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ANTIMICROBIAL PROPERTIES OF EDIBLE SEAWEEDS, *RHODOMENIA PALMATATA* AND *SARGASSUM SWARTZII* FROM THE GULF OF MANNAR SOUTH COAST, MANAPPAD, SOUTH INDIA

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Keywords:

Rhodomenia palmata, *Sargassum swartzii*, Disc diffusion method, MIC, MBC

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ABSTRACT: Nowadays, intensive use of several forms of synthetic antibiotics to treat bacterial infections has resulted dramatically in emergence of resistant pathogenic microorganisms, often due to indiscriminate use of these substances. The present study was carried to evaluate the antibacterial and antifungal activities of different concentrations of methanol extracts of *Rhodomenia palmata* and *Sargassum swartzii* collected from Manappad, the Gulf of Mannar Biosphere Reserve, Tamil Nadu, India. The methanol extracts of selected two marine macro algae were screened against *Bacillus subtilis*, *B. pumilus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Proteus vulgaris*, *Shigella flexneri*, *Salmonella enterica*, *Vibrio cholerae* and five yeast viz., *Candida albicans*, *C. glabarata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *Merozoma guilliermondii* and *Candida lusitaniae* and nine mould fungi namely *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *A. clavatus* four dermatophytes viz., *Trichophyton rubrum* and *T. mentagrophytes* strains. The antimicrobial activity was determined the extent of inhibitory zones using disc diffusion method, Minimum inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC). The mean zones of inhibition ranged from 7.1 to 21.5 mm. The MIC values of the methanol extracts of the tested organisms ranged between 62.5 and 250 µg/ml, while the MBC and MFC values were between 250 and 500 µg/ml. The highest antibacterial activity was recorded in the ethyl acetate extract of *R. palmata* than other extracts. The overall results have established antimicrobial activities of *R. palmata* and *S. swartzii* could be used against several diseases and, in the food processing industry, to preserve foods.

INTRODUCTION: Each year, over 13 million deaths worldwide are attributed to the emergence of new infectious diseases or the re-emergence of previously controlled pathogens.

Bacterial infections pose a significant threat to public health, as many pathogenic bacteria quickly develop resistance to multiple antibiotics¹.

This increased antibiotic resistance is driven by several factors, including over prescription by healthcare providers and self medication. The widespread use of antibiotics contributes to both acquired and natural resistance, resulting in numerous therapeutic failures globally. Excessive usage of antibiotic is destructive to human health, ecosystem, and environment.

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It could also increase the incidences of drug-resistant pathogens. Antibiotics resistance is a worldwide major problem which is rapidly increasing in both hospitals and the community involved in morbidity, mortality, and health-care²⁶.

In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants³.

The marine environment is an excellent reservoir of bioactive natural products possessing structural features that are not found on land¹³. As a result, marine organisms contain highly bioactive metabolites that could lead to the development of new pharmaceutical agents. Marine macroalgae are a various group of marine organisms that have been widely investigated for their secondary metabolites content. They represent one of the richest sources of natural antioxidants among marine resources¹⁷.

Edible seaweeds, including algae from the Protista orders: Laminariales (brown), Chlorophyta (green) and Rhodophyta (red) have a long history of use in the diets of Pacific and Asian cultures compared to those of Europe and the Canadian and US Maritimes. These seaweeds include red algae in Japanese and Korean cuisine ['Nori' or 'Kim', 'Laver' (*Porphyra tenera*) or European/ North American diets ['Dulse' (*Palmaria palmata*)]; brown kelps in Japanese cuisine ['Hijiki' (*Hijikia fusiformis*), 'Wakame' (Sea Mustard, *Undaria pinnatifida*), 'Makonbu' (Sea Tangle, *Laminaria japonica*)] or Chinese cuisine ['Hai dai' (*Laminaria* sp.)]; green algae in Hawaiian cuisine ['*Limu palahalaha*' (*Ulva* sp.)]. Within the traditional Japanese diet, seaweeds are commonly used as sushi wrappings, seasonings, condiments and

vegetables and can thus constitute between 10% and 25% of food intake by most Japanese. Within the food ingredient industry, seaweeds have been traditionally viewed as sources of soluble and insoluble dietary fibres such as gums (i.e. carrageenans, agar), storage polysaccharides (i.e. b-1, 3-glucan) and floridean starch²².

