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Active ingredients fatty acids as antibacterial agent from the brown algae *Padina pavonica* and *Hormophysa triquetra*

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PEER REVIEW

ABSTRACT

Objective: To estimate the fatty acids content in the brown algae *Padina pavonica* (*P. pavonica*) and *Hormophysa triquetra* (*H. triquetra*) and evaluate their potential antimicrobial activity as bioactive compounds.
Methods: The fatty acid compositions of the examined species were analyzed using gas chromatography−mass spectrometry. The antimicrobial activity of crude and fatty acids was assessed using the agar plug technique.
Results: The fatty acids profile ranged from C8:0 to C20:4. Concentration of saturated fatty acids in *P. pavonica* was in the order palmitic>myristic>stearic whereas concentration of the unsaturated fatty acids was oleic acid>palmitoleic>9−cis−hexadecenoic>linoleic acid>α−linolenic>arachidonic> elaidic acid. *H. triquetra* contained high concentration of saturated fatty acids than those of *P. pavonica* which was in the order as follows: palmitic>margaric>myristic>nonadecyclic>stearic>caprylic>tridecylic>pentadecylic>lauric while the unsaturated fatty acids consisted of oleic>nonadecenoic>non adecadienoate>margaroleic. The crude and fatty acid extracts of *H. triquetra* and *P. pavonica* were biologically active on the tested pathogens. *H. triquetra* exhibited a larger inhibitory zone than *P. pavonica*.
Conclusions: The brown algae *P. pavonica* and *H. triquetra* have high efficient amount of fatty acids and showed strong antibacterial activity, especially *H. triquetra*.

1. Introduction

Seaweeds are the most accessible marine resource of the coastal zone that occupy potential important source of biochemical compound of secondary metabolites characterized by a broad spectrum of biological activities[1]. The functions of these secondary metabolites are formed defense mechanism against herbivores, fouling organisms and pathogens[2]. Compounds with cytostatic, antiviral, antihelmintic, antitussive, antihypertensive, antitumour, anti−diarrhea, antifungal and antibacterial activities have been detected in green, brown and red algae[3,4]. Selective utilization of marine algae as potential source of drug development in pharmaceutical industry has been increasing in recent years[5,6].

Although most of the antibiotics found in terrestrial sources were used as therapeutic agents to treat various diseases, the oceans have enormous biodiversity and

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potential to provide novel compounds with commercial value[7,8]. Marine algae have been generally considered as an inexhaustible source of novel antibiotics and agrochemicals[9,10]. There is a number of studies on antimicrobial activity of marine seaweeds against several pathogens[11–13]. Many of the seaweeds possess bioactive components which inhibit the growth of some of the Gram positive and Gram negative bacterial pathogens[14]. These bioactive components include terpenoid, phlorotannins, acryl acid, phenolic compounds, steroid, halogenated ketone and alkaline, cyclic polysulfides and fatty acid[15]. Of these components, fatty acids are a vital constituent of algae and nutritionally essential for most of the marine species, affecting their growth and condition[16–18].

Antimicrobial properties of fatty acids were reported as early as 1960[19]. In recent years, microbicidal properties of oleic acid from red algae were reported[20,21]. Johnston[22]mentioned that the fatty acids in seaweeds are of the same type as those found in higher plants. Of the saturated–acid series, palmitic (C16) was found in the highest concentration, followed by myristic (C14) and stearic (C18). Among the unsaturated acids, oleic (C18) represented the greatest component followed by palmitoleic (C16) and then arachidic acid (C20).

Polyunsaturated fatty acids (PUFA) are of the utmost importance for human metabolism. They are the major components of cell membrane phospholipids[23], and may also present in cellular storage oils[24]. In addition, PUFA are used in the biosynthesis of eicosanoids and hormone–like signaling molecules, which include thromboxanes, prostaglandins and leukotrienes[25]. Beside their fundamental role in metabolism, PUFA had beneficial properties as antibacterial[26], anti–inflammatory[27], antioxidant[28], prevention of cardiac diseases[29], and tumor progression inhibitor[30]. Such properties are indicative of the potential of PUFA for nutraceutical and pharmaceutical purposes.

