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Evaluation of antibacterial activity of the brown Seaweed *Turbinaria ornata* (Turner) J. Agardh from Egypt

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ABSTRACT

Objective: To investigate the potential antibacterial activities of ethanol extracts of *Turbinaria ornata* (*T. ornata*), Oleic acid (OA) and palmitic acid (PA) extracted from *T. ornata* as well as mixtures of OA and PA (1:1) against some bacterial species.

Methods: Brown seaweed *T. ornata* was collected from Hurghada shores, Red Sea coast of Egypt. OA and PA were extracted from *T. ornata*. Ethanol extracts of *T. ornata*, OA, PA and mixtures of these two fatty acids (1:1) were tested for their antibacterial activities against *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Staphylococcus aureus* by the disc diffusion method.

Results: Ethanol extracts of *T. ornata*, OA, PA and mixtures of OA and PA (1:1) showed antibacterial activities that increased significantly (least significant difference at 0.05 level) with increasing concentrations against all tested bacteria. Different concentrations of ethanol extracts of *T. ornata* and extracted OA showed its highest activity against *Bacillus subtilis*, while PA and mixtures of PA and OA (1:1) showed its highest activity against *Bacillus cereus*. The maximum inhibition activities were shown for mixtures of OA and PA (1:1). Scanning electron microscope showed that mixtures of OA and PA (1:1) caused plasmolysis and reduction in cell size of *Escherichia coli*.

Conclusions: Different concentrations of *T. ornata* and its fatty acids showed activities against all tested bacteria. Therefore, it is a potential source of natural antimicrobial compounds.

1. Introduction

Selective utilization of marine algae as a potential source of pharmaceutical agents has been increasing in recent years. Many of the seaweeds possess bioactive components which inhibit the growth of some pathogenic bacteria and fungi. Bioactive brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, sulphated polysaccharides and peptides substances were isolated from marine algae. The sulphated polysaccharides presenting in some marine algae were highly effective in preventing plaque formation by interfering with glucan deposition[1]. More than 600 secondary metabolites werev isolated from nearly 3 600 seaweeds. Among these, the diterpenoids constitute the most abundant group with profound ecological and pharmacological significance[2].

The Red Sea is a rich and diverse ecosystem. The rich diversity is in part due to the 2 000 km of coral reef extending along its coastline. Over 500 species of seaweeds have been recorded in the Red Sea[3]. Macroalgae in the Red Sea are adapted to living in an environment with specific demands. The temperature is often above 35 ˚C and the specific nutrients available define their cellular compositions.

Some bacteria become resistant to available antibacterial agents which could be used to treat them previously, and so antibiotics have lost the ability to be effective against these bacteria. Intensive research efforts are needed for the isolation of new antibacterial compounds from nature. Seaweeds are under persistent threat of infections by bacteria in the marine environment, and in response, they have evolved complex organic compounds with antibacterial activities to protect themselves[4].

Seaweeds used in our study, *Turbinaria ornata* (*T. ornata*) is belonging to the family Sargassaceae that belongs to the Fucales, an order of marine brown algae[5]. The seaweed extracts of *Turbinaria* showed activities against positive and negative bacteria as compared with standard ampicillin. Hexane, chloroform, methanol,

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chloroform: methanol (2:1 v/v) and petroleum ether extracts of *Turbinaria conoides* from the coast of Mandapam showed *in vitro* antibacterial activities against *Escherichia coli* (*E. coli*), *Salmonella typhi, Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* and *Proteus mirabilis*[6]. *T. aornata* extract was active against Gram-positive and Gram-negative bacteria; *Aeromonas hydrophila*, *Enterococcus faecalis*, *E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *S. aureus*[7]. Among marine algae, brown algae have been reported to contain higher phlorotannin contents as marine phenolic compounds[8]. Chauhan and Kasture[9] showed that seaweeds provide a rich source of structurally diverse secondary metabolites such as terpenes, acetogenins, alkaloids, steroids, polysaccharides, fatty acids and polyphenolics with many of these compounds being halogenated and then having antimicrobial activities.

The present study was carried out to investigate the potential antibacterial activity of ethanol extracts of *T. ornata*, Oleic acid (OA) and palmitic acid (PA) extracted from *T. ornata* as well as mixtures of OA and PA (1:1) against some bacterial species.

2. Materials and methods

2.1. Harvest and drying of brown seaweed T. ornata

T. ornata was collected along the semi-exposed shores of Hurghada, Red Sea coast of Egypt (Figure 1). The latitude was 27° 13' N and the longitude was 33° 45' E. *T. ornata* was identified according to the method of Guiry and Guiry[10]. *T. ornata* was washed with tap water followed by distilled water. The wet seaweed was dried by spreading in the atmospheric air dried away from the sun and then kept in an oven at 60 ˚C for 4 h. Finally, it was ground and stored in polyethylene bags at room temperature for further assays.

