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ORIGINAL RESEARCH ARTICLE

Comparitive Studies on Antimicrobial Activity of *Ulva reticulata* and *Ulva lactuca* against Human Pathogens

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ABSTRACT

Marine algae are not only the primary and major producers of organic matter in the sea, but they also exert profound effects on the density and distribution of other inhabitants of the marine environment. The present study was conducted to compare the antimicrobial activity of *Ulva reticulata* and *Ulva lactuca* solvent extracts against human pathogenic bacteria and fungi. The seaweeds *Ulva reticulata* and *Ulva lactuca* were collected and powdered. The powdered material was extracted using the organic solvents *viz*., methanol, acetone, chloroform, hexane and ethyl acetate. Antimicrobial activity of *Ulva reticulata* and *Ulva lactuca* solvent extracts was determined by Disc diffusion method. Among the solvents tested, methanol extract showed maximum inhibitory activity than other solvents. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful source of new antimicrobial agents.

Keywords: *Ulva reticulata*, *Ulva lactuca*, Solvent extracts, antimicrobial activity and minimum inhibitory concentration (MIC).

1. INTRODUCTION

Seaweeds are eukaryotic organisms that lives in salty water in the ocean and is recognized as a potential source of bioactive natural products [1]. They contain compounds ranging from sterols, terpenoids to brominated phenolic, which shows bioactive against microorganisms [2]. Seaweeds are rich and varied source of bioactive natural products and have been studied as potential biocidal and pharmaceutical agents. In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti inflammatory, antitumoric, cytotoxic and antimitotic activities [3,4]. Presently seaweeds constitute commercially important marine renewable resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations [5]. Marine seaweeds are the simplest group of marine algae where they are present nearby seashore and in rocky regions of beaches. These marine algal members possess bioactive substances, which are antibacterial, antiviral, antifungal in nature and it is an universally known fact that marine algae has got rejuvenating properties where they have been used as source of nutrients in many countries. These marine algae are present in coastal regions of Tamil Nadu starting of coast of Mandapam to Kanyakumari. Seaweeds are plant-like ocean organisms that are botanically classified as macrophytic marine algae. Edible seaweeds are often called "sea vegetables." Seaweeds come in an amazing variety of beautiful shapes, colors and sizes, and are found in all of the world's oceans. They are most abundant in shallow rocky coastal areas, especially where they are exposed at low tide.

Many species of algae have been investigated for antibacterial and antiviral properties in the highly volatile fractions of the genera Asparagopsis, Bonnemaisonia and Pcilonia, belonging to the family Bonnemaisoniaceae a great variety of halogenated alkanes, saturated and unsaturated ketones, aldehyde, alcohols, epoxides and halogenated derivatives of acetic and acrylic acids have been deducted for antibiotic activity against *Bacillus subtilis*, *Staphylococcus* sp., *Fusarium* sp. and *Vibrio* sp. was shown for the halogenated heptanones [6]. Many polysaccharides are recovered from seaweeds. The most important of them are agar, alginic acid, laminarin, fucoidin, galactans, carrageenan, xylene and mannans. The

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seaweeds for medicinal purpose were begun in the orient as early as 3000 BC. Japanese and Chinese had used then for treatment of goiter and Romans used them for variety of purposes including prevention of disease like scurvy. Algal constituents include acids, alkaloids, amine, antibacterial, antifungal, antiviral substances, lipids, sterols, steroids, fatty acids, phenolic compounds, phyto-chromes, pigments, proteins, peptides, amino acids, sugar, alcohols and vitamins.

Seaweeds offer a wide range of therapeutic possibilities both internally and externally. The term seaweeds refer only to macrophytic marine algae, both wild and cultivated, growing in saltwater. Botanically, seaweeds are classified as green, brown, or red. A particular seaweed's placement in one of these groups is determined first by its photosynthetic pigments, then its reproductive mode, then its micro and macro morphologies, and finally by its phycopolymers. In the last three decades the discovery of metabolites with biological activities from macroalgae has increased significantly. However, despite the intense research effort by academic and corporate institutions, very few products with real potential have been identified or developed. The present study was aimed to compare the antimicrobial efficacy of *Ulva reticulata* and *Ulva lactuca* against human pathogens.