Fatty acids can be released from lipids, typically by enzyme action, to become free fatty acids (FFAs). FFAs have the diverse and potent biological activities such as the ability to kill or inhibit the growth of bacteria[31]. The antibacterial properties of FFAs are used by many organisms such as seaweeds to defend against parasitic or pathogenic bacteria[31,32]. The antibacterial actions of FFAs are typically broad and of potencies comparable to natural antimicrobial peptides *in vitro*[33]. The antibacterial activity of each FFA is influenced by its structure and shape. This, in turn, is a function of the length of the carbon chain and the presence, number, position and orientation of double bonds[34,35].

The use of brown algae in medicine dates back over a thousand years in several ancient Chienese medical books including Compendium of Materia Medica in 1593 and Shennong′s Classic of Materia Medica in 1st century AD. Recently, brown algae can help in medical research and have economic value. *Padina pavonica (P. pavonica)*, a widespread alga in the world, has been known with its antibacterial activity[36]. *Hormophysa triquetra* (*H. triquetra*) was found in different regions such as Red Sea, Africa, South–West Asia, South–East Asia, Australia, New Zealand and Pacific Islands. Heiba *et al.* determined the fatty acid compositions in the brown alga *H. triquetra*[37]. The fatty

acids of latter species were not detected as antimicrobial agents in comparison with *P. pavonica*. The objective of this study was to estimate the fatty acids content in the two species of brown algae, *P. pavonica* and *H. triquetra* and evaluate their potential antimicrobial activity as bioactive compounds against five species of pathogenic bacteria and one species of actinobacteria.

2. Materials and methods

2.1. Algal sample collection

The selected brown macroalgae *P. pavonica* (Linnaeus) Thivy and *H. triquetra* (C. Agardh) Kützing were collected from Al Shoaiba, Red Sea, Saudi Arabia (20° 48–20° 51′ N, 39° 24′–39° 28′ E) from summer 2011 to spring 2012. These species were identified by method of Womersley and Papenfuss[38,39]. The samples were washed thoroughly with sea water and then distilled water to eliminate the epiphytes, coarse sand and other calcareous impurities. Samples were dried at room temperature. Crude dried seaweed materials were blended to obtain homogeneous samples, and pulverized before representative samples were taken for analysis.

2.2. Extraction of plant material

A definite quantity (150 g) of dried algal powder was soaked in acetone overnight at room temperature. Algal material was filtered using Whatman filter paper No. 1 fitted with a Buchner funnel. The algal extracts were concentrated in a rotary evaporator to a suitable volume. The crude extract was stored at −20 °C for the identification of the isolated fatty acids and antimicrobial test.

2.3. Lipid extraction and preparation of fatty acid methyl esters (FAME)

The crude extract was evaporated till dryness in rotary evaporator and then redissolved in 10% KOH in methanol, and refluxed on water bath for 4 h at a temperature not higher than 50 °C. The reaction mixture should not be allowed to go to dryness, until the salts have been converted to acids[40]. The saponified extract was exposed to evaporation of its methanol content and then a suitable volume of distilled water was added. The aquatic extract was extracted with ether for several times, to free the extract from any terpenoids and steroids contents. The alkaline aquatic extract was acidified by adding sulphuric acid to obtain the FFAs. The acidic mother liquor extract was then extracted with ether for several times and was concentrated in rotary evaporator. The FFAs present in ether fraction were subjected to the methylation method[41]. A total of 0.5 g of the fatty acids was dissolved in 3 mL pure methanol containing 3% HCl and refluxed on water bath for one and half hour. The reaction mixture was diluted with water and extracted with ether. The ether extract was washed for several times with distilled water till neutral to litmus paper, then dried over anhydrous calcium chloride and evaporated

till dryness. The resulting fatty acid methyl esters were subjected to gas chromatography−mass spectrometry in Faculty of Agriculture of Cairo University. The instrument was HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector. It was equipped with capillary column TR−FAME (Thermo 260 M142 P) (30 m, 0.25 mm inner diameter, 0.25 μm film) (70% cyanopropyl−polysilphenylene siloxane). The temperature programming was the injector temperature (200 °C), the temperature transfer line (250 °C), initial rate (80 °C/2 min) and ramp (3 °C/min) (temperature 230 °C/5 min). The carrier gas was He$_2$ (1.5 mL/min). The amount of sample injected was about 1 μL (5 μL/1 mL solvent) and the ionization energy was 70 eV. Qualitative and quantitative identification of the different constituents were performed by comparison of their mass spectrum with those of authentic reference compounds.

