The Effectiveness of Sargassum Polycystum Extract Against Streptococcus Mutans and Candida Albicans as Denture Cleanser

Mohammad Dharmautama[1], Ikhriahni[1]*, Marianti A. Manggau[2], Richard Tetelepta[3], Adam Malik[1], Meriyam Muchtr[1], Magfirah Amiruddin[1], Rustan Ambo Asse[1], Sitti Arfa[1]

1.Departments of Prosthodontic, Faculty of Dentistry Hasanuddin University, Makassar, Indonesia
2.Faculty of Pharmacy Hasanuddin University, Makassar, Indonesia
3. Department of Prosthodontics, Naval Dental Hospital Ladokgi Yos Sudarso, Makassar, Indonesia

Abstract

To find out the effect of *Sargassum polycystum* extract in inhibiting the growth of *Streptococcus mutans* and *Candida albicans* which used denture cleaning preparations. The extraction were using macerated method with 96% methanol as solvent. *Sargassum polysistum* extract activity were tested with several concentration of 1,25 %, 2,5%, 5%, 7,5% and 10%. *Streptococcus mutans* which incubated for 24 hours and *Candida albicans* for 48 hours. Control grup were using sodium perborate denture cleanser. *Sargassum polycystum* seaweeds extract showed antibacterial and antifungal activities with 2,5% minimal inhibitory concentration of *Streptococcus mutans* and 1,25% for *Candida albicans*. *Sargassum polycystum* extract inhibiti the growth of *Streptococcus mutans* and *Candida albicans* and can be developed into a denture cleanser.

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Introduction

Acrylic resin has been commonly used as denture base. As denture base, acrylic resin has some advantages and also some dissanvantages.[1] Arylic surfaces that face the mucosal or intaglio tissue, usually have a pit and porosity that are ideal for deposition of food waste accumulate microorganisms. The accumulation of these micro organisms are difficult to clean and known as denture plaque.[2] Plaque which attached to the denture is one of the factor that cause inflammation on the palatal mucosa and causing the denture stomatitis. Some Factors that cause the denture stomatitis are Candida albicans, bacterial infections, lack of denture hygiene, salivary flow and nutrients.[3]

A Research conducted by Daniel et al (2016) reported that on artificial teeth can be found some bacteria such *as Candida albicans* 65.5%, *Staphylococcus aureus* 34.4%, and *Streptococcus mutans* 53.3%.[4] *Streptococcus sp*. is the first bacteria that attached to the denture base and form a colony. *Streptococcus mutans (S.mutans)* can produces a extra cellular polysaccharide (PSE) which is not possessed by other bacteria. The substrate can open an access for other microorganisms to attach to the denture base.[5] *Candida albicans (C.albicans)* can penetrate acrylic resins and grow on the surface of denture so as to infect soft tissue.[6] *C.albicans* can release endoktoksin that damage the oral mucosa and cause denture stomatitis. therefore disinfection of denture is an important factor to be done.[7]

Denture cleaning can be done with mechanical and chemical techniques. Mechanical cleaning is done by using a toothbrush and ultrasonic.[8] Chemical cleaning can be done by immersing dentures in disinfectant solutions such as alkali peroxide, alkali hypochlorite, chlorhexidine, sodium hypochlorite, enzymes and herbs. Chemical based denture cleanser is more effective than mechanical, so it needs cleanser agents that have bactericide and fungicide effects, easy to use, and compatible with all denture materials.[9]

Natural ingredients that can be developed as an alternative to denture cleanser is the type of seaweed brown algae (*Phaeophyta*). Choudhury et al that examined the methanol

Corresponding author:
Ikhriahni,
Department of Prosthodontic
Faculty of Dentistry
Hasanuddin University
Makassar, Indonesia
E-mail: ikhriahni12@gmail.com

extract from three classes of seaweed class green algae (*Chlorophyta)*, brown algae (*Phaeophyta*), red algae (*Rhodophyta)*, type of brown algae (*Phaeophyta*) had the highest antibacterial activity.[10] A species of brown algae is *Sargassum polysyctum (S.Polycystum)*. *S.Polycystum* reportedly used for eczema, scabies, ulcer, and lung diseases,viral hepatitis, and antioxidant.[11] Composition of the active compounds *S.polysyctum* contains of flavonoids, alkoloid, saponin, phenol, and trapezoid serves as antibacterial, antiviral, and anti-fungal.[12] Tannin can be locally applied on wounds at the throat and oral cavity, the later especially in stomatitis. Tannin has a physiological action against bacteria growth.[13] As a detoxification agent, tannin can precipitate protein and form a specific compound interacting with protein and saliva pellicle to inhibit the attachment of *S. mutans* as well as reducing it.[14] A research which conducted by Rina et al suggest that the extract of *S. polysyctum* has an inhibitory effect on the antifungal activity of *C.albicans* at 100% consentration.[12] Based on this background, this research was conducted to determine the effectiveness of *S. polysyctum* extract against *S.mutans* and *C. albicans* at concentration 10% until 1,25% which can be used as basis for denture cleanser preparations.