2. MATERIALS AND METHODS

2.1. Collection of seaweeds

The seaweed *Ulva reticulata and Ulva lactuca* were collected from East coast of Mandapam, Tamil Nadu, India. The seaweed was taxonomically identified at the CAS in Marine Biology, Annamalai University and voucher specimens were deposited at the Department of Zoology, Annamalai University.

2.2. Preparation of seaweed extracts

The collected *Ulva reticulata and Ulva lactuca* samples were cleaned and the necrotic parts were removed. The seaweeds washed with tap water to remove any associated debris and shade dried at room temperature (28±2^{o}C) for 5-8 days or until they are brittle easily by hand. After completely drying, the seaweed materials (1.0 kg) were ground to a fine powder using Electrical blender. Forty gram of powdered sea weeds were extracted successively with 200 mL of solvents (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

2.3. Disc preparation

2.3.1. Preparation of algal disc for antibacterial activity

5 mm diameter discs were prepared using sterile Whatmann No.1 filter paper. The solvent (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) extracts of seaweed *Ulva reticulata and Ulva lactuca* were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent extracts of *Ulva reticulata and Ulva lactuca* at two different concentrations ranging 2.5mg/ml and 5mg/ml to check their antibacterial activity. The Ampicillin (5mg/ml) was used as positive control and the 5% DMSO was used as a blind control.

2.3.2. Preparation of algal disc for antifungal activity

5 mm diameter discs were prepared using sterile Whatmann No.1 filter paper. The solvent (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) extracts of *Ulva reticulata and Ulva lactuca* were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 20µl of different solvent extracts of *Ulva reticulata and Ulva lactuca* at 10 mg/ml to check their antifungal activity. The Flucanozole (100 units/ml) was used as positive control and the 5% DMSO was used as a blind control.

2.4. Collection of test microbial cultures

Eleven different bacterial cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh. *Staphylococcus aureus* (MTCC 3160), *Streptococcus epidermis* (MTCC 889), *Streptococcus pyogenes* (MTCC 1926), *Bacillus subtilis* (MTCC 1427), *Bacillus cereus* (MTCC 7417), *Escherichia coli* (MTCC 1195), *Pseudomonas aeruginosa* (MTCC 7093), *Vibrio cholerae* (MTCC 3904), *Salmonella typhi* (MTCC 3215), *Klebsiella pneumoniae* (MTCC 4032) and *Enterobacter aerogenes* (MTCC 6804). Six different fungal isolates were used in this present study. The fungal cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh. *Aspergillus flavus* (MTCC 1883), *Aspergillus niger* (MTCC 4285), *Aspergillus fumigatus* (MTCC 4964), *Saccharomyces cerevisiae* (MTCC 2627), *Candida albicans* (MTCC 7315) and *Candida glabrata* (MTCC 3983).

2.5. Determination of Antibacterial activity of *Ulva reticulata and Ulva lactuca*

2.5.1. Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards and then used for the determination of antibacterial activity.

2.5.2. Disc diffusion method

The antibacterial activity of *Ulva reticulata and Ulva lactuca* extract were determined by Disc diffusion method proposed by Bauer *et al*. (1966)[7]. Petri plates were prepared by pouring 20 mL of Mueller Hinton agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 mL of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates allowed drying for five minutes. After drying the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The Ampicillin (5mg/disc) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

2.5.3. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the *Ulva reticulata and Ulva lactuca* extracts against bacterial isolates were tested in Mueller Hinton broth by Broth macro dilution method. The seaweed extracts were dissolved in 5% DMSO to obtain 128mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Muller Hinton broth for bacteria to get a concentration of 80, 40, 20, 10, 5, 2.50 and 1.25 mg/ml for *Ulva reticulata and Ulva lactuca* extracts and 50*m*l of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of *Ulva reticulata and Ulva lactuca* extracts. The culture tubes were incubated at 37^{o}C for 24 hours. The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

