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The Biotechnological Potential of *Artemia salina* Fatty Acids

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In the study, fatty acids of *Artemia salina* that is commonly used in both aquarium fishing and culture fishing due to its high nutritional content were investigated against fish and human pathogenic microorganisms to determine biotechnological potential in feed/food and pharmaceutical industry. Analysis of fatty acids by gas chromatography revealed the presence of higher amounts of unsaturated fatty acids than saturated fatty acids. To evaluate the antimicrobial activity of *A. salina* fatty acids, disc diffusion and microdilution broth methods were used. The antimicrobial activities of the fatty acids were determined against 4 different fish pathogens. The antimicrobial activity assay results showed that the fatty acids were the most active against *Vibrio alginolyticus*. The fatty acids were also tested against clinical and foodborne pathogen microorganisms (thirteen bacteria and one yeast). The antimicrobial test results showed that the fatty acids of *A. salina* showed varying degrees of antimicrobial activity on the tested microorganisms except for *Escherichia coli* O157:H7, *Micrococcus luteus* NRRL B-4375 and *Salmonella enteritidis* ATCC 13076. The results presented here may suggest that the fatty acids of *A. salina* possess antimicrobial properties against both fish and human clinical and foodborne pathogens, and therefore can be used as a natural preservative ingredient in the feed and/or pharmaceutical industry.

Key words: Antibacterial activity, Antifungal activity, Fish pathogens, Human pathogens.

Natural fats and dietary oils contain fatty acids. Fatty acids are major nutritious materials and metabolites in living organisms[1]. And also fatty acids have chemical defense against pathogenic microorganisms[2-4]. As it is known fatty acids have antibacterial and antifungal effects[5].

Microbial diseases are major limiting factor in the production of both of these properties in wild and cultured fishes[6]. Infectious diseases in aquaculture lead to significant economic losses causing significant problems in the development of the sector[7]. Due to continuous use of antimicrobial agents in the aquatic environment has evolved more resistant bacterial strains8. Besides, continual use of synthetic antibiotics create a threat to nontarget organisms, consumer health, and the environment[8,9]. The utilization of heavy antibiotics in the aquaculture needs to be reduced and replaced with an alternative process for treating fish diseases[10].

A. salina is a species of Crustacea that live in halophilic ecosystems. Due to its high nutritional value, it is used worldwide as live feed for aquarium fishes. Its eggs turn into a cyst form under unsuitable conditions and can stay in the said form for a long period of time. When conditions become favorable, eggs are hatched and then outcome new individuals[11,12]. It is economically an important living; *Artemia* culture pools are established and cysts are put up for sale in packages around the world. Crustaceans are

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covered with a structure called carapax on the outer surface of their bodies. The said carapax structure contains chitin[13]. *Artemia* cysts have been established to have chitin content and antimicrobial characteristics in previous studies[14]. There is no previous study concerning the antimicrobial activity of the fatty acid composition of *A. salina*.

The present study was carried out to evaluate the antibacterial efficacy of fatty acids of *A. salina* as an alternative to commonly used antibiotics in aquaculture; particularly against bacterial disease. The fatty acids were also investigated for their antimicrobial potentials against a total of 14 reference clinical and food borne pathogen microorganisms.

MATERIALS AND METHODS

Field survey

Adult *A. salina* samples living in Lake Tersakan located in Salt Lake Basin were collected individually by using clamps in July 2012 and were brought to the laboratory environment in a 500 ml plastic bottle. The depth of the lake is ranged between 65 to 75cm. Water temperature was established to be 22°C and electrical conductivity was found to be 148 mS/cm.

Fatty acid analysis

Samples were extracted with chloroform/ methanol (2:1 v/v) according to Folch *et al*.[15]. The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF3 (v/v) in methanol[16].

Fatty acid methyl esters (FAMEs) were prepared from ten samples from each species. The FAMEs were analyzed on a HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted with a HP-88 capillary column (100 m, 0.25 mm i.d. and 0.2 µm). Injector and detector temperatures were 240ºC and 250ºC, respectively. The oven was programmed at 160ºC initial temperature and 2 min initial time. Thereafter the temperature was increased to 185ºC at 4ºC/min, then increased to 200ºC at 1ºC/min and held at 200ºC for 46.75 min. Total run time was 70 min. The carrier gas used was helium (1 ml/min). GC analysis of FAMEs was performed in three replications.

