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SCREENING FOR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES IN SOME MARINE ALGAE FROM THE FUJIAN COAST OF CHINA WITH THREE DIFFERENT SOLVENTS*

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Abstract Three different solvents viz ethanol, acetone and methanol-toluene (3:1) were used to extract antibiotics from 23 species of marine algae belonging to the Chlorophyta, Phaeophyta and Rhodophyta. Their crude extracts were tested for antibacterial and antifungal activities. Among them, the ethanol extract showed the strongest activity against the bacteria and fungi tested. Four species of the Rhodophyta (*Laurencia okamurai*, *Dasya scoparia*, *Grateloupia filicina* and *plocamium telfairiae*) showed a wide spectrum of antibacterial activity. Every solvent extract from the four species was active against all the bacteria tested. The test bacterium *Pseudomonas solancearum* and the fungus *Penicilium citrinum* were most sensitive to the extracts of marine algae. In general, the extracts of seaweeds inhibited bacteria more strongly than fungi and species of the Rhodophyta showed the greatest activity against the bacteria and fungi tested.

Key words: marine algae, antibacterial activity, antifungal activity, solvent extracts

INTRODUCTION

Since the finding of antibacterial and antifungal activities in many species of marine algae from different parts of the world and the isolation of some active compounds from them (Hornsey and Hide, 1974; Reichelt and Borowitzka, 1984), marine algae have become recognized as potential sources of antibiotic substances. China has a long coastline and abundant natural resources of marine algae with very high species diversity, but there are only few reports on the screening of Chinese marine algae for antibacterial and antifungal activities (Ma and Tan, 1983; Chen et al., 1992). The number of species so far screened is only a very small percentage of the total. In most previous studies (Caccamese and Azzolina, 1981; Sreenivasa Rao and Parekh, 1981; Pesando and Caram, 1984; Ballesteros et al., 1992; Padmakumar and Ayyakkannu, 1997), only one kind of solvent was used to screen seaweeds for their antimicrobial activities. This study was aimed to determine the efficiency of ethanol, acetone, and methanol-toluene for extracting antibiotics from seaweeds, and to obtain species with highly active antibacterial and antifungal compounds. In addition, we extended our screening for the routine test-microorganisms to the crop pathogenic bacteria and fungi.

MATERIAL AND METHODS

Twenty-three species of marine algae occurring in the intertidal zone were collected from the coast along Fujian, China, in May 1996. After collection, the algal samples were thoroughly washed to remove all attached materials and then dried under shade.

Ethanol, acetone and methanol-toluene (3:1) were used for the preparation of different extracts. Ten grams of each algal sample were chopped into small pieces and soaked overnight in 30 ml

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Table 1 Antibacterial and antifungal activities of three solvent extracts from 23 seaweeds

