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Microbiological profile of cultured seaweeds *kappaphycus alvarezii*

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Abstract

Seaweeds are widely used for food consumption of humans and domesticated animals. In the practice of eating the seaweeds fresh and raw, it is important to examine its microbiological aspect as it may contain fecal and other human pathogens. This study aimed to analyze the microbiological profile of fresh edible seaweeds. Brown and green strains of *Kappaphycus alvarezii* collected from three different sampling stations were examined for the presence of *Escherichia coli* and *Salmonella* and for the total aerobic plate count, coliform count, and *Staphylococcus aureus*. The results of the microbiological analysis revealed that *Salmonella* and *E. coli* were absent in all samples. Colony-forming units for total plate count, coliform and *S. aureus* did not exceed the limits of the food quality standards set by JECFA, FDA and Food standards of Australia and New Zealand. This study concludes that fresh *K. alvarezii* had a microbiological profile that conforms to the requirement for food quality standards.

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Introduction

Consumption of seaweeds can be traced back in ancient times. People during those times harvest wild-grown edible seaweeds mainly for food. In recent years, the use of seaweeds is no longer limited to a source of food, but also as a medicine, an additive in processed foods and as raw material for making new products. The discovery of its uses has prompted the people around the world to establish seaweed farms to facilitate the supply of constant demand for seaweeds.

Varieties of seaweeds can be found along the coastal areas of Lanao del Norte, especially in Panguil Bay, regarded as the highest producer of fresh seaweeds in Northern Mindanao since 2009. Communities living near the area, mostly rely on the sea ecosystem for their food and livelihood. The *Kappaphycus alvarezii* is among the abundant seafood farmed in this coastal area.

Seaweeds are known for its versatility. It is widely used for food consumption of humans and domesticated animals. It is also an ingredient of global food and cosmetics industries and used as fertilizer and animal feed additive (McHugh, 2003). *K. alvarezii* is an edible species of red seaweeds. Aside from eating processed foods made from seaweeds, people love eating it raw or mixed with other fresh vegetable salads. Its green and brown strains, as shown in figure 1, are of interest in this study since these two are commonly cultivated in this area. It is one of the main sources of carrageenan in the Philippines accounting for 98% of the total Philippine production, making it the most economically important seaweeds variety (Boquiren and Idrovo, 2014).

Researches on *K. alvarezii* have been done globally to evaluate its biochemical composition and concentration levels of essential elements. Monitoring of the concentration of heavy metals in the seaweeds has also been done. Most of the studies have found appreciable levels of biochemical compositions depending on the type of species and the environment from where they are cultured. Microbiological analysis on *K. alvarezii* have also been conducted worldwide and studies have found that some microorganisms living on the surface of seaweeds are capable of initiating complex chemical reactions with algae producing bioactive compounds with biotechnological potential (Martin *et al.*, 2014).

However, with the practice of eating fresh and raw seaweeds, it is important to examine its microbiological aspects as it may contain fecal and other human pathogens.

While increasing numbers of researches on seaweeds are conducted globally, the information regarding the microbiological composition of edible seaweeds in the Philippines is scarce. In this study, the microbiological profile of edible seaweeds obtained from the coastal areas of Panguil Bay in Lanao del Norte was assessed. This will help to ensure the sustainability of edible seaweeds in the market for direct human consumption and food safety reasons.

Materials and methods

Collection of sample

Three sampling stations were chosen as the study area as shown in figure 2. Samples of fresh *K.alvarezii* were randomly collected from the seaweed farms in Barangays Simbuco (8°09' North latitude 123°83' East latitude), Manga (8°09' North latitude 123°83' East latitude) and Tabigue (8°09' North latitude 123°84' East latitude) in the municipality of Kolambugan, Lanao del Norte during the month of July 2018.

Sample preparation

Collected samples were washed thoroughly with seawater and rinsed several times with distilled water, placed in sterile plastic containers, labeled, preserve in an ice bucket and transported to the laboratory (Gutierrez Jr. *et al.*, 2014).

Sample analysis

The fresh samples prepared were immediately brought to the laboratory for analyses.

The analysis of Total Plate Count (TPC) and Coliform Count were done following the methods of Pour Plate test. The presence of *Escherechia coli* was determined following the test method of Indole—Methyl red-Voges-Proskaver-Citrate (IMViC) utilization tests.

