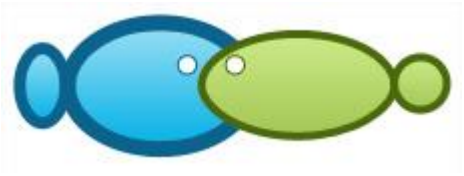

Antibacterial activity of liquid soap with combined *Sargassum* sp. and *Eucheuma* sp. seaweed extracts

Nurhana J. Sabaani, Myrtle Ayn E. Peñaredondo, Melbert C. Sepe

Department of Biological Sciences, College of Science and Mathematics, Western Mindanao State University, Zamboanga City, Philippines. Corresponding author: M. C. Sepe, melbert.sepe@wmsu.edu.ph

Abstract. Many medicinal plants have been reported to possess organic based compounds that can kill harmful microbes more effectively than synthetic chemicals. Due to the undesirable adverse effects of synthetic substances, it is preferable to use medicinal soap products. This study attempted to develop liquid soaps that have combined *Sargassum* sp. and *Eucheuma* sp. seaweed extracts and to determine their antibacterial activity against skin bacteria. The physico-chemical properties of the formulated liquid soaps were evaluated in terms of pH values and foam retention capacity. The antibacterial activity of the treatments was examined using the disc diffusion method. Results showed that the pH of all formulated liquid soaps ranged between 8.5 and 10.5, and therefore are safe to use. The soap base possessed the longest duration of foam retention capacity compared with the formulated treatments. All the formulated liquid soaps with *Sargassum* sp. and *Eucheuma* sp. crude extracts were found to be efficient in inhibiting bacteria development and have the same effect as the commercial antibacterial liquid soap. Furthermore, the combination of 25% *Sargassum* sp. extract and 75% *Eucheuma* sp. extract was the most effective antibacterial formulation, as evidenced by the greatest mean zone of inhibition, effective 12 hours more compared with the commercial product. This may suggest that, in this combination, the seaweed extracts exhibit the highest synergism to provide antibacterial properties against skin bacteria.
Key Words: aseptic technique, disc diffusion test, *Eucheuma*, *Sargassum*, synergistic effect.

Introduction. Bacterial infections and the emergence of antibacterial resistance have become a global health problem, for they cause high mortality rates in human populations (Al-Saif et al 2014). Even though many treatments have been discovered and many antibiotics have been produced, microorganisms have developed adaptive mechanisms against the action of antimicrobial drugs. Thus, there is an urgent need to develop new antibacterial products to control the spread of pathogens that have gained resistance against current antibiotics and to provide treatments against chronic and severe diseases. Medicinal soaps are a simple variation of the normal soaps, where synthetic or natural bioactive ingredients are added into the basic soap medium to give a vast variety of biological activities to the final product (Wijetunge & Perera 2016). Most of the hand wash available in the market is made from synthetic chemicals and has side effects like dryness of skin or rashes (Londhe et al 2015). Due to the undesirable adverse effects of synthetic substances, it is preferable to avoid the use of harmful synthetic chemicals from medicinal soap products (Ribeiro et al 2015). Many medicinal plants have been found to possess bioactive compounds that can effectively kill harmful microbes and are considered to be less toxic and more free from side effects than synthetic chemicals.

The diverse phyla of marine macroalgae (seaweeds) are rich sources of bioactive compounds, as they are able to produce various secondary metabolites, such as phenols, flavonoids, glycosides and sterols that have important biological roles, which include antifungal, anti-diabetic, anti-inflammatory, antimalarial, antioxidant, antiviral, and antibacterial activities. The *Eucheuma* sp. is a highly commercial red alga, being in demand as a good source for rough carrageenan used in different industries (Kasim et al 2016). It is also known to have the bioactive compound androstan-11-one, 3-

(acetyloxy)-17-iodo-(17α), a steroid containing iodium, which has antibacterial activity against both Gram positive and Gram negative bacteria (Anggadiredja 2011). The *Sargassum* spp. are marine brown algae abundant in tropical and subtropical regions. *S. macrocarpum* contains a recently discovered compound, named Sargafuran (Kamei et al 2009). This compound has low toxicity and the time-kill study showed that Sargafuran was bactericidal and completely killed *Propionibacterium acnes* (Kamei et al 2009). This is a superior property, because bactericidal activity minimizes the chance of resistance development.

