Screening of phytochemicals and antimicrobial activity of some seaweeds from the east coast of Tripoli / Libya

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Abstract

In this study, crude methanolic extracts of six algal species, two chlorophyta *Enteromorpha linza* and *Codium tomentosum* , three phaeophyta *Padina pavonica* ,*Sargassum vulgare* and *Cytociera sp.* and one rhodophyta *Gelidium sesquipedale*, collected from the east coast of Tripoli, were evaluated for antimicrobial activity against four species of bacteria, two gram positive *Staphylococcus aureus* and *Bacillus subtilis* and two gram negative *Escherichia coli* and *Salmonella typhi* , and two fungal species *Aspergillus sp.* and *Penicillum sp.*. Most of the extracts showed a significant antimicrobial against all four bacteria species. Among tested algae, green algae namely *Enteromorpha linza* exhibited the highest antibacterial activity, meanwhile the most effective algal extract against the two fungal species was the red algae *Gelidium sesquipedale.*

The study showed that the residual content of the dry matter of these algal samples were varied andthe highest was obtained from the brown algae *S. vulgare* .

key words :Algal Phytochemicals extracts, antibacterial, antifungal

1. Introduction

Marine algae species have been used in a wide range of usual remedies as they provide a fine source of antimicrobial analysis. Many metabolites are isolated from algae and have shown to posses bioactive components that exhibit biochemical and antimicrobial properties (Sreenivasa *et.al* 2009) (Arunkumar *et.al* 2005). They are rich sources of bioactive compounds such as carotenoids, proteins, essential fatty acids, vitamins and minerals. there has been increasing interest in secondary metabolites in seaweeds, these metabolites include flavonoids which are widely distributed in plants. Reports between 1977 and 1985 showed that more than 1700 compounds been described from marine organisms, and 48% of these compounds were of algal origins (Ito and Hori 1989). A study showed that the green algae *Caulerpa scalpelliformis* contains 12 elements and compounds (Simpson and Hayes 1958). It is known that flavonoids and other secondary metabolites are produced under stress of microbial infection.

In this study algal samples of six species collected from Tripoli shore, 2 green, 3 brown and one red, were subjected to extraction of their phytochemical compounds. The crude extracts were tested for the presence of secondary metabolites, namely, antheraquinones, tannins, saponins, coumarins, terpenoids, flavonoids and alkaloids. The crude extract will be tested of their effect as antibacterial and antifungal on some samples of positive and negative bacteria as well as on samples of fungi.

2. Materials and methods

2.1- Collecting and processing of algal samples

The fresh algal species were collected from the coastal area of Tripoli on the 15th of June 2014. The samples were collected and thoroughly washed with distilled water to get rid of holdfast and epiphytes. The water was drained off from the samples and spread on blotting paper to remove the excess water and then shade dried. The dried material was crushed in an electric mixer to obtain coarse powder, and they were stored in darkened containers to prevent sunlight.

2.2- Preparation of crude extracts

Extracts were prepared by soaking 5g of coarse powdered material in 50 ml of methanol (99 %) with intermittent shaking. after 48 hours, the extract were filtered by a filter paper (No.1). The organic extracts were concentrated using rotary evaporator till solvent free by evaporation at 40 C°.

The percentage of yields was calculated as follows:

$$\% = \frac{weight\ of\ residue}{weight\ of\ sample} \times 100$$

2.3- Phytochemical analysis

Phytochemical screening determines the biologically active compounds that are present in these genera samples under study. All extracts were tested for the presence of different phytochemicals namely, alkaloids, tannins, flavonoids, saponins, terpenoids, coumarins and anthraquinones.(Fadeyi *et.al* 1989), (Abulude 2007).

2.3.1- Test for alkaloids

For alkaloids, the test was carried out by subjecting 1g methanolic extract in 10 ml 1% HCl , boiled, filtered and Mayer's reagent was applied.

2.3.2- Test for tannins

The test was carried out by subjecting 1g of the algal coarse powder in 10 ml of 99% methanol, then evaporated by the rotary evaporator till nearly dry, and then about 10 ml of NaCl (0.9%) warm solution was added , then divided in two portions each in a test tube. Adding to the first one a few drops of gelatin (1.0 %), and to the other test tube was added a few drops of $FeCl_2$. If the gelatinous residue was formed then the test is positive. In the other tube, if the solution turned blue, dark blue, green or dark green in colour, then the test is positive.