Figure 1. Map showing sampling site (Hurghada, Egypt).

2.2. Preparation of ethanol extracts of T. ornata

One hundred grams of the air-dried *T. ornata* powder were extracted in a Soxhlet extractor for 8 h with 1 L of ethanol (95%). These extracts were then decolorized on activated charcoal and filtered. The filterate was taken to dryness under reduced pressure at 40 ˚C. Thick residues were obtained, parts of which were dissolved in ethanol to make concentrations of 100, 300, 500, 800 and 1 000 µg/mL from these residues and then screened for its antibacterial activities.

2.3. Extraction of OA and PA from T. ornata

First, lipids content in 200 g pre-dried powder of T. ornata was determined according to Van Wychen et al.[11]. The lipids were separated into major classes according to American Society for Microbiology[12] by passing through a column (0.8 × 3 cm) of silicic acid (100 mesh, Mallinckrodt Chemical Works, St. Louis, Mo. USA). The neutral lipids were eluted with 20 mL of chloroform, and the polar lipids were subsequently eluted with 20 mL of methanol. The polar lipids were chromatographed on a column (0.8 × 3.5 cm) of silica gel impregnated with $AgNO_3$. Elution was carried out with 15 mL portions of different concentrations of diethyl ether increased from 1% to 8% in hexane. Elution of the saturated fatty acids was carried out with 1% to 2% diethyl ether in hexane, whereas the unsaturated fatty acids are eluted with 4% to 8% diethyl ether in hexane. The efficiency of the chromatographic method was confirmed with the standard PA and OA. Nearly the entire PA was eluted from the column with 1% diethyl ether in hexane, whereas the OA was eluted with 4% to 6% diethyl ether in hexane.

2.4. Determination of concentrations and purity of OA and PA

Concentrations and purity of OA and PA were chromatographically determined by reversed-phase high performance liquid chromatography, (Agilent Technologies 1 200 series, Alexandria, Egypt) equipped with UV detector. The column was Eclipse XDB-C 18 (4.6 × 150 mm). An appropriate mobile phase was 100% methanol with a flow rate of 0.5 mL/min. The detection of OA and PA was at wave length of 254 nm.

2.5. Preparation of different concentrations of OA and PA and preparation of bacterial inoculum

OA and PA were dissolved and diluted in ethanol to make concentrations of 100, 300, 500, 800 and 1 000 µg/mL.

Test bacteria were obtained from Bacteriology Laboratory, Faculty of Science, Damietta University, Egypt, and comprised two genera (3 species) of Gram-positive bacteria [*Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*) and *S. aureus*] and two species of Gram-negative bacteria [*E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*)]. The 24–48-hour-old cultures were used in the determination of antibacterial activity of *T. ornata*. Portion of each bacterium to be tested was inoculated into 10 mL sterile water (saline solution) to prepare the inoculum.

2.6. Antibacterial assay

Antibacterial activities of different concentrations of ethanol extracts of *T. ornata*, OA and PA extracted from *T. ornata* as well as mixtures of OA and PA (1:1) were assayed using the paper disc diffusion method[13]. In paper disc diffusion method, 1 mL of the

bacterial suspension was transferred to Petri dishes with nutrient agar media which consisted of 5 g glucose, 5 g peptone, 5 g sodium chloride, 3 g beef extract, 15 g agar and 1 L distilled water. Bacterial suspension must be spread thoroughly on the media to obtain uniform inoculum.

The test extracts (different concentrations of algal extracts, OA and PA as well as mixtures of of OA and PA) were then dropped (50 µL/piece) onto sterile filter paper discs (5 mm in diameter) under aseptic conditions. As for the control, ethanol was used. These discs were then placed onto the surface of the solidified culture media containing the bacterial inoculum in Petri dishes (5 piece/dish). The discs were then incubated at 37 °C for 18 h. After the incubation period, the inhibition zones around the discs were measured in millimeters and the mean values were recorded. Finally, the inhibition zones around the discs in control dishes were subtracted and results were recorded. The antibacterial activities assays were replicated in triplicated and the mean values were recorded in tables.

E. coli affected by the mixture of 50 µg/mL OA and PA (1:1) were scanned using Jeol, JSM-5300 scanning electron microscope at 20 000× magnification. Also, control *E. coli* were scanned using the same device for comparison.

