masses. Seaweeds have been consumed as food in various parts of the world. Seaweed products like alginate, agar, and carrageen are used as food additives. They are used primarily in the medical industry and also for edible packaging, bioremediation, ocean afforestation (carbon removal), and fertilizer.[14] *Sargassum* is a genus of brown algae that has over 300 species. *Sargassum polycystum* is an edible brown seaweed. *Sargassum* and *Turbinaria* belong to the class Phaeophyceae.[15] In *Turbinaria* species that are often preferentially consumed by herbivorous fishes and echinoids, there



Figs 1A to C: Prepared seaweed tablets. (A) Group II, combination of *Turbinaria conoides* and *Sargassum polycystum* seaweeds (double strength tablets), (B) Group III, *Sargassum polycystum* alone (single strength tablets), and (C) Group IV, *Turbinaria conoides* alone (single strength tablets)

is a relatively low level of phenolics and tannins. Genus *Turbinaria* is extraordinarily promising antiproliferative, antipyretic, anti-inflammatory, antidiabetic, antiviral, antimicrobial, and hepato-protective seaweed algae. These activities are represented by diverse classes of compounds, including sterols, amino acids, fatty acids, alcohols, halocarbons, hydrocarbons, carbohydrates, esters, and cyclic tetrapyrrole compounds.[14,15]

Previously, studies have been done using extracts of *S. polycystum* seaweeds[16] but powder form has still not been used. In this study, the powder form of both seaweeds was tested individually and in combination of seaweeds. In the present *in vitro* study, commercially available denture cleanser was compared with seaweeds *T. conoides and S. polycystum* for its antifungal activity against *C. albicans* and antibacterial efficacy against *Streptococcus mutans* adherent to acrylic denture base resins.

Materials and Methods

The *in vitro* experimental controlled trial was presented to the institutional review and ethics board, and approval was granted for the same (Ref. number: IGIDSIEC2021NRP05PGGSPRI). This *in vitro* study was conducted in the Indira Gandhi Institute of Dental Sciences for a period of 1 year from 2021 to 2022. The standard strains of *C. albicans* and *S. mutans* were acquired and tested at Apex Biotechnology and Research Centre, Chennai, India. Serial dilution was done. The spread plate technique was used for counting the microorganisms.

The current study assessed the efficacy of four denture cleansers (group I samples were to be treated by Fittydent, group II samples were treated by combination of *T. conoides* and *S. polycystum* seaweed (double strength), group III samples were treated by *S. polycystum* alone (single strength), and group IV samples were treated by *T. conoides* alone (single strength) against both *S. mutans* and *C. albicans*. Each group of denture cleanser tested 21 samples for *C. albicans* and 21 samples for *S. mutans* summing up to a total of 168 samples distributed in the four groups. A comparison was done between group Fittydent and the remaining three groups.

Collection and Preparation of *Sargassum polycystum* and *Turbinaria conoides* Seaweeds

Turbinaria conoides and *S. polycystum* seaweed were collected from the coastal areas of Mandapam, Gulf of Mannar, Rameswaram, Tamil Nadu, India. All the seaweeds were washed thoroughly with running water to remove the sand particles, animal casting and then sundried. A total of 500 g of *S. polycystum* and 500 g of *T. conoides* sundried seaweed were ground to a coarse powder using a mortar and pestle, and tablet preparation was done.

