SEA CUCUMBER AS INHIBITOR OF BACTERIAL ADHESION IN DENTAL PLAQUE: WOULD THIS BE A POSSIBLE REALITY?

Nurjehan Mohamed Ibrahim
Aspalilah Alias
Nor Nazifahani Noraini
Siti Sakinah Kamarudin
Abd. Rashid Li
Mohd Kamal Nik Hasan

ABSTRACT

In Malaysia, sea cucumber or Gamat is used as traditional medicine to relieve muscle pain and itchiness, and to treat cuts and wounds. It is made into ointment, cream, oil or gel and is also used as food supplement to boost energy. Stichopus horrens is one of the well-known sea cucumber species around indo-Pacific Ocean including Malaysia. This study assessed the ability of polysaccharide and protein extracts of Stichopus horrens to inhibit the adherence of Streptococcus mutans on tooth surface. Streptococcus mutans was grown inside eight test tubes kept in brain-heart infusion (BHI) broth containing 1% starch solution. Eight sound extracted premolars were autoclaved. Three different concentrations of both polysaccharide and protein extracts (25, 50 and 100mg/mL) were used to coat six sterilised premolars. Two other teeth acted as positive (chlorhexidine liquid coating) and negative (nil coating) control. All teeth were inserted into a different prepared test tube and incubated at 37ºC. After 48 hours, a swab taken from occlusal surface of each tooth was cultured on BHI agar. The inhibition effects were recorded by counting the S. mutans colonies in the agar. Polysaccharide extracts showed decreased counts of S. mutans with increasing concentrations of polysaccharide ranging from 13 to 2 colonies. In contrast, protein extracts of Stichopus horrens exhibited increment in S. mutans colony which ranged from 1 to 35 colonies with increasing concentrations of protein. The outcome of this research showed that polysaccharide extract of sea cucumber Stichopus horrens had the potential to be further expanded into a beneficial substance with therapeutic feature that could be used in preventive and restorative dentistry.

INTRODUCTION

Dental caries

Dental caries is the most common global oral health problem which is recognized as the primary cause of oral pain and tooth loss that affects both children and adults. Despite the preventive management done worldwide, there are still approximately 2.43 billion people (36% of the population) with dental caries in their permanent teeth. While, in baby teeth it affects about 620 million people or 9% of the population (Yadav K et al., 2016).

Dental caries is defined as a disease of demineralization of hard tissue of tooth structure by organic acid produced by bacteria in dental plaque. It is a multi-factorial disease, occurs when presence of four main factors including fermentable carbohydrate (sugar), bacterial acid (from dental plaque), susceptible tooth and time. However, other risk and modifying factors including genetics and biology, social environment, physical environment, health influencing behaviors and medical care, also influence the occurrence of dental caries (Fisher-Owens et al., 2007).

The process of caries commences within dental plaque. Bacterial biofilms develop due to the interactions between micro-organisms, host factors (salivary flow rate and buffering capacity) and a diet containing fermentable carboyhdrates. The bacterial composition of the biofilm reacts to changes in the local environment. These changes may lead to an imbalance in composition of the biofilm, change the overall metabolic activity of the biofilm and subsequently lead to disease (Marsh, 2012). The first step in the development of the biofilm is the formation of a thin layer containing mainly salivary proteins and bacterial enzymes like glycosyltransferases (Gtfs) that are able to bind some oral bacteria. Gtfs are also able to synthetize glucan on the pellicle which further increases the adherence of bacteria (Bowen W.H et al., 2011). *Streptococcus mutans* and sucrose have been observed to be the main modulators of the development of cariogenic biofilms (Paes Leme A.L et al., 2006).

Various substances with antimicrobial activity have been developed to inhibit bacterial adherence on teeth surfaces such as phenolic compounds, cetylpyridinium chloride (CPC) and clorhexididne (W. Krzyściak et al., 2014). CPC acts as an antibacterial substance which affecting the cell membrane of a microorganism, thus inhibiting the development of biofilm. Current application of CPC in products of oral hygiene is such as toothpastes and mouthwashes, and in dental materials, such as varnishes and restorative materials (W. Krzyściak et al., 2014).

Whilst there are varieties of modern medications and pharmaceutical products with evidences of efficient efficacies, many studies are being carried out to look for alternative treatment and prevention methods which are safe, economically beneficial and effective. The uses of naturally occurring organic molecules derived from terrestrial plants and/or animals (Taiyeb-Ali et al., 2003) as therapeutic agent in pharmaceutical and medical field becoming more popular and easily accepted by many people on the assumption that natural means safe. Therefore, it is undoubtedly beneficial if a replacement of imported synthetic biomaterials in dentistry can be developed by using natural-based ingredients that can be obtained from the environment.

