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*Original article*

# Antibacterial Activity of Extracts from Three Marine Seaweeds (*Gongolaria barbata*, *Laurencia obtusa*, and *Cladophora glomerata*) Collected from the Al-Hamama Coast, Libya

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**Abstract**
The spread of bacteria that no longer respond to antibiotics has made the discovery of alternative antibacterial agents of natural origin a pressing priority. In this work, three macroalgae gathered in June 2025 from the Al-Hamama coast of northeastern Libya, namely *Gongolaria barbata* (formerly *Cystoseira barbata*), *Laurencia obtusa*, and *Cladophora glomerata*, were screened for antibacterial activity. Powdered dry biomass of each alga was extracted with aqueous methanol, aqueous ethanol, and diethyl ether, and each extract was assayed by agar well diffusion on clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella* sp., and *Escherichia coli*. Disk diffusion was used to establish how the same isolates responded to amoxicillin–clavulanic acid, ciprofloxacin, and doxycycline. Every extract gave a zone wider than the well itself, although in several cases only marginally, and within each isolate the zone diameters varied significantly with the solvent ($p < 0.05$). For the two Gram-positive isolates, the strongest inhibition came from the diethyl ether extracts, whereas for *Salmonella* sp. the aqueous methanol extracts ranked first in all three algae. Of the three species, *L. obtusa* was the most potent; its diethyl ether extract yielded the widest zone recorded (16.50 mm, including the 6 mm well), against *S. pyogenes*. The aqueous ethanol extracts were, in general, the weakest. Susceptibility was recorded for ten of the 12 isolate-antibiotic pairings, and all four isolates were susceptible to ciprofloxacin. Taken together, the findings point to these macroalgae, and above all the diethyl ether extract of *L. obtusa*, as candidate sources of antibacterial compounds that deserve further study.
**Keywords.** Seaweeds; Antibacterial activity; *Gongolaria barbata*; *Laurencia obtusa*; *Cladophora glomerata*.

## Introduction

Seaweeds, or marine macroalgae, are photosynthetic inhabitants of coastal waters that add considerably to the biodiversity and productivity of the sea. Based on their pigments and biochemical make-up, they fall into three principal divisions: the green algae (Chlorophyta), the brown algae (Phaeophyceae), and the red algae (Rhodophyta). Interest in these organisms has grown markedly over the past years because they yield many biologically active substances of possible pharmaceutical value. Among the secondary metabolites they produce are phlorotannins, terpenoids, alkaloids, halogenated molecules, and sulfated polysaccharides, to which antibacterial, antifungal, antiviral, and antioxidant properties have been ascribed [1,2]. At the same time, bacterial resistance to antibiotics is spreading quickly and now ranks among the most serious threats to public health worldwide, as it erodes the efficacy of the antimicrobial drugs in routine use. Because resistant strains occur among Gram-positive as well as Gram-negative species, there is a growing urgency to look to other natural sources for novel antimicrobial substances [3].

Several seaweed extracts are known to suppress pathogens of clinical importance, *Staphylococcus aureus* and *Escherichia coli* among them [4-6]. As an illustration, methanol extracts of the brown alga *Cystoseira barbata* collected in the Aegean Sea were active against a wider range of bacteria than those of the other algae examined in the same study [4]. How strongly a seaweed extract inhibits bacteria is determined both by the species of alga and by the solvent chosen, because solvents differing in polarity recover different classes of compounds [7]. Little work has so far addressed the antibacterial properties of marine algae from Libya [8]. Accordingly, this study examined aqueous methanol, aqueous ethanol, and diethyl ether extracts prepared from three macroalgae of the Al-Hamama coast, northeastern Libya, namely *Gongolaria barbata* (formerly *Cystoseira barbata*), *Laurencia obtusa*, and *Cladophora glomerata*, for their activity against clinical isolates of *S. aureus*, *Streptococcus pyogenes*, *Salmonella* sp., and *E. coli*. For comparison, the response of the same isolates to standard antibiotics was also assessed.