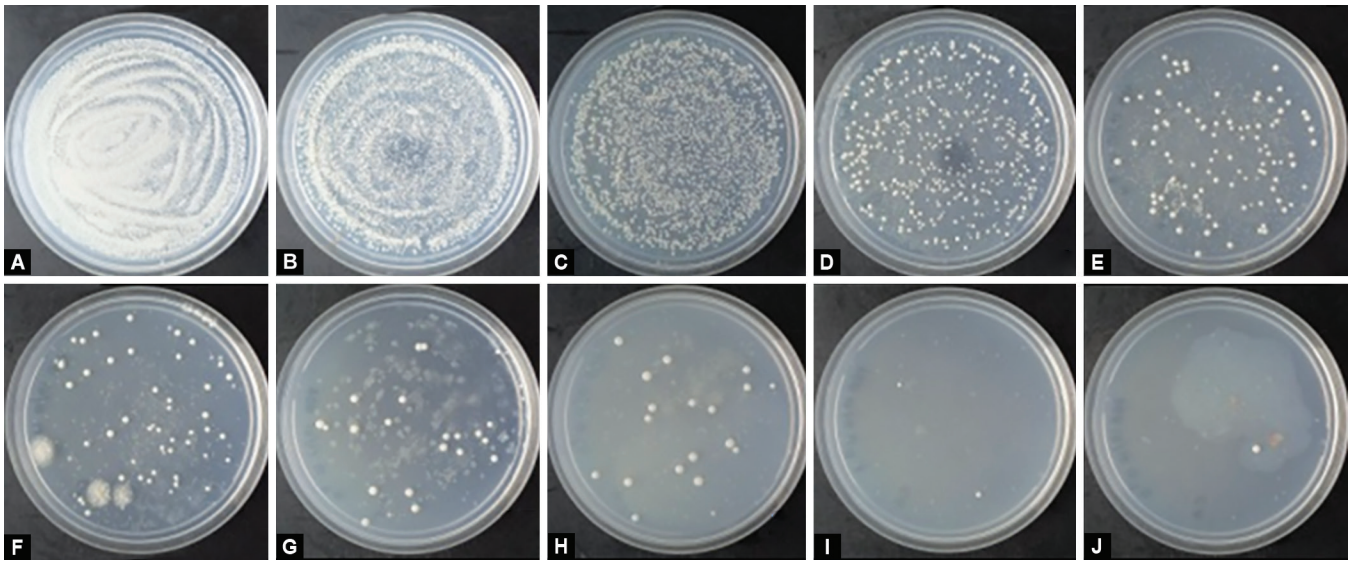
Tablet Preparation

The active pharmaceutical ingredient was seaweed powder, 200 mg of *S. polycystum* and 200 mg of *T. conoides* seaweed in double strength seaweed tablet group II and 400 mg of *S. polycystum* seaweed alone were used for group III, 400 mg of *T. conoides* seaweed alone was used for group IV. Other ingredients including citric acid of 151 mg, sodium bicarbonate of 383 mg, tartaric acid of 248 mg, and magnesium stearate of 16 mg were mixed with the active pharmaceutical ingredient using binder and diluent as required.

The dry granulation method had been used for mixing the seaweed powder with the remaining ingredients and tablets were punched using a tablet punching machine. A tablet of 1.2 g was punched using a tablet punching machine. Figure 1A depicts the group II combination of *T. conoides* and *S. polycystum* seaweed (double strength tablets), Figure 1B depicts the group III *S. polycystum* alone (single strength tablets), and Figure 1C depicts the group IV *T. conoides* alone (single strength tablets).

Preparation of Acrylic Specimens

A metal die of 10 mm diameter and 2 mm thickness was prepared to be used as a mold for the preparation of acrylic specimens. For this preparation, a wax pattern was made using inlay wax measuring 10 mm in diameter and 2 mm in thickness. Addition silicone putty (DPI Photosil soft putty) index was made for the wax pattern, which was used as an index for preparing more metal dies with the same dimensions and casting was done. It was later finished



Figs 2A to J: Plates containing *Candida albicans* after treatment from (A) 10^{-1} dilution; (B) 10^{-2} dilution; (C) 10^{-3} dilution; (D) 10^{-4} dilution; (E) 10^{-5} dilution; (F) 10^{-6} dilution; (G) 10^{-7} dilution; (H) 10^{-8} dilution; (I) 10^{-9} dilution; (J) 10^{-10} dilution

and polished. Flasking was done with type IV gypsum (goldstone, die stone) as a base, and in addition silicone putty was used over it. Metal discs were carefully placed over the addition of silicone putty, and the counterpart was closed using type IV gypsum (die stone) and tightened with a clamp. Heat cure powder and liquid were mixed and packed into flasks and short curing cycle was used for the curing of acrylic samples. This procedure was used and 168 samples were made.

Preparation of Broth and Agar

Potato dextrose agar consists of dextrose and dehydrated potato infusion. This serves as a very good medium for the growth of fungi. For every 1 mL of distilled water, 0.039 gm of potato dextrose agar was diluted and poured into a conical flask containing a funnel with filter paper. It was autoclaved for 15 minutes at 121 psi. Loops of *C. albicans* were incorporated into this broth. It was then placed in an incubator at 37°C for 24 hours. For the preparation of agar, 1.5 gm of potato dextrose agar was taken for every 1 mL of distilled water, and agar-agar was added. This solution was autoclaved for 15 minutes at 121 psi as such, without filtering.