2.6. Determination of Antifungal activity of *Ulva reticulata and Ulva lactuca*

2.6.1. Disc diffusion method

The antifungal activity of *Ulva reticulata and Ulva lactuca* extracts were determined by Disc diffusion method proposed by Bauer *et al*. (1966)[7]. Petri plates were prepared by pouring 20 mL of Sabouraud's dextrose agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 mL of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates allowed drying for five minutes. After drying the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The flucanozole (100 units/ml) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 28°C for 48 hours (yeasts) and 72 hours (molds). The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

2.6.2. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the *Ulva reticulata and Ulva lactuca* extracts against fungal isolates were tested in Sabouraud's dextrose broth by Broth macro dilution method. The *Ulva reticulata and Ulva lactuca* extracts were dissolved in 5% DMSO to obtain 128mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Sabouraud's dextrose broth for fungi to get a concentration of 64, 32, 16, 8, 4, 2 and 1 mg/ml for *Ulva reticulata and Ulva lactuca* extracts and 50*m*l of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of seaweed extracts. The culture tubes were incubated at 28^{o}C for 48 hours (yeasts) and 72 hours (moulds). The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

3. RESULTS AND DISCUSSION

Marine algae are not only the primary and major producers of organic matter in the sea, but they also exert profound effects on the density and distribution of other inhabitants of the marine environment. An understanding of the wide range of behavioral relationships that exist among organisms would provide us with clues to substances of biomedical interest. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds, and

other marine organisms. The host organisms biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment. Some of these secondary metabolites offer avenues for developing cost-effective, safe and potent drugs. Nearly 50 lakhs species available in the sea are virtually untapped sources of secondary metabolites. Those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation [8] apart from their potential ecological/industrial significances such as controlling reproduction, biofouling and feeding deterrents [9].

In the present study, antibacterial activity of marine seaweeds extract *Ulva reticulata* and *Ulva lactuca* was investigated against Gram positive (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermis, Bacillus subtilis, Bacillus cereus*) and Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes*). The zone of inhibition of *Ulva reticulata* and *Ulva lactuca* extracts against Gram positive and Gram negative bacteria ranged between 7mm to 16mm at 5.0 mg/ml. The zone of inhibition of the seaweeds at 2.5 mg/ml concentration was relatively low when compared to 5.0 mg/ml of seaweed extracts.

The methanol extract of *Ulva reticulata* (5.0mg/ml) showed highest mean zone of inhibition (15 ± 0.6mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Staphylococcus aureus* (13 ± 0.3mm), *Streptococcus epidermis* (12 ± 0.6mm), *Bacillus subtilis* (11 ± 0.6mm) and *Bacillus cereus* (10 ± 0.5mm). For Gram negative bacteria, maximum zone of inhibition was recorded in methanol extract of *Ulva reticulata* against *Klebsiella pneumoniae* (13 ± 0.6mm) followed by *Escherichia coli* (12 ± 0.8mm), *Enterobacter aerogenes* (11 ± 0.3mm), *Pseudomonas aeruginosa* (10 ± 0.3mm), *Vibrio cholerae* (9 ± 0.6mm) and *Salmonella typhi* (9 ± 0.3mm). The zone of inhibition obtained from the Hexane extract of seaweed *Ulva reticulata* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO control and the positive control Ampicillin showed zone of inhibition ranging from 14 ± 0.8 mm to 20 ± 0.8mm against the test bacterial pathogens (**Table 1**). The Minimum inhibitory concentration (MIC) value of *Ulva reticulata* against bacteria was ranged between 2.50*m*g/ml to 80*m*g/ml. The lowest MIC (2.50 *m*g/ml) value was recorded against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae* and *Enterobacter aerogens* (**Table 2**).

