Screening of Seaweeds Potential Against Oral Infections

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ABSTRACT

This research is designed to screen the beneficial point of Caulerpa lentillifera (C. lentillifera) and Kappaphycus alvarezii (K. alvarezii) seaweeds. The extracts of both seaweeds were tested for their antibacterial property against the common causative agents for oral infections; the Staphylococcus aureus (S. aureus) and Streptococcus mutans (S. mutans). The collected seaweeds from the sea of Sabah were extracted using aqueous and methanol extraction methods. The extracted substances from aqueous and methanol extractions from each seaweed were subjected to separate drying process and kept at 55 ± 2ᴼC. Since K. alvarezii was more suitable with another aqueous extraction method, it was therefore excluded from the experiment. Fresh seaweed samples were prepared at concentration of 25 to 200 mg/ml. For disc diffusion method, each tested extract was impregnated onto a disc and placed on Mueller Hinton agars that were inoculated with S. aureus and S. mutans separately. After 24 hours incubation, the zones of inhibition were measured. C. lentillifera and K. alvarezii methanol extracts showed antibacterial activity against S. aureus and S. mutan as well as the C. lentillifera aqueous extracts. The results demonstrated that the C. lentillifera and K. alvarezii extracts produced antibacterial activities against S. aureus and S. mutans using disc diffusion method.

Keywords: Caulerpa lentillifera; Kappaphycus alvarezii; oral infection; zone of inhibition

INTRODUCTION

A complex composition of microflora including Staphylococcus aureus (S. aureus) harbors the oral cavity [37]. In the general systemic health, S. aureus is known as a common medical pathogen for many years [15]. In the oral cavity however, S. aureus is recognized as a normal flora but with opportunistic in nature. It was evident that S. aureus was isolated not only from ill patients but also from healthy individuals of various age groups including the infants up to the elderly [29]. In the pathogenesis of oral infection, it was noted that in the ill patients, particularly the elderly who were receiving parenteral nutrition and in patients with advanced malignant diseases, the significant reduction in saliva flow rate is an important contributing factor [29]. The mouth became dry as the lubricating effect of the saliva was lacking. More importantly, the cleaning effect of saliva and the antimicrobial factors it contained were reduced. Presence of lysozyme in the saliva for example, is believed to keep and maintain the harmony of normal flora in the oral cavity. When lysozyme is less, healthy normal flora composition was disturbed and this provides opportunity for oral infection to occur. In conditions where the immune system were compromised such as during ill and with the reduced saliva flow rate, the opportunistic bacteria will multiply and causing infection. In favorable condition, the intraoral S. aureus has caused angular cheilitis [33], parotitis [7], staphylococcal mucositis [11] and acute dento-alveolar infections [9]. Besides, with the presence of prosthetic devices within the oral cavity, there is marked increase of S. aureus population and the affected individual are more prone towards oral infection [30]. The causal agent of oral infections is not solely the opportunistic microflora but also...
caused by oral pathogen or oral pathogenic bacteria.

Presence of oral pathogen on the tooth surface in dental plaque has proven to cause caries, the commonest infectious and transmissible dental infection [19]. In fact, caries have significantly contributed to the decay, missing and fillings (DMFT) [4]. Oral Streptococci such as Streptococcus mutans (S. mutans) and Porphyromonas gingivalis (P. gingivalis) are some of the oral pathogens that are commonly associated with dental plaque, the formation of caries and inflammation of oral tissue [19]. Although the involvement of oral pathogenic bacteria does not necessarily result in caries, the involvement of cariogenic bacteria does [37].

Cariogenic bacteria including the S. mutans ferments carbohydrate particles and produces organic acids that is able to dissolve tooth minerals which later results in caries [14]. In the disease process of any oral or dental infection, the initiation of bacterial involvement begins with the attachment of the bacteria to any components of the oral cavity such as the enamel, tongue, gums or being present in the saliva [10]. Since direct involvement of any bacteria is crucial in the pathogenesis of any bacterial infection, therefore it is ideal to investigate the possibility of controlling the activities of the bacteria in order to prevent the infection.

In the pharmaceutical world, commercial antibiotics are always available to treat medical and oral diseases of bacterial origins. However, as the awareness upon the threat of antibiotic resistance increased [37], the need for alternatives is becoming a demand. Therefore, the search for antibacterial properties from natural resources particularly from herbal family emerged. The quest for antibacterial agent from natural sources was then shifted towards the potential of marine plants including the seaweeds [23]. Based on the historical perspective, seaweed were mainly used as food source. The seaweeds are therefore not alien to us and in fact, have been used in Asian countries since 5000 years ago as functional foods and some even claimed seaweed as medicinal herbs [20]. Today, the beneficial point of seaweeds is not limited as food source for coastal population but has extended further when it has been demonstrated to contain substances of human health benefits [16].

