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Screening of Antibiosis Activity from Green Algae (Chlorophyta) from Drini Beach, Yogyakarta: a Preliminary Study

Penentuan Daya Antibiosis Beberapa Rumput Laut Hijau (Chlorophyta) dari Pantai Drini, Yogyakarta: Sebuah Kajian Awal

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Abstrak

Penelitian ini bertujuan untuk menguak keberadaan dan nilai penting dari senyawa alamiah yang terkandung di dalam beberapa jenis rumput laut di Indonesia, khususnya yang tergolong sebagai anggota rumput laut hijau (Chlorophyta). Tujuan khusus penelitian ini adalah untuk mencari kemampuan antibiosis dari rumput laut hijau yang ditemukan di pantai Drini. Empat jenis rumput laut hijau yang diketemukan memperlihatkan kemampuan antibiosis yang bervariasi dibandingkan dengan perlakuan kontrol. Salah satu jenis, bahkan, menunjukkan daya hambat yang lebih kuat terhadap pertumbuhan mikroorganisme uji, E. coli, *daripada larutan penisilin murni (10 μg).*

Kata kunci: daya antibiosis, rumput laut, Chlorophyta, Drini

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Introduction

Ancient practices from maritime peoples from Japan and China have used seaweeds as medicinals, for example to cure goiter disease. In addition to that, early Chinese pharmacopeia wrote about the usage of seaweeds for diverse maladies such as doopsy, menstrual difficulties, abscesses, and even cancer (Ruggieri, 1976). Seaweeds extracts were reported to have effects on certain pests and diseases from agricultural plants. Plants treated with seaweed extracts shown more resistant condition to infection by fungus and aphids (especially potato aphid *Myzus persicae*) (Stephenson, 1965).

Most of the green algae which are tropical are abundantly and widely distributed in the continental shelf habitat (Paul and Fenical, 1987). Several genera of Chlorophyta are found to be widely used by human beings. In Indonesia, some member of green algae are utilised both as food and agar producers, such as *Gelidium* spp and *Gracillaria* spp (Suharni *et al.*, 1992; Hatta & Purnomo, 1994). However, little is known about their antibiosis activity reported in the country.

Michanek (1979) noted that Indonesia has been able to produce 3000 tons of seaweeds along the Java sea in 1973 and in 1974 it became tripled into 8400 and 8800 tons in 1975 and 1976. Therefore, Indonesia has a very big chance to explore the diversity of the seaweeds growth in the saline waters in the coastal area and to explore its usage with special regard to industrial and medicinal aspects. In addition to that, this will become the new source of economic income for the country.

New drug discoveries are very important too for every local government in the country at this time. After the declaration of the Decree No. 22/1999, it is stated that every local government should be able to be independent from national government with special regard to financial aspect. It means that local governments have to find new sources of economic support by their own capacity and

ability to fulfill their financial needs. One of the possibility is to utilise its natural resources such as marine natural resources, more specifically seaweeds. In Indonesia seaweeds are actually have been utilised as an ultimate source of agar or carrageenan which is then used as basic substances for cosmetics, medicines, and foodstuffs as well. Therefore, to alleviate the economic value of the seaweeds in the country it still needs to be explored further by doing continual research in order to find their bioactive substances to be produced in pharmaceutical and medicinal industries.

The specific objective of the research are to identify and determine the green seaweeds belong to Chlorophyta groups and to reveal the antibiotics activity of the seaweeds' extracts found at Drini beach. Identification and determination of the green seaweeds are done in order to select the best seaweeds which contains the most effective extracts against harmful microorganisms known to human health condition, such as *E. coli* and *Bacillus subtilis*. In addition to that, *in vitro* tests will be conducted and the result will be compared to common antibiotics used to cure diseases, for instance penicillin and streptomycin. It is also hoped that the result of the research can give an alternative income for the people lived near Drini beach by cultivating the selected seaweeds in a bigger scale.

Research Methods

Seaweed samples were collected from Drini beach during low tide and then stored in a cold box. Upon reaching the laboratory, the samples were divided into two parts. The first part was identified and determined under Chlorophyta groups based on the work of Hoppe (1979) and Bonotto (1979), while the second part was washed for several times using tap water and then are chopped off into small pieces. These pieces were blend and extracted with petroleum ether (PE) for 8 hrs following the method of Henriques *et al*. (1979, with minor modification). Antibiosis activity assay of the sample extracts were done utilising standard agar plate method to some human pathogenic bacteria such as *E. coli* and *Bacillus subtilis*. The agar plates were incubated at 37 ^{o}C for 24 hrs. Zone of inhibitions were then measured. The results were compared to common antibiotics sell in the market place, such as penicillin and streptomycin in pure concentration (10 μg). In order to examine the significant result between treatments ANOVA statistical computation was applied (Gomez and Gomez 1984).