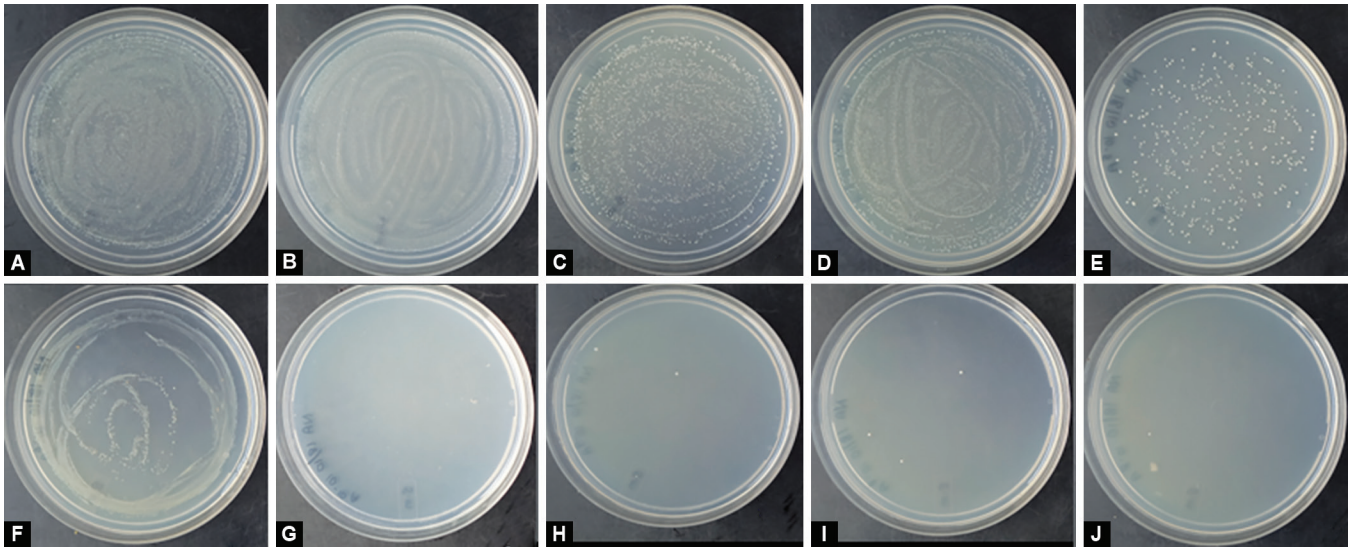
Streptococcus mutans was incubated in Mueller Hinton broth for 24 hours at 28°C. The Mueller Hinton broth contains dehydrated infusion of beef, casein hydrolysate, and starch. Loops of *S. mutans* were incorporated into this broth. It was then placed in an incubator at 37°C for 24 hours. For the preparation of agar, 38 g of Mueller Hinton agar powder in 1 l of distilled water was mixed with the broth and was sterilized by autoclaving at 121°C for 15 minutes. The liquid was poured into the Petri dish. These Petri dishes were allowed to solidify at room temperature. The dehydrated medium was stored at 10 to 30°C.

Treatment of Acrylic Samples with Denture Cleansers

After the broth preparation, the acrylic plates were placed in the broth and allowed to incubate for 24 hours in the incubator. Acrylic samples were incubated with *C. albicans* ($n = 21$) and *S. mutans* ($n = 21$) individually. Acrylic discs incubated with *C. albicans* were placed in a microplate containing 24 wells and *Streptococcus* in other 24-well microplates separately. The formation of biofilm was confirmed after 24 hours. Before intervention, the colony-formation units (CFU) were counted. The samples were divided into four groups. Samples belonging to group I were to be treated by Fittydent, group II samples were treated by a combination of *T. conoides* and *S. polycystum* seaweed (double strength), group III was treated by *S. polycystum* alone (single strength), and group IV was treated by *T. conoides* alone (single strength). One denture cleanser tablet of Fittydent (group I) was dissolved into a beaker containing 100 mL of distilled water and 1 mL of denture cleanser solution was pipetted from the beaker. One milliliter of Fittydent denture cleanser was placed on all 21 acrylic samples in a microplate containing *C. albicans* and all the 21 acrylic samples in another microplate containing *S. mutans*. It was incubated for 8 hours. After 8 hours, the acrylic samples were removed from the broth and washed with saline to remove any microorganisms not adherent to the acrylic sample. The same procedure was done for all the denture cleansers (groups II, III, and IV) and then serial dilution method was used for counting the number of microorganisms.