Streptococcus mutans

Normal oral flora of a man consists of *Streptococcus* species as the major group of microorganism (Igarashi T. et al., 1998). Formation of dental plaque is one of the etiological factors of dental caries. This is due to the ability of the bacteria to form multi-dimensional complex structure known as biofilm. *Streptococcus mutans* is capable of forming bacterial biofilm through the mechanism of adhesion to the tooth surface. The discovery of long filamentous structures on Streptococcus surface determines the pathogenicity of biofilm structure (Kreikemeyer et al., 2011). These structures act as adhesive properties which play a role in adhering to host cells and tissues, as well as in biofilm formation. Increased virulence bacteria forming biofilm can be associated with a higher tolerance to low pH (W. Krzyściak et al., 2014). It has been recorded that the species of *Streptococcus mutans* is the major type of microorganisms that initiates the process of dental caries development on smooth surface of teeth (Kristofersson K. et al., 1985) and also well-adapted to tolerate an acidic environment and other stresses (Xiao et al., 2012).

Sea cucumbers

Our earth consists of land, air, water and life. More than 70% of earth surface is covered by water and oceans accounts for 96.5% of all earth's water, making the aquatic or marine ecosystem as the largest ecosystem of the earth. Similar with other ecosystem, marine ecosystem also performs certain functions that are critically important for organism. One of the functions is the production of plant biomass from sunlight and nutrients as the primary producer, which represents the basic food source for all oceans' life and for human ultimately. Besides, it acts as the home for marine animals such as protozoans, marine invertebrates (echinoderms, mollusks, segmented and non-segmented worms, jellies, coral, sea anemones, hyroids) marine vertebrates (fishes, birds, mammals), and plankton (phyto and zooplankton).

Sea cucumbers are one of the marine animals that are popular for its great benefits for nutritional, medicinal and pharmacological sources. They are soft-bodied echinoderms comprising a diverse group of flexible, elongated, worm-like organisms, with a leathery skin and gelatinous body, looking like a cucumber. Habitually, they tend to live on the sea floor in deep seas (Conand C et al., 1990). In Malaysia, nearly 42 species of sea cucumbers were identified where it contain high therapeutic capabilities including polysaccharides and protein (Bordbar et al., 2011).

Whilst there is a lot of studies done related to sea cucumber, there is no study that has observed the benefits of sea cucumber directly to the teeth. In dentistry, sea cucumber had been tested to have antibacterial ability against oral bacteria such as *Streptococcus salivarius* and *Streptococcus mutans* (Kiani N et al., 2014) and promote significant improvements in gingival disease due to its anti-inflammatory and healing properties. Nevertheless, there was no previous study done to look at the capability of sea cucumber in inhibition of bacteria on teeth surface or stimulating healing and repair of dental hard tissue.

The control of dental plaque can be achieved by mechanical oral hygiene procedures, but in many cases an adjunct chemical plaque control is needed. Antimicrobial and antiplaque product such as chlorhexidine digluconate, triclosan, and thymol are commonly incorporated into mouthwashes and toothpastes to give a chemotherapeutic property to reduce dental plaque formation. However some of these materials may give adverse effects and can be toxic (Forssten SD et al., 2010).

There are many previous studies that observed the benefits of sea cucumber as a potential natural product to be used in healthcare and patient treatment. Nevertheless, there is still no study done looking at the effects of sea cucumber directly on teeth. Sea cucumber species *Stichopus horrens* is one of the most common type of sea cucumber in the region of Malaysian ocean.

Thus, it is the aims of this study to evaluate the potential of the *Stichopus horrens sp* extract (polysaccharide and protein extracts) in three different concentrations inhibiting the adherence of bacteria *Streptococcus mutans* on the tooth surface. There is no obvious weakness in the already developed and established oral hygiene care products like antibacterial mouthwash, antibacterial toothpaste and fluoride gel that have been proven to eliminate bacterial accumulation on teeth. The purpose of initiating an observation into the sea cucumber extracts in this study was to record the possibility of these extracts to inhibit bacteria *Streptococcus mutans* from accumulating on teeth surface. Having known that *Streptococcus mutans* is the bacteria responsible for producing acids from sugar utilisation that resulting in development of dental caries, it is of great benefit that observation of possibility of this particular species of sea cucumber in inhibiting *Streptococcus mutans*, is made. Positive result promises suitability of this species of sea cucumbers to be further studied as possible anti-bacterial materials that could be commercialized as a material that is able to prevent the development of dental caries. The future sea cucumber based material could be an adjunct and alternative to the present antibacterial mouthwash and toothpaste that are used in our daily routine of oral hygiene care, with a possibility of less inexpensive market price since the material is based on a natural marine live.

MATERIALS AND METHOD

Materials

- Sea cucumbers' (*Stichopus horrens sp.*) protein and polysaccharide extracts
- 8 sound extracted premolar teeth

- Brain Heart Infusion (BHI) broth and agar
- Starch
- Liquid chlorhexidine (CHX)
- Bacteria *Streptococcus mutans* (*S.mutans*)

Methods

Sample collection

Samples of sea cucumber *Stichopus horrens* (Figure 1 and 2) were collected from Pulau Pangkor, Perak. The sea cucumber samples were cut along the ventral part of the body wall while all the internal organs were removed (Figure 3) and washed thoroughly with water. After that, they were cut into 4-5 pieces each prior to storing at -86 ° C.

