Original Research Article

Antimicrobial and anti-inflammatory effects of two different species marine red algae isolated from Quseir, Red Sea, Egypt.

ABSTRACT

Aims: This study aimed to analyze the bioactivities of two different species of marine red algae crude extracts; *Acanthophoraspicifera(M.Vahl) Borgese* and *Digenea simplex (Wulfen) C.Agardh*.
Place & duration of study: Sample: Quseir city, Red Sea, Egypt at (April, 2015), extraction and biological study; pharmacognosy department, summer 2015,*Kasr El-Eini Teaching Hospitals, Cairo University, Egypt*.
Methodology: The crude extract screened against some human pathogenic bacteria including ten Gram positive, ten Gram negative strains and tenunicellular and filamentous fungi. The antimicrobial activity was done through the agar well diffusion method. The anti-inflammatory effect of the crude extract on inflammed liver cells was evaluated by measuringSOD, MDA, catalase, GSH, Il-6 and TNF-alpha in the serum of treated Wister albino rats compared to positive and negative control groups
Results: The antimicrobial study showed that both *Acanthophoraspicifera* and *Digenea simplex* extracts showed the highest zone of inhibition against *Streptococcus agalactiae* with clear zones (22.5±0.58 mm and 23.1±0.58 mm) as Gram positive bacteria compared to Ampicillin as positive control. But with Gram negative bacteria Acanthophora spicifera showed highest zone of inhibition against *Pseudomonas aeruginosa* (22.3±2.1 mm) and *Digenea simplex* showed highest zone of inhibition against *Serratiamarcescens* (24.1±0.58 mm) compared to Gentamycin as positive control. Finally, the effect of both *Acanthophoraspicifera* and *Digenea simplex* against fungi showed highest zone of inhibition (23.8±0.63 mm and 22.4±2.1 mm) against *Geotricumcandidum.* On the other hand results revealed that all methanol extracts had equally potent anti-inflammatory effects on inflammated liver rats as it observed significant decreased in the levels of SOD, Catalase and GSH. Also observed that significant increase in the inflamed liver state of MDA, IL-6 and TNF-AlphaAfter treatment of inflamed liver rats with crude extracts observed that the difference in the parameters levels relatively equal to the normal control results.
Conclusion: *Acanthophoraspicifera(M.Vahl) Borgese*and *Digenea simplex (Wulfen) C.Agardh* might be a good source of anti-inflammatory and antibacterial compounds activity.

Keywords: Marine red macro-algae, Antimicrobial, Anti-inflammation.

1. INTRODUCTION

Marine macro algae are one of nature's most biologically active resources, as they hold a wealth of bioactive compounds. Many compounds isolated from marine macroalgae have demonstrated various biological activities, such as antibacterial activity, antioxidant potential, anti-inflammatory properties, anticoagulant activities, antiviral activities, apoptotic activities, and prebiotic activity [1-6]. Marine macro algae are commonly classified into three main groups based on their pigmentation; Phaeophyta (brown algae), Chlorophyta (green algae), and Rhodophyta (red algae). Macroalgae was considered as a rich source of dietary fiber, minerals, lipids, proteins, omega-3 fatty acids, essential amino acids, polysaccharides, and vitamins A, B, C, and E. Various bioactive compounds from marine organisms have been experimentally tested to comprehensively study the biological effects of recently developed drugs [7]. Studies on the bioactivities of marine algae have revealed numerous health-promoting effects, including anti-oxidative, anti-inflammatory, antimicrobial, and anti-cancer effects. Seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids have exhibits different biological activities, relaying on their solubility and polarity, different solvents shows different antimicrobial activity.Chemical compounds should be extracted from different seaweeds in order to optimize their antibacterial and antifungal activity by selecting the best solvent system [8]. Oxidative stress plays important role in endothelial dysfunction because the molecular disintegration in reactive oxygen species (ROS)metabolities which plays a main resone in the pathogenesis of it by a loss of nitric oxide (NO) bioavailability[9], lung disease [10], gastrointestinal dysfunction [11], and atherosclerosis [12], all of which involve inflammatory reactions. Many marine natural products that contain antioxidants

wereknown to have anti-inflammatory effects [13-15]. This study was carried out to figure the antimicrobial and anti-inflammatory effects of 2 different algal species.Finally marine algae considered as the main of the food chain in the areas overlooking the ocean and enhance about more than two-thirds of the world's populations. In addition to its importance, algae are responsible for about half of global photosynthetic activity [16].

