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Research Article

Antimicrobial Properties of Deep-Sea Water Towards *Escherichia coli* and *Staphylococcus aureus*

Govinraj Ravi Chandran^a, Daniel Joe Dailin^{a,b}, Nor Hasmaliana Abdul Manas^{a,b}, Hesham Ali El-Ensashy^{a,b}, Mustafa Man^c, Zehra Edis^{d,e}, Widya Fatriasari^{f,g}, and Nur Izyan Wan Azelee^{a,b*}

^a Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia.

^b Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia.

^c Faculty of Ocean Engineering Technology & Informatics, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

^d Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Ajman University, Ajman P.O. Box 346, United Arab Emirates.

^e Center of Medical and Bio-Allied Health Sciences Research, Ajman University, Ajman P.O. Box 346, United Arab Emirates.

^f Research Center for Biomass and Bioproducts, National Research and Innovation Agency (BRIN), Jl Raya Bogor KM 46 Cibinong, Bogor 16911, West Java, Indonesia.

^g Research Collaboration Center for Biomass-Based Nano Cosmetics, Mulawarman University, Samarinda 75119, East Kalimantan, Indonesia.

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ABSTRACT

With the quick commercial expansion, demand for cosmetics made with natural materials has been rising steadily over time. A potential replacement that is substantially safer than the chemical ingredients would be a deep-sea water (DSW) based cosmetic that mostly consists of organic and biomaterial elements. This research attempts to demonstrate that DSW can be a good alternative to chemical cosmetics by examining its antibacterial capabilities. The antibacterial properties of DSW were ascertained using the well diffusion method and the Mueller Hinton Agar plate technique. *Escherichia coli* and *Staphylococcus aureus*, two of the most prevalent bacteria on human skin, were used in the antimicrobial tests. The hollow zones that were produced as a result of the inhibition zones were assessed to demonstrate their potential as a replacement for numerous chemical-based products. In this research, the concentrated deep sea water showed bigger inhibition zone (12.5% and 12.75%) compared to pure deep-sea water (7.2% and 0%) for *E. coli* and *S. aureus*, respectively. This study will serve as a foundation for the widespread use of DSW, which promises to be a safe and sustainable ingredient, in future cosmetics.

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INTRODUCTION

Deep sea water (DSW) is the name for the water that makes up the seas, which make up over 70% of the Earth's surface. On average, 96.5% of seawater is composed of clean water, 2.5% salt, with small portion containing trace amounts of particles, dissolved inorganic and organic chemicals, and atmospheric gases. DSW may contain a variety of various

chemical elements with substantial commercial relevance. Both vast quantities of bromine and a significant fraction of the magnesium present on Earth are removed from saltwater (Balboa et al., 2015). Desalinated seawater can also be used to create an endless supply of potable water.

*Corresponding Author

E-mail address: nur.izyan@utm.my

DOI address

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Several large desalination facilities have been built in dry areas to help with freshwater shortages (Duxbury, 2020).

Electrolytes, important minerals like calcium, magnesium, and potassium, as well as other nutrients, are still present in the liquid after the DSW has been desalinated and the salt has been removed. These vitamins, minerals, and other nutrients are essential for supporting human biological and physical health. Consuming DSW has been shown to have various benefits, including lowering blood pressure, lowering the risk of heart disease, and encouraging fat and cholesterol reduction. In actuality, the cleanest source of water for human consumption is deep sea water (Danny, 2018).

The term "antimicrobial activity" refers to all active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial colonies, and occasionally result in the death of microorganisms. According to Walsh Medical Media in 2022, antimicrobial activity is the process of eliminating or suppressing germs that cause illness. Numerous antibacterial medications are used to achieve this such as penicillin, cephalosporins, tetracyclines and macrolides. Antimicrobials have properties that include antibacterial, antifungal, and antiviral. Various strategies have been employed to kill the microbes. For instance, antimicrobials that target bacteria's cell walls include penicillin and cephalosporins, while polymyxins damage the cell membrane. In addition, antibiotics like macrolides, tetracyclines, and aminoglycosides function by blocking bacterial protein synthesis, which stops the pathogen from procreating. Depending on the type of microorganism and the severity of the illness, these techniques may be utilized singly or in combination. It is crucial to remember that in order to guarantee the greatest results and stop the emergence of antibiotic resistance, the selection and use of antimicrobials should always be overseen by a healthcare professional. Based on previous published studies, DSW have demonstrates promising antibacterial activity (Kawada and Takeuchi, 2013).

There are several factors influencing antimicrobial activities of deep-sea water. Among the important factors is the pH where the antimicrobial activities is normally higher at low pHs (ionized amino groups) (Fluhr et al., 2012). Secondly, is the temperature where low temperature will enhance the presence of bacteria. These two are known as environmental factors (Kulikov et al., 2014).

