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Effect of *Ulva lactuca* and *Sargassum fluitans* extracts on growth of some bacteria

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Abstract
This study investigated the effect of extracts from the seaweeds *Ulva lactuca* and *Sargassum fluitans* on the growth of four bacterial species: two Gram-negative, Salmonella and Escherichia coli, and two Gram-positive, *Staphylococcus epidermidis and Staphylococcus aureus.* The results showed that the extract exhibited inhibitory activity against three of the four bacterial species: *E. coli, Staphylococcus epidermidis* (-323) and *Staphylococcus aureus* (HS). However, no inhibitory activity was observed against *Salmonella*. The results also showed that the aqueous extract of *Ulva lactuca* gave a higher inhibition rate than the alcoholic extract, whereas for *Sargassum fluitans*, the alcoholic extract gave a higher inhibition rate than the aqueous extract.

Keywords: *Ulva lactuca*, *Sargassum Fluitans*, *Salmonella*, *Escherichia Coli*, *Staphylococcus Epidermidis*, *Staphylococcus Aureus*

Introduction
The scientific researches on microalgae and seaweeds, and their economic, medicinal and commercial applications. Algae are currently used in many different household and industrial products, and are also used as a source of chemicals, plant extracts and active ingredients in numerous applications (such as pharmaceuticals and cosmetics) (Wilson *et al.,* 1989).
Extracts are solutions prepared using organic solvents such as methanol or water from dried algae, where active compounds such as alkaloids, flavonoids, tannins, steroids, saponins and phenolic compounds, polysaccharides, fatty acids, terpenoids, vitamins and minerals and anthraquinones (Nguyen *et al*., 2024) [5] (Herrero *et al*, 2006) [2]
Supercritical CO_2 extraction uses compressed carbon dioxide at high temperatures and pressures, which is suitable for extracting heat-sensitive compounds such as carotenoids and lipids (Herrero *et al.,* 2006) [2]
Extracts of Sargassum and Cladophora have shown efficacy against MRSA Methicillin-Resistant Staphylococcus aureus and various other bacterial species, due to their high phenolic content. It has been shown that Ulva extracts inhibit the growth of Bacillus cereus and Staphylococcus aureus at concentrations ≤ 500 µg/ml (Moubayed *et al.,* 2006)
In the last decades, there were an increase in infectious diseases and the use of chemically based antibiotics, leading to a rise of antibiotic-resistant microorganisms. Therefore, it has become essential to look for new antimicrobial compounds from natural sources such as plants and seaweeds, which represent good alternative due there save use and their widespread availability (Usov, 1998) [7]

Materials and Methods
Collection of Algae Samples
Algae samples were collected from the beaches of Tajoura, Zaitoun and Qarqash during April–May 2025. They were washed with tap water to remove sand and impurities. The samples were dried at laboratory temperature; once dry, they were ground into a powder using an electric grinder, placed in glass containers and stored in a refrigerator at 4 °C.

Extraction
Extraction was carried out using two methods: water and an organic solvent (90% methanol). Then each extract was weighed as indicated in table 1.

Table 1: Shows the weights of the concentrated extracts

Weight/g	extract
41.451	*Ulva lactuca* Aqueous extract
20.91	*Ulva lactuca* alcoholic methanol extract
39.98	*Sargassum fluitans* Aqueous extract
20.69	*Sargassum fluitans* alcoholic methanol extract

Bacteria Used
The bacterial specimens were obtained from the Libyan Centre for Biotechnology Research, which are.
1. *Salmonella sp*.
2. *Escherichia coli*.
3. *Staphylococcus epidermidis* (-323)
4. *Staphylococcus aureus* (HS).