2. MATERIAL AND METHODS

2.1. Collection and Identification of marine macroalgae

The fresh algal species were collected from the inter-tidal region of Quseir city (Figure 1) which located on the west coastal area of Red Sea shore between longitude 34° 17`E and latitude 26° 06`N during the spring year 2015. Collected sample was immediately brought to the laboratory in new plastic bags containing pond water to prevent evaporation. Algal material was washed thoroughly with tap water and distilled water to remove extraneous materials and shade-dried for 5 days and oven dried at 60°C until constant weight was obtained, then was grind into fine powder using electric mixer and stored at 4°C for future use. Algal species were identified according to [17-20].

Fig.1 Map show the studied area [Quseir city, Red Sea, Egypt]

2.2. Extraction of selected algal species:

Powdered marine algae (500 g, each) were extracted with 70% methanol [21] , because some of the active components in the crude extracts are polar and thus dissolved readily in the methanol (polar solvent), by percolation till exhaustion. The alcoholic extract in each case was evaporated under reduced pressure to obtain a semisolid residue.

2.3. Antimicrobial activity (The microorganisms used are from the RCMB collection)

The hydro alcoholic extracts of the selected species were investigated for their antimicrobial activities using Agar well diffusion and MullerHenton against gram positive and gram negative bacteria and used Sab. dextrose agar against some fungi. All tested microorganisms were kindly supplied from Biotechnology Research Center, Al-Azhar University (for boys), Cairo, Egypt.

2.3.1. Gram positive bacteria (the microorganisms used are from the RCMB collection) :

Staphylococcus aureus (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024), *Streptococcus sanguis*(RCMB 01001 71-3), *Streptococcus pyogenes* (RCMB 01001 74-2), *Streptococcus agalactiae*(RCMB 01001 73-2), *Bacillus subtilis* (RCMB 01001 69-3), *Enterococcus faecalis*(RCMB 01001 54-2),*Corynebacteriumdiphtheriae*(RCMB 01001 26-7), *Micrococcus luteus*(RCMB 01001 76-9), *Methicillin-resistant Staphylococcus aureus MRSA* (RCMB 01001 94-5).

2.3.2. Gram negative bacteria (the microoganisms used are from the RCMB collection) :

Escherichia coli (RCMB 01002 52-6), *Proteus mirabilis* (RCMB 01002 54-2), *Acinetobacterbaumannii* (RCMB 01002 82-9),*Klebsiella pneumonia*(RCMB 01002 23-5), *Pseudomonas aeruginosa* (RCMB 01002 43-5), *Serratiaplymuthica*(RCMB 01002 75-3), *Serratiamarcescens*(RCMB 01002 75b-8), *Salmonella tophi*(RCMB 01002 15-4), *Enterobacter cloacae* (RCMB 01002 64-5), *Shigelladysenteriae*(RCMB 01002 41-8).

2.3.3. Unicellular fungi & Filamentous fungi (the microorganisms used are from the RCMB collection):

Aspergillus fumigatus (RCMB 02568), *Syncephalastrum racemosum* (RCMB 05922), *Geotricum candidum* (RCMB 05097), *Candida albicans* (RCMB 05036), *Aspergillus niger* (RCMB 02724), *Cryptococcus neoformans* (RCMB 05642), *Candida trobicalis* (RCMB05239), *Penicillium expansum* (RCMB 01924), *Microsporum canis* (RCMB 0834),*Trichophyton mentagrophytes (RCMB 0925).*

2.3.4. Methods of measuring antimicrobial activity:

2.3.4.1. Well-diffusion method for anti-bacterial activity: (Mueller Henton)

The solution of 50 mg/ml of each sample in DMSO (Dimethyl sulphoxide) was prepared for testing against bacteria. Centrifuged pellets of bacteria from 24h old culture containing approximately 104-106 CFU/ml (Colony forming unit per ml) were spread on the surface of Nutrient agar (type tone 1%, Yeast extract 0.5%, agar 1%, 100 ml of distilled water, PH 7.0) which autoclaved under 121ºC for at least 20 min. Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45ºC. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100µl of the tested samples (100 mg/ml) were loaded into the wells of the plates. All samples were prepared in DMSO. DMSO was loaded as control. The plates were kept for incubation at 37°C for 24h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. Ampicillin and Gentamycin were used as antibacterial standard drugs[22].

