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Bioactivity of the crude extract from *Padina japonica* Yamada (Phaeophyta, Dictyotales)

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ABSTRACT

The search for alternative sources of antibiotics from marine organisms has been motivated by the exhaustion of traditional sources which are mostly terrestrial plants. This study focuses on analyzing crude extract from *Padina japonica*, a brown seaweed, for any bioactive compounds that can inhibit bacterial growth. Crude extracts were prepared separately from blade and holdfast parts of *Padina* using four types of solvents, namely, acetone, methanol, ethanol and seawater. These extracts were added to each of the clinical strains of bacteria, namely, *Escherichia coli* and *Staphylococcus aureus* grown on nutrient agar plates, incubated overnight and the zone of bacterial inhibition measured. Crude extract obtained using acetone showed a higher bacterial activity (mean MI = 0.372) than those using other solvents, although differences were not significant ($P>0.05$). Antimicrobial activity of the acetone extract was higher on *E. coli* (MI= 0.472) than on *S. aureus* (MI = 0.271). Crude extract from *Padina* blades exhibited higher microbial activity than holdfast extract. The strength of antibacterial activity of the crude blade extract was compared with that of a commercial antibiotic, chloramphenicol, as control. Results showed that the minimum inhibitory concentration (MIC) of the blade extract has a breakpoint of 250 µl for *E. coli* and 500 µl for *S. aureus*, while chloramphenicol has a breakpoint of 10 µg ml^{-1} and 30 µg ml^{-1}, respectively for the two test bacteria. Despite the difference in concentrations, there is no significant difference ($P>0.05$) between the antibiotic and the crude extract in terms of inhibiting the two test bacteria. Thus, either the commercial antibiotic or the crude extract of *P. japonica* can be used to inhibit bacterial growth.

Keywords: Bioactivity, crude extract, antibacterial activity, inhibition, *Padina japonicum*

INTRODUCTION

Bioactive compounds occur typically in food in small quantities and are being intensively studied in order to determine their effects on human health (Etherton, et al., 2002). The most frequently studied bioactivities are cytolytic, antifungal, antiviral, growth promoter, anticoagulant, antioxidant, antihelmintic, antibacterial properties of an organism (Ara, 2001). Microbial populations have been shown to change due to exposure to antibiotics and antibacterial agents. Some diseases that were susceptible to a variety of

antibiotics are now untreatable. Microbes become resistant to antibiotics due to environmental pollution, antibacterial agents and overuse of antibiotics (Callahan, 1999), and this resistance is outpacing our ability to synthesize new drugs that can eliminate them. A classic example is the use of sulfonamides in 1936 to treat gonorrhea which resulted in the susceptibility of all strains of gonococci but six years later, the majority of the strains became resistant to (Jawets, *et al.,* 1982). Cases such as this motivated researches to look for alternative sources of medicines and antibiotics that are affordable for low income societies.

There is a broad spectrum of marine organisms studied for their bioactivity, from cyanobacteria (Thajuddin and Subramanian, 2005), bacteria (Frick, *et al.,* 2003), seagrass (Kumar, *et al.,* 2008), mollusk (Pereira, *et al.,* 1998), soft corals (Kerr, 2002), sponges (Sennet, *et al.,* 2000), and seaweeds. Active antibacterial properties were extracted from 13 seaweeds using acetone as the solvent (De Lara-Isassi, *et al.,* 1998). In a collection of 20 seaweed species from Linamon, Lanao del Norte and Naawan, Misamis Oriental, it was found that not all seaweeds can inhibit bacteria whether the extract used is in aqueous or alcoholic solution (Garcia, 1979). The concentration of bioactive compounds also depends on the growth period of the species (Uy and Largo, 2004).

The brown alga *Padina japonica* thrives in many coastal areas of the Philippines but its utility as a source of bioactive compounds has not been studied locally nor reported in other countries. Some related studies on *Padina* species are on *P. tetrastomatica* (Paula, 2002) and *P. boergesenii (*De Lara-Isassi et al., 1998)*)*. Once the antibacterial property of *Padina* is discovered, further studies and refinement of the crude extract could be undertaken to possibly produce a synthetic drug to treat certain diseases in humans and other animals. For example, bioactivite compounds from *P. japonica* may contribute to fish health and improvement of yield from aquaculture or an alternative herbal medicine in treating wounds as an antibacterial agent. This study was undertaken to evaluate the ability of *P. japonica* crude extract in inhibiting the growth of common bacteria such as *Escherichia coli* and *Staphylococcus aureus.* Various modes of extracting bioactive compounds from the seaweed were also evaluated in terms of efficiency and optimal effect.

