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# Antimicrobial and Anti-inflammatory Activities of Some Marine Brown and Red Algal Species Collected from Safaga Seashore, Red Sea, Egypt

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Estimation of the biological activities of 3 algal crude extracts for anti-inflammatory and antimicrobial activities.
**Methodology:** Three marine algae were collected from Safaga seashores, the Red Sea in Egypt, extracted using 70% hydroalcoholic methanol. The antimicrobial activities were carried out using agar disc diffusion method, in addition to the In vivo anti-inflammatory investigation.
**Results:** The three algal extracts showed significant antimicrobial activity against most of the microbial strains. Moreover, the three extracts showed explicit anti-inflammatory effects by determination different inflammation parameters as *Superoxide dismutase (SOD), Malondialdehyde (MDA), Catalase, Glutathione (GSH), Interleukin-6(IL-6) and Tumor necrosis factor ( TNF-α).*
**Conclusion:** The Three algae, has a notable antimicrobial and anti-inflammatory activity, that may be due to their unique bioactive secondary metabolites pattern.

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*Keywords: Marine algae; antimicrobial; anti-inflammatory; Cystoseira barbata; Hydroclathrus clathratus; Liagora farinosa.*

## 1. INTRODUCTION

*Macro algae* have shown to play an important role in discovering new bioactive metabolites, that may play efficient tools in the treatment of different types of diseases [1]. Saking of several biological characteristics of algae, it was known as prolific and contains a normal fortune of bioactive components, that may be present in large amounts to be utilized as practical elements for human and health purposes [2-3]. There are considerable numbers of brown algae in the world; some of them have been widely plowed [4]. The red algae were regarded the most vital source due to their contents of plentiful biologically active substances compared to other algal species [5]. Seaweeds showed abundant resources of lipids, omega-3 fatty acids, proteins, and polysaccharides as well as including a large number of bioactive materials such as minerals, polysaccharides and various kinds of vitamins" A, B, C and E [6]. Consequently, seaweeds became a fantastic possibility as a precious source for the isolation of complexes. Due to the prolific amount of bioactive components contained in algae, various biological activities have been detected such as antimicrobial and anti-inflammatory [7-8]. This work aims to screen for the antimicrobial and anti-inflammatory activities of three collected algal species related to brown and red algae.

## 2. MATERIALS AND METHODS

### 2.1 Studied Area

Three fresh algal species were collected from Safaga seashores (Fig. 1) along 53 Km South of Hurghada at the Red Sea in Egypt during the spring period, April 2015, at the depth of 1-3 m under the sea surface.

### 2.2 Collection and Identification

The fresh algal species were collected from the inter-tidal region of Safaga city which located in 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. The 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℃ until constant weight was obtained, then was grind into a fine powder using an electric mixer and stored at 4℃ for future use. Algal species were identified [9-10].

### 2.3 Extraction of the Selected Algal species

All algal powders, *Cystoseira barbata, Hydroclathrus clathratus and Liagora farinosa* (400, 300 and 500 gram) respectively, were extracted using 70% of hydroalcoholic mathanol, then dried under reduced pressure using rotary-evaporators and kept for further biological investigations.

### *2.*4 Antimicrobial Activity

#### 2.4.1 Tested microorganisms used

Thirty microorganisms were used for testing, 10 Gram Positive, 10 Gram Negative bacteria and 10 fungi as showed.

*2.4.1.1 Gram-negative*

*Escherichia coli* (RCMB 01002 52-6), *Proteus mirabilis* (RCMB 01002 54-2), *Acinetobacter baumannii* (RCMB 01002 82-9), *Klebsiella pneumonia* (RCMB 01002 23-5), *Pseudomonas aeruginosa* (RCMB 01002 43-5), *Serratia plymuthica* (RCMB01002 75-3), *Serratia marcescens* (RCMB 01002 75b-8), *Salmonella typhi* (RCMB 01002 15-4), *Enterobacter cloacae* (RCMB 01002 64-5), *Shigella dysenteriae* (RCMB 01002 41-8).

*2.4.1.2 Gram-positive*

*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), *Corynebacterium diphtheriae* (RCMB 01001 26-7) ,*Micrococcus luteus* (RCMB 01001 76-9), *Methicillin-resistant Staphylococcus aureus (MRSA)* (RCMB 01001 94-5).