Table 1: Antibacterial activity of crude extracts of *Ulva reticulata*

Microorganisms	Zone of inhibition (mm) mg/ml										
	Methanol		Acetone		Chloroform		Hexane		Ethyl acetate		Positive control*
	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	5
Staphylococcus aureus	11±0.8	13±0.3	10±0.5	13±0.2	8±0.4	10±0.3	7±0.5	9±0.4	8±0.3	12±0.2	16±0.5
Streptococcus pyogenes	13±0.4	15±0.6	10±0.3	12±0.8	8±0.5	10±0.3	7±0.6	9±0.7	9±0.6	13±0.6	18±0.3
Streptococcus epidermis	10±0.6	12±0.6	10±0.5	12±0.7	8±0.3	10±0.5	7±0.4	9±0.2	9±0.5	11±0.8	14±0.8
Bacillus subtilis	9±0.4	11±0.6	8±0.8	10±0.5	7±0.6	9±0.2	6±0.3	8±0.8	7±0.6	9±0.3	19±0.6
Bacillus cereus	8±0.3	10±0.5	8±0.5	10±0.7	7±0.3	9±0.6	6±0.4	8±0.6	17±0.	9±0.6	17±0.5
Escherichia coli	9±0.5	12±0.8	9±0.5	12±0.8	8±0.6	10±0.5	7±0.5	9±0.4	8±0.6	11±0.5	17±0.3
Pseudomonas aeruginosa	9±0.5	10±0.3	8±0.6	11±0.7	6±0.6	8±0.2	7±0.4	9±0.5	7±0.3	10±0.5	18±0.7
Vibrio cholera	7±0.2	9±0.6	6±0.5	9±0.3	6±0.5	8±0.5	6±0.6	9±0.7	7±0.3	10±0.4	16±0.5
Salmonella typhi	8±0.5	9±0.3	7±0.5	10±0.6	7±0.4	9±0.6	6±0.5	8±0.6	6±0.6	8±0.3	19±0.6
Klebsiella pneumonia	9±0.6	13±0.6	11±0.5	11±0.8	6±0.6	8±0.5	6±0.8	8±0.4	8±0.6	10±0.9	20±0.8
Enterobacter aerogenes	9±0.3	11±0.3	9±0.6	12±0.4	7±0.8	9±0.5	5±0.2	7±0.4	8±0.4	10±0.5	17±0.4

Mean ±S.D, *Ampicillin

Table 2: Minimum inhibitory concentration of crude extracts of *Ulva reticulata*

Microorganisms	Minimum inhibitory concentration (mg/ml)					
	Hexane	Methanol	Acetone	Chloroform	Ethyl acetate	Positive Control*
Staphylococcus aureus	20	2.50	5	20	5	5
Streptococcus pyogenes	40	5	10	20	10	10
Streptococcus epidermis	40	5	10	20	10	10
Bacillus subtilis	20	2.50	5	10	5	10
Bacillus cereus	20	2.50	5	10	10	10
Escherichia coli	40	5	10	20	20	5
Pseudomonas aeruginosa	40	5	10	20	10	5
Vibrio cholerae	40	10	20	40	20	20
Salmonella typhi	80	10	20	40	40	20
Klebsiella pneumoniae	40	2.50	5	20	10	10
Enterobacter aerogenes	40	2.50	2.50	20	10	10

***Ampicillin**

The *Ulva lactuca* methanol extract (5.0 mg/ml) showed maximum zone of inhibition (16 ± 0.3mm) against the Gram positive bacilli *Bacillus cereus* followed by *Bacillus subtilis* (16 ± 0.2 mm), *Streptococcus pyogenes* (14 ± 0.3 mm), *Staphylococcus aureus* (13 ± 0.6 mm) and *Streptococcus epidermis* (12 ± 0.3 mm). For Gram negative bacteria, highest zone of inhibition was recorded in methanol extract of *Ulva lactuca* against *Enterobacter aerogens* (15 ± 0.7mm) followed by *Klebsiella pneumoniae* (15 ± 0.6 mm), *Escherichia coli* (15 ± 0.2 mm), *Pseudomonas aeruginosa* (13 ± 0.6 mm) and *Vibrio cholerae* (10 ± 0.2 mm). The zone of inhibition obtained from the Hexane extract of seaweed *Ulva lactuca* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin showed zone of inhibition ranging from 14 ± 0.8 mm to 20 ± 0.8mm against the test bacterial pathogens (**Table 3**). The minimum inhibitory concentration (MIC) value of *Ulva* lactuca against bacteria was ranged between 1.25 to 80*m*g/ml. The lowest MIC (1.25 *m*g/ml) value was recorded against *Klebsiella pneumoniae* (**Table 4**).

