A PRELIMINARY REPORT ON THE SCREENING OF MARINE ALGAE FOR ANTIBACTERIAL PROPERTIES

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Especially today with the current energy crisis and economic restraints, there has been a growing need and awareness for the identification and utilization of local medicinal plants as substitutes for expensive pharmaceutical chemicals and as sources of new drugs. Newspapers and periodicals have popularized articles that deal with this search (*Bulletin Today,* 1-29-77; *The Carillon,* March 1977; *Bulletin Today,* 10-11-77). But even before this current interests, Quisumbing (1951) has written his voluminous *Medicinal Plants of the Philippines* and a number of workers (Masilungan, *et al.,* 1953; Masilungan, et al., 1959; Masilungan, *et al.,* 1963) have tested and screened these reported medicinal plants for antimicrobial substances. These works, though, were concentrated on the higher plants. Nevertheless there were some investigations on the antimicrobial properties of fungi and lichens (listed in Nemenzo's compilation, 1969) and regarding the algae, Allen and Dawson (1960) reported the production of antibacterial substances by benthic tropical marine algae and Velasquez (in SEC, 1971) noted that *Caulerpa sertularioides, C. serrulata* and *C. racemosa* are being tested for some active chemical constituents and possible traces of antibiotics.

This paper then seeks to add further information as to what other kinds of benthic algae there are that would exhibit inhibitory activity on the test organisms *Staphylococous aureus* and *Escherichia coli.*

Materials and Methods

Twenty different species of marine benthic algae were collected from the littoral and infratidal zones of Linamon, Lanao del Norte and Naawan, Misamis Oriental. They were transported to the laboratory in

plastic bags with a small amount of sea water and immediately processed for testing. Extra samples were preserved in formaldehyde or pressed and dried then mounted. These were then identified and classified.

Preparation of Extract. The algae were rinsed in tap water and then two 5-gram samples were weighed. Each of the 5-gram samples were pounded in a mortar, then transferred together with a bit of solvent (tap water or 95% ethyl alcohol, as the case may be) from a measured volume of 20 cc into an Erlenmeyer flask. The rest of the solvent was added by flooding the mortar with it and then pouring the washing into the same flask. The flask was corked and allowed to stand with occasional shaking for 24 hours. The resulting supernatant was then used in testing for the presence of an antibacterial substance. The aqueous extracts were first autoclaved before they were used for testing.

Filter paper-disc Test. The supernatants were tested for antibacterial activity by the filter paper-disc method. The test organisms were *Escherichia coli* (Migula) Castellani & Chalmers and *Staphylococcus aureus* Rosenbach taken from the stock culture of the MSU Department of Biology which were originally obtained from the UP Culture Collection of Microorganisms through Dr. Flordeliz Uyenco.

The Petri dishes were sterilized first. Tubes of sterilized 15-ml nutrient agar (prepared according to Pelczar & Chan, 1972) were melted and allowed to cool to about 45°C. They were then seeded with the test organism by transferring to them two loopfuls from a 24-hour broth culture of the test organism and vigorously rotating them with the palms of the hands. The seeded nutrient agar was then poured into the sterile Petri dish, utilizing aseptic procedures. The Petri plate was gently rotated to allow even distribution of the nutrient agar, then allowed to solidify on a level surface.

The filter discs, 13.5 mm. in diameter, were made by cutting Whatman filter paper No. 1 with a cork borer. They were wrapped with clean paper in lots of four, autoclaved, then dried over the stove.

The sterile filter paper was then grasped with a sterile pair of forceps and immersed into the test supernatant. The excess liquid was allowed to drain by allowing the lower edge of the disc to touch the

side of the flask. Then the disc was laid in the center of the quadrant to which the plate was divided. Three replicates were made for each algal supernatant and placed into three separate plates. Thus, in a Petri plate, there are four sectors, three of which contain the discs dipped into three different algal extracts and the fourth dipped into sterile sea water or 95% ethyl alcohol. There are then three of such plates.

The test plates were incubated at 37°C for 24 hours and then observed for any zone of inhibition around the disc which will indicate antibacterial properties.

Results and Discussions

The results of the screening of 20 marine benthic algae are presented in Table 1. Aqueous extracts of eight species exhibited slight inhibitory action on *E. coli,* 2. on *S. aureus.* Alcoholic extracts of two species exhibited distinct inhibitory action on *S. aureus,* one species clear inhibitory action, two species slight inhibitory action, and one species doubtful inhibitory activity. Alcoholic extracts of no species exhibited inhibitory action on *E. coli.*

From the results obtained, it can be shown that there are quite strong antibacterial substances acting against *S. aureus* which can be extracted by alcohol in species of *Caulerpa* and *Halimeda.* There seems to be greater solubility of antibacterial substances in water than in alcohol.

It must be noted that the tests are qualitative and do not portray the actual reaction of the active substances against the test organisms. The quantitative amount and useful evaluation of any antibacterial substance can only be known after it has been isolated, and purified and tested.

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Table 1. Inhibitory action of aqueous and alcoholic extracts of benthic marine algae on bacterial growth

Coll. No.	Alga	Aqueous extract		Alcoholic extract	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
	Blue green:				
20	*Lyngbya* sp.	+	—	—	—
	Green:				
13	*Caulerpa serrulata* (Forsk.) J. Agardh	+	—	—	+++
14	*C. sertulatioides* (Cmel.) Howe	—	—	—	+++
17	*Filamentous siphonaceous*	—	—	—	+
11	*Halimeda discoidea* Decaisne	+	—	—	++
	Brown:				
8	*Dictyota bartayresii* Lamouroux	+	—	—	—
6	*D. ciliplata* Kutzing	—	—	—	—
3	*Padina arborescens* Holmes	—	—	—	—
21	*Padina* sp.	—	—	—	—
1	*Sargassum confusum*	—	—	—	—
2	*S. filicinum* Harvey	+	—	—	—
5	*Turbinaria ornata* J. Agardh	—	—	—	—
10	*Amphiroa dilatata* Lamouroux	+	+	—	—
15	*Chondrococcus hornemanni* (Martens) Schmitz	—	—	—	+
19	*Corallina* sp.	—	—	—	—

16	*Eucheuma striatum*	+	±	−	±
12	*Gelidiella acerosa* (Forsk.) Feldmann & Hamel	−	−	−	−
22	*Gracilaria verrucosa* (Huds.) Papenfuss	+	−	−	−
9	*Mastophora rosea*	−	±	−	−

Legend: +, slight inhibitory activity
++, clear inhibitory activity
+++, distinct inhibitory activity
±, doubtful inhibitory activity

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