*2.4.1.3 Fungi*

*Aspergillus fumigates* (RCMB 02568), *Syncephalastrum racemosum* (RCMB 05922),

*Geotricum candidum* (RCMB 05097), *Candida albicans* (RCMB 05036), *Aspergillus niger* (RCMB 02724), *Cryptococcus neoformans* (RCMB 05642), *Candida trobicalis* (RCMBA 05239), *Penicillium expansum* (RCMB 01924), *Microsporum canis* (RCMB 0834) , *Trichophyton mentagrophytes* (RCMB 0925) .

### 2.4.2 Methods

#### 2.4.2.1 Well-diffusion method for antibacterial activity

The solution of 50 mg/ml of each sample in dimethyl sulfoxide (DMSO) was prepared for testing against bacteria. Centrifuged pellets of bacteria from 24/h old culture containing approximately $10^4$-$10^6$ CFU (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 sterile metallic bores and then cooled down to 45ºC. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100 µ1 of the tested samples were loaded into the wells of the plates. DMSO was loaded as control. The plates were examined for the formation of the zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. Gentamycine, Ampicillin and Vancomycine were used as positive control [11].

#### 2.4.2.2 Well-diffusion method for antifungal activity

The antifungal activity was investigated by agar well diffusion method as follow: 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 sterilized petridish in laminar flow and left for 20 min to form the solidified sabourad dextrose agar plate. These plates were inverted and kept at 30ºC in the incubator to remove the moisture and check for any contamination. Antifungal assay: Fungal strains was grown in 5 ml sabourad dextrose broth (glucose: Peptone; 40:10) for 3-4days to achieve $10^5$ CFU/ml cells. The fungal culture (0.1 ml) was spread out uniformly on the sabourad dextrose agar plates. Now small wells of size (4 mm x20 mm) were cut into the plates with the help of well cutter and bottom of the wells were seales with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100 µ1 of the tested samples (10 mg/ml) were loaded into the wells of the plates. All samples were prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 30ºC for 3-4 days and then the plates were examined for the formation of the 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 drugs [11].

## 2.5 Anti-Inflammatory Activity

Anti-inflammatory effect of hydroalcoholic methanolic extracts of a two brown and a single red marine alga was examined at Kasr El Aini Hospital laboratory. Wister albino rats (140-200 gm) were obtained from animal house of National Research Centre, Dokki – Giza, Egypt. Rats were fed on a standard diet and free access to tap water [12]. The study was approved by Institutional Animal Ethics Committee of National Research Center that the animal doesn't suffer at any stage of experiment and maintained in accordance with the Guide for the Care and Use of Laboratory Animals. Inflamed rats induction was according to [13]. Rats are classified into three groups, six rats in each one, as follows: Group I, Normal (control); Group II, diseased group (inflamed liver rats); Group III, Treated liver rats with methanolic extracts. 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℃ for further determinations. The test was utilized to determine the biological properties of some marine algae by exhibiting the inflammation that results in dangerous damages and to determine various pharmacological actions by using various parameters kits such as SOD, MAD, CATALASE, GSH, IL-6, AND TNF-alpha. By measuring the different parameters in each group, the anti-inflammation can be achieved.

### 2.5.1 Statistical work

The results for the anti-inflammatory activity was compared by using analysis of variance and tukey test at $P$ <0.0001 using statistical software spss windows ( by using one-way anova) version 20.


**Fig. 1. Safaga location map, Rea Sea, Egypt**

## 3. RESULTS AND DISCUSSION

### 3.1 Sampling and Identification

The three marine macroalgae were collected were identified, as 2 species belonging to Ochrophyta (*Phaeophyceae*, brown algae) and to Rhodophyta (red algae) respectively, (*Cystosiera barbata*, *Hydroclathras clathratus* and *Liagora farinosa*). Phaeophyceae was showed at 1-2 m while Rhodophyta manifested at 1-6 m and more. The three algal species was shown in Fig. 2.