There are two factors affecting the antimicrobial activities of deep-sea water which are molecular weight and degree of acetylation (DOA). High molecular weight of compounds in deep sea water will stack on the bacterial surface and will block nutrient transport which will result in cell death. Whereas, lower molecular weight could penetrate membrane surface and bind with DNA, thus inhibiting synthesis of mRNA. Meanwhile a higher positively charged sample is associated with the degree of acetylation. A 30-40% degree of acetylation produced the highest antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* (Kulikov et al., 2014).

Furthermore, types of microorganisms also influence the efficacy of antimicrobial activities of DSW. Comprises of peptidoglycan and teichoic acid responsible for structural constancy of cell wall, it is suggested that DSW samples possess the strongest bactericidal effect on Gram-negative bacteria. On the other hand, gram-negative bacteria which have cell walls with thick peptidoglycan layer that has a

highly negative charge proves to be more resistance (Kulikov et al., 2014) towards degradation. Hence, this study focuses on investigating antimicrobial activities of deep-sea water against *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative).

MATERIALS AND METHOD

In order to prove that DSW samples play an important role in antimicrobial properties, the DSW samples was obtained locally from an area in the East Cost of Peninsular Malaysia. The exact location was the Peraian Kelantan. The samples were tested without any fungus or bacteria extraction. The method of experiment was conducted by using pure and concentrated DSW. Antimicrobial properties of the samples were determined using Mueller Hinton (MH) Agar plate technique and well diffusion method. The antimicrobial tests were run on two selected microbial strains which are *Escherichia coli* and *Staphylococcus aureus*.

Materials

Deep sea water, Mueller Hinton Agar, distilled water, bacterial strain of *E. coli* and *S. aureus*, autoclave, micropipette, beakers are the chemicals and materials used in the study.

Antimicrobial Testing

Sample Preparation

A 200 mL of pure DSW samples was poured into a 500 mL of sterilized and autoclavable collection bottles. This was considered as Sample 1. Sample 2 was prepared by boiling 500 mL DSW. This boiling water was left for evaporation at 80 °C and 500 rpm until the total volume reached 50 mL. The sample 2 was considered as 10 times concentrated by volume.

Preparation of Agar Plates and Bacterial Inoculation

Muller Hinton (MH) broth and agar-agar powder were mixed with distilled water. The solution was mix thoroughly until the powder is completely dissolved. The mixture was made up the volume to 500 mL and was autoclaved at 121 °C and 1 bar for 90 mins before plating it. The cooled agar mixture was poured in sterile plates about 20-24 mL. Then the agar plates were left to solidify at room temperature and stored in refrigerator.

E. coli and *S. aureus* were sub-cultured a day before in nutrient agar. A sterile cotton swab was used to contact one or two isolated colonies of the tested microorganisms. The bacteria were suspended in 10 mL distilled water (Chen, Alexander, and Baki 2016). The solution was mixed until a cloudy solution formed. The turbidity of the solution was compared to 0.5 Mc Farland standard (Cockerill, 2012).

Preparation of Agar Well Diffusion Assay

The wells were created by striking holes in the inoculated MH agar plates with a sterile cork borer. The inoculum of the bacterial were spread on the MH plates. After the inoculum were dried, the prepared samples, positive and negative controls were poured into the well. The inoculated agar plates were incubated at 37 °C for 24 hours.

Antimicrobial Testing

After, 24 hours of incubation, the antimicrobial properties of samples were evaluated by calculating the zone of inhibition (a circular clear zone) with a ruler to the nearest millimeters.

RESULTS AND DISCUSSION

Antimicrobial activities of the samples were tested against *S. aureus* and *E. coli* at different concentrations to test the efficiency of the samples. From the results obtained, higher concentrations of the samples lead to bigger inhibition zone diameters that were measured using a ruler.

There are many types of chemical elements found in the DSW. Minerals such as Magnesium, Calcium, Chlorine, Sodium, Potassium, and Vanadium are available in DSW. DSW contains higher concentration of minerals than seawater near the surface (Sheu et al., 2013). DSW is a good nutritional source and potentially be regarded as a nutrient provider due to the numerous health benefits of the minerals it contains. For instance, magnesium is necessary for the body's multiple physiological processes, such as enzyme and energy metabolism. Mg has the capacity to reduce fat accumulation in the aorta of individuals to ingest high levels of cholesterol (MgGuire et al., 2013). Additionally, it can reduce the risk of diabetes, obesity, and asthma. Drinking water with a high Mg concentration has demonstrated increased inhibitory effects in adipocyte differentiation, suggesting that Mg can slow down fat cell formation (Hwang et al., 2009). Similar readings were found for the concentrations of chemical elements from the DSW sample used in this study.