Rhodomenia palmata (or) *Palmaria palmata* (Linnaeus) Kuntze, also called dulse, dillisk or dilsk (from Irish/Scottish Gaelic duileasc/duileasg), red dulse, sea lettuce flakes or creathnach, is a red alga, Rhodophyta previously referred to as *Rhodymenia palmata* (Linnaeus) Greville. Dulse grows attached by its discoid holdfast to the stipes of Laminaria or to rocks. It has a short stipe, the fronds are variable and vary in color from deep-rose to reddish-purple and are rather leathery in texture. The flat foliose blade gradually expands and divides into broad segments ranging in size to 50 cm long and 3 cm–8 cm in width which can bear flat wedge-shaped proliferations from the edge.

Sargassum swartzii belong to the family Phaeophyceae. Disc-shaped holdfast; main branch flat, 3 mm wide, without spines, secondary lateral alternate at intervals of 2-3 cm; leaves thick, lanceolate to linear-lanceolate, apex acute, base broad cuneate, up to 4 cm long and 6 mm wide, phyllotaxis 1/2, midrib inconspicuous, reaching the apex, margin with acute teeth or irregular small teeth, cryptostomata forming two rows on both sides of midrib; vesicle, cryptostomata few on the vesicles.

Androgynous receptacles terete or compressed, about 5 mm long, with small spines on apical part, often forked and cymosely arranged. The bioactive compounds held by seaweed are responsible for the different bioactivities already researched by many authors. Numerous studies and reviews have referred to the antioxidant²⁰ antimicrobial, anti-fungal^{1, 30} anti-inflammatory²⁸, anti-cholesterol¹¹, anti-neurodegenerative¹⁶, anti-tumor²⁹ and prebiotic²⁴ properties of these bioactive compounds extracted from seaweeds. On industrial and commercial levels, while seaweed bioactives remain relatively unexploited, efforts are being undertaken to promote the use of seaweeds in food ingredient applications. In recent years there has been an increasing interest in the use of natural

substances against infectious diseases¹⁴. The development of antibiotic resistance and undesirable side effects due to excessive use of synthetic drugs has, necessitated the search for new plant products to treat bacterial and fungal infections (Bolivara et al., 2011). Recently, the dramatically increasing resistance of microorganisms towards these conventional antibiotics and food preservatives and their interactions with the food chain urging the need to develop additional natural antimicrobial agents as a public health priority⁸. Hence, the present investigation reports antibacterial and antifungal, activities of methanol extracts of *R. palmata* and *S. swartzii*.

MATERIALS AND METHODS:

Algae Sample Collection: *Rhodomenia palmata* (Rhodophyceae) and *Sargassum swartzii* (Phaeophyceae) marine algae were collected by hand picking from the submerged marine rocks at Manappad village, (Lat. 8°30'N; Long. 78°8'E), Tuticorin district, the Gulf of Mannar Marine biosphere, Tamil Nadu, India. Seaweeds collections were made from the month of during December 2024. The museum specimens are deposited in the Department of Botany, Annamalai University, Annamalainagar.

Extract Preparation: Algae samples were cleaned of epiphytes and extraneous matter and necrotic parts were removed. Plants were washed with seawater and then in fresh water. The seaweeds were transported to the laboratory in sterile polythene bags and were kept in an ice box containing slush ice and transported to the laboratory. The samples were rinsed with sterile distilled water and were shade dried, cut into small pieces and powdered in a mixer grinder. Five hundred grams of powdered samples were packed in Soxhlet apparatus and extracted with methanol for 6 hours. The extracts were pooled and the solvent were evaporated under vacuum in rotary evaporator (Heidolph, Germany) at 40 °C and the dried extracts were stored at 4 °C in refrigerator until further assay.