Serial Dilution and Enumeration of Colonies

The spread plate method was used in the present study. The acrylic sample was placed in saline, and 1 mL of this was used for serial dilution. For serial dilution, sterile microcentrifuge tubes were used. Each microcentrifuge tube was filled with 1 mL of saline. For each sample, 10 serial dilutions were made. For each sample evaluation, 0.1 mL of acrylic sample with microorganism broth was placed in the first microcentrifuge tube with a micropipette and vortexed. Then, from the first microcentrifuge tube, 0.1 mL was transferred into the second, and the same was continued until the 10th dilution. All the microcentrifuge tubes were vortexed. And then 0.1 mL of each microcentrifuge tube was transferred on to the potato dextrose agar for *C. albicans* and the Mueller Hinton agar for *S. mutans*. After placing 0.1 mL on the agar medium, it was spread on the agar plate with a sterile bent glass rod uniformly, and any bubbles that were formed on the plates were removed with a metal loop. This procedure was done for all the samples. These spread plates were incubated for 24 hours, and then the colonies were counted on each plate and enumerated using the viable counting method. Before treatment of the acrylic samples, *C. albicans* and *S. mutans* were counted. Figure 2 shows spread plates with *C. albicans* after



Figs 3A to J: Plates containing *Streptococcus mutans* after treatment from (A) 10^{-1} dilution; (B) 10^{-2} dilution; (C) 10^{-3} dilution; (D) 10^{-4} dilution; (E) 10^{-5} dilution; (F) 10^{-6} dilution; (G) 10^{-7} dilution; (H) 10^{-8} dilution; (I) 10^{-9} dilution; (J) 10^{-10} dilution

Table 1: Comparison of antifungal efficacy between the groups after treatment at 10^{-5} dilution

Dependent variables	*Group (I)*		*Group (J)*		*Mean difference (I–J)*	*Std. error*	*Sig. (p-value)*
CFU 5 ($\times10^{-5}$)	I	Fittydent	II	*Sargassum polycystum* and *Turbinaria conoides*	45.667	35.946	1.000
			III	*Sargassum polycystum*	–90.000	35.946	0.086
			IV	*Turbinaria conoides*	223.524	35.946	0.001*
	II	*Sargassum polycystum* and *Turbinaria conoides*	III	*Sargassum polycystum*	–135.667	35.946	0.001*
			IV	*Turbinaria conoides*	177.857	35.946	0.001*
	III	*Sargassum polycystum*	IV	*Turbinaria conoides*	313.524	35.946	0.001*

Post hoc Bonferroni analysis, **p*-value is significant if the value is <0.05

treatment and Figure 3 shows spread plates with *S. mutans* after treatment. In this study, viable counting method was done. The plate was divided into nine parts, and each part was counted. If more than 2000 CFU/mL are present, it was difficult to count them on each plate, and this was mentioned as being too numerous to count (NTC). And less than 10 CFU/mL are too less to count but still counted and noted.

Statistical Analysis

Differences in percentages of *Candida and Streptococcus* were analyzed using descriptive statistics, analysis of variance (ANOVA), and *post hoc* Bonferroni analysis. All these statistical analyses were done using SPSS® (Statistical Package for the Social Sciences) version 17.

Results

Enumeration of CFU was done before and after intervention with different denture cleansers. Before intervention, all the dilutions (10^{-1} to 10^{-10}) have shown NTC. The counting that showed numerous microorganisms was considered as NTC. After intervention, the enumeration of CFU was done, and values had been noted under different dilutions from 10^{-1} to 10^{-10}. Before intervention, there were numerous microorganisms, while after treating with denture cleanser, there was a countable number of microorganism, which shows that there was a decrease in the amount of microorganism, while using denture cleanser. But the first four dilutions showed NTC, and hence, for statistical analysis, the dilutions from 10^{-5} to 10^{-10} alone had been considered.

Antifungal Efficacy

A one-way ANOVA of antifungal efficacy for different denture cleansers (groups I to IV) was done for data at 10^{-5} and 10^{-10} dilution. Among the groups treated with denture cleansers, the mean count of *C. albicans* at 10^{-5} and 10^{-10} dilution was higher in group III followed by groups I, II, and IV. The mean counts of all groups were statistically significant, as the *p* value was 0.001.