2.3.3- Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H_2So_4. A yellow colouration will be observed if flavonoid is present. The presence of flavonoids can be detected by adding a few drops of concentrated HCl to 5ml of the extract with fragments of Mg in the test tube, the solution will turn pink-red if the test is positive.

1.3.4- Test for saponins

Crude extract is mixed with 5 ml of distilled water in a test tube and shaken vigorously.

The formation of stable foam is an indication of the presence of saponins.

2.3.5- Test for terpenoids

5 ml of the extract were mixed with 2 ml of chloroform and 3 ml of concentrated H_2 So_4 were carefully added to form a layer. formation of a reddish brown colour is an indication of the presence of terpenoids.

2.3.6- Test for coumarins

For coumarins detection, a piece of filter paper was moistened in NaOH and placed and kept on the top of test tube containing a boiling crud extract. If the filter paper showed any yellow fluorescent under UV light , is an indication of positive presence of coumarins.

2.3.7- Test for anthraquinones

By the addition of 10 ml of KOH (0.5N) to 1g of the algal coarse powder, then add 1 ml of hydrogen peroxide (0.5%), then left for 5 minutes in a boiling water bath, after that 1 ml of acetic acid (pH = 5) and 5 ml of toluidine were added, then shake the solution in a separation funnel for a while, two layers will be formed, separate the upper layer and add to it 4 ml of KOH (0.5 N), a red colour will form if anthraquinones are present.

2.4. Anti-microbial activity test

Bacteria and fungi cultures were obtained from department of microbiology University of Zawia, these bacteria was two gram positive *Staphylococcus aureus* and *Bacillus subtilis*, and gram negative *Escherichia coli* and *Salmonella typhi,* while the fungi species were *Aspergillus sp.* and *penicillum sp* . The crude extracts of the seaweeds were subjected to microbial assay in *vitro* by " the hole-plate diffusion method " . Using sterile cork borer 8mm wide well was made on each plate. The sterile nutrient agar plates and potato dextrose agar were prepared and inoculated with respective bacterial and fungal cultures. A volume of 100 µl of the six different extracts were introduced to respective wells and allowed to diffuse for 30 minutes. The plates with bacteria were incubated at 37 C^0 for 24 hours, while the plates of fungi were incubated at 25 C^0 for 48 hours, the results were recorded at two periods, after 24 hours and 48 hours. After incubation the inhibition zones formed around the holes were measured. considering the clear zone greater than 10 mm as positive results.

3-Results and Discussion

3.1 Crude extract

The percentage of the crude extracts were determined for each algae and the most amount of crude was extracted from the brown algae *Sargassum vulgare* with a percentage of 79% and the least was *Enteromorpha linza* which was less than 60%, the others were *Padina pavonica* 71%, *Gelidium sesquipedale* 70%,*Cystoseira sp.* and *Codium tomentosum* 65% .

3.2-Phytochemical analysis

The purpose of this process is to detect the presence of bioactive compounds which might have antimicrobial potency. The presence of alkaloids, tannins, saponins, flavonoids, terpenoids,antheraquinones and coumarins was investigated and it is shown in [Table 1].

Table 1 Preliminary phytochemical screening of crude extracts of red , brown and green algal species

	Alkaloids	tannins	saponins	flavonoids	terpenes	antheraquinones	coumarins
				chlorophyta			
E. linza	+	-	+	+	-	+	-
C. tomentosum	+	++	++	+	+	-	-
				phaeophyta			
Cystoseira sp.	+	+	-	++	+	-	-
S. vulgare	+	++	+	++	+	-	-
P. pavonica	-	+	+	-	+	-	-
				rhodophyta			
G. sesquipedales	+	+	-	+	-	-	-

++ ve: moderate, + ve : poor , - ve : absent

Alkaloids were present in all algal species except *Padina pavonica* , and all in poor amounts. Tannins were present in moderate amounts in two alga, *Codium tomentosum* and *Sargassum vulgare*, and three of them showed poor amounts, *Cystoseira sp., P. pavonica* and *Gelidium sesquipedales* , while one tested negative *Enteromorpha linza*. For saponins only one species *C. tomentosum* showed moderate presence, while *E. linza, S. vulgare* and *P. pavonica,* tested poor, and the rest were tested poor. Flavonoids were present in moderate amounts in two alga *Cystoseira sp.,* and *S. vulgare,* three had poor amounts, *E. linza, C. tomentosum* and *G. sesquipedales*. While *P. padina* tested negative. Test for terpenoids proved either negative as in *E. linza* and *G. sesquipedales,* or poor in the rest of species. For antheraquinones only proved to be found in *E. linza* in poor amounts. Tests for comarins proved negative for all species. These results were in agreement with previous studies which showed presence of these bioactive compounds in most of marine algae. (Wang *et.al* 1998) , (Guven *et.al* 2010).

