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ANTIMICROBIAL ACTIVITY OF SOME MARINE ALGAE OF SRI LANKA

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Abstract: Thirty five species of seaweeds were screened for antibacterial (against *Staphylococcus aureus* and *Escherichia coli*) and antifungal (against *Cladosporium cladosporioides* and *Candida albicans*) activity. Twenty six species exhibited antibacterial and/or antifungal activity. Extracts with pronounced activity were obtained from *Chondrococcus hornemanni* (Rhodophyta) and the active component was found in a mixture containing dihalogenated monoterpenes. Acrylic acid appears to be responsible for the antimicrobial activity of *Gracilaria corticata* (Rhodophyta) and *Ulva lactuca* (Chlorophyta).

1. Introduction

Marine flora are known to contain biologically active substances including antimicrobial compounds. A variety of marine algae collected mostly from Caribbean and Pacific waters have been screened for antimicrobial activity.[2,6]

Marine algae have been found abundant particularly along the Northern and Southern coastal regions of Sri Lanka. The algal vegetation along the coasts shows distinct floristic associations and seasonal variations. In a floristic survey in 1961, the presence of 315 species of algae distributed among the orders Chlorophyta, Phaeophyta and Rhodophyta has been recorded from the coastal areas in Sri Lanka.[7] Here we present the results of screening of 35 marine algae collected in Sri Lanka for their antimicrobial activity against *Cladosporium cladosporioides*, *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* and chemical fractionation of the extracts from *Chondrococcus hornemanni*, which was found to be the most active seaweed.

2. Materials and Methods

2.1 Algal Specimens

Algal specimens were collected from shallow waters of Mandativu, Mankumban, Keeramalai and Thiruvadinilayam in the Jaffna peninsula (Northern Coast) and also at Kirinda and Tangalla along the Southern Coast of Sri

Lanka in 1980 (May, Oct.), 1981 (June, Oct.), 1982 (July, Aug) and 1983 (Feb, Sept.) (Table 1). Voucher specimens were deposited at the University Herbarium, Department of Botany, University of Peradeniya, Sri Lanka. The plant material was washed in fresh water, transferred into bottles (10 1.— 20 1.) and sufficient methanol added to completely cover the plant material. The bottles were transported to the laboratory.

2.2 Extraction

Methanol was decanted from the bottles which contained the plant material 1 to 4 weeks after collection and storage. The algal material was reduced in size by chopping and fresh methanol was introduced into the bottle to completely cover the plant material. The mixture was allowed to stand at room temperature for two days. This was repeated twice with fresh methanol. The plant material was then extracted successively with cold light petroleum (40—60^0 C) and cold chloroform for 2 days, three times. With the algae collected in 1980 and 1981, dichloromethane was used as the solvent instead of chloroform, as a shortage of chloroform was experienced during this time. The methanol, petrol and chloroform/dichloromethane solubles were combined separately, and concentrated to dryness on a rotavapor at temperatures below 40^0 C. A separate extraction of *C. conoides* was carried out with light petroleum (40—60^0 C) under reflux conditions for two days and the petrol solubles were concentrated on a rotavapor at temperatures below 40^0 C. The resulting solvent-free extractives were subjected to antimicrobial screening tests as described below.

2.3 *Cladosporium*-TLC-Bioassay

The foregoing solvent-free extracts (2 mg) were spotted on TLC plates (silica gel 60 $PG_{254-366}$, 0.5mm x 20cm x 20cm) and the plates were developed in ethyl acetate: light petroleum (1:1). The plates, after air-drying overnight, were sprayed with a suspension of conidia of *Cladosporium cladosporioides* in Czapek-Dox nutrient solution and incubated in a moist chamber at 26 ± 2^0 C for 48 h.[11] The regions in which the growth was inhibited, appeared light coloured against a background of green mycelia. The diameter of the zones of inhibition was measured in mm; the zones of inhibition were usually approximately circular. The extracts which showed inhibition are given in Table 1 with R_f value (distance travelled by the spot/distance travelled by the solvent front) and the diameter of the zone of inhibition. Benlate (0.2 mg in MeOH; 50% active ingredient methyl 1—(butylcarbonyl)-2-benzimidazolecarbamate, Du Pont, USA) was spotted on each TLC plate as the standard and the diameter of the resultant inhibition area was measured. Some of the extracts were not tested for *Cladosporium*-TLC bioassay (Table 1).