2.4. Antibacterial activity assay

Antibacterial test was accomplished in Suez Canal University Centre for Environmental Studies and Consultation. The 6 mm discs were concentrated by the crude and fatty acids extracts separately and analyzed using agar plug technique[42]. Samples were tested against the following indicator strains: Gram negative bacteria [*Escherichia coli* (NCMB 11943) (*E. coli*), *Pseudomonas aeruginosa* (NCMB 8295) (*P. aeruginosa*), *Salmonella typhimurium* (NCMB 74) (*S. typhimurium*), *Shigella boydii* (ATCC 9207) (*S. boydii*)], Gram positive bacteria [*Staphylococcus aureus* (NCMB 6571) (*S. aureus*) and actinobacteria [*Streptomyces antibioticus* (wild type) (*S. antibioticus*)]. Control was maintained with solvent (acetone) alone. All the assays were carried out in duplicate. The zones of inhibition (mm) were measured after 24 h of incubation at 28 °C for bacteria and 5 d for actinobacteria.

2.5. Statistics

The statistical analysis was performed using the software MINITAB version 16.1.1. to evaluate the Pearson's correlation coefficients ($\varrho<0.005$) in antimicrobial activity between the crude and fatty acid extracts of *P. pavonica* and *H. triquetra*.

3. Results

3.1. Fatty acids composition

The fatty acid composition obtained from the selected algae is summarized in Table 1. Nineteen fatty acids were separated and identified according to the data base of gas chromatography−mass computer search libraries and their characteristic molecular weights. Gas chromatography mass spectrum was illustrated for fatty acids composition from *P. pavonica* (Figure 1) and *H. triquetra* (Figure 2). The active principles revealed that the mixture of fatty acids ranged from C8:0 to C20:4. The total concentration of fatty acids in *P. pavonica* was 70.20% whereas in *H. triquetra* it was

65.36%. Palmitic and oleic acids formed the bulk of the total fatty acids content in *P. pavonica* and were more than that estimated in *H. triquetra*. The other fatty acids detected in the algal extracts occurred in smaller quantities.

Table 1

Fatty acids composition as methyl esters (concentration %) in *P. pavonica* and *H. triquetra*.

Isomer	Mol. Wt. m/e	Fatty acids Type	Trivial name	Systematic name	*P. pavonica* concentration %	*H. triquetra* concentration %
C8:0	200	SFA	Caprylic	Octanoic	–	2.98
C12:0	214	SFA	Lauric	Dodecanoic	–	0.79
C13:0	228	SFA	Tridecylic	Tridecanoic	–	2.26
C14:0	242	SFA	Myristic	Tetradecanoic	4.46	4.60
C15:0	256	SFA	Pentadecylic	Pentadecanoic	–	0.86
C16:0	270	SFA	Palmitic	Hexadecanoic	28.10	19.81
C16:1 trans	268	USFA	Palmitoleic	Hexadecenoic	4.70	–
C16:1 cis	268	USFA	Palmitoleic	9−cis−hexadecenoic	3.51	–
C17:0	284	SFA	Margaric	Heptadecanoic	–	9.70
C17:1	282	SFA	Margaroleic	Heptadecenoic	–	0.54
C18:0	298	SFA	Stearic	Octadecanoic	2.47	3.53
C18:1	296	USFA	Oleic	Octadecenoic	19.80	8.94
C18:1 trans	296	USFA	Elaidic		0.88	–
C18:2	294	USFA	Linoleic	Octadecadienoic	3.27	–
C18:3	292	USFA	α−Linolenic		1.59	–
C19:0	312	SFA	Nonadecylic	Nonadecanoic	–	3.93
C19:1	310	USFA		Nonadecenoic	–	6.44
C19:2	308	USFA		Non adecadienoate	–	0.98
C20:4	318	USFA	Arachidonic	Eicosatetraenoic	1.42	–
Σ Fatty acids					70.20	65.36
Σ SFA					35.03	49.00
Σ USFA					35.17	16.36

