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STUDY THE EFFECTIVENESS OF DIFFERENT SEASONS OF EXTRACTS COLPOMENIA SINUOSA (MERTENS EX ROTH) DERBES ET SOLIER AGAINST SOME TYPES OF HUMANS PATHOGENIC BACTERIA

Fatimah. M. Omar[1*], Massoud, M. Godeh[1] and Alaa, A. Said[2]
*[*1]Botany Department, Faculty of science, Benghazi University, Elmarj, Libya*
[2]Botany Department, Faculty of science, Zagazig University, Egypt

***[*]Corresponding Author**:-*
***E-mail:-** Fatema.mohammed1984@gmail.com*

Abstract:-

Previous studies and researches had already showed that, the brown macroalgae Colpomenia sinuosa Crude extracts were prepared using four organic solvent which are ethanol, methanol, acetone, Diethyl ether, in addition to distilled water from the seaweeds and screening for their antibacterial activity against five bacterial pathogens were also carried out. The test bacterial strains were Escherichia coli, Klebsilla pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis. The Diethyl ether elicited remarkable antibacterial activity against all pathogenic bacteria screened, the summer extractions were the most effective on Bacillus subtilis bacteria where was June month stronger on all bacteria then May month then August month then September month then July month and April month.

Keywords:- *Colpomenia sinuosa, Brown macro algae; Antibacterial Activity; Extract*

INTRODUCTION

Algae's are very large and significant aquatic diverse groups found in marine and fresh water. Algae are divided into three classes, chlorophyceae (green algae), phaeophyceae (brown algae) and Rhodophyceae (red algae) [1]. In recent years there has been an increase of the resistance of microorganisms to antibiotics usually used in the treatment of some diseases. To overcome this problem, new therapeutic drugs from natural products have been explored [2]. Thus, marine organisms appear as an efficient alternative source of new drugs, and algae have been extensively documented for their capacity to provide a rich source of primary and secondary metabolites [3]. Actually, there are several substances obtained from algae that are already in use in traditional medicine for a long time.]4[

Materials and Methods

Collection of Seaweeds

The seaweeds were collected from El-Sabry coasts in Benghazi, east of Libya, during April to September, 2012. The algal samples were authenticated at the Botany Department, Faculty of Science, and Benghazi University. The collected samples were carefully cleaned with seawater and sterile double distilled water to remove all epiphytes and dried in room temperature. Then cut into small pieces and then ground in a tissue grinder until reach fine powder form and properly stored in sealed bottles.5 [

Preparation of Algae Extracts

Typically, we took 5 g from algae powder in 100 ml from each organic solvent at 100 rpm in a shaker incubator for 24 h at room temperature. Then solution was filtered through considerable what man number 1 sterile filter paper [6; 7; 8]. At that point, the extracts were evaporated by dryness by Clock bottles in the air at room temperature [9]. It was dissolved in 2.5 ml of the solvent used for extraction and the extracts were placed in sealed glass tubes for preservation and kept in the refrigerator at a temperature of 4° C until use.]12; 11 ;10 ;8 ;7[

Antibacterial Activity Determination

To objectively evaluate the antimicrobial activity of the seaweed extracts, the following microrganisms were tested: Gram negative - Escherichia coli and Klebsilla pneumoniae and Pseudomonas aeruginosa; Gram positive - Basillus subtilis and Staphylococcus aureus. The bacterial strains, which obtained from Microbial Wealth Center - Cairo – Egypt, were cultivated and stored in Nutrient Agar (NA). The Muller-Hinton agar medium was used for antibacterial assay. The agar diffusion method was used to accurately assess the antimicrobial activity of the extracts Equip the bacterial suspension by taking from 3-5 colonies of bacteria and put in 3-4 ml Normal saline. Then, we took from suspension 100 µl and put in all agar plates by sterile cotton swab containing bacterial cultures incubated for 24 hours at 37° C [13; 14]. Then, seaweed extracts were applied directly on agar plates using the drop method (100 µL), [8; 15]. Next, the prepared extracts were poured in to the well in the standard concentration (100 µL). All the plates were incubated for 24 hours at 37° C. Subsequently, the presence of the zone of inhibition could be measured on the plates. All tests which performed in triplicate and clear zones greater than 7 mm were considered as positive results because Cork borer was 7 mm in diameter.]16; 8[