The seaweeds are belonging to algae group. Biologically, based on their nutritional value and chemical composition, the seaweeds are classified as Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae) [6]. In general, there were reports on the antioxidant properties of the brown, red and green algae [5] while other researcher discovered antibacterial activities of some seaweeds extracts against pathogen of general health interest [31], antifungal [26] and anti-inflammatory [1] activities.

Caulerpa lentillifera (C. tentillifera) and Kappaphycus alvarezii (K. alvarezi) are belonging to the Chlorophyta and Rhodophyta algae respectively. These edible seaweeds are abundantly available in the sea of Sabah. C. lentillifera was claimed to be consumed as food, eaten raw commonly as salad and used in general medicine [36]. Besides, it possessed antidiabetic properties by stimulating insulin secretion and increase glucose uptake in adipocyte [28] and its polysaccharide compound had demonstrated immunostimulatory activity on macrophage cells [21]. The Caulerpa species itself were reported to be used as vitamin and mineral sources [2] and were associated with antihypertensive property [18]. On the other hand, K. alvarezii is known for its ability to produce carrageenan [25]. The carrageenan is the hydrophilic colloid that can be obtained by aqueous extraction of K. alvarezii or other carrageenan seaweed species [32] which were used as food thickener, agar and stabilizer in cosmetics creams [13]. The K. alvarezii also have been reported to demonstrate antibacterial properties against plant bacteria [35], possess antioxidant properties [17] and has the ability to bind to mutagenic amines [24].

In order to investigate the antibacterial activity of seaweeds extracts, antimicrobial susceptibility testing (AST) was carried out. The disc diffusion method is one of the methods of choice to screen the potential of antibacterial properties [27] of particularly new agent such as from plant extracts, including the seaweeds. In addition, the disc diffusion method has been the mainstay for AST in most clinical microbiological laboratories since Bauer, Kirby et al., first described this technique in the 1960s [12]. It is a well-established method that explained the appearance of zones of inhibition in determining the susceptibility of antibiotics or antimicrobial agents against a particular tested bacterium. The zones of inhibition are the area on the agar plate where the growth of bacterial lawn around the disc is prevented by antibacterial agent that was placed on the disc, on top of the agar [12].

2. Objectives:

The purpose of this research was to screen the antibacterial potential of C. lentillifera and K. alvarezii extracts against S. aureus and S. mutans using disc diffusion method.

3. Materials and Methods

3.1. Preparation of seaweed extracts:

Seaweeds obtained from the sea of Sabah were cleansed and sundried. Each dried seaweed was placed in mortar and ground to smaller pieces. For aqueous extraction, 10g of each dried seaweed were separately stirred at 60 °C for 2 hours in 100 ml distilled water. The aqueous extract obtained from each seaweed was then filtered through Whatman No. 1 filter paper and later freeze-dried for its final aqueous product.
In another experimental setup, 10g of each dried seaweed were separately stirred for 2 hours in 200 ml of 90% methanol (Sigma Aldrich, USA). The crude methanol extract obtained was then filtered through Whatman No. 1 filter paper and subjected to evaporation under vacuum at 40°C using rotary evaporator and later freeze-dried. The resultant freeze-dried seaweed extracts were stored at −20°C until further use. Fresh seaweed extracts were prepared using Mueller-Hinton broth for each experiment.

3.2. Preparation of inoculums:

Staphylococcus aureus and Streptococcus mutans were maintained on Trypsin Soy Agar (TSA) and kept at 4°C. Bacterial inoculum was prepared by sub-culturing each bacterium on separate bijou bottles containing 15 ml of Mueller-Hinton broth. The bacterial inoculums were then incubated for 24 hours before used.

3.3. Disk Diffusion Susceptibility Test:

Using an aseptic technique, a sterile swab was dipped into the broth culture of S. aureus and S. mutans containing 10^8 CFU/ml separately. The amount of organism used was determined by turbidity test using visual approximation. A spectrophotometer was used and the wavelength was set at 625nm. A sample of cultured broth should obtain optical density value of 0.08 to 0.10 which suggest that the sample contained 10^5 CFU/ml bacteria. Dilution using sterile Mueller-Hinton broth was done in the case if the reading was higher that the range. Each cultured broth was then spread onto a Mueller-Hinton agar (MHA) plate using sterile swab separately to form bacterial lawn. The plates were allowed to dry for about 5 minutes. In determining the antibacterial potential of a tested sample, filter paper was used as the discs. Whatman No. 1 filter paper was cut into standard disc with diameter of 6 mm. Each disc was labelled accordingly. Fresh seaweed extracts were prepared by dissolving the freeze-dried aqueous and methanol extracts of the seaweeds in Mueller-Hinton broth separately at various concentrations of 25, 50, 100 and 200 mg/ml. Each seaweed extract was then filtered through 0.20 μm filter disc. Subsequently, each disc was impregnated with 5 μl of C. lentillifera and K. alvarezi aqueous and methanol extracts of concentrations from 25 to 200 mg/ml separately and allowed to dry. The discs of C. lentillifera aqueous and methanol extracts were placed and pressed gently onto the prepared agars containing the S. aureus and S. mutan bacterial lawns separately. The discs of K. alvarezi methanol extracts were also placed accordingly. All the plates were then inverted and incubated for duration of 24 hours at 37 °C. After the 24 hours incubation, the diameters of the zones of inhibition that appeared were measured using digital caliper. The AST of each seaweed extract on each bacterium tested were performed in triplicates. Filter discs impregnated with 5 μl of Mueller-Hinton broth were used as negative controls.

