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Bioactivity of selected seaweeds from gulf of Mannar, South-east coast of India

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PEER REVIEW

Peer reviewer

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Comments

This is an applicable research on algal seaweeds extracts that can be continued and completed by new research on freshwater and seawater algae. Also, this study suggests that *T. conoides* may possess active compounds for the treatment of TB.

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ABSTRACT

Objective: To evaluate antimicrobial and antimycobacterial activity of three seaweeds [*Gelidiella acerosa* (*G. acerosa*), *Turbinaria conoides* (*T. conoides*) and *Sargassum wightii* (*S. wightii*)] from Gulf of Mannar.

Methods: Three seaweeds *G. acerosa*, *T. conoides* and *S. wightii* were collected from Gulf of Mannar, South-East Coast of India. Solvent extraction of the selected seaweeds was done using hexane and ethanol. These extracts were tested for antibacterial activity against four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*) by disc diffusion method. The active extracts of *T. conoides* were tested for antimycobacterial activity by luciferase reporter phage assay at two different concentrations (100 µg/mL and 500 µg/mL).

Results: Among the tested seaweeds, ethanol extract of *T. conoides* showed maximum antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* followed by hexane extract at concentration of 1 mg/disc. The remaining two seaweeds (*G. acerosa* and *S. wightii*) did not show any activity. The ethanol extract of *T. conoides* exhibited maximum antimycobacterial activity (87.33%) followed by hexane extract (74.68%) against *Mycobacterium tuberculosis* H37Rv at 500 µg/mL.

Conclusions: Ethanol extract of *T. conoids* showed both antibacterial and antimycobacterial activity. Further photochemical studies are needed to identify the active antimycobacterial agents.

KEYWORDS

Seaweeds, Antibacterial, Antimycobacterial activity

1. Introduction

Tuberculosis (TB) is a common and deadly infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). It remains a major global health problem in humans since antiquity. Over one-third of the world's population harbour the TB bacterium in their bodies and new infections occur at a rate of one per second[1]. One productive cough can release as many as 3000 droplet nuclei which, when inhaled by another individual, results in infection. In the year 2012, World Health Organization estimated that 8.6 million people developed TB and 1.3 million died from the disease[2]. At present, because of uncontrolled use of anti-TB drugs, there is an increasing prevalence of both multidrug and extensively drug resistant strains[3]. Hence there is an urgent need to develop alternative and effective drugs that shorten the course of chemotherapy and to restrict the spread of drug resistant TB.

Plants and marine species are the source of variety of microorganisms viz. fungi, actinomycetes and bacteria, and constitute the backbone of the traditional medicine. Although the terrestrial organisms have been extensively screened as important sources of secondary metabolites, marine species also represent a rich source of biologically active compounds. More than 15000 marine natural compounds have been isolated, and these compounds were suggested to play an important role in defence mechanism against biotic and abiotic stress[4]. Seaweed is one of the medicinally and economically important marine living resources that grow almost exclusively in shallow waters at the edge of the oceans. It has been a source for the production of a variety of major metabolites such as polysaccharides, lipids, proteins, carotenoids, vitamins, sterols, enzymes, antibiotics, and many other fine chemicals[5,6]. The nutritional value of protein and lipid in seaweed is comparatively higher than that in any other vegetables. So far seaweeds extracts have been screened for different biological

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activities, including, antitumor[7], antiprotozoal[8], antiviral[9], antioxidant[10], cytotoxic activity against the human cancer cell lines[11], and antimicrobial activity[12].

However, only a small proportion of seaweeds have been thoroughly investigated for their medicinal properties and undoubtedly there are many more novel biologically active compounds yet to be discovered. In this regard the present study is aimed at screening and evaluating seaweeds against some bacterial strains and *M. tuberculosis* H37Rv using luciferase reporter phage (LRP) assay for identification of the potent seaweed having antibacterial activity.

2. Materials and methods

2.1. Test materials seaweeds

Based on the knowledge and experience of traditional usage and literature survey, three seaweeds [*Gelidiella acerosa* (*G. acerosa*), *Turbinaria conoides* (*T. conoides*) and *Sargassum wightii* (*S. wightii*)] were selected for antimicrobial study. These seaweeds were collected from Mandapam coastal area, Tamil Nadu, India during December 2006 and the species were identified with help of field experts.

