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Antibacterial activity of four marine seaweeds collected from the coast of Gaza Strip, Palestine

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Abstract - Four commonly occurring marine seaweeds; *Ulva lactuca*, *Enteromorpha compressa* (Chlorophyta), *Padina pavonica* (Phaeophyta) and *Jania rubens* (Rhodophyta) were collected from the coast of Gaza strip, Palestine. Crude extracts were prepared using the solvent methanol and evaluated for antibacterial activity by well diffusion method against both Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis).* The crude methanolic extract of *U. lactuca* inhibited the growth of all the test organisms except *E. coli*. Seaweed extract of *E. compressa* was found to be effective against two of the examined bacteria. Algae belonging to Chlorophyta showed higher antibacterial activity than other members of the algae tested in the present investigation. The methanol extracts of brown and red algae did not show any significant effect on the growth of tested bacteria. *E. coli* was resistant to all the extracts. Results of the present study confirmed the potential use of seaweed extracts as a source of antibacterial compounds.

Key words: Antibacterial activity, marine macroalgae, Gaza Strip.

Introduction

In recent years, the development of microbial resistance to common antibiotics due to indiscriminate use of commercial antibiotics forced researchers to search for novel antimicrobial substances from various sources.

Although the most important sources of antimicrobial substances are the terrestrial microorganisms (e.g. fungi, actinomycetes and bacteria) and higher plants, however, marine organisms also present a rich source of biologically active compounds. Reports showed that more than 15,000 marine natural products have been isolated in the period from 1965 to 2005 (Blunt *et al.,* 2007). These compounds were suggested to play an important role in defense mechanism against biotic and abiotic stress.

Since the early days of marine natural product discovery, sessile marine organisms have dominated as the major contributing organisms of novel bioactive compounds. It is believed that these compounds have a lot of important roles. For example, they keep them from being eaten by other organisms (Amade and Lemeé, 1998), they allow them to compete for space on the substrate with other sessile organisms, and they help them to ensure reproductive success and enhance their ability to combat infection from ambient pathogenic microorganisms such as bacteria, fungi and viruses (Potin *et al.,* 2002).

Like other sessile organisms, macroalgae (seaweeds) are responsible for producing large number of secondary metabolites that prevent attachment and growth of ubiquitous planktonic bacterial colonizers (Maximilien *et al.,* 1998).

Several studies have shown that seaweeds or its extracts have different biological activities, including, antitumor (Xu *et al.,* 2004), antiprotozoal (Allmendinger *et al.,* 2010), antiviral (Kim *et al.,* 1997), antioxidant (Cox *et al.,* 2010) and cytotoxic activity against the human cancer cell lines (Taskin *et al.,* 2010). Seaweed extracts were also reported to exhibit antimicrobial activity (Ballesteros *et al.,* 1992; Gonzalez del val *et al.,* 2001; Kandhasamy and Arunachalam, 2008; Karthikaidevi *et al.,* 2009; Kolanjinathan and Stella, 2009; Lavanya and Veerappan, 2011; Osman *et al.,* 2010; Sreenivasa-Rao, 1991; 1995; Seenivasan *et al.,* 2010; Tuney *et al.,* 2006; Vallinayagam *et al.,* 2009).

Active compounds from seaweeds were found to be active against human bacterial pathogens (Kolanjinathan and Stella, 2009), fish bacterial pathogens (Bansemir *et al.,* 2006; Kolanjinathan *et al.,* 2009), leaf spot disease of plant (Kumar *et al.,* 2008) and marine pathogenic microorganisms (Engel *et al.,* 2006).

The antimicrobial activities of the macroalgae have been attributed to the presence of biologically active compounds with antibacterial potential such as Cycloeudesmol, Lyengaroside A, meroditerpenoid, neoirietetraol, diterpene-benzoate, polybrominated indoles, halogenated sesquiterepene alcohol, Lanosol enol ether, diterpenebenzoic acids, callophycoic acids, halogenated diterpene-phenols, callophycols and eicosanoids (El Gamal, 2010).

Many researchers have reported on the antibacterial activity of seaweeds from different localities around the world (Freile-Pelegrin and Morales, 2004; Gonzalez del val *et al.,* 2001; Ibtissam *et al.,* 2009; Lavanya and Veerappan, 2011; Osman *et al.,* 2010; Tuney *et al.,* 2006). However, reports on the antibacterial activity of seaweed extracts from Palestinian coasts are absent. Hence, the objective of the present study was to evaluate the antibacterial activities of the methanol extract from four seaweeds collected from the coast of Gaza Strip (Mediterranean Sea, Palestine).