2.7. Statistical analysis

One-way ANOVA and SPSS 21.0 were used to test for differences between different concentrations of treatments. The least significant difference (LSD) at 0.05 was used.

3. Results

3.1. Antibacterial activities of T. ornata extracts

The average limit of five different concentrations of *T. ornata* ethanol extracts over the five tested bacteria was ranged between 0.5 mm against *E. coli* to 5.5 mm against *B. subtilis* mm (Figure 2). Antibacterial activities of ethanol extracts of *T. ornata* increased significantly (LSD at 0.05 level) with increasing concentrations against all tested bacteria.

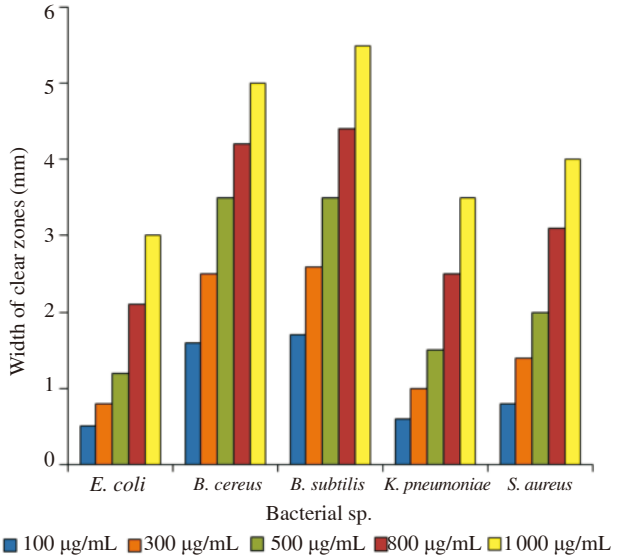

Figure 2. Variations in antibacterial activities of ethanol extracts of *T. ornata.*

3.2. Antibacterial activity of OA

The antibacterial activities of OA showed that the average limit of different concentrations of OA extracted from *T. ornata* over the five tested bacteria were ranged between 0.5 mm against *S. aureus* to 5 mm against *B. subtilis* (Figure 3). The antibacterial activity of OA extracted from *T. ornata* increased significantly (LSD at 0.05 level) with increasing concentrations against all tested bacteria.

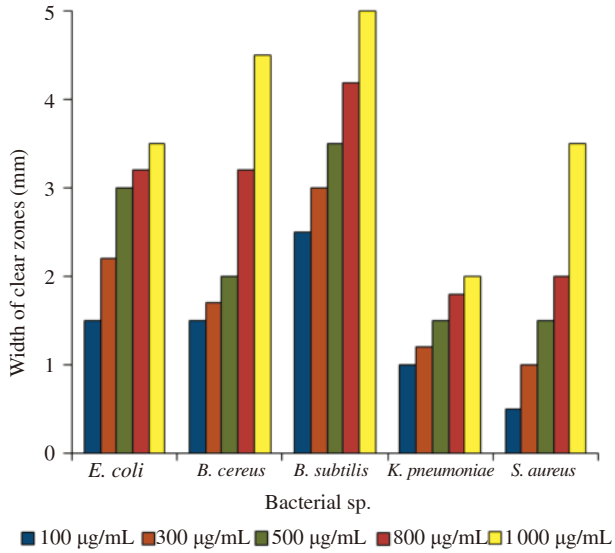

Figure 3. Variations in antibacterial activities of OA extracted from *T. ornata.*

3.3. Antibacterial activity of PA

The Antibacterial activities of PA showed that the average limit of different concentrations of PA extracted from *T. ornata* over the five tested bacteria were ranged between 0.5 mm against *E. coli* and *K. pneumoniae* to 5 mm against *B. cereus*. PA with the concentration of 100 µg/mL of had no activity against *S. aureus* (Figure 4). Antibacterial activities of PA extracted from *T. ornata* increased significantly (LSD at 0.05 level) with increasing concentrations against all tested bacteria.

3.4. Antibacterial activities of mixtures of OA and PA (1:1)

Antibacterial activities of mixtures of OA and PA (1:1) showed that the average limit of mixtures of both OA and PA (1:1) extracted from *T. ornata* over the five tested bacteria were ranged between 2 mm against *K. pneumoniae* and *S. aureus* to 9.5 mm against *B. cereus* (Figure 5). Antibacterial activities of mixtures of OA and PA (1:1) showed significant (LSD at 0.05 level) increase with increasing concentrations against all tested bacteria.

Scanning electron microscope showed that mixtures of OA and PA (1:1) caused plasmolysis and reduction in cell size of *E. coli* as shown by comparison between Figures 6 and 7.