Results and Discussion

By tracking the growth of Colpomenia Sinuosa in 6 months, the average length in April was 6.3 cm and in May and June increased to 9.7 cm (the highest length). In July, August and September, the growth fell to 8.5 cm and 7 cm and 3 cm respectively. This shows that the best growth of algae was in May and June and the lowest growth of algae was in September. The effect of diethyl ether extracts on C. Sinuosa was studied on the growth of 5 types of bacteria E. Coli and K. pneumonia and P. aeruginosa and B. Subtilis and S. Aureus during 6 months in 2012 and 2013 in the three seasons of spring, summer and autumn, to determine the extent of the effect of crude extracts on the growth of some bacteria and change the concentration of active substances in the alfalfa extract by changing the surrounding environmental conditions. The results shown in table (1) indicate that the highest average diameter of the bacterial growth inhibition zones was for algae collected in the summer, followed by spring and autumn. The highest average diameters of B. subtilise inhibitors and lowest average diameters of S. aureus .

The study also showed that the average diameters of the E. coli growth regions for spring, summer and autumn extracts were close. The average diameter of the growth inhibiting regions of E. coli in April was 14.39 mm. The impact increased in May and June where the areas of inhibition in May was 16.45 mm and the month of June was 17.45 mm and decreased the impact in July to 14.44 mm and then increased influence again in August and September by close rates where the regions of the areas of inhibition in August 15.62 mm and the month of September is 15.40 mm.

The results showed the average diameters of the areas of inhibition in the seasons were close. In spring, April and May, the mean areas of the inhibition zones were 15.422 mm, while in the summer, June, July and August, were 15.83 mm but in the fall, September, was 15.40 mm.

K. pneumonia was the average of diameters of spring, summer, and autumn damping regions. The average diameter of the inhibition zones in April was 22.39 mm. The effect in May increased to 24.26 mm. The effect increased in June to 26.42 mm. The effect in July, August and in September the average diameter was close to 22.49 mm, in August, 22.56 mm and 21.62 mm in September. This indicates that the mean diameters for the growth of these bacteria in the spring season (April and May) were 23.33 mm and in the summer months (July and August) were 23.82 mm but in the fall (September) decreased to 21.62 mm.

The average diameter of the regions of *P. aeruginosa* inhibition of spring, summer and autumn extracts was also convergent. The average diameter of the growth inhibiting regions for these bacteria in April was 12.49 mm. The effect in May increased to 16.39 mm and also increased in June to 27.45 mm. In July, August and September was 14.46 mm and in August was 14.44 mm and 14.52 mm in September. This indicates that the average diameter of the regions inhibition of the growth of these bacteria in the spring, April and May, 14.44 mm, in a quarter summer was 18.78 mm and in the fall, September, was 14.52 mm.

The average diameter of the inhibitory regions for the growth of *B. subtilis* bacteria for spring, summer and autumn extracts was close. The average diameter of the growth inhibitory regions for these bacteria in April was 26.37 mm. The effect in May increased to 29.63 mm. In June, it increased to 36.64 mm. The effect in July, August and September was close to 26.42 mm in July, 26.57 mm in August and 26.54 mm in September. This indicates that the average diameter of the areas inhibition of growth of the bacteria in the spring, April and May, 28.00 mm but in the summer, June and July and August, it was 29.87 mm and in the fall, September, it was 26.54 mm.

The mean diameters of S. aureus for the growth of *S. aureus* for spring, summer and autumn extracts were also convergent. The average diameter of the growth inhibiting regions for these bacteria in April was 14.44 mm. The effect was increased in May and June, where the diameter of the inhibition zones was 15.43 mm in May. In July, it was 15.44 mm. The effect in July, August and September decreased by a small percentage. The diameter of the inhibition zones in July was 14.41 mm, in August was 14.35 mm and in September was 14.45 mm. In the spring, April and May, it was 14.93 mm, but in the summer, Jun, July and August, it was 14.74 mm and in the autumn, September, it was 14.45 mm. (Figure 1)