Microorganisms: The bacterial strains viz., *Bacillus subtilis* (MTCC 2063), *B. pumilus* (MTCC 1640), *Micrococcus luteus* (MTCC 106), *Staphylococcus aureus* (MTCC 737), *Klebsiella*

pneumoniae (MTCC 109), *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439), *Streptococcus pyogenes* (MTCC 442), *Proteus mirabilis* (MTCC 425), *Proteus vulgaris* (MTCC 426), *Shigella flexneri* (MTCC 1457), *Salmonella enterica* (MTCC 3231), *Vibrio cholerae* (MTCC 3906) and five yeast viz., *Candida albicans* (MTCC 3017), *C. glaberrata* (MTCC 3019), *C. tropicalis* (MTCC 184), *C. krusei* (MTCC 9215), *C. parapsilosis* (MTCC 2509), *C. guilliermondii* (NCIM 3126), *Merozomya guilliermondii* (MTCC 3955) and *Candida lusitanae* (MTCC 1001) and nine mould fungi namely *Aspergillus niger* (MTCC 282), *A. flavus* (MTCC 277), *A. fumigatus* (MTCC 343) and *A. clavatus* (MTCC 1323) four dermatophytes viz., *Trichophyton rubrum* (MTCC 296) and *T. mentagrophytes* (MTCC 8476), were used in this study. These standard bacterial and fungal strains were obtained from Microbial Type Culture Collection and Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Gene Bank, Biochemical Sciences Division, National Chemical Laboratory, Pune, India. The stock cultures were maintained on Nutrient Agar for bacteria and Sabouraud Dextrose Agar for fungi at 4 °C.

In-vitro antibacterial activity was determined by using Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB). *In-vitro* antifungal activity was determined by using Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB) (for mycelial fungi) and Yeast Nitrogen Base (YNB) (for yeast) and they were obtained from Himedia Ltd., Mumbai

Disc Diffusion Method: The disc diffusion method⁵ was followed for antibacterial and antifungal susceptibility test. Petri plates were prepared by pouring 20 ml of MHA for bacteria and SDA for yeast and filamentous fungi. Then the plates were allowed to solidify and used in susceptibility test. The standard inoculum using bacterial suspensions containing 10⁸ colony forming units (CFU) per ml, yeast suspensions containing 10⁶ colony forming units (CFU) per ml and mould fungal suspensions containing 10⁴ colony forming units (CFU) per ml were swabbed on the top of the solidified respective media and allowed to dry for 10 minutes.

The methanol extract was dissolved in 10 per cent Dimethyl sulfoxide (DMSO) and under aseptic conditions, sterile Himedia discs (6 mm) were impregnated with 20 µl of different concentrations (500, 250 and 125 µg/disc). The discs with methanol extract were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Methicillin (5 µg/disc) for MRSA, Ampicillin (10 µg/disc) for bacteria and Amphotericin-B (100 units/disc) for Yeast and Ketoconazole (5µg/disc) for *Aspergillus* and dermatophytes were used as positive control and 10 per cent DMSO was used as blind control in C for 24 h for all bacterial strains all the assays. Finally, the inoculated plates were incubated at 37 °C for 24 - 48 h for MRSA and plates were incubated at 28 °C for 24 hours yeast and 30 °C and 35 for 72-96 h for *Aspergillus* and 4-7 days for dermatophytes. The zone of inhibitions was observed and measured in millimeters. The assay in this experiment was repeated three times.

Microdilution Broth Assay:

Antibacterial Elvaluation: Minimum inhibitory concentration (MIC) of the methanol extract, a modified Reassuring microtitre plate assay was used as reported by Sarker *et al.* (2007)²⁷ for bacteria sterile MHA and MHB for bacteria, MHB supplemented with 4% sodium chloride for MRSA 50 µl of respective MHB for bacteria, MHB supplemented with 4% sodium chloride for MRSA were transferred in to each well of a sterile 96-well microtiter plate.

The methanol extract was dissolved in 10% DMSO to obtain 2000 µg/ml stock solution. 50 µl of methanol extract stock solution was added into the first well. After fine mixing, the methanol extract was transferred to the second well and in this way the dilution procedure was continued to a twofold dilution to obtain concentrations like 1000 to 15.625 µg/ml of the methanolic extracts in each well. To each well, 10 µL of resazurin indicator solution was added for bacteria. Finally, 10 µl of the standardized bacterial and fungal suspensions to each well to achieve a concentration of approximately 5×10^5 CFU/mL were transferred in to all wells. Each plate had a set of controls: a column with all solutions with the exception of the crude extracts; a column with all solutions with the exception of the bacterial solution adding 10 µl of

MHB instead and a column with 10% DMSO solution as a negative control. The control well contained only sterile water. The plates were incubated at 37 °C for 24 h for all bacterial strains. The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). The lowest concentration at which color change occurred was taken as the MIC value for bacteria.