Table 1. Antimicrobial activity of marine algae

	Collection		Activity[a]			
	Site	Date	*Cladosporium cladosporioides*[b]	*Candida albicans*[c]	*Staphyloccocus aureus*[c]	*Escherichia coli*[c]
CHLOROPHYTA (Green algae)						
Caulerpa chemitzia (Esper)Web.V.Bosse (Caulerpaceae)	Mankumban	Sept. 1983	+(petrol) [0.68,20]	—	—	—
Caulerpa racemosa (Forssk.)Web.V.Bosse (Caulerpaceae)	Mandativu	Sept. 1983	+(petrol) [0.97,5]	—	—	—
Caulerpa taxifolia (Vahl)Ag. (Caulerpaceae)	Mandativu	Oct. 1980	NT	—	—	—
Cladophora sp. (Cladophoraceae)	Mandativu	Oct. 1981	NT	+(petrol) [12]	+(MeOH) [19]	+(MeOH) [14]
Codium adherens Anderson (Codiaceae)	Mankumban	Sept. 1983	—	—	—	—
Dictyophaeria favulosa (Ag.)Decaisne (Valoniaceae)	Mandativu	Oct. 1980	NT	—	—	—
Halimeda macroloba (Ag.)Decaisne (Codiaceae)	Kirinda	Feb. 1983	+(MeOH) [0.73,18] +(petrol) [0.57,12]	—	—	—

Table 1 contd.

Microdictyon agardhianum Decaisne (Valoniaceae)	Mankumban	Sept. 1983	+(MeOH) [0.84,12] +(petrol) [0.84,10]	–	–	–
Ulva fasciata Delile (Ulvaceae)	Kirinda	Feb. 1983	+(MeOH) [0.94,15] +(petrol) [0.63,14]	–	–	–
Ulva lactuca Linnaeus (Ulvaceae)	Keerimalai	Oct. 1981	NT	–	+(MeOH) [40]	+(MeOH) [20]
Valoniopsis pachynema (Martens)Boergesen (Valoniaceae)	Mankumban	Sept. 1983	+($CHCl_3$) [0.82,8]	–	–	–
	Mandativu	Sept. 1983	+(petrol) [0.81,5] +($CHCl_3$) [0.81,10]	–	–	–
	Kirinda	May 1980	NT	+(petrol) [12]	+(MeOH) [16] +(CH_2Cl_2) [14]	+(MeOH) [20]
PHAEOPHYTA (Brown algae)						
Chnoospora fastigiata J. Agardh (Chnoosporaceae)	Kirinda	Feb. 1983	+(MeOH) [0.83,20] +(petrol) [0.58,25]	–	–	–

Table 1 contd.

Cystoseira trinodis (Forssk)C.Ag. (Cystoseiraceae)	Mandativu	Sept. 1983	–	–	–	–
Cystoseira triquetra L. (Cystoseiraceae)	Mandativu	Sept. 1983	–	–	+(MeOH) [13] +(petrol) [12]	–
Stoechospermum marginatum J.Ag. (Dictyotaceae)	Mandativu	Oct. 1980	NT	–	+(MeOH) [14]	–
		Sept. 1983	–	–	–	–
Sargassum cristaefolium J.Ag. (Sargassaceae)	Kirinda	Feb. 1983	–	–	–	–
Sargassum sp. (Sargassaceae)	Mandativu	Oct. 1980	NT	+(MeOH) [12]	+(MeOH) [14]	+(MeOH) [12]
Turbinaria conoides Kuetz (Sargassaceae)	Mandativu	Oct. 1981	NT	+(CH_2Cl_2) [18] +(petrol)[d] [50]	+(CH_2Cl_2) [18] +(petrol) [20]	+(CH_2Cl_2) [11] +(petrol) [11]
	Mandativu	Sept. 1982	+(petrol) [0.75,15]	–	–	–
	Mandativu	Sept. 1983	–	–	–	–
	Mankumban	Sept. 1983	–	–	–	–

Table 1 contd.