The results of the test of the effect of different concentrations on inhibiting the growth of the tested bacteria showed a positive relationship. The higher the concentration of the extract, the greater the diameter of the inhibitory halo formed. The concentrations used in the study were limited to 25µl-50µl-75µl-100µl-125µl-150µl, (table 2) which is consistent with [17]. It showed the diameter of the inhibition increased by increasing the concentration of the extracts of some brown algae such as *Dictyota dichotoma,Padina tetrastromatica and Chnoospora bicanaliculata.* [18] Explained that the diameter halo circuit inhibition of extracts of red kappa species increased with increased concentration. This may be due to the low concentration of inhibitors of bacterial growth at dilute concentrations and increased concentration of these substances in concentrated concentrations. (Figure 2)

As for the effect of the three seasons on the extracts of *C. sinuosa* extract, it produced different effect on inhibiting the growth of bacteria, based on the chapter in which the moss was collected in accordance with many previous studies [11; 19; 20; 21] revealed, the difference in results by different seasons may be due to the fact that seasonal changes have an impact on the life cycle of algae and the concentration of active substances of secondary metabolites contained in algae.In general, most of the previous studies (available to us) investigated the efficacy of algae extract in inhibiting bacterial growth have confirmed that gram positive bacteria are most affected by the action of marine algae extracts from gram-negative bacteria [11; 7; 8; 22; 23; 24; 25] This may be due to the difference in the structure of the cell wall where the extract is extracted from the cell membrane. Raw organic analysis of the bacterial cell wall [26; 27]. In general, most of the raw extracts of C. sinuosa have proved efficient in inhibiting the growth of the tested bacteria. This may be due to the fact that the algae extracts contain many chemical compounds of the nature that act as inhibitors or antibiotics [28. 29]

Table 1. Effect of monthly changes of diethyl ether extracts for *C. sinuosa* on bacterial growth

September	August	July	June	May	April	Months / Bacteria
15.41 ± 0.30 ***	15.62 ± 0.24 ***	14.44 ± 0.28 ***	17.46 ± 0.29 ***	16.45 ± 0.29 ***	14.39 ± 0.27 ***	***Escherichia coli***
21.63 ± 0.30 ***	22.57 ± 0.30 ***	22.50 ± 0.28 ***	26.42 ± 0.80 ***	24.27 ± 0.23 ***	22.40 ± 0.25 ***	***Klebsiella pneumonia***
14.53 ± 0.22 ***	14.44 ± 0.25 ***	14.46 ± 0.26 ***	27.45 ± 0.28 ***	16.40 ± 0.28 ***	12.50 ± 0.28 ***	***Pseudomonas aeruginosa***
26.54 ± 0.28 ***	26.57 ± 0.26 ***	26.42 ± 0.30 ***	36.64 ± 0.28 ***	29.64 ± 0.20 ***	26.38 ± 0.23 ***	***Bacillus subtilis***
14.45 ± 0.24 ***	14.36 ± 0.28 ***	14.42 ± 0.25 ***	15.45 ± 0.27 ***	15.43 ± 0.26 ***	14.45 ± 0.30 ***	***Staphylococcus aureus***

Diameter of the well 7 mm - average diameter of the inhibition zones in mm (at least 6 replicates) - * = high significance at P <0.05 - ± = standard deviation.**

Table 2. Effect of different concentrations of diethyl ether for *C. sinuosa* extracts on bacterial growth

150µl	125µl	100µl	75 µl	50 µl	25 µl	Concentrations / bacteria
34.51 ± 0.36 ***	32.55 ± 0.22 ***	17.74 ± 0.16 ***	17.4 ± 0.22 ***	17.28 ± 0.28 ***	16.55 ± 0.22 ***	*Escherichia coli*
27.33 ± 0.30 ***	26.59 ± 0.31 ***	26.49 ± 0.29 ***	12.41 ± 0.32 ***	12.40 ± 0.37 ***	11.41 ± 0.24 ***	*Klebsiella pneumonia*
30.44 ± 0.32 ***	28.49 ± 0.23 ***	27.47 ± 0.29 ***	17.47 ± 0.23 ***	16.31 ± 0.17 ***	15.43 ± 0.32 ***	*Pseudomonas aeruginosa*
37.55 ± 0.30 ***	36.65 ± 0.26 ***	36.51 ± 0.32 ***	28.64 ± 0.25 ***	21.56 ± 0.31 ***	19.36 ± 0.22 ***	*Bacillus subtilis*
16.63 ± 0.25 ***	16.45 ± 0.25 ***	15.34 ± 0.24 ***	14.27 ± 0.21 ***	13.49 ± 0.20 ***	12.37 ± 0.26 ***	*Staphylococcus aureus*