METHODS

Fresh specimens of *Padina japonica* were collected from the littoral area fronting the Mindanao State University-Naawan ($8^0$25'34.2", $124^0$17'17.2") in Naawan, Misamis Oriental (Fig. 1). Collection of samples was done during low tide on January 24-29, 2008 and the seaweeds were placed in plastics bags filled with seawater and brought to the MSU-IFRD Microbiology Laboratory for immediate processing. The specimens were rinsed with filtered seawater to remove any sediment, attached particles and other impurities, then each thallus was separated into the holdfast and blade components.

Preparation of crude extract

Crude extracts from *Padina* were prepared based on the method described by Uy and Largo (2004). The seaweed parts were cut and shredded separately in a blender each with a different solvent, namely, acetone, methanol, ethanol and seawater. The blended

materials were each placed on properly labeled test tubes and stored at room temperature for 24 hours. Each tube sample was centrifuged then the supernatant liquid was filtered and the resulting crude extracts were placed in separate burettes where they were left to stand and form layers for 5 h. Prior to usage, each layer was separated from each other and placed on test tubes.

Figure 1. Type specimen of *Padina japonica* Yamada *(Photo credit:* Taiju Kitayama (2011), National Museum of Nature and Science, Tokyo).

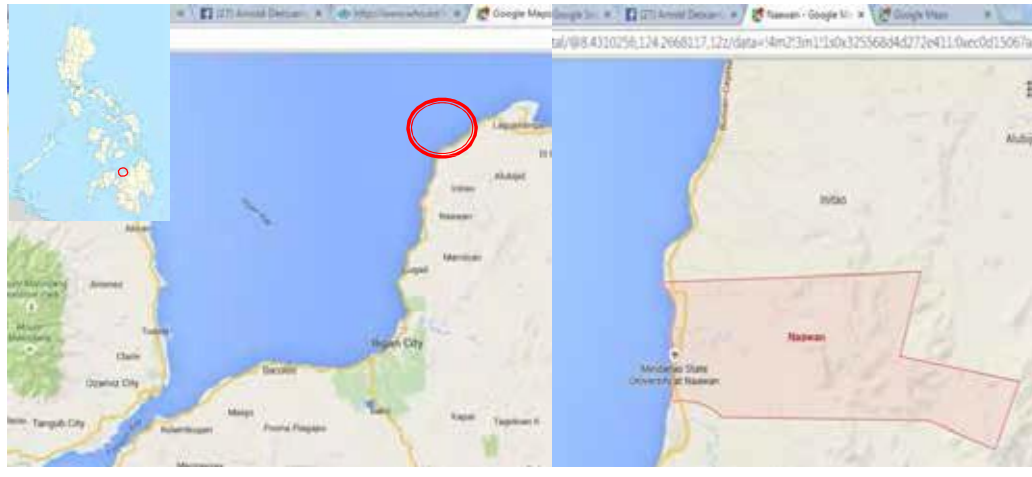

Figure 2. Map showing the collection site in Naawan, Misamis Oriental.

Test bacteria culture

Pure cultures or clinical strains of the bacteria *Escherichia coli* and *Staphylococcus aureus* were obtained from Siliman University Medical Center in Dumaguete City. Fresh cultures of each strain were prepared a day before seeding into the agar plates and their turbidity was compared with the MacFarland standard. The bacterial strains were carefully seeded on nutrient agar plates following the agar overlay

technique described by Fankhauser (2005) to ensure that the resulting plates had a homogenous lawn of bacteria.