2.2. Preparation of extracts

The seaweeds were thoroughly washed with sea water to remove epiphytes and allowed to dry in the shade for ten to fifteen days. The dried samples were thoroughly washed with distilled water to remove the salts on surface and dried on the blotting paper to remove the excess moisture and powdered using electric blender. A total of 50 g of seaweed powder was packed in Soxhlet apparatus and extracted with 250 mL of hexane and ethanol (1:5) for 8 h at temperature 50-65 °C. The extracts were concentrated and solvent free residues were obtained. Stock solutions (100 mg/mL) were prepared by using dimethylsulfoxide and water, and stored in sterile dark bottles for subsequent experiment. Different concentrations were prepared with distilled water and sterilized through filtration using the membrane with pore size of 0.45 µm.

2.3. Screening of extracts for antibacterial activity

Microorganisms were obtained from the Department of Microbiology, Jaya College of Arts and Science, Chennai. Two Gram negative bacteria *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and two strains of Gram-positive bacteria *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) were used.

The culture media (Muller Hinton agar) were prepared as per manufacturer's instructions. The antibacterial activity of the seaweeds was evaluated by the disc diffusion method. It was performed using bacterial culture grown for 18 h at 37 °C in 10 mL of Mueller Hinton broth. About 500 µL inoculate of each bacterial culture were spread over Muller Hinton agar plates using sterile cotton swab in order to get a uniform growth on both control and test plates. Under aseptic conditions, empty sterile discs (Whatman no. 5, 6 mm in diameter) were loaded with different concentrations (2 mg, 1 mg, 0.5 mg, 0.25 mg/disc) of extracts and placed on the agar surface. Paper discs moistened with aqueous dimethylsulfoxide were placed on each seeded Petri plate as a control. A standard disc containing chloramphenicol (10 µg/disc) was used as reference control. The plates were incubated at 37 °C for 18 h. After the incubation period, the zone of inhibition was measured. Experiments were performed in triplicate, to identify the potent extracts against the bacterial strains.

2.4. Screening of active seaweed against *M. tuberculosis* by LRP assay

Due to the higher antibacterial activity, hexane and ethanol extracts of *T. conoides* were selected for antimycobacterial activity. Standard strain, *M. tuberculosis* H37Rv, a drug sensitive reference strain grown and maintained on Lowenstein Jensen medium in the Department of Bacteriology, National Institute for Research in Tuberculosis, Chennai, was used for the study. Two concentrations, 500 µg/mL and 100 µg/mL were prepared by using double distilled water and the test compound was sterilized by filtration using membrane filter with pore size of 0.45 µm. In addition, 2 µg/mL concentration of rifampicin was prepared and used as assay control.

Antimycobacterial activity of active extracts was evaluated by LRP assay[13]. About 100 µL of *M. tuberculosis* cell suspension was added to 400 µL of G7H9 with desired concentration of test compound 100 µg/mL and 500 µg/mL. For each sample, one reference control rifampicin (2 µg/mL), a drug free control plain media and a solvent control [dimethylsulfoxide 1% (v/v)] were prepared. This set up was incubated for 72 h at 37 °C, and after incubation 50 µL of the high titre LRP (phAE 129) and 40 µL of 0.1 mol/L CaCl₂ was added to all the vials and this setup was incubated at 37 °C for 4 h. After incubation 100 µL of the mixture was taken from each tube into a cuvette and 100 µL of working D-Luciferin solution was added. The relative light units (RLU) was measured in luminometer with integration time of 10 seconds.

The percentage reduction in the RLU was calculated in test sample compared with control as follows:

$$\text{RLU reduction (\%)} = \frac{\text{Control RLU} - \text{sample RLU}}{\text{Control RLU}} \times 100$$

2.5. Thin-layer chromatography (TLC) profile of active extract

A total of 10 µL of each active extracts was mixed with 1 mL of ethyl acetate. About 5 µL of test solution were loaded on a pre-coated silica gel 60 F₂₅₄ TLC plate (Merck) of uniform thickness (0.2 mm) and eluted with hexane: ethyl acetate (8:2) solvent system. After spraying anisaldehyde-sulphuric acid reagent and heating the plate at 110 °C for 5 min the R_f values were recorded.