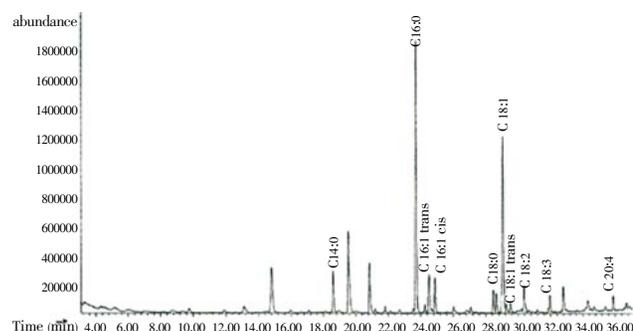SFA: saturated fatty acids; USFA: unsaturated fatty acids; Mol. Wt.: Molecular weight.

Figure 1. Gas chromatography mass spectra recorded for fatty acids extract of *P. pavonica*.
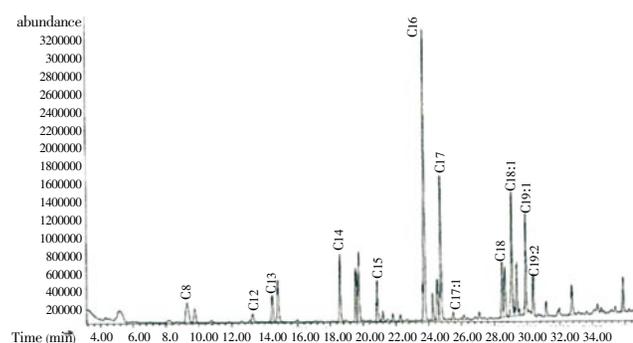

Figure 2. Gas chromatography mass spectra recorded for fatty acids extract of *H. triquetra*.

The FAME composition of *P. pavonica* showed that the concentration of saturated fatty acids (SFA) (35.03%) were

nearly equal to unsaturated fatty acids (USFA) (35.17%). The concentration order of saturated fatty acids were palmitic (28.10%)> myristic (4.46%)>stearic (2.47%), whereas the concentration order of USFA were oleic acid (19.80%)> palmitoleic (4.70%)>9−cis−hexadecenoic (3.51%)>linoleic acid (3.27%)>α−linolenic (1.59%)>arachidonic (1.42%)> elaidic acid (0.88%). In *H. triquetra*, the SFA showed higher concentration (49.00%) than that recorded in *P. pavonica* (35.03%) as follows: palmitic (19.81%)>margaric (9.70%)>myristic (4.60%)>nonadecyclic (3.93%)>stearic (3.53%)>caprylic (2.98%)>tridecylic (2.26)>pentadecylic (0.86%)>lauric (0.79%). The USFA in *H. triquetra* (16.36%) were less than *P. pavonica* (35.17%), the concentration order is as follows: oleic (8.94%)>nonadecenoic (6.44%)> non adecadienoate (0.98%)>margaroleic (0.54%).

When analyzing the fatty acid profile of the extracts, it was observed that palmitic acid (C16:0) was the most abundant SFA in *P. pavonica* (28.10%) and *H. triquetra* (19.81%), whilst the minimum one was elaidic acid (0.88%) in *P. pavonica* and margaroleic (0.54%) in *H. triquetra*.

It was noticed that, the concentration percentage of SFA to the total FAME in *P. pavonica* was 46.38%, which was lower than those in *H. triquetra* (74.11%). *P. pavonica* contained high concentration percentage level of USFA (53.62%), whereas *H. triquetra* had the low concentration percentage (25.89%) of USFA level. Regarding USFA, it was found that *P. pavonica* contained the essential fatty acids linoleic (an omega−6 fatty acid) (3.27%), α−linolenic (an omega−3 fatty acid) (1.59%) and oleic (an omega−9 fatty acid) (19.80%). *H. triquetra* had only one essential fatty acids oleic acid (8.94%) whereas neither linoleic nor α−linolenic acids were found.

