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Research Article
Antimicrobial, Antioxidant, Anticancer Activity and Phytochemical Analysis of the Red Alga, *Laurencia papillosa*

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Abstract

Background and Objective: Marine macro-algae contain bioactive compounds and can produce a great variety of secondary metabolites useful to human health. The present study was meant to evaluate the biological activities of extracts from the red alga, *Laurencia papillosa* (*L. papillosa*). **Materials and Methods:** The dichloromethane, dichloromethane:methanol (1:1 v/v), methanol and water were used to obtain the algal extract. The antimicrobial activity of the different extracts was evaluated against gram-positive bacteria (*Staphylococcus aureus* (*S. aureus*), *Staphylococcus saprophyticus* and *Streptococcus agaloctiae*) and gram-negative bacteria (*Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus mirabilis* (*P. mirabilis*)) and two fungal strains (*Candida albicans* and *Candida tropicalis* (*C. tropicalis*)) by the well diffusion method. The algal extract was assayed for DPPH radical scavenging activity and MTT cell viability using HCT-116 colon cancer. The phytochemical composition of *L. papillosa* was studied. The FT-IR and GC-MS analysis were performed for the most effective algal extract. **Results:** The dichloromethane:methanol (1:1) extract revealed a wide range of antimicrobial activity against tested pathogens, which mostly inhibited the growth of *P. aeruginosa, S. aureus*, *C. tropicalis*, *E. coli* and *P. mirabilis* with inhibition zones range of 13-15 mm. The algal extracts exhibited bactericidal effect against the tested strains at the ratio of MBC/MIC $\geq$2.0. Among the algal extracts, dichloromethane: methanol (1:1) exhibited the highest antioxidant and anticancer activities with a very low IC_{50} (110.8 and 196 μg mL^{-1}, respectively). The pigments, protein, carbohydrates, lipid, total phenolic, flavonoid and tannin contents of alga were assayed. The FTIR analysis of extract revealed the presence of alcohols, phenols, ester and alkanes. The results indicated the presence of long chain unsaturated and saturated fatty acids with the dominance of palmitic acid (53.3%). **Conclusion:** The results indicated that *L. papillosa* contains different biologically active compounds and can act as a promising antioxidant, antimicrobial, anticancer agents and may represent an application in the therapeutic uses.

Key words: Algal extracts, pathogenic organisms, disc diffusion, tolerance ratio, 2,2-Diphenyl-1-picrylhydrazyl, HCT-116 colon cancer, fatty acids

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microorganisms elaborate different management to avoid the effect of antibiotics, leading to bacterial strains with multiple drug-resistant. So, there is a priority to discover and evolve cheaper and efficient natural antimicrobial agents with high potential, less toxicity, fewer side effects than antibiotics and good availability[1].

Marine macroalgae are large groups of organisms which have a broad range of biologically active metabolites. The extract of marine macroalgae showed antibacterial and antifungal activities[2,3]. The different types of biologically active compound in algae can be extracted by using different solvents with different polarity[4]. These bioactive metabolites have the ability to inhibit the growth of microbes through binding to their cell walls. The bioactive compounds which recorded biological activity were acrylic acid, fatty acids, phenolic compounds, steroids, terpenoids, ketones, phlorotannins and alkanes[5].

Reactive oxygen species (ROS) are known to be derived from normal metabolic activity in the human body or from external sources like smoking, air pollutants, radiation, ozone and industrial chemicals[6]. ROS are highly reactive, unstable and can damage cells by chain reactions, such as lipid peroxidation or formation of DNA adducts that could cause cancer. The ROS is the major cause of human cancer, cardiovascular disease aging processes and brain dysfunction and this can be reduced by increased consumption of antioxidants[7]. The utilization of antioxidants plays an important role against lipid peroxidation. Antioxidants are substances that inhibit the process of oxidation by scavenging the free radicals in body cells and may decrease mutations and thereby prevent cancer and heart disease[8]. So, the finding of antioxidants from natural origins has grown more and more. The macroalgae extracts and fractions have different types of antioxidants[9]. Phytochemical screening of algae extract showed antioxidant activity for alkaloids, flavonoids, phenols, tannins, phlorotannin, terpenoids, pigments, glycosides and steroids[10,11]. The algal polysaccharides play important roles as antioxidants and as inhibitors for oxidative damage in living organisms[12].

Cancer is a major disease today and attacks the modern society globally. The lifestyle, radiation and exposure to various carcinogenic/mutagenic agents lead to cancer and death in the world population. Researchers are looking for new treatment for cancer with low side-effects on the immune system[13,14]. The *in vitro* assay indicates that the extracts of marine macroalgae are the significant source of a noble anticancer agent[15]. Certain compounds isolated from algae show interesting therapeutic properties, including anticancer[16,17]. Bioactive molecules such as terpenoids and polysaccharides were detected in brown algae and are promising for anticancer medicine[18]. Unsaturated fatty acids were reported in marine algae to block the spread of breast cancer[19]. Red and brown algae produce halogenated hydrocarbons such as phenols, terpenes, fatty acids and sulfated polysaccharides which have antitumor activity[20]. Therefore, the present study may help to increase the knowledge about the anticancer property of algae.