Materials and Methods

Collection of algae samples:

Marine algae samples were collected from two locations along the coastline of the Mediterranean coast of Gaza strip, Palestine (Fig. 1). Four algal species, three of which namely *Ulva lactuca*, *Enteromorpha compressa* (Chlorophyta) and *Jania rubens* (Rhodophyta) were collected by hand from the submerged marine rocks from Gaza city coast in November 2010 and *Padina pavonica* (Phaeophyta) was collected from the drifted algae along the coast of Khan Younis city in June 2010.

Algae samples were rinsed thoroughly with tab water to remove any associated debris such as sand and shells, then the samples were dried under sunshade for two weeks. After drying the samples were cut into small pieces, ground to powder form in a mixer grinder and stored in plastic cups in the dark at room temperature until utilization.

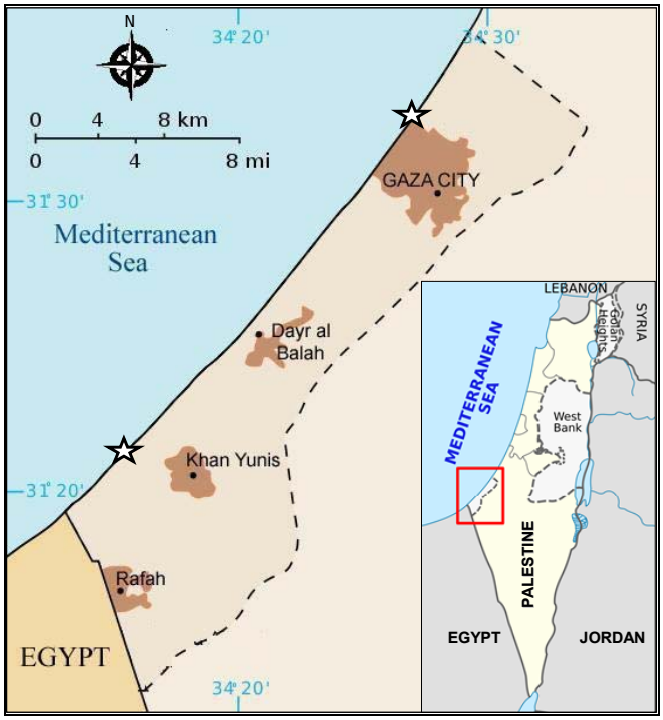

Figure 1. Map of Gaza Strip: Seaweeds were collected from Gaza city and Khan Younis coasts.

Preparation of algae extract:

A modification of previously described procedures (Elmanama *et al.*, 2011; Sreenivasa-Rao and Parekh, 1981) was followed to prepare crude extracts of algae as follows: 10 g of each algae powder was extracted with 150 ml of 100% methanol using a Soxhlet extraction apparatus at 60 ºC for 24 h. The obtained extracts were, then, placed in an oven at 50 ºC, so that the methanol gets evaporated. The residues (crude extracts) were collected and stored at –20 °C in airtight vials until further use. The extract was weighted and the percentage of extract yield was calculated in terms of the initial algal material used for extraction.

Microorganisms:

Screening for the antibacterial activity of all seaweed extracts under investigation was performed against six bacterial species: gram positive; *Bacillus subtilis*, *Staphylococcus aureus*, and gram negative, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae* The bacteria were subcultured and routinely maintained on Difco nutrient agar medium. All bacteria used for the tests are of clinical origin except for *B. subtilis* which was an environmental isolate. The bacterial strains used in the present study were kindly provided by the Medical Technology Department, faculty of Science, Islamic University-Gaza.

Preparation of inoculums:

To activate the bacterial strains before inoculation, they were cultured individually on nutrient broth (Difco) and incubated at 37°C for 24 h. For inoculums preparation and assay of antibacterial activity, Muller-hinton agar was used. Approximately, 20 ml of the autoclaved medium were dispensed into sterile plates and allowed to solidify under aseptic conditions. Then, bacterial strains were inoculated and spread with a sterile swab on the surface of agar plates.