2.6. TLC-bioautography

For direct bioautographic testing, agar overlay assay as described by Slusarenko et al.[14] was used with minor modification for the localization of some of the active compounds in the active crude extract against microbial strains. About 10 µL of each active extract was spotted on ready-made chromatographic silica gel 60 plates. Only one solvent system (hexane: ethyl acetate, 8:2) was used. Developed TLC plates were carefully dried for complete removal of the solvents and overlaid on agar seeded with an overnight culture of the test bacteria. Plates were incubated at 37 °C for 24 h. The zone of inhibition of bacterial growth could be seen around the active chromatogram spot. Inhibition zones indicated the presence of active compounds. Duplicates of the chromatograms used in the bioautographic method were used for the identification of active bands (compounds) after spraying an aqueous solution of anisaldehyde sulphuric acid reagent.

3. Results

Antibacterial activity of hexane and ethanol crude extracts of three seaweeds against four bacterial species is summarized in Table 1. The activity was found in varying degrees being dose dependent. Among the three seaweeds tested, *T. conoides* showed antibacterial

activity against *B. subtilis*. Maximum activity was observed in hexane extract of *T. conoides* at 2 mg/disc concentration. On the other hand, *S. wightii* and *G. acerosa* failed to inhibit the bacterial growth.

Table 1

Zone of inhibition (mm) of selected seaweeds against bacterial strains by disc diffusion method.

Bacteria	<i>G. acerosa</i>				<i>T. conoids</i>				<i>S. wightii</i>			
	2	1	0.5	0.25	2	1	0.5	0.25	2	1	0.5	0.25
	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc	mg/ disc
Hexane extract	<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-
	<i>P. aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-
	<i>B. subtilis</i>	-	-	-	-	18	15	11	8	-	-	-
	<i>S. aureus</i>	-	-	-	-	13	11	8	-	-	-	-
Ethanol extract	<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-
	<i>P. aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-
	<i>B. subtilis</i>	-	-	-	-	13	10	8	-	-	-	-
	<i>S. aureus</i>	-	-	-	-	11	9	-	-	-	-	-

-: No activity.

Laboratory reference strain *M. tuberculosis* H37Rv was used for screening hexane and ethanol extract of *T. conoides* for antimycobacterial activity and the results are summarized in Table 2. Among the two extracts tested, ethanol extract showed maximum antimycobacterial activity (87.33%) followed by hexane extract (74.68%) at 500 µg/mL concentration. Notably, ethanol extract of *T. conoides* was found to show good activity even at a lower concentration of 100 µg/mL (75.10%).

Table 2

Antimycobacterial activity of hexane and ethanol extracts of *T. conoides* against *M. tuberculosis* using LRP assay.

Agents	% RLU reduction	
Hexane extracts	100 µg/mL	32.14
	500 µg/mL	74.68
Ethanol extracts	100 µg/mL	75.10
	500 µg/mL	87.33
Rifampicin		82.50
Solvent control		13.98

TLC-bioautography of ethanol extract of *T. conoides* was performed against *B. subtilis* for determining the active fraction. Bioautography test showed single inhibitory zones ($R_f=0.25$ to 0.33). This result clearly indicated the presence of active antibacterial compound in ethanol extract.

4. Discussion

In many countries, seaweeds are used for food and medicine. They are also increasingly used in pharmaceutical industries particularly as a source of bioactive compounds as antiviral, antibacterial, and antifungal substances. In this study, the antibacterial activities of seaweeds were studied using disc diffusion method. The advantage of this method is the requirement of very little sample for screening and the possibility of testing five or six compounds against a single microorganism on a single plate.

T. conoides extracts showed maximum activity against *B. subtilis*. Other two seaweeds did not show any activity. It may be due to seasonal and geographical variation. In 1974, Hornsey and Hide reported that seaweeds had no activity when alga undergoes deterioration[15]. Hence the bacteria are not inhibited during the deterioration stage. Seaweeds contain a rich and largely untapped source of a vast assortment of biologically active substances[16]. It has been reported that the efficacy of seaweed extracts against pathogenic microorganism is mostly influenced by factors such as location and seasonality[17]. *T. conoides* exhibited activity against

Gram positive bacteria only. Earlier reports stated that some of the seaweeds show antibacterial activity only against Gram positive bacteria but not against Gram negative ones due to the presence of some factors in Gram negative bacteria[18].