Identification of fatty acids was carried out by comparing sample the FAME peak relative retention times with those obtained for Alltech (Carolean Industrial Drive, Satate Collage, PA) standards. Results were expressed as FID response area relative percentages. The results are given as mean ± SD in Table 1.

Determination of Antimicrobial Activity

Microbial Strains

Thirteen bacteria (*Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* RSKK 863, *Micrococcus luteus* NRRL B-4375, *Bacillus subtilis* RSKK 244, *Escherichia coli* ATCC 11229, *Escherichia coli* ATCC 35218, *Escherichia coli* O157:H7, *Salmonella enteritidis* ATCC 13076, *Salmonella enteritidis* RSKK 171, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* Mu:57, *Yersinia enterocolitica* NCTC 11175) and one yeast (*Candida albicans* ATCC 10231) were used in this study. Tryptic Soy Agar (TSA) and Nutrient agar (NA) were used to cultivate bacteria while YPD was used for the cultivation of the yeast. The bacterial strains were incubated at 37 ºC for 24 h. The yeast culture was grown at 30 ºC for 48 h.

The following fish pathogenic bacteria were also used in the screening of antibacterial activity: *Lactococcus garvieae, Yersinia ruckeri*, *Vibrio anguillarum* (M1 and A4 strains, from two different companies) and *Vibrio alginolyticus*. *Y. ruckeri* and *L. garviae* were grown on TSA. *V. anguillarum* and *V. alginolyticus* were cultured in TSA supplemented with 2% NaCl. The bacterial cultures were incubated at 25ºC for 24 h.

Inhibitory Effect with the Disc Diffusion Method

Fatty acids of *A. salina* were dissolved in dimethyl sulfoxide (DMSO) and sterilized by 0.45 µm Milipore filter and used as stock solution. The disc diffusion method was employed for the definition of the antimicrobial activity[17]. The culture suspensions were adjusted with comparing with 0.5 McFarland. One hundred microlitres of suspension of the test microorganisms were spread on solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 20 µl fatty acid (250 µg fatty acids/disc), and then placed on the inoculated plates. Then, they were kept for 2 hours in a refrigerator to enable prediffusion of the extracts into the agar. Then, the inoculated plates were incubated for 24 h and 48 h for bacterial and

yeast strains respectively. Antibiotic discs of Ampicillin (Amp, 10 µg/disc), Gentamicin (CN, 10 µg/disc), and Amikacin (AK, 30 µg/disc) were also used as positive controls. DMSO was used as negative controls. The diameters of inhibition zones (mm) were used as a measure of antimicrobial activity, and each assay was repeated twice.

Determination of Minimal Bactericidal (MBC) or Fungicidal (MFC) concentration

MBC or MFC values of extracts were determined with a 2-fold serial dilution method with some modifications[18], and studied for the microorganisms which are sensitive to the extracts in the disc diffusion assay. The test samples were added to growth broth medium to obtain a final concentration of 12.50 mg/ml and serially diluted to reach 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20 mg/ml. The final volume in each tube was 100 µl. 2.5 µl of standardized suspension (adjusted to 0.5 McFarland) of each tested microorganism was transferred to each tube. A positive control (2.5 µl inoculum and 100 µl growth medium) and a negative control (2.5 µl of extract, 100 µl growth medium without inoculum) were studied for each test. The contents of the tubes were mixed by pipetting and they were incubated for 24 h. 5 µl samples from all tubes were spread onto solid growth medium. The MBC and MFC were known as the lowest concentration of the substance that did not permit any visible bacterial and fungal colony growth on the solid growth medium after incubation[18]. The concentrations of the fatty acids that prevent the growth of a microorganism on the agar plate were recorded as MBC or MFC values.