Antibacterial assays

The disc-diffusion technique (Enriquez, et al., 1995) was used to evaluate the antimicrobial activity of the *Padina* extract. Instead of paper disc, cylinder cups were used to increase the amount of extract that was introduced to the test bacteria. Exactly 100 µL of the extract was pipetted into the cups then placed on the bacteria culture in three replicates each for all tests. The plates were labeled and incubated at 35^0C for 24 h. Antibacterial activity of the extract is indicated by the growth-free zone of inhibition on the bacteria culture. The zone of inhibition was measured in millimeter and its microbial index (MI) was calculated.

An important test of antibacterial effectivity is measurement of minimum inhibitory concentration (MIC) or the lowest concentration of an antimicrobial extract that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001). The MIC of different *Padina* extracts was determined based on the method employed by Scott and Bailey (1966) and was made only on the extract that showed the greatest inhibition for the test bacteria. The strength of antibacterial activity of the crude extract was compared with that of a commercial antibiotic, chloramphenicol, serving as a control. The antibiotic was weighed and dissolved by ethanol then diluted to produce concentrations of 5, 10, 30, 75, and 100 µg and its breakpoint referred to the correlative MIC breakpoints for food-borne pathogens.

Data management

The two-way ANOVA (Walpole, 1997) was used to compare a) microbial index of the different solvents used in extraction; b) MI among blade and holdfast extracts and (c) MIC of the crude extracts and chloramphenicol control. One-way ANOVA, t-test and Tukey's HSD test were used for results that showed significant differences.

RESULTS AND DISCUSSION

Bioactivity of the different solvents

Among the four solvents (i.e. acetone, methanol, ethanol and seawater) used in extracting antibacterial compounds from the seaweed *P. japonica*, acetone yielded the highest mean MI value (0.372) and thus, may be considered the most efficient solvent. For specific actions on the test bacteria the highest MI was obtained on *E. coli* by acetone-based (0.472) and seawater (0.472) extracts while the highest mean MI on *S. aureus* was obtained from extract using the solvent ethanol (0.314). Results suggest that bacterial growth inhibition is influenced by the type of solvent used to extract the bioactive compounds and that any solvent is probably compound-specific.

Comparative bioactivity of *Padina japonica* parts

After allowing to settle for 5 h the blade extract formed two layers in a burette. The first layer (Blade L-1) was characterized by small solid red and green particles, presumably, tiny particles of the blade that passed through the filter paper while the second layer (Blade L-2) was only green liquid. Extract from the holdfast did not form any layers. In the antibacterial assays the diameter of inhibition zone (DIZ) on the bacteria-agar plate from the Blade L-1 was much wider compared to that from Blade L-2 and the holdfast extract. Consequently the assay showed that Blade L-1 extract produced the higher MI values for *E. coli* (0.417) and *S. aureus* (0.308) while the Blade L-2 extract obtained lower MI for *E. coli* (0.344) and *S. aureus* (0.256) (Table 1). The holdfast extract produced the lowest MI for both *E. coli* (0.271) and *S. aureus* (0.167) (Fig. 3). In pure 95% acetone solvent which was used as negative control, no inhibition was produced. During the final test, there was no interaction observed between the acetone solvent and the test bacteria. This means that whether the acetone extracts of *Padina japonica* will be layered or not, the effect is most likely the same.

Table 1. Diameter of inhibition zone (DIZ) and microbial index **(**MI) of the different extracts from holdfast, blade layer-1, and blade layer-2 of *Padina japonica*.

Solvent	Bacteria	Average DIZ (mm)	MI	Mean MI
Holdfast	*Escherichia coli*	7.6	0.27	0.22
	Staphylococcus aureus	7.0	0.17	
Blade layer 1	*Escherichia coli*	8.5	0.42	0.36
	Staphylococcus aureus	7.9	0.31	
Blade layer 2	*Escherichia coli*	8.1	0.34	0.30
	Staphylococcus aureus	7.5	0.26	