Materials and methods

Seeweed material

S. polycystum brown algae is obtained from the coastal waters of Punaga, Takalar district, South Sulawesi. The algae was washed using sea water and then washed again with clear flowing water to remove salt, epiphytes and other suspended materials. Algae that have been cleaned then dried 5-6 days in a way aired and should not be directly exposed to the sun.

Preparation of brown algae extraction *S. polycystum*

This research was conducted at the Laboratory of Pharmaceutical Chemistry and Biofarmaka Laboratories Research Centre of Hasanuddin University, Makassar, Indonesia. The simplicia powder *S. polycystum* (1,7 kg) was immerted in 1500 ml of methanol for 3x24 hours, then filtered to obtain the filtrate. Futher, the filtrate was evaporated using a rotary evaporator at temperature 50ºC until the methanol solvent condentation was condensed in the condensor.

Breeding Media

The Making of Culture Stock

S. mutans (it was directly brought to Microbiology Laboratory, Pharmacy Faculty of Hasanuddin University) and *C. albicans* (ATCC 10231). One bacteria colony of *S. mutans* was collected using sterile oleal wire, then implanted on the Nutrient agar medium by tilting it, then incubated at 37°C for 1x24 hours, while *C. albicans* culture stock performed the same way as in *S.Mutans*, but the medium replaced by potato dextrose broth (PDB) and the broth incubated at 28ºC for 2x24 hours.

Preparation of Inoculum

From the growing stock of *S. Mutans* cultures taken with sterile osseous wire then suspended in a reaction tube containing 5 ml of 0.9% sodium chloride solution to obtain a bacterial suspension turbidity equal to the standard Mc Farland, solution turbidity. means the concentration of bacterial suspension is 10^8 CFU / ml. After that, dilution with 0.1 ml of bacterial suspension (10^8 CFU/ml) was inserted into a sterile tube and 0.9% sodium chloride solution was added and the volume was adjusted to 5 mL and then homogenized. The preparation of *C. albicans* inoculum was done together with *S. mutans* bacteria.

Determination of MIC (Minimum Inhibitory Consentration)

Determination of MIC is done by liquid dilution method. The liquid dilution method for *S. mutans* were wearing Nutrient broth (NB), for *C.albicans* and using PDB. The preparation of the extracts stock by weighing 2 grams of extracts dissolved in 10 ml DM50 10%. Then the 5 ml extracts stock was inserted into a 5ml (5% dilution NB as the first tube) reaction tube carried out 2x workmanship/replication. Furthermore, the dilution were done with removal of 5 ml of solution from the first tube to the last tube until the concentration of 10%, 7.5%, 5%, 2.5% and 1.25% obtained. Each concentration was

undergo the twice replication This test was done by dripping bacterial inoculum of 20 microliter into each tube. Then the whole test tube is good tube testing *S.mutans, C.albicans.* Then incubated for *S.Mutans* 24 hours 37C, for *C. albicans* 28 C for 48 hours. and the control group (+) effervesent tablet sodium carbonate (Polident) the way of making the concentration were equal to all of the treatment groups and only dissolved with aquades. Based on the observation, the incubation reaction tube did not show the effect of the suspension because of its solid color, further tested then be done by scratching each concentration of NA/MHA to the dilution medium on the petri dish for the concentration of 10%, 7.5%, 5%, 2.5% and 1.25% both for *S. mutans* and *C. albicans*

Results

After 24 h of incubation at 37ºC, the visual test in Figure. 1 showed a clear surface at a concentration of 2.5% to 10%. This suggests the presence of colony-resistant strains of *S. mutans* bacteria. At the concentration of 1.25% showed a visible growth of bacterial colonies. When compared to the control group the results obtained were similar to the seeding medium in the treatment group. Figure 2. *C. albicans* that had been incubated for 48 hours on the seeding medium did not show the growth of the colony. All showed the growth restriction of mushrooms with concentrations ranging from 1.25% to 10%. Figure 3 and the control group had the same result as the treatment group. Figure 4.

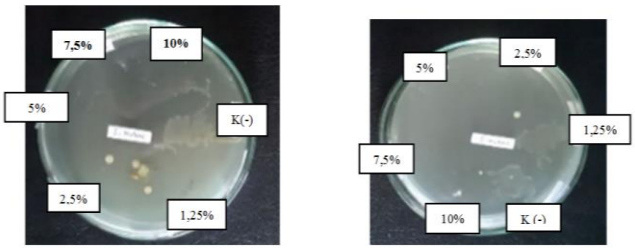

Figure 1. MIC of Sargassum Polycystum extract on the growth of Streptococus mutans.