Figure 1: Dorsal part of *Stichopus horrens sp*

Figure 2: Ventral part of *Stichopus horrens sp*

Figure 3: Sea cucumbers after removal of internal organs

Preparation of extracts

Samples were thawed from deep freezer after two weeks, cut into smaller pieces and the weight was measured.

Extraction of protein-rich fraction and polysaccharides fraction

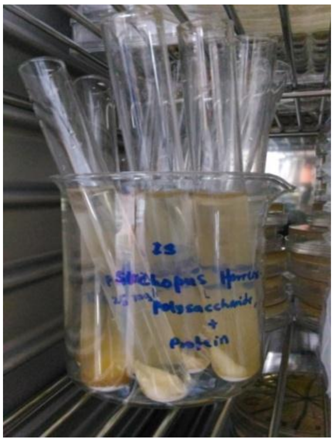
1500g of fresh sea cucumber were suspended in 1.5L of distilled water and boiled under reflux for 2 hours. The water extract was collected by filtration and cooled at room temperature. The insoluble residue was reused again by mixing with 2L distilled water and boiled under reflux for another 2 hours. The protein-rich fraction was obtained by collection of supernatant precipitation from suspension of filtered sea cucumber water with Seavag reagent (4: 1 ratio of Chloroform: Butanol). The collected precipitate was centrifuged and freeze-dried. The processes were repeated to get about 3.0g of dried protein-rich fraction.

Next, 3 volumes of ethanol was added to remaining water extract to precipitate the polysaccharide-rich fraction. The precipitate was collected by centrifugation and freeze-dried to give 2.0 g of dried polysaccharide-rich fraction.

Teeth samples preparations and *Stichopus horrens* extracts testing

Eight sound extracted premolar teeth were autoclaved. Teeth with caries or enamel defects are excluded. For this study eight test tubes were prepared to mimic intra-oral saliva (Bjorklund et al., 2011) containing 1% starch solution and 2mL of BHI broth. The BHI broth contained *Streptococcus mutans* bacteria that was cultured earlier with No.2 McFarland standard which equal to $6x10^8$ colony forming units (CFU/mL). Dried polysaccharides and protein extracts of sea cucumber *Stichopus horrens* was measured with analytical balance machine to get 100mg, 50mg and 25mg and were diluted with 1mL distilled water. Each of this concentration was applied as coating agent on the crown surfaces of the autoclaved teeth. Three teeth were coated with for 100mg/mL, 50mg/mL, and 25mg/mL protein extracts while another three teeth were coated with 100mg/mL, 50mg/mL, and 25mg/mL polysaccharides extract respectively. The remaining two teeth were used as control in this study where chlorhexidine liquid mouthwash was applied as positive control and no coating for negative control tooth sample. All of these prepared teeth were suspended and labelled in eight different prepared test tubes containing starch solution and *Streptococcus mutans* bacteria. Each test tube was then incubated for 48 hours in the incubator with temperature of 37°C (Figure 4).

Figure 4: Eight prepared test tubes with teeth samples.



Bacterial culture

After 48 hours, each tooth was removed from the test tube and rinsed under running distilled water without contaminating the occlusal surface of the tooth. Inoculation was done and the bacteria were swabbed from the fissure and occlusal surface of the tooth with one stoke using inoculation ring (Figure 5). Bacteria were cultured in the BHI agar and incubated for 2 days (Figure 6). Along the procedure, all instruments used were sterilized to avoid any contamination for the bacterial culture.

Figure 5: Bacteria were swab from the occlusal surface of the tooth.

