



## Antibacterial and antioxidant activities of Phlorotannins extracted from *Sargassum linifolium* brown alga

Mai kamal<sup>1</sup>, Saly Gheda<sup>2</sup>, Abeer Abdel Hamid<sup>3</sup>, Tarek M Mohamed<sup>4</sup>

1. Biochemistry Research Student, Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt

2. Assistance professor of phycology, Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt.

3. Lecturer of Biochemistry, Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt

4. Professor of Biochemistry, Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt

### KEY WORDS

### ABSTRACT

*Sargassum*  
*linifolium*;  
 brown alga;  
 phlorotannins;  
 polyphenol;  
 extraction  
 method;  
 identification.

Phlorotannins are a group of complex polymers of phloroglucinol which are unique compounds from marine brown algae. In the present study, Phlorotannins polyphenol compounds have been extracted from *Sargassum linifolium* brown alga and identified by FT-IR and UPLC-MS/MS. Moreover, the total phenolic content was determined by the Folin–Ciocalteu assay. The results demonstrated the presence of poly phenolic compounds in the extract with higher levels about (22.19 ± 3.42) mg / gram gallic acid. In addition *S. linifolium* phlorotannins extract showed higher antioxidant activity about 63.56 mg/g dry weight. Also, the bactericidal activity of phlorotannins extract has been examined against food-borne pathogenic bacteria. The result showed that phlorotannins has higher bacteriostatic activity, which lead to bacterial growth inhibition and bacterial death. These results suggested that *S.linifolium* phlorotannins extract could be utilized as a good source of antimicrobial agent in pharmaceutical industry.

### Introduction

Marine algae contain a large variety of components that may be involved in the prevention and treatment of health diseases by various mechanisms (Délérís *et al.*, 2016). Phlorotannins are a class of polyphenol compounds produced by brown seaweed as secondary metabolites and biosynthesized via the acetate malonate pathway (Li *et al.*, 2017). They

are present in the algae in free form or forming complexes with different components of the cell walls, such as alginic acid (Montero *et al.*, 2016). Phlorotannins are essential to the physiological integrity of alga and involved in a number of important secondary roles such as chemical defense, protection against oxidative

damage that occurs in response to changes in nutrient availability and UV radiation, interactions with other organisms or the abiotic environment, as well as being integral components of cell wall (Li *et al.*, 2017).

Phlorotannins have attracted considerable research interest for their broad health benefits and potential uses in a range of therapeutics (Montero *et al.*, 2016). The present study was undertaken to elucidate the characteristic of phlorotannin that extracted from Egyptian *Sargassum linifolium* brown alga. Also, antioxidant and antibacterial activity on different strains of bacteria of Phlorotannin were studied (Pérez *et al.*, 2016).

## 2. Materials and methods

### 2.1. Preparation of phlorotannin from *S. linifolium* brown alga

Phlorotannin has been extracted from *S. linifolium* brown alga, (collected from Tamar cruise beach, Ras Sidr Area, Egypt, and it has been identified according to (Guiry, 2018). Seaweed has been air dried at room temperature (25-30 °C), ground into powder and extracted with hexane, then it centrifuged at 3200 rpm for 3 minutes (Koivikko *et al.*, 2007). The pellet was treated with 70% acetone and centrifuged at 3200 rpm for 6 minutes. Finally, the supernatant was concentrated to dryness in an incubator at 40 °C and stored at 4 °C for analysis.

### 2.2. Identification of extracted polyphenol.

*Fourier transforms infrared (FT-IR) analysis:*

Dried Seaweed phlorotannin extract were powdered and analyzed as potassium bromide (KBr) pellets using FT-IR (Model-JASCO FT-IR 4100 LE, made in Japan; Range: 4000–400 cm<sup>-1</sup>, in micro analytical unit, Faculty of Science, Tanta University, Egypt).

