Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(2): 609 – 630 (2024)
www.ejabf.journals.ekb.eg

Antimicrobial and Antioxidant Activities of Some Selected Seaweeds Species from the Western Coast of the Northern Egyptian Red Sea

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ARTICLE INFO

Article History:
Received: March 9, 2024
Accepted: March 22, 2024
Online: April 13, 2024

Keywords:
Red Sea seaweeds,
Antibacterial activity,
Antioxidant,
Pathogenic bacteria

ABSTRACT

The present investigation was carried out to evaluate the antimicrobial and antioxidant activities of four seaweed species gathered from the western coast of the northern Egyptian Red Sea using two different solvents: dichloromethane/ methanol (1:1 v/v) and ethyl acetate. These species are *Turbinaria ornata*, *Padina pavonica* (Phaeophyta), *Actinotrichia fragilis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta). The antimicrobial activity of the crude extracts was assessed *in vitro* against three microorganisms: Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus*) and the yeast *Candida albicans* by the disc diffusion method. Both extracts of *T. ornata* and *P. povonica* showed a wide spectrum of antibacterial activity, however, the fungal entity *Candida albicans* was not inhibited in any way by any of the examined seaweed extracts. The efficacy of both solvents was discussed. The antioxidant activity was detected using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The highest activity was recorded in the dichloromethane/ methanol extract of the brown seaweed *T. ornata*. On the other hand, the lowest activity was recorded in the ethyl acetate extract of the green seaweed *Ulva lactuca*. These findings revealed the potential of the Red Sea brown seaweeds as a promising candidate to act as antibacterial and antioxidant agent in medical applications. Further work is required to identify and separate the exact active compounds responsible for the biological activity.

INTRODUCTION

Antibiotic resistance is a major global problem **(Liu, 2017)**. The emergence of human pathogens strains resistant to antibiotics has been associated with the intensive use of antimicrobial drugs for prophylactic and therapeutic purposes **(Pradhan *et al.*, 2022)**. *Escherichia coli* is one particular kind of bacteria that can cause infection. Though it normally poses no danger to humans, it can develop into a pathogen that is resistant to multiple medications **(Ahlstrom *et al*., 2018; Warsidah *et al*., 2022)**. The Gram-positive *Staphylococcus aureus* is another frequent pathogenic bacterium that produces biofilms. It is generally linked to hospital-acquired illnesses causing numerous biofilm-related infections globally. The World Health Organization defines it as a member of a group that is highly prioritized in the search for innovative treatment approaches **(WHO, 2017; Rima *et al*., 2022)**.

On the other hand, during metabolism, reactive oxygen species (ROS) with superoxide, peroxyl and hydroxyl radicals are produced inside the cell **(Hamza *et al.*, 2015; Santos-Sánchez *et al.*, 2019)**. Such extensive oxidation causes numerous alterations in enzymes and cellular structures, as well as health issues including cancer, diabetes, cardiovascular issues, Alzheimer's, and Parkinson's disease **(Leopold, 2015; Pradhan *et al.*, 2022)**. However, synthetic antioxidants and antimicrobial agents are subject to strict regulation due to the potential adverse effects. Hence, it is of great interest to search for natural antimicrobials and antioxidants as substitutes for synthetic alternatives **(Amorim *et al.*, 2012; Rattaya *et al.*, 2015)**.

Studies focusing on the preparation and chemical composition of seaweed extracts revealed a broad range of secondary metabolites including phenolic compounds, sterols and terpenes. These compounds are characterized by a wide spectrum of biological activities with antitumor, anti-inflammatory, antimicrobial, antioxidants, antidiabetic, antivirus and neuroprotective activities **(Cox *et al.*, 2010; Martins *et al.*, 2018; Lopez-Santamarina *et al.*, 2020)**. Investigations on the biological activity of seaweed species encountered in the Red Sea have been done extensively. **El-Shoubaky and Salem (2014)** assessed the fatty acids of two seaweeds (*Padina pavonica* and *Hormophysa trquetra*) collected from Saudi Arabia's Red Sea shoreline for potential antimicrobial activity. **Ward and Deyab (2016)** investigated the potential antibacterial activity of *Turbinaria ornata* harvested from the Red Sea coast of Egypt against some bacterial species. In the same context, four brown seaweeds species (*Cystoseira myrica*, *Turbinaria turbinata*, *Sargassum cinereum* and *Hormophysa cuneiformis*) and two red species (*Actinotrichia fragilis* and *Laurencia papillosa*) from Egypt's Red Sea coast were analyzed as potent anticancer agents in the study of **Osman *et al.* (2020)**. **Alkhalaf (2021)** analyzed the properties of the red seaweed (*Chondrus crispus*) from the southeast shore of Jeddah, Saudi Arabia in terms of chemical composition, cytotoxic, anti-inflammatory and antioxidant properties. In view of the above, intensive research efforts are needed for screening new antimicrobial and antioxidant agents from natural sources. It is indisputable that the Egyptian Red Sea coastlines are abundant in both variety and quantity of algae **(Chiffings, 2003)**. However, none are utilized for commercial purposes with an obvious limited researches regarding their potential as functional food and bioactive agents **(Shanab, 2007; El-Manawy, 2008; Osman *et al.*, 2011; El-Manawy *et al.*, 2019)**. Consequently, the present work aimed to investigate some of the abundant species of seaweeds in the waters of the western coast of the northern Red Sea in terms of their antimicrobial and antioxidant activities. Algal extracts were obtained using two different solvents: dichloromethane/ methanol and ethyl acetate.