RESULTS

Fatty Acids

The fatty acid composition of *A. salina* was characterized by four major fatty acids, including palmitic (C16:0), stearic (C18:0), oleic (C18:1 ω9) and vaccenic acid (C18:1 ω7) (Table 1). These fatty acids comprised between 10.07 and 22.25% of total fatty acids. The total saturated fatty acids (SFA) were 49.83%. Within these fatty acids the major fatty acids were C16:0 and C18:0. The mono (MUFA) and poly unsaturated fatty acids (PUFA) were found to be 32.38% and 17.79%, respectively. Hence, the percentage of total unsaturated fatty acids (UFA) was 50.17%. The most abundant PUFA in *A. salina* was eicosapentaenoic acid (EPA, C20:5 ω3) with a ratio of 6.82%. The total amount of linoleic (C18:2 ω6) and ±-linolenic acid (C18:3 ω3), which are essential fatty acids (EFA), was found to be 8.29%. The ω3,

Table 1. Fatty acid composition of *Artemia salina*

Fatty Acids	*Artemia salina*
C 8:0	0.48±0.01
C 10:0	0.13±0.01
C 11:0	0.15±0.01
C 12:0	0.36±0.05
C 13:0	0.17±0.01
C 14:0	2.72±0.20
C 15:0	0.61±0.01
C 16:0	22.25±0.08
C 17:0	1.07±0.11
C 18:0	20.74±0.10
C 20:0	0.34±0.04
C 21:0	0.81±0.01
ΣSFA	49.83±0.07
C 14:1 ω5	0.82±0.00
C 15:1 ω5	0.47±0.01
C 16:1 ω7	8.07±0.05
C 17:1 ω7	1.18±0.05
C 18:1 ω9	10.07±0.00
C 18:1 ω11	11.68±0.06
C 20:1 ω9	0.09±0.00
MUFA	32.38±0.05
C 18:2 ω6	4.22±0.01
C 18:3 ω6	0.33±0.01
C 18:3 ω3	4.07±0.01
C 20:2 ω6	0.03±0.00
C 20:4 ω6	2.32±0.00
C 20:5 ω3	6.82±0.01
ΣPUFA	17.79±0.02
ΣUFA	50.17
ΣEFA	8.29
Σω3	10.89
ω6	6.90
ω3/ω6	**1.58**

[a] Values reported are means ± S.D. of three parallel measurements

[b] SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, UFA: Unsaturated fatty acids, EFA: Essential fatty acids

ω6 and ω3/ω6 ratios of *A. salina* were 10.89, 6.90 and 1.58, respectively.

Antimicrobial activity

The results of the antimicrobial screening assay against fish pathogens of A. *salina* fatty acids are shown in Table 2. The disc diffusion assay results showed that the fatty acids were the most active against V. *alginolyticus* (14.12 mm) followed by *V. anguillarum* (A4 and M1 strains, respectively) and *Y. ruckeri*. The weakest inhibitory activity was determined against *L. garviae* (8.10 mm). The fatty acids of *A. salina* showed better antibacterial activities against 3 out of 5 bacteria when compared with standard Ampicillin (Table 2). MBC values for the microorganisms which were sensitive to the fatty acids of *A. salina* were in the range of 1.56-6.25 mg/ml (Table 2). V. *alginolyticus,* the most susceptible against the fatty acids in the disc diffusion assay, showed the lowest MBC value (1.56 mg/ml).

In this study, fatty acid of *A. salina* was also screened for antimicrobial activity against 14 clinical and food borne pathogens. According to the results given in Table 3, most of the test

Table 2. Antibacterial activity of *Artemia salina* fatty acids against different bacterial fish pathogens

Test microorganisms	MBC[a] (mg/ml)	Inhibition zone diameter[b] (mm)	Antibiotics Inhibition zone diameter[b] (mm)		
			Amp	CN	AK
L. garviae	6.25	8.10±0.32	33.10±0.12	15.19±0.10	10.30±0.08
Y. ruckeri	6.25	8.84±0.12	32.30±0.15	18.85±0.05	18.69±0.12
V. anguillarum M1	3.13	9.20±0.09	9.02±0.04	12.38±0.09	9.46±0.12
V. anguillarum A4	3.13	12.10±0.21	9.40±0.11	15.13±0.15	12.07±0.13
V. alginolyticus	1.56	14.12±0.34	13.57±0.09	15.06±0.07	15.03±0.03

[a]: Minimal Bactericidal Concentration (MBC)
[b]: Diameter of the inhibition zone including disc diameter.
Values are reported as means ± SD of two separate experiments.