### Chemical characterization of Seaweed extract by Ultra Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) technique

This technique was performed in the Center of Drug Discovery Research and development, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The mass spectrometer was operated in positive ion & negative ions acquisition mode which carried out on a XEVO TQD triple quadrupole instrument (Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer) using the following condition : Column : ACQUITY UPLC - BEH C18 1.7 μm - 2.1 × 50 mm Column , Flow rate : 0.2 mL/min , Solvent system : consisted of Water containing 0.1 % formic acid and Methanol containing 0.1 % formic acid , capillary voltage of 3 Kv, temperature of 440 °C and the mass range was set from 100 to 1000.

### 2.3. Quantification of total phenolic content (TPC) by the Folin–Ciocalteu assay.

Total phenolic content was determined by the Folin – Ciocalteu method (Baba and Malik, 2015). Briefly 20μl of seaweed extract put in tube then 1.58 mL water was added. Then 100μl of Folin-Ciocalteu reagent was added, and mixed well. Then 300μl of sodium carbonate solution was added, After 5 minutes the

mixture was left at 20 °C for 2 hours. Finally the absorbance was determined at 760 nm against the blank. Phenolic content was expressed as gallic acid equivalent per gram (GAE/g) of extract (Alhakmani *et al.*, 2013).

#### 2.4. Determination Total antioxidant activity

Total antioxidant capacity was measured according to the method of (Prieto, 1999). Phosphomolybdate assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of a green phosphate Mo (V) complex at acidic pH (Singh and Singh, 2008). Briefly 900 µl of distilled water was added to 100 µl of extract. Then 3 mL reagent (0.6M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate) was added and mixed well. The reaction mixture was incubated at 95 °C in the water bath for 90 minute. Finally the absorbance of mixture was measured at 695 nm and ascorbic acid was used as stander.

#### 2.4. Antibacterial activity

The antibacterial activities were determined by conventional agar diffusion assay. The stock solution of phlorotannis was dissolved in water and different concentration of extract was prepared (50-300 mg/mL) each disk contains 10 µl of phlorotannis extract. The experiment included three bacteria species, two types of Gram-positive bacteria, [*Staphylococcus aureus* and *Bacillus cereus*] and one

type of Gram-negative bacteria [*Escherichia coli*]. Bacterial cultures were made at Department of Microbiology, Faculty of Science, Tanta University, Egypt, and optimal growth conditions and purity were done, after incubation of cultures for 24 hours at 37°C. The bacterial growth inhibition zone was measured and minimum inhibitory concentration (MIC) was determined. MIC was determined as the lowest concentration of poly phenol extracts inhibiting the visual growth culture on agar plate (Teh *et al.*, 2017). Doxacyclin and Cephaloxin were used as control antibiotic.

### 3. Results:

#### 3.1. Fourier transforms infrared (FT-IR) analysis

The functional groups which detected by FT-IR spectrum (400 and 4000 cm<sup>-1</sup>) of isolated phlorotannin were illustrated in figure (1).

#### 3.2. UPLC-MS/MS

Furthermore UPLC-MS/MS of extract developed in this work indicate that 33 compounds were separated from *Sargassum linifolium*. The majority of compounds at peak (31, 52, 68, 78) were tentatively identified as phlorotannis figure (2) and table (1).

#### 3.3. Folin-Ciocalteu assay:

Phlorotannin extract from *Sargassum linifolium* brown alga had highest total phenolic content with the value 22.19 ± 3.42 mg gallic acid equivalents/ g.

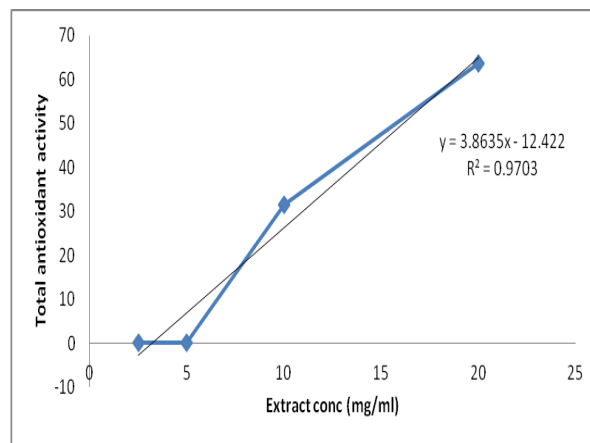
#### 3.4. Total antioxidant activity assay:

Total antioxidant capacity of isolated phlorotannin was also evaluated by

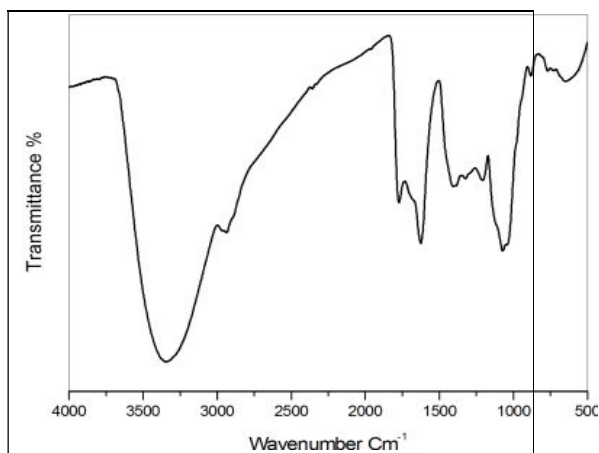
reference to standard curve of ascorbic acid. The total antioxidant capacity was increased by increasing concentration. It was indicated that 20 mg/mL phlorotannin extract give the highest total antioxidant capacity about 63.56 mg/g dry weight Figure (3).

### 3.5. Antibacterial activity:

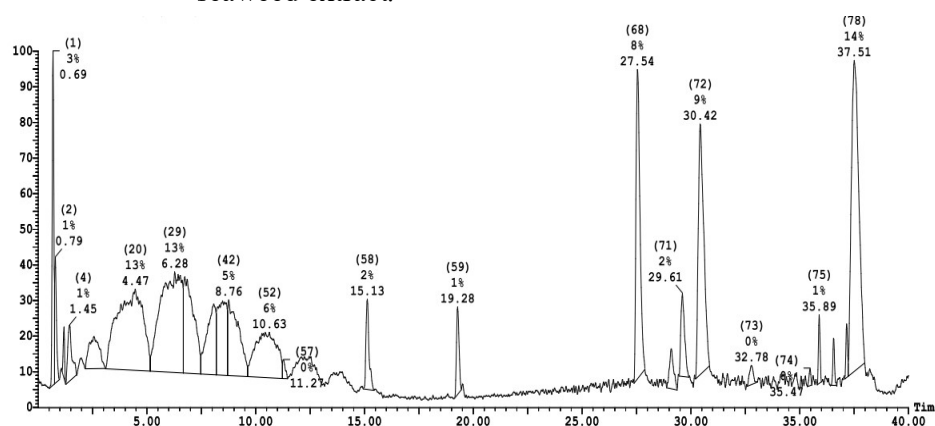
Phlorotannins extract showed bacteriostatic activity against species [*Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*] using serial dilution of extract (50 to 300 mg/mL), Table 2, 3.



**Figure (3):** Total antioxidant activity of *Sargassum* extract poly phenol



**Figure (1):** FTIR spectroscopy of *Sargassum* seaweed extract.



**Figure (2):** UPLC-MS/MS analysis of *Sargassum* seaweed extract

**Table (1):** Tentative peak assignment of the compounds separated by UPLC /MS found in *Sargassum* extract

Peak	Total RT(min)	[M-H] <sup>-</sup>	MS/MS fragments	Identification	Reference
20	4.47	690.5	633.4 , 587.3 243.1	Not identified	
29	6.28	783.9	735 , 378.1 , 271.9	Carmalol derivatives	(Lopes <i>et al.</i> , 2018)
31	6.74	671.4	647.4 , 343.3 , 335.3	Dihydroxy pentafulhalol	(Montero <i>et al.</i> , 2016)
52	10.63	801.5	788 ,763.5 , 404.3 , 393.4	Trihydroxy hexafulhalol	(Montero <i>et al.</i> , 2016)
68	27.54	524.6	353.3 , 271.3 , 240.3	Hydroxy tetrafulhalol	(Montero <i>et al.</i> , 2016)
72	30.24	566.6	397.3 , 41.4 381.3, 249.3	Not identified	
78	37.51	874.7	871.7	Phlorotannis-7 phloroglucinol	(Lopes <i>et al.</i> , 2018)