The analysis of *Staphylococcus aureus* was done following the method of Spread Plate test and the presence of *Salmonella* was determined following the conventional test method. Gathered data were compared with a specific standard of food requirement set by Joint FAO/WHO Expert Committee on Food Additive (JECFA), Food and Drug Administration of the Philippines (FDA) and Food Standards of Australia and New Zealand.

Results and discussion

The results of the microbiological analysis for brown and green strains of *K. alvarezii* are summarized in Table 1 and 2. *Salmonella* and *E. coli* were found to be absent in all samples. The total aerobic plate count, coliform and *S. aureus* for brown seaweeds are 108 cfu/g, 5 cfu/g and 5 cfu/g, respectively as presented in Table 1.

Table 1. Microbiological profile of brown strain *K. alvarezii* from Kolambugan, Lanao del Norte.

Site	Total Plate Count (CFU/g)	Coliform Count (CFU/g)	*E. coli*	*S. aureus* count (CFU/g)	*Salmonella* (per 25g)
1	60	5	Negative	5	Negative
2	120	5	Negative	5	Negative
3	145	5	Negative	5	Negative
Mean	108	5	Negative	5	Negative

For green seaweeds, the total aerobic plate count, coliform and *S. aureus* are found to be 197 cfu/g, 5 cfu/g and 42 cfu/g, respectively as presented in Table 2.

Table 2. Microbiological profile of green strain *K. alvarezii* from Kolambugan, Lanao del Norte.

Site	Total Plate Count (CFU/g)	Coliform Count (CFU/g)	*E. coli*	*S. aureus* count (CFU/g)	*Salmonella* (per 25g)
1	125	5	Negative	90	Negative
2	400	5	Negative	30	Negative
3	65	5	Negative	5	Negative
Mean	197	5	Negative	42	Negative

Table 3. Microbiological Quality Standards for Seaweeds.

Parameters	JEFCA	FDA-PHILS	Food Standards-Au&Nz
Aerobic Plate Count	≤ 5000 cfu/g	250cfu/g	-
Coliform Bacteria	-	-	-
Escherichia coli	Negative in 1.0g	Negative	<3 cfu/g
Salmonella spp.	Negative per test	Negative	Not detected in 25.0g
S. aureus	-	-	$<10^2$ cfu/g

The test for the total aerobic plate count will determine the amount of bacterial population in fresh edible seaweeds. A study on cultured *Caulerpa lentillifera* obtained from Cebu and Bohol revealed an aerobic plate count to be ranging from $1.4\text{x}10^5$cfu/g – $2.6\text{x}10^6$cfu/g (Delan *et al.*, 2015). This shows a much higher level compared to the result of this study. This difference could possibly be due to its habitat and seaweed variety.

The number of coliform bacteria indicates an unsanitary condition of the food and its environment.

The samples in this study taken from three different sampling stations are found to contain the same amount <10 cfu/g.

This result is comparable to a study conducted on *Ulva Lactuca*, a red seaweed obtained from Turkey, having coliform bacteria of 4.40 cfu/g (Karacalar and Turan, 2008). *E. coli*, *Salmonella* and *S. aureus* are considered as pathogenic bacteria and the result of this study has revealed that these two pathogenic bacteria are not present in all samples. As for the *S. aureus*, it was found that brown and green seaweeds contain <10 cfu/g and 42 cfu/g, respectively. A study on the level of *E. coli* in cultured *C. lentillifera* obtained from Cebu and Bohol was found to be <10 cfu/g (Delan *et al.*, 2015). A study conducted on the microbial activity of the crude extract of *K. alvarezii* obtained in Malaysia has revealed that *K. alvarezii* was not effective against gram-positive and gram-negative bacteria (Chuah *et al.*, 2017).

Fig. 1. K. *alvarezii* strains - brown (left) and green (right).