## Methods

### Study area and sample collection

Collecting seaweeds by hand is a long-established practice that is still widely used for species occurring naturally along the shore [9]. The present work, carried out in 2025, examined three marine macroalgae for antibacterial activity: *Gongolaria barbata* (Stackhouse) Kuntze [formerly *Cystoseira barbata* (Stackhouse) C. Agardh], *Laurencia obtusa* (Hudson) J.V. Lamouroux, and *Cladophora glomerata* (Linnaeus) Kützing. The algae were hand-picked in June 2025 at depths of 0.5–2 m in the shallow subtidal zone of the Al-Hamama coast (32°54'52" N, 21°37'43" E). This site lies on the Mediterranean shore of northeastern Libya, in the Al-Jabal Al-Akhdar region, some 20–25 km north of the city of Al-Bayda. Its shoreline is dominated by rocky substrates and related coastal habitats, which offer macroalgae suitable surfaces for attachment.

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### Sample preparation and taxonomic identification

To free the collected algae of plankton and debris, they were rinsed several times in seawater and then with water [10]. After cleaning, the material was left to dry in air for seven days and was subsequently placed in an oven at 40 °C for 20–30 min so that any remaining moisture was driven off. An electric mill was used to reduce the dry biomass to a fine powder, which was kept at −20 °C until required [11]. Taxonomic identification of the specimens relied on [12], [13], [14], [15], and [16], and the species names were checked against Algae Base [17]. This work was done in the Department of Botany of the Faculty of Science at Omar Al-Mukhtar University.

### Preparation of algal extracts

For each species, 20 g of dried algal powder was extracted separately in 100 mL of each of three solvents: aqueous methanol (methanol:water, 80:20, v/v), aqueous ethanol (ethanol:water, 80:20, v/v), and diethyl ether. The flasks were tightly sealed and agitated at 100 rpm in a shaker incubator at room temperature for 24 h. Each mixture was then filtered through Whatman No. 1 filter paper, and the solvent was removed from the filtrate at 35 °C using a rotary evaporator under reduced pressure until a dry residue remained. The resulting crude extracts were individually redissolved in 5 mL of their respective original extraction solvents and stored at −20 °C until further use in the antibacterial assays [8].

### Test microorganisms

Each algal extract was assayed individually against four pathogenic bacteria, two of them Gram-positive (*S. aureus* and *S. pyogenes*) and two Gram-negative (*Salmonella* sp. and *E. coli*). All four were clinical isolates supplied by the Department of Microbiology, Al-Bayda Hospital, Libya; their identity had been established by a specialist microbiologist using standard microbiological and biochemical methods.

### Agar well diffusion assay

The agar well diffusion technique [18], [19] was used to assess antibacterial activity. Each test bacterium was spread with a sterile swab across the whole surface of a Mueller-Hinton agar plate. Wells of 6 mm diameter were then cut in the agar with a sterile cork borer, and each well received 50 µL of extract. The plates were incubated for 24 h at 30 °C, after which a clear zone surrounding a well was taken to indicate antibacterial activity. Zone diameters were recorded in millimetres and included the diameter of the well.

### Antibiotic susceptibility testing

Disk diffusion was used to determine how the bacterial isolates responded in vitro to three antibiotics (Table 1). To prepare each inoculum, well-separated colonies were picked from a nutrient agar plate grown overnight and suspended in 2 mL of tryptone soya broth (TSB). After adjustment of the suspension, a sterile cotton swab was immersed in it, turned several times, and squeezed against the inside of the tube above the liquid so that surplus inoculum drained off. The whole surface of a Mueller-Hinton agar plate was swabbed three times in total, and the plate was turned between applications so that the inoculum was spread uniformly. Once the plates had dried for 5 min, a disk dispenser was used to apply the antibiotic disks to the seeded agar, and incubation followed at 37 °C for 18–22 h. Inhibition zone diameters were then read with a ruler to the nearest millimetre, and each isolate was classed as susceptible (S) or resistant (R) following [20].