This study aimed to evaluate the potential application of the red alga, *Laurencia papillosa* as antimicrobial, antioxidant and anticancer agents. The chemical constituent of alga was analyzed.

MATERIALS AND METHODS

Algal material: The red alga, *Laurencia papillosa*, was collected from the coastal area of the Red Sea of Jeddah, Saudi Arabia. The algal material was cleaned in running taps water and finally in distilled water. The samples allowed to dry in air. Air dried samples were ground and stored at room temperature.

Preparation of algal extract: Successive extractions were carried out by soaking the dried powder of *L. papillosa* in dichloromethane, dichloromethane: methanol (1:1,v/v), methanol (Merck) and distilled water within a conical flask and then kept on a shaker at 120 rpm for 3 days at room temperature (25°C). The mixture was filtered through Muslin cloth followed by Whatman No.1 filter paper. The obtained filtrates were taken to dryness under reduced pressure at 50°C. The crude extract was stored at -20°C for further studies.

Test organisms: The tested organisms were the fast growing and antibiotic resistant strains obtained from American Type Culture Collection (ATCC). The gram-positive bacteria (*Staphylococcus aureus* ATCC 33591, *Staphylococcus saprophyticus* ATCC 15305 and *Streptococcus agalactiae* group B), the gram-negative bacteria (*Escherichia coli* ATCC 8239, *Pseudomonas aeruginosa* ATCC 27853 and *Proteus mirabilis* ATCC 35659) and the fungal strains (*Candida albicans* ATCC CA 10231 and *Candida tropicalis* ATCC CT 2697) were used in the present study. All bacteria were cultivated and stored in nutrient agar and for the fungal strains that used either Sabouraud broth or agar at 4°C.

Antimicrobial assay: Antimicrobial activity was carried out *in vitro* using the agar well diffusion technique[21]. The cultures of bacteria were prepared in liquid nutrient broth media with bacterial density 1.5×10^8 CFU mL^{-1}. A sterile cotton swab was dipped in the bacterial culture and spread onto Muller Hinton agar plate surfaces. A sterile 0.5 cm cork borer was used to cut wells from the plate. The different algal crude extracts were dissolved in dimethylsulfoxide (DMSO) at concentrations of 100 mg mL^{-1} and then filtered through 0.22 µm Millipore filter (Millipore, Billerica, MA). Then 50 µL algal extract was introduced into each well and the plates were incubated at 37°C for 24 h for bacteria. Antibiotic chloramphenicol (l00 mg mL^{-1}) was used as positive control.

The above procedure is allowed for fungal assays, the Saboraud dextrose agar media were used. The fungal colonies were diluted in sterilized water to 0.5 MacFarland scale turbidity standard (10^7 spores mL^{-1} suspension). The antibiotic amphotericin at a concentration of 100 mg mL^{-1} was used as a standard. The plates were incubated at 30°C for 48 h. The diameter of the growth inhibition halos (mm) caused by the different algal extracts was measured. All tests were performed in triplicate and clear zones greater than 10 mm were considered as positive results[22].

Determination of MIC, MBC and tolerance ratio: The lowest level of antimicrobial agent that inhibits growth was defined as MIC[23]. MIC was determined by the broth dilution method. Serial dilutions of the algal extract were inoculated into broth media. The test organism was added to each dilution of algal extract. The growth of organism was checked after overnight incubation at 37°C.

The MBC is usually an extension of the MIC and demonstrates the lowest level of antimicrobial agent that kills 99.9% of the organism. The organisms are quantitatively subcultured from MIC tubes on the agar medium to indicate the minimum concentration where no viable organism was present in the culture[24].

The degree of tolerance was calculated from MBC/MIC as explained by Sader *et al.*[25]. When the ratio of MBC/MIC is $\leq$2.0, the extract is considered bacteriostatic and otherwise bactericidal. If the ratio is $\geq$16.0 the extract is considered ineffective.

Analysis of antioxidant activity "DPPH radical-scavenging activity": The algal extract was assayed for DPPH radical scavenging activity as described by Ratty *et al.*[26]. In a flat bottom 96 well-microplate, a total test volume of 200 µL was used. In each well, 20 µL of different concentrations (0-500 µg mL^{-1} final concentration) of the tested samples were mixed with 180 µL of 0.1 mM DPPH/absolute ethanol and incubated for 30 min at 37°C. Triplicate wells were prepared for each concentration and the average was calculated. Then a photometric determination of absorbance at 520 nm was performed by microplate ELISA reader. A serial dilution of ascorbic acid in concentrations ranging from 0-50 µg mL^{-1} in distilled water was used to obtain a standard calibration curve. Every sample was treated in triplicate (n = 3). The DPPH radical scavenging activity was calculated using the following Eq:

$$\text{Scavenging effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of the control (DPPH solution without sample), A_{sample} is the absorbance of the test sample (DPPH solution plus test sample). The value IC_{50} which is the concentration of substrate that causes 50% loss of the DPPH activity was calculated.