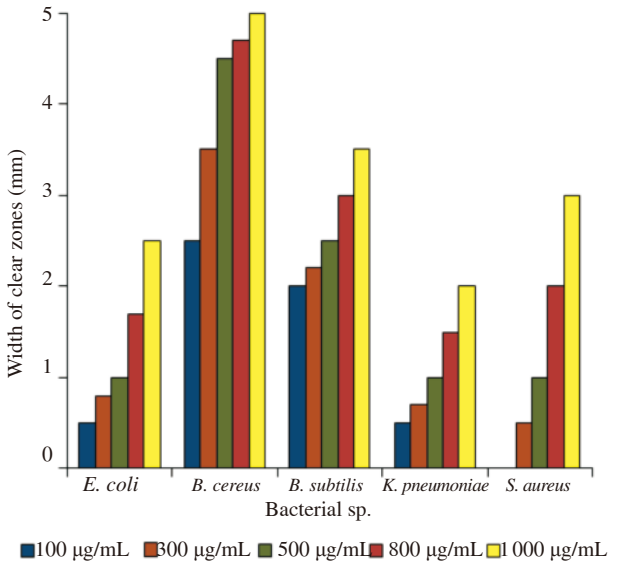

Figure 4. Variations in antibacterial activities of PA extracted from *T. ornata*.

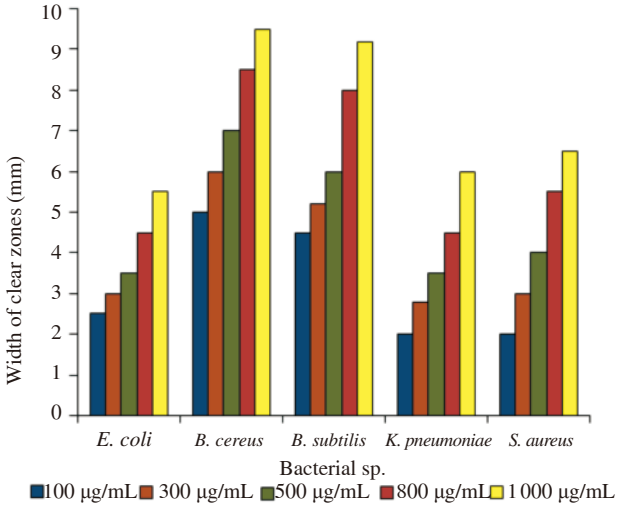

Figure 5. Variations in antibacterial activities of mixtures of OA and PA extracted from *T. ornata* (1:1).

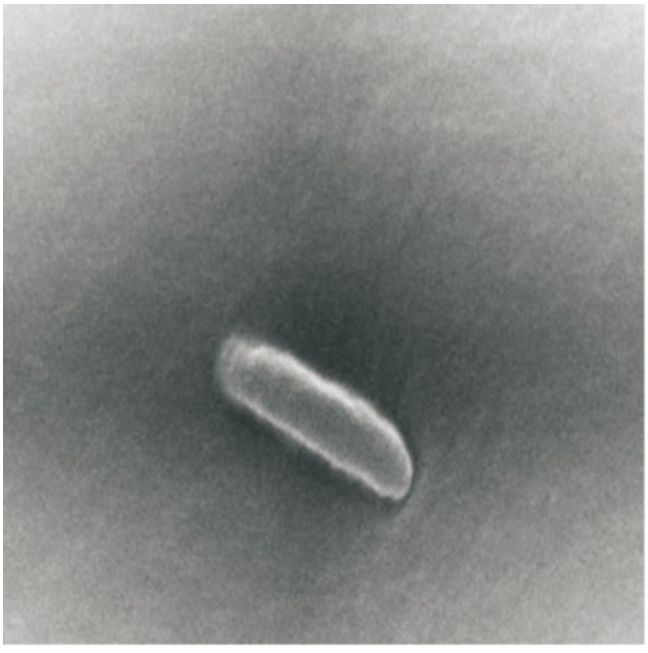

Figure 6. Control *E. coli* using electronic microscope.