Table-3: Antibacterial activity of crude extracts of *Ulva lactuca*

Microorganisms	Zone of inhibition (mm) mg/ml										
	Methanol		Acetone		Chloroform		Hexane		Ethyl acetate		Positive control*
	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	5
Staphylococcus aureus	11±0.5	13±0.6	10±0.8	13±0.3	7±0.3	9±0.4	6±0.2	10±0.7	7±0.4	10±0.3	16±0.5
Streptococcus pyogenes	11±0.3	14±0.3	11±0.3	13±0.5	6±0.4	10±0.3	5±0.6	8±0.6	8±0.6	11±0.5	18±0.3
Streptococcus epidermis	10±0.5	12±0.3	10±0.4	13±0.7	7±0.3	11±0.5	6±0.8	9±0.5	8±0.2	12±0.4	14±0.8
Bacillus subtilis	13±0.6	16±0.2	10±0.8	13±0.4	7±0.4	11±0.4	7±0.4	10±0.3	7±0.3	9±0.6	19±0.6
Bacillus cereus	13±0.5	16±0.3	10±0.5	14±0.2	6±0.5	9±0.5	6±0.5	8±0.4	6±0.5	9±0.5	17±0.5
Escherichia coli	11±0.2	15±0.2	11±0.8	14±0.4	8±0.5	12±0.6	7±0.6	10±0.3	7±0.4	10±0.4	17±0.3
Pseudomonas aeruginosa	9±0.4	13±0.6	10±0.3	13±0.5	8±0.3	11±0.5	6±0.5	9±0.2	7±0.6	10±0.3	18±0.7
Vibrio cholerae	8±0.6	10±0.2	8±0.3	11±0.3	8±0.5	12±0.4	8±0.3	11±0.4	6±0.3	9±0.5	16±0.5
Salmonella typhi	10±0.5	13±0.3	9±0.2	12±0.4	6±0.4	9±0.3	7±0.5	9±0.6	6±0.6	9±0.6	19±0.6
Klebsiella pneumoniae	12±0.4	15±0.6	12±0.5	14±0.6	8±0.6	13±0.2	7±0.6	9±0.3	8±0.6	11±0.7	20±0.8
Enterobacter aerogenes	11±0.2	15±0.7	11±0.2	13±0.3	8±0.4	12±0.5	6±0.5	9±0.5	8±0.5	12±0.8	17±0.4

Mean ± S.D, *Ampicillin

Table 4: Minimum inhibitory concentration of crude extracts of *Ulva lactuca*

Microorganisms	Minimum inhibitory concentration (mg/ml)					
	Hexane	Methanol	Acetone	Chloroform	Ethyl acetate	Positive Control*
Staphylococcus aureus	40	2.50	5	20	10	5
Streptococcus pyogenes	80	5	10	40	10	10
Streptococcus epidermis	40	5	10	20	10	10
Bacillus subtilis	20	2.50	5	10	5	10
Bacillus cereus	20	2.50	5	10	10	10
Escherichia coli	20	2.50	5	10	5	5
Pseudomonas aeruginosa	40	5	10	20	10	5
Vibrio cholera	80	10	20	40	40	20
Salmonella typhi	80	20	20	80	40	20
Klebsiella pneumonia	20	1.25	2.50	10	5	10
Enterobacter aerogenes	20	2.50	2.50	10	5	10

***Ampicillin**

Vallinayagam *et al*. (2009)[10] screened the antibacterial activities of two important seaweeds namely *Ulva lactuca* and *Gracilaria edulis* were screened against human bacterial pathogens *Staphylococcus aureus, Vibrio cholerae, Shigella dysentriae, Shigella boydii, Salmonella paratyphi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The maximum activity was recorded from the extract of *Gracilaria edulis* against *Staphylococcus aureus* and minimum by *Ulva lactuca* against *Pseudomonas aeruginosa*.