Antifungal Elvaluation: MIC of each extract was determined by using broth micro dilution technique according to Guidelines of the NCCLS for yeasts (M27-A2) (CLSI 2008) and for filamentous fungi (M 38 A) (CLSI 2002). The minimum inhibitory concentration values were determined in RPMI-1640 (Himedia, Mumbai) with L – glutamine without sodium bicarbonate, pH 7.0 with Morpholine – sulfonicacid (MOPS). 20 µl of a stock solution (1mg/ml) of each algae extract in 10% DMSO was dissolved with 980 µl of RPMI-1640 made a solution 1000 µl (2mg/ml). From that, the two fold serial dilutions in the range from 15.7 to 1000 µg/ml were prepared. 100µl of solution was poured into first well of 96 well microtitre plates and then, 50µl were transformed to the next well containing 100 µl of RPMI-1640. The same procedure was performed for all wells. 10µl of fungal standardized inoculum suspensions containing $1-5 \times 10^3$ for yeast $0.4-5 \times 10^4$ for dermatophytes colony forming units (CFU) per ml was transferred to each well. The control well contained only sterile water and devoid of inoculum. The microtitre tray plates were incubated at 28 °C for 24 hours with yeast and 30 °C for 72-96h for *Aspergillus* and 4-7 days for the dermatophytes. MIC of the extracts was recorded as the lowest concentration of extracts that inhibited the growth of the *Candida* and dermatophytes as compared to that of control.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC):

MBC and MFC were determined by plating loop full samples from each MIC assay well with growth inhibition in to freshly prepared MHA for bacteria and SDA for fungal strains. The plates were incubated at, 37 °C for 24 h for all bacterial strains, 35 °C for 24 - 48 h for MRSA, 28 °C for 24 hours for yeasts and 30 °C for 48 h for *Aspergillus* and 4 -7 days for

dermatophytes. The MBC and MFC were recorded as the lowest concentration of the methanolic extracts that did not permit any visible bacterial and fungal growth after the period of incubation.

Statistical Analysis: The results are expressed as the mean±SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant difference between methanol extracts for *in-vitro* antibacterial and fungal assays. Comparison of means for *in-vitro* antibacterial and fungal assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. P value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

Antibacterial Activity: The present study was made to screen the methanol extracts of *R. palmata* and *S. swartzii* against gram positive bacteria such as *B. subtilis*, *B. pumilus*, *M. luteus*, *S. aureus* (MTCC 737), VRE and *S. pyogenes* and gram negative bacteria such as *S. enterica*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *P. vulgaris*, *S. flexneri* and *V. cholera*. The mean zones of inhibition ranged from 7.1 to 21.3 mm. The MIC values of the methanolic extracts of *Rhodomencia palmate* and *Sargassum swartzii* tested ranged between 62.5 and 250 µg/ml, while

the MBC values were between 125 and 1000 µg/ml. The highest mean zone of inhibition (21.3 mm) was against *B. subtilis* and lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) values were obtained with the methanol extracts of *R. palmata* and *S. swartzii* against *B. subtilis* and the results are tabulated in **Table 1**.

Same results were observed in methanol extracts of both seaweeds *S. wightii* and *T. ornata* were tested against various *B. subtilis*, *E. coli*, *Enterococcus faecalis*, *P. aeruginosa*, *Aeromonas hydrophila*, *P. vulgaris*, *K. pneumoniae*, *S. flexneri* and *S. aureus*³³. The brown seaweeds, *Turbinaria ornata* and *Sargassum wightii* extracts were active against nine pathogens such as *A. hydrophila*, *B. subtilis*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *S. flexneri* and *S. aureus*. This may be due to active components which are present in the seaweed extracts. These results indicate that the extracts contained different antibacterial substances and reflect the variety of secondary metabolites³². The *Gelidium acerosa* extracts of seaweeds are prepared with three different solvents like ethanol, methanol and acetone and tested against bacteria like *S. aureus*, *Bacillus cereus*, *M. luteus*, *K. pneumoniae*, *P. aeruginosa*, fungus like *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *C. albicans* and *C. tropicalis*³⁴.