Media Preparation
Two types of culture medium were prepared: one for reviving the bacteria and the other for bacterial growth. Revival/Activation of the bacteria after long-term storage was achieved by inoculating them onto a solid culture medium containing agar to restore their ability to grow and divide.
Then the bacteria were inoculated in a nutrient agar and incubated at 37°C for 18–24 hours until the bacterial colonies appear.
The bacteria are then transferred from a solid medium (agar) to a liquid culture medium (broth) and incubated at 37°C for 12 to 24 hours until the medium become turgid as a result of bacterial growth. Then the bacteria from liquid media were transferred to solid medium and in incubated at 37°C for 24 hours.

The Wells Diffusion Method
Five wells in each plate were made using a sterile cork borer with a diameter of 6–8 mm, and distributed at

approximately equal intervals, four wells were used for the extracts, and the fifth used as a control in which the antibiotic ceftriaxone was used.

A bout 1000 µl of each extract at a concentration of 100% was placed in its designated well, with the name of the bacterium written on the back of the plate. Each plate was left for 5 to 10 minutes to allow the agar to absorb the extracts, and then the plates were incubated upside down at 37°C for 24 hours.

Results

The antimicrobial activity of algal extracts against the four bacterial strains was tested using the Wells Diffusion Method, the result showed formation of circular inhibition zones around the wells, indicating that the extract successfully inhibited bacterial growth.

The diameter of the inhibition zones was measured and the average of the three replicates for each algal extract was calculated (tale 2).

Table 2: shows the average diameter of the inhibition zones in millimeters (mm)

***Staphylococcus aureus* (HS)**	***Staphylococcus epidermidis* (-323)**	***E. coli***	***Salmonella***	**Diameter of inhibition zone (mm) / Extract**
8	10	12	0	*Ulva lactuca* Aqueous extract
7	11	9	0	*Ulva lactuca* alcoholic methanol extract
7	11	8	0	*Sargassum fluitans* Aqueous extract
10	12	13	0	*Sargassum fluitans* alcoholic methanol extract
12	0	0	30	CEFTRLAXONE