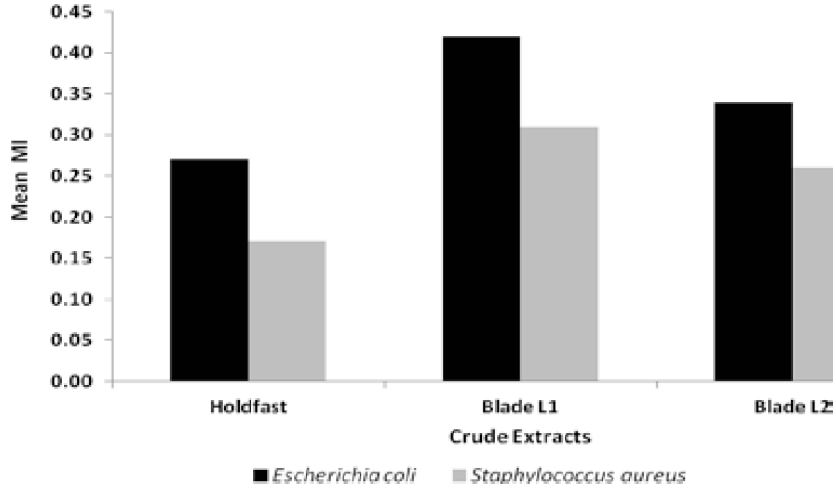

Figure 3. Microbial index of crude blade and holdfast extracts of *Padina japonica* on *E. coli* and *S. aureus.*

Minimum inhibitory concentration (MIC) breakpoint

The MIC breakpoint of the commercial antibiotic chloramphenicol was at lower concentrations for both *E. coli* and *S. aureus* as compared with the first layer of the blade of *P. japonica* extract (Fig. 4). Only 10 µg ml^{-1} of chloramphenicol was needed to inhibit *E. coli* growth and 30 µg ml^{-1} to inhibit *S. aureus*, however, it will require 250 µl and 500 µl of crude extract, respectively to inhibit the two bacteria. Based on the MIC breakpoints standard, chloramphenicol has intermediate effect on the test bacteria *E. coli* and S. *aureus,* thus, the level of antimicrobial susceptibility results in an indeterminate outcome.

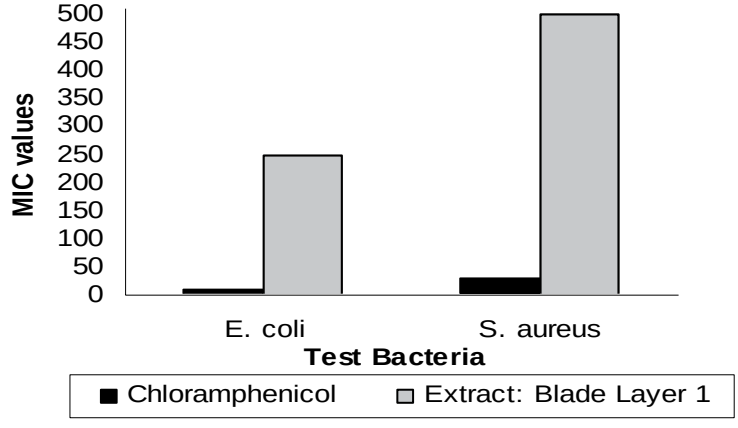

Figure 4. Minimum inhibition concentration (MIC) values of the chloramphenicol (control variable) and the first layer extract of the blade on *Escherichia coli* and *Staphylococcus aureus.*

Statistical analysis showed no significant difference (P>0.05) between the MIC breakpoint of the commercialized antibiotic and the *P. japonica* extract in terms of efficacy in eliminating the test bacteria. However, a greater volume of crude extract is needed to eliminate the same number of bacteria compared to the lower amount needed if the commercial antibiotic is used. This means that either the chloramphenicol or the *Padina japonica* extract could be used to inhibit *E. coli* and *S. aureus*, but at varying concentrations.

CONCLUSIONS AND RECOMMENDATIONS

This study provides a preliminary profile on the ability of crude extract from the brown seaweed *Padina japonica* to present growth of pathogenic bacteria. Positive bioactivity was present in both blade and holdfast extracts but at variable levels of efficacy and at higher levels of concentration than the commercial antibiotic chloramphenicol. These findings can have useful applications for agriculture, aquaculture and public health. *Padina* is often used in fertilizer and cattle feeds and may help control bacterial growth in farmland or in the intestinal tract of cattle. More intensive research is

recommended to verify these results particularly in optimizing extraction and concentration of bioactive compounds to increase their efficacy on inhibiting microbial growth. Lastly it is important to conduct an analysis of the bioactive substance from *Padina*, synthesize and produce it for human use.

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