***Table 1. Antibiotics used in the susceptibility tests.***

| Antibiotic | Spectrum | Symbol | Disk content (µg/disk) | Mode of action |
|---|---|---|---|---|
| Amoxicillin–clavulanic acid | Broad spectrum | AMC | 20/10 | Inhibits cell wall synthesis (clavulanic acid inhibits β-lactamase) |
| Ciprofloxacin | Broad spectrum | CIP | 30 | Inhibits DNA gyrase and topoisomerase IV (DNA synthesis) |
| Doxycycline | Broad spectrum | DOX | 30 | Inhibits protein synthesis (binds the 30S ribosomal subunit) |

### *Statistical Analysis*

Three replicates were run for every experiment, and values are reported as the mean ± standard error of the mean (SEM, n = 3). For each bacterial isolate, the inhibition zone diameters (mm) obtained with the three extraction solvents were compared using one-way analysis of variance (ANOVA), and Tukey's honestly significant difference (HSD) test was then applied as the post hoc procedure [21].

## Results

### Antibacterial activity of seaweed extracts

[Table 2] summarizes the results of the agar-well diffusion assay performed using aqueous ethanol, aqueous methanol, and diethyl ether extracts of the three seaweed species (*G. barbata*, *L. obtusa*, and *C. glomerata*) against the four bacterial isolates.

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***Table 2. Comparison of inhibition zone diameters (mm) among the three seaweed species for each bacterial isolate and extraction solvent.***

| Bacterial isolate | Solvent extract | *G. barbata* | *L. obtusa* | *C. glomerata* |
|---|---|---|---|---|
| ***Escherichia coli*** | Aqueous ethanol | 6.80 ± 0.06 $^{b}$ | 7.20 ± 0.05 $^{a}$ | 6.50 ± 0.03 $^{c}$ |
| | Aqueous methanol | 8.77 ± 0.03 $^{b}$ | 9.50 ± 0.04 $^{a}$ | 7.90 ± 0.04 $^{c}$ |
| | Diethyl ether | 7.03 ± 0.03 $^{b}$ | 11.10 ± 0.06 $^{a}$ | 7.10 ± 0.05 $^{b}$ |
| ***Staphylococcus aureus*** | Aqueous ethanol | 8.47 ± 0.03 $^{b}$ | 9.10 ± 0.03 $^{a}$ | 7.80 ± 0.05 $^{c}$ |
| | Aqueous methanol | 10.10 ± 0.06 $^{b}$ | 12.30 ± 0.05 $^{a}$ | 9.40 ± 0.03 $^{c}$ |
| | Diethyl ether | 13.10 ± 0.06 $^{b}$ | 14.20 ± 0.04 $^{a}$ | 11.20 ± 0.06 $^{c}$ |
| ***Streptococcus pyogenes*** | Aqueous ethanol | 7.50 ± 0.06 $^{b}$ | 8.40 ± 0.06 $^{a}$ | 7.00 ± 0.04 $^{c}$ |
| | Aqueous methanol | 11.40 ± 0.06 $^{b}$ | 13.00 ± 0.03 $^{a}$ | 10.10 ± 0.05 $^{c}$ |
| | Diethyl ether | 15.10 ± 0.06 $^{b}$ | 16.50 ± 0.05 $^{a}$ | 12.80 ± 0.04 $^{c}$ |
| ***Salmonella* sp.** | Aqueous ethanol | 9.27 ± 0.03 $^{b}$ | 10.10 ± 0.04 $^{a}$ | 8.50 ± 0.06 $^{c}$ |
| | Aqueous methanol | 12.10 ± 0.06 $^{a}$ | 11.80 ± 0.05 $^{b}$ | 11.00 ± 0.03 $^{c}$ |
| | Diethyl ether | 8.17 ± 0.03 $^{b}$ | 9.00 ± 0.03 $^{a}$ | 7.80 ± 0.04 $^{c}$ |

*Data are means ± SEM (n = 3) and include the 6 mm well diameter. Within a row, means followed by different letters differ significantly among species (one-way ANOVA with Tukey's HSD, $p < 0.05$).*