MATERIALS AND METHODS

Seaweeds samples collection

Based on a previous survey conducted at three sites on the western coast of the northern Egyptian Red Sea located in Suez (NIOF: 29° 55′ 29″ N, 32° 28′ 31″ E),

Hurghada (NIOF: 27° 17′ 07″ N, 33° 46′ 17″ E) and Marsa Alam (Port Ghalib: 25° 32′ 41″ N, 34° 38′ 30″ E) (Fig. 1), four seaweed species were selected as commonly abundant species. These are: Phaeophyta; *Padina pavonica* (Linnaeus) Thivy 1960 and *Turbinaria ornata* (Turner) J. Agardh 1848, Chlorophyta; *Ulva lactuca* (Linnaeus) 1753, and Rhodophyta; *Actinotrichia fragilis* (Forsskål) Børgesen 1932. Samples were harvested manually by snorkeling from the intertidal zone in the summer of 2022 during high tide (Plate 1). Seaweeds were identified morphologically based on several keys (**Lipkin, 1972; Aleem, 1993; Abbott, 1999)**.

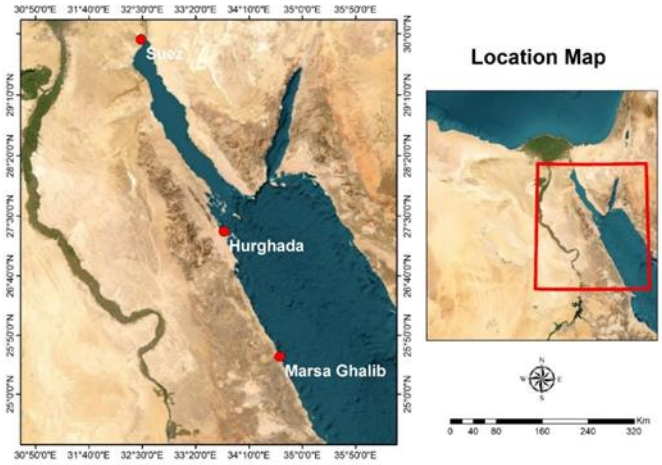

Fig. 1: Location of the selected sites along the Red Sea.

The collected seaweeds were thoroughly washed using seawater in the field to eliminate contaminants and to remove any sand particles and epiphytes. To preserve the samples and avoid water loss during transportation to the laboratory, they were placed in sterile plastic bags with sea water. Then they were completely cleaned with tap water and and rinsed in distilled water. Subsequently, samples were chopped by using scissors into small pieces, spread on paper sheets until dried at room temperature in the shade to prevent thermal degradation. *T. ornata* was difficult to cut, therefore samples were left to dry then broken into small pieces. The dried samples were grounded into very fine powder by using an electric blender and stored in tightly sealed polyethylene bags for further investigations.

Plate 1. Fresh and dried seaweed species: **(A)** *Turbinaria ornate*, **(B)** *Padina pavonica*, **(C)** *Ulva lactuca*, and **(D)** *Actinotrichia fragilis*

Preparation of algal extracts

Two solvents, dichloromethane/ methanol (DCM) (1:1 v/v) and ethyl acetate (E.A) were chosen for the extraction process. 25g of dried samples were soaked in 250ml of solvent in a conical flask wrapped in aluminum foil to prevent photolysis and kept overnight on a rotary shaker at 120rpm. The extraction procedure was repeated under the same conditions until the extract became clear. The samples were filtered out of the extract using Whatman No. 1 filter paper then concentrated using a rotary evaporator at 40ºC with reduced pressure and then completely dried at 40ºC in the oven. A

resuspension was made in dimethyl sulfoxide (DMSO), followed by storage in airtight dark bottles at 4°C in refrigerator.

The yield extract percentage was calculated following the equation of **Maisuthisakul and Pongsawatmanit (2004)**, as follows:

$$\text{Yield}\% = \frac{\text{Extract dry weight}}{\text{Seaweed dry weight}} * 100$$

Antimicrobial analysis of seaweed extracts

The analysis of algal extracts was conducted on three pathogens representing Gram-positive bacteria *Staphylococcus aureus* ATCC25923, Gram-negative bacteria *Escherichia coli* ATCC25922 and the yeast *Candida albicans* ATCC10231. These pathogens were obtained from the Center of Environmental Studies and Consultations at Suez Canal University, Egypt. All chemicals used were of an analytical grade, and the antimicrobial assay was performed in sterile plastic petri dishes (nerbe plus 90x16.5mm).

Subculturing of pathogens

Pathogen cultures were grown in nutrient broth media (HIMEDIA) at 37ºC and shaken at 121rpm for 24h for the bacteria and 48h for the yeast. The bacterial cells were collected using aseptic centrifugation (7min at 4000rpm). Then, they were resuspended in sterile saline solution to match the 0.5 McFarland standard density, which is approximately 1.5×10^8CFU/ mL, while a heavy suspension of harvested cells was prepared in sterile saline for the yeast **(Behravan *et al.*, 2019; Patil *et al.*, 2020)**.