Determination of antibacterial activity:

Antibacterial activity of the above mentioned extracts was evaluated using agar well diffusion method as described by Patra *et al.* (2009) with some modification. Briefly, five wells of 8.0 mm diameter were aseptically made on the assay plates seeded with target microorganism using the wide end of sterile 1 ml tips. In order to avoid the high toxicity of methanol on the test strains, the crude extracts were reconstituted in dimethylsulfoxide (DMSO) to a final concentration of 50 mg/ml. The wells were then filled with 100 µl of each extract. The DMSO was used as negative control. The plates were left for 2 h for complete diffusion and then incubated overnight at 37±1 °C. The diameter of inhibition zones, including the diameter of the well (8 mm) was measured and compared to that of negative control. Each test was made in triplicate and the average of the three replicates for each extract was calculated.

Statistical analysis:

The data were expressed as mean ± SD (standard deviation) and analyzed by one way ANOVA followed by the least significant difference test (L.S.D.). Differences were considered significant when $P \leq 0.05$. All calculations were carried out using the SPSS 13.0 program for Windows (SPSS Inc., Chicago IL, USA).

Results

The percentage of extracted material (g of crude extract/g of the dry weight of starting material × 100) obtained from the different algal species using methanol as solvent and 60 °C as extraction temperature is shown in (Table 1). Among these algae, *U. lactuca* yielded maximum extractable matter (17%), followed by *E. compressa* (7.2%), *P. pavonica* (5.2%) and *J. rubens* (1.2%).

Table 1. The extraction yields (percentage dry weight of starting material) obtained from the different algal species using methanol.

Seaweed	Yield (%)
U. lactuca	17.0
E. compressa	7.3
P. pavonica	5.2
J. rubens	1.2

The results of the antibacterial activity of crude extracts of the four marine seaweeds species are shown in (Figures 2-8). The averages ±SD (standard deviation) of diameters of inhibition zones of the methanolic extracts of the four marine seaweeds namely, *U. lactuca*, *E. compressa*, *P. pavonica*, *J. rubens* against the tested bacteria were given in (Figure 2). Figures (3-8) shows images of inhibition zones observed for the methanolic extracts of the four seaweeds against tested microorganisms; *S. aureus*, *B. subtilis*., *P. vulgrais*., *P. aeruginosa*, *K. pneumoniae* and *E. coli.*

The extract of *U. lactuca* exhibited the strongest antibacterial activity ($p < 0.05$) against all examined microorganisms (Figures 3-7) except *E. coli* (Figure 8). In terms of the difference between the averages of diameters of inhibition zones of each seaweed extract and that of the solvent (DMSO), *U. lactuca* produced zone differences of 9.8, 9.3, 5.8, 4.8 and 3.3 mm to *K. pneumoniae* (Figure 3), *S. aureus* (Figure 4), *P. vulgaris* (Figure 5), *B. subtilis* (Figure 6) and *P. aeruginosa* (Figure 7), respectively.

E. compressa was the second active extract with highest inhibition activity ($p < 0.05$) against *K. pneumoniae* followed by *P. aeruginosa,* with