The objective of this study is to develop a liquid soap with combined *Sargassum* sp. and *Eucheuma* sp. seaweed extracts and to determine its antibacterial activity against common skin bacteria. Specifically, to evaluate the quality of the soap base and to determine which combination of seaweed extracts as a liquid soap will yield a significantly greater zone of inhibition. This study could benefit many people, not just ecologically, but also economically. It can be a commercial product that can be produced in large amounts, due to the rich availability of *Sargassum* sp. and *Eucheuma* sp. in the Philippines. In addition, it can serve as a source of information for the development of antimicrobial products based on seaweeds.

Material and Method

Seaweed samples. As shown in Figure 1(A), *Sargassum* sp. has a yellow-brown thallus about 300-500 mm height, arising from small discoid holdfasts, a very short, cylindrical main axis, of 2-3 mm length and 2 mm width, giving rise to several primary branches and secondary branches alternately arranged, beset with branchlets that have copious leaves, vesicles and receptacles. In Figure 1(B), the *Eucheuma* sp. has a multi-axial structure and is composed of spine-like branches narrowing to acute tips. The branches are densely covered with 1-8 mm long branchlets.

Collection of seaweed samples. Five kilograms of fresh *Sargassum* sp. samples were collected from Caragasan Beach area, Barangay Recodo, Zamboanga City, while five kilograms of fresh cultured *Eucheuma* sp. samples were collected at Tictabon Island, Zamboanga City.

Preparation of seaweed powder. The collected seaweeds were cleaned of epiphytes and extraneous matters, necrotic parts were removed, and washed with clean water. After washing, the seaweeds were subjected to bleaching, using 0.1% Clorox solution as the bleaching agent to clean and sterilize the seaweeds (Hanen 2013). The seaweed samples were air dried at room temperature for two weeks, cut into small portions and subjected to electrical grounding, accomplished in the Zamboanga State College of Marine Science and Technology Laboratory Food Innovation Center, Zamboanga City, Philippines.

Preparation of seaweed extracts. The extract of powdered seaweeds was prepared using sequential extraction by soaking the 50 grams of *Sargassum* sp. powder in 500 mL of 70% methanol solvent for 24 hours, at room temperature. The solution was decanted and filtered with Whatman No. 4 filter papers. The solvent from the extract was removed using a vacuum on a rotary evaporator, at 60°C, to collect the crude extracts produced. The same procedure was performed for 50 grams of *Eucheuma* sp. powder.

Figure 1. The seaweed samples of (A) *Sargassum* sp. and (B) *Eucheuma* sp.

Preparation of Liquid Soap. For the formulation of the soap, the method described by Debnath et al (2011) was adapted applying some modifications. The hot process method was used for the formulation of the liquid soap, since it is considered to be more suitable for laboratory and industrial preparation. The lye-water solution was prepared by mixing 93.48 grams of potassium hydroxide (KOH) with 311.6 mL of distilled water. In a beaker, 150 mL of glycerine was heated. At the same time, 360 mL of commercial virgin coconut oil was heated in a separate beaker. Once the heated oil reached the temperature of 60°C, the lye-water solution was added, followed by the heated glycerine. The solution was continuously stirred in a mechanical mixer until it formed a vaseline-like, thickened substance. This served as the soap paste. The 100 mL of soap paste was diluted in 900 mL of distilled water to obtain 1000 mL of soap. About 10 grams of Borax powder was added to the liquid soap, which served as a preservative and buffer. The treatments were prepared are as follows:

Treatment 1 (T1): was prepared by adding 10 mL *Sargassum* sp. extract and 30 mL *Eucheuma* sp. extract to 160 mL of soap base.

Treatment 2 (T2): was prepared by adding 20 mL *Sargassum* sp. extract and 20 mL *Eucheuma* sp. extract to 160 mL of soap base.

Treatment 3 (T3): was prepared by adding 30 mL *Sargassum* sp. extract and 10 mL *Eucheuma* sp. extract to 160 mL of soap base.

Treatment 4 (T4): was prepared by adding 40 mL *Sargassum* sp. extract to 160 mL of soap base.

Treatment 5 (T5): was prepared by adding 40 mL *Eucheuma* sp. extract to 160 mL of soap base.

Treatment 6 (T6): was prepared using 200 mL of soap base without the combined seaweed extracts, serving as the negative control.

Treatment 7 (T7): is the commercial antibacterial liquid soap (ingredients: water, sodium laureth sulfate, sodium chloride, cocamidopropyl betaine, glycerine, benzophenone 4, citric acid, tetrasodium editate, triclosan, methylisothiazolinone), which served as positive control.