Results and Discussions

The present research found 4 (four) Chlorophyte species and identified as *Chaetomorpha crassa* (Ag.) Kultzing (Kadi 1996) for extract sample B, *Ulva fasciata* L (Kadi 1996) for extract sample A, *Enteromorpha intestinalis* (Linn.) Link (Trono, Jr. and Ganzon-Fortes 1980) for extract sample C, and an unidentified seaweed for extract sample D.

Drini beach, with its rocky substrate, seems to be convenience environment for the growth of the species found in the area. These four species were found to be abundance in this beach. The most abundance species, however, is *Ulva fasciata*, that dominate almost the entire area.

Determination and identification of the seaweeds found in the area of research are shown in Table 1.

The results showed that seaweed extract of the samples have an inhibition activities to two (2) test microorganisms, i.e. *B. subtilis* and *E. coli*. In other words the seaweed's extract showed potential antibiosis activities to human pathogenic microorganisms used as bioassay test.

There are, however, varieties result in term of strength of inhibition. For example, the results utilising *B. subtilis* as test microorganism it is noted that all extract samples show significant differences compare to control treatment (K). The strongest inhibition to *B. subtilis* was still recorded from pure penicillin (10 microgram) treatment (P) and followed respectively by pure streptomycin (10 microgram) treatment (S), treatments of extract seaweed C and D, and treatments of extract seaweed A and B.

The results above similar to some earlier findings reported by, for instance, Diaz-Piferrer

(1979) and Nizawa (1979). Though the seaweeds' species utilised were different from this previous research, i.e. *Chondria littoralis* (Diaz-Piferrer 1979).

Inhibition activities were also revealed for *E. coli* as bioassay test microorganism (Table 2). Pure streptomycin (10 microgram) and seaweed extract D showed the strongest inhibitions and followed respectively by pure penicillin (10 microgram), seaweed extract C, seaweed extract A, and seaweed extract B. One interesting thing from this result was that seaweed extract D showed stronger inhibition than pure penicillin (10 microgram) treatment to *E.coli* and even have similar inhibition zone to pure streptomycin (10 microgram) treatment.

This present results were in line with earlier reports such as Diaz-Piferrer (1979), whose report concluded that the extract of *Sargassum natans* and *Chondria littoralis* showed strong antibiosis activity against *E. coli*. Furthermore, Nisizawa (1979) also reported that some marine algae extracts from Japanese waters also showed antibiotic activity to *E. coli*.

The results, however, are still need to be verified since the extracts were in the crude forms. It was not clear enough whether the antibiosis activities were due to the active substances found from the seaweeds or other impurities mixed within the crude extracts.

Conclusions and Recommendations

The present research concluded that all seaweed extracts show antibiosis activities in variety of inhibition zones to the growth of test microorganisms. There is one seaweed sample (unidentified seaweed sample) that is more potent in term of antibiosis activity compare to pure penicillin treatment.

It is recommended that purification (utilizing column chromatography) of the extract in order to reveal the active substance need to be done for further research. Other test microorganisms such as fungus, microalgae, protozoa, etc might be utilised to know the potential inhibition of each of the seaweed extracts.

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Table 1. Determination and identification of the seaweed samples found in the research area

Sample Code	Determination	Identification
A	Thalli wide (up to 5 cm) and long (up to 12 cm) attached to the substrates such as rocks and corals by its discoid holdfast, green in color, usually becomes dominant seaweeds in intertidal areas	*Ulva fasciata* L
B	Thalli cylindrical like hairs, ± 2 cm long, formed a bundle of thalli, green in color, growth and attach to (or epiphyte) on solid substrates, sometimes becomes abundance and creates problems to other seaweeds cultivation, distributed widely in Indonesian seas	*Chaetomorpha crassa* (Ag.) Kultzing
C	Thalli up to 17 cm long, bright green when fresh, whitish when dried, attached to the substratum by a small discoid holdfast, can be observed at exposure intertidal areas on solid substrates like rocks and pieces of corals	*Enteromorpha intestinalis* (Linn.) Link
D	Thalli are fine, cylindrical, and long (± 7 cm), dark green in color, usualy formed a group of thalli, attached to substratum by its holdfast on rocks and corals	Unidentified

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Table 2. Average inhibition zone (cm^2) of seaweed's extracts to B. subtilis *and* E. coli

Sample	***B. subtilis***	***E. coli***
A	0.133^{a}	0.166^{h}
B	0.100^{a}	0.066^{g}
C	0.266^{b}	0.233^{i}
D	0.233^{b}	0.400^{k}
P	3.000^{c}	0.300^{j}
S	0.500^{d}	0.400^{k}
K	0.000^{e}	0.010^{f}

Note:
A, B, C, D : Seaweed's sample extracts
P : Penicillin 10 µg
S : Streptomycin 10 µg
K : Control (Petroleum Ether)
a – k : Represent the significant difference between treatments in the same columns