At 10^{-5} dilution, the reduction of *C. albicans* in group I was compared with groups II, III, and IV individually using *post hoc* Bonferroni analysis (Table 1). Group IV was statistically significant in comparison to group I. Among seaweeds, group IV followed by group II were statistically significant in reducing *C. albicans.*

At 10^{-10} dilution, groups II and IV were equally effective to group I. Group I had higher antifungal efficacy on *C. albicans* and statistically significant in comparison to group III alone. Group II was

Table 2: Comparison of antifungal efficacy between the groups after treatment at 10^{-10} dilution

Dependent variables	*Group (I)*		*Group (J)*		*Mean difference (I–J)*	*Std. error*	*Sig. (p-alue)*
CFU 10 ($\times10^{-10}$)	I	Fittydent	II	*Sargassum polycystum* and *Turbinaria conoides*	3.048	7.094	1.000
			III	*Sargassum polycystum*	–41.619	7.094	0.001*
			IV	*Turbinaria conoides*	3.286	7.094	1.000
	II	*Sargassum polycystum* and *Turbinaria conoides*	III	*Sargassum polycystum*	–44.667	7.094	0.001*
			IV	*Turbinaria conoides*	0.238	7.094	1.000
	III	*Sargassum polycystum*	IV	*Turbinaria conoides*	44.905	7.094	0.001*

Post hoc Bonferroni analysis, *p value is significant if the value is <0.05

Table 3: Comparison of antibacterial efficacy between the groups after treatment at 10^{-5} dilution

Dependent variable	*Group (I)*		*Group (J)*		*Mean difference (I–J)*	*Std. error*	*Sig. (p-value)*
CFU 5 ($\times10^{-5}$)	I	Fittydent	II	*Sargassum polycystum* and *Turbinaria conoides*	214.952	29.937	0.001*
			III	*Sargassum polycystum*	3.857	29.937	1.000
			IV	*Turbinaria conoides*	297.095	29.937	0.001*
	II	*Sargassum polycystum* and *Turbinaria conoides*	III	*Sargassum polycystum*	–211.095	29.937	0.001*
			IV	*Turbinaria conoides*	82.143	29.937	0.045*
	III	*Sargassum polycystum*	IV	*Turbinaria conoides*	293.238	29.937	0.001*

Post hoc Bonferroni analysis, *p value is significant if the value is <0.05

Table 4: Comparison of antibacterial efficacy between the groups after treatment at 10^{-10} dilution

Dependent variable	*Group (I)*		*Group (J)*		*Mean difference (I–J)*	*Std. error*	*Sig. (p-value)*
CFU 10 ($\times10^{-10}$)	I	Fittydent	II	*Sargassum polycystum* and *Turbinaria conoides*	96.286	16.580	0.001*
			III	*Sargassum polycystum*	–106.857	16.580	0.001*
			IV	*Turbinaria conoides*	102.571	14.359	0.001*
	II	*Sargassum polycystum* and *Turbinaria conoides*	III	*Sargassum polycystum*	–203.143	16.580	0.001*
			IV	*Turbinaria conoides*	6.286	14.359	1.000
	III	*Sargassum polycystum*	IV	*Turbinaria conoides*	209.429	14.359	0.001*

Post hoc Bonferroni analysis, *p value is significant if the value is <0.05.

superior in its antifungal efficacy against *C. albicans* in comparison group III (Table 2).

On comparison of commercially available denture cleanser with seaweed, *S. polycystum* and *T. conoides* combination and *T. conoides* are equally effective to Fittydent for its antifungal efficacy against *C. albicans*. Fittydent was statistically significant in comparison to *S. polycystum* alone for its antifungal efficacy against *C. albicans*.

Among the seaweeds, *S. polycystum* and *T. conoides* combination and *T. conoides* are equally effective for their antifungal efficacy against *C. albicans*. *Sargassum polycystum* and *T. conoides* combination was statistically significant in comparison to *S. polycystum* alone for its antifungal efficacy against *C. albicans*.

Antibacterial Efficacy

Among the groups treated with denture cleansers on *S. mutans*, the mean count at 10^{-5} and 10^{-10} dilution was higher in group I followed by groups III, II, and IV. The mean counts of all groups were statistically significant, as the p value was 0.001.

At 10^{-5} dilution, group IV had higher antibacterial efficacy in comparison to all the groups, which was statistically significant. Group II had higher antibacterial efficacy in comparison to group I, which was statistically significant. Groups III and I are equally effective. Group II had higher antibacterial efficacy in comparison to group III, which was statistically significant (Table 3).

At 10^{-10} dilution, groups II and IV had higher antibacterial efficacy in comparison to group I, which was statistically significant. Group I had higher antibacterial efficacy in comparison to group III alone, which was statistically significant. Group II had higher antibacterial efficacy in comparison to group III, which was statistically significant. Groups II and IV are equally effective. Group IV had higher antibacterial efficacy in comparison to group III, which was statistically significant (Table 4).

On comparison of commercially available denture cleanser with seaweed, *S. polycystum* and *T. conoides* combination and *T. conoides* have higher antibacterial efficacy on *S. mutans* in comparison to Fittydent, which was statistically significant. Fittydent was statistically significant in comparison to *S. polycystum* alone for its antibacterial efficacy against *S. mutans*.