The antibacterial activity varied among the three investigated seaweed species depending on the bacterial isolate and extraction solvent (Table 2). Descriptive comparisons showed that *L. obtusa* generally exhibited higher inhibition-zone diameters than the other two species. Specifically, *L. obtusa* produced numerically larger inhibition zones than *G. barbata* in 11 of the 12 bacterial isolate–solvent combinations and higher values than *C. glomerata* in all 12 combinations. The only combination in which *G. barbata* showed a higher value than *L. obtusa* was the aqueous methanol extract against *Salmonella* sp., with inhibition-zone diameters of 12.10 and 11.80 mm, respectively. The greatest observed inhibition zone was recorded for the diethyl ether extract of *L. obtusa* against *S. pyogenes* (16.50 mm). Similarly, against *E. coli*, the diethyl ether extract of *L. obtusa* produced an inhibition zone of 11.10 mm, whereas the corresponding extracts of *G. barbata* and *C. glomerata* produced smaller and similar values (7.03 and 7.10 mm, respectively). Overall, *C. glomerata* generally exhibited the lowest observed inhibition-zone diameters, although its value was slightly higher than that of *G. barbata* in the diethyl ether treatment against *E. coli*. As a descriptive summary across the 12 bacterial isolate–solvent combinations, the overall mean inhibition-zone diameters were 11.02 mm for *L. obtusa*, 9.82 mm for *G. barbata*, and approximately 8.93 mm for *C. glomerata*. These numerical differences describe the responses of the tested extract preparations and should not be interpreted as statistically confirmed differences among the seaweed species

**Antibiotic susceptibility**

Table 3 shows how the bacterial isolates responded, in the disk diffusion test, to three antibiotics in common use: amoxicillin–clavulanic acid (AMC), ciprofloxacin (CIP), and doxycycline (DOX).

***Table3. Antibiotic susceptibility patterns of the tested bacterial isolates.***

| Antibiotic | Symbol | Disk content (µg/disk) | *S. aureus* | *S. pyogenes* | *Salmonella sp.* | *E. coli* |
|---|---|---|---|---|---|---|
| **Amoxicillin–clavulanic acid** | AMC | 20/10 | S | S | S | R |
| **Ciprofloxacin** | CIP | 30 | S | S | S | S |
| **Doxycycline** | DOX | 30 | S | S | R | S |

*S = susceptible; R = resistant (NCCLS, 2001). Antibiotic susceptibility results are presented descriptively because inhibition-zone diameters were not recorded.*

The isolates were largely susceptible to the tested agents, with 10 of the 12 isolate antibiotic combinations classified as susceptible. The two Gram-positive isolates, *S. aureus* and *S. pyogenes*, were susceptible to all three antibiotics. Among the Gram-negative isolates, *Salmonella* sp. was susceptible to AMC and CIP but resistant to DOX, whereas *E. coli* was susceptible to CIP and DOX but resistant to AMC. Ciprofloxacin was the only antibiotic to which all four isolates were susceptible. None of the isolates was resistant to more than one antibiotic.

## Discussion

The antibacterial activity of the investigated seaweeds was influenced by the extraction solvent, although the observed effects varied depending on the bacterial isolate and algal species. Diethyl ether extracts consistently produced the largest inhibition zones against the two Gram-positive bacteria, *S. aureus* and *S. pyogenes*, across all three seaweed species. In contrast, aqueous methanol extracts exhibited the highest activity against *Salmonella* sp. for all three species. Against *E. coli*, aqueous methanol produced the largest inhibition zones for *G. barbata* and *C. glomerata*, whereas the diethyl ether extract of *L. obtusa* showed the highest activity. Aqueous ethanol generally produced the smallest inhibition zones,

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recording the lowest values in nine of the twelve bacterial isolate–solvent comparisons. These findings indicate that the extraction solvent plays an important role in determining the antibacterial activity of seaweed extracts, possibly through differences in the types and amounts of compounds recovered. The observed solvent-dependent activity may be related to the chemical diversity of antibacterial metabolites reported in marine macroalgae, including fatty acids, phenolic compounds, terpenoids, pigments, and halogenated secondary metabolites. Their occurrence and abundance vary among brown, red, and green seaweeds. For example, phlorotannins are characteristic phenolic compounds of brown algae and have been associated with antibacterial activity, whereas red and green algae have been reported to contain various terpenoids, fatty acids, and halogenated metabolites [22,23,24]. Previous studies have also demonstrated that solvent selection can substantially influence the antibacterial activity of seaweed extracts [22,24,25]. However, solvent polarity alone cannot fully explain the observed pattern.