Disc diffusion assay

The paper disc assay method was used to evaluate the antimicrobial activity of the algal extracts **(Tendencia, 2004)**. Discs of Whatman No. 1 filter paper with a 6mm diameter were autoclaved for 15min to sterilize them at 121°C. Subsequently, they were impregnated with two concentrations of algal extracts (1000, 2500µg/ disc). 25ml of melted Muller Hinton agar (HIMEDIA) was put into a sterilized petri dish and allowed to cool and solidify. 100µl of each suspension was spread on the surface of the solidified medium. The impregnated discs were distributed over the inoculated medium spaced appropriately from each other and from the plate wall.

Plates were placed for 20min at 4°C in the refrigerator to allow the extracts to diffuse then incubated for 24hrs at 37°C. Tetracycline disc (TE 30- Oxoid) was used as a positive control for bacterial pathogens, while the DMSO impregnated disc was used as a negative control. After incubation, the plates were photographed, and the inhibition zones were measured using ImageJ ij152 software. The assays samples were prepared in triplicate, and data were expressed as the mean diameter of inhibition zones in ranges of millimeters ± standard deviation. Analysis of variances in inhibition activity among the tested seaweed extracts by each solvent against the tested microbes was performed by ANOVA test using Minitab 17 program.

Antioxidant assay

The antioxidant activity of algal extracts was conducted at the Regional Center of Mycology and Biotechnology at Al- Azhar University, Egypt, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. DPPH is a stable free radical that turns color from red to yellow when scavenged. When antioxidants interact with DPPH, they reduce it to DPPH-H, causing the absorbance to decrease. Thus, the degree of discoloration is an indicator of the potential scavenging of the antioxidant compound regarding its ability to donate hydrogen **(Prior *et al.*, 2005)**. The following formula represents the scavenging reaction between an antioxidant (H-A) and DPPH:

$$\mathbf{(DPPH) + (H\text{-}A) \rightarrow DPPH\text{-}H + (A)}$$

Freshly prepared DPPH methanol solution (0.004%w/ v) was prepared and stored in the dark at 10°C. A methanol solution was prepared from the algal extracts at concentrations ranging from 500 to 5000µg. 3ml of DPPH solution was mixed with 40µL aliquot of the methanol solution. Absorbance measurements were immediately recorded using a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). Data were continuously recorded at 1min intervals to determine the reduction in absorbance at 515nm until the absorbance stabilized (16min). Additionally, the absorbance of the reference compound ascorbic acid and the DPPH radical without extract (control) were assessed. The plotted graph of extract concentrations against inhibition percentage was used to calculate the extract concentration providing 50% inhibition (IC50).

All the measurements were carried out in triplicates and then averaged. The following formula was used to calculate the percentage inhibition (PI) of the DPPH radical **(Yen & Duh, 1994)**.

$$\mathbf{PI = [\{(AC - AT)/ AC\} \times 100]}$$

Where:
AC = Absorbance of the control at t = 0 min and
AT = Absorbance of the sample + DPPH at t = 16 min

RESULTS

1. Yield extract of seaweeds

The yield extract values ranged from 1.4 to 4.1% for dichloromethane/ methanol extracts and 0.56 to 1.04% for ethyl acetate extracts. The extract with the highest yield was found in dichloromethane/ methanol extract of *P. pavonica* (4.2%), followed by *A. fragilis* (2.52%). In contrast, the lowest yield was recorded in the ethyl acetate extracts of *A. fragilis* and *U. lactuca* (0.56%) (Fig. 2).

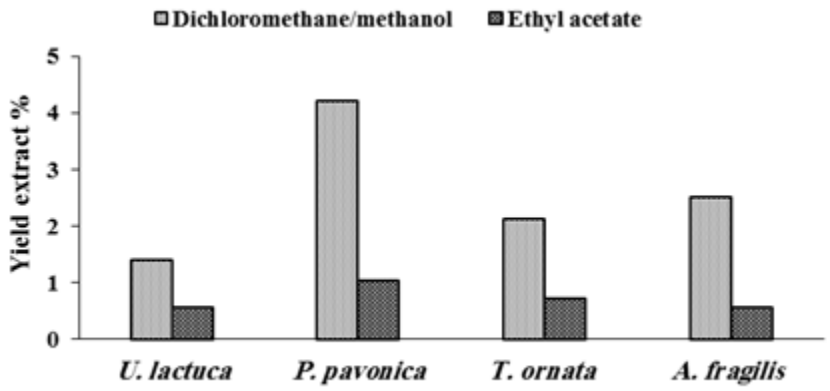

Fig. 2: Seaweeds yield extracts.

2. Antimicrobial activity of the seaweed extracts

Different extracts of *T. ornata*, *P. pavonica*, *A. fragilis* and *U. lactuca* were tested using the disc diffusion method to assess their potential antibacterial efficacy against three strains of pathogenic microbes. There was no obvious impact of any extract by the two solvents on the growth of the fungal pathogen *C. albicans*. The results are presented in Table (1) and Plate (2).

Comparing the crude extracts' antibacterial activity with that of the reference antibiotic (Tetracycline 30µg), all tested extracts were less potent than tetracycline. However, DCM extract of the brown seaweed *P. pavonica* was more active than tetracycline against the pathogenic bacteria *E. coli* with an inhibition zone of 18.89± 0.67mm (Table 1).