Diameter of the well 7 mm - average diameter of the inhibition zones in mm (at least 6 replicates) - * = high significance at P <0.05 - ± = standard deviation - concentration, A = 25 concentration, B = 50 concentration, 100 concentration, E = 125 concentration F = 150 .**

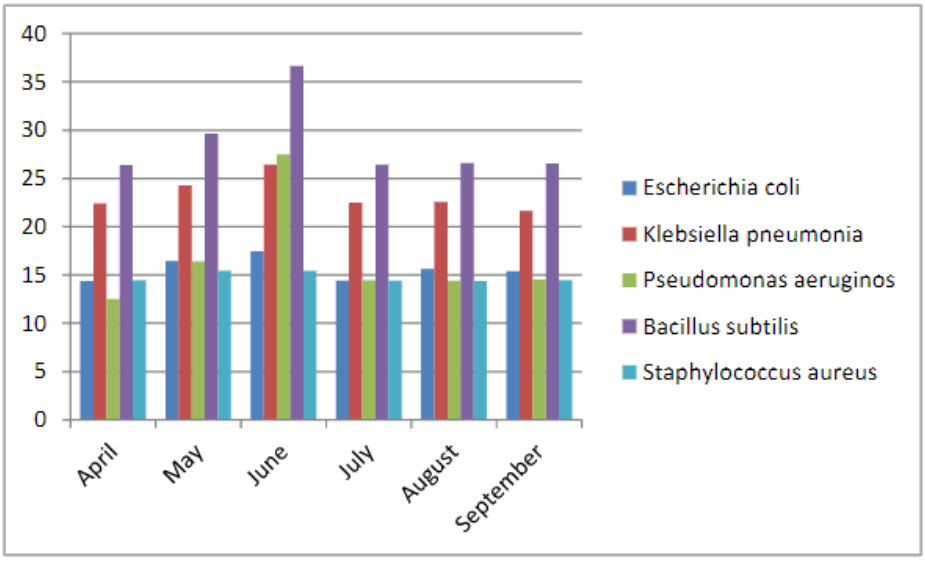

Figure (1): Effect of monthly changes of Colpomenia sinuosa Extract on the growth of the bacteria

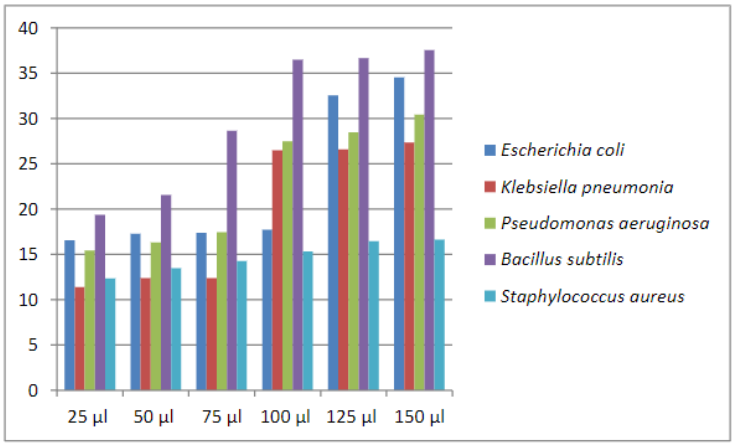

Figure (2): Effect of monthly changes of *Colpomenia sinuosa* Extract on the growth of the bacteria

Conclusions

From the study, it can be concluded that the antibacterial activity of seaweeds di-ethyl-ether extracts in June was found to be high for gram positive and gram negative strains. so this results can conclude that the activity varies according to the seaweeds species and the type of solvents used for extraction. Different solvents with different polarity may result in extraction of different types of biologically active compound from seaweeds. These bioactive compounds may go and bind to the cell wall of the icrobes leading to inhibition of its growth.

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