Among the seaweeds, *S. polycystum* and *T. conoides* combination had higher antibacterial efficacy in comparison to *S. polycystum*

which was statistically significant. *Sargassum polycystum* and *T. conoides* combination and *T. conoides* are equally effective for their antibacterial efficacy against *S. mutans*. *Turbinaria conoides* had higher antibacterial efficacy in comparison to *S. polycystum*, which is statistically significant for its antibacterial efficacy against *S. mutans*.

Discussion

This study compared the efficacy of seaweeds *T. conoides* and *S. polycystum* with a commercially available denture cleanser Fittydent for their antifungal and antibacterial activity. Using seaweed at a single strength has previously been evaluated by Dharmautama et al. using extract,[16] but none had used powder and combined it with other seaweed algae powder and tested it for its efficacy against various organisms. In the present study, a mixture of *S. polycystum* and *T. conoides* has been used to evaluate its efficacy against the most common organism, *C. albicans*, and the next most common organism, *S. mutans*, which cause problems in dentures and lead to denture stomatitis.

Every denture cleanser has both advantages and disadvantages. Immersion type of denture cleansers was found to be effective in reducing the colony count of microorganisms on the surface of the dentures.[17] In this study, Fittydent is used, which is an effervescent type containing 450 mg of sodium perborate monohydrate. The active composition of these denture cleansers, like EDTA, sodium perborate, and sodium bicarbonate has been efficient in reducing the biofilm, but these biofilms are associated with antifungal resistance, causing reduced efficacy.[17] So, there is a need for a denture cleanser to address the antifungal resistance.

A study conducted earlier by Ribeiro Rocha et al. has suggested chlorhexidine mouthwash as an adjuvant but it was not statistically proven.[18] In a previous study by Uludamar et al., different alkaline agents like Fittydent were compared with mouthwashes against *C. albicans* and it was shown that mouthwashes have better efficiency.[19] In this study, Fittydent has been compared with different seaweed algae. At 10^{-5} dilution, *T. conoides* had higher antibacterial efficacy in comparison to all the groups, which was statistically significant. At 10^{-10} dilution, *S. polycystum* and *T. conoides* combination and *T. conoides* were equally effective to Fittydent. Fittydent had higher antibacterial efficacy on *C. albicans* and statistically significant in comparison to *S. polycystum* alone.

Various herbal and natural extracts have been used as denture cleaners in recent times. A study conducted by Faot et al., using citric acid as a denture cleanser found that the *C. albicans* biofilm formed on the denture surface was comparatively reduced, but the recolonization could not be prevented.[20] In the present study, though citric acid was added to the tablet form of the seaweeds for its antibacterial and antifungal effects, the recolonization was not studied. In the present study, *S. polycystum* and *T. conoides* combination and *T. conoides* were equally effective to Fittydent. Long-term care elderly people in hospitals who cannot brush their dentures were considered, and Denture Brite, Polident, and Efferdent cleansers were used for denture cleaning in a previous study by Nevalainen et al.[21] It was seen that the use of denture cleansers significantly reduced the number of microorganisms on dentures in a hospitalized geriatric population. There were no significant differences among the cleansers in the reduction of *C. albicans* or *S. mutans*. The reduction in the number of *S. mutans* was significantly greater with Efferdent than with water.[21] In this study, at 10^{-5} dilution, *T. conoides* had higher antibacterial efficacy in comparison to all the groups which is statistically significant.

In a previous study by Beyari, three types of commercially available denture cleansers Fittydent, Corega, and Clorox (sodium hypochlorite solution) were used to evaluate the effect of the denture cleansers on the oral mucosa clinically and colony-forming units bacteriologically on *S. mutans*. The continuous decrease in the total colony forming units and *S. mutans*, together with the improvement in mucosal condition, suggests that the use of denture cleansers provides better oral health. Hypochlorite cleanser was less effective in comparison to Fittydent.[22] In the present study, Fittydent for comparison with the seaweed and found that at 10^{-10} dilution *S. polycystum* and *T. conoides* combination and *T. conoides* had higher antibacterial efficacy in comparison to Fittydent, which was statistically significant. Fittydent had higher antibacterial efficacy in comparison to *S. polycystum*, which was statistically significant. However, this is an *in vitro* study and it does not include any clinical parameters.