Evaluation of physico-chemical properties

a) Foam Retention Capacity. A volume of 1 mL of the formulated liquid soap was added to 49 mL of water in a graduated cylinder. Using the palm of the hand, the graduated cylinder was covered and it was shaken vigorously 10 times. The disappearance rate of the foam was measured using a timer. The same process was carried out for the other treatments, including the positive and negative controls in separate graduated cylinders.

b) Test of pH. A volume of 1 mL of the formulated liquid soap obtained was dissolved in 100 mL distilled water and shaken vigorously. The pH of the soap solution was measured by using a calibrated pH meter. The pH of the liquid soap must be in accordance with the standard test method for pH of aqueous solutions set by the ASTM E70 (American Society Testing and Materials 2015). The pH should be between 8.5 and 10.

Preparation of broth culture. In a 500 mL Erlenmeyer flask, 1 gram of beef extract and 0.6 grams of peptone were added to 200 mL of distilled water. It was subjected to sterilization in an autoclave at 121°C, for 15 minutes.

Preparation of test organisms. A letter of consent was signed by the selected respondent. Hand washing with soap was done before the respondent was sampled. A sterile wet cotton stick was used to swab the surface of the palm of the respondent and in between fingers, then swirled and placed in the Erlenmeyer flask containing the nutrient broth. It was incubated for 24 hours at 37°C. The flask was shaken occasionally to aerate and promote growth. The cloudy appearance of the nutrient broth indicates bacterial growth.

Preparation of MH agar test plate. The Mueller Hinton (MH) agar was utilized, since it is the usual type of medium used in antimicrobial susceptibility testing by the disc diffusion method. It was prepared by dissolving 38 grams of MH agar powder in 1000 mL of distilled water. It was sterilized by subjecting it in an autoclave at 121°C for 15 minutes. The spread plate method was applied, 10 mL of MH agar being added to each of the sterile 70 petri plates. There are 10 petri plates that serve as replicates for each treatment prior to antibacterial assay. The agar was allowed to stand for a few minutes, until it solidified. 100 µL of nutrient broth with test organisms were transferred into the petri plate by using a micropipette. A sterilized L-tube was used to spread the added inoculum thoroughly.

Preparation of filter paper disc. Whatman No. 4 filter paper was cut into discs of about 6 mm in diameter. These were wrapped in an aluminum foil and were sterilized in an autoclave for 15 minutes, at 121°C. These filter paper discs were aseptically dipped in the different treatments of liquid soap solutions.

Test of antibacterial property using disc diffusion method. The antibacterial property of the formulated antibacterial liquid soaps was examined using the disc diffusion method. Each of the filter paper discs dipped in the different liquid soaps were placed in the center of various MH agar plates containing the cultured bacteria through aseptic technique. Zones of inhibition were measured in millimeters, using a Vernier caliper, within 4 hours intervals, until the growth of the bacteria was again proliferated. The standard diameter for the zones of inhibition was based within the recommended intervals by the CLSI (Clinical Laboratory Standard Institute).

Statistical analysis. All results were analyzed using One-Way Analysis of Variance (ANOVA) to determine if there is a significant difference in the mean zones of inhibition between treatments, and Post-Hoc test (Tukey HSD) to compare how each treatment differs in terms of the mean zones of inhibition after 4, 8, 12 and 16 hours of observation, using SPSS 17.0. Results with $p<0.05$ were considered statistically significant.

Results and Discussion. In terms of pH, T2 has the highest pH value of 9.5 and T7 (the positive control) has the lowest pH value of 8.4 among the treatments. The pH values of all formulated liquid soaps were all safe to use, since the normal pH range for liquid hand soap is between 8.5 and 10.5 (Narkhede 2010). The highest pH value for T2 resulted in lower zones of inhibition. This may be correlated with the studies of Sacks & Pence (1958) and Wiegand et al (2015), in which the increase in pH led to a significant increase of bacterial growth. The increase of skin pH irritates the physiologic protective 'acid mantle', changes the composition of the cutaneous bacterial flora and the activity of enzymes in the upper epidermis (Gfatter 1997).

Another important attribute of a liquid soap is the foam retention capacity. Table 1 shows that T6 (negative control) possessed the longest foam retention capacity, which reached 215 minutes, while T3 had the shortest foam retention capacity, which only lasted 81 minutes. The formulated liquid soap without crude extracts (negative control) possessed the longest foam retention capacity, which may be attributed to the amount of fatty acids that have been retained, since no other bioactive compounds were added. Fatty acids present in the coconut oil are responsible for the foaming property of the soap (Oghome et al 2012). Several studies have suggested that longer foam retention provides a greater microbial reduction (Fischler et al 2007; Fuls et al 2008; Jensen et al 2015; Lowbury & Lilly 1973; Ojajärvi 1980). However, this study proved that formulations with lower foam retention can exhibit greater antibacterial activity in terms of zones of inhibition.