Although aqueous ethanol has intermediate polarity relative to aqueous methanol and diethyl ether, it generally produced the smallest inhibition-zone diameters in this study. This suggests that the selectivity of the extraction solvent and its ability to recover particular antibacterial constituents may be as important as its overall polarity. The stronger inhibition produced by diethyl ether extracts against the Gram-positive bacteria is consistent with previous reports describing the greater susceptibility of some Gram-positive bacteria to marine algal extracts [12]. One possible explanation is the difference in cell-envelope structure between Gram-positive and Gram-negative bacteria. Gram-negative bacteria possess an additional outer membrane that may restrict the entry of certain antimicrobial compounds, particularly some hydrophobic substances [26,27]. Nevertheless, this pattern was not uniform across all bacterial isolates. *Salmonella* sp. responded particularly well to aqueous methanol extracts, suggesting that relatively polar constituents may also contribute to the antibacterial activity. Phenolic compounds and other methanol-soluble metabolites have previously been associated with antibacterial effects against both Gram-positive and Gram-negative bacteria [25,28]. The different responses of *Salmonella* sp. and *E. coli*, despite both being Gram-negative, may reflect differences in their cell-envelope properties or susceptibility to particular antimicrobial constituents; however, these possible mechanisms were not investigated in the present study. Among the three investigated seaweeds, *L. obtusa* generally exhibited the highest observed antibacterial activity. Its extracts produced numerically larger inhibition zones than those of *G. barbata* in eleven of the twelve bacterial isolate–solvent combinations and higher values than *C. glomerata* in all twelve combinations. The largest inhibition zone in the study was recorded for the diethyl ether extract of *L. obtusa* against *S. pyogenes* (16.50 mm). This relatively high activity may be associated with the production of secondary metabolites characteristic of the genus *Laurencia*, particularly halogenated compounds such as brominated sesquiterpenes, which have been reported to possess antibacterial properties [29].

Nevertheless, the contribution of these compounds to the observed activity remains hypothetical because the chemical composition of the extracts was not determined. The differences among the three seaweed species should also be interpreted descriptively, since only one extraction batch was prepared for each species–solvent combination. Regarding antibiotic susceptibility, ten of the twelve bacterial isolate antibiotic combinations were classified as susceptible. Both Gram-positive isolates, *S. aureus* and *S. pyogenes*, were susceptible to all three antibiotics, whereas *Salmonella* sp. was resistant to doxycycline and *E. coli* was resistant to amoxicillin–clavulanic acid. All four isolates were susceptible to ciprofloxacin. Interestingly, the algal extracts also produced inhibition zones against the two isolates classified as resistant to particular antibiotics. This observation indicates that the tested seaweed extracts retained in vitro inhibitory activity against these isolates under the experimental conditions. However, it does not demonstrate that the extracts overcome antibiotic-resistance mechanisms or that they are more effective than the conventional antibiotics, since the chemical composition, concentration, and diffusion characteristics of the algal extracts differ from those of the standard antibiotic preparations.

## Conclusion

Crude extracts of *Gongolaria barbata*, *Laurencia obtusa*, and *Cladophora glomerata* exhibited varying levels of antibacterial activity depending on the extraction solvent and bacterial isolate. Among the tested extracts, the diethyl ether extract of *L. obtusa* showed the highest observed activity, producing the largest inhibition zones against *S. pyogenes* (16.50 mm) and *S. aureus* (14.20 mm). These results suggest that marine seaweeds, particularly *L. obtusa*, warrant further investigation as potential sources of antibacterial compounds.

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***Conflicts of Interest***

The authors declare no conflicts of interest.

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