Karthigai Devi *et al.* (2009)[11] evaluated for antibacterial activity of commonly occurring green algae *Codium adherens*, *Ulva reticulata* and *Halimeda tuna* by agar diffusion method. Seven different solvents namely acetone, methanol, chloroform, diethyl ether, ethyl acetate, ethanol and petroleum ether were used for extraction. The zone of inhibition was compared and the ethanol extract shows the better result for the other extracts. Some extracts found more effective than the commercial medicine. The maximum antibacterial activity was noted in ethanol extracts showed activity against *Staphylococcus* sp. and the minimum was recorded in methanol extracts against *Escherichia coli*, *Staphylococcus* sp., *Proteus* sp., *Streptococcus* sp. and *Enterococci* sp. The findings of the present study showed that the methanol extract recorded better results than other

extracts and the results of the present study was completely in contrast with their results.

The antifungal activity of marine seaweeds extract *Ulva reticulata* and *Ulva lactuca* was investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The zone of inhibition of *Ulva reticulata* and *Ulva lactuca* extracts against fungal pathogens ranged between 6mm to 13mm at 10 mg/ml. The methanol extract of *Ulva reticulata* (10mg/ml) showed highest mean zone of inhibition (13 ± 0.6mm) against *Aspergillus niger* followed by *Aspergillus fumigatus* (10 ± 0.3mm), *Candida glabrata* (9 ± 0.6mm), *Candida albicans* (9 ± 0.4mm) and *Aspergillus flavus* (8 ± 0.5mm). No zone of inhibition was recorded against *Saccharomyces cerevisiae*. The zone of inhibition obtained from the Hexane extract of seaweed *Ulva reticulata* against fungal pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole showed zone of inhibition ranging from 12 ± 0.7 mm to 15 ± 0.6mm against the test fungal pathogens (**Table 5**). The Minimum inhibitory concentration (MIC) value of *Ulva reticulata* against fungi was ranged between 4*m*g/ml to 64*m*g/ml. The lowest MIC (4*m*g/ml) value was recorded against *Candida albicans* and *Candida glabrata* (**Table 6**).

Table 5: Antifungal activity of crude extract of *Ulva reticulata*

	Zone of inhibition (mm) /10mg/ml					
Microorganisms	**Hexane**	**Ethyl Acetate**	**Acetone**	**Methanol**	**Chloroform**	**Positive Control ***
Aspergillus flavus	6±0.3	8±0.5	10±0.3	13±0.6	8±0.3	15±0.6
Aspergillus niger	6±0.3	7±0.6	8±0.5	8±0.5	7±0.4	13±0.4
Aspergillus fumigatus	5±0.5	6±0.3	8±0.4	10±0.3	6±0.5	14±0.7
Saccharomyces cerevisiae	5±0.4	6±0.7	6±0.4	7±0.6	5±0.6	10±0.6
Candida albicans	6±0.5	8±0.4	9±0.4	9±0.4	6±0.4	12±0.7
Candida glabrata	6±0.6	8±0.3	8±0.5	9±0.6	7±0.3	13±0.6

Mean ± S.D, * Flucanozole

Table 6: Minimum inhibitory concentration of crude extracts of *Ulva reticulata*

	Minimum inhibitory concentration (mg/ml)					
Microorganisms	**Hexane**	**Methanol**	**Acetone**	**Chloroform**	**Ethyl acetate**	**Positive control***
Aspergillus flavus	32	8	8	32	16	8
Aspergillus niger	64	8	16	32	32	8
Aspergillus fumigatus	32	8	8	32	16	8
Saccharomyces cerevisiae	64	16	32	32	16	32
Candida albicans	32	4	8	16	8	4
Candida glabrata	32	4	8	16	16	4