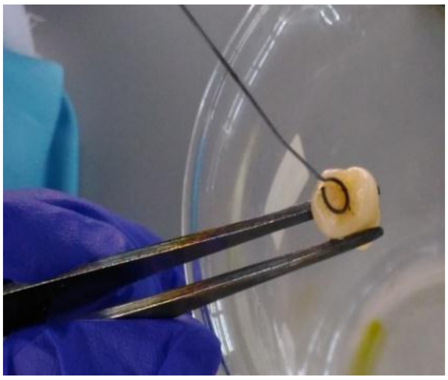
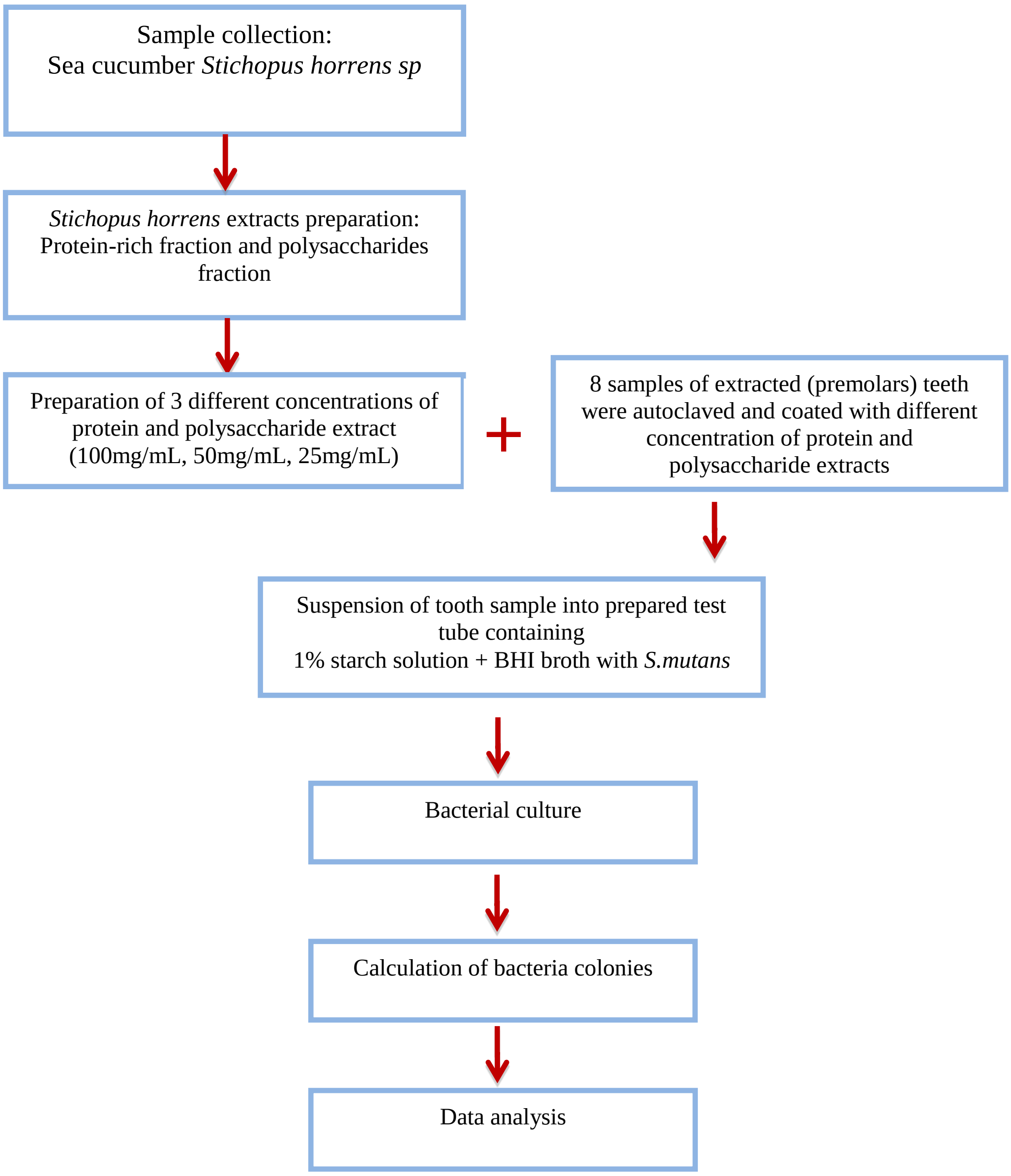


Figure 6: Cultured bacteria were incubated at 36°C for 2 days.

Evaluation procedure

After 2 days of incubation, presence or absence of *Streptococcus mutans* colonies were observed. The number of colonies was calculated and recorded. Data was tabulated into a table and graphs.

Flow chart of data collection process for research



RESULTS

From the bacterial culture, number of *S.mutans* colonies that grows was calculated. Polysaccharide extracts showed decreased counts of *S. mutans* with increasing concentrations of polysaccharide ranging from 13 to 2 colonies (Figure 7). In contrast, protein extracts of *Stichopus horrens* exhibited increment in *S. mutans* colonies which ranged from 1 to 35 colonies with increasing concentrations of protein (Figure 8).

Figure 7: Decreasing counts of *S.mutans* of 13, 11 and 2 colonies in increasing polysachharide concentrations of 25, 50 and 100mg/mL respectively.

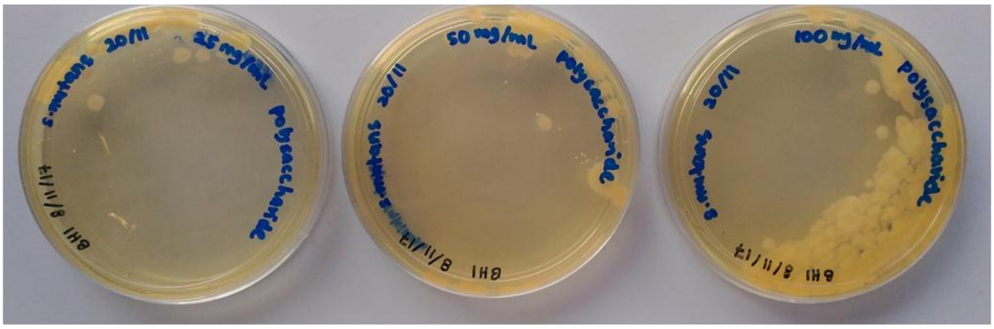
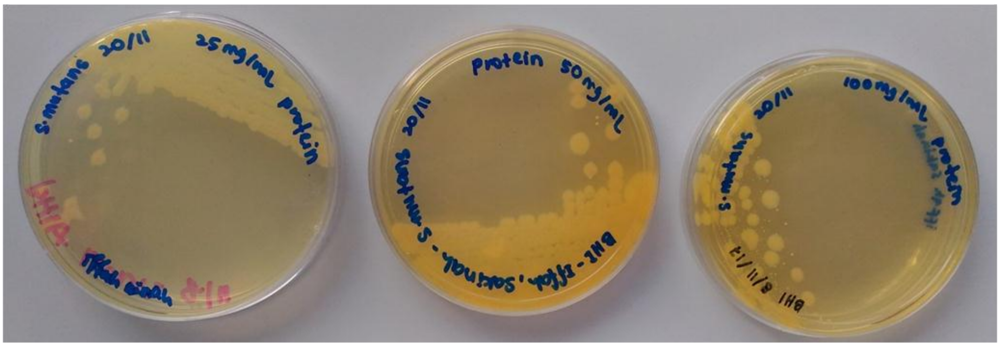