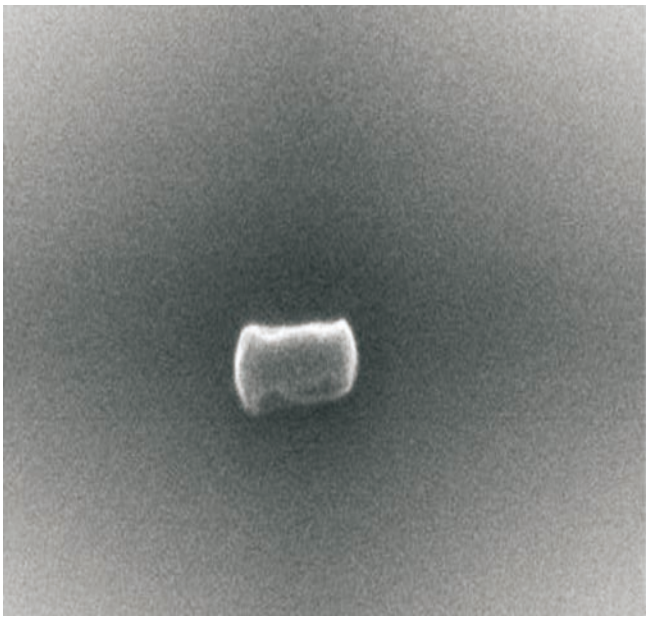

Figure 7. *E. coli* affected by mixture of OA and PA (1:1) using electronic microscope.

4. Discussion

Competition between marine organisms for nutrients and space leads to production of bioactive products. Seaweeds produce secondary metabolites to protect themselves against different ecological pressure and environmental stresses. Some of these secondary metabolites have antibacterial activities. Lipids of marine macroalgae possess antibacterial, antiviral, antitumor, anti-inflammatory, antiproliferative and antioxidant activities[14]. The present investigation revealed that ethanol extracts of *T. ornata*, OA and PA extracted from *T. ornata* and mixtures of these two fatty acids (1:1) were effective against the different tested bacteria. Different concentrations of ethanol extracts of *T. ornata* and its two fatty acids showed activities against Gram-positive bacteria (*B. cereus*, *B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *K. pneumonia*e). As mentioned by Heo *et al.*[15], the antimicrobial activity of ethanol extract of *T. ornata* might be due to its content of antimicrobial substances such as phenolic compounds and terpenes. The volatile mixture identified in methanol extract of *T. ornata* was comprised of hydrocarbons, acids, aldehydes, ketones, esters, ethers, alcohols, halogenated and aromatic compounds[16]. In *Chaetoceros* sp., polysaccharides and fatty acids are the major candidate molecules contributing to the antibacterial activity[17].

Different concentrations of ethanol extract of *T. ornata* and extracted OA showed its highest antibacterial activity against *B. subtilis*. The antibacterial effect of OA extracted from *T. ornata* against *B. subtilis* was explained by Strauch *et al.*[18] that unsaturated fatty acids inhibited one of the protein kinases KinA that affected the initiation of sporulation in *B. subtilis*. Moreover, Furusawa and Koyama[19]found that addition of unsaturated fatty acids such as OA caused the instantaneous depolarization of the membrane potential of the bacterium, *Bacillus* which appeared to result in the drastic decrease of viability.

Different concentrations of PA showed its highest antibacterial activity against *B. cereus*. This high activity was related to the ability

of PA to inhibit the spores of *B. cereus*[20]. The antibacterial activity of mixtures of OA and PA (1:1) extracted from *T. ornata* was higher than the activity of OA or PA alone. Its maximum antibacterial activity was against *B. cereus* (inhibition zone 9.5 mm).

The results of the present study revealed that *T. ornata* showed a higher activity against Gram-positive bacteria as compared to its activity against Gram-negative bacteria. Saeid and Rahil[21] also reported that Gram-negative bacteria were more resistant than Gram-positive bacteria to the algal extracts. Differences in the inhibitory effect of algal extracts between Gram-positive and Gram-negative bacteria may be due to the differences in their cell wall structures and compositions. The bioactive compounds derived from macroalgae may be of phenolic nature, which solubilized the lipopolysaccharide layer of the bacterial cell wall, leading to the entry of the inhibitory molecules[22]. In Gram-negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics. The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors[23]. In other studies, some algal extracts showed higher activities against Gram-negative bacteria than Gram-positive bacteria. This might be due to the production of bioactive compounds related to the seasons, habitat, organic solvents used for extraction of bioactive compounds and differences in assay methods. Scanning electron microscope showed that mixtures of OA and PA (1:1) caused plasmolysis and reduction in cell size of *E. coli*. It may be attributed to the osmotic stress on the cells or the failure of cell membrane regulation.

In the present study, different concentrations of *T. ornata* and its fatty acids affected the growth of different bacterial species. *T. ornata* showed a higher activity against Gram-positive bacteria as compared to its activity against Gram-negative bacteria. Further investigation is needed to isolate different antibacterial compounds from brown seaweeds.

Conflict of interest statement

We declare that we have no conflict of interest.

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