Table 2. Mean of inhibition zone diameter (mm) ±SD of the selected seaweed extracts against selected pathogenic microbial strains. **TE 30:** Tetracycline 30µg, ***E. coli:*** *Escherichia coli* (ATCC2592), ***St. aureus:*** *Staphylococcus aureus* (ATCC25923), ***C. albicans:*** *Candida albicans* (ATCC10231).

Solvent			Ethyl acetate			Dichloromethane/methanol		
Algal group	**Algal species**	**Conc. µg/disc**	**Microbial strain**					
			E. coli	***St. aureus***	***C. albicans***	***E. coli***	***St. aureus***	***C. albicans***
			Inhibition zone (mm)					
Brown	***T. ornata***	1000	-	13.25±0.8	-	11.14±2.1	8.99±0.7	-
		2500	-	**14.72±0.1**	-	-	12.08±1.4	-
	P. pavonica	1000	-	-	-	**16.56±0.6**	-	-
		2500	-	-	-	**18.89±0.7**	8.47±1.02	-
Green	***U. lactuca***	1000	9.21±0.9	-	-	-	-	-
		2500	9.66±1.3	7.89±0.4	-	-	13.43±2.4	-
Red	***A. fragilis***	1000	10.74±0.4	-	-	-	-	-
		2500	-	7.96 ± 0.7	-	-	10.99±1.2	-
TE 30			18.5±0.7	29.63 ± 1.1		18.5±0.7	29.63 ± 1.1	
***p*- value**			0.000***	0.000***		0.001***	0.000***	

*** significant at $p<0.05$

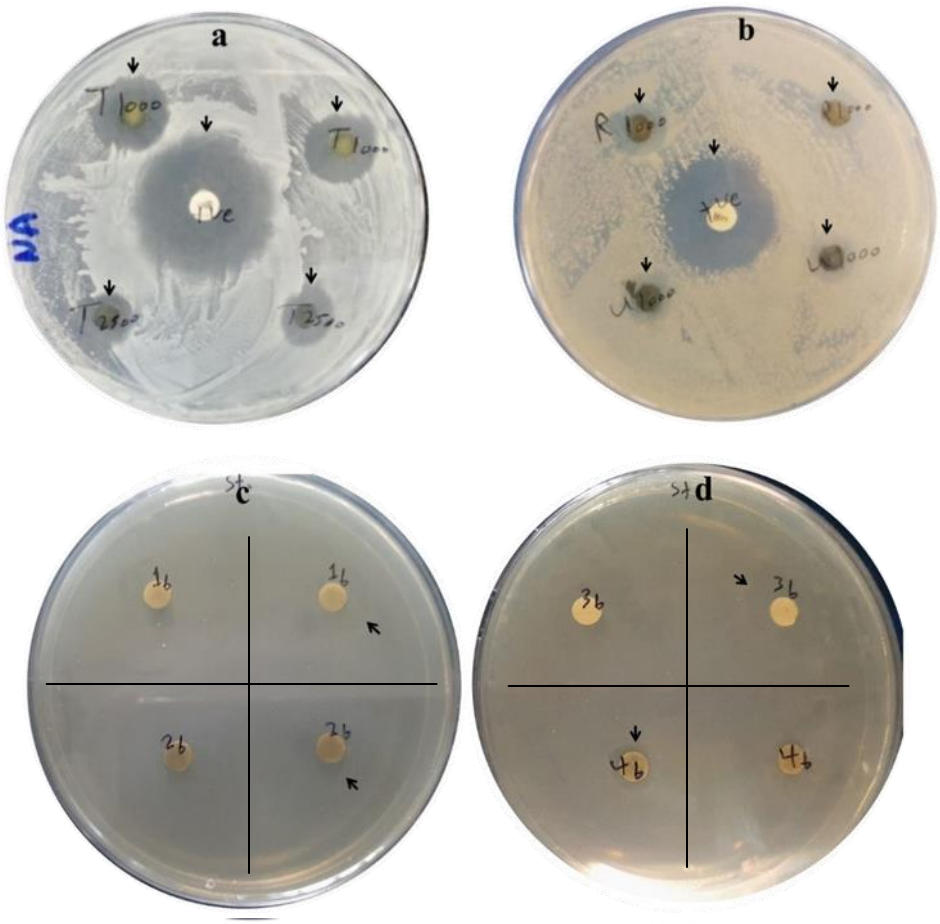

Plate 2: Inhibition zones of the seaweeds extracts against selected pathogenic microbial strains; two concentrations of EA extract of *T. ornata* against *St. aureus* **(a)**, EA extract of *U. lactuca* and *A. fragilis* against *E. coli* **(b)**, DCM extract of *T. ornata* and *P. pavonica* against *St. aureus* **(c)**, *U. lactuca* and *A. fragilis* DCM extract against *St. aureus* **(d)**. Arrows indicate the inhibition zones.

2.1. Ethyl acetate extracts

Turbinaria ornata extract exhibited the maximum antibacterial activity against *St. aureus* with inhibition zones of 13.25± 0.83 and 14.72± 0.12mm at concentrations of 1000 and 2500µg/ disc, respectively (Table 1). No inhibitory activity was detected for ethyl acetate extract of *P. pavonica* against the tested microbes. *E. coli* was more resistant to both brown seaweed extracts. Conversely, the extract from *A. fragilis* was the most effective against *E. coli*, with an inhibition zone of around 10.74mm. According to ANOVA analysis, there is a significant difference (P= 0.000) in the inhibition activities between the ethyl acetate extract of tested seaweeds against *E. coli* and *St. aureus* (Table 1).