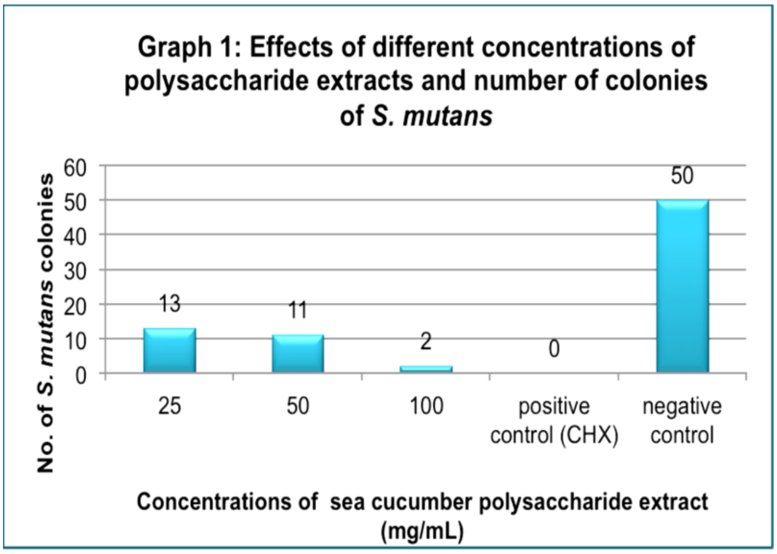
Figure 8: Increasing counts of *S.mutans* of 1, 3 and 35 colonies in increasing protein concentrations of 25, 50 and 100mg/mL respectively.

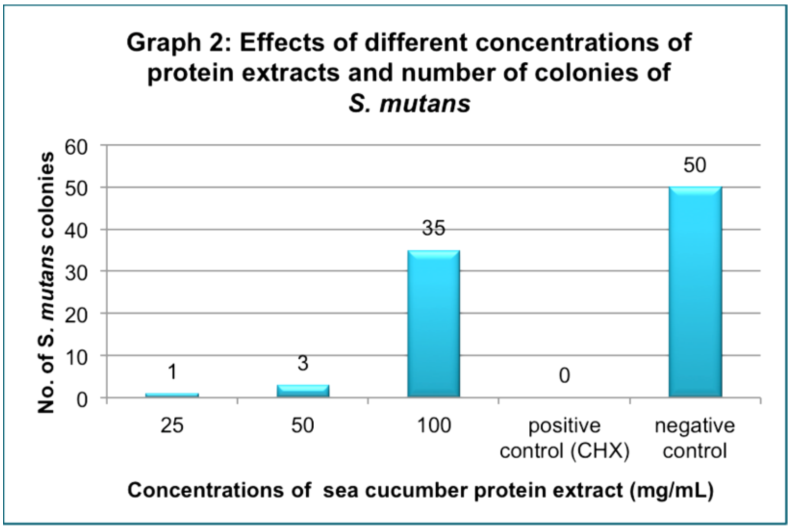


All of the data were tabulated into a table and graphs.

Table 1: Number of *S. mutans* colonies in different concentrations of polysaccharides and protein extracts

Concentration (mg/mL)	Number of colonies	
	Polysaccharide	Protein
25	13	1
50	11	3
100	2	35





DISCUSSION

Sea cucumbers are also known as trepang in Indonesia, beche-de-mer in French, namako in Japan, balatan in Philippines and gamat in Malaysia (Lovatelli A et al., 2004). In Malaysia, it is used commercially for the preparation of traditional medicinal products and for the medicinal properties of its coelomic fluid or "gamat" and with medicinal purposes in China. These raw products are traditionally processed into gamat oil and gamat water, and recently into medicated balm, toothpaste and soap (Conand C, 2006). Sea cucumbers have long been utilized in food and folk medicine systems of Asia and Middle East community (Huizeng , 2001).

A number of unique biological and pharmacological activities including anti-angiogenic, anticancer, anticoagulant, anti-hypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor and wound healing have been ascribed to various species of sea cucumbers (Bordbar et.al, 2011).