Table 1

Physico-chemical properties of formulated liquid soaps with combined *Sargassum* sp. and *Eucheuma* sp. extracts, liquid soap without the extracts and commercial antibacterial liquid soap

Physico-chemical Parameters	*Treatments*						
	T1	T2	T3	T4	T5	T6	T7
Foam retention capacity	91 minutes	149 minutes	81 minutes	125 minutes	168 minutes	215 minutes	120 minutes
pH	8.6	9.5	9.3	8.6	8.8	9.3	8.4

Note: T1 - 10 mL *Sargassum* sp. + 30 mL *Eucheuma* sp. + 160 mL soap base; T2 - 20 mL *Sargassum* sp. + 20 mL *Eucheuma* sp. + 160 mL soap base; T3 - 30 mL *Sargassum* sp. + 10 mL *Eucheuma* sp. + 160 mL soap base; T4 - 40 mL *Sargassum* sp. + 160 mL soap base; T5 - 40 mL *Eucheuma* sp. + 160 mL soap base; T6 - soap base (negative control); T7 - commercial antibacterial liquid soap (positive control).

Table 2 shows that T1 with 10 mL *Sargassum* sp. and 30 mL *Eucheuma* sp. crude extracts has a greater mean zone of inhibition, 28.30±9.62 mm, after 12 hours compared with all formulated liquid soaps and the commercial liquid soap product. Figure 2 demonstrates that T1 has the highest mean zone of inhibition after 12 hours among all formulated liquid soaps. It indicates that T7 (positive control) has a similar effect in terms of mean zone of inhibition as all formulated liquid soaps and T6 (negative control) after 8 hours. It also appears that T2 has the lowest zone of inhibition among all of treatments.

Table 2

The mean±SD (mm) for the zone of inhibition of the treatments exposed after 4 hours, 8 hours, 12 hours and 16 hours of observation

Treatments	*After 4 hours*	*After 8 hours*	*After 12 hours*	*After 16 hours*
T1	10.50±0.85	19.90±4.23	28.30±9.62	25.60±12.08
T2	10.60±1.08	18.10±3.32	20.30±3.50	14.00±5.75
T3	11.20±1.69	19.50±5.15	22.60±3.13	18.50±4.55
T4	10.50±0.71	17.50±5.14	20.60±4.81	18.20±8.80
T5	9.60±2.01	20.30±4.40	21.30±5.79	19.00±7.04
T6	11.00±1.41	21.60±4.38	21.50±5.44	22.40±10.74
T7	17.60±6.42	21.30±4.92	19.90±3.70	19.90±5.20

Note: T1 - 10 mL *Sargassum* sp. + 30 mL *Eucheuma* sp. + 160 mL soap base; T2 - 20 mL *Sargassum* sp. + 20 mL *Eucheuma* sp. + 160 mL soap base; T3 - 30 mL *Sargassum* sp. + 10 mL *Eucheuma* sp. + 160 mL soap base; T4 - 40 mL *Sargassum* sp. + 160 mL soap base; T5 - 40 mL *Eucheuma* sp. + 160 mL soap base; T6 - soap base (negative control); T7 - commercial antibacterial liquid soap (positive control).