2.2. Dichloromethane/ methanol extracts

Escherichia coli appeared to be the most sensitive pathogen for *P. pavonica* extract at both concentrations 1000 and 2500µg/ disc, with inhibition zones of 16.56± 0.60 and 18.89± 0.67mm, respectively (Table 1). However, *U. lactuca* extract showed the greatest activity against *St. aureus* at the concentration of 2500µg/ disc, with 13.43± 2.38mm inhibition zone, followed by *T. ornata* extract, with an inhibition zone of 12.08±

1.42mm at the same concentration. DCM extracts of *U. lactuca* and *A. fragilis* did not show any noticeable activity against *E. coli* pathogen. Similar to ethyl acetate, there is a significant difference (*P*= 0.001- 0.000) in the inhibition activities of the dichloromethane/ methanol extracts against both microbes (Table 1).

3. Antioxidant activity of seaweed extracts

The scavenging activity varied depending on the concentration of each crude extract, confirming that all seaweed extracts have different levels of antioxidant activity (Fig. 3). It was obvious that the scavenging activity of the tested extracts showed a dose-dependent pattern, which increased as the extract concentration increased.

As observed in Fig. (3), the DCM extract of *T. ornata* exhibited the maximum percentage of DPPH scavenging activity 96.78± 0.64%, followed by *P. pavonica* 94.61± 1.02%, which was comparable with that recorded for the reference control ascorbic acid (98.65 ±0.1%). Both *U. lactuca* extracts showed a contrast in their antioxidant activity; DCM extract exhibited a high percentage of DPPH scavenging activity of 93.82± 0.14%, while the EA extract showed the lowest activity of 68.17± 0.61%. A relatively similar antioxidant activity was obtained by *A. fragilis* with both DCM and EA solvents 75.91± 0.37 and 72.39± 1.45%, respectively. The extracts of the brown seaweeds *T. ornata* and *P. pavonica* showed the lowest IC50 value of 404.66± 6.79 and 905.11± 17.84µg/ ml, respectively, while the highest IC50 value was obtained with the ethyl acetate extract of *A. fragilis* and *U. lactuca* of 3397.28± 61.92 and 3887.29± 78.23µg/ ml, respectively (Fig. 4).

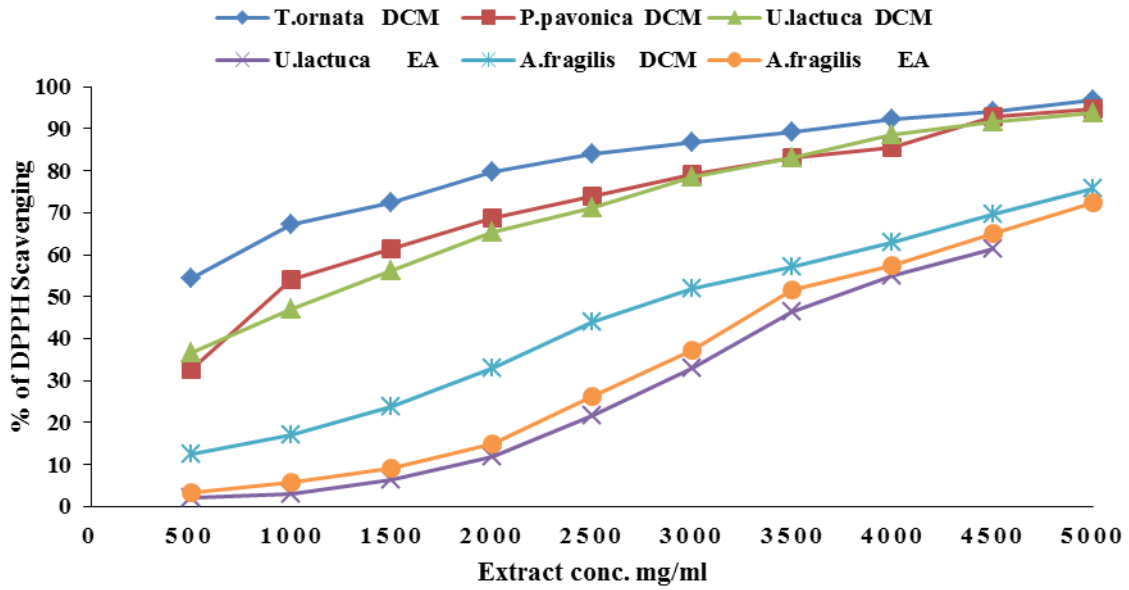

Fig. 3: DPPH radical scavenging activity of tested seaweeds.**(DCM):** Dichloromethane/methanol, **(EA):** Ethyl acetate.

