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Antibacterial Effects of Brown Algae Extracts of *Cystoseira* Sp. Collected From Tukra Beach, Mediterranean Sea Coast of Libya

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***Abstract*—** Two species of brown algae *Cystoseira compressa* and *Cystoseira crinita* were studied in this project collected from Mediterranean coast of Tukra beach and that was in order to study their inhibition effects on growth of five different species of bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, and Escherichia coli*). Five different organic solvents (ethanol, methanol, acetone, ethyl acetate and hexane) were used with both algal species and the bacteria species. Discs of the antibiotic Azithromycin 15 were used as a positive control and dimethyl sulfoxide (DMSO) as a negative control. Nutrient Agar was used to reactivate colonies of bacteria that were used in this study and Muller Hinton (M.H) media was used for the sensitivity test. Well diffusion method was also used in the sensitivity test for both species of Cystoseira which were extracted by applying a cold extraction method. Both *Cystoseira spp*. showed vary inhibition effects on gram-positive bacteria *S. aureus* and *S. pyogenes*. Highest inhibition zones were found in colonies of *S. aureus* and *S. pyogenes* by using of *Cystoseira compressa* ethanol extracts. Extracts of both species of Cystoseira had no inhibition effect on gram-negative bacteria.

Keywords— Brown algae, Antibacterial activity, Algae natural products, Algae extraction, Libyan Mediterranean Sea coast algae.

I. INTRODUCTION

For more than 4000 years, algae have been used in the world safely for many economic benefits such as food and feed for humans and animals. There are more than 160 species of algae in Asia are used as food. Some algae are source of antibiotics, such as *Chlorella* sp. [1]. which produce chlorine antibiotics which inhibiting growth of bacteria and other algae from around as way of defence [2]. There are about 2400 products isolated from red, brown and green algae which have been actively characterized by antimicrobial activity. This is an indicator for detection of valuable pharmaceutical capacity of algae and their using in development of new antibiotics. Marine macroalgae contain large amounts of proteins, vitamins, minerals, alkaloids, phenolic compounds and carotenes [3]. The antimicrobial drugs are very important adventure have been used since the last century. A lot of researches have been performed on extraction of algae secondary metabolites and testing them for antimicrobial activity [4], [5]. Locally, many research studies for the marine algal inhibitory effects on growth of pathogenic bacteria and fungi were performed by algae extracts [6], [7], [8], [9].

In Libya, many research studies were done on different algae species. Only two species of *Cystoseira* sp. were studied for antibacterial activity ; *Cystoseira montagnani* [10] and *Cystoseira barbata* [11], while the current study is aimed to test the effect of two species; *Cystoseira compressa* and *Cystoseira crinita* against growth of gram-positive pathogenic bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*), and gram-negative pathogenic bacteria (*Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli*) using five organic solvents, ethanol, ethyl acetate, methanol, hexane and acetone.

II. MATERIALS AND METHODS

Sample collection

Cystoseira algae were collected from Tukra beach, Mediterranean Sea coast of Libya at the tidal zone during summer 2017. Samples were well washed with sea water and collected in plastic bags with little of sea water to prevent evaporation. In the lab, samples washed again by fresh water several times to remove any salts and planktons. Samples were dried in air at room temperature and finally in oven at 37° C until obtaining the constant weight and then powdered in an electric blender. The milled samples were stored in refrigerator. Until the extraction step, some samples were fixed on herbarium sheets and deposited in Botany department, Faculty of science, University of Benghazi. *Cystoseira* sp. were identified to *Cystoseira compressa* and *Cystoseira crinita* by M.M. Godeh in the botany department.

a b

Figure 1: a: *C. compressa*, b: *C. crinita*

Preparation of algae extract

Five grams of algae dried powder were added to 100 ml of each solvent: ethyl acetate ethanol, acetone, methanol and hexane in closed conical flask at room temperature. Flasks mixed on a shaker at 100 c/min [12] for 21 days, then the samples were filtered by Whatman filter paper (no. 1) to separate the filtrate and the extracts were evaporated by rotary evaporator to be free from solvents. The extracts dissolved in 2.5 ml of dimethyl sulfoxide (DMSO).

Antibacterial assay

Bacterial suspensions were prepared by injection of 4 to 8 colonies in 3 ml of normal saline solution and compared to the standard barium chloride to determine the appropriate concentration. The sensitivity test of bacteria to antibiotics was done based on [13] method. Bacterial sensitivity to algae extract was done by the well diffusion method. Algae extracts were added at 200 µl concentration in the holes. The petri dishes incubated for 24 h at 37°C, the inhibition zones formed around the holes were measured. Dimethyl Sulphoxide (DMSO) was used as a negative control and discs of Azithromycin (AZM 15) were used as a positive control.

Bacterial strains used

Five strains of bacteria that cause human diseases were obtained from the Benghazi Children's hospital and identified by phoenix device. Two strains gram-positive (*Streptococcus pyogenes* and *Staphylococcus aureus*) and three strains gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*).