**Table (2):** Antibacterial activity of poly phenol extract by disc diffusion method.

Clinical bacterial isolates		Zone of inhibition (mm) of bacterial isolates			
		Conc. of <i>Sargassum</i> poly phenolic extract (mg/mL)			
Gram +ve bacteria	<i>Staphylococcus aure</i>	300	200	100	50
				18.6 ± 0.57	11.3 ± 0.57
	<i>Bacillus cereus</i>	17 ± 1.0	15.3 ± 0.57	12 ± 1.0	0 ± 0.0
Gram -ve bacteria	<i>Escherichia coli</i>	20.6 ± 0.57	16 ± 1.0	13 ± 0.57	9.6 ± 0.57

Values are mean inhibition zone (mm) ± S.D of three replicate, 0 = no inhibition zone

**Table (3):** Antibiotic effect on the isolated bacteria.

Antibiotics	Zone of inhibition (mm) of bacterial isolates		
	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Escherichia coli</i>
Doxacyclin (30 µg /disc)	25.6 ± 1.52	20 ± 1.0	10.6 ± 0.57
Cephaloxin (30 µg /disc)	14 ± 1.0	15.3 ± 0.57	7 ± 1.0

#### 4. Discussion

In the present investigation, crude phlorotannin which isolated from *Sargassum linifolium* brown alga was detected by rapid and simple methods. Due to the sensitivity of bioactive compounds in marine organisms, the choice of solvents and extraction methods are critical for evaluation of these compounds' biological activities. Extraction was performed by adding acetone (Koivikko *et al.*, 2007). The use of acetone for the extraction of polyphenol was increased the yield of polyphenols by inhibiting the interaction between polyphenols and proteins during extraction and by breaking the hydrogen bonds of polyphenol-protein complexes. FT-IR technique was performed as highly specific methods for phlorotannin identification. The functional groups which detected by FT-IR spectrum ( $4000$  and  $400\text{ cm}^{-1}$ ) were displayed and illustrated in (Fig. 1), the functional groups showed that the extracted compound contains a broad band at  $3352\text{ cm}^{-1}$  which indicated hydroxyl group (OH), band at  $2939\text{ cm}^{-1}$  that confirm the presence of (C-H) alkyl group. The peak at  $1326\text{ cm}^{-1}$  may possible presence of C-N stretch, peak at  $1771\text{ cm}^{-1}$  may possible presence of (C=O), peak at  $1208\text{ cm}^{-1}$  and sharp peak observed at  $1073\text{ cm}^{-1}$  may be due to the glycosidic linkage vibrations of C-O-C and C-OH, indicating the presence of some carbohydrates in the sample (Leyton *et al.*, 2016).

Also obtained data showed that phlorotannin which has been extracted from *Sargassum linifolium* brown alga have antibacterial and antioxidant activity. This confirmed by the inhibition zone which formed around the strain under study and by formation of a green color with Phosphomolybdate assay. The obtained data were in agreement with the published data by (Shiyamala, 2011). Phlorotannin can inhibit bacterial growth by inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane functions and increased

permeability of cell membrane causing structural and functional damage of bacteria (Coppo and Marchese, 2014). Moreover, the antioxidant properties of phlorotannins result from their chemical structure as phlorotannins are bi-polar in nature and have multiple phenolic groups (Sathya *et al.*, 2017).

#### 5. Conclusion

The phlorotannin extract of *Sargassum linifolium* possessed noticeable activity against both Gram positive and Gram negative bacteria, in addition to that the extract showed antioxidant activity. when compared with standard antibiotic. These results demonstrate a critical role of seaweed active metabolites as pharmaceutical agents in the treatment of human infections by bacteria.

#### 6. Acknowledgement

The authors wish to thank Dr Amira R. El Barky for her kind co-operation during this work.

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