Table 3. Antimicrobial activity of *Artemia salina* fatty acids against test microorganisms

Test microorganisms MFC[b]	MBC[a] or zone diameter[c] (mg/ml)	Inhibition (mm)	Antibiotics Inhibition zone diameter[c] (mm)		
			Amp	CN	AK
B.cereus RSKK 863	3.13	10.78±1.01	37.68±0.03	18.02±0.11	18.72±0.07
E. coli O157:H7	-	-	25.92±0.15	18.37±0.17	22.58±0.09
S. sonnei Mu:57	3.13	14.93±1.09	38.43±0.16	19.49±0.05	27.07±0.04
M. luteus NRRL B-4375	-	-	34.65±0.12	13.48±0.22	19.55±0.14
Y. enterocolitica NCTC 11175	3.13	9.46±1.27	11.58±0.09	16.17±0.11	21.19±0.07
E. coli ATCC 11229	3.13	9.84±0.14	27.99±0.14	14.98±0.12	19.81±0.13
P. aeruginosa ATCC 27853	6.25	10.73±0.57	-	15.89±0.05	19.71±0.08
S. aureus ATCC 25923	6.25	8.98±0.19	34.82±0.06	15.52±0.14	19.46±0.16
E.coli ATCC 35218	6.25	9.62±0.81	25.78±0.19	12.17±0.21	20.03±0.09
S. enteritidis ATCC 13076	-	-	29.49±0.15	16.38±0.17	17.27±0.11
L. monocytogenes ATCC 7644	6.25	10.12±0.64	25.13±0.06	20.63±0.16	20.52±0.21
B. subtilis RSKK 244	1.56	13.05±0.23	37.57±0.08	15.35±0.04	16.86±0.07
S.enteritidis RSKK 171	3.13	10.93±0.12	31.27±0.11	12.05±0.14	15.67±0.09
C. albicans ATCC 10231	1.56	10.28±0.14	-	-	-

[a]: Minimal Bactericidal Concentration (MBC)
[b]: Minimal Fungicidal Concentration (MFC)
[c]: Diameter of the inhibition zone including disc diameter.
Values are reported as means ± SD of two separate experiments. -: no zone of inhibition

microorganisms were sensitive to the fatty acids. However, *S. sonnei* Mu:57, *B. subtilis* RSKK 244 and *S. enteritidis* RSKK 171 were more sensitive than the other test microorganisms. The inhibition zones of disc and MBC or MFC values for test microorganism strains in this study which were sensitive to the fatty acids were in the range of 8.98-14.93 mm and 1.56–6.25 mg/ml, respectively (Table 3). The fatty acid showed no antimicrobial activity against *E. coli* O157:H7, *M. luteus* NRRL B-4375 and *S. enteritidis* ATCC 13076. Of the other microorganisms, *B. subtilis* RSKK 244 and *C. albicans* ATCC 10231 showed the lowest MBC and MFC values (1.56 mg/ml).