TABLE 1: ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF RHODOMENIA PALMATA AND SARGASSUM SWARTZII

| | | Mean zone of inhibition ^a (mm) ^b | | | | | | <i>Rhodomencia palmata</i> | | <i>Sargassum swartzii</i> | | |
|--------|-------------------------------|--|----------|----------|------------------------------------|----------|----------|----------------------------|-----------|---------------------------|-----------|-----------|
| S. no. | Name of the microorganisms | Concentration of the disc (µg/disc) | | | Concentration of the disc(µg/disc) | | | Control (Cip/Am p) | MIC µg/ml | MBC µg/ml | MIC µg/ml | MBC µg/ml |
| | | <i>Rhodomencia palmata</i> | | | <i>Sargassum swartzii</i> | | | | | | | |
| | | 1000 | 500 | 250 | 1000 | 500 | 250 | | | | | |
| 1 | <i>Bacillus subtilis</i> | 21.5±1.3 | 14.8±1.3 | 10.3±1.0 | 19.5±1.3 | 13.3±1.0 | 10.3±1.0 | 31.5±2.4 | 62.5 | 125 | 62.5 | 125 |
| 2 | <i>Bacillus pumilus</i> | 19.0±1.4 | 14.8±1.0 | 10.0±0.8 | 16.3±1.3 | 12.0±0.8 | 10.0±0.6 | 34.3±2.1 | 62.5 | 125 | 62.5 | 125 |
| 3 | <i>Micrococcus luteus</i> | 20.5±1.7 | 15.3±1.5 | 11.5±1.3 | 19.3±1.3 | 11.3±1.3 | 9.5±1.0 | 30.5±2.4 | 62.5 | 125 | 62.5 | 125 |
| 4 | <i>Staphylococcus aureus</i> | 20.0±1.4 | 14.5±1.3 | 10.5±1.0 | 18.5±1.3 | 13.5±1.2 | 9.3±1.0 | 29.0±1.8 | 62.5 | 125 | 62.5 | 125 |
| 5 | <i>Pseudomonas aeruginosa</i> | 15.8±1.0 | 11.0±0.8 | 9.0±0.8 | 15.3±1.0 | 10.5±0.6 | 9.3±0.5 | 30.0±1.8 | 125 | 500 | 250 | 500 |
| 6. | <i>Klebsiella pneumoniae</i> | 15.0±1.0 | 10.0±0.8 | 8.5±0.8 | 12.3±1.0 | 10.3±0.6 | 9.3±0.5 | 32.5±1.7 | 250 | 250 | 250 | 500 |
| 7 | <i>Escherichia coli</i> | 16.5±1.3 | 13.8±1.0 | 9.0±0.8 | 13.3±1.0 | 10.8±0.6 | 8.5±0.6 | 30.3±1.7 | 125 | 250 | 250 | 500 |
| 8 | <i>Streptococcus pyogenes</i> | 14.3±1.0 | 13.0±0.8 | 8.8 ±0.8 | 12.0±0.8 | 10.1±0.6 | 7.5 ±0.5 | 17.5±1.3 | 250 | 500 | 250 | 500 |
| 9 | <i>Shigella flexneri</i> | 12.0±0.8 | 9.8 ±0.5 | 8.5 ±0.6 | 11.5±0.6 | 9.8 ±0.5 | 7.3 ±0.5 | 18.8±1.3 | 250 | 500 | 250 | 500 |
| 10 | <i>Vibriochloerae</i> | 12.0±0.8 | 10.5±0.6 | 8.3 ±0.5 | 11.8±0.5 | 9.5 ±0.6 | 7.0 ±0.5 | 17.3±1.0 | 250 | 500 | 250 | 500 |
| 11 | <i>Salmonella typhimurium</i> | 15.8±1.0 | 10.0±0.8 | 8.5 ±0.6 | 13.0±0.8 | 10.5±0.6 | 9.5 ±0.6 | 18.3±1.0 | 250 | 500 | 250 | 500 |
| 12 | <i>Proteus mirabilis</i> | 14.0±0.8 | 10.0±0.8 | 9.5 ±0.6 | 13.5±0.8 | 10.3±0.5 | 8.6 ±0.5 | 15.3±1.0 | 250 | 500 | 250 | 500 |