2.3.4.2. Well-diffsion method for anti-fungal activity: (Sabourad dextrose)

The antifungal activity was investigated by agar well diffusion method by the following procedure: Sabourad dextrose agar plates: A homogenous mixture of glucose-peptone-agar (40: 10: 15) was sterilized by autoclaving at 121ºC for 20 min. The sterilized solution (25 ml) was poured in each sterializedpetridish in laminar flow and left for 20 min to form the solidified sabourad dextrose agar plate. These plates were inverted and kept at 30 ºC in incubator to remove the moisture and check for any contamination. Antifungal assay: Fungalstrain was grown in 5ml Sabourad dextrose broth (glucose: peptone; 40: 10) for 3-4 days to achieve 105 CFU/ml cells. The fungal culture (0.1 ml) was spread out uniformly on the Sabourad dextrose agar plates. Now small wells of size (4mm×20mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100µl of the tested samples (10mg/ml) were loaded into wells of the plates. All samples was prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 30 ºC for 3-4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Amphotericin B was used as antifungal standard drug [22].

2.4. Anti-inflammatory activity

Animal: Wister albino rats weighing between (140-200 g) were obtained from animal house of National Research Center, Dokki – Giza, Egypt. They were housed in well protected polypropylene cages and maintained in standard laboratory conditions in an air conditioned area at 25±2°C in 10:14

hr light dark cycle and allowed to take standard laboratory feed and tap water [23]. The study was approved by Institutional Animal Ethics Committee of National Research Center that the animal doesn't suffering at any stage of experiment and maintained in accordance with the Guide for the Care and Use of Laboratory Animals. The inflamed liver was induced in rats according to the described method[24].The animals were divided into three groups consisted of six animals; Normal control (A), Diseased or inflammated Liver cancer (positive control receiving saline) (B), Inflammated liver rats were treated by injection with methanol extract of the first extract (*Acanthophoraspicifera)*(C) and the second extract (*Digenea simplex*)(D).The methanol extract (0.1 g of each sample, separately) mixed or dissolved with 1ml of phosphate buffer and every day injected 100 µm to each rat for 15 days. After 15 days, rats were sacrificed and blood samples were collected by puncture the sublingual vein in clean and dry test tube. Allow clotting for 10 minutes before centrifuging at 3000 rpm for serum separation. The separated serum was stored at -80°C for further determinations of the following tests: SOD, MDA, catalase, GSH, Il-6 and TNF-alpha. The anti-inflammatory activity was observed on Wister albino rats after injection by the extracts of the marine algal species (Table 4) by measuring the parameters in the normal and diseased states.

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3. RESULTS AND DISCUSSION

3.1. Collection and Identification

Isolated algal species: Two algal samples of red algae*Acanthophoraspicifera(M.Vahl) Borgese*(Fig.2)and *Digenea simplex(Wulfen) C.Agardh*(Fig.3)were collected fromRed Sea, Egypt(Quseir city).