DISCUSSION

Due to the cysts and nauplii of *Artemia* being a vital food in aquaculture, determining its fatty acid and biochemical composition is very important[19]. Watanabe *et al*.[20] divided *Artemia* cysts into 2 categories as freshwater-type and marine-type according to fatty acid composition. The first one is characterized with high linolenic acid and low EPA concentration, but the second one had higher EPA and lower linolenic acid concentration. Lake Tersakan located in the Salt Lake Basin is also a salt-water lake. According to data obtained as a result of our study, EPA was established to be higher than linolenic acid in the fatty acid composition of *Artemia*. As can be seen here, the results conform to the findings of researchers. In addition, EPA was the most plentifully found PUFA in our study. EPA has numerous beneficial biological activities such as its major role in eicosanoid synthesis[21] and its reducing the risk of coronary diseases[22]. Previous studies showed that fatty acid profiles of *Artemia* and other zooplanktons reflected the fatty acid compositions of their nutrients [23,24]. Therefore, the fatty acid composition of *Artemia* provides us information on the fatty acid profiles of algal creatures that live in the same environment. Ruiz *et al*.[25] investigated the cysts of marine- and freshwater-types of *Artemia* that comprise the Argentinian population in terms of fatty acid profile. Researchers found 16:0, 16:1 ω7, 18:1 ω9, 18:1ω7, 18:2 ω6, and 18:3 ω3 fatty acids as major fatty acids in freshwater-type population. EPA was lower than linolenic acid (18:3 ω3) in freshwater-type. Although the rates of fatty acids of marine-type Artemia were different from freshwater-type, a high proportion of total fatty acids was the same fatty acids. However, in a different manner, EPA was higher than linolenic acid, as expected. In a study by Ruiz *et al*.[25], while SFA rate demonstrated a distribution in a range of 17.4-38.4% in freshwater-type and 18.4-31.4% in marine-type, total unsaturation was above 60% in both. These percentages were established to be 49.83% (SFA) and 50.17% (UFA) in our study. In another study, fatty acid compositions of *Artemia* cyst, nauplii and metanauplii were investigated and 16:0, 16:1 ω7, 18:1 ω9, 18:1 ω7, 18:2 ω6, and 20:5 ω3 (EPA) were established to be main components [26]. In the same study, the total ω3 and ω6 of cyst, nauplii and metanauplii were found to be ranging between 11.6-12.1% and 11.9-12.8%, respectively. Also, ω3/ω6 was at the levels of 1.3 (cyst), 1.2 (nauplii), and 1.3 (metanauplii). In our study, total ω3, ω6, and ω3/ω6 rates were determined as 10.89, 6.90, and 1.58, respectively. In the study of Naceur *et al*.[27] that was investigated biochemical characteristics of *Artemia* cysts belonging to Tunisian *A. salina* population, 16:1 ω7 in fatty acid profile was found to be major fatty acid at a rate of 20.55%. Other fatty acids had similar results with our study. However, EPA became the third abundantly found fatty acid with its quite high level (14.73%) following 16:0 (16.88%). One of the most comprehensive studies on the fatty acid profile of *Artemia* was the study by Zhukova *et al*.[28] in which they compared fatty acid compositions of *Artemia*, and some microalgae (*Isochrysis galbana*, *Phaeodactylum tricornutum,* and *Nannochloropsis oculata*) and yeast that commonly constitute its nutrient with fatty acid composition of *Artemia* fed with them. When compared in the study the fatty acid compositions of cultured *Artemia nauplii* and *Artemia* fed with these nutrients, the saturation rate that was 21.7% initially was observed to have increased to about 22.7-27.8%. In addition, while total ω6 value of nauplii of 15.2% reduced to 7.4-12.6% a result of feeding practice, and total É3 level was obtained in higher levels (18.9-29.0%) compared to what was established initially (18.2%).

Fatty acids have been used for years as antimicrobial substance[29-32]. In the present study, we investigated the growth inhibitory effect of

A. salina fatty acids on one gram-positive bacterium (*L. garvieae*) and three gram-negative bacteria (*Y. ruckeri*, *V. anguillarum* (two strains) and *V.alginolyticus)*. These pathogens commonly occur in the aquaculture industry and they can cause significant diseases and mortality in fish[33].

Gram-negative bacteria are generally known more resistant than gram-positive ones against fatty acids and essential oils because of their cell wall lipopolysaccharide[34,35,5], but this situation is not always true[36, 37]. Here, the gram positive *L. garviae* was found to be the most resistant to the fatty acids, which is a result similar to that found by Benkendorff *et al*.[37]. They also demonstrated that a gram negative bacterium *V. harveyi* was the least resistant to fatty acids. Similarly, *V. alginolyticus* was the most susceptible bacterium among the tested fish bacteria in our study. The results obtained from the disc diffusion method indicated that the fatty acid can be used as natural antimicrobial against *V. anguillarum* (M1 and A4 strains) and *V. alginolyticus* pathogenic strains which have a higher zone inhibition.