| | | | | | | | | | | | | |
|----|------------------------------|----------|----------|----------|----------|----------|----------|----------|-----|-----|-----|-----|
| 13 | <i>Salmonella enterica</i> | 14.0±0.8 | 11.0±0.8 | 9.5 ±0.6 | 13.3±0.8 | 11.5±0.5 | 8.0 ±0.5 | 16.5±1.3 | 250 | 500 | 250 | 500 |
| 14 | <i>Enterococcus faecalis</i> | 15.8±1.0 | 10.3±1.0 | 8.9 ±0.8 | 13.8±0.8 | 10.8±0.5 | 8.8 ±0.5 | 15.5±1.0 | 250 | 500 | 250 | 500 |

a- Diameter of zone of inhibition (mm) including disc diameter of 6 mm, b- Mean of four assays, ± standard deviation, Cip–Ciprofloxacin antibacterial drug(5µg/disc), Amp–Amphotericin-Bantifungal drug(100 units/disc)

In present results indicated of the methanol extracts of *R. palmata* and *S. swartzii* possessed antibacterial activity against all the bacterial strains tested. Similar results were observed²⁵ reported notable antimicrobial activity of marine algal extracts. The methanolic extract of *Ulva intestinalis* and the ethanolic extract of *Hypnea musciformis* were effective, though Gram-positive bacteria showed resistance to the methanolic extract of *U. intestinalis*. *H. musciformis* inhibited *Staphylococcus aureus* (16 ± 2.7 mm) and *S. epidermidis* (12.5 ± 1.9 mm). The ethanolic extract of *I. stellata* inhibited *Escherichia coli* (10 ± 2.9 mm). Antifungal activity was observed with the ethanolic extract of *U. intestinalis* against *Fusarium oxysporum* (13 ± 3.3 mm) and *Aspergillus flavus* (15±1.9mm), while *C. decorticatum* showed activity against *A. niger* (15±0.9mm)²¹. Regarding *E. coli*, *P. aeruginosa*, and *Klebsiella pneumoniae*. Nevertheless, in some research, like that by Demirel et al. (2009)¹², antibacterial activity of *C. Fragile* extract against *E. aerogenes*, *E. coli*, and *B. subtilis* was noted⁴.

The ethanol extract of *C. decorticatum* effectively inhibited both grampositive (*S. aureus* and *S. epidermis*) and gramnegative (*E. coli*) bacteria. Swapnil Patil and Nakade (2025)³⁰ reported that the antimicrobial activity of extracts from the red seaweed *Ceramium diaphanum* was evaluated against pathogenic bacteria and fungi using the agar diffusion method. The extracts exhibited strong inhibitory activity against all tested microorganisms. Notably, the ethanol and chloroform extracts showed significant antibacterial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, and methicillin-resistant *Staphylococcus aureus* (MRSA). The results of the present study revealed that Gram positive organisms were more susceptible than gram negative bacteria to the crude extracts of *R. palmata* and *S. swartzii* Taskin et al.³¹, also reported that Gram-positive bacteria were more effectively controlled by the extracts of algae used

in their study compared to Gram-negative bacteria. Taskin et al. (2001)³¹, also made similar observations, indicating that the more susceptibility of Gram-positive bacteria to the algal extract was due to the differences in their cell wall structure and their composition. Leakage of intracellular constituents and impairment of microbial enzyme systems can then occur⁷ and extensive loss of the cell contents will cause the death of cell. Furthermore, there are some generally accepted mechanisms of antimicrobial interaction for synergy: sequential inhibition of a common biochemical pathway, inhibition of protective enzymes, combinations of cell wall active agents, and use of cell wall active agents to enhance the uptake of other antimicrobials³⁵. Therefore, it is necessary to clarify the specific modes of action of plant extracts constituents with antimicrobial properties on the metabolic pathway of microorganisms^{15, 36} screened around 20 algae using methanol and ethanol along Idinthakarai coast and they reported that *B. subtilis* and *Staphylococcus* sp. were highly susceptible to most of the algal extracts.