Sodium hypochlorite and sodium hydroxide cleaners have better antimicrobial activity but can also corrode and tarnish metal dentures. Effervescent cleaners can be used to effectively clean metal dentures but can degrade denture lining materials.[23] In a previous study by Murata et al., denture cleansers were tested on tissue conditioners, and the results showed that peroxide cleansers with enzymes were better compared with alkaline peroxide based on roughness evaluation, but the microbial efficacy of these cleansers was lower.[24] In this study, tissue conditioners are not used, but there is a need for denture cleanser that does not affect the surface properties and has improved antimicrobial properties. Household denture cleansers are also used often by the patients, and a study by Kumar et al., compared Fittydent and diluted vinegar for their efficacy on *C. albicans* and concluded that diluted vinegar was not effective in reducing the microorganism.[25]

Based on an earlier study by Shibu and Dhanam, it has been shown that *T. conoides* has an antifungal effect on *C. albicans*.[26] In this study, at 10^{-10} dilution, *S. polycystum* and *T. conoides* combination and *T. conoides* were equally effective to Fittydent. Fittydent had higher antibacterial efficacy on *C. albicans* and statistically significant in comparison to *S. polycystum* alone. More studies are needed to find the main components of these seaweeds and combine them to have an effective action against various microorganisms.

There is no literature evidence where *T. conoides* and *S. polycystum* have been compared and there is no literature regarding combining two seaweeds and using them as denture cleanser. Hence, in this study, the aim was to see the results of a comparison of seaweed as well as a comparison with the combined seaweed denture cleanser. This would open the door to an alternative for using *S. polycystum* as a denture cleanser. As observed in the study, *T. conoides* has proven to be effective against *S. mutans*. Two kinds of seaweed combined (double strength) were found to be more effective compared to single strength on *C. albicans*.

In future, more research is necessary to test the active component of *T. conoides* and *S. polycystum* that is responsible for its antifungal and antibacterial efficacy. Further *in vivo* studies need to be carried out to know more about clinical parameters. Also, studies related to the effect of prolonged use of these denture cleansers on the properties of denture base resins and liners need to be done.

Limitations of the Study

As the colony count above 2,000 is categorized as too NTC, the difference in the first four serial dilutions could not be evaluated.

Patient-related factors were not considered in the present study as it is an *in vitro* study. The study does not test the surface characteristics of denture base resins.

Conclusion

Within the limitations of this *in vitro* study, the following can be concluded:

Sargassum polycystum and *T. conoides* seaweeds combination and *T. conoides* seaweeds have statistically significant higher antibacterial efficacy against *S. mutans* and also equally effective to Fittydent for their antifungal efficacy against *C. albicans*. Within the seaweeds, the antifungal and antibacterial efficacy of *T. conoides* is more effective followed by *S. polycystum* and *T. conoides* combination in comparison to *S. polycystum* alone.