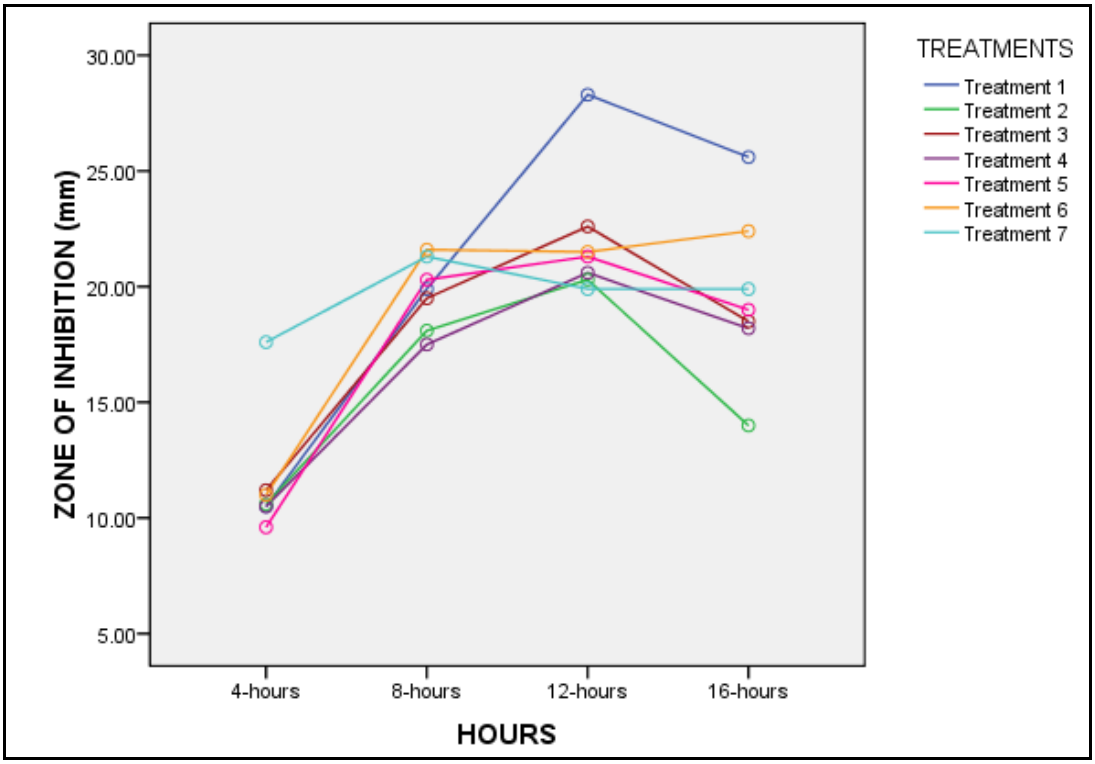

Figure 2. The mean zone of inhibition (mm) exhibited by each treatment.

The One-way Analysis of Variance (ANOVA) at α=0.05 level of significance, shown in Table 3, has a highly significant difference of p=0.0000** between and within treatments, after 4 hours. There is also a significance regarding the mean zones of inhibition after 12 hours and 16 hours, with values of p=0.0170* and p=0.0480*, respectively. However, there was no significant difference ($p=0.3560^{ns}$) between and within groups after 8 hours of observation. The Post-Hoc analysis using Tukey B homogeneous subsets is presented in Table 4. It shows that T7 is significantly higher than other treatments in terms of mean zone of inhibition after 4 hours. However, after 8 hours, there is no significant difference in terms of mean zone of inhibition in all treatments. It was also noted that T1 is significantly higher in terms of mean zone of inhibition among all the treatments after 12 hours and after 16 hours of observation. This

indicates that T1 has a greater antibacterial activity compared with the commercial antibacterial liquid soap.

Table 3

One-Way Analysis of Variance of the mean zones of inhibition of the different liquid soaps after 4, 8, 12 and 16 hours of observation

		Sum of Squares	*df*	*Mean Square*	*F*	*Significance*
After 4 hours	Between Groups	439.343	6	73.224	9.778	0.0000**
	Within Groups	471.800	63	7.489		
	Total	911.143	69			
After 8 hours	Between Groups	139.971	6	23.329	1.129	0.3560^{ns}
	Within Groups	1301.400	63	20.657		
	Total	1441.371	69			
After 12 hours	Between Groups	500.143	6	83.357	2.808	0.0170*
	Within Groups	1870.500	63	29.690		
	Total	2370.643	69			
After 16 hours	Between Groups	787.971	6	131.329	2.264	0.0480*
	Within Groups	3653.800	63	57.997		
	Total	4441.771	69			

Note: * - significant if $p<0.05$; ** - highly significant if $p<0.0000$; ^{ns} - not significant if $p>0.05$.

Table 4

Post-Hoc Analysis using Tukey's B Homogenous Subsets on the mean zone of inhibition (mm) of the different liquid soaps as tested for antibacterial activity after 4, 8, 12 and 16 hours

Treatments	*After 4 hours*		*After 8 hours*		*After 12 hours*		*After 16 hours*	
	1	2	1	2	1	2	1	2
T1	10.50		19.90			28.30		25.60
T2	10.60		18.10		20.30		14.00	
T3	11.20		19.50		22.60		18.50	
T4	10.50		17.50		20.60		18.20	
T5	9.60		20.30		21.30		19.00	
T6	11.00		21.60		21.50		22.40	
T7		17.60	21.30		19.90		19.90	

Note: Subset for α=0.05 level of significance. T1 - 10 mL *Sargassum* sp. + 30 mL *Eucheuma* sp. + 160 mL soap base; T2 - 20 mL *Sargassum* sp. + 20 mL *Eucheuma* sp. + 160 mL soap base; T3 - 30 mL *Sargassum* sp. + 10 mL *Eucheuma* sp. + 160 mL soap base; T4 - 40 mL *Sargassum* sp. + 160 mL soap base; T5 - 40 mL *Eucheuma* sp. + 160 mL soap base; T6 - soap base (negative control); T7 - commercial antibacterial liquid soap (positive control).