In contrast, the absence of *E. coli* and *Salmonella* in *K. alvarezii* of this study indicates that this seaweed has a strong antibacterial activity. In terms of *S. aureus*, although the result of this study shows low levels of these bacteria, it implies that *K. alvarezii* has indeed a weak antimicrobial activity against *S. aureus*. But the effectiveness of antimicrobial activity of *K. alvarezii* against *S. aureus, E. coli* and *Salmonella* were confirmed by several studies conducted in India on the extracts of acetone, ethanol, methanol, ethyl acetate, chloroform and isoamyl alcohol (Prabha *et al.*, 2013; Prasad *et al.*, 2013; Pushparaj *et al.*, 2014). Another study on red seaweeds obtained from India has revealed its moderate antibacterial activity of methanol extract against *S. aureus* and *E. coli* (Abirami and Kowsalya, 2012). Comparing the results of this study to the microbiological quality standards of food established by JECFA, FDA Philippines and Food Standards of Australia and New Zealand as seen in Table 3, it was found that the results of this study conform to the standards set by these three agencies.

It is evident from the result of this study that cultured *K. alvarezii* in Kolambugan Lanao del Norte has passed the microbiological standards for food quality and is, therefore, safe for human and animal consumption with regards to its microbial safety.

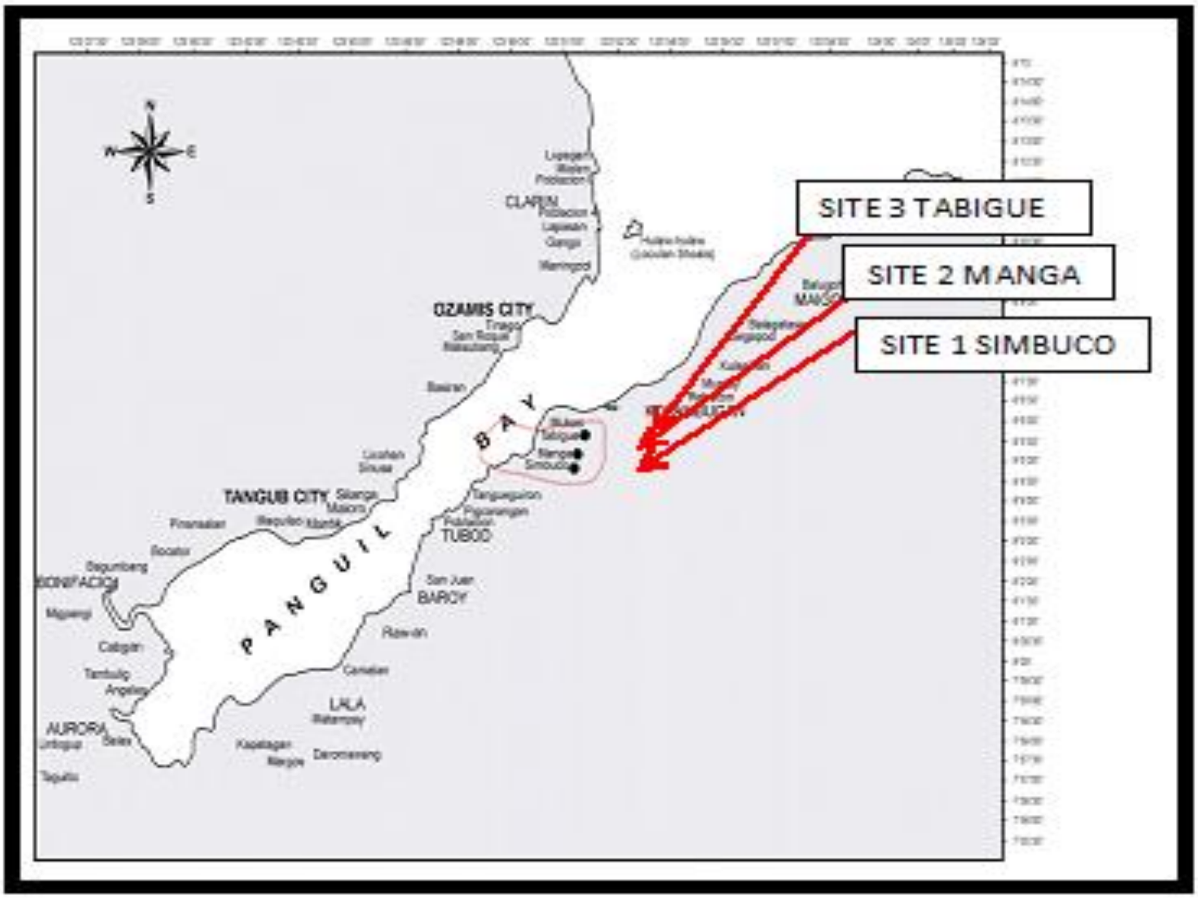

Fig. 2. A map showing the three sampling stations (*Source: Google map*).