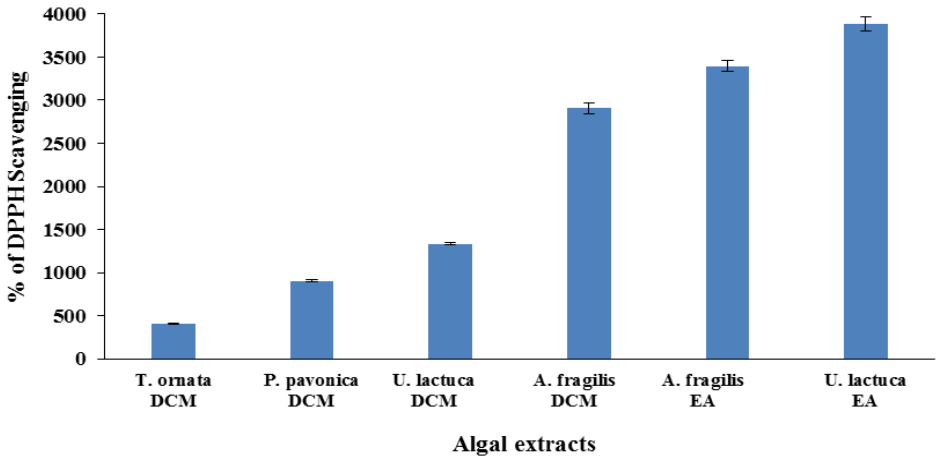

Fig. 4: IC50 value of DPPH scavenging of the algal extracts. (DCM): Dichloromethan/methanol, (EA): Ethyl acetate.

DISCUSSION

1. Yield of seaweed extracts

The yield of crude extracts differed considerably between the various seaweeds and solvents. DCM extract of *P. pavonica* recorded the highest yield of 4.2%, it indicates that 4.2g of active compounds were obtained from every 100g of seaweed biomass, followed by the DCM extract of the red seaweed *A. fragilis* of 2.52%. This result agree with that of **Abdullah *et al.* (2012)**, who reported that the methanolic yield extract of Rhodophyta species from Kedah, Malaysia, is the lowest compared to that of Pheophyta. **Oktaviani *et al.* (2019)** stated that, *T. ornata* ethyl acetate extract collected from Karapyak beach, Indonesia, yielded 3.4% higher than the green seaweed *Chaetomorpha antennina* 2%, which is in accordance with our findings. **Helal *et al.* (2023)** estimated the yield of the methanolic extract of *Sargassum dentifolium* collected from Hurghada, the Red Sea as 2.5%. These variations in extracts yield may depend on various factors, such as the seaweed's composition, temperature, extraction approach and extraction conditions **(Terblanche *et al.*, 2017; Afrin *et al.*., 2023)**. The estimation of seaweeds yield extract in industrial applications is a crucial aspect of assessing the effectiveness and cost viability of bioactive compounds that can be extracted from algae biomass, as suggested by **Helal *et al.* (2023)**.

2. Antimicrobial activity

2.1. Extraction solvents

In the present study, screening for antimicrobial activity of the four selected seaweeds prepared by two solvents exhibited variable inhibition levels on the tested microbial growth. According to our findings, the dichloromethane/ methanol extracts exhibited a broad spectrum of antibacterial activity. These results are in close agreement with those obtained by **Demirel *et al.* (2009)** who reported that, dichloromethane extracts

of five brown seaweeds caused better inhibition zones than methanol extracts for all bacterial strains tested from the Aegean Sea in Turkey. Similarly, **Oumaskour *et al.* (2012)** studied the antimicrobial properties of 27 seaweeds species belonging to the Chlorophyta and Phaeophyta in Morocco and observed that the best activity was in methanolic extracts, followed by acetone extracts, and those prepared by dichloromethane/ methanol extracts. According to **Shanab (2007)** and **Hamza *et al*. (2015)**, dichloromethane/ methanol extracts of species from the Chlorophyta, Rhodophyta and Phaeophyta groups in the Suez Canal demonstrated an antibacterial activity against the pathogens *St. aureus* and *E. coli*. Additionally, differences in antimicrobial assay techniques and variations in the extraction capacity approaches to isolate bioactive compounds may lead to varying susceptibilities of the target strains **(Cox *et al*., 2010; Salem *et al*.., 2011)**.

The dichloromethane/ methanol extract of *P. pavonica* recorded the greatest activity than the commercial antibiotic tetracycline against *E. coli*. In addition, it was noted that the inhibitory activity of *P. pavonica* was only observed in the extract with dichloromethane/ methanol. Therefore, variation in results may be attributed to the solubility of the bioactive metabolites of species, which varies depending on the solvent in which they are soluble, as suggested by **Karthikaidevi *et al*. (2009)** and **Salem *et al.* (2011)**.

2.2. Seaweed extracts

Antibacterial activities of tested seaweeds varied among species from the different groups. In our study, the brown algae *P. povonica* and *T. ornata* were more active against the tested pathogenic bacteria compared to other algal groups. Similarly, **Omar *et al*. (2012)** tested the activity of different seaweeds belonging to the Chlorophyta, Phaeophyta and Rhodophyta collected from the coast of Jeddah, the Red Sea. Their findings showed that *P. pavonica* and *T. triquetra* exhibited the maximum inhibition activity against various Gram-positive and Gram-negative pathogenic bacteria. Our results are also in accordance with those recorded for the highest level of antibacterial activity of brown seaweeds recorded by **Chandrasekaran *et al*. (2014**) in India, **Kolsi *et al.* (2015)** in Tunisia, and **Madkour *et al*. (2019)**, who reported the strong antibacterial activity of *T. ornata* from Wadi El-Gemal, the Red Sea against *St. aureus* and *E. coli*. On the other hand, **Salem *et al.* (2011)** recorded a higher antibacterial activity from the extracts of *Padina gymnospora* and *Sargassum dentifolium* than those of the red alga *Actinotrichia fragilis* in Hurghada, Egypt. However, this was in contrast to the findings of **Koz *et al*. (2009)** who stated that, extracts of the Chlorophyta had the greatest level of antimicrobial activity, followed by the Rhodophyta and the Phaeophyta in Turkey. Additionally, **Shanab (2007)** elucidated that, the red algal species *Jania corniculata* and *Laurencia papillosa* demonstrated a greater activity than the brown alga *Sargassum dentifolium* from the Suez Canal, Egypt.