To determine antimicrobial activity against reference clinical and food borne pathogens, the fatty acids of A. *salina* were also tested against five gram-positive bacteria (*L. monocytogenes* ATCC 7644, *S. aureus* ATCC 25923, *B. cereus* RSKK 863, *M. luteus* NRRL B-4375, *B. subtilis* RSKK 244), eight gram-negative bacteria (*E. coli* ATCC 11229, *E. coli* ATCC 35218, *E. coli* O157:H7, *S. enteritidis* ATCC 13076, *S. enteritidis* RSKK 171, *P. aeruginosa* ATCC 27853, *S. sonnei* Mu:57, *Y. enterocolitica* NCTC 11175) and a yeast (*C. albicans*). The two gram negative bacteria (*E. coli* O157:H7 and *S. enteritidis* ATCC 13076) were unaffected by fatty acids at 250 µg concentration. This was to be expected since several other studies[34,38,39] reported that gram negative bacteria were resistant to the inhibitory effects of fatty acids and their derivatives. However, *M. luteus* NRRL B-4375, a gram positive bacterium, was not affected the fatty acids at the same concentration, which is a result similar to antimicrobial activity against the fish pathogens used in this study (Table 2). Also, the fatty acids showed the highest inhibitory effect on a gram positive bacterium (*S. sonnei* Mu:57). Similar results have been reported by Kim *et al*.[40] who found that *L. monocytogenes* (gram-positive) was more resistant to the inhibitory effects of eleven essential oil constituents than the gram-negative bacteria tested under the same conditions.

C. albicans is a commensal fungus of the human oral, vaginal, gastrointestinal, and mucosal surfaces[41]. *C. albicans* can cause mortality or some problems because of the use of drugs and antibiotics that suppress the useful bacterial flora[42]. The search for new natural antifungal drugs should be a priority because of the the development of resistance during treatment to the synthetic antifungal drugs[43].

In our study, *C. albicans* ATCC 10231 derived from a man with bronchomycosis showed the lowest MFC value against the fatty acids. Therefore, the fatty acids may be used as a natural antifungal alternative to synthetic drugs. Toxicity studies should also be done to determine their safety.

The antimicrobial activity of fatty acids has been reported to be dependent on the unsaturation degree and chain length[37, 44]. Fatty acids, have <8 carbon atoms, are considered short chain. >16 carbon atoms of fatty acids are referred as long chain[45]. The fatty acids from *A. salina* were determined as medium and long chain fatty acids. The antimicrobial activity may be due to this content because antimicrobial activity of medium and long chain fatty acids has been reported in several researches[46, 47]. Unsaturated fatty acids are more effective against microorganisms than the saturated fatty acids. The inhibitory activity increase with the number of double bonds of the molecule[48]. Therefore, in this study the antimicrobial activity of the fatty acids may be due to a higher total unsaturated fatty acid content (50.17%) than total saturated fatty acids of *A. salina*.

Benkerdorf *et al*.[37] and Galbraith *et al*.[47] indicated that palmitic (C16:0) and stearic acids (C18:0) such as saturated fatty acids and oleic acid (C 18:1) such as unsaturated fatty acid, which are the dominant fatty acids of *A. salina* in this study, showed antibacterial activity against gram negative and gram positive. Palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2) and arachidonic (C20:4) acids are known to show antimicrobial activity against human pathogens and aquatic pathogenic bacteria[34,37]. Benkendorff *et al*.[37] reported that palmitic acid was more effective against *V. harveyi* and *V. alginocolyticus* than *L. garvieae* and *V.*

anguillarium. Benkendorff *et al*.[37] also found Oleic acid to be active against these aquatic pathogenic bacteria at the concentration of 0.01 mg/ml[37]. *V. alginolyticus* was found to be the most susceptible bacteria among the tested fish pathogens in our study. Similarly, this result may be due to these fatty acids which are found in high amounts in *A. salina* fatty acid content. Kabara *et al*.[49] also reported that palmitoleic acid and linoleic acid, highly abundant among fatty acids of *A. salina,* showed antifungal activity.

CONCLUSIONS

Fatty acid composition obtained from *A. salina* that lives in halophilic ecosystems and is used as feed in both aquarium and culture fishing was established, and these fatty acids were found to be effective on fish and human pathogens. Fatty acids are attractive as antimicrobial agents for various applications in medicine, agriculture, aquaculture and food preservation etc. Therefore, fatty acids of *A. salina* can be used as a natural preservative ingredient in the feed and/or pharmaceutical industry.

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