3.2. Antibacterial activity of crude and fatty acid extracts

The bioactivity of crude and fatty acid extracts of *P. pavonica* and *H. triquetra* were evaluated *in vitro* against six resistant pathogens (Figure 3). The crude and fatty acid extracts of *H. triquetra* recorded higher antibacterial activity than the fatty acid extract of *P. pavonica* (ρ<0.005).
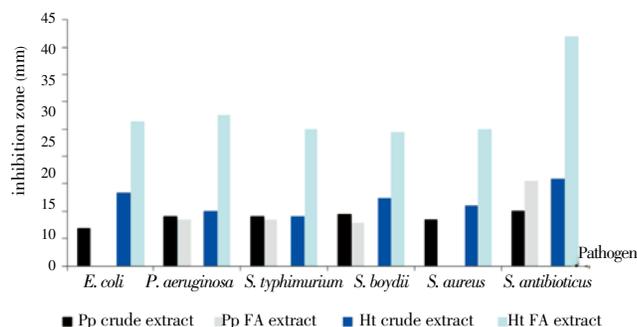
Figure 3. Antibacterial activity of crude and fatty acid extracts from *P. pavonica* (Pp) and *H. triquetra* (Ht) against six pathogens. FA: fatty acids.

The crude extract of *P. pavonica* exhibited inhibition zones against all selected pathogen strains whereas the fatty acids extract did not record inhibition activity against Gram negative bacteria *E. coli*, and Gram positive bacteria *S. aureus*. The crude extract showed the highest activity (10 mm) against the actinomycete *S. antibioticus* (ρ<0.005) while the lowest one was observed against *E. coli* (7 mm).

The maximum inhibition zone for fatty acids extract of *P. pavonica* was observed against the actinobacteria *S. antibioticus* (ρ<0.005). Figure 4 shows the antibacterial activity of fatty acids extract of *P. pavonica* against the four inhibited pathogens (*P. aeruginosa*, *S. typhimurium*, *S. boydii* and *S. antibioticus*).
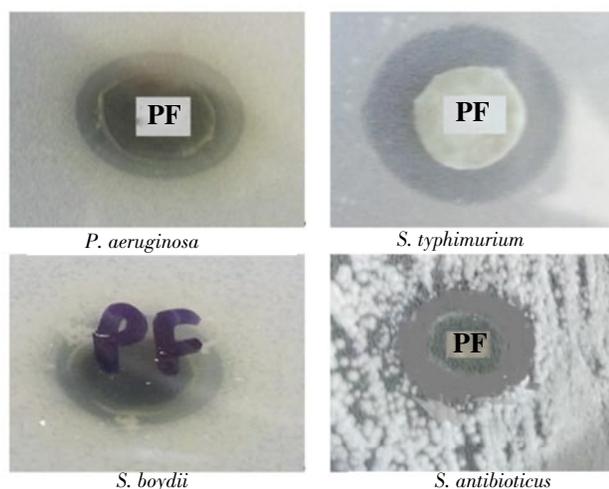
Figure 4. Antibacterial activity of fatty acids extract of *P. pavonica* (PF) against the pathogens.

In the present study, the crude and fatty acids extracts of *H. triquetra* exhibited the broadest and highest activity which successfully inhibited six resistant pathogens (Figure 3). The maximum inhibition zone against the actinobacteria *S. antibioticus* by crude and fatty acid extracts (ρ<0.005) was 16 mm and 42 mm, respectively. The minimum inhibition zone was 9 mm against *S. typhimurium* by *H. triquetra* crude extract and 24.5 mm against *S. boydii* by fatty acids extract (ρ<0.005). Figure 5 illustrates the antibacterial activity of fatty acids extract of *H. triquetra* against the six pathogens.
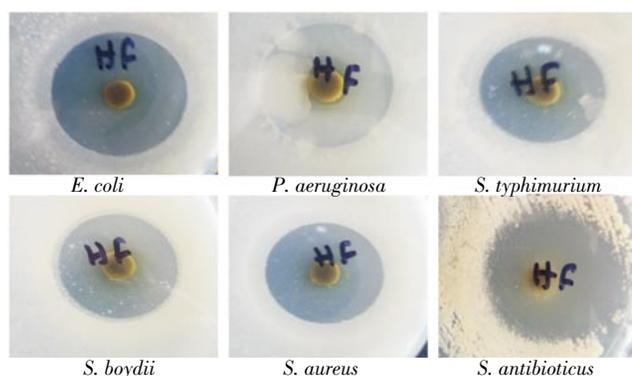
Figure 5. Antibacterial activity of fatty acids extract of *H. triquetra* (HF) against six pathogens.