3.4. Data analysis:

The data on diameters of zones of inhibition were analyzed using SPSS program for Windows. The mean value of inhibition effect produced at each concentration of extracts among the triplicate of each bacterium were determined and presented as mean ± standard deviation (SD).

Results and Discussion

Based on dry weight basis, the C. lentillifera aqueous extraction produced 30% yield compared to 15% yield from the methanol extraction while K. alvarezi methanol extraction produced 10% yield. These percentage yields are in agreeable with Matajun et al., [22]. The aqueous extraction was employed to mimic the decoction method used by the old folks in preparing their traditional remedies. Furthermore, the aqueous extraction would extracts water soluble or polar compounds. For K. alvarezi, hot aqueous extraction may result in production of the carrageenan and agar liked compound may be obtained [8]. When the compound is freeze-dried, upon reconstituting for fresh seaweed sample, the dry seaweed extract would absorbed the solution rather than dissolve within it. This was due to the fact that carrageenan featured liquid absorbing properties that when it is incorporated in wound dressing, it will keep the wound dry [3]. Therefore, since aqueous extraction of K. alvarezi was more suitable using another technique, it was excluded from the experiment.

On the other hand, extraction using methanol as solvent enabled us to obtain the lipid soluble and the non-polar compounds as it is more penetrable to the cell membrane [34]. In instant, the aqueous and methanol solvents extracted different substances that may contain different compound and potential.

There were present of zones of inhibition around the discs of tested C. lentillifera and K. alvarezi extract samples on the plates inoculated with S. aureus (Table 1) and S. mutans (Table 2) while all control disc did not show any inhibition effect. The antibacterial activities against S. aureus and S. mutans are varied with zones of inhibition between 9.8 to 16.3 mm. The zones of inhibition produced however, did not showed dose dependent patterns. A trend of biggest inhibition zone against S. aureus was recognized at concentration of 50 mg/ml for both aqueous and methanol extracts of C. lentillifera and the methanol extracts of K. alvarezi. It was observed that the methanol extracts of C. lentillifera seemed to produce greater antimicrobial effect against S. mutans at all concentrations compared to aqueous extracts of C. lentillifera and the methanol extracts of
K. alvarezii. The greatest antibacterial activity against S. mutans was produced by methanol extract of C. lentillifera at concentration of 50 mg/ml. It appeared that the extracts tested showed more potency against S. mutans compared to the S. aureus. The results obtained indicate that C. lentillifera and K. alvarezii extracts are able to inhibit the activity of common oral infection causal agents.

For future research, determination of bactericidal ability of the potential substance in the extracts and isolation of the active compound is worthy to be carried out to investigate the molecular mechanism of the action of seaweed in the infection control of the oral cavity.

Table 1: Zone inhibition of C. lentillifera and K. alvarezii extracts on S. aureus.

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<th>Seaweeds extracts</th>
<th>Zone of inhibition (mm) based on concentrations of extracts</th>
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<td>25 mg/ml</td>
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<td>Aqueous extracts of C.</td>
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<td>Methanol extracts of C.</td>
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<td>lentillifera</td>
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<td>Methanol extracts of K.</td>
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The results are means ± SD (n=3)

Table 2: Zone inhibition of C. lentillifera and K. alvarezii extracts on S. mutan.

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The results are means ± SD (n=3)

5. Conclusion:
The results of the present study demonstrated that the Malaysian Chlorophyta and Rhodophyta seaweeds; the C. lentillifera and K. alvarezii respectively possessed antibacterial activities against common causal agent for oral infections; the S. aureus and S. mutans using disc diffusion method. The antibacterial activities suggest the possibility of therapeutic value of seaweed against oral infection. Water and methanol were suitable solvents for C. lentillifera extraction while for K. alvarezii, the methanol was a better choice.

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Authors’ Contribution:
Faezah Sabirin handled paper write up and submission, Dr. Kazi Ahsan Jamil had important role in the material and discussion. Irman Shahir Ibrahim and Muhammad Mu’az Abd Rashit performed the methods and analyzed the results.

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