Figure 2.*Acanthophoraspicifera*Figure 3.*Digenea simplex*

3.2. Antimicrobial activity

Results summarized in the tables (1-3) showed the antimicrobial screening of the crude extracts of two macro algae against some human pathogenic bacteria as well as some unicellular and filamentous fungi.Both *A.spicifera* and *D.simplex* extracts showed similar potent inhibitory growth activities against three Gram +ve bacteria [*Streptococcus agalactiae*, *Streptococcus pyogenes* and *Streptococcus sanguis*] with zone of inhibition ranging from [23.1±0.58 to 20.6±0.63 mm] and showed moderate activities with [*Corynebacteriumdiphtheriae*, *Bacillus subtilis* and *Staphylococcus aureus*] with inhibition zones ranging from [20.1±1.5 to 16.3±2.1 mm]. Also the crude extracts were found to be more active than the positive control Ampicillin ,(22.3±1.5 mm), against *Streptococcus agalactiae* which showing inhibition zone [22.5±0.58 mm with *A.spicifera* and 23.1±0.58 mm with *D. simplex*].*Staphylococcus epidermidis*, *Enterococcus faecalis*, *Micrococcus luteus* and *Methicillin-resistant Staphylococcus aureus* are resistance to the activity of the algae compared to the positive control Ampicillin and Vancomycin, this was on the contorary of previously published activity of *A.spicifera* against MRSA [25]. On the other hand the two algal species were showing activities against seven Gram –ve bacteria [*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Serratiaplymuthica*, *Serratiamarcescens* and *Enterobacter cloacae*] with zones of inhibition ranging from [17.2±1.5 to 24.1±0.58 mm] similar to previously reported [26]. Both algal extracts showed higher selective activity against *Pseudomonas aeruginosa* with zone of

inhibition [22.3±2.1 mm with *A.spicifera* and 22.9±0.63 mm with *D.simplex*] compared to the positive control Gentamycine (20.6±1.5 mm) and against *Serratiamarcescens* with zone of inhibition [22.3±1.5 mm with *A.spicifera* and 24.1±0.58 mm with *D.simplex*] compared to the same positive control with (20.4±0.58 mm of inhibition zone).*Acinetobacterbaumannii*, *Salmonella typhi* and*Shigelladysenteriae* are resistance to the effect of the selected algae compared to the positive control Gentamycin. Previous results were different from the reported one that showed the crude e*xtract of Digenea simplex* as a weak growth inhibition zones ranging from 6 to 8 mm against *Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa* [27].Discussing the antifungal activity, the algal extracts were found to be active on the selected species of filamentous fungi against six fungi [*Aspergillusfumigatus*, *Syncephalastrumracemosum*, *Geotricumcandidum*, *Aspergillusniger*, *Candida trobicalis* and *Microsporumcanis]* with zones of inhibition ranging from [18.6±0.63 to 23.8±0.63 mm]. *Geotricumcandidum and Aspergillusniger* are the most sensitive fungal species against both *A.spicifera* with clear zones [23.8±0.63 and 20.6±0.58 mm] and *D.simplex* with clear zones [22.4±2.1 and 21.2±0.63 mm] compared to the positive control Amphotericin B with zone of inhibition [20.3±1.5 and 20.3±0.58 mm].*Candida albicans*, *Cryptococcus neoformans*, *Penicilliumexpansum* and *Trichophytonmentagrophytes* were resistance to the selected algae compared to the positive control Amphotericin B, on the other hand this result was differ from previous reported where the inhibition zone (6 mm) was recorded in methanol extract against *C. albicans* [28].
The presence of flavonoids [29, 30, 31, 32], terpenoides [33], saponins[34] and the strong presence of tanins [35] in the extract showed significant antimicrobial activity.

Table 1.Antibacterial activity against gram + bacteria from red algae extracts.

Tested microorganisms	***Acanthophora spicifera***	***Digenea simplex***	**St.**
	zone of inhibition in mm		
Gram positive bacteria			Ampicillin
Staphylococcus aureus	16.3±2.1	17.1±2.1	22±1.0
Staphylococcus epidermidis	NA*	NA	23±1.0
Streptococcus sanguis	20.6±0.63	21.2±0.63	21.7±1.5
Streptococcus pyogenes	21.4±1.2	21.6±1.2	22.7±1.5
Streptococcus agalactiae	22.5±0.58	23.1±0.58	22.3±1.5
Bacillus subtilis	18.3±0.63	19.3±1.2	25.3±1.5
Enterococcus faecalis	NA	NA	19.3±0.58
Corynebacterium diphtheria	19.3±1.2	20.1±1.5	20±1.0
Micrococcus luteus	NA	NA	19.6±1.5
Methicillin-resistant microorganisms			vancomycine
Methicillin-resistant Staphylococcus aureus MRSA	NA	NA	21.6±2.1 mm

*NA: No activity.