Turbinaria ornata J.Ag. (Sargassaceae)	Mandativu	Oct. 1980	NT	+(CH_2Cl_2)[e] [11] +(petrol) [12]	+(MeOH)[e] [14] +(petrol) [15] +(CH2Cl2) [12]	+(petrol)[e] [11]
	Mankumban	Sept. 1983	—	—	+(petrol) [15]	—
Tangalla		Feb. 1983	—	—	—	—
RHODOPHYTA (Red Algae)						
Acanthophora delilei Lamaroux (Rhodomelaceae)	Mandativu	Aug. 1982	+(petrol) [0.68,30]	—	—	—
	Mandativu	Oct. 1981	NT	—	—	—
Bryocladia thwaitesii Harvey (Rhodomelaceae)	Kirinda	June 1981	NT	—	—	—
Chondrococcus hornemanii (Mert)Schmitz (Rhizophyllidaceae)	Thiruvadini–layam	Aug. 1982	+(MeOH) [0.87,35; 0.70,14] +(petrol) [0.87,38; 0.70,15] +($CHCl_3$) [0.87,26]	+(MeOH) [27] +(petrol) [22] +($CHCl_3$) [19]	+(MeOH) [26] +(petrol) [20] +($CHCl_3$) [17]	+(MeOH) [20] +(petrol) [18] +($CHCl_3$) [18]

Table 1 contd.

Chrysemania uvaria Boergesen (Rhodymeniaceae)	Kirinda	June 1981	NT	–	–	–
Corynomorpha prismatica J.Ag. (Halymeniaceae)	Kirinda	Feb. 1983	+(petrol) [0.71,20]	–	+(MeOH) [13]	–
Gellidiella acerosa (Forssk.)Felman et Himel (Gellidiellaceae)	Mandativu	Oct. 1980	NT	+(MeOH) [12]	+(MeOH) [14]	+(MeOH) [11]
Gracilaria corticata J.Ag. (Gracilariaceae)	Kirinda	Feb. 1983	+(MeOH) [0.83,18; 0.27,10] +(petrol) [0.83,25]	–	+(MeOH) [13]	+(MeOH) [13]
Gracilaria edulis (Gmel)Silva (Gracilariaceae)	Mandativu	Oct. 1980	NT	–	–	–
Gracilaria fergusonii J.Ag. (Gracilariaceae)	Kirinda	May 1980	NT	–	–	–
	Kirinda	Feb. 1983	+(petrol) [0.74,20]			

Table 1 contd.

Gymnogonyrus pygmaeus (Greville)J.Ag. (Phyllophoraceae)	Kirinda	June 1981	NT	–	+(MeOH) [20]	+(MeOH) [23] +(CH_2Cl_2) [13]
Laurencia papillosa (Forssk.)Greville (Rhodomelaceae)	Mandativu	Aug. 1982	–	–	–	–
Liagora sp. (Helminthocladiaceae)	Mandativu	July 1982	+(petrol) [0.68,20] +($CHCl_3$) [0.67,10]	–	+(MeOH) [16] +($CHCl_3$) [13]	–
Polyopes ligulata (Harv.)Schmitz (Grateloupiaceae)	Kirinda	Feb. 1983	+(MeOH) [0.77,35; 0.26,10] +($CHCl_3$) [0.26,10]	–	–	–
Sarcodia ceylanica Harv. (Sarcodiaceae)	Mandativu	Oct. 1981	NT	–	–	+(MeOH) [18]
	Kirinda	Feb. 1983	+(MeOH) [0.93,35] +($CHCl_3$) [0.55,40]	–	+(MeOH) [14]	–

Table 1 contd.

Spyridia aculeata J.Ag. (Ceramiaceae)	Kirinda	Feb. 1983	+(petrol) [0.48,35]	+(petrol) [18]	+(MeOH) [14] +(petrol) [16]	–
Vancoorstia spectabilis Harv. (Delesseriaceae)	Mandativu	Oct. 1980	NT	+(CH_2Cl_2) [18] +(petrol) [20]	+(CH_2Cl_2) [14]	–
Benlate			+ [0.15,38±2]			
Nyastatin				+ [36±2]		
Penicillin					+ [32±2]	+ [30±2]

a + indicates extract derived using the solvent given in parenthesis is active; - , all extracts tested found inactive; NT, not tested.
b TLC bioassay; R_f value(s) of the active spot(s) and the diameter of the active spot (in mm) are indicated within [ ]
c Filter paper disc bioassay; diameter of the zone of inhibition around the paper disc (in mm) are indicated within [ ]
d hot petrol extract.
e 6 mm discs used.