Rapid methods are needed especially for high throughput screening for antituberculous compounds against *M. tuberculosis*. The LRP assay has great promise in this regard, since the assay uses very small drug quantity to screen for antituberculous activity. The availability of rapid assay allows large scale screening and encourages the search for new classes of antituberculosis agents urgently needed to control this disease[12]. LRP assay is already reported to be capable of rapidly assessing antibiotic susceptibilities with high sensitivity and specificity, in a timely and relatively 'low-tech' manner[19]. In addition, phage based assays have the advantage of being less expensive and technologically simpler than molecular and broth based tests[20].

Ethanol extract of *T. conoides* showed maximum antimycobacterial activity. Shower *et al.* screened 480 plant extracts using both luciferase and colorimetric broth dilution assays[21]. There was an overall agreement of 99 percent between these two methods. *Asparagopsis taxiformis* and *Cymopolia barbata* were the species with the strongest activities against the broadest spectrum of target microorganisms. All the species with the antibacterial activity were active against Gram-positive bacteria; whereas only two species, *Asparagopsis taxiformis* and *Osmunda hybrida*, were active against mycobacterial[22].

Bioautography is a very convenient and simple way of testing crude extracts and pure substances for their effect on both human and plant pathogens. It can be employed in the target-directed isolation of active constituents. The bioautography method was further improved for screening of plant extracts, allowing identification of even small amounts of antimicrobial active compounds in complex compound mixtures. In the present study TLC bioactive method was employed which revealed one individual band of ethanol extract of *T. conoides* exhibiting antibacterial activity.

Several seaweeds are used in traditional medicine for the treatment of bacterial and viral diseases in many parts of the world. This preliminary study points to a possible role of *T. conoides* in the treatment of TB, although further studies are required to isolate and characterise the active compound. This study reiterates that seaweeds could be of great value in the continuing struggle to control pathogenic organisms.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Today algal extracts has many drug applications, and antimicrobial activity of algae has been recognized among some groups of algal seaweeds. Seaweed is a vast source of biological compounds, in this decades there is an attention to evaluate antimicrobial activity of algal seaweeds. The antimicrobial substances produced by algae could be used not only as a defense agent (against pathogens) but also as pharmaceutical bioactive natural compounds. Though much

information about the chemistry and the antimicrobial action of several phytochemicals is known, very few reports are available on the possible mechanism of action.

Research frontiers

The present research shows antimicrobial and antimycobacterial activity of three seaweeds (*G. acerosa*, *T. conoides* and *S. wightii*). Solvent extract of the selected seaweeds was prepared using hexane and ethanol, then these extracts were tested for antibacterial activity against four bacterial strains (*E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*) by disc diffusion method.

Related reports

Algae are potential sources of biologically active compounds with antiviral, antibacterial, antifungal, and anticancer activities. The effect of different solvents, including methanol, acetone, and water on the total phenolic, flavonoid, antioxidant and antibacterial activities of algal extracts were evaluated. Organic extracts (with methanol or acetone) of the tested species actively inhibited the growth of bacteria compared to aqueous extracts.

Innovations and breakthroughs

Ethanol, methanol and hexane extracts of algal seaweeds has been reported before used as drug or antibacterial compounds but antimycobacterial compounds is less studied, and the present research reported the antimycobacterial effects of these seaweeds.

Applications

This survey proposes the possible role of *T. conoides* in the treatment of TB, although further studies are required to isolate and characterise the active compound.

Peer review

This is an applicable research on algal seaweeds extracts that can be continued and completed by new research on freshwater and seawater algae. Also, this study suggests that *T. conoides* may possess active compounds for the treatment of TB.