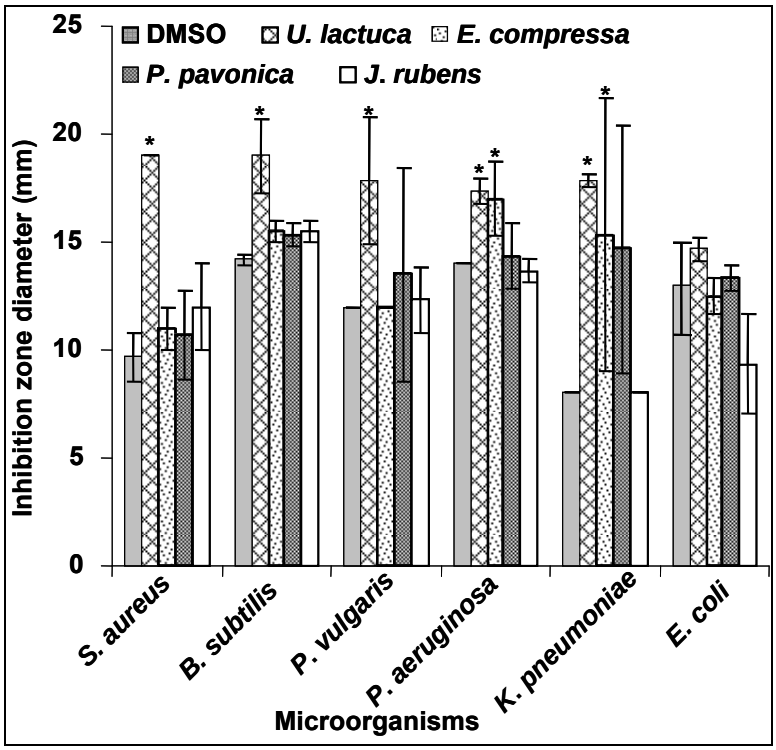

Figure 2. Average diameter (±SD) of inhibition zones (including the diameter of the wells) in mm of the methanol extracts of *U. lactuca*, *E. compressa*, *P. pavonica*, *J. rubens* and negative control (DMSO) against the six tested bacterial species.
*$P < 0.05$, compared to the value of the control.

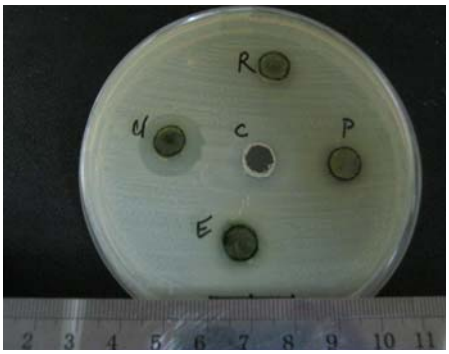

Figure 3. *Klebsiella pneumoniae*

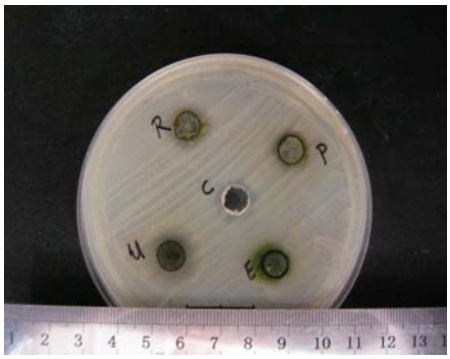

Figure 4. *Staphylococcus aureus*

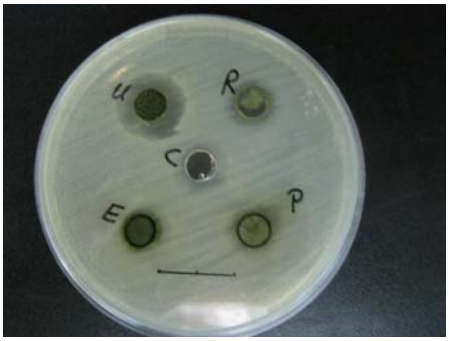

Figure 5. *Proteus vulgaris*

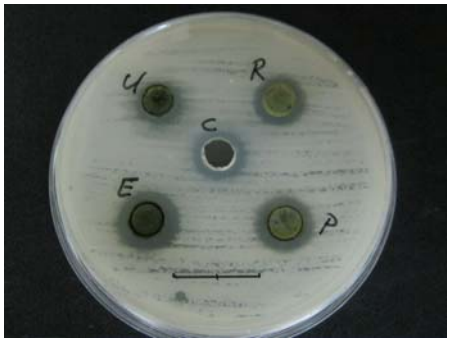

Figure 6. *Bacillus subtilis*

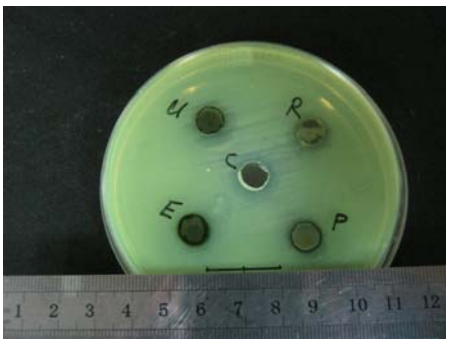

Figure 7. *Pseudomonas aeruginosa*

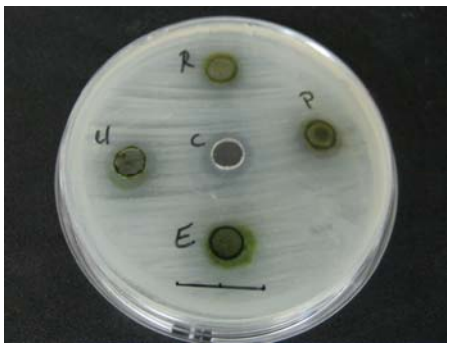

Figure 8. *Escherichia coli*

Figures 3-8. Inhibition zones obtained by well diffusion method for *U. lactuca* (U), *E. compressa* (E), *P. pavonica* (P), *J. rubens* (R) methanolic extracts and carrier solvent (DMSO) (c) against against the six tested strains of bacteria. Images chosen where those closest to the average of *U. lactuca*.