Table 1 Typical chemical elements found in deep-sea water

Chemical Elements	Amount (mg/L) or (ppm)
Sodium	496.000
Magnesium	172.000
Potassium	87.400
Calcium	63.400
Aluminum	0.004
Strontium	2.490
Mercury	0.000

Based on the chemical analysis test conducted at the Universiti Malaysia Terengganu, there are many compounds were found from the screening of the chemical contents from 10 mL DSW sample in the study. From the analysis result obtained, sodium chloride recorded the highest reading which is 496 (mg/L). In this sample, the amount of mercury in the DSW sample obtained is recorded as zero where it proves that this sample is safe to be consumed for industrial uses.

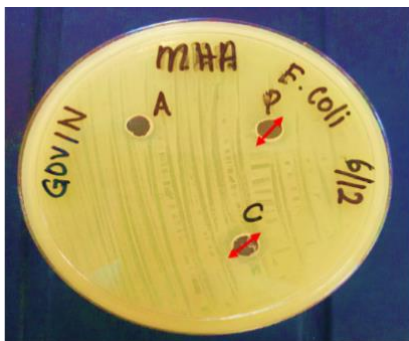


Figure 1 Antimicrobial properties of deep-sea water towards *E. coli* (A-Negative control, B- Positive Control, C- 10x concentrated DSW, P-Pure DSW)

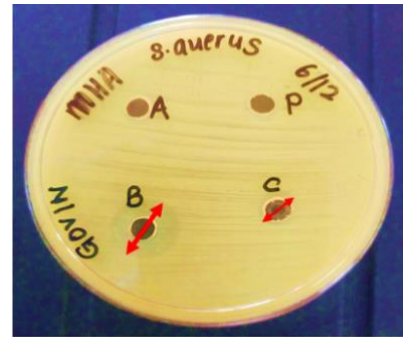


Figure 2 Antimicrobial properties of deep-sea water on *S. aureus* (A-Negative control, B- Positive Control, C- 10x concentrated DSW, P-Pure DSW)

From **Figure 1** and **Figure 2**, the diameters of inhibition zones are visible in various types of solution. **Figure 1** shows the antimicrobial properties of pure DSW for the *E. coli* strained agar plate. The diameter of pure DSW sample is smaller compared to the concentrated DSW. While for *S. aureus* strained agar plate, no inhibition zone is visible for pure DSW. The diameter of inhibition zone for both *E. coli* and *S. aureus* strains were biggest when the 10 times concentrated DSW samples were used. This is due to the density of the deep-sea water which is rich with sea salt.

Table 2 Diameter of inhibition zone (in mm)

Type of Sample	<i>E. coli</i>	<i>S. aureus</i>
Negative Control (A)	0	0
Positive Control (B)	-	16.5
Pure DSW (P)	7.2	0
10x Concentrated DSW (C)	12.5	12.75

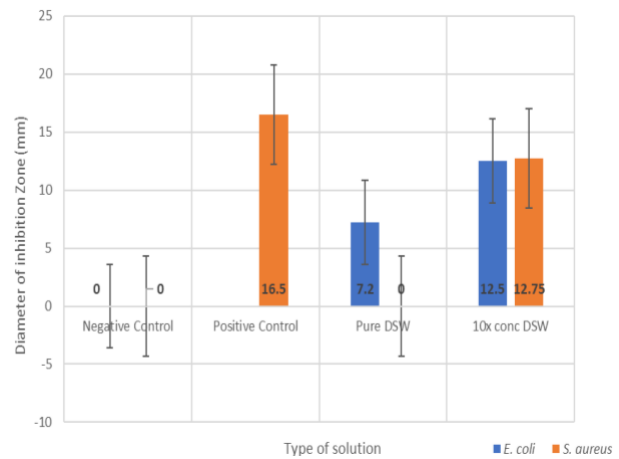


Figure 3 Graph of the inhibition zone diameter against different types of solution

Previously, a research have proved that DSW exhibits antimicrobial properties which was shown by the inhibition zone of bacterial strain called *Helicobacter pylori* (Kawada and Takeuchi, 2013). **Figure 3** and **Table 2** shows the diameter of inhibition zone measurement in mm. The negative control for each strain was distilled water where the result shows zero inhibition zone. For *E. coli* the positive control was expected to use streptomycin as the *E. coli* is a gram-negative bacterium. However, due to the time constraint and lack of existing positive control disk in the lab, this research was unable to run with a positive control for the *E. coli* antimicrobial analysis. Meanwhile for the *S. aureus*, the positive control used was penicillin as it is a

gram-positive bacterium (Li et al., 2018). By delivering the anticipated outcome, positive controls are used to evaluate the test validity of the experimental protocol or apparatus. The absence of reagents or other elements required for a successful analyte detection characterizes negative controls.