Algae	Solvent	Bacteria				Fungi			
		E.c	B.s	S.a	P.s	P.c	A.n	F.o	A.d
[**Rhodophyta**] *Laurencia chinensis* Tseng	MT	+	+	−	+ +	−	−	−	−
	E	+	+	+	+	−	−	+ +	−
	A	+	+	+	+	−	−	+	−
L. okamurai Yamada	MT	+ + +	+ +	+	+	+	+	+	+
	E	+ + +	+ +	+ + + + + +		+	+	−	+ +
	A	+ + +	+ +	+ +	+ + +	−	+	+	+ +
Gracilaria blodgetii Harvey	MT	tr	tr	−	−	+	−	−	−
	E	+ +	+	+	+	+ +	−	−	+
	A	−	−	tr	−	−	−	−	−
Gelidium amansii Lamouroux	MT	−	−	−	−	−	−	−	−
	E	+	+	tr	+	tr	−	+ + +	+
	A	−	−	−	−	−	−	−	−
Gloiopeltis furcata (Postels et Ruprecht) J. Agardh	MT	−	−	−	−	−	−	−	−
	E	−	−	−	+	−	−	−	−
	A	−	−	−	−	−	−	−	−
Dasya scoparia Harvey ex J. Agardh	MT	+ + +	+ +	+ +	+	+	+	−	−
	E	+ +	+	+ +	+	−	−	+	+
	A	+ + +	+	+	+	+	−	+ +	+
Grateloupia filicina (Lamouroux) C. Agardh	MT	+	+	+	tr	−	−	−	−
	E	+ +	+	tr	+	tr	−	+ +	tr
	A	+ +	+ +	+ +	+ +	+	−	+ +	tr
Gigartina intermedia Suringar	MT	+	−	−	+	−	−	−	−
	E	+ +	+	−	+	+	−	−	tr
	A	−	tr	−	−	+	−	−	+
Chondria crassicaulis Harvey	MT	−	−	−	−	+	−	+	−
	E	tr	−	−	tr	−	−	−	−
	A	+ +	−	−	+	−	−	−	tr
Gymnogomgrus flabeliformis Harvey	MT	+	−	−	−	−	−	−	−
	E	+	tr	+	+	+	−	+	−
	A	−	−	−	−	−	−	−	−
Plocamium telfairiae (Hooker et Harvey) Harvey	MT	+ + +	+	+	+ +	+	−	−	−
	E	+ +	+	+ +	+ +	+	−	+	+
	A	+ +	+	+ +	+ +	tr	−	−	−
Hypnea cervicornis J. Agardh	MT	+	+	tr	+ +	+	−	−	−
	E	+	+	+	−	+	−	−	−
	A	+	tr	+	+	+	−	tr	−
[**Phaeophyta**] *Ishige okamurai* Yendo	MT	+	−	−	−	−	−	−	−
	E	+	tr	+	+	+	−	+	−
	A	−	−	−	−	−	−	−	−
I. folicacea Okamura	MT	−	−	+	tr	−	−	−	−
	E	tr	−	+	−	−	−	−	−
	A	−	tr	−	tr	−	−	− −	−
Colpomenia sinuosa (Mertens ex Roth) Derbes et Solier	MT	tr	+	−	+ +	tr	−	−	−
	E	−	−	−	tr	−	−	−	−
	A	+	+	+	+	tr	−	−	−
Dictyota cervicornis Kützing	MT	+ +	+	tr	+	+	−	+	−
	E	+	tr	−	−	−	−	−	−
	A	+	+	+	+	−	−	−	−
Pachydictyon coriaceum (Holmes) Okamura	MT	−	−	−	−	−	−	−	−
	E	+	+	tr	+	−	−	−	+ +
	A	+	+	+	+	−	−	−	−
Dictyoptris latiuscula (Okamura) Okamura	MT	+	tr	−	+	−	−	−	−
	E	tr	−	−	+	tr	−	−	−
	A	−	−	−	−	−	−	−	−
Spatoglossum pacificum Yendo	MT	−	−	−	−	+		− −	−
	E	−	−	−	+	+	−	+	+ +
	A	+	tr	+ +	tr	−	−	−	−
Sargassum fusiforme (Harvey) Setchell	MT	−	+	−	−	−	−	−	−
	E	−	−	−	−	−	−	−	−
	A	−	−	−	−	−	−	−	−
S. thunbergii (Mertens ex Roth) Kuntze	MT	+ +	+	−	−	−	−	−	−
	E	−	tr	+	tr	+	−	+	−
	A	−	+	−	−	−	−	−	−
[**Chlorophyta**] *Ulva pertusa* Kjellman	MT	−	−	−	−	−	−	−	−
	E	+	−	−	+	−	−	−	−
	A	−	+	+	+	−	−	−	−
Enteromorpha prolifera (Muller) J. Agardh	MT	−	−	−	+	−	−	−	−
	E	−	−	−	−	−	−	−	−
	A	−	−	−	−	−	−	−	−

Note: − : No activity; tr: trace; MT: methanol-toluene; E: ethanol; A: acetone; + 1.0 < Zone width < 2.5 (mm); + + 2.5 < Zone width < 4.0(mm); + + + Zone width > 4.0(mm)

of the respective solvents. Then they were transferred to a blender and homogenized for 2 min. The solvent extracts were filtered under pressure and all filtrates were evaporated to dryness in vacuum. The dried extracts were respectively dissolved in the above three solvents to give 50 mg/ml aqueous extracts for storage in airtight bottles in a refrigerator before testing.

Four bacteria, *Escherichia coli* (E.c), *Bacillus subtilis* (B.s), *Staphylococus aureus* (S.a) and *Pseudomonas solanacearum* (P.s) and four fungi, *Penicilium citrinum* (P.c), *Aspergillus niger* (A.n), *Fusarium oxyssporam* (F.o) and *Alternaria dianth* (A.d), were used as test microorganisms (crop pathogenic bacterium and fungi obtained from the Plant Protection Department of Fujian Agricultural University). The bioassay was conducted with 6 mm diameter filter paper discs using an agar diffusion technique and tests were made in duplicate. A suspension of each bacterium and fungus tested was prepared from the slides and mixed with 150 ml melting agar medium at 40 – 45℃. After agitation, the seeded agar was poured into ten pairs of sterilized plates and allowed to solidify. Two sterilized discs impregnated with 20 μl of aqueous extract of each seaweed were placed aseptically on the seeded agar plates. Controls were made with the three solvents used. The plates of bacteria were incubated at 37℃ for 24 h and that of fungi at 28℃ for 48 – 72 h. The zones of inhibition around the discs were measured.

RESULTS

Antibacterial and antifungal activities of the extracts prepared in three solvents from 23 species of marine algae are summarized in Table 1. In general, the seaweeds extracts inhibited bacteria more strongly than fungi, and species of the Rhodophyta showed the greatest antibacterial and antifungal activities.