There are many different species of sea cucumbers that have been proven to be medicinally important bioactives (Sara B. et al., 2011). Among the beneficial bioactive compounds were triterpene glycoside (saponin), sulfated triterpene glycosides, cerberoside, chondroitin sulfates, glycosaminoglycan, lectin, sulfated polysaccharide, sterol, glycoprotein and steroidal sapogenins. *Stichopus horrens* is found in the Pacific Ocean from Malaysia to the Society Islands, around French Polynesia, and from southern Japan and Hawaiito New Caledonia. The body of *S. horrens* is grey-brown with irregular grey-white spots

(Massin C et al., 2002). It is cryptic and lethargic by day, and can be found in cracks, caves and crevasses in the rocky substrate at night. In China, this type of sea cucumber is used as food (Chen J, 2003).

Sea cucumbers are claimed as the most important commercial and medicinal group among echinoderms which are popular for its great benefits for nutritional, medicinal and pharmacological sources. Sea cucumbers have an impressive profile of valuable nutrients such as as Vitamin A, Vitamin B1 (thiamine) and Vitamin B2 (riboflavin), Vitamin B3 (niacin), and minerals, especially calcium, magnesium, iron and zinc (Bordbar et.al, 2011). Pharmacologically, it was proven to have significant anti-angiogenic, anticancer, anticoagulant, anti-hypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor and wound healing activities which depends on various species of sea cucumbers. These pharmacological effects and medicinal benefits are linked to the presence of a wide array of bioactives especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulphated polysaccharides, sterols (glycosides and sulfates), phenolics, cerberosides, lectins, peptides, glycoprotein, glycosphingolipids and essential fatty acids (Boardbar S et al., 2011).

A high amount of good-quality protein in sea cucumber is linked with its beneficial effects on serum triglyceride levels (Taboada et.al, 2003). According to a study done by Qin Z. et.al, sea cucumber protein, especially produced from body wall, is rich in glycine, glutamic acid and arginine which have remarkable function in immune regulation. Glycine can stimulate production and release of IL-2 and B cell antibody thus, enhance phagocytosis. Glycine and glutamic acid are essential components for cells to synthesize glutathione which can stimulate activation and proliferation of NK cell. Arginine can enhance cell immunity by promoting activation and proliferation of T-cell. Besides, about 70% of sea cucumber body wall protein is comprised of collagen. Collagen is recognized as a valued component in the connective tissues, due to its usefulness and specific distribution (Qin Z et al., 2008).

In 2003, a study of sea cucumber toothpaste called 'gamadent' was carried out. It has been reported that participants who used a toothpaste containing sea cucumber extract showed significantly greater improvements in several markers of gum health such as plaque buildup and bleeding which may be beneficial to people with gum disease and topical exposure of gingival tissue to sea cucumber extract over 3 months was reported to be safe (Taiyeb-Ali et al., 2003). Antifungal activity of sea cucumber against oral Candida sp. was proved by reduction of the number of oral Candida in elderly individuals in a nursing home who took *S. japonicas* jelly thrice-daily (Yano A et al., 2013).

Another research recorded that sea cucumbers (*Actinopyga mauritiana*, *Holothuria scarba*, *Bohadschia marmorata* and *Holothuria leucospilota*) had protein as the highest compound followed by carbohydrates and a low fat contents. The bioactive compounds in sea cucumber describe its efficacy in tissue regeneration and inflammatory diseases (Omran, 2013).

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages and on hydrolysis give the constituent monosaccharides or oligosaccharides. They range in structure from linear to highly branched.

Both polysaccharides and protein extracts of sea cucumber *Stichopus horrens* have been observed to have positive antibacterial effects towards *S. mutans* (NM Ibrahim et al., 2016). Sea cucumber contains high amount of sulphated polysaccharides known as fucosylated chondroitin sulphate glucose aminoglycan (GAG) which is high in sulphated glycan. This sulphated glycan can be divided into three fractions which are fucose, fucoidan and amino sugar with glucoronic acid. The antibacterial properties of sea cucumber mainly come from the fucoidan fraction, which consist of linear polysaccharides with repeated glycosidic linkages of L-fucose and sulphate ester group (EA Siahaan et al., 2017).

In this study polysaccharide extract was shown to be able to inhibit *S. mutans* adhesion on teeth surface. The functional group of hydroxyl ion (OH) and amino ion (NH) in polysaccharide formed a binding with the outer layer of *S. mutans* that led to failure of the bacteria to multiply. In contrast, number of *S. mutans* is increased in increasing concentrations of protein extract. The amino acid histidine in protein extract of *Stichopus horrens* becoming the substrate for multiplication of *S. mutans*, therefore assisting in multiplication of *S. mutans*.

CONCLUSION

In conclusion, polysaccharide extract of sea cucumber *Stichopus horrens* possesses antibacterial effects towards *S. mutans* while the protein extract may acts as a nutrient source for the *S. mutans* to grow. This was shown in this study by the difference of *S. mutans* number on the BHI agar counted by direct visualization. Polysaccharide component of *Stichopus horrens* is therefore can be concluded as potentially beneficial as an additional material or medicament to be used in the preventive and restorative management of caries. Nevertheless, contaminations of the BHI agar by other bacteria or possible candida strains were also noted in this study.

