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Antibacterial Activity of the Sea hare, Dolabella auricularia, Egg String Extracts Against the Escherichia coli

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Abstract

An assay was conducted to determine the antibacterial activity of various concentrations of the Sea hare, *Dolabella auricularia*, egg string extracts against the bacterium, Escherichia coli, using disk diffusion method. The study was conducted at the Davao Oriental State College of Science and Technology (DOSCST), City of Mati, Davao Oriental. Escherichia coli showed susceptibility to the extract from a mixture of 0.6g powdered, dried-green, and dried-brown *D. auricularia* egg strings per ml ethanol. Extract obtained from the freshly-laid green strings showed a mean of 8.51 mm zone of inhibition while the one from brown-dried egg strings had a mean of 7.71 mm. Results suggest that *D. auricularia* egg string extracts contain antibacterial factors which were evident if extracts are taken from dried egg strings and applied at a higher level of concentration of 0.6g/ml. The freshly-laid green egg strings showed to have more antibacterial factors than the mature, brown egg strings.

Keywords: antibacterial property, Sea hare, *Dolabella auricularia*, egg string extracts, Pujada Bay

Introduction

The discovery of antimicrobial agents during the 20th century has substantially reduced the threat posed by infectious diseases. The use of these "wonder drugs", combined with improved sanitation, housing, and nutrition, and the advent of widespread immunization programs, has led to a dramatic drop in deaths from diseases that were previously widespread, untreatable, and frequently fatal. Over the years, antimicrobials have saved the lives and eased the suffering of millions of people (WHO, 2002).

However, there has been an emergence and spread of antimicrobial resistance among pathogenic bacteria (Courvalin, 2005) which caused severe consequences in the community in the past few decades. Infections caused

by resistant microbes result in prolonged illness and greater risk of death. Treatment failures increased the numbers of infected people moving in the community and thus, exposed the general population to the risk of contracting a resistant strain of infection. Increasing concern about the prevalence of these resistant-bacteria led to continuing search and development of new drugs from various kinds of sources.

For the last 20 years, several studies established that seahares are important source of antimicrobial peptides (Blunden, 2001: Pettit, R. K. Pettit, G.R., and Hazen, 1998: lijima, Kisugi, & Yamazaki, 2002). In the sea hare, Dolabella auricularia antimicrobial factors such as dolastatin B2, isolated from the body wall of *D. auricularia*, and dolabellaninA, purified from the animal's albumen gland, showed antimicrobial properties. The presence of antimicrobial activity has also been reported from the purple ink and egg strings ofsea hares (Melo, Fonsea, Vasconcelos, & Carvalho, 1998).

Ayag (unpublished thesis, 2006) indicated that the sea hare, *D. auricularia*, as well as their egg strings are abundant in some areas of Pujada Bay, Mati, Davao Oriental. Importantly, residents of Mati are fond of collecting sea hare egg strings and consume the egg strings as food.

Since egg strings are abundant in the area and the people used the egg strings as food, it is appropriate to determine the antimicrobial activity of the seahare egg strings from along Pujada Bay, Mati, Davao Oriental.

Thus, this study aimed to determine if *D. auricularia* egg strings contain antibacterial properties against the pathogenicentero-bacteria*, Escherichiacoli.*

Objectives

Specifically, this study determined:

1. the efficacy of extracts from various concentrations ofegg strings blended as fresh green or brown in ethanol, against the E. coli.
2. the efficacy of extracts from various concentrations of egg strings blended as dry either green or brown in ethanol, against E coli.

Materials and Methods

Study Area

The study was conducted at the Davao Oriental State College of Science and Technology (DOSCST), City of Mati, Davao Oriental (Fig. 1) on December 1, 2008 to January 15, 2009.

Sampling and Sampling Schedule

Sea hare egg strings were collected from along the coast of Pujada Bay at Guang-Guang in the City of Mati, Davao Oriental, which is approximately 1.0 km away from DOSCST (Fig. 1). Collection of egg string samples for the dry method was conducted on December 4, 2008 while egg strings for the fresh method was on December 9, 2008. Sampling of egg strings were done at 6:00 in the morning.

The gathered egg strings were placed in a well cleaned plastic bucket with

seawater and crushed ice to prevent the egg strings from dehydration. The egg strings were transported to the DOSCST Chemistry Laboratory approximately, 1.0 km away from the sampling area. Samples were cleaned with tap water, drained for 10 minutes and segregated according to developmental stages — mature (brown) and freshly-laid (green). It was observed during sampling that the freshly laid greenish egg strings were more abundant than the mature, brownish egg strings. There were 900 g of brown, and 1900 g of green egg strings collected.