Antibacterial activity

One or more extracts obtained from 23 species of seaweeds by using three different solvents showed activity against at least one of four bacteria tested. Four species of the Rhodophyta (*Laurencia okamurai*, *Dasya scoparia*, *Grateloupia filicina and Plocamium telfairiae*) showed a wide spectrum of antibacterial activity; each of their solvent extracts was active against all the bacteria tested. Among the actively antibiotic species, the three solvent extracts of *Laurencia okamurai*, the methanol-toluene and acetone extracts of *Dasya scoparia* and the methanol-toluene extracts of *Plocamium telfairiae*, strongly inhibited *Escherichia coli*. Strong antibacterial activities (against *Staphylococus aureus* by the ethanol extract of *L. Okamurai*, and against *Pseudomonas solanacearum* by the ethanol and acetone extracts of *L. okamurai*) were observed. None of the extracts from *Gloiopeltis furcata* and *Enteromorpha prolifera* exhibited any antibacterial activity against the tested bacteria except *P. Solanacearum*; and all solvent extracts from *Sargassum thunbergii* were inactive against the tested bacteria except *B. subtilis*. Of the 23 species of algae screened for antibacterial activity, 22 species were active against *P. solanacearum*, 20 species against *E. coli*, and *B. subtilis* and 17 species against *S. aureus*, which may indicate that the bacterium *P. solanacearum* was the most and that *S. areus* was the least, sensitive to the extracts of seaweeds.

Among the solvents used for the extraction in the present screening test, the ethanol extracts showed the strongest inhibition against the bacteria tested, followed by the acetone extracts, whereas the methanol-toluene extracts showed the weakest inhibition.

Antifungal activity

Of the 23 species of seaweeds screened, 17 showed activity against *P. citrinum*, 13 against *F. oxyssporam*, 9 against *A. dianth* and 2 against *A. niger*, which indicated that the fungus *P.*

citrinum was the most, and that *A. niger* was the least, sensitive to the extracts of seaweeds. Strong antifungal activity was observed in the ethanol extract of *Gelidium amansii* which strongly inhibited the growth of *F. oxyssporam*. No antifungal activity was found in all solvent extracts from the following species: *Gloiopeltis furcata*, *Ishige foliacea*, *Sargassum thunbergii*, *Ulva pertusa* and *Enteromorpha prolifera*.

The antifungal test revealed that the ethanol extracts had the highest, and that the methanol-toluene extracts had the lowest, inhibitory effect against the fungi tested.

DISCUSSION

The antimicrobial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of the plant, experimental methods, etc., which may explain the variations in antimicrobial activity in the findings by different workers. Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kind of solvent is the most effective and suitable for extraction of seaweeds. A few workers tried using different solvents for screening the antimicrobial activity of seaweeds and made comparisons. Parekh et al. (1984) reported that extracts obtained with acetone, ethyl alcohol and ether showed higher antibacterial activity than that of extracts obtained with chloroform. Rosell and Srivastava (1987) found similar antibacterial activity when they screened brown algae from Canada with acetone, chloroform, ethyl-ether, methanol and acetic acid. Sastry and Rao (1994) carried out a successive extraction using benzene, chloroform and methanol and reported the chloroform extract exhibited the strongest antibacterial activity. It can be seen from the above reports that the efficiency of chloroform in the extraction of seaweeds remains uncertain.

The results from the present screening revealed that the strongest antibacterial and antifungal activities were exhibited by the ethanol extract and the least by the methanol-toluene extract. In some species (such as *Gelidium amansii*) the inhibitory activity was only observed in the extract obtained with one kind of solvent but not in extracts obtained with other solvents, which may suggest that a particular solvent is required to extract some antimicrobial substances within the algal plant and therefore the percentage of inhibitory activity will go up when several solvents are used in the screening.

Antibacterial and antifungal activities of seaweeds also varied with the species from different division. Rao and Parekh (1981) and Padmakumar and Ayyakkannu (1997) reported that the species of Rhodophyta showed the highest antibacterial activity, whereas Caccamese and Azzolina (1979) and Pesando and Caram (1984) found that the highest antibacterial activity was exhibited by the species of Phaeophyta. The reason for this was not explained by these workers but it was suggested that more species have to be screened before coming to a definite conclusion. In the present study, the species of Rhodophyta showed the strongest activities against the test bacteria and fungi, which was in agreement with the findings of Sreenivasa Rao and Parekh (1981) and Padmakumar and Ayyakkannu (1997). It may probably be due to the tested seaweeds' vertical distribution. Red algae mostly occur in the intertidal zone lower region, which may be of advantage for the protection of the active compounds within the algal plant from degradation.

The active compounds in the species that showed strong antibacterial and antifungal activities in our study remain to be identified.

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