Antifungal Activity: In the present investigation, the methanolic extracts of *R. palmata* and *S. swartzii* against all the fungal strains tested such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. lusitaniae*, *A. clavatus*, *A. niger*, *A. flavus*, *A. fumigatus*, *T. rubrum*, *T. mentagrophytes*. The mean zones of inhibition ranged from 7.1 to 17.5 mm. The lowest MIC (125 µg/ml) and MFC (250 µg/ml) values were obtained with the methanolic extracts of *R. palmata* and *S. swartzii* and the results are presented in **Table 2**. Zovko et al. (2012) obtained the same results against fungal strains with a high activity of algal extracts against *C. albicans*. Gao et al. (2011)²⁹ showed that a few extracts of marine algae have not only an antifungal activity but toxicity towards cancer cells. Tuney et al. (2006)³⁷ found that ethanolic extract of *Padina pavonica* were active against *C. albicans*. Kolanjinathan and Stella (2009)¹⁹ reported that antifungal activity of

marine seaweeds extracts of *Ulva reticulata* and *Ulva lactuca* against *A. niger*, *A. flavus*, *A. fumigatus*, *Saccharomyces cerevisiae*, *C. albicans* and *C. glabrata*. It is expected that the antifungal activity found by us to be done in the presence of bioactive molecules, as phenolic compounds (phlorotannins, terpenes, alkaloids), polysaccharides or fatty acids, many of these structures being identified as antimicrobials (Hanaa et al. 2008). Pierre et al. (2011) also reported the antimicrobial effect sulfated of galactan from *Chaetomorpha aerea* against the human bacterial pathogens such as *S. aureus*, *Salmonella enteritidis*, *P. aeruginosa*, *E. faecalis*, *B. subtilis*, *M. luteus* and *C. glabrata*.

In the present investigation, methanolic extracts of *R. palmata* and *S. swartzii* no activity against *C. glabrata*, *C. tropicalis*, *C. lusitaniae* and *Cryptococcus neoformans*. The activity and inactivity of marine algae against microorganisms could be due to the reproductive state and seasonality (Ely et al. 2004). The extraction protocol and the harvest period are other important factors (Rajasulochana et al. 2009). Moubayed et al. (2017)²³ reported that the methanolic and acetone extracts were tested against gram positive, gram

negative bacteria and *Candida albicans* in an attempt to be used as an alternative to commonly used antibiotics. Both brown seaweed species *Sargassum latifolium* and *Sargassum platycarpum* methanolic extracts were found to be active against gram positive than gram negative; however, *S. latifolium* acetone extract gave the highest inhibitory activity against *Salmonella* sp. On the other hand, *Cladophora socialis* organic extract demonstrated higher antibacterial activity than the fresh extract but both *C. socialis* extracts revealed decreased activity compared to *Sargassum* extracts. *Cladophora* methanolic extract showed an obvious effect on methicillin resistant *Staphylococcus aureus* (MRSA).

Khanzada et al. (2015)¹⁸ reported *A. flavus* was subjected to 75% Codium inhibitory activity in ethyl acetate extract and 72% in methanol extract. However, the results of the current investigation indicated that *C. decortatum* was marginally more effective at resisting *A. niger* than *A. flavous*. *C. intricatum* methanol extract was tested for its antibacterial properties against a variety of bacterial infections. *C. intricatum* demonstrated a wide range of inhibitory action against Methicillin-Resistant *Staphylococcus aureus* (MRSA)⁴.