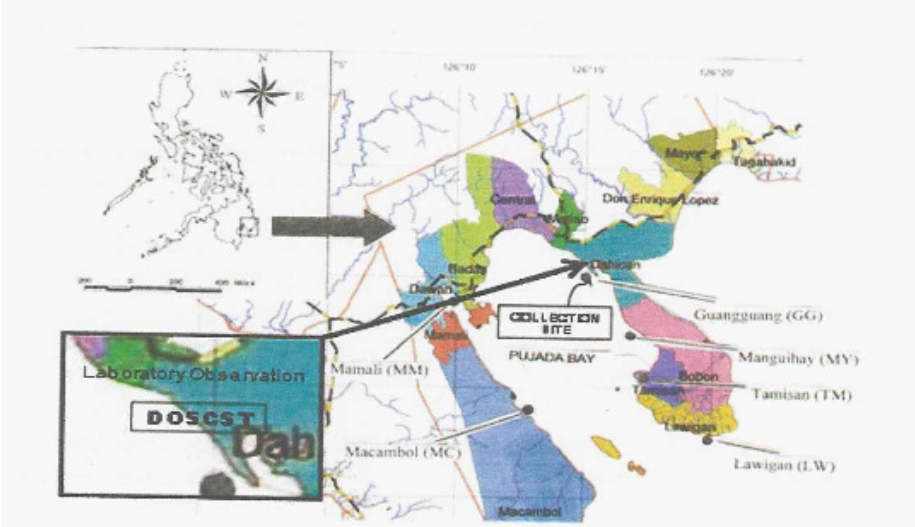

Figure 1. Vicinity map of sampling sites in Pujada Bay

With the dry method, the egg strings were air-dried on suspended nets at the Chemistry Laboratory for five days. After drying, the final weight of each green and the brown egg strings samples were measured.

Moisture Loss

Final weight of green egg strings was 949 g while the weight of the brown egg strings was reduced to 420 g. Moisture loss was computed following the formula:

$$\text{Moisture loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}}$$

$$\text{Moisture Loss in Green-Dried Egg String} = \frac{1900\text{g} - 949\text{g}}{1900\text{g}} \times 100 = 50\%$$

$$\text{Moisture Loss in Brown-Dried Egg String} = \frac{900\text{g} - 420\text{g}}{900\text{g}} \times 100 = 50\%$$

Preparation of Extracts and Extraction Method

Using an electric blender, 270 g of dried green samples of *D. auricul*aria egg string were ground and divided into weight of 60 g, 90 g, and 120 g, respectively. The weighed samples were soaked separately for 24 hours in 200 ml of ethanol in sealed beakers. Thereafter, the mixtures were each filtered through Whatman filter paper# 1 into properly-labeled sterile 500-ml glass bottles. The same procedure was carried out for dried brown egg strings, fresh green egg strings and fresh brown egg strings.

The filtrates Were bought to the Research Center of the University of Immaculate Concepcion (IJIC) in Davao City for extraction in a rotary evaporator.

Preparation of Paper Discs

The paper discs used were made from Whatman filter paper#l which was cut using an office puncher 6.2 mm in diameter. These were wrapped in a foil and sterilized in the oven.

There were 117 paper discs used in 13 treatment combinations (Table 1). During the assay, nine paper discs were immersed in every treatment; three paper discs in every replicate.

Preparation of Culture Media

Nutrient agar was used in the culture Of *E. coli*. Preparation was as follows:

1. Suspend 23g of the medium in one liter of distilled water.
2. Mixed well and left to stand until the mixture was uniform.
3. Heated with gentle agitation and boiled for one to two minutes, or until completely dissolved.
4. Dispensed or poured 20-25ml into sterile Petri plates and sterilized at 121^{o}C for 15 minutes.
5. Culture medium was cooled and allowed to solidify after sterilization.
6. The agar plates were placed in upside down position in the inoculating chamber for 24hr to minimize moisture.

Antibacterial Assay - Disk Diffusion Test

The assay was carried out using disk diffusion test, alsocalledKirby-Bauer method.

A sterile inoculating loop was used to pick colonies from pure culture of *s* to inoculate a nutrient broth. The culture broth was incubated for 24hr at 37^{0}C until it became slightly turbid. Sterile cotton swabs were dipped into the culture broth

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Treatments and Treatment Combinations

Table 1. Treatment combinations

Treatment	Treatment Combination		
	Developmental Stage	Preparation Method	Concentration Level g per ml
1 Control	-------	------	-------
2 GFT1	Green	Fresh	0.30
3 GFT2	Green	Fresh	0.45
4 GFT3	Green	Fresh	0.60
5 GDT1	Green	Dry	0.30
6 GDT2	Green	Dry	0.45
7 GDT3	Green	Dry	0.60
8 BFT1	Brown	Fresh	0.30
9 BFT2	Brown	Fresh	0.45
10 BFT3	Brown	Fresh	0.60
11 BDT1	Brown	Dry	0.30
12 BDT2	Brown	Dry	0.45
13 BDT3	Brown	Dry	0.60

GFT = Green Fresh Treatment
GDT = Green Dry Treatment
BFT = Brown Fresh Treatment
BDT = Brown Dry Treatment

was incubated for 24hr at 37^0C until it became slightly turbid. Sterile cotton swabs were dipped into the culture broth to evenly inoculate the surfaces of the agar plates. After 5 minutes when the agar surface in the plates was dried, the appropriate three paper discs previously immersed in a treatment combination were placed on the surface at equidistant position. The treated plates of E. coli colony were incubated at 37^0C for 24hr. At the 24-hr incubation, the inhibition zone formed around each disc in all the treatments was measured in millimeter using a vernier caliper.