Overall, the results affirm that all of the formulated liquid soaps with *Sargassum* sp. and *Eucheuma* sp. crude extracts were efficient in inhibiting the bacteria and have the same effect as the commercial antibacterial liquid soap. However, it is revealed that the formulated liquid soap of T1, with a combination of 10 mL *Sargassum* sp. and 30 mL *Eucheuma* sp. crude extracts, was considered the most effective antibacterial agent, having a mean zone of inhibition zone greater than that of the commercial one, effective until 12 hours. This may suggest that the combined 25% *Sargassum* sp. and 75% *Eucheuma* sp. crude extracts concentration has synergistic antibacterial effects against the common skin bacteria.

The antibacterial performance of liquid soap with combined crude extracts, as indicated by the zones of inhibition, was substantially higher than the data obtained in

other liquid soaps with pure extracts. These suggest that a synergism effect may occur between compounds in the whole extract samples compared with the single compound. As stated by Foucquier & Guedj (2015), combination therapies exploit the chances for better efficacy, decreased toxicity and reduced development of drug resistance. Owing to these advantages, they have become a standard for the treatment of several diseases and continue being a promising approach in unmet medical needs.

In bacteria, the important targets of antimicrobial action are the cell wall, the cytoplasmic membrane, the biosynthetic processes of protein synthesis and nucleic acid synthesis. The secondary metabolites such as Sargafuran, glycoside, polyphenol, saponin, sterols, triglycerides, fatty acids and volatile oil present in the crude extract of *Sargassum* sp. (Coronado & Dionisio-Sese 2014) attack the skin bacteria in various ways (Kamei et al 2009). Primarily, they bind to the bacterial cell wall leading to the inhibition of its growth. Carotenoids present in both *Sargassum* sp. and *Eucheuma* sp. are lipid-soluble, natural pigments composed of eight units of five carbons, tetraterpenoids, with up to 15 conjugated double bonds. The antimicrobial mechanism proposed for carotenoids could lead to the accumulation of lysozyme, an immune enzyme that digests bacterial cell walls (Perez et al 2016). Phenolic compounds found in *Eucheuma* sp. are characterized by an aromatic ring, with one or more hydroxyl groups. These compounds have the capability to alter the microbial cell permeability and destroy the internal macromolecules. They can also interfere with the membrane function and remove the cellular integrity, eventually leading to bacterial cell death (Abu-Ghannam & Rajauria 2013).

The *Eucheuma* sp. contains a steroid with an iodium compound, androstan-11-one, 3-(acetyloxy)-17-iodo-, (17α) or 3-acetoxy-17α -iodo-androstan-11-one, which is known to inhibit both Gram positive and Gram negative bacteria (Anggadiredja 2011). Also, *Eucheuma* sp. is rich in C20 polyunsaturated fatty acids (PUFAs), mainly arachidonic (C20:4n-6) and eicosapentaenoic (C20:5n-3) acids. *Sargassum* sp. is a brown alga which exhibits both. Oxylipins are the oxygenated products of fatty acids and are mainly derived from C16, C18, C20 and C22 PUFAs, which also confer innate immunity in response to biotic and abiotic stress, such as pathogenic bacteria and herbivores (Kumari et al 2013). This might be the reason of higher zones of inhibition exhibited by the combined liquid soap with higher concentration of *Eucheuma* sp. crude extract. Moreover, the presence of saponin in the seaweed extracts (Wijetunge & Perera 2016) makes them ideal for use in medicinal soap preparations, since the saponin itself can act as a natural foaming agent.

The great variation observed in the potential antimicrobial components in seaweeds could be due to external environmental factors like herbivory, light, depth, salinity and nutrients (Zubia et al 2008). All of these factors could act on the spatiotemporal regulation on metabolic expression of the active compounds leading to marked qualitative and quantitative variations among seaweed species. The methanol solvent used was also useful in extracting the antibacterial bioactive compounds exhibited by *Sargassum* sp. and *Eucheuma* sp. seaweeds, as it is considered as the best solvent in some previous studies (Pierre et al 2011; Rusli et al 2016) and it is found to be less toxic in human erythrocytes (Mole & Sabale 2014). Thus, this might be the reason that led to the higher bacteriostatic activity of *Sargassum* sp. and *Eucheuma* sp. seaweeds collected in two coastal areas of Zamboanga City.