Anticancer assay: The MTT cell viability assay [3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] was used to test the activity of algal extract against HCT-116 colon cancer. MTT assay is based on the ability of dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form an insoluble dark blue formazan crystal which is impermeable to cell membranes, resulting in its accumulation within healthy cells. The level of soluble formazan dark blue color is directly proportional to the number of viable cells. The reduction of MTT was estimated by measuring the absorbance at 570 nm[27]. The HCT-116 cells in serum-free media were plated in a flat bottom 96-well microplate (0.5×10^5 cells/well) and treated with 20 µL of different concentrations of algal extract in a humidified 5% CO_2 atmosphere at 37° C for 48 h. The media were removed after incubation and 40 µL MTT solution (5 mg mL^{-1} MTT in 0.9% NaCl)/well were added and the plate was shacked and incubated for 4 h at room temperature. The absorbance was measured by using microplate ELISA reader at 570 nm. The average of triplicate repeats was calculated for each concentration. Data were expressed as the percentage of relative viability:

$$\text{Relative viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Then, the value IC_{50} was calculated from the equation of the dose-response curve.

Phytochemical analysis

Estimation of chlorophyll and carotenoids: The pigments were extracted and measured according to Mackinney[28]. For extraction of chlorophylls a and carotenoids, water-free methanol was added to dry algae. The tube was incubated in water bath at 55°C for 15 min and then centrifuged at 6000 rpm for 10 min. The supernatant was separated and the content of pigments was measured against blank of free methanol at the absorbance 452 nm (A_{452}), 650 nm (A_{650}) and 665 nm (A_{665}). The results obtained for quantification of pigments were an average of three determinations. The concentrations of chlorophyll a and carotenoids (μg mL^{-1}) were obtained by using the following equations and the results were estimated as mg g^{-1} of dry weight:

$$\text{Chlorophyll a} = 10.3\ A_{665}\text{-}0.918\ A_{650}$$

$$\text{Carotenoids} = 4.2\ A_{452}\text{-}0.0246\ \text{Chlorophyll a}$$

Estimation of phycobiliprotein: Phycobiliprotein was extracted according to Bennett and Bogorad[29]. A known weight of algae was extracted by repeated freezing and thawing using 0.05 M phosphate buffer (0.03 g monosodium phosphate and 0.08 g disodium phosphate, pH 7.0). The absorbance of the supernatant was read at 562 nm (A_{562}), 615 nm (A_{615}) and 652 nm (A_{652}) and phycobilins were estimated. The phycobilins pigments were calculated using the following formula and expressed as mg g^{-1}:

$$\text{Phycocyanin (PC)} = \frac{[(A_{615})\text{-}(0.474 \times A_{652})] \times \text{Volume of extract}}{(5.34 \times \text{Weight of sample})}$$

$$\text{Allophycocyanin (APC)} = \frac{[(A_{652})\text{-}(0.208 \times A_{615})] \times \text{Volume of extract}}{(5.09 \times \text{Weight of sample})}$$

$$\text{Phycoerythrin (PE)} = \frac{[(A_{562})\text{-}(2.41 \times \text{PC})\text{-}(0.849 \times \text{APC})] \times \text{Volume of extract}}{(9.62 \times \text{Weight of sample})}$$

Estimation of total soluble protein: After removal of pigment, the cells were extracted with 1 N NaOH in a boiling water bath for 2 h as described by Payne and Stewart[30]. A sample of 0.1 mL extract was mixed with 3 mL solution C and left for 10 min and then 0.25 mL of solution D was added. The mixture was left to stand at room temperature for 30 min and the absorbance was measured at 750 nm. Reagent A: 20 g sodium carbonate and 0.5 g sodium tartrate were dissolved in little warm water, then 100 mL of 1 N NaOH was added and finally the solution was completed up to 1000 mL with distilled water. Reagent B: About 1 g copper sulfate ($CuSO_4.5H_2O$) was dissolved in 1 L of water (freshly prepared). Reagent C: 9 volumes of reagent A and 1 volume of reagent B. Reagent D: consists of one volume of Folin-Ciocalteu's reagent diluted with two volumes of water.

Estimation of total soluble carbohydrates: The phenol-sulphuric acid method was used to determine the total carbohydrates[31]. One mililiter of 5% phenol was added to 0.5 mL sample (1N NaOH extract) in a culture tube and then 5 mL concentrated sulphuric acid (95.5%) was added directly to the surface of culture tube. The sample was allowed to stand at room temperature for 30 min. The absorption was measured against blank at 490 nm. The carbohydrate concentration of the unknown solution was estimated after preparation of a calibration curve using glucose as a standard.

Lipid extraction: The dry weight of the sample was homogenized and lipid was extracted with a mixture of chloroform: methanol (1:2, v/v), filtered and reextracted with chloroform[32]. The chloroform layer was evaporated to dryness, weighed and the percent total lipids was calculated.

Estimation of total phenolic: The total phenolic content of alga was determined according to Singleton *et al.*[33]. About 0.5 mL of 1 mg mL^{-1} algal extract was mixed with 2.5 mL Folin-Ciocalteu's reagent (10%) dissolved in water followed by 2.5 mL $NaHCO_3$ (7.5%). The sample was incubated at 45°C for 45 min. The absorbance of the formed blue color was measured at 765 nm. Gallic acid was used for calibration curve (concentration range: 0-12 g mL^{-1}). The total phenolic was calculated from the calibration line and was expressed in terms of gallic acid equivalent (mg gallic acid/g extract).