Polysaccharide extract of *S. horrens* may become an additional substance to be incorporated into the already established materials of oral hygiene care including mouthwash and toothpaste. Knowing that the polysaccharide component is able to prevent accumulation of *S. mutans* on teeth, it would be an upgrade to the features of varieties of mouthwashes and toothpastes used today, if polysacharide is incorporated as one of the substance in those products, as this may enhance the prevention of plaque (bacteria) from sticking on the teeth surface. Since sea cucumber is one of the natural products derived from the marine lives, a possible adverse effect due to toxicity or non-compatibility of a product can be reduced.

In addition to that, polysaccharide component of *Stichopus horrens* can be further evaluated to create a possible lining material for teeth. Having known that sea cucumbers have anti-inflammatory and antibacterial feature, and also able to assist in healing of mild cuts and burns, it is not impossible that this creature may also have the ability to stimulate dentine repair. Human's teeth have the natural reflection of self-repair when caries start to progress and extends from enamel into the dentine layer of teeth. When caries (bacteria) enters the dentine layer, the inner dentine is stimulated to produce more dentine (secondary and tertiary dentine) in order to protect the pulp (blood vessels and nerves) of the tooth from bacteria invasion. The process of secondary and tertiary dentine layers development slows down the caries penetration into pulp. However, caries progression is only eliminated when it is removed from the tooth during a restorative procedure by a dental clinician. Natural products like sea cucumber extracts (polysachharide) are a great potential source for therapeutic agents that can be used to enhance secondary or tertiary dentine development.

Further studies related to polysaccharide component of *Stichopus horrens* are necessary in order to look at the capability of this particular component of sea cucumber to be commercialized as a possible therapeutic and prophylactic property in dentistry. Bacterial count that was recorded in this study was calculated by means of direct vision. A more accurate manner of observing increment or reduction of bacterial count is necessary in future work, like using the scanning electron microscope (SEM) to explore the biofilms collected from teeth surface.

ACKNOWLEDGEMENT

The authors would like to thank Universiti Sains Islam Malaysia for providing the grant Geran Jangka Pendek PPP/USG – 0115/FPG/30/12815.

REFERENCES

Björklund, M., Ouwehand, A. C., & Forssten, S. D. (2011). Improved artificial saliva for studying the cariogenic effect of carbohydrates. *Current Microbiology*, 63(1), 46-49.
Bordbar S, Anwar F, Saari N. (2011). High-value components and bioactives from sea cucumber for functional foods-a review: *Journal of Marine Drugs*, 1761-805.
Bowen, W. H., & Koo, H. (2011). Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. *Caries Research*, 45(1), 69-86.
Chen, J. (2003). Overview of Sea Cucumber Farming and Sea Ranching Practices in China. *SPC Beche-de-mer Information Bulletin*, 18, 18-23.
Conand C. (1990). The Fishery Resources of Pacific Island Countries Part 2: Holothurians. Food and Agriculture Organization of the United Nations; Rome, Italy: *FAO Fisheries Technical Paper*, 2722, 143.
Conand, C. (2006). Harvest and Trade: Utilization of Sea Cucumber; Sea Cucumber Fisheries; Current International Trade; Illegal, Unreported and Unregulated Trade; By-catch; and Socio-economic Characteristics of the Trade in Sea Cucumbers. In: *Proceedings of the CITES Workshop on the Conservation of Sea Cucumbers in the Families Holothuriidae and Stichopodidae, 1–3 March, 2004, Kuala Lumpur, Malaysia A. Bruckner, (ed). NOAA Technical Memorandum*, NMFS-OPR-34, 51–73.
EA Siahaan, R Pangestuti, H Munandar, SK Kim. (2017). Cosmeceuticals Properties of Sea Cucumbers: Prospects and Trends: *Cosmetics*, 4(3), 26.
Fisher-Owens SA, Gansky SA, Platt LJ, Weintraub JA, Soobader MJ, Bramlett MD. (2007). Influences On Children's Oral Health: A Conceptual Model. *Pediatrics*,120, 510-520.
Forssten SD, M Bjorklund M, AC Ouwehand AC. (2010). Streptococcus mutans, Caries and Simulation Models: *Nutrients*, 2(3), 290-298.
Huizeng, F. (2001). Sea Cucumber: Ginseng of sea. *Zhongguo Mar. Med*, 82(4), 37-44.
Igarashi T. Yamamoto A., Goto N. (1998). Polymerase chain reaction for identification of oral streptococci: Streptococcus mutans, Streptococcus sobrinus, Streptococcus downei and Streptococcus salivarius. *J. Microbiol Methods*, 34, 81-91.
Aas JA, Griffen AL, Dardis SR. (2008). Bacteria of Dental Caries in Primary and Permanent Teeth in Children and Young Adults. *J Clin Microbiol*, 46(4), 1407–1417.
Kiani N, Heidari B, Rassa M, Kadkhodazadeh M, Heidari B. (2014). Antibacterial activity of the body wall extracts of sea cucumber (Invertebrata; Echinodermata) on infectious oral streptococci. *J Basic Clin Physiol Pharmacol*, 25(4), 367–373.
Kreikemeyer B, Gámez G, Margarit I, Giard JC, Hammerschmidt S, Hartke A, Podbielski A. (2011). Genomic organization, structure, regulation and pathogenic role of pilus constituents in major pathogenic Streptococci and Enterococci. *Int J Med Microbiol.* 301(3), 240–251.
Kristofersson K, Grondahl JG, Bratthall D. (1985). The more Streptococcus mutans, the more caries on approximal surfaces. *J Dent Res,* 64, 58-61.
Lovatelli A, Conand C. (2004). Advances in sea cucumber aquaculture and management. *FAO fisheries technical paper United Nations Food & Agriculture ORG*, 58, 463.
Manan WW, Mahalingam SR, Arshad K, Bukhari SI, Ming LC. Safety and efficacy of sea cucumber containing products. (2016). *Arch Pharma Pract,* 7(1), 48-52.
Marsh, P. D. (2012). Contemporary perspective on plaque control. *British Dental Journal*, 212(12), 601-606.
Massin C, Zulfigar Y, Hwai TSH, Boss RSZ. (2002) The genus *Stichopus* (Echinodermata: Holothuroidea) from the Johore Marine Park (Malaysia) with the description of two new species. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie*, 72, 73–99.
Mourão PA. (1991). Proteoglycans, glycosaminoglycans and sulfated polysaccharides from connective tissues. *Mem. Inst. Oswaldo Cruz*, 86(3),13-22.