zone differences of 7.3 and 3.0 mm respectively. *P. pavonica* and *J. rubens* were found to be the least effective extracts. Although they showed notable activity against some of the tested microorganisms, the statistical analysis (ANOVA) between the diameter of inhibition zones of the extracts of both seaweeds and the carrier control solvent (DMSO) were not significant ($p > 0.05$). The highest activity of *P. pavonica* extract was observed against *K. pneumoniae*, while that of *J. rubens* was against *S. aureus*, with zone differences of 6.6 and 2.3 mm respectively. On the other hand,, none of the algae extract evaluated in the current study inhibited the growth of *E. coli* (Figures 2 & 8), where all zone differences were found to be ≤ 2.0 mm ($p > 0.05$).

Discussion

The main objective of the present study was to evaluate the ability of different seaweed from Gaza Strip coast to inhibit the growth of some clinically important bacteria.

In the present study, the methanol was chosen for extraction procedure because it was reported in different occasions that seaweeds extracts obtained with methanol have higher antibacterial activity than that of extracts obtained with other organic solvents (Febles *et al.,* 1995; Sidharta *et al.,* 1997; Kumar *et al.,* 2008; Seenivasan *et al.,* 2010; Lavanya and Veerappan, 2011). It was also reported that the methanol extract of seaweeds contain phenolics, alkaloids and amino acids which may responsible for the antimicrobial activity (Devi *et al.,* 2008; Meenakshi *et al.,* 2009; Cox *et al.,* 2010; Srivastava *et al.,* 2010).

As can be observed, the extraction yields obtained from green algae were higher than those obtained from the red and brown one (Table 1). Thus, it seems that the compounds formed in those algae are more soluble in the solvents employed (methanol), at the conditions used, than those found in other algae.

The present study demonstrated that extracts of seaweeds belonging to Chlorophyta namely *U. lactuca* and *E. compressa* were the most active against tested bacteria species with *U. lactuca* proved to be the most effective. These results agreed with the results of previous studies using other test microorganisms such as *Candida albicans*, *Aspergillus niger* (Ballesteros *et al.* 1992), *Micrococcus luteus*, *Enterobacter faecalis*, *Streptococcus faecalis* (Kandhasamy and Arunachalam, 2008) or *Salmonella typhi* (Osman *et al.,* 2010).

The reported antibacterial activities of green algae against studied bacteria have been attributed to the distribution of these algae, where they are mostly occur in the intertidal zone lower region, which may be advantage for the protection of the active compounds within the algal plant from degradation (Karthikaidevi *et al.,* 2009).

The crude extract of the genus *Ulva* has been subjected to numerous studies. Perez *et al.* (1990) observed that the extract of *U. lactuca* had no antibacterial activity. In contrast, results of our study showed that *U. lactuca* inhibited all the test organisms except *E. coli*. This is also in accordance with the results obtained from previous studies, indicating that the extracts of the genus *Ulva* was the most active (Tuney *et al.,* 2006;

Kandhasamy and Arunachalam, 2008; Seenivasan *et al.,* 2010). The high activity of *Ulva* may be due to active components which are present in its extracts.

Our results and those obtained by Tuney *et al.* (2006) demonstrated that the methanol extract of *P. pavonica* had no antimicrobial activity. Earlier reports indicated that the methanol extract of *P. pavonica* exhibited antimicrobial activity only against *B. subtilis* (Gonzalez del val *et al.,* 2001). Kandhasamy and Arunachalam (2008) however, indicated that the methanol extract of *Padina tetrastromatica* inhibited the growth of *Klebsiella pnemoniae*, *Enterobacter aerogens*, *Micrococcus luteus*, *Enterobacter faecalis*, *S. aureus*, *Pseudomnas aeruginosa* and *Bacillus subtilis*. This difference may be attributed to species variation.

The present study indicated that red algae *J. rubens* had no antimicrobial activity against the tested bacteria. These results are in agreement with those obtained by Tuney *et al.* (2006) and Gonzalez del val *et al.* (2001). Previous screening studies (Salvador *et al.,* 2007; Osman *et al.,* 2010) of antimicrobial activities from the same species however have detected antibacterial activities that were not detected in our screening. Furthermore, other studies reported that the red algae have the highest antimicrobial activity against tested bacteria than the brown and green algae (Kolanjinathan *et al.,* 2009; Padmakumar and Ayyakkannu, 1997; Sreenivasa-Rao and Parekh, 1981; Vallinayagam *et al.,* 2009).