### 3.2 Biological Activities Investigations

#### 3.2.1 Antimicrobial results of the selected algae

The antimicrobial results were showed in Tables 1-3. All the algal extracts showed strong activities against six *Gram -ve* bacteria (Table 1), {*Escherichia Coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi and Shigella dysenteriae*} with activity range from (20.3±0.85 to 27.2±2.1 mm of inhibition zone) compared to the positive control. Nevertheless, *H. clathratus* was found to be more active than the positive control, (22.4 ± 1.5), against *Serratia marcescens* where the positive control (20.4±0.58). On the other hand the three algal extracts showed powerful strong activities against seven *Gram +ve* bacteria, (Table 2), (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus sanguis, Streptococcus agalactiae, Bacillus subtilis,* and *Corynebacterium diphtheria*) with activity range from (19.3±0.58 to 23±1.0 mm of the inhibition zone) compared to the positive control as well as moderate activities againist *Methicillin-resistant Staphylococcus aureus* (MRSA). The algal extracts were active as antifungal against four fungi, (Table 3), (*Aspergillus fumigatus, Geotricum candidum, Aspergillus niger, Microsporum canis*) with activity range from (20.3±0.58 to 25.7±1.5) compared to the positive control. The extracts showed no activities of 4 *Gram–ve* except *Serratia marcescens* microoraganism which was sensitive to *H.clathratus* which showed a zone of inhibition r (22.4 ± 1.5) compared to the positive control "gentamycine" (20.4±0.58). Furthermore, the three algal extracts showed no activities against 3 *Gram+ve* (*Streptococcus pyogenes, Enterococcus faecalis, Micrococcus luteus*). Moreover, all extracts were inactive against 4 fungi (*Syncephalastrum racemosum, Cryptococcus neoformans, Candida trobicali* and *Trichophyton mentagrophytes*). On the contrary, *Candida albican* and *Penicillium expansum* were sensitive to the extracts of *C. barbata* and *H. clathratus*. Table 1 showed that the crude extract of *H. clathratus* was the most active one as antimicrobial agent followed by *L. farinosa* and *C.barbata* extracts. Moreover, the *H. clathratus* extract showed higher activity against *Pseudomonas aeruginosa* (26.4±1.2 mm), even more than the positive control. Table 3 showed that *H.clathratus* extract was significantly active against *MRSA* followed by *L. farinosa* and *C. barbata*.

Previous results may be correlated to the different bioactive profiling of different algal species [14-15]. In this study, *C. Barbata* showed significant antimicrobial activity against *Pseudomonas aeruginosa, staphylococcus epidermis, bacillus subtilis* and *candida albicans*, this was the first report of these activities. On the other hand, it showed an activity against *E.coli, Salmonella typhi,* and *Streptococcus aureus*, with no activity against *Enterobacter cloacae* as previously reported [16]. *H. clathratus* extract showed significant inhibitory activity against *E.coli*, *P.aeruginosa, S.aureus, c. albicans, klebsiella* and *B.subtilis*. Furthermore, *L. farinose* extract showed significant activity against *S.aureus* and *B. subtilis* but with no activity against *candida albicans*, on the contrary of what published previously by Ibrahim et al [7].

### 3.2.2 Anti-inflammatory activity

Table 4 and Fig. 3 showed the SOD, MAD, CATALAS, GSH, TNF-ALPHA and IL-6 levels in liver tissue homogenates of normal control and treated groups. In response to the inflamed liver state, the different parameters showed significant different degrees in their levels as compared to normal control. As is observed, SOD, Catalase, and GSH showed a remarkable decrease in their levels compared to normal control. On the other hand, a significant increase in MAD, IL-6 and TNF-Alpha levels compared to normal control. Inconsiderable change has been showed in the different parameters as a result of treatment of inflamed liver rats with different algal extracts using a dose of 500 mg/kg compared to normal control group.

Previous results showed that all the algal extracts were potent to treat the inflammation and normalize the tissue necrotic parameters. This may be due to the different compositions of these extracts as previously mentioned in the literature [17]. Oxidative stress has implicated in many diseases such as lung disease, atherosclerosis as well as gastrointestinal dysfunction which are all related to inflammatory reactions [18- 20].

Active antibacterial extracts from different marine algae have been found to be made up of saturated and unsaturated fatty acids with a predominance of myristic, palmitic, oleic and eicosapentaenoic acids [21] and Terpenoid [22]. Also, Flavonoids comprise a large group of naturally occurring compounds widely distributed