NM Ibrahim, FM Rafie, SNZ Mohd Zahid, RM Hanafiah, SA Abd Ghafar, AR Li, A Alias. (2016). Antibacterial effects of *Stichopus horrens* (sea cucumber) against *Streptococcus mutans*. *Proceedings of a PDAA conference 2016 Tokyo, Japan,* 156.
Omran NS. (2013). Nutritional value of some Egyptian sea cucumbers. *African Journal of Biotechnology,* 12(35), 5466-5472.
Paes Leme, A. F., Koo, H., Bellato, C. M., Bedi, G., & Cury, J. A. (2006). The role of sucrose in cariogenic dental biofilm formation--new insight. *Journal of Dental Research,* 85(10), 878-887.
Qin Z., Jing-feng W, Yong X, Yi W, Sen G, Min L, Chang-hu X. (2008). Comparative study on the bioactive components and immune function of three species of sea cucumber. *J. Fish. Sci. China,* 23, 1661-1670.
Salli KM, Forssten SD, Lahtinen SJ, Ouwehand AC. (2016). Influence of sucrose and xylitol on an early *Streptococcus mutans* biofilm in a dental simulator. *Archives of Oral Biology*, 70, 39-46.
Sara B, Farooq Anwar, Nazamid Saari. (2011). High-Value Components and Bioactives from Sea Cucumbers for Functional Foods – A Review. *Mar Drugs*, 9(10), 1761-1805.
Taboada MC, Gonzalez M, Rodriguez E, Value and effects on digestive enzymes and serum lipids of the marine invertebrate Holothuria forskali. (2003). *Nutr. Res*, 23, 1661-1670.
Taiyeb-Ali TB, Zainuddin SLA, Swaminathan D, Yaacob H. Efficacy of 'Gamadent' toothpaste on the healing of gingival tissues: a preliminary report. (2003). *Journal of Oral Science,* 45(3), 153-159.
W. Krzyściak, A. Jurczak, D. Kościelniak, B. Bystrowska, and A. Skalniak. (2014). The virulence of Streptococcus mutans and the ability to form biofilms. *Eur J Clin Microbiol Infect Dis,* 33(4), 499–515.
Xiao, J., Klein, M. I., Falsetta, M. L., Lu, B., Delahunty, C. M., Yates, J. R., 3rd, et al. (2012). The exopolysaccharide matrix modulates the interaction between 3D architecture and virulence of a mixed-species oral biofilm. *PLoS Pathogens*, 8(4), e1002623.
Yadav K, Prakash S. (2016). Dental caries: A review. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 6(53), 01-07.
Yano A, Abe A, Aizawa F, Yamada H, Minami K, Matsui M, Kishi M. (2013). The Effect of Eating Sea Cucumber Jelly on Candida Load in the Oral Cavity of Elderly Individuals in a Nursing Home. *Mar. Drugs,* 11, 4993-5007.

Nurjehan Mohamed Ibrahim
Faculty of Dentistry
Universiti Sains Islam Malaysia
Email: nurjehan.mohamed@gmail.com

Aspalilah Alias
Faculty of Dentistry
Universiti Sains Islam Malaysia

Nor Nazifahani Noraini
Faculty of Dentistry
Universiti Sains Islam Malaysia

Siti Sakinah Kamarudin
Faculty of Dentistry
Universiti Sains Islam Malaysia

Abd. Rashid Li
Department of Phytochemical Forest Research Institute of Malaysia

Mohd Kamal Nik Hasan
Department of Phytochemical Forest Research Institute of Malaysia