Conclusions. In this study, all the formulated liquid soaps with *Sargassum* sp. and *Eucheuma* sp. crude extracts, including the negative control, have pH values within the normal range of liquid soap, which guarantee its safe application to human skin. The formulated liquid soap without the combined crude extracts possessed the longest foam retention capacity, which may be attributed to the amount of fatty acids retained, since no other bioactive compounds were added. The formulated liquid soap with combined 25% *Sargassum* sp. extract and 75% *Eucheuma* sp. extract significantly inhibits the growth of bacteria compared with the commercial antibacterial liquid soap, lasting 12 hours. The current study suggests that a potential antibacterial synergistic effect may occur between the crude extracts of *Sargassum* sp. and *Eucheuma* sp. seaweeds.

Acknowledgements. The authors are thankful to the Regional Animal Disease Diagnostic Laboratory (RADDL), Zamboanga City, for the conduct of the antibacterial assay; to the Chemistry Department in College of Science and Mathematics, Western Mindanao State University, Zamboanga City, for the aid in using the rotary evaporator on the crude extraction; to Professor Soledad P. Salazar, for the invaluable guidance to this study; to Dr. Arnold V. Hallare, for the technical assistance of this manuscript; to Alia J. Sabaani and Isnijol K. Sabaani, for the advice and support; and especially to the DOST-SEI (Department of Science and Technology - Science Education Institute), for the scholarship grant.

References

Abu-Ghannam N., Rajauria G., 2013 Antimicrobial activity of compounds isolated from algae. In: Functional Ingredients from Algae for Foods and Nutraceuticals. Domínguez H. (ed), Woodhead Publishing, Cambridge, UK, pp. 287-306.

Al-Saif S. S. A., Abdel-Raouf N., El-Wazanani H., Aref I., 2014 Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. Saudi Journal of Biological Sciences 21(1):57-64.

American Society for Testing and Materials, 2015 ASTM E70 Standard test method for pH of aqueous solutions with the glass electrode. American Society Testing and Materials International, West Conshohocken, Pennsylvania, United States, 6 p.

Anggadiredja J. T., 2011 Diversity of antibacterial compounds from *Eucheuma serra, Halimeda opuntia,* and *Hydroclathrus clathratus.* Jurnal Teknologi Lingkungan 12(2):131-142.

Coronado A. S., Dionisio-Sese M. L., 2014 Antimicrobial property of crude ethanolic extract from *Sargassum crassifolium*. Asian Journal of Microbiology, Biotechnology & Environmental Sciences 16(3):471-474.

Debnath S., Babu M. N., Bharathi M. D., Jyothsna T., Revathi D., Kishore Kumar T. S., 2011 Formulation and evaluation of liquid soap containing herbal antimicrobial agent. Research Journal of Pharmacognosy and Phytochemistry 3(5):225-231.

Fischler G. E., Fuls J. L., Dail E. W., Duran M. H., Rodgers N. D., Waggoner A. L., 2007 Effect of hand wash agents on controlling the transmission of pathogenic bacteria from hands to food. Journal of Food Protection 70(12):2873-2877.

Foucquier J., Guedj M., 2015 Analysis of drug combinations: current methodological landscape. Pharmacology Research & Perspectives 3(3):e00149, DOI: 10.1002/prp2.149.

Fuls J. L, Rodgers N. D., Fischler G. E., Howard J. M., Patel M., Weidner P. L., Duran M. H., 2008 Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. Applied and Environmental Microbiology 74(12):3739-3744.

Gfatter R., 1997 Effects of soap and detergents on skin surface pH, stratum corneum hydration and fat content in infants. Dermatology 195(3):258-262.

Hanen N., 2013 Colour removal of raw carrageenan by using sodium hypochlorite as bleaching agent. Bachelor thesis, Faculty of Chemical & Natural Resources Engineering. University Malaysia Pahang, 64 p.

Jensen D. A., Danyluk M. D., Harris L. J., Schaffner D. W., 2015 Quantifying the effect of hand wash duration, soap use, ground beef debris, and drying methods on the removal of *Enterobacter aerogenes* on hands. Journal of Food Protection 78(4):685-690.

Kamei Y., Sueyoshi M., Hayashi K., Terada R., Nozaki H., 2009 The novel anti-*Propionibacterium acnes* compound, Sargafuran, found in the marine brown alga *Sargassum macrocarpum.* The Journal of Antibiotics 62(5):259-263.