In addition, the pure DSW sample shows a positive result towards the *E. coli* strain (Arandia-Gorostidi et al., 2023). The diameter for the inhibition zone obtained from the study is about 7.2 mm. While the ten times concentrated DSW shows the highest inhibition zone about 12.5 mm and 12.75 mm for *E. coli* and *S. aureus*, respectively. This is because the samples that have been investigated contain high amount of sodium. Sodium can act as an antimicrobial agent in various industries. For concentrated sample, the solution is much denser compared to the pure sample. This concentrated sample was prepared by evaporation method where the 500 mL pure DSW solution was left boil until the final volume reached 50 mL. The volume of water decreased but the amount of salt in the sample is kept constant. Since the amount of water available decreases, sodium chloride is best employed as a microbial inhibitor, which implies that it stops or inhibit the growth of bacteria. As a result, the bacterium does not have as much of a chance to grow and thrive (Truong and Whitlock, 2021).

Overall, the diameter of inhibition zone of DSW sample against *S. aureus* strains is bigger compared to *E. Coli* strains. Not only that, highest concentration of DSW also leads to bigger inhibition zone diameter. **Figure 1** and **Figure 2** shows that there is a small inhibition zone at lower concentration of sample for *E. coli* and no inhibition in *S. aureus* microbial strains. In addition, the zone of inhibition (ZOI) for *S. aureus* (Gram +ve) is bigger compared to *E. coli* (Gram -ve) (Bannerman et al., 2004). The ZOI refers to the circumscribed region where bacterial growth is suppressed or prevented by an antibiotic disc or other source of antimicrobial agent. When an antibiotic or antiseptic is administered to a bacterial culture on an agar plate, the antimicrobial agent diffuses into the surrounding agar. If the bacteria are receptive to the substance, they will stop growing in the vicinity of the disc or other substance source, establishing a definite zone of inhibition. The ZOI area can be used to estimate how sensitive the tested bacterial strain is to the antimicrobial agent. Greater sensitivity to the agent is typically indicated by larger zones of inhibition, whereas greater sensitivity or resistance may be indicated by smaller zones. In comparison to *E. coli*, *S. aureus* is thought to be more susceptible to a wider variety of antimicrobial agents. This is partly because their cell walls and membranes have different compositions and structures. *S. aureus* lacks an outer membrane and has a larger peptidoglycan layer, which renders it more vulnerable to some antimicrobial drug classes that attack the cell wall, such as beta-lactam antibiotics like penicillin. On the other hand, *E. coli*'s outer membrane serves as a defense against many antimicrobial substances and may increase its resistance to some medications. As a result, when exposed to the same quantity of an antimicrobial drug, *S. aureus* frequently exhibits a greater zone of inhibition than *E. coli*. It's vital to remember that other factors, including the specific antimicrobial agent being tested and the concentration utilized, might also affect the size of the ZOI.

In addition, gram-positive bacteria have extensive coatings of peptidoglycan in their cell walls. Gram-negative bacteria have a cell wall made up of thin layers of peptidoglycan. Therefore, gram positive bacteria have higher resistance to the samples compared to gram negative bacteria (Rohde, 2019). Most bacteria's cell wall is primarily made up of a complex polymer called peptidoglycan. It is made up of a network of long chains of the amino sugars N-acetylglucosamine and N-acetylmuramic acid, alternated with short peptide chains that serve as cross-links. The peptidoglycan layer gives the cell wall strength and stiffness while shielding the bacterium from outside osmotic pressures. Different bacterial species may exhibit differences in the thickness and structure of the peptidoglycan layer, which may affect how susceptible they are to various therapies. For instance, some bacteria have a thick layer of peptidoglycan covering them, which indicates that the molecule is well organized and present in this layer. As a result, the cell wall is more resistant to penicillin and other beta-lactam antibiotics, which attack the peptidoglycan layer. However, some bacteria have peptidoglycan layers that are thinner and less well-organized, which makes them more vulnerable to these antibiotics. This is because the peptidoglycan layer's structure can be more easily disrupted by the antibiotics' ability to more readily penetrate the cell wall and attach to it, causing the cell to burst (Rohde, 2019).

In this study, well diffusion method was used to determine the antimicrobial properties. This is because well diffusion method can accommodate more sample compared to disc diffusion method. In addition, disc diffusion method also has several disadvantages such as qualitative results and it requires large inoculum size while well diffusion method does not (Arandia-Gorostidi et al., 2023).

In the future, it is expected that the DSW may have a lot of potentials in many industries especially in the cosmeceuticals. Further research can be carried out to find the availability and efficiency of DSW in various applications.

CONCLUSION

In conclusion, it is proven that the DSW in various concentrations exhibit antimicrobial properties against *E. coli* and *S. aureus*. The concentrated DSW shows superior antimicrobial activity having larger inhibition zones. For future use, the use of DSW can be widened in multi applications with the benefit of enhancing the environment safety by developing an organic antimicrobial product especially for cosmetic applications.

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