References

1. Peltzer K, Hewlett S, Yawson AE, et al. Prevalence of loss of all teeth (edentulism) and associated factors in older adults in China, Ghana, India, Mexico, Russia and South Africa. Int J Environ Res Public Health 2014;11(11):11308–11324. DOI: 10.4103/jfmpc.jfmpc_1181_19.
2. Al-Rafee MA. The epidemiology of edentulism and the associated factors: a literature review. J Family Med Prim Care 2020;9(4): 1841–1843. DOI: 10.4103/jfmpc.jfmpc_1181_19.
3. Sadig W. The denture hygiene, denture stomatitis and role of dental hygienist. Int J Dent Hyg 2010;8(3):227–231. DOI: 10.1111/j.1601-5037.2009.00413.x.
4. Baena-Monroy T, Moreno-Maldonado V, Franco-Martínez F, et al. *Candida albicans*, *Staphylococcus aureus* and *Streptococcus mutans* colonization in patients wearing dental prosthesis. Med Oral Patol Oral Cir Bucal 2005;10(Suppl. 1):E27–E39.
5. Salles MM, Badaró MM, Arruda CNF, et al. Antimicrobial activity of complete denture cleanser solutions based on sodium hypochlorite and *Ricinus communis* – A randomized clinical study. J Appl Oral Sci 2015;23(6):637–642. DOI: 10.1590/1678-775720150204.
6. Jain SG, Magdum D, Karagir A, et al. Denture cleansers: A review. OSR J Dent Med Sci 2015;14(2):94–96. DOI: 10.9790/0853-14249496.
7. Kalekar A, Hipparkar M, Karande S, et al. Herbal denture cleanser: Examples and recent developments. J Bio Innovat 2022;11(2);538–549. DOI: 10.46344/JBINO.2022.v11i02.26.
8. Remya RR, Samrot AV, Suresh Kumar S, et al. Bioactive potential of brown algae. Adsorp Sci Technol 2022;13. DOI: 10.1155/2022/9104835.
9. Cervino G, Fiorillo L, Herford AS, et al. Alginate materials and dental impression technique: A current state of the art and application to dental practice. Mar Drugs 2018;17(1):18. DOI: 10.3390/md17010018.
10. Stengel DB, Connan S, Popper ZA. Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. Biotechnol Adv 2011;29(5):483–501. DOI: 10.1016/j.biotechadv.2011.05.016.
11. Anil S, Venkatesan J, Chalisserry EP, et al. Applications of seaweed polysaccharides in dentistry. In: Seaweed polysaccharides; 2017. pp. 331–340. DOI: 10.1016/B978-0-12-809816-5.00017-7.
12. Balasubramaniam A, Arumugham IM, Nathan PS, et al. Emerging technologies and potential applications of algae in dentistry – A critical review. J Biotechnol 2022;360:1–10. DOI: 10.1016/j.jbiotec.2022.09.021.
13. Lomartire S, Gonçalves AMM. An overview of potential seaweed-derived bioactive compounds for pharmaceutical applications. Mar Drugs 2022;20(2):141. DOI: 10.3390/md20020141.
14. Jensen A. Present and future needs for algae and algal products. Fourteenth International Seaweed Symposium Hydrobiologia 1993. p. 15–23. DOI: 10.1007/978-94-011-1998-6_2.
15. Nazarudin MF, Alias NH, Balakrishnan S, et al. Chemical, nutrient and physicochemical properties of brown seaweed, *Sargassum polycystum* C. Agardh (Phaeophyceae) collected from Port Dickson, Peninsular Malaysia. Molecules 2021;26(17):5216. DOI: 10.3390/molecules26175216.
16. Dharmautama M, Manggau MA, Tetelepta R, et al. The effectiveness of *Sargassum polycystum* extract against *Streptococcus mutans* and *Candida albicans* as denture cleanser. J Int Dent Med Res 2019;12(2):528–532.
17. Anthony DH, Gibbons P. The nature and behavior of denture cleansers. J Prosthet Dent 1958;8:796. DOI: 10.1016/0022-3913(58)90100-8.
18. Ribeiro Rocha GDS, Neves Duarte T, de Oliveira Corrêa G, et al. Chemical cleaning methods for prostheses colonized by *Candida* spp.: A systematic review. J Prosthet Dent 2020;124(6):653–658. DOI: 10.1016/j.prosdent.2019.10.004.
19. Uludamar A, Ozkan YK, Kadir T, et al. In vivo efficacy of alkaline peroxide tablets and mouthwashes on *Candida albicans* in patients with denture stomatitis. J Appl Oral Sci 2010;18(3):291–296. DOI: 10.1590/s1678-77572010000300017.
20. Faot F, Cavalcanti YW, Mendonça e Bertolini Md, et al. Efficacy of citric acid denture cleanser on the *Candida albicans* biofilm formed on poly(methyl methacrylate): Effects on residual biofilm and recolonization process. BMC Oral Health 2014;14:77. DOI: 10.1186/1472-6831-14-77.
21. Nevalainen MJ, Nahri TO, Ainamo A. Oral mucosal lesions and oral hygiene habits in the home-living elderly. J Oral Rehabil 1997;24: 332–337. DOI: 10.1046/j.1365-2842.1997.d01-298.x.
22. Beyari MM. Tissue inflammatory response and salivary *Streptococcus mutans* count with three different denture cleansers. Afr J Microbiol Res 2011;5:965–974. DOI: 10.5897/AJMR10.083.
23. Abelson DC. Denture plaque and denture cleansers. J Prosthet Dent 1981;45(4):376–379. DOI: 10.1016/0022-3913(81)90094-9.
24. Murata H, Chimari H, Hong G, et al. Compatibility of tissue conditioners and denture cleansers: Influence on surface conditions. Dent Mater J 2010;29(4):446–453. DOI: 10.4012/dmj.2009-135.
25. Kumar MN, Thippeswamy HM, Raghavendra Swamy KN, et al. Efficacy of commercial and household denture cleansers against *Candida albicans* adherent to acrylic denture base resin: An in vitro study. Indian J Dent Res 2012;23(1):39–42. DOI: 10.4103/0970-9290.99036.
26. Shibu A, Dhanam S. In vitro antifungal activity of *Turbinaria conoides* collected from Mandapam Coast, Tamil Nadu, India. Life Sci Leaflet 2016;74:12–17. DOI: 10.19071/jes.2016.v7.3057.