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ANTIBACTERIAL ACTIVITY OF LIBYAN SEAWEED EXTRACTS

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ABSTRACT: Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. The Libyan marine biodiversity including macroalgae remains partially unexplored in term of their potential bioactivities. **Method:** The phytochemical analysis of the alcoholic extracts of some commonly occurring seaweed *Cystoseira compressa*, *Enteromorpha intestinalis*, *Corallina* and *Ulva lactuca* and their evaluated for antibacterial activity by well diffusion assay were studied. Four different solvents namely water, ethanol 99 %, methanol 99 %, and methylated spirit 95 % were used for extraction. **Results:** The phytochemical analysis revealed the presence of Carbohydrates, Steroids, Tannin & Phenols, Saponins, Proteins, and glycosides. The extracts were subjected for study of antibacterial activity. The zone of inhibition ranged between 8 to 16 mm in aqueous extract and up to 16 mm in methanol extract. The maximum activity (16 mm) was recorded from methanol extract of *Ulva lactuca* against *Staphylococcus aureus* and, minimum activity (8mm) recorded by *Cystoseira compressa* against *Staphylococcus aureus*.

INTRODUCTION: Marine organisms are rich sources of structurally new and biologically active metabolites ¹. There are about 2400 natural products have been isolated from macroalgae belonging to the classes Rhodophyceae, Phaeophyceae and Chlorophyceae ². The antimicrobial activity was regarded as an indicator to detect the potent pharmaceutical capacity of macroalgae for its synthesis of bioactive secondary metabolites ³.

Marine algae are widely distributed in the coastal regions of continents. Several works have been carried out on the extracts from marine algae. Extracts of marine algae were reported to exhibit antibacterial activity ^{4, 5, and 6}. Antimicrobial activities on bacteria and fungi were reported by Helliouet *al* ⁷.

In recent years, there have been many reports of macroalgae derived compounds that have a broad range of biological activities, such as antifungal ⁸, antiviral ^{9, 10}, antitumors ^{11, 12} antioxidant ^{13, 14, 15} and anti-inflammatory activity ^{16, 17, 18}. The use of antimicrobial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produce. These limitations demand for improved pharmacokinetic properties which necessitates continued research for the

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search of new antimicrobial compounds for the development of drugs. This study was designed after initial observations showed that Libyan seaweeds have antimicrobial activities against wide varieties of microorganisms. The increase of microbial resistance to the most commonly used antibiotics led to testing many other natural resources as alternative, therefore it is became an essential to do more screening on some available seaweeds that have wide distribution on Libyan coast.

The present study was undertaken to examine the effect of different extracts of 4 species of marine benthic algae including *Cystoseira compressa*, *Enteromorpha intestinalis*, *Corallina* and *Ulva lactuca*, collected from the Mediterranean Benghazi coasts, against pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIAL AND METHODS:

Collection of Algae samples: Four species of macro algae have a wide spread on Benghazi - Libya coast including *Cystoseira compressa*, *Enteromorpha intestinalis*, *Corallina* and *Ulva lactuca* were nominated for this study. Samples were collected by hand picking (within the latitude 32° 8'27.79" N and longitude 20° 4'37.73" E) from Benghazi coast (Alsabri area) in spring time, samples were then cleaned from sand, epiphytes and animal and then washed thoroughly in sterile distilled water to remove salt.

The collected samples were identified by Phycology experts at Botany Department, Faculty of Science, University of Benghazi. All samples were dried for 48 hours naturally at 25°C. The dried seaweeds were crushed in an electrical mill until a fine powder was obtained, and stored in bottles at room temp in cool dry place until use.

Preparation of organic algae extracts: Four solvents including water, ethanol 99%, methanol 99%, and methylated spirit 95% have been used for extractions by mixing 5 g of seaweed's powder with 100 ml solvent. The mixtures were placed in water bath shaker incubator at 25 °C for 24 hours then have filtered to remove the insoluble materials, The extract were concentrated by evaporated the solvent

to dryness and the solid residue then dissolved in 2 ml by the same solvent and stored at 4°C

Qualitative phytochemical screening:

Preliminary phytochemical tests for identification of Carbohydrates, Steroids, Tannin & Phenols, Saponins, Proteins, and anthraquinones glycosides were carried out for all the extracts using standard qualitative methods that have been described previously^{19, 20}

Test Pathogens: Three bacterial strains including *Staphylococcus aureus* ATCC. 25923, *Pseudomonas aeruginosa* ATCC 17933 and *Escherichia coli* ATCC 25922 were obtained from Microbiology department, Benghazi Medical Center, Benghazi City, Libya were used in antimicrobial assay. All cultures were preserved on on Mac Conkey agar and nutrient agar and stored at 4°C.

Antibacterial activity was performed using agar-well diffusion method. Strains concentrations on plates were carried out using modified BD quality control procedures^{21, 22}. Briefly, fresh colonies from Nutrient agar plates suspended in to normal saline (pH 7). Microbial concentrations were adjusted by the turbidity, after 15 minutes the inoculum was collected by dipping a sterile cotton swab into the suspension, swab rotated several times on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swabs then applied on Mueller-Hinton agar (pH 7.4), plates then left for at least 3 min to allow inoculum to be absorbed. 100µl of algal extracts was poured into 6 mm diameter wells and left for 2hours for complete diffusion. Solvent without algal extract was used as a negative control. Plates were incubated at 37 °C for 24 hours.

Determination of Antibacterial Activity: Antibacterial efficacy was estimated via inhibition zone appeared around the wells which measured in millimeters (including the well diameter) using caliper. The antibacterial activity was prescribe as inactive when the inhibition zone was 7 mm or less, whereas a diameter less than 9 mm was interpreted as trace active (+), a diameter between 10 and 14 was interpreted as moderately active (++) , a

diameter more than 14 was interpreted as a highly active (+++).