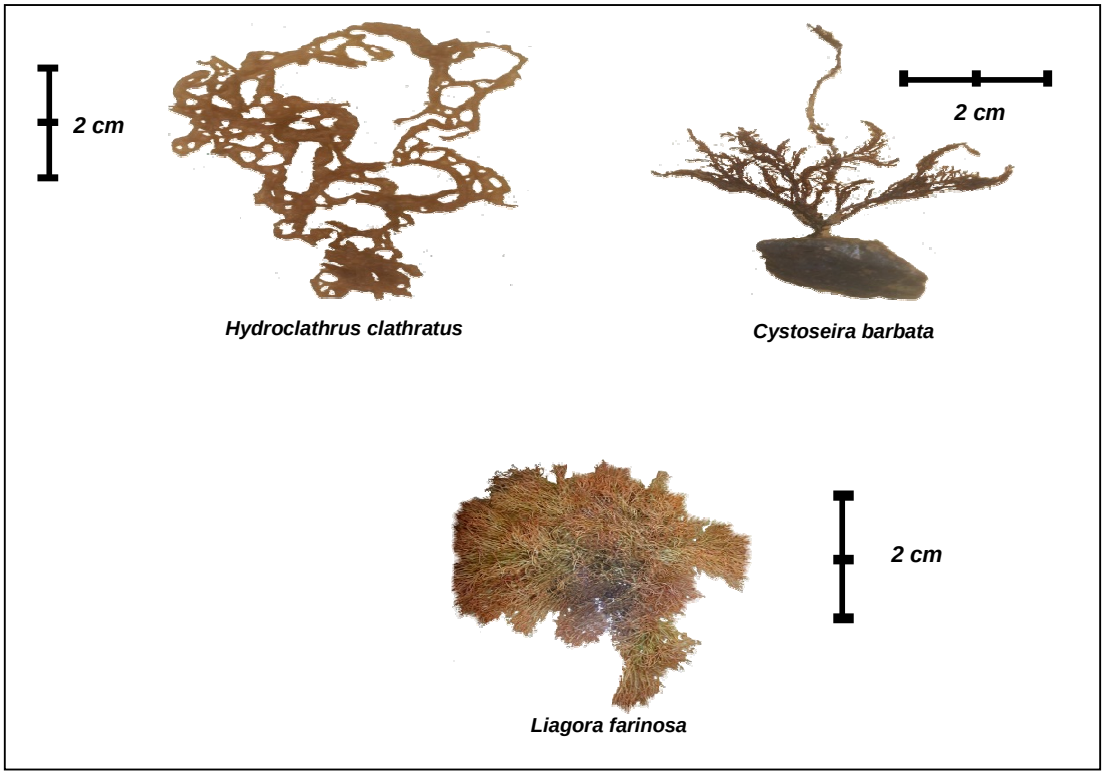


**Fig. 2. Algal species collected from the Red Sea**

**Table 1. Antimicrobial activity of marine algae crude extracts against gram negative**

| Sample / Tested microorganisms | *Cystoseira barbata* | *Hydroclathrus clathratus* | *Liagora farinosa* | St. |
|---|---|---|---|---|
| **Gram negative Bacteria** | | | | **Gentamycine** |
| *Escherichia coli (RCMB 01002 52-6)* | 19.6± 1.2 | 22.1± 0.58 | 21.3 ± 2.1 | 20.3± 0.85 |
| *Proteus mirabilis (RCMB 01002 54-2)* | 17.2± 1.5 | 20.8± 2.1 | 19.6± 1.2 | 21.2± 1.2 |
| *Acinetobacter baumannii (RCMB 01002 82-9)* | NA | NA | NA | 23.4± 1.2 |
| *Klebsiella pneumonia (RCMB 01002 23-5)* | 20.4 ± 1.2 | 23.6 ±0.72 | 22.4 ±0.58 | 27.2± 2.1 |
| *Pseudomonas aeruginosa (RCMB 01002 43-5)* | 21.3± 1.5 | 26.4± 1.2 | 25.4 ± 1.2 | 20.6± 1.5 |
| *Serratia plymuthica (RCMB 01002 75-3*) | NA | NA | NA | 22.3± 0.58 |
| *Serratia marcescens (RCMB 01002 75b-8)* | NA | 22.4 ± 1.5 | NA | 20.4±0.58 |
| *Salmonella typhi (RCMB 01002 15-4)* | 16.4 ± 1.2 | 21.2 ± 0.58 | 20.9±0.58 | 21.1± 0.72 |
| *Enterobacter cloacae (RCMB 01002 64-5)* | NA | NA | NA | 22.4± 2.1 |
| *Shigella dysenteriae (RCMB 01002 41-8)* | 18.3± 1.5 | 21.8± 1.5 | 21.4 ±1.2 | 21.3± 1.5 |