Determination of flavonoids: The content of flavonoids was estimated in alga as described by Quettier-Deleu *et al.*[34]. The sample contained 1 mL of methanol extract (1 mg mL^{-1}) and 1 mL of 2% $AlCl_3$ solution dissolved in methanol were incubated for an hour at room temperature. The absorbance was measured at 415 nm. The mean value of triplicate for the sample was obtained. The standard solution of rutin was used to obtain the calibration curve. The concentration of flavonoids (mg mL^{-1}) was obtained on the calibration line. The flavonoids content was expressed as mg of RU g^{-1} of extract.

Estimation of tannin: The total content of tannin was measured according to Julkunen-Tiitto[35]. The algal extract (50 μL) was mixed with 1.5 mL vanillin (40% prepared with

methanol) and then 750 µL HCl was added. The mixture vigorously shacked and left to stand in darkness for 20 min at room temperature. The absorbance was measured at 500 nm. The standard curve was obtained by using catechin. The samples were prepared in triplicate and the tannin content was expressed as mg g^{-1}.

FT-IR spectroscopic analysis: The frequency of different components in the algal extract was analyzed by using JASCO, FT-IR 6100, made in Japan.

Estimation of fatty acid by GC/MS: Gas chromatograph system (HP 6890) with mass selective detector (HP 5973) was used for estimation of fatty acids in the algal extract. Fatty acid was represented as percentage dry weight.

Statistical analysis: All data were expressed as mean values±SD. The mean values were analyzed by one-way ANOVA. Significant differences between the means of parameters were determined ($p<0.05$).

RESULTS AND DISCUSSION

Antimicrobial activity: The antibacterial and antifungal activities of different extracts (dichloromethane, 1:1 dichloromethane:methanol, methanol and water) of *L. papillosa* were tested against various microorganism (Table 1). Among these extracts, 1:1 dichloromethane: methanol extract showed the maximum activity against the experimental bacteria and fungi with inhibition zones in the range of 11-15 mm, followed by water extract (11-14 mm) and finally the dichloromethane extract (9-14 mm). The methanol extract of *L. papillosa* showed the lowest antimicrobial activity with inhibition zones ranged between 8 and 11 mm. The dichloromethane:methanol (1:1) extract showed the highly significant activity against *P. aeruginosa, S. aureus* and *C. tropicalis* with inhibition zones ranged between 14 and 15 mm. The water extract of *L. papillosa* recorded highly significant effect against *P. aeruginosa* (14 mm), *C. tropicalis* (14 mm) and *E. coli* (13 mm). However, the greatest inhibition zones with dichloromethane extract was obtained against *P. aeruginosa* (14 mm), *C. tropicalis* (14 mm) and *S. aureus* (13 mm). It can be noticed the different algal extracts showed highly significant effect against *P. aeruginosa* and *C. tropicalis*. Cortes *et al*.[36] found that the dichloromethane of the red alga, *Ceramium rubrum* presented 100% bacterial inhibition on *Yersinia ruckeri* at approximately 500 µg mL^{-1}. Bansemir *et al*.[37] observed that the dichloromethane extract of *Laurencia chondrioides* (2 mg/disk) was active on *Pseudomonas anguilliseptica* with an inhibition of 15 mm but it was not active on *Yersinia ruckeri*. However, the dichloromethane extract (2 mg/disk) obtained from *Gracilaria cornea* was active on *Y. ruckeri* with inhibitions of 9.0 mm. Rodrigues *et al*.[38] showed that the dichloromethane extract of the red alga, *Sphaerococcus coronopifolius* induced inhibition of *S. aureus* and *C. albicans* growth. Kim *et al*.[39] tested methanol extracts of marine macroalgae against pathogenic bacteria and fungi and recorded the antimicrobial activity of the crude extracts and fractions against *E. coli*, *P. aeruginosa*, *Salmonella enteritidis*, *B. subtilis*, *S. aureus*, *Vibrio cholera*, *Klebsiella* sp. and *Aspergillus niger*. The difference may due to the efficiency of the extraction methods to recover the active metabolites, solvents used and susceptibility of strains[4].

MIC, MBC and tolerance ratio: The results of MIC and MBC of the different extracts of *L. papillosa* against the tested strains are shown in Table 2. The MIC values of tested algal extracts were ranged between 0.2-1.0 mg mL^{-1} against bacteria and

Table 1: Antimicrobial activity of different extracts of the red alga, *Laurencia papillosa* (100 mg mL^{-1})

Organisms	Zone of inhibition (mm) D	1: 1 D: M	M	W	Positive control
Gram (+)					
Staphylococcus aureus	13±0.83	14±0.56	11±0.26	12±0.47	16±0.15
Staphylococcus saparlyticus	12±0.69	12±0.74	11±0.51	11±0.38	19±0.34
Staphylococcus agaloctiae	9±0.26	11±0.48	8±0.64	12±0.64	18±0.65
Gram (-)					
Escherichia coli	11±0.57	13±0.62	11±0.42	13±0.78	20±0.63
Psevdomenas aeruginosa	14±0.49	15±0.87	11±0.55	14±0.46	21±0.43
Proteus mirabilis	12±0.65	13±0.39	11±0.32	11±0.62	19±0.28
Fungi					
Candida albicans	11±0.72	12±0.26	9±0.17	12±0.77	17±0.21
Candida tropicalis	14±0.66	14±0.84	11±0.21	14±0.59	19±0.36