***** Flucanozole

The *Ulva lactuca* methanol extract (10mg/ml) showed highest mean zone of inhibition (13 ± 0.5mm) against *Candida glabrata* followed by *Candida albicans* (12 ± 0.6mm), *Aspergillus flavus* (12 ± 0.5mm), *Aspergillus fumigatus* (11 ± 0.3mm) and *Aspergillus niger* (9 ± 0.5mm). No zone of inhibition was recorded against *Saccharomyces cerevisiae*. The zone of inhibition obtained from the Hexane extract of seaweed *Ulva lactuca* against fungal pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole showed zone of inhibition ranging from 10 ± 0.6 mm to 15 ± 0.6mm against the test fungal pathogens (**Table 7**). The Minimum inhibitory concentration (MIC) value of *Ulva lactuca* against fungi was ranged between 4*m*g/ml to 32*m*g/ml. The lowest MIC (4*m*g/ml) value was recorded against *Candida albicans* and *Candida glabrata* (**Table 8**).

Table-7: Antifungal activity of crude extracts of *Ulva lactuca*

	Zone of inhibition (mm) /10mg/ml					
Microorganisms	**Hexane**	**Ethyl Acetate**	**Acetone**	**Methanol**	**Chloroform**	**Positive Control ***
Aspergillus flavus	6±0.3	10±0.3	10±0.3	12 ±0.5	8±0.4	15±0.6
Aspergillus niger	6±0.6	8±0.5	8±0.4	9±0.5	7±0.3	13±0.4
Aspergillus fumigatus	6±0.3	8±0.5	9±0.5	11±0.3	8±0.3	14±0.7
Saccharomyces cerevisiae	6±0.4	7±0.4	7±0.5	8±0.2	7±0.1	10±0.6
Candida albicans	7±0.5	10±0.5	11±0.3	12±0.6	8±0.3	12±0.7
Candida glabrata	9±0.3	7±0.4	11±0.6	13±0.5	7±0.5	13±0.6

Mean ± S.D, * Flucanozole

Table 8: Minimum inhibitory concentration of crude extracts of *Ulva lactuca*

	Minimum inhibitory concentration (mg/ml)					
Microorganisms	**Hexane**	**Methanol**	**Acetone**	**Chloroform**	**Ethyl acetate**	**Positive control***
Aspergillus flavus	32	8	8	16	16	8
Aspergillus niger	32	8	16	32	16	8

Aspergillus fumigatus	32	8	8	16	16	8
Saccharomyces cerevisiae	64	16	16	32	32	32
Candida albicans	16	4	8	16	8	4
Candida glabrata	32	4	8	16	16	4

***** Flucanozole

Roberta Paulert *et al*. (2007)[12] studied the antifungal activity of cell-wall polysaccharides and crude extracts from the seaweed *Ulva fasciata* against filamentous fungi and yeast. The antifungal activity was assessed by agar diffusion assay and by means of the broth dilution method estimating the minimal inhibitory concentration (MIC). MIC was determined for the fungi *Colletotrichum lindemuthianum* (plant pathogen), *Trichophyton mentagrophytes* and *Microsporum canis* (dermatophyte pathogens). The methanol-insoluble extract inhibited the growth of *Trichophyton mentagrophytes* at concentration of 2 mg/ml. In contrast, ulvans did not show any *in vitro* activity towards all test organisms. In this present study, the minimum inhibitory concentration (MIC) value of *Ulva reticulata* and *Ulva lactuca* against fungi was ranged between 4*m*g/ml to 64*m*g/ml. The lowest MIC (4*m*g/ml) value was recorded against *Candida albicans* and *Candida glabrata.*

4. CONCLUSION

The study of antimicrobial activity of seaweeds *Ulva reticulata* and *Ulva lactuca* extracts showed promising antimicrobial activity against bacterial and fungal human pathogens. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful source of new antimicrobial agents.

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