TABLE 2: ANTIFUNGAL ACTIVITY OF METHANOL EXTRACT OF RHODOMENIA PALMATATA AND SARGASSUM SWARTZII

| Mean zone of inhibition ^a (mm) ^b | | | | | | | | | <i>Rhodomenia palmata</i> | | <i>Sargassum swartzii</i> | |
|--|----------------------------|-------------------------------------|----------|----------|-------------------------------------|----------|----------|-------------------|---------------------------|-----------|---------------------------|-----------|
| S. no. | Name of the microorganisms | Concentration of the disc (µg/disc) | | | Concentration of the disc (µg/disc) | | | Control (Cip/Amp) | MIC µg/ml | MBC µg/ml | MIC µg/ml | MBC µg/ml |
| | | <i>Rhodomenia palmata</i> | | | <i>Sargassum swartzii</i> | | | | | | | |
| | | 1000 | 500 | 250 | 1000 | 500 | 250 | | | | | |
| 15 | <i>Candida albicans</i> | 17.5±1.3 | 13.8±1.3 | 10.3±1.0 | 15.3±1.3 | 12.3±1.0 | 10.3±1.0 | 31.5±2.4 | 125 | 250 | 125 | 250 |
| 16 | <i>C. tropicalis</i> | 17.0±1.4 | 13.5±1.0 | 10.0±0.8 | 14.3±1.3 | 11.0±0.8 | 10.8±0.6 | 34.3±2.1 | 125 | 250 | 250 | 500 |
| 17 | <i>C. krusei</i> | 16.5±1.7 | 12.3±1.5 | 9.5±1.3 | 14.5±1.3 | 10.3±1.3 | 10.5±1.0 | 30.5±2.4 | 125 | 250 | 250 | 500 |
| 18 | <i>C. parapsilosis</i> | 15.0±1.4 | 13.5±1.3 | 9.5±1.0 | 13.5±1.3 | 9.0±1.2 | 11.3±1.0 | 29.0±1.8 | 125 | 250 | 250 | 500 |
| 19 | <i>C. guilliermondii</i> | 15.3±1.0 | 12.0±0.8 | 8.0±0.8 | 12.3±1.0 | 10.5±0.6 | 9.3±0.5 | 30.0±1.8 | 250 | 500 | 250 | 500 |
| 20 | <i>C. lusitaniae</i> | 15.1±1.0 | 11.0±0.8 | 8.8±0.8 | 12.3±1.0 | 10.5±0.6 | 9.3±0.5 | 32.5±1.7 | 250 | 500 | 250 | 500 |
| 21 | <i>Aspergillus niger</i> | 14.5±1.3 | 12.8±1.0 | 8.0±0.8 | 13.3±1.0 | 9.5±0.6 | 10.5±0.6 | 30.3±1.7 | 250 | 500 | 250 | 500 |
| 22 | <i>A. flavus</i> | 14.3±1.0 | 13.0±0.8 | 8.5±0.8 | 12.0±0.8 | 10.5±0.6 | 9.3±0.5 | 17.5±1.3 | 250 | 500 | 250 | 500 |
| 23 | <i>A. fumigatus</i> | 12.0±0.8 | 10.8±0.5 | 7.1±0.6 | 11.5±0.6 | 9.3±0.5 | 7.3±0.5 | 18.8±1.3 | 250 | 500 | 250 | 500 |
| 24 | <i>A. clavatus</i> | 12.5±0.8 | 11.5±0.6 | 8.3±0.5 | 12.8±0.5 | 9.5±0.6 | 6.8±0.5 | 17.3±1.0 | 250 | 500 | 250 | 500 |
| 25 | <i>A. synowii</i> | 15.8±1.0 | 12.0±0.8 | 10.0±0.6 | 12.5±0.8 | 10.5±0.6 | 9.5±0.6 | 18.3±1.0 | 125 | 250 | 125 | 250 |
| 26 | <i>Trichophyton rubrum</i> | 14.0±0.8 | 13.0±0.8 | 7.5±0.6 | 13.3±0.8 | 10.0±0.5 | 8.8±0.5 | 15.3±1.0 | 250 | 500 | 250 | 500 |
| 27 | <i>T. mentagrophytes</i> | 14.0±0.8 | 11.1±0.8 | 7.8±0.6 | 12.8±0.8 | 10.1±0.5 | 8.8±0.5 | 16.5±1.3 | 250 | 500 | 250 | 500 |
| 28 | <i>Microporum gypseum</i> | 13.8±1.0 | 11.3±1.0 | 7.3±0.8 | 12.5±0.8 | 10.8±0.5 | 8.8±0.5 | 15.5±1.0 | 250 | 500 | 250 | 500 |

a- Diameter of zone of inhibition (mm) including disc diameter of 6 mm; b- Mean of four assays; ± standard deviation; Cip – Ciprofloxacin antibacterial drug (5 µg/disc); Amp – Amphotericin-B antifungal drug (100 units/disc)

CONCLUSION: The results of the present study indicated that antimicrobial effective of *S. swartzii*

and *R. palmata* is one of the most examined features, important for both food preservation and

control of human and animal diseases of microbial origin. Numerous reports suggest strong antimicrobial activities of a wide range of *S. swartzii* and *R. palmata*, especially those belonging to the Phaeophyceae and Rhodophyceae family. *S. swartzii* and *R. palmata* they may lead to an efficient lead for the discovery of new drug molecules against several pathogens causing infectious diseases.

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