References

- [1] World Health Organization. Tuberculosis. Geneva: World Health Organization; 2014. [Online] Available from: <http://www.who.int/mediacentre/factsheets/fs104/en/> [Accessed on 14th July, 2014]
- [2] World Health Organization. Global tuberculosis report 2013. Geneva: World Health Organization; 2013. [Online] Available from: http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf [Accessed on 21st June, 2014]
- [3] Raviglione M. XDR-TB: entering the post-antibiotic era? *Int J Tuberc Lung Dis* 2006; **10**(11): 1185-1187.
- [4] Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. Marine natural products. *Nat Prod Rep* 2013; **30**: 237-323.
- [5] Stein J, Borden C. Causatine and beneficial algae in human disease condition: a review. *Phycologia* 1984; **23**: 485-501.
- [6] Paul VJ, Fenical W. Toxic feeding deterrents from the tropical marine alga *Caulerpa bikiensis* (Chlorophyta). *Tetrahedron Lett* 1982; **23**: 5017-5020.
- [7] Xu NJ, Fan X, Yan XJ, Tseng CK. Screening marine algae from China for their antitumor activities. *J Appl Phycol* 2004; **16**: 451-456.
- [8] Allmendinger A, Spavieri J, Kaiser M, Casey R, Hingley-Wilson S, Lalvani A, et al. Antiprotozoal, antimycobacterial and cytotoxic potential of twenty-three British and Irish red algae. *Phytother Res* 2010; **24**: 1099-1103.
- [9] Kim JH, Huang AM, Bannister K, Choi TJ, Towers GHN, DeWreede RE, et al. Biological activities of seaweed extracts from British Columbia, Canada, and Korea. I. Antiviral activity. *Can J Bot* 1997; **75**(10): 1656-1660.
- [10] Cox S, Abu-Ghannam N, Gupta S. An assessment of the antioxidant and antimicrobial activity of six edible Irish seaweeds. *Int Food Res J* 2010; **17**(1): 205-220.
- [11] Taskin E, Caki Z, Ozturk M, Taskin E. Assessment of *in vitro* antitumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean sea. *Afr J Biotechnol* 2010; **9**(27): 4272-4277.
- [12] Prabuseenivasagan S, Kumar, Shanmugam N, Ignacimuthu S. Rapid screening of selected plant essential oils against *Mycobacterium tuberculosis* using luciferase reporter phage (LRP) assay. In: Balagurunathan R, Radhakrishnan M, editors. *Recent trends in microbial biotechnology*. Kanchipuram: Sri Sankara Arts & Science College; 2006, p. 107-114.
- [13] Karthik Kumar K, Prabu Seenivasan S, Kumar V, Mohan Das T. Synthesis of quinoline coupled [1,2,3]-triazoles as a promising class of anti-tuberculosis agents. *Carbohydr Res* 2011; **346**(14): 2084-2090.
- [14] Slusarenko AJ, Longland AC, Whitehead IM. A convenient, sensitive and rapid assay for antibacterial activity of phytoalexins. *Bot Helv* 1989; **99**: 203-207.
- [15] Hornsey IS, Hide D. The production of antimicrobial compounds by British marine algae I. Antibiotic-producing marine algae. *Br Phycol J* 1974; **9**: 353-361.
- [16] Cannell RJP. Algae as a source of biological active products. *Pestic Sci* 1993; **39**: 147-153.
- [17] Vidyavathi N, Sridhar KR. Seasonal and geographical variations in the antimicrobial activity of seaweeds from the Mangalore Coast of India. *Bot Mar* 1991; **34**: 279-284.
- [18] Rao PS, Parekh KS. Antibacterial activity of Indian seaweeds extracts. *Bot Mar* 1981; **24**: 577-582.
- [19] Carriere C, Riska PF, Zimhony O, Kriakov J, Bardarov S, Burns J, et al. Conditionally replicating luciferase reporter phages: improved sensitivity for rapid detection and assessment of drug susceptibility of *Mycobacterium tuberculosis*. *J Clin Microbiol* 1997; **35**: 3232-3239.
- [20] Pai M, Kalantri S, Pascopella L, Riley LW, Reingold AL. Bacteriophage-based assays for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a meta-analysis. *J Infect* 2005; **51**: 175-187.
- [21] Shawar RM, Humble DJ, Van Dalfsen JM, Stover CK, Hickey MJ, Steele S, et al. Rapid screening of natural products for antimycobacterial activity by using luciferase-expressing strains of *Mycobacterium bovis* BCG and *Mycobacterium intracellulare*. *Antimicrob Agents Chemother* 1997; **41**: 570-574.
- [22] Gonzalez del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol* 2001; **4**(1): 35-40.