Table 2.Antibacterial activity against gram - bacteria from red algae extracts (zone of inhibition in mm)

Sample Tested microorganisms	*Acanthophora spicifera*	*Digenea simplex*	St.
Gram negative bacteria			Gentamycine
Escherichia coli (RCMB 01002 52-6)	18.9 ± 1.2mm	19.1± 1.2 mm	20.3± 0.85 mm
Proteus mirabilis (RCMB 01002 54-2)	17.2± 1.5 mm	17.8± 1.5 mm	21.2± 1.2 mm
Acinetobacterbaumannii (RCMB 01002 82-9)	NA	NA	23.4± 1.2 mm
Klebsiella pneumonia (RCMB 01002 23-5)	21.4 ± 1.2 mm	21.6 ± 0.72 mm	27.2± 2.1 mm
Pseudomonas aeruginosa (RCMB 01002 43-5)	22.3 ± 2.1 mm	22.9± 0.63 mm	20.6± 1.5 mm
Serratiaplymuthica (RCMB 01002 75-3)	20.2 ± 0.72 mm	20.8 ± 2.1 mm	22.3± 0.58 mm
Serratiamarcescens (RCMB 01002 75b-8)	22.3 ± 1.5 mm	24.1 ± 0.58 mm	20.4± 0.58 mm
Salmonella typhi (RCMB 01002 15-4)	NA	NA	21.1± 0.72 mm
Enterobacter cloacae (RCMB 01002 64-5)	21.3 ± 0.58 mm	21.8± 1.5 mm	22.4± 2.1 mm
Shigelladysenteriae (RCMB 01002 41-8)	NA	NA	21.3± 1.5 mm

RCMB: Regional Center of Mycology and Biotechnology Antimicrobial unit test organism.
NA: No activity.

Table 3. Antibacterial activity against fungi from red algae extracts (zone of inhibition in mm).

Sample Tested microorganisms	***Acanthophora spicifera***	***Digenea simplex***	**St.**
Fungi			Amphotericin B
Aspergillus fumigatus (RCMB 02568)	21.3±1.2 mm	22.4±0.58 mm	25.7±1.5 mm
Syncephalastrumracemosum(RCMB 05922)	19.3±1.2 mm	20.3±1.2 mm	24.3±1.2 mm
Geotricumcandidum (RCMB 05097)	23.8±0.63mm	22.4±2.1 mm	20.3±1.5 mm
Candida albicans (RCMB 05036)	NA	NA	21.3±1.5 mm
Aspergillusniger (RCMB 02724)	20.6±0.58mm	21.2±0.63mm	20.3±0.58mm
Cryptococcus neoformans (RCMB 05642)	NA	NA	21±1.0mm
Candida trobicalis (RCMB 05239)	18.6±0.63mm	20.3±1.2mm	23.7±2.0 mm
Penicilliumexpansum (RCMB 01924)	NA	NA	21.7±2.0mm
Microsporumcanis (RCMB 0834)	20.3±1.2mm	21.9±0.58mm	23.3±1.5 mm

Trichophytonmentagrophytes(RCMB 0925)	NA	NA	21.3±1.5mm

Data in table 1,2,3 are represented as mean zone of inhibition ± standard deviation.
RCMB: Regional Center of Mycology and Biotechnology Antimicrobial unit test organism.
NA: No activity.

3.3. Anti-inflammatory activity

In this study table and figure no. 4 represent the levels of SOD, MDA, Catalase, GSH, IL-6 and TNF-Alpha in the liver tissue homogenates of the normal and treated groups. Each parameter showed significant different degree in the disease state compared to the normal 'control' state. As it observed significant decreased in the levels of SOD from (72.03±6.49 to 5.93±0.31 U/ml), Catalase from (75.68±2.6 to 6.96±0.31 U/ml) and GSH from (58.85±3.6 to 5.03±0.29µmol/ml). Also observed that significant increase in the inflamed liver state of MDA from (20.31±0.90 to 133.56±5.10nmol/ml), IL-6 from (15.51±1.04 to 144.1±5.3Pg/ml) and TNF-Alpha from (23.89±1.07 to 257.56±11.85Pg/ml). After treatment of inflamed liver rats with crude extracts using a dose of 500mg/kg of each drug, observed that the difference in the parameters levels relatively equal to the normal control results.