D: Dichloromethane, D:M: Dichloromethane: methanol, M: Methanol, W: Water extract. Chloramphenicol and amphotericin were taken as positive control for antibacterial and antifungal activity, respectively at a concentration of 100 mg mL^{-1}, Data are the mean of three replicates±SD

Table 2: MIC and MBC (mg mL^{-1}) of different extracts of the red alga, *Laurencia papillosa* against pathogenic bacteria and fungi

	MIC (mg mL^{-1})				MBC (mg mL^{-1})				MBC/MIC			
Organisms	D	1:1 D:M	M	W	D	1:1 D:M	M	W	D	1:1 D:M	M	W
Staphylococcus aureus	0.4	0.4	0.8	0.4	0.6	0.4	1.6	0.8	1.5	1.0	2.0	2.0
Staphylococcus saparlyticus	0.6	0.4	0.8	0.6	0.8	0.6	1.6	0.8	1.3	1.5	2.0	1.3
Staphylococcus agaloctiae	1.0	0.6	1.0	0.6	1.8	0.8	2.0	0.8	1.8	1.3	2.0	1.3
Escherichia coli	0.8	0.2	0.8	0.4	1.4	0.2	1.6	0.6	1.7	1.0	2.0	1.5
Psevdomenas aeruginosa	0.4	0.2	0.8	0.4	0.6	0.2	1.6	0.6	1.5	1.0	2.0	1.5
Proteus vulgaris	0.8	0.4	1.0	0.8	1.6	0.6	1.8	1.4	2.0	1.5	1.8	1.7
Candida albicans	1.2	1.0	1.4	1.2	2.8	2.4	3.6	2.6	2.3	2.4	2.6	2.1
Candida tropicalis	0.8	1.0	1.2	1.0	2.0	2.2	3.0	2.2	2.5	2.2	2.5	2.2

D: Dichloromethane, D:M: Dichloromethane:methanol, M: Methanol, W: Water extract, MIC: Minimum inhibitery concentration, MBC: Minimum bactericidal concentration

Table 3: Radical scavenging activity and cytotoxic effect against HCT-116 cells of different extracts of the red alga, *Laurencia papillosa*

Solvent	Antioxidant activity IC_{50} (μg mL^{-1})	Anticancer activity IC_{50} (μg mL^{-1})
D	310.2±0.86	215.2±11.45
1:1 D:M	110.8±0.15	196.0±8.84
W	225.7±0.42	644.5±18.56

D: Dichloromethane, D:M: Dichloromethane: methanol, W: Water extract, Data are the mean of three replicates±SD

0.8-1.4 mg mL^{-1} against fungi. A minimum value of MIC as 0.2 mg mL^{-1} was observed for *E. coli* and *P. aeruginosa* with1:1 D:M extract of *L. papillosa*. The MBC values of algal extracts were ranged between 0.2-2.0 mg mL^{-1} against bacteria and 2.0-3.6 mg mL^{-1} against fungi. Salem *et al.*[40] found that MICs of macroalgae extracts were ranged from 5-50 mg mL^{-1}. Meanwhile, Nor Afifah *et al.*[41] showed that the hexane extract of *Halimeda discoidea* was active against eight bacteria with MIC values ranged between 0.25-1.00 μg mL^{-1}.

As for determination of antibiosis, the different extracts of *L. papillosa* exhibited bactericidal effect against the present strains at the ratio of MBC/MIC ≥2.0 (Table 2). However, the algal extracts can be considered as fungi statics agents against the tested fungal strains where the MBC/MIC ratios were more than 2 as regarded by Sader *et al.*[25].

Antioxidant activity: The algal extracts with the greatest antimicrobial activity were chosen to study the antioxidant and anticancer activity. The radical scavenging activity of dichloromethane, dichloromethane:methanol (1:1 v/v) and water extracts were summarized in Table 3. The results showed a significant difference between the means of antioxidant activity of different algal extracts. Among the three algal extract, dichloromethane:methanol (1:1 v/v) extract exhibited the greatest antioxidant activity with a very low IC_{50} (110.8 μg mL^{-1}) followed by water extract (225.7 μg mL^{-1}) and finally dichloromethane extract (310.2 μg mL^{-1}). In accordance with these results, Kokabi *et al.*[42] clarified that the different solvent extracts of seaweeds exhibited different antioxidant activities. However, Jassbi *et al.*[43] found that the most antioxidant activity was obtained with the water extract of red and brown algae. The extracts of red algae species were found to have antimicrobial and antioxidant activities as obtained by El-Din and El-Ahwany[44]. The pigments, vitamins and phenolic compounds in marine algae play important roles as antioxidant agents[45]. Vijayabaskar *et al.*[46] reported that macroalgae antioxidative molecules were polysaccharides, protein, organic acid etc. Different solvents have been used in screening algae for antimicrobial and antioxidant activity but the most effective solvent is unclear[47].