3.3- Anti-bacterial activity of the crude extract

Table 2. Anti-bacterial activity of methanol marine algae extracts.

Inhibition zone in mm

TG		Gram positive		Gram negative	
		Staphylococcus aureus	*Bacillus subtillis*	*Escherechia coli*	*salmonella typhi*
C	*C.tomentosum*	10 ± 0.0	10 ± 0.0	23± 0.0	24±0.0
	E. linza	*22*	12	30	22
P	*S.vulgare*	-	10	30	24
	P.pavonica	-	-	21	24
	Custoseira sp.	17	25	-	20
R	*G.sesquipedales*	12	10	30	21

TG= taxonomic group, C = chlorophyta, P = phaeophyta, R = rhodophyta.

Table 2 shows the inhibitory effect of crude extracts on bacteria species been used in this study. The algal methanolic extracts displayed different degrees of anti-bacterial activities against different bacteria. *C. tomentosum, E. linza* and *G.sesquipedales* were active against all tested bacteria, these results were in agreement with other reports (Alghazeer *et.al* 2013). While other algal extracts showed no activity against some tested strains of bacteria.

3.4-Anti- fungal activity of the crude extracts

Table 3. Anti-fungal activity of methanol algal extracts

Growth inhibition zone in mm					
TG		*Aspergillus sp.*		*Penicillum sp.*	
		After 24 hours	After 48 hours	After 24 hours	After 48 hour
C	*C.tomentosum*	32.5 ±0.0	30 ±0.0	-	10 ± 0.0
	E.linza	20.5	20	-	13
P	*S.vulgare*	20	20	-	12
	P.pavonica	23.5	22	-	10
	Cystoseira sp.	22.5	25	-	14
R	*G.sesquipedales*	33.5	35	-	15

TG= taxonomic group, C=chlorophyta, P=phaeophyta, R= rhodophyta

All crude extracts of the algal species under study showed inhibitory effect on *Aspergillus sp.*at 24 and 48 hours, while inhibition did not happen at 24 hours in case of *Penicillum sp.* . However, the inhibition was greater in case of *Aspergillus sp.,* than that of the case of *Penicillium sp.*. The inhibition effect of algal species was varied and the highest was crude extract of *G.sesquipedales.*

Conclusion

In conclusion, the results indicate that the algal species under study represent a rich source of phytochemical compounds, which have a significant capacity of antimicrobial activities, therefore screening of their natural products will be of great interest and further studies should be taken under consideration.

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الملخص

في هذه الدراسة تم إستخلاص المواد الكيمونباتية بإستخدام الميثانول من 6 أنواع من الطحالب البحرية التي تم تجميعها من شاطئ طرابلس. الطحالب كانت من الخضراء وعددها إثنان وهما *Enteromorpha linza* و*tomentosum* والبنية وعددها ثلاثة وهما *Codium* *Padina pavonica* و *Sargassum vulgare* و *Cystoceira sp.* وطحلب أحمر *Gelidium sesquipedale*. ولقد تم إختبار هذه المستخلصات على أربعة أنواع من البكتيريا إثنتان من الجرام الموجبة *Staphylococcus aureus* و *Bacillus subtilis* وإثنتان سالبة الجرام *Escherichia coli* و *Salmonella typhi* وتم الإختبار أيضا على نوعين من الفطريات *Aspergillus sp.* و *Penicillum sp.* . وقد بينت النتائج تفاوت عملية تثبيط نمو كل من البكتيريا والفطريات بالمستخلصات وأن الطحلب الأخضر *E. linza* كان له الأثر الأكبر على تثبيط نمو البكتيريا، وأن الطحلب الأحمر *Gelidium sesquipedale* له الأثر الأكبر على تثبيط نمو الفطريات. كما بينت الدراسة أن المستخلصات تتباين في نسبتها من الوزن الجاف لطحالب الدراسة حيث كان أعلاها في طحلب *S. vulgare* .