2.4 Screening against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*

Aqueous ethanolic solutions (1 ml) of the extracts (15 mg) were absorbed into ten 6 mm paper discs. After ovendrying (42^0C) the discs were aseptically placed on Muller Hintant Agar (MHA) plates seeded with *S. aureus* (Oxford stain) and *E. coli*, and on Saboured Agar plates seeded with *C. albicans*. Tablets of Penicillin (10 i.u., DIFCO, Detroit, U.S.A.), standard for *E. coli* and *S. aureus* assay, and Nystatin (25 μg, DIFCO, Detroit, U.S.A.), standard for *C. albicans* assay, were also placed on a separate set of seeded MHA and Saboured Agar plates, respectively.[1] The plates were incubated at $38^0 \pm 2^0$C for 48h and the diameter of the Zone of inhibition on agar around the paper disc/tablet was measured. The results are given in Table 1.

2.5 Fractionation of the extractives of *Chondrococcus hornemanni*

A large sample (dry wt. 1.5 kg) of *C. hornemanni* was collected in Thiruvadinilayam and stored in chloroform:methanol (1:1) mixture for 2 weeks. The extract was filtered and the filtrate on standing separated into two layers and these were treated separately. The aqueous layer was washed with chloroform and the washings were combined with the organic layer. The combined organic layer was washed with water, dried over anhydrous $MgSO_4$ and concentrated to obtain a brown oil (14.1 g). Flash chromatography (silica gel GF_{254} Merck, light petroleum and $CHCl_3$) of the brown oil afforded a white crystalline solid (57 mg), m.p. 52-55^{0}C, $[\alpha]_D$;+18.7^0 (*c*, 1.34, $CHCl_3$). IR ν^{KBr}_{max} cm^{-1}: 2920, 1620, 1450, 1100, 900, 700. ^{1}H-NMR (60 MHz, CCl_4): δ (ppm) 5.82(IH,D, J = 3HZ), 5.62(IH,d, J = 3Hz), 4.03 (IH,d, J = 10Hz), 3.80(2H,s), 2.9-1.9(4H,m), 1.80(3H,s), 1.68(3H,s). MS: *m/z* (relative intensity) 366(M^+-Cl, 6%), 285(26), 249(16), 205(18), 203 (22), 169(15), 167(29), 127(32), 125(34), 113(19), 105(20), 103(26), 91(30), 79(36), 77(100). Further elution of the column gave a colourless oil (766 mg) which was homogeneous on TLC. IR ν^{neat}_{max} cm^{-1}: 2920, 1440, 1250, 1200, 675. ^{1}H-NMR (CCl_4): δ (ppm) 5.83-5.50(m), 5.10(s,br), 4.90 (s,br), 4,17(s), 4.10(s), 4.04(s), 3.97(s), 3.91(s), 2.30(s), 2.25(s), 1.83(s), 1.80(s). MS: *m/z* 252, 250, 248, 209, 207, 205, 191, 171, 169, 133, 129, 127, 103, 102, 79, 75, 69, 68, 67, 65.

3. Results and Discussion

Cold methanol, petroleum and chloroform/dichloromethane extracts of 35 algae belonging to Chlorophyta (11 species), Phaeophyta (8 species) and Rhodophyta (16 species) were examined for antimicrobial activity against *C. cladosporioides*, *C. albicans*, *S. aureus* and *E. coli*. The results (Table 1) show that 26 species exhibited activity against one or more microorganisms.