It can also be a potential source of antimicrobial substances (Abirami and Kowsalya, 2011; Martin *et al.*, 2014; Pal Singh and Reddy, 2014; Karthick and Mohanraju, 2018).

Conclusion

From the results of this study, it can be concluded that the microorganisms found in *K. alvarezii* such as *E. coli, Salmonella, S. aureus* and bacterial load do not pose potential risks to human health since it did not exceed the limits of the food quality standards.

It is recommended that further studies on the microbiological profile of associated seawater be done to validate the food safety of these seaweeds for human and animal consumption.

It is also recommended that further studies on seaweed-microbial interactions be conducted for possible antimicrobial molecules and bioactive compounds that may be used for medical purposes.

References

Abirami RG, Kowsalya S. 2011. Nutrient and nutraceutical potentials of seaweed biomass Ulva lactuca and Kappaphycus alvarezii. Journal of Agricultural Science and Technology **5(1),** 109-112.

Boquiren J, Idrovo J. 2014. Value chain analysis and competitiveness strategy: Carrageenan seaweed Mindanao, Issue: October 2014. Mindanao Regions: Department of Agriculture-Philippine Rural Development Project.

Food Standard Australia New Zealand. 2016. Compendium of Microbiological Criteria for Food (PDF File). Food Standards Australia New Zealand. Retrieved from: https:///www.foodstandards.gov.au

Chuah XQ, Mun W, Teo SS. 2017. Comparison study of anti-microbial activity between crude extract of Kappaphycus alvarezii and Andographis paniculata. Asian Pacific Journal of Tropical Biomedicine **7(8),** 729-731.

Delan GG, Legados JA, Pepito AR, Cunado VD, Rica RL, Abdon HC, Ilano AS. 2015. The influence of habitat on the quality characteristics of the green macro alga Caulerpa lentillifera Agardh (Caulerpaceae, Chlorophyta). Tropical Technology Journal **19(1),** 10. http://dx.doi.org/10.7603/S40934-015-0010-4.

Gutierrez Jr PM, Naranjo NB, Santos MFL. 2014. Phytoextraction of Mercury by Seaweeds Kappaphycus sp. And Sargassum sp. in the Contaminated Sites of Murciellagos bay, Northern Mindanao, Philippines. Cebu Normal University Journal of Higher Education **8,** p 162-173.

Karacalar U, Turan G. 2008. Microbiological assays on edible seaweed Ulva Lactuca (L.) cultured in outdoor tanks. Journal of Applied Biological Sciences **2(2),** 27-30, 20083.

Karthick P, Mohanraju R. 2018. Antimicrobial potential of epiphytic bacteria associated with seaweeds of Little Andaman, India. Frontiers in Microbiology **9,** 611. http://dx.doi.org/10.3389/fmicb.2018.00611

Martin M, Portetelle D, Michel G, Vandenbol M. 2014. Microorganisms living on macroalgae: diversity, interactions, and biotechnological applications. Applied Microbiology and Biotechnology **98,** 2917-2935. http://dx.doi.org/10.1007/s00253-014-5557-2.

McHugh DJ. 2003. A guide to the seaweed industry. FAO Fisheries Technical Paper. Paper Rome: Food and Agriculture Organization of the United Nations **441,** p 7.

Pal Singh R, Reddy CRK. 2014. Seaweed-microbial interactions: key functions of seaweed-associated bacteria. Federation of European Microbiological Sciences **88,** 213-230. http://dx.doi.org/10.1111/1574-6941.12297

Prabha V, Prakash DJ, Sudha PN. 2013. Analysis of bioactive compounds and antimicrobial activity of marine algae Kappaphycus alvarezii using three solvent extracts. International Journal of Pharmaceutical Sciences and Research **4(1),** 306-310.

Prasad MP, Shekhar S, Babhulkar AP. 2013. Antibacterial activity of seaweed (Kappaphycus) extracts against infectious pathogens. African Journal of Biotechnology **12(20),** p 2968-2971. http://dx.doi.org/10.5897/AJB12.2307

Pushparaj A, Raubbin RS, Balasankar T. 2014. Antibacterial activity of Kappaphycus alvarezii and Ulva lactuca extracts against human pathogenic bacteria. International Journal of Current Microbiology and Applied Sciences **3(1),** 432-436.