Anticancer activity: The effect of dichloromethane, dichloromethane:methanol (1:1 v/v) and water extracts of *L. papillosa* on the proliferation of HCT-116 cells were studied after 48 h of incubation (Table 3). The dichloromethane:methanol (1:1) extract showed the highest anticancer activity, with an IC_{50} value of 196.0±8.84 μg mL^{-1} followed by the dichloromethane extract, with IC_{50} value of 215.2±11.45 μg mL^{-1}. While the water extract had low activity against HCT-116 cells, with IC_{50} value of 644.5±18.56 μg mL^{-1}. According to Hyun *et al.*[48], marine algal extract at a concentration of 100 μg mL^{-1} was able to increase apoptosis in HCT-15 colon cancer cells. Whereas, Kim *et al.*[49] showed that 5-20 μg mL^{-1} algal extract stimulate apoptosis of HCT-116 cells. In addition, they reported that algal fucoidan activated caspases, resulting in the induction of apoptosis through both deaths receptor-mediated and mitochondria-mediated apoptotic pathways. The marine algae contain an abundance of biologically active substances with novel chemical structures and can successfully treat the colorectal cancer[50]. Farooqi *et al.*[51] suggested that the algal bioactive compounds induced the apoptosis in cancer cells through inhibition of cancer cell growth, invasion and metastasis.

Table 4: Phytochemical compositions of the red alga, *Laurencia papillosa*

Chl. a (mg g^{-1})	Carot. (mg g^{-1})	Phycoc. (mg g^{-1})	Phycoe. (mg g^{-1})	Prot. (%)	Carb. (%)	Lipid (%)	Phen. (mg g^{-1})	Flav. (mg g^{-1})	Tan. (mg g^{-1})
0.162±0.038	0.056±0.032	0.267±0.020	0.527±0.021	9.74±0.55	26.91±0.89	4.67±0.65	3.14±0.71	2.67±0.31	1.94±0.22

Chl. a: Chlorophyll a, Carot.: Carotenoid, Phycoc.: Phycocyanine, Phycoe.: Phycoerythrine, Prot.: Proteins, Carb.: Carbohydrate, Phen.: Phenolics, Flav.: Flavonoids and Tan.: Tannin. Data are the mean of three replicates±SD

Phytochemical analysis: The phytochemical composition of *L. papillosa* is shown in Table 4. The photosynthetic pigments, chlorophyll a, carotenoid, phycocyanine and phycoerythrine were estimated and represented by 0.162±0.038, 0.056±0.032, 0.267±0.020 and 0.527±0.021 mg g^{-1}, respectively in *L. papillosa*. Torres *et al.*[52] found that the red alga, *Gracilaria* sp. contained 665.22 µg g^{-1} chlorophyll a and 162.93 µg g^{-1} carotenoids. However, Arunkumar *et al.*[53] showed that phycocyanin and phycoerythrin in the red alga, *Gracilaria corticata* were represented by 0.09 and 0.48 mg g^{-1}, respectively. Pigments such as chlorophyll and carotenoids play important roles as antioxidant agents[54].

The red alga *L. papillosa* was found to contain high carbohydrate content (26.91%), protein (9.74%) and low lipid composition (4.67%) (Table 4). A similar result of carbohydrate content was previously obtained by Matanjun *et al.*[55] for various marine macroalgae. Carbohydrates in algae are stored in different forms like starch in Floridian, starch in Rhodophyta as food reserves and energy. Concerning marine algae, Dawczynski *et al.*[56] showed that the protein content was typically 10-30% dry weight. In agreement with the present result, Rameshkumar *et al.*[57] found that the lipid content of macroalgae accounts for 1-6% dry weight. Lower levels of lipid content (1.7-2.9% dry weight) were reported in the red algae by McDermid and Stuercke[58] and Hong and Hien[59]. Colombo *et al.*[60] reported that the total lipid in seaweeds was ranged from 2.8-33.0 mg g^{-1} dry weight. Macroalgae are known to contain protein, carbohydrate, elements, dietary fibers, vitamins, essential amino acids and essential fatty acids[61]. The phytochemical contents of macroalgae vary between species, habitats, levels of maturity and seasons[62].

In this study, *L. papillosa* was found to contain phenolic, flavonoids and tannin compounds of 3.14±0.71, 2.67±0.31 and 1.94±0.22 mg g^{-1}, respectively (Table 4). The phenolic compounds such as phenol, tannin and flavonoids have been found to be present in the maximum amount in red and brown seaweeds[63]. The total phenol, flavonoid and tannin content were 0.37, 0.12 and 13.33 mg g^{-1}, respectively, as recorded in the red alga, *Corallina mediterranea*[44]. It has been reported that phenolic compounds have a wide range of chemical and biological activities, including free radical scavenging properties and used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents[64].

Previous studies showed that variations in the phytochemical contents of algae depend on season and geography[62], nutrient content of the environment[65], light density and quality, photoperiod and temperature[66].

FT-IR spectrum of algal extract: The FTIR analysis of dichloromethane extract of *L. papillosa* revealed the presence of different functional groups (Fig. 1a). The analysis gives a medium peak at 3445 cm^{-1}, which indicated the presence of OH stretching in algal extract depicting the presence of alcohols and phenols. Sharp peak also obtained in the extract for alkanes, C-H stretching at 2930 cm^{-1}. The absorption peak at 1710 cm^{-1} is assigned to C=O stretching vibration in carbonyl compounds. Moreover, the peak generated at 1480 cm^{-1} represents CH_2 bending. The peak for the C-O stretch at 1250 cm^{-1} revealed the presence of phenol or ester, C-O stretching at 1050 cm^{-1} attributed to alcohol or ether.