According to **Hafez *et al*. (2022)**, certain environmental variables, viz. time and location of samples collection, herbivory, light, salinity and nutrients have a major impact

on the effectiveness of seaweed extracts against microorganisms. In addition to the aforementioned variables, extraction factors such as extraction method and the used solvents **(Zubia *et al.*, 2008; Silva *et al*., 2013)** have a role in determining the efficacy of seaweed extracts. Most of the brown seaweeds that exhibited antibacterial activity belong to an intermediate or susceptible category forming inhibition zones greater than 15mm **(Akremi *et al*., 2017; Silva *et al*., 2021)**. The strong activity of brown seaweeds could be related to the presence of tannins, terpenoids and phenolic compounds which inhibit microbial growth based on their concentration and composition **(Karthikaidevi *et al.*, 2009; Chandrasekaran *et al*., 2014)**.

2.3. Microbial strains

The study indicated that *St. aureus* was more susceptible to the algal extracts than the Gram-negative bacteria *E. coli*. In contrast, **El-Manawy *et al.* (2019)** reported that *Pseudomonas aeruginosa,* Gram-negative bacteria, was found to be more vulnerable to the alga that was collected from the Red Sea coast in Hurghada. Our results are in line with that of **Salem *et al.* (2011)** who reported that, *St. aureus* and *B. cereus* were the most susceptible to different eight seaweed extracts from the Red Sea coast of Hurghada, while the tested Gram-negative bacteria (*E. coli, Salmonella* sp., *E. faecalis,* and *P. aeruginosa*) were the most resistant. In the same context, the findings of **Abdel-Raouf *et al.* (2017)**, who evaluated the antibacterial activity of *C. barbata* from Red Sea, Safaga, Egypt and **Madkour *et al*. (2019)**, who studied the bioactivity of three brown seaweeds namely: *P. pavonica, C. myrica* and *T. ornata* from the Egyptian Red Sea coast had confirmed the sensitivity of Gram-positive bacteria to the tested extracts. Moreover, **Oumaskour *et al.* (2012)** observed the sensitivity of *St. aureus* to most algal extracts. **Demirel *et al.* (2009)** stated that the sensitivity of a specific group of bacteria was mainly due to the variation in their composition and cell wall structure. In Gram-negative bacteria, the cell's outer membrane serves as a barrier against many environmental substances preventing the entry of the inhibitors and antibiotics **(Tortora *et al.*, 2007; Mendes *et al.*, 2013; El-Manawy *et al*., 2019)**.

On the other hand, none of the tested seaweeds extracts inhibited the fungal pathogen *C. albicans*. Our observation confirms the findings of **Hamza *et al.* (2015)** who revealed that, the dichloromethane/ methanol extracts of seaweeds from the Suez Canal had no effect against any of the tested fungal strains *C. albicans* and *Aspergillus niger*. Similarly, **EL-Sheekh *et al*. (2022)** observed that *C. albicans* was more resistant to three brown seaweeds extracts *S. cinereum, C. Myrica* and *P. boergesenii* collected from the Red Sea coast, Hurghada using diverse solvents (ethanol, acetone and methanol). Generally, the antifungal efficacy of bioactive chemicals from seaweed extracts is primarily based on both the type of extraction solvent and the algae species **(Lotfi *et al*., 2021)**. On a comparative perception, Table (2) presents the results of our study in comparison with previous works on the antimicrobial activity of seaweeds worldwide. Our findings pointed out the potent impact of the various algal groups extracts on the growth of *St. aureus* pathogen which was confirmed by the previous results of many

authors. In addition, DCM is not widely used as an extraction solvent. Nevertheless, our findings revealed that DCM extracts obtained from several types of seaweeds exhibited a potentially effective antibacterial effect.

3. Antioxidant activity

DPPH radical scavenging activity assay has been extensively used as a stable free radical for screening antioxidant capacity of algae and is a useful reagent to assess the compounds' ability to scavenge free radicals **(Parthiban *et al*., 2013; Asaduzzaman *et al*., 2020)**. It is considered a simple, rapid and inexpensive method to measure antioxidant capacity of algae since it is sensitive enough to detect active ingredients at low concentrations in a short time **(Ganesan *et al*., 2011; Ismail, 2017)**. The activity was expressed as scavenging percentage and IC50 value, which is the concentration required to scavenge 50% of the DPPH radical. A lower IC50 value indicates a higher antioxidant activity **(El- shafay *et al*., 2021)**. Based on the present result, all of the investigated seaweed extracts have reduced the stable DPPH radical depending on the species and solvent utilized. Furthermore, as indicated by **Ibrahim *et al*. (2016)**, the antioxidant activity demonstrated a dose-dependent manner as the extract's concentration increased, hence improving the DPPH radical scavenging activity.