3.1 Chemical fractionation of the extractives of *Chondrococcus hornemanni*

The extracts of *C. hornemanni* inhibited the growth of all four microbes tested, and the observed activities were comparable to those of a standard antibiotic Penicillin and standard fungicides, 'Benlate' and 'Nystatin'. The active extract of *C. hornemanni* was partitioned on a silica gel column using combinations of light petroleum and $CHCl_3$ with increasing polarity. A white crystalline solid (*1*) and a colourless oil (*2*) were obtained. The IR spectrum of the solid showed a band at 1620 cm^{-1} corresponding to an olefinic double bond. The ^{1}H-NMR spectrum of this compound indicated the presence of two tertiary methyl groups (δ 1.80 and 1.68), three hydrogens on halogen bearing carbon atoms (δ 4.03,IH,d, J = 10 Hz and δ 3.80,2H,s) and two olefinic hydrogens which are geminally coupled (δ 5.83,IH,d, and J = 3Hz and δ 5.62,IH,d, J = 3HZ). The mass spectrum showed two peaks at *m/z* 366 and 285 corresponding to M-Cl and M-Cl-HBr, respectively. High resolution mass spectral data indicated the molecular formula of the ion at *m/z* 285 to be $C_{10}H_{14}Cl_2Br$. This suggested that compound *1* was identical to 6-bromo-3-bromomethyl -2,3,7-trichloro-7-methyl-1-octene previously isolated from *C. hornemanni.*[3] This compound was found to be inactive against *C. cladosporioides.*

The colourless oil (*2*) obtained from the above column was homogeneous on TLC although GLC and HPLC analysis suggested the presence of more than one compound in the oil. The oil was not fractionated into more than one component by either silica gel/$AgNO_3$ impregnated silica gel chromatography (plates or column) eluted with *n*-hexane or HPLC (Whatman Partisil-10 ODS 2, aqueous MeOH). The oil (*2*) corresponded to the inhibition zone at R_f 0.87 in the *Cladosporium*-TLC-bioassay. Mass spectral fragments at *m/z* 252, 250, 248(M^+), 209, 207, 205(M-43), 171, 169(M-Br), 133(M-Br-Cl-H), 129, 127(M-Br-43+H) and 69(base peak) suggested that the active fraction contained mainly dihalogenated monoterpenes.[10] The presence of several halogenated monoterpenes in *C. hornemanni* has been reported previously.[3,10,15] Structurally related compounds isolated from *Delisea fimbriata* (Rhodophyta) have also displayed antimicrobial activity.[12,13]

3.2 Screening for antimicrobial activity

The extracts of *Cladosphora* sp , *Valoniopsis pachynema*, *Turbinaria conoides*, *Corynomorpha prismatica*, *Gracilaria corticata*, *Liagora* sp., *Sarcodia ceylanica* and *Spyridia aculeata* also showed antibacterial and antifungal activity but to a lesser extent than *C. hornemanni*. The methanol extract of *G. corticata* produced two inhibition areas in the *Cladosporium*-TLC-bioassay plate (petrol:EtOAc:1:1) corresponding to low polar (R_f 0.83) and high polar (R_f 0.27) active constituents. The R_f value of the inhibition area produced by the high polar compound was identical to that of acrylic acid.

It is noteworthy that the antimicrobial principle of *G. folifera* and *G. varrucosa* has been reported to be acrylic acid.[9]

The extracts of *Caulerpa chemitzia, Caulerpa racemosa, Halimeda macroloba, Ulva fasciata, Microdictyon agardharanum, Chnoospora fastigiata, Acanthopora delilei, Gracilaria fergusonii* and *Polyopes ligulata* inhibited only *C. cladosporioides*. In a previous survey on fungistatic properties of marine algae,[14] *C. racemosa* was, however, found to be particularly active against *Candida albicans* and *Cryptococcus neoformans.*

Only antibacterial properties were detected in the extracts of *Ulva lactuca, Cystoseira triquetra, Stoechospermum marginatum, Turbinaria ornata, Cymnogyrus pygmaeus* and *Sarcodia ceylanica*. Our results of *U. lactuca* are in agreement with those of a previous study,[2] and the active compound is probably (TLC) acrylic acid.[8] The antibacterial constituents of *S. marginatum* has been identified as a spatol acetate.[5]

The extracts of *Caulerpa taxifolia, Codium adherens, Dictyosphaeria favulosa, Cystophyllum muricatum, Sargassum cristaefolium, Bryocladia thwaitesii, Chrysemania uvaria, Gracilaria edulis* and *Laurencia papillosa* did not inhibit the growth of the microbes tested. A homogenized preparation of *L. papillosa* has, however, been found to be a potent inhibitor of some pathogenic fungi in a previous survey.[14]

Varying activities of the extracts were observed with different collections of algal specimens, e.g. *V. pachynema, S. marginatum, T. conoides, T. ornata* and *S. ceylanica*. This may be attributed partly to seasonal variations[2] and ecotypic variations from different locations.[4]

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