The result of FTIR analysis of 1:1 dichloromethane: methanol extract gives sharp peak at 3445 cm^{-1}, which indicated the presence of OH stretching in depicting the presence of alcohols and phenols (Fig. 1b). Also, the extract showed the presence of medium band for alkanes: C-H stretching at 2925 cm^{-1}, medium C-H bending for alkanes at peak 1450 cm^{-1}. While peaks obtained at 1630 cm^{-1} showed C=O stretching for carboxylic acids. Another peak at 1255 cm^{-1} C-O stretching indicates the presence of phenol or ester, whereas, C-O stretch at 1083 cm^{-1} in extracts indicated alcohol or ether.

The IR spectrum of water extract of *L. papillosa* revealed the presence of a sharp peak at 3445 cm^{-1}, which indicated the presence of OH stretching in the algal extract suggests the presence of alcohols and phenols (Fig. 1c). The extract also showed the presence of C-H bending for alkanes at peaks 2920 cm^{-1}. The band for -C=C- stretch at 1654 cm^{-1} in algal extract revealed the presence of terpenes. The peaks at 1375 cm^{-1} indicate the presence of sulfate groups and at 1255cm^{-1} equivalent to S=O stretch. The IR spectra identify a basic polysaccharide backbone with an intense peak centering 1035 cm^{-1} representing the stretching vibrations of the glycoside bridge (C–O–C). The band observed at 770 cm^{-1} shows the skeletal bending of galactose rings.

The study of Dhinakaran *et al.*[67] indicated the presence of bioactive compounds by using FTIR in the red alga, *Hypnea*

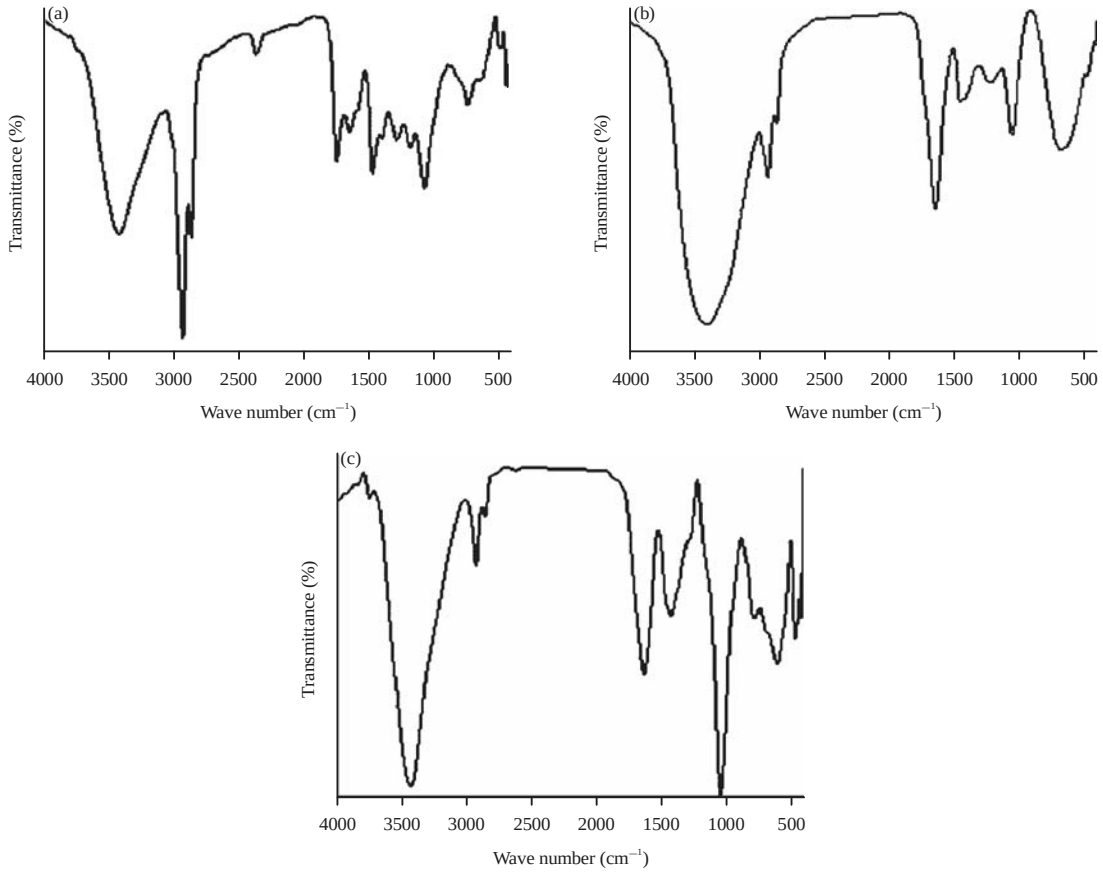

Fig. 1(a-c): FTIR spectrum of different extracts of the red alg, *L. papillosa*, (a) Dichloromethane, (b) 1:1 dichloromethane:methanol and (c) Water extract

musciformis. It includes polysaccharides, terpenes, alkenes, sterols etc. The major polysaccharides in red algae are galactans that include agar, carrageenan, floridean starch and xylan[68]. Galactose is the major monosaccharide found in red algae that build up galactans (agar and carrageenan).