Statistical analysis

One-way ANOVA statistical analysis from SPSS version 21 was used to indicate the significant of variance between the different solvents and pathogenic bacteria by Tukey test at $p \leq 0.01$.

III. RESULTS AND DISCUSSION

Antibacterial activities of two species of seaweed *C. crinite* and *C. compressa* tested against bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Streptococcus pyogenes.* The results of tests are summarized in table 1&2. The extracts of *C. crinita* showed effects on growth of the gram-positive bacteria only, and the inhibition differed among solvents also. Ethanol extracts was the highest inhibitor on *S. pyogenes* and *S. aureus*. The *S. aureus* was more sensitive than *S. pyogenes* while *P. aeruginosa, K. pneumonia* and *E. coli* were more resistance. One Way Anova statistical analysis Tukey test ($p \leq 0.01$) showed that there are high significant differences between *C. crinita* extracts and the used bacteria.

Table 1: Antibacterial activity of *C. crinita* extracts against five pathogenic bacterial strains.

	Bacterial strains				
solvents	*S. aureus*	*S. pyogenes*	*E.coli*	*K. pneuomoniae*	*P. areuginosa*
Methanol	11.3 ± 0.58**b**	10.3± 0.57**bc**	na	na	na
Ethanol	20.6 ± 0.58**a**	19.6± 0.57**a**	na	na	na
Acetone	10.6 ± 0.58**bc**	00 ± 000 **e**	na	na	na
Ethyl Acetate	9.3± 0.58**cd**	10.0± 000 **bc**	na	na	na
Hexane	10.6 ± 0.58**bc**	8.3± 0.57**d**	na	na	na
DMSO	0	0	na	na	na
AZM15	38.3	36.3	-	-	-

Inhibition zones in (means ±SD) mm, na: no activity, DMSO as negative control, AZM15 as positive control. **a,b,c,d,e** indicate to significant differences in each column ($p \leq 0.01$).

The extract of *C. compressa* showed effects on growth of the gram-positive bacteria only as well, and the inhibition differed among solvents. Ethanol extracts was the highest inhibitor on *S. pyogenes* and S. The S. aureus was more sensitive than *S. pyogenes* while *E. coli*, *P. aeruginosa*, and *K. pneumonia* were more resistance. One Way Anova statistical analysis Tukey test at ($p \leq 0.01$) showed that there are high significant differences between *C. compressa* extracts and the used bacteria.

Table 2: Antibacterial activity of *C. compressa* extracts against five pathogenic bacterial strains.

	Bacterial strains				
Solvents	*S. aureus*	*S. Pyogenes*	*E.coli*	*K. pneuomoniae*	*P. areuginosa*
Methanol	20.0 ± 0.00**c**	20.3± 0.57**c**	na	na	na
Ethanol	29.0 ± 0.00**a**	23.3± 0.57**b**	na	na	na
Acetone	16.0 ± 1.00**d**	12.3 ± 0.57**e**	na	na	na
Ethyl Acetate	21.0± 1.00**c**	20.3± 0.57**c**	na	na	na
Hexane	10.0 ± 1.00**f**	11.6± 0.57**ef**	na	na	na
DMSO	0	0	na	na	na
AZM15	38.3	36.3	-	-	-

Inhibition zones in (means ±SD) mm, na: no activity, DMSO as negative control, AZM15 as positive control. **a,b,c,d,e,f** indicate to significant differences in each column ($p \leq 0.01$).

This study showed that most of the used extracts have produced inhibitory results on growth of the gram-positive bacteria (*S. pyogenes* and *S. aureus*) in varied levels. Ethanol was the most effective in extraction, these results are compatible with many researchers such as [14]and [15].

Results were obtained by [16] showed the high inhibition efficiency of *C. mediterranea* and *C.barbata* by using four organic solvents against growth of Gram-positive and negative bacteria. The sensitivity or resistance of bacteria to the inhibitory substances may be due to the composition of the plasma membrane [17], [18], [19]. It may be due to mutations and continues structure changing and then producing resistance strains [20], [21], [22].

It is important to mention that some researchers have another explanation through their positive results which confirm that algae used in current study have an inhibition effect on growth of gram-negative bacteria such as *P. aeruginosa*, *E. coli*, and *K. pneumonia* [3] [11], [16], [23]. Algae characterized by their containing on huge amounts of secondary metabolized products such as terpenoids, alkaloids, phenolic compounds, fatty acids. These products work as microbial inhibitors [24], furthermore, these substances can be as defensive technique by algae in their environment [25], [26], [27], [28].

IV. CONCLUSION

The current study showed that *Cystoseira compressa* and *Cystoseira crinita* extracts have valuable products that can eliminate growth of pathogenic bacteria. The ethanol extract was the most effective antibacterial activity. All the both Cystoseira species extracts have broad spectrum activity on gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), while there were no effects on gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*).

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