Table 4.Anti-inflammatory activity of the studied algal species.

parameter **Group**	**SOD (U/ml)**	**MAD (nmol/ml)**	**Catalase (U/ml)**	**GSH (µmol/ml)**	**IL-6 (Pg/ml)**	**TNF-Alpha (Pg/ml)**
Group A	72.03±6.49	20.31±0.90	75.68±2.6	58.85±3.6	15.51±1.04	23.89±1.07
Group B	5.93±0.31	133.56±5.10	6.96±0.31	5.03±0.29	144.1±5.3	257.56±11.85
Group C *(Acanthophoraspicifera)*	69.18±0.51	16.61±0.30	70.46±0.40	56.01±0.58	18.35±1.03	29.08±1.04
Group D *(Digenea simplex)*	70.18±0.73	18.25±0.29	72.3±0.36	56.63±0.33	19.63±1.02	26.58±1.95
F-Prob	P<0.0001	P<0.0001	P <0.0001	P<0.0001	P<0.0001	P<0.0001
LSD at5%	9.68	7.67	3.98	5.43	8.26	15.16
LSD at1%	13.20	10.46	5.43	7.41	11.27	22.32

Data in table 4 expressed as Mean ± S.E.M = Mean values ± Standard error of means of six experiments. Number of animals in each group is six.
Statistical evaluation of results was performed using the one-way ANOVA and least significant difference was determined at the level of LSD at 5% and LSD at 1%.
Test drugs: significant from normal control, P < 0.0001.
SOD: Superoxide Dismutase, MAD: Malonialdehyde, GSH: Glutathione, IL-6: Interleukin-6, TNF-α:Tumor necrosis factor.
Group A: Normal control, Group B: Diseased or inflammated Liver cancer (positive control receiving saline), Group C and D:Inflammated liver rats were treated by injection with methanol extract of the first drug (Acanthophoraspicifera) and the second drug (Digenea simplex).

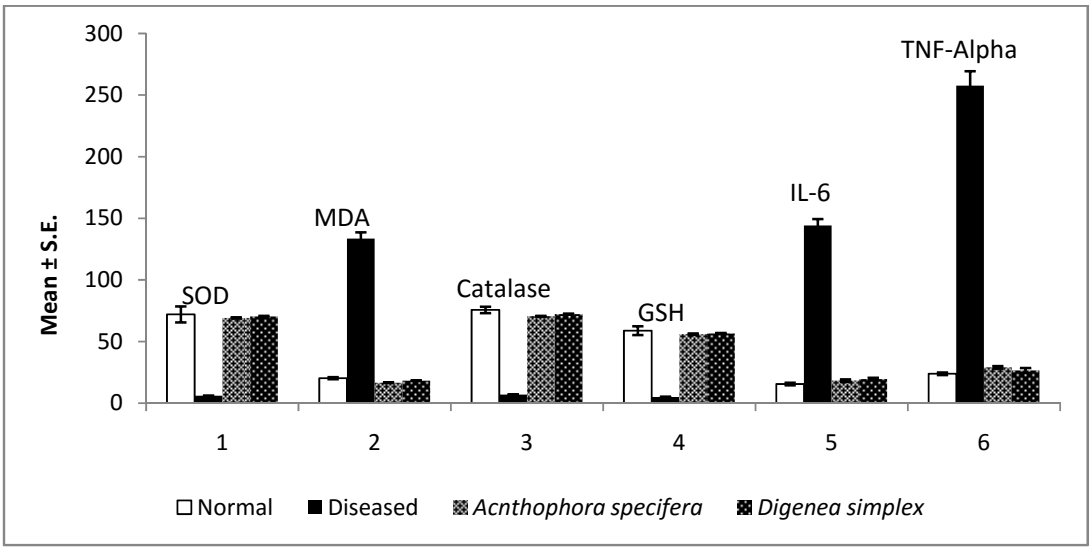

Fig. 4: Anti-inflammatory activity of *Acanthophora spicifera* and *Digenea simplex* on inflamed liver Wister Albino rats.

4. CONCLUSION

The bioactive components from seaweeds crude extract have antimicrobial activity on some human pathogenic bacterial species and unicellular and filamentous fungi and anti-inflammatory effect which might protect the human health against some oxidative stress which attack DNA, proteins and membrane lipids and induced cellular damage.

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