**Table 2. Antimicrobial activity of marine algae crude extracts against gram positive**

| Sample / Test microorganism | *Cystoseira barbata* | *Hydroclathrus clathratus* | *Liagora farinosa* | St. |
|---|---|---|---|---|
| **Gram Positive Bacteria** | | | | **Ampicillin** |
| *Staphylococcus aureus* (RCMB 010027) | 19.3±1 | 23.3±1.5 | 21.3±1.2 | 22±1.0 |
| *Staphylococcus epidermidis* (RCMB 010024) | 20.1±1.5 | 21.2±0.58 | 20.3±0.58 | 23±1.0 |
| *Streptococcus sanguis* (RCMB 01001 71-3) | 17.8±0.58 | 20.4±0.63 | 19.3±1.5 | 21.7±1.5 |
| *Streptococcus pyogenes* (RCMB 01001 74-2) | NA | NA | NA | 22.7±1.5 |
| *Streptococcus agalactiae*(RCMB 01001 73-2) | 19.4±1.5 | 22.3±1.2 | 20.9±2.1 | 22.3±1.5 |
| *Bacillus subtilis* (RCMB 01001 693) | 13.1±2 | 16.3±2.1 | 14.3±2.1 | 25.3±1.5 |
| *Enterococcus faecalis* (RCMB 01001 54-2) | NA | NA | NA | 19.3±0.58 |
| *Corynebacterium diphtheriae*(RCMB 01001 26-7) | 17.9±0.58 | 21.8±1.2 | 19.6±0.58 | 20±1.0 |
| *Micrococcus luteus* (RCMB 01001 76-9) | NA | NA | NA | 19.6±1.5 |
| Methicillin-resistant microorganisms | | | | vancomycine |
| *Methicillin-resistant Staphylococcus aureus MRSA (RCMB 01001 94-5)* | 12.2±1 | 16.4±2.1 | 14.3±1 | 21.6±2.1 |

**Table 3. Antimicrobial activity of marine algae crude extracts against Fungi**

| Samples / Tested microorganisms | *Cystoseira barbata* | *Hydroclathrus clathratus* | *Liagora farinosa* | St. |
|---|---|---|---|---|
| **Fungi** | | | | ***Amphotericin B*** |
| *Aspergillus fumigatus* (RCMB 02568) | 21.4±0.63 | 22.3±0.58 | 14.2±1.2 | 25.7±1.5 |
| *Syncephalastrum racemosum* (RCMB 05922) | NA | NA | NA | 24.3±1.2 |
| *Geotricum candidum* (RCMB 05097) | 19.4±1.2 | 25.4±0.63 | 12.2±2.1 | 20.3±1.5 |
| *Candida albicans* (RCMB 05036) | 21±1 | 22.3±1.2 | NA | 21.3±1.5 |
| *Aspergillus niger* (RCMB 02724) | 20.9±1.2 | 21.3±0.58 | 14.7±0.58 | 20.3±0.58 |
| *Cryptococcus neoformans* (RCMB 05642) | NA | NA | NA | 21±1.0 |
| *Candida trobicalis* (RCMBA 05239 ) | NA | NA | NA | 23.7±2.0 |
| *Penicillium expansum* (RCMB 01924) | 18.6±0.63 | 20.4±1.2 | NA | 21.7±2.0 |
| *Microsporum canis* (RCMB 0834) | 17.6±2.1 | 17.3±1.5 | 10.3±1.2 | 23.3±1.5 |
| *Trichophyton mentagrophytes* (RCMB 0925) | NA | NA | NA | 21.3±1.5 |

*In Tables 1, 2 and 3. Data are expressed in the form of the mean zone of inhibition in mm ± Standard deviation. Well diameter: 6.0 mm, (100µl was tested), RCMB: Regional Center for Mycology and Biotechnology Antimicrobial Unit test organism, *NA: No activity*

in the marine algae and some of these compounds [23]. So, the antibacterial activities of the algae tested could be attributed to the type and amount of free fatty acids which have a role in the overall defense against the studied pathogenic Gram-positive and Gram-negative

bacteria [24]. According to the previous reports in literature, marine algae are rich sources of dietary fiber, minerals, proteins and vitamins, and anti-oxidant activity of these seaweeds would elevate their value in the human diet as food and pharmaceutical supplements. The results of the present investigation on selected species of marine algae indicated scope for deriving biologically active compounds which are effective in inhibiting the growth of the pathogenic bacteria both Gram-positive and Gram-negative. Further, the Red sea marine environment of the Red Seashore has potential to return pharmaceutically useful seaweeds which can be harnessed for the development of drugs for use in the management of human pathogens, cancer, tumor, AIDS and many human degenerative diseases. There is great scope for further investigations toward drug development.