Kasim M., Mustafa A., Munier T., 2016 The growth rate of seaweed (*Eucheuma denticulatum*) cultivated in longline and floating cage. AACL Bioflux 9(2):291-299.

Kumari P., Kumar M., Reddy C. R., Jha B., 2013 Algal lipids, fatty acids and sterols. In: Functional Ingredients from Algae for Foods and Nutraceuticals. Domínguez H. (ed), Woodhead Publishing, Cambridge, UK, pp. 87–134.

Londhe J., Jagtap S., Doshi C., Jagade D., 2015 Formulations of herbal hand wash with potential antibacterial activity. International Journal of Research in Advent Technology. In: Special Issue National Conference on Advances and Challenges in Green Technology, Savitribai Phule Pune University, pp. 11-15.
Lowbury E. J. L., Lilly H. A., 1973 Use of 4% chlorhexidine detergent solution (hibiscrub) and other methods of skin disinfection. British Medical Journal 1(5852):510-515.
Mole M. N., Sabale A. B., 2014 Antimicrobial, antioxidant and haemolytic potential of brown macro-alga *Sargassum.* World Journal of Pharmacy and Pharmaceutical Sciences 3(8):2091-2104.
Narkhede D. B., 2010 Formulation and evaluation of coconut oil liquid soap. International Journal of Pharma World Research 2(3):1-15.
Oghome P., Eke M., Kamalu C. I., 2012 Characterization of fatty acid used in soap manufacturing in Nigeria: laundry, toilet, medicated and antiseptic soap. International Journal of Modern Engineering Research 2(4):2930-2934.
Ojajärvi J., 1980 Effectiveness of handwashing and disinfection methods in removing transient bacteria after patient nursing. Journal of Hygiene 85(2):193-203.
Pérez M. J., Falqué E., Domínguez H., 2016 Antimicrobial Action of Compounds from Marine Seaweed. Marine Drugs 14(3), pii: E52, DOI: 10.3390/md14030052.
Pierre G., Sopena V., Juin C., Mastouri A., Graber M., Maugard T., 2011 Antibacterial activity of a sulfated galactan extracted from the marine alga *Chaetomorpha aerea* against *Staphylococcus*. Biotechnology and Bioprocess Engineering 16(5):937-945.
Ribeiro A. S., Estanqueiro M., Oliveira M. B., Lobo J. M., 2015 Main benefits and applicability of plant extracts in skin care products. Cosmetics 2(2):48-65.
Rusli A., Metusalach M., Tahir M. M., Salengke S., Syamsuar S., 2016 Analysis of bioactive compounds of *Caulerpa recemosa, Sargassum sp.* and *Gracillaria verrucosa* using different solvents. Jurnal Teknologi 78(4-2):15-19.
Sacks L., Pence J., 1958 The influence of pH on the antibacterial action of Subtilin A. Journal of General Microbiology 19:542-550.
Wiegand C., Abel M., Ruth P., lsner P., Hipler U., 2015 pH Influence on antibacterial efficacy of common antiseptic substances. Skin Pharmacology and Physiology 28(3):147-158.
Wijetunge W. M. A. N. K., Perera B. G. K., 2016 Preparation of medicinal soap products using the leaf extracts of *Punica granatum* (pomegranate). International Journal of Pharmacy and Biological Sciences 6(2):7-16.
Zubia M., Payri C., Deslandes E., 2008 Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornate* (Phaeophyta: Fucales), from Tahiti French Polynesia. Journal of Applied Phycology 20(6):1033-1043.

Received: 2 April 2019. Accepted: 11 June 2019. Published online: 25 September 2019.
Authors:
Nurhana Jalali Sabaani, Department of Biological Sciences, College of Science and Mathematics, Western Mindanao State University, Zamboanga City, 7000 Philippines, e-mail: nanasabaani@gmail.com
Myrtle Ayn Empinado Peñaredondo, Department of Biological Sciences, College of Science and Mathematics, Western Mindanao State University, Zamboanga City, 7000 Philippines, e-mail: pmyrtleayn@gmail.com
Melbert Cabañog Sepe, Department of Biological Sciences, College of Science and Mathematics, Western Mindanao State University, Zamboanga City, 7000 Philippines, e-mail: melbert.sepe@wmsu.edu.ph

How to cite this article:
Sabaani N. J., Peñaredondo M. A. E., Sepe M. C., 2019 Antibacterial activity of liquid soap with combined *Sargassum* sp. and *Eucheuma* sp. seaweed extracts. AACL Bioflux 12(5):1514-1523.