RESULTS AND DISSECTION:

Phytochemical screening: The qualitative phytochemical screening of the alcoholic extract of four algae was carried out in order to assess the presence of bioactive compounds which might have antibacterial potency. The presence of Carbohydrates, Steroids, Tannin & Phenols,

Saponins, Proteins, and glycosides was investigated (**Table 1**). The presence of Phenols and flavonoids in all tested algae is interesting because of their possible use as natural antioxidants and antimicrobials. Many reports revealed the presence of flavonoids in marine algae and some of them have been investigated for their biological activity. The antifungal, antiviral and antibacterial activities of saponins are well documented^{23, 24}.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF ALCOHOLIC EXTRACT OF 4 DIFFERENT ALGAE

Carbohydrates	Alkaloids	Saponins	Flavonoids	Glycosides	Protien
<i>Cystoseira compressa</i>	++	++	+	+++	+
<i>Enteromorpha intestinala</i>	++	+	++	++	+
<i>Corallina</i>	++	++	++	+++	+
<i>Ulvalactuca</i>	++	++	+	+++	+

The antibacterial activities of algal extracts: Antibacterial activities of algal extracts of four dominant marine macroalgae species from Benghazi coast were evaluated against both Gram

positive and Gram negative human pathogens bacteria by agar well diffusion method and the results are shown in **Table 2**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF 4 DIFFERENT ALGAE

Solvent	Algae	Bacteria		
		<i>E.coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
Water	<i>Cystoseira compressa</i>	—	+	+
	<i>Enteromorpha intestinal</i>	—	+	++
	<i>Corallina</i>	—	+	++
	<i>Ulva lactuca</i>	—	++	++
Methanol (99%)	<i>Cystoseira compressa</i>	—	++	++
	<i>Enteromorpha intestinal</i>	—	++	++
	<i>Corallina</i>	—	+++	+++
	<i>Ulva lactuca</i>	—	+++	+++
Ethanol (99%)	<i>Cystoseira compressa</i>	—	+	++
	<i>Enteromorpha intestinal</i>	—	++	++
	<i>Corallina</i>	—	++	+++
	<i>Ulva lactuca</i>	—	+++	++
Methylated spirit (95%).	<i>Cystoseira compressa</i>	—	+	+
	<i>Enteromorpha intestinal</i>	—	+	++
	<i>Corallina</i>	—	++	++
	<i>Ulva lactuca</i>	—	+++	++

Table (2) shows the effects of four seaweeds on three bacterial strains using four different solvents. Water extract of the four experimented seaweeds have trace and moderate bioactivity against *S. Aureus* and *P. Aeruginosa* and narrow spectrum of action to *E. coli*. Methanolic seaweeds extracts have introduced no bioactivity on *E. coli*; in contrast, from moderate to high bioactivity with broad spectrum bactericidal was recorded on *S. aureus* and *P. aeruginosa*. The use of ethanolic and methylated spirit seaweeds extractions did not

show antimicrobial activity on *E. coli*; on the other hand, trace, moderate and high antimicrobial effectiveness obtained on *S. aureus* and *P. aeruginosa*. Methanol extracts of all seaweeds test exhibited broad spectrum of antibacterial activity. Screening of antibacterial activity in marine green and brown Macroalgae from the coast of Morocco in (2009) study found that the methanolic extract of *Cystoseira compressa* does not inhibit to *S. Aureus*²⁵. In our study all solvents extracts of

Cystoseira compressa produced an inhibiting effect against *S. aureus*.²⁶

In present study the zone of inhibition ranged between 8-13mm in aqueous extract and 10-16mm in methanolic extract. The results have a proved that methanolic extraction of *U. lactuca* has the highest antimicrobial activity compare to other experimented sea weeds.

The *S. aureus* was found to be the most sensitive (widest inhibition zone) among the tested bacteria to all used algal extracts. *Escherchia coli* alone were resistant to all the seaweed extracts. It was reported that the Gram-positive bacterial strains were more susceptible to seaweeds extract than Gram-negative bacterial strains²⁷.

In order to determine the minimum lethal concentration for the most sensitive strain, several methanolic concentrations including (5 %, 10 %, 15 %, 20 % and 25 %) were made from all experimented seaweeds. The results showed that seaweeds methanolic extraction killing of *S. aureus* was very concentration independent with 10% or more showing killing ability (**Figure 1**). In fact with a concentration 10% or more the bacteria were vanished in the plates.



FIGURE 1: METHANOLIC EXTRACT OF U. LACTUCA

To analyze the minimum bactericidal concentration of *E. coli* and *P. aeruginosa* several algal extraction concentrations including (5 %, 10 %, 15 %, 20 % and 25 %) were made, the results has represented all seaweeds extractions had no activity killing for both strains. Although algal extracts efficacy obtained using 5 %, however, no further killing activity gained on *P. aeruginosa* using higher concentrations.

In order to characterize seaweed's components stability, algal extracts were also tested of the time factor on their effectiveness; the results showed all seaweed have stable killing activity on *S. Aureus* up to one year.

CONCLUSION: Seaweeds collected from Benghazi (Libyan) Mediterranean coasts have been shown to possess a specific antimicrobial activity. The most interesting species were *Ulva lactuca* and *Carolina*. These observations showed their importance as a potential source for biological active compounds such as antibacterial substances. Further research studies are being carried out on the other species of seaweeds from the same habitat in order to provide complete data of the antimicrobial potential seaweeds along the coast of Libya. It is also necessary for successful separation, purification and characterization of biologically active compounds using chromatographic and spectroscopic techniques for the synthesis novel antibiotics.

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