**Table. 4 *In vivo* anti-inflammatory activity of the crude extracts *Cystoseira Barbata, Hydroclathrus clathratus and liagora farinosa***

| Parameter / Group | SOD (U/ml) | MAD (nmol/ml) | Catalase (U/ml) | GSH ( µmol/ml) | IL-6 (Pg/ml) | TNF-Alpha Pg/ml |
|---|---|---|---|---|---|---|
| Group A | 66.73±3.61 | 20.43±1.02 | 76.18±2.71 | 59.2±3.57 | 15.68±1.01 | 22.75±0.97 |
| Group B | 5.86±0.32 | 133.75±5.56 | 7.03±0.30 | 5.01±0.29 | 144.61±5.42 | 259.4±12.96 |
| *Cytoseira barbata* | 59.73±4.20 | 27.68±0.7 | 66.85±3.65 | 51.20±1.15 | 19.85±1. 46 | 29.98±1.46 |
| *Hydroclathrus clathratus* | 57.60±1.38 | 27.13±1.08 | 66.01±2.24 | 54.2±4.60 | 20.85±2. 08 | 25.68±1.91 |
| *Liagora Farinosa* | 57.5±0.90 | 23.73±0.98 | 61.70±1.66 | 54.56±3.02 | 17.36±0. 85 | 25.88±1.34 |
| F-Prob | P>0.0001 | P>0.0001 | P >0.0001 | P>0.0001 | P>0.0001 | P>0.0001 |
| LSD at5% | 7.55 | 8.24 | 6.97 | 8.71 | 7.99 | 17.31 |
| LSD at1% | 10.21 | 11.15 | 9.43 | 11.78 | 10.81 | 23.42 |

*In Table 4, Data are represented as Mean ±SE (Standard errors). Number of animals in each group is 6. SOD: Superoxide Dismutase; MAD: TBARS; GSH: Glutathione; IL-6: Interleukin-6; LSD: Least significance of difference Group A: negative control group (normal); group B: positive control Inflamed liver group (Diseased); group C: hydroalcoholic extract treatment group*

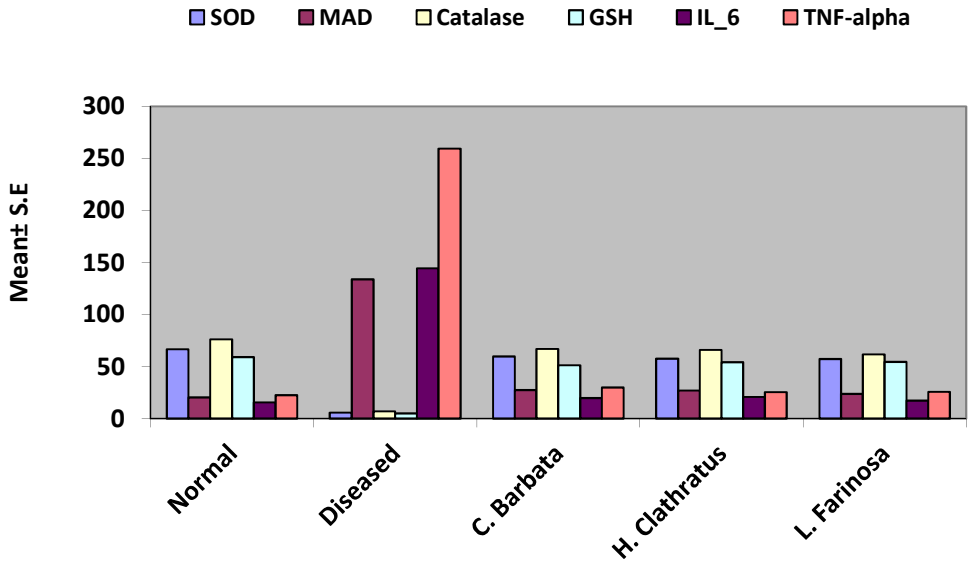


**Fig. 3 Anti-inflammatory activity of *Cystoseira barbata, Hydroclathrus clathratus* and *Liagora farinosa* methanolic extract in alcoholic inflamed liver Wister albino rats**

*SOD: Superoxide Dismutase; MAD: Malondialdehyde; Catalase; GSH: Glutathione; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor*

## 4. CONCLUSION

The current study showed that the three crude algal extracts have potent anti-microbial and anti-inflammatory activity. The degree of potency varied due to the different biochemical profiling that needs further investigations.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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