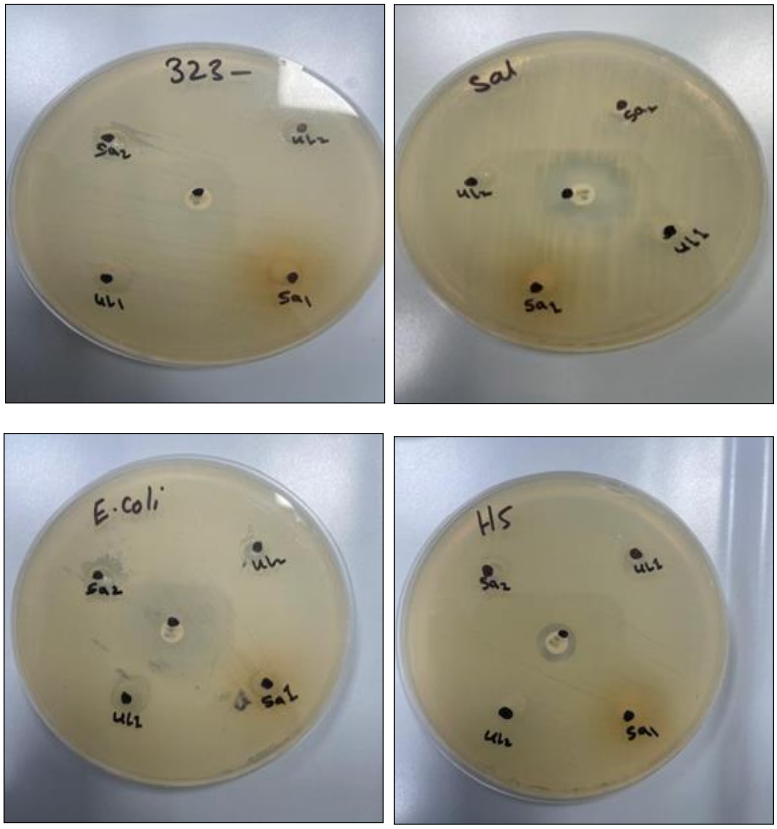

Fig 1: Shows the inhibition zones around the boreholes

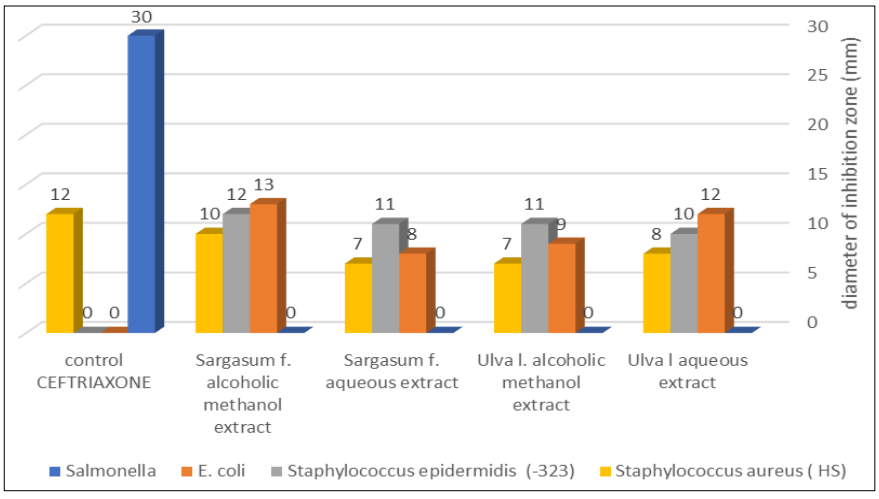

Fig 2: Shows the antibacterial activity of the algal extracts

The results showed that the extracts exhibited an antibacterial activity against three out of the four bacterial species examined, which are *E. coli, Staphylococcus epidermidis* (-323) and *Staphylococcus aureus* (HS). No inhibition zone was observed around the wells for *Salmonella sp,* indicating that it was not affected by the extracts used.

Furthermore, as shown in Figure 1, the aqueous extract of *Ulva lactuca* exhibited the largest inhibition zone compared to the alcoholic extract, whilst for *Sargassum fluitans*, the alcoholic extract exhibited stronger inhibitory activity than the aqueous extract as shown in figure 2.

Discussion

The results show that the extracts used in this research possess growth-inhibiting activity, which is agreed with the study of Sasikala *et al.*, (2022) [6]. The presence of inhibition zones around the spots indicates the ability of the active compounds in the extract—such as phenols, flavonoids, or polyphenols to inhibit bacterial growth (Al-Soll, 2005; Hassan and Abdullah, 2015) [1] (Moubayed *et al*, 2006), and the response varies depending on the bacteria species; some species are sensitive to the extract, whilst others may show partial or complete resistance.

The study showed that the *Ulva* and *Sargassum* extracts (alcoholic and aqueous) showed antibacterial activity against three of the tested species, whilst didn't affect the fourth one,

This variation may be due to differences in the degree of the sensitivity of each bacterial species against active compounds present in the extracts, and the variation in the sensitivity can be explained by differences in the composition of the bacterial cell wall.

Gram-positive bacteria have a cell wall consisting primarily of a layer of peptidoglycan, which is easily penetrated by many active compounds, whereas Gram-negative bacteria possess a lipid-rich outer membrane and a layer of lipopolysaccharide (LPS) that acts as an effective barrier, preventing the entry of many compounds

These results indicate that the efficacy of the extracts depends on:

1. Type of bacterial species or strains and the composition of cell wall
2. Type of solvent and its ability to solve the active compounds
3. The ability of certain species to resist the active compounds in the algal extracts

Conclusion

Based on the results obtained, it can be concluded that algal extracts show promising potential as natural antimicrobials against certain species of bacteria, and marine algae can be a source of bioactive compounds with antibacterial activity, particularly against susceptible strains.

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