Dichloromethane/ methanol extracts of *T. ornata* and *P. pavonica* showed the maximum activity to scavenge free radical at the highest concentration (5000µg/ ml). This activity was comparable with that recorded for the reference control ascorbic acid (98.65 ±0.1%). Recently, **El-Shafay *et al*. (2021)** found that the methanol extract of *P. pavonica* obtained from Abu Qir in Alexandria, Egypt, recorded the greatest DPPH radical scavenging activity. Close results were obtained by **Shanab (2007)** who reported that, dichloromethane extracts of the brown seaweed *S. dentifolium* from the Suez Canal, Egypt, had the greatest free radical scavenging activity. Moreover, **El-Manawy *et al.* (2019)** confirmed the antioxidant activity of ethyl extracts of brown seaweed *Hormophysa cuneiformis* from Hurghada coast, the Red Sea. **Farghl *et al.* (2021)** stated that, *T. ornata* collected from the Egyptian Red Sea shore had a higher DPPH scavenging activity compared to other tested species *Polycladia indica*, *Laurencia obtusa* and *Sarconema scinaioides*.

On the other hand, our result showed that the lowest activity was observed in the ethyl acetate extract of the green seaweed *U. lactuca*. This result is in accordance with that of **El-Manawy *et al.* (2019)** who reported that, the lowest activity was recorded in the ethyl extract of the green seaweed *Caulerpa racemose*. In contrast, in Abu Qir Bay, Alexandria, Egypt, **Ismail (2017)** reported that green algae *Ulva fasciata* recorded higher antioxidant activity than the brown algae *Sargassum linifolium*. The IC50 values of DPPH scavenging activity of the seaweed extracts of the present study ranged from 0.4 to 3.89mg/ ml which was lower than that recorded by **Afrin *et al*. (2023)** (1.82 to 5.09mg/ ml) for six different seaweeds *Padina tetrastromatica, Sargassum muticum, Hydroclathrus clathratus, Botryocladia wrightii, Porphyra* sp., and *Gracilaria parvispora* collected from Saint Martin's Island of Bangladesh.

As observed, brown seaweeds appeared to have greater potential for antioxidant activity than green and red seaweeds species. These findings agree with those published by **Wang *et al*. (2009)**, **Cox *et al*. (2010)**, **Indu and Seenivasan (2013)** and **Sarojini *et al*. (2016)**. They suggested that the presence of polyphenols such phlorotannins may be the reason behind brown seaweeds' highest antioxidant activity. It functions as an antioxidative substance to assist the algae overcoming various environmental oxidative stresses **(Farghl *et al.*, 2021; Kerzabi-Kanoun *et al.*, 2021)**.

Tabe. 2. Comparative results of the antimicrobial activity of the different seaweeds studied worldwide. **E.A**: ethyl acetate, **DCM**: dichloromethane/ methanol, **A**: *Escherichia coli*, **B**: *Staphylococcus aureus*, **C**: *Candida albicans*. **+ve**: positive activity, **-ve**: negative activity, - : no data.

Reference	Region	Green seaweeds						Brown seaweeds						Red seaweeds					
		E. A			DCM			E. A			DCM			E. A			DCM		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
EL-Sheekh *et al.*, 2019	Red Sea	-	-	-	-	-	-	+ve	+ve	-	-	-	-	-	-	-	-	-	-
Salem *et al.*, 2011	Red Sea	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-
Abd El Hafez *et al.*, 2020	Red Sea	-ve	-ve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Omar *et al.*, 2012	Red Sea	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-
Chandrasekaran *et al.*, 2014	India	-	-	-	-	-	-	-	+ve	-	-	-	-	-	+ve	-	-	-	-
Pushparaj et al., 2014	India	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-
Lavanya and Veerappan, 2011	India	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-	+ve	+ve	-	-	-	-
Warsidah *et al.*, 2022	Indonesia	-	-	-	-	-	-	+ve	-	-	-	-	-	-	-	-	-	-	-
Hamza *et al.*, 2015	Suez Canal	-	-	-	+ve	+ve	-ve	-	-	-	-	-	-	-	-	-	+ve	-	-ve
Shanab, 2007	Suez Canal	-	-	-	-	-	-	-	-	-	+ve	+ve	+ve	-	-	-	+ve	+ve	+ve
Kolsi *et al.*, 2015	Tunisia	+ve	+ve	-	-	-	-	-ve	+ve	-	-	-	-	-	-	-	-	-	-
Oumaskour *et al.*, 2012	Morocco	-	-	-	-ve	-ve	-	-	-	-	+ve	+ve	-	-	-	-	-	-	-
Demirel *et al.*, 2009	Turkey	-	-	-	-	-	-	-	-	-	+ve	+ve	-ve	-	-	-	-	-	-
Present study	Red Sea	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve

CONCLUSION

The current study demonstrated the potential bioactivity of the studied seaweeds species that are commonly present along the western coast of the northern Egyptian Red Sea. Among the tested extracts, dichloromethane/ methanol extracts *T. ornata* and *P. povonica* were found to be the most effective antibacterial and antioxidant agents, which could be utilized in pharmaceutical industries. However, further researches need to be achieved to determine and assess the active compounds responsible for the biological activity.

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