In the current work, the *Ulva* extract was active against both Gram-negative and Gram-positive bacteria. Interestingly, the Gram-negative *E. coli* was the most resistant to the antimicrobial effect of all extracts tested. Several authors obtained similar results, supporting the hypothesis that algae extracts are active mainly against Gram-positive bacteria (Ozdemir *et al.,* 2006; Tuney *et al.,* 2006; Salvador *et al.,* 2007; Kandhasamy and Arunachalam, 2008; Ibtissam *et al.,* 2009; Taskin *et al.,* 2010). Previous studies have suggested that the differences in cell wall structure and composition between Gram-positive and Gram-negative bacteria might be the reason (Paz *et al.,* 1995). Some authors suggest that the Gram-negative bacteria have an outer membrane acting as a barrier to many environmental substances, including antibiotics (Tortora *et al.,* 2001; Tshikalange *et al.,* 2005).

Over the last decades several studies have reported on the antibacterial activity of marine algal extracts. Although the majority of these studies indicated variable activities against tested microorganisms, however, it is difficult to compare the results from these studies because the antimicrobial activity of algae extracts may be influenced by a number of factors including algal species (Ibtissam *et al.,* 2009) extraction method, testing methodology, solvent used in extraction (Karthikaidevi *et al.,* 2009 ; Tuney *et al.,* 2006), season or time at which samples were collected (Marechal *et al.,* 2004; Salvador *et al.,* 2007), place of sample collection (Salvador *et al.,* 2007) and the thallus regions used for extraction (Freile-Pelegrin and Morales, 2004).

Finally it can be concluded from the study that the methanol-based extracts of green macroalgae from the coast of Gaza strip are potential sources of bioactive compounds with antibacterial properties and should be investigated for natural antibiotics. It also worthwhile to mention that the

present investigation represents a preliminary screening on antibacterial activity of seaweeds from Gaza strip and work is progressing for the isolation and identification of the active compounds. Crude extracts of other seaweed species will also be evaluated against bacteria and fungi. The most active seaweeds will be fractionated with solvents of different polarity and the fractions will be re-assayed. In the sequence of this work, the active compounds will be isolated and their minimum inhibitory concentrations (MIC) will be also determined.

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الفعالية المضادة للبكتريا لمستخلصات أربع أعشاب بحرية جمعت من ساحل قطاع غزة، فلسطين

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المستخلص - تم جمع أربع أعشاب بحرية و تشمل الطحالب الخضراء *Ulva lactuca* و *Enteromorpha compressa* والطحلب البني *Padina pavonica* والطحلب الأحمر *Jania rubens* من شاطئ قطاع غزة في فلسطين. تم إعداد المستخلصات الخام باستخدام مذيب الميثانول وتقييم الفعالية المضادة للبكتريا لها باستخدام طريقة الانتشار حول الحفر تجاه كلا من البكتريا السالبة لصبغة كرام (*Pseudomonas aeruginosa* و *Escherichia coli* و *Klebsiella pneumoniae* و *Proteus vulgaris*) و البكتريا الموجبة لصبغة كرام (*Staphylococcus aureus* و *Bacillus subtilis*). أظهرت النتائج أن مستخلص الميثانول الخام لعشبة *U. lactuca* ثبط نمو كل أنواع البكتيريا التي تم اختبارها باستثناء *E. coli* . أما مستخلص *E. compressa* فقد كان فعالا تجاه نوعين فقط من البكتيريا. كما لوحظ أن مستخلصات الطحالب الخضراء كانت الأكثر فعالية تجاه البكتيريا المختبرة من الأنواع الأخرى من الطحالب التي تم استخدامها في هذه الدراسة. فمستخلص الميثانول للطحلب البني والأحمر لم يظهرا أي أثر معنوي تجاه نمو البكتيريا المختبرة.كما أظهرت النتائج أن بكتيريا *E. coli* كانت مقاومة لكل المستخلصات. تؤكد نتائج الدراسة الحالية على إمكانية استخدام مستخلصات الأعشاب البحرية كمصدر لمركبات مضادة للبكتيريا.