4. Discussion

In the present study, gas chromatographic analysis showed the presence of nineteen fatty acids in the brown algae *P. pavonica* and *H. triquetra*. Palmitic acid formed the bulk of the total fatty acid content in the selected species. The other fatty acids were detected in smaller quantities. The results of active principles revealed that the mixture of fatty acids ranged from C8:0 to C20:4. SFA in *P. pavonica* were in the order palmitic>myristic>stearic, whereas the USFA were oleic acid>palmitoleic>9–cis–hexadecenoic>linoleic acid>α–linolenic>arachidonic>elaidic acid. *H. triquetra* contained SFA in more concentration than *P. pavonica* which were in the order palmitic>margaric>myristic>non adecyclic>stearic>caprylic>tridecyclic>pentadecylic> lauric while the USFA consisted of oleic>nonadecenoic>non adecadienoate>margaroleic. The dominance of SFA in *H. triquetra* is in agreement with Mendes *et al.* who noticed that, in general, all algae extracts presented higher amounts of SFA when compared with the PUFA[43]. This also accords with the findings of Bhaskar *et al.* and Pereira *et al*[44,45]. Bhaskar *et al.* reported that such high content of SFA was probably due to the influence of moderate water temperature of the location from which the algae was harvested[44]. Mendes *et al.*[43] mentioned that although lipid content in marine algae is usually low (less than 4%)[46], their PUFA content is superior to that of terrestrial vegetables[47].

Little is known about the chemical composition of *H. triquetra*. Heiba *et al.*[37] found that *H. triquetra* from the coastal zones of Qatar contained twenty one fatty acids from C12:0 to C20:4. They mentioned that the main fatty acids were palmitic>oleic>eicosadienoic then arachidonic whereas our fatty acids were palmitic>oleic>nonadecenoic. The difference in fatty acid composition between the present study and that mentioned by Heiba *et al.*[37] could be attributed to the different localities of examined alga; however, the most dominant fatty acids were the same.

In our study, *P. pavonica* contained fatty acids with 14–20:4 carbon atoms and the main acid in *P. pavonica* was palmitic followed by oleic and palmitoleic. Kamenarska *et al.*[48] mentioned that fatty acids, containing 14–22 carbon atoms have been identified in different *Padina* species[49]. They revealed that *P. pavonica* contained fatty acids with 12–22 carbon atoms, a profile similar to other brown algae. Kanias *et al.* referred that the main acid in *P. pavonica* was palmitic acid followed by oleic and myristic[50]. It is more similar to the composition of the Red Sea *P. pavonica* in which palmitic, myristic and stearic acids comprised 80% of the fatty acids[51]. In Aegean Sea, the fatty acid composition of *P. pavonica* differed a lot from that recorded in the present study, where oleic acid

was found to be the main fatty acid, and palmitic acid occurred in low concentration[50]. The C18:0 and C20:0 PUFA are characteristic of brown algae[52]. In contrary to *Padina boryana* Thivy from the Saudi Arabian coast, El–Naggar found high contents of C20:4[52].

Seaweeds act as potential bioactive compounds for pharmaceutical applications[53]. This capability of seaweeds can be attributed to synthesis of various bioactive secondary metabolites which are biologically active against different types of pathogenic bacteria[54]. The majority of the compounds isolated from marine algae are responsible for the antimicrobial activity. These compounds are lipidic in nature[55,56].

The bioactivity of crude and fatty acid extracts of *P. pavonica* and *H. triquetra* were tested *in vitro* against six resistant pathogens. The crude and fatty acid extracts of *H. triquetra* successfully inhibited the six resistant pathogens. Fatty acids extract of *P. pavonica* showed no inhibition effect against *E. coli* and *S. aureus*. This may attributed to other bioactive components which inhibit the growth. Fatty acid and crude extracts of *H. triquetra* than *P. pavonica* showed significant strong antibacterial activity. The maximum activity was recorded with *H. triquetra* and *P. pavonica* fatty acids extract against the actinobacteria *S. antibioticus*.