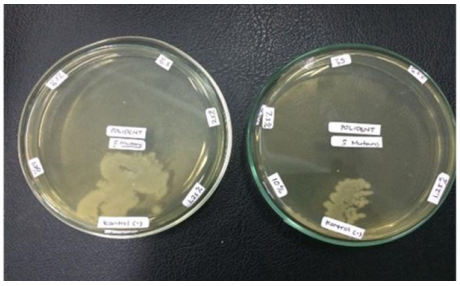

Figure 2. Positive control (Polident) on the growth of Streptococus mutans.

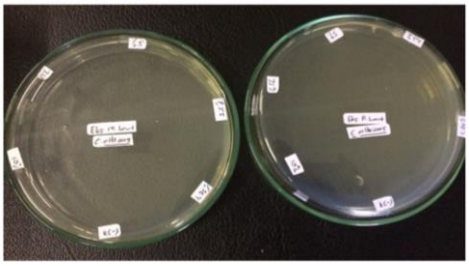

Figure 3. MIC of Sargassum Polycystum extract on the growth of Candida

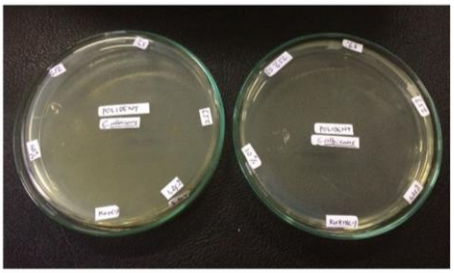

Figure 4. Positive control (Polident) on the growth of Candida albicans.

Discussion

The results showed that the extract of *S. polycystum* gave the antibacterial effect on *Strreptococus mutans* by measuring the MIC values. *S.polycystum* methanol extract with a concentration of 2.5%. was a minimal concentration and able to provide its effectiveness. It was all because when the extract was in contact with the cell it diffused on the cell membrane of *Strreptococus mutans* and produce anti-bacterial effects In addition the phenolic content of *S.polycystum* was able to inhibit the growth by destroying the membrane and denaturing the cell protein. Methanol extract from *Sargassum sp* shows very strong antioxidant activity and the antimicrobial against gram-positive and gram-negative bacteria such as *Bacillus subtilis, Escherichia coli* and *Staphylococcus aerus.*[15] However, that

Sargassum polyscystum cannot inhibit the growth of *Escheichia coli* and *Staphylococcus aureus* bacteria, this may be due to differences in the use of solvents in the manufacture extracts of n hexane and ethanol.[16]

At the concentration of 1.25%, there was no visible inhibitory effectiveness observed. One of the factors affecting the size of the inhibition area is the speed of agar diffusion and a thing which affects the speed of the agar diffusion is the concentration of the microorganism.[17] Compared to the observed MIC test the positive control group also exhibits the same effectiveness in the treatment group, this indicates that the antibacterial effectiveness of the material *S. polycytum* can be used as the ingredients of gentle desenfection drugs.

The results of *Candida albicans* colonies observation showed that all variations of *S. polycystum* concentration give the inhibitory effect. The polyphenol content of *S. polycystum* is also known to inhibit the growth of *C.albicans* colonies. This result support by Rina et al.[12] who obtained *S. polycystum* and *Sargassum polycrossfolium* extracts have an antifungal effect against *Candida albicans.*[12] Phenol compounds can damage the cell walls and cell membranes, precipitate proteins, and play a role in enzyme inactivation. The triterpenoid compound in the *Sargassum Sp* extract is lipophilic which can cause an obstruction of the fungi membrane and dissolve the lipid contained in the cell membrane.[18]

Compounds belonging to the flavonoid group polyphenols contained in the anthocyanin, tannin and saponin. The antifungal properties of tannins are known from the ability of tannins to disrupt cell membrane structures and inhibit the process of vegetative reproduction of *C.albicans.* Tannin can inhibit the biosynthesis of ergosterol which is the main sterol in the *Candida cell* membrane. This sterol is responsible for membrane fluidity and permeability, so that if sterol is not formed then the Candida cell membrane will be disrupted. There are some enzymes in the *Candida albicans* cell membrane which used in the synthesis of *C.albicans* cell wall. Tannins can bind to these enzymes so that they can not perform their function in the synthesis of *C.albicans* cell wall.[19] This has the same result with the Akiyama18 study which states that tannins have antimicrobial effects.[18,20]

Conclusions

Sargassum polycystum seaweed extract is able to inhibit the growth of *S. mutans*, *C. albicans* with the minimum inhibitory concentration value of Streptococcus mutans at 2.5% concentration, Candida albicans at concentration 1.25% and can be developed into a denture cleanser.

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Declaration of Interest

All the authors hereby declare that there is no conflict of interest.

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