The results based on spectral data of FTIR of the red alga, *Kappaphycus alvarezii* revealed the presence of aliphatic constituents containing carbon, ketones, alkyl halides and hydroxyl groups[69]. The spectrum study of the methanolic extract of *Acanthophora specifera* confirmed the presence of phenol, alkane, alkene, carboxylic acid, aromatic compound, nitro compound, alcohol, benzene and bromo alkane compounds[70].

Pereira *et al.*[71] showed that *Laurencia obtusa* presents a complex agar like sulfated galactan. These polysaccharides belong to the agar group, being agarose derivatives with a rather high content sulfate groups and with a reduced amount of 3,6-Anhydro-L-galactose residues (700-950 cm^{-1})[72].

GC-mass analysis of extract: The GC-MS analysis of the most biologically active extract in *L. papillosa* (1:1 D: M) was recorded in Table 5. The results indicated the presence of long chain unsaturated and saturated fatty acids. The most abundance chemical constituent of the extract was palmitic acid (53.3%) followed by isostearic acid (10.24%), isomyristic acid (6.57%), linoleic acid (6.32%), oleic acid (5.84%), pentadecanoic acid (3.76%), linoelaidic acid (3.30%),14-Methyl palmitic acid (3.00%), lignoceric acid (1.5%), behenic acid (1.09%) and finally paullinic acid (0.68%). Similar results were obtained by Cortes *et al.*[36], who showed that palmitic acid (25.8%) was the major components in the extract of the red alga, *Ceramium rubrum.* The biological activities of the algal extracts were reported due to the presence of palmitic, lauric, linoleic, linolenic, stearic, myristic, oleic and acids[73]. It was found that lipids obstruct microbes by distracting the cellular membrane[74] of fungi, yeasts and bacteria. It has been found that fatty acids with a chain length greater than 10 carbon atoms enhance the degeneration of bacterial protoplasts[75]. These fatty acids may further distress the expression of bacterial virulence, which is significant for establishing infection. Palmitic acid was identified as a selective cytotoxic substance from the marine red algal extract and induced

Table 5: Composition of 1:1 dichloromethane: methanol extract of the red alg, *Laurencia papillosa* as investigated by GC-MS

Peak	Systematic name	Common name	Structural formula	Retention time (min)	Molecular weight (g mol^{-1})	Peak area (%)
1	Methyl 12-Methyl-tridecanoate	Isomyristic acid methyl ester	$C_{15}H_{30}O_2$	18.30	242.40	6.57
2	Methyl penta-decanoate	Pentadecanoic acid methyl ester	$C_{16}H_{32}O_2$	20.30	256.24	3.76
3	Methyl hexa-decanoate	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	22.36	270.46	53.30
4	Methyl 14-Methyl hexadecanoate	14-Methyl palmitic acid methyl ester	$C_{18}H_{36}O_2$	24.03	284.48	3.00
5	Methyl 16-Methyl heptadecanoate	Isostearic acid methyl ester	$C_{19}H_{38}O_2$	25.79	298.51	10.24
6	Methyl 9-Octa-decenoate	Oleic acid methyl ester	$C_{19}H_{36}O_2$	26.23	296.50	5.84
7	(E, E)- Methyl 9, 11-Octa-decadienoate	Linoelaidic acid methyl ester	$C_{19}H_{34}O_2$	29.03	294.48	3.30
8	Methyl 9-cis, 11-trans octadecadienoate	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	29.66	294.48	6.32
9	Methyl docosanoate	Behenic acid methyl ester	$C_{23}H_{46}O_2$	32.05	354.62	1.09
10	Cis-13-Eicosenoic acid	Paullinic acid	$C_{20}H_{38}O_2$	33.47	310.52	0.68
11	Methyl tetracos-anoate	Lignoceric acid methyl ester	$C_{25}H_{50}O_2$	35.17	382.67	1.50

apoptosis in the human leukemic cell line MOLT-4[76]. They also reported that one molecular target of palmitic acid in tumor cells is DNA topoisomerase I, suggesting that palmitic acid may be a lead compound of anticancer drugs.

CONCLUSION

These results indicated that the different biological activity of the red alga, *L. papillosa* extract may be returned to the presence of various phytochemical contents such as phycobiliproteins, carotenoids, phenol, flavonoids, tannin, alkaloids and polyunsaturated fatty acids. Among the *L. papillosa* crude extracts, 1:1 dichloromethane: methanol exhibited the highest antimicrobial, antioxidant and anticancer potential. The red alga, *L. papillosa* can act as a promising therapeutic antioxidant, antimicrobial and anticancer agents. The potent biological activities of marine macroalgae may represent an interesting advance in the search for novel functional applications in the pharmaceuticals uses.

SIGNIFICANCE STATEMENT

This study discover the possible application of the red alga, *Laurencia papillosa* extract as a biologically active compound that may be applied for treatment of pathogenic organisms and cancer. The study help the researchers for future drug discovery from marine algae.

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