Among the bacterial test strain, the Gram positive bacteria were more susceptible to the extract than Gram negative bacteria except *Streptomyces* species. This may be due to the more complex structure and composition of Gram negative bacterial cell wall[57]. Mendes *et al.*[43] showed that the outer membrane and the thick murein layer of Gram negative bacteria act as a barrier, preventing the entrance of environmental substances such as antibiotics and inhibitors[58].

In this study, the lipid profile showed that the extracts especially *H. triquetra* extracts were rich in fatty acids, in particular SFA, which may indicate their probable role in the antimicrobial activity. Fatty acids including palmitic and stearic acids are known to have the same effect[59]. In contrast, *P. pavonica* has increase in USFA. The antimicrobial activity of fatty acids has been more attributed to long–chain USFA (C16–C20) such as palmitoleic, oleic and linolenic acids[43]. The result of *P. pavonica* is in agreement with Plaza *et al.* and Kayalvizhi *et al.*[59,60] who mentioned that the high content of USFA in the analyzed algae plays a major role in the antimicrobial properties of the algal extracts.

Our study revealed that acetone extracts of tested brown seaweeds showed strong antimicrobial activity against both Gram positive and Gram negative bacteria. The acetone extract of brown seaweed was recorded as highly effective

solvent against bacteria and fungi when compared to other solvents[60]. Other authors used acetone solvent for extracting the bioactive antimicrobial compounds which gave the highest antimicrobial activity against different pathogens[61].

Desbois and Smith[32] reported that the antibacterial activity of each fatty acid is influenced by its structure and shape. They explained the mechanism of antibacterial activity as a function of the length of the carbon chain and the presence, number, position and orientation of double bonds[62]. The ‾OH group of the carboxyl group seems to be important for the antibacterial activity of FFAs as methylated FFAs often have reduced or had no activity[63]. In general, USFA tend to have greater potency than SFAs with the same length carbon chain[64]. Some of the detrimental effects of fatty acids on bacterial cells can be attributed to the detergent properties of fatty acids on account of their amphipathic structures. This allows them to interact with the cell membrane to create transient or permanent pores of variable size. The higher concentrations of detergents, such as fatty acids, can solubilize the membrane to such an extent that various membrane proteins or larger sections of the lipid bilayer are released[32]. Fatty acids are potent inhibitors of diverse enzymes and USFA usually have greater inhibitory activity than saturated ones[65]. Antibacterial fatty acids may be applied to the treatment of bovine mastitis[66] and in the control of honeybee infections[67].

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Seaweeds are the most marine resources with biochemical compound of secondary metabolites characterized by a broad spectrum of biological activities. Fatty acids are one of these compounds that known for their diverse and potent biological activities such as the ability to kill or inhibit the growth of bacteria. Thus, the use of seaweeds as antibacterial agent for many pathogenic bacteria became wide spread.

Research frontiers

The present study estimates the fatty acids content of two species of brown algae occurring in Red Sea, Saudi Arabia. Authors evaluated the potential antimicrobial activity of the crude and fatty acids extracts against five species of pathogenic bacteria and one species of actinobacteria using disc technique.

Related reports

Fatty acids are the vital constituent of algae. The fatty acids in seaweeds are of the same type as those found in higher plants. In recent years, antimicrobial properties of fatty acids from brown and red algae have been reported.

Innovations and breakthroughs

The lipid profile of acetone extracts of the brown algae *P. pavonica* and *H. triquetra* showed the presence of nineteen fatty acids. The concentration of SFA in *H. triquetra* was higher than that in *P. pavonica*. The study showed good inhibitory result of tested brown seaweeds on both Gram–positive and Gram–negative bacteria. This indicates the good antimicrobial activity of algae and proved their role as therapeutic agents.

Applications

The findings of this study support the use of fatty acids extracts of these algae as antibacterial agents for some pathogenic bacteria.

Peer review

In the present work, the authors give significant information about fatty acid profile of the two brown seaweeds, *P. pavonica* and *H. triquetra* which spread worldwide. The authors make study valuable by evaluating the potential antibacterial activity of fatty acids extracts of the tested algae and compare their effect with that of crude extracts. Materials and methods are well planned. Findings are sound and discussion contains good explanations.

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