Results and Discussion

Only the extract from the highest concentration mixture of 0.6 g dried and blended, either freshly laid (green) or mature (brown) egg strings, per ml of ethanol had inhibited the growth of *E. coli* (Table 2). All the other treatments showed no inhibition of bacterial growth.

Likewise, it was evident that green (freshly-laid) egg strings contain more antibacterial factors than the brown (mature) egg strings. This suggests that antibacterial property of *D. auricularia* egg string extracts varied with the developmental stages of the egg strings.

Results imply that in order to be an effective inhibiting agent against *E. coli*, Anti-bacterial substance from Sea hare egg strings must be extracted at a higher concentration (0.6g/ml) using the dry method. Drying the egg strings reduced their

moisture content that resulted in a more concentrated extract than those prepared from freshly collected wet egg strings.

Table 2. Inhibition zones (diameter in mm) of extracts from methanol filtrate of varying mixtures of egg string powdered dry or fresh.

Concentration (g/ml) / Treatment Level		Replication 1	2	3	Total	Mean
Control	0	0	0	0	0	0
GFT1	0.30	0	0	0	0	0
GFT2	0.45	0	0	0	0	0
GFT3	0.60	0	0	0	0	0
GDT1	0.30	0	0	0	0	0
GDT2	0.45	0	0	0	0	0
GDT3	0.60	8.467	8.833	8.233	25.533	8.511
BFT1	0.30	0	0	0	0	0
BFT2	0.45	0	0	0	0	0
BFT3	0.60	0	0	0	0	0
BDT1	0.30	0	0	0	0	0
BDT2	0.45	0	0	0	0	0
BDT3	0.60	7.300	7.667	8.167	23.134	7.711

GFT = Green Fresh Treatment
GDT = Green Dry Treatment
BFT = Brown Fresh Treatment
BDT = Brown Dry Treatment

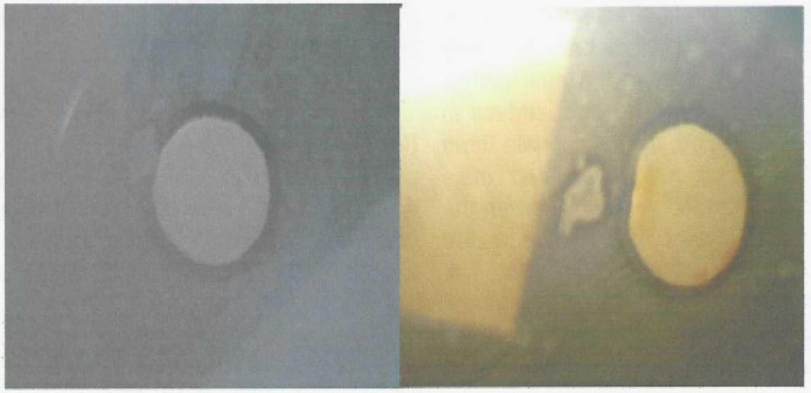

Fig. 2. Inhibition zones by extracts from Green-Dry and Brown-Dry D. auricularia egg string against E. coli

Results affirm the study Of Kamiya, and others (1987) that the antibacterial

activity of egg mass was kept during the trochophore stage and disappeared by tle time embryonic larvae assumed their characteristic veliger forms.

According to Bantayan (2009) green egg strings contain pre-hatched embryo including the trochophore stage, and the brown egg strings mostly were consisting of empty capsule suggesting that the embryos had hatched from and assumed the veliger form. The present finding substantiates reports that marine mollusks protect their eggs with natural products (Benkendorff and others, 2001: Murugan and others, 2005). This explains why extract from freshly laid (green) dried eggs showed wider inhibition zone than those from mature (brown) dried eggs string.

Studies conducted by Blunden (2001), Pettit and others (1987) and lijima and others (2002) indicated that sea hares and their egg strings are important sources of antimicrobial peptides. These studies support that D. auricularja egg strings contain antimicrobial factors that were effective against E. coli at a concentration of 0.6gJml.

Dolastatin, and Dolabellanin are antimicrobial peptides extracted from the sea hare.

Nutrient analysis of *Dolabella auricularia* egg strings by Morales (2008) showed 23.87% protein per 100 g sample of egg strings.

Conclusion

Extract from the newly laid (green) egg string of the sea hare, *Dolabella auricularia* contain antibacterial factors that inhibited the growth of the entero-bacterium, Escherichia coli. Its antibacterial property is more effective if extracted from dried egg strings at a concentration of O .6g powder per ml ethanol. Drying the egg strings reduces the moisture content therefore, resulting in a more concentrated extract than those prepared from web fresh egg strings.

Mature (brown) egg string had lesser zone of inhibition that suggests it has less antibacterial activity than the newly laid eggs.

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