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Original article

Antioxidant Properties, Phytochemical Screening of Posidonia oceanica in Sabratha Beach, Libya

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Abstract
This study investigates the antioxidant qualities of Posidonia oceanica, a type of seagrass that is widespread in the Mediterranean Sea, especially near Libya's Sabratha coast. Analyzing P. oceanica's phytochemicals related to any health advantages is the main goal of this study. A considerable amount of phenolic chemicals, particularly flavonoids, which are recognized for their antioxidant properties, were found by phytochemical screening. The extracts showed a great potential to reduce oxidative stress by demonstrating a notable capacity to scavenge free radicals. The importance of Posidonia oceanica as a source of bioactive antioxidant properties is highlighted in this study, which also suggests possible uses in the nutraceutical and pharmaceutical sectors.
Keywords: Antioxidant, Phytochemical, Posidonia oceanica, Libya

Introduction

To fight off harmful microbes, marine organisms create secondary metabolites and bioactive chemicals [1]. Seagrasses that are native to the Mediterranean Sea, such as Posidonia oceanica (L.) Delile, are important supplier of these substances [2]. Because it stabilizes the seafloor and provides marine life with food and shelter, P. oceanica is essential to marine ecosystems [3]. According to studies, P. oceanica includes substances such as flavonoids and phenols that have a variety of biological actions, including anti-cancer properties [4]. For example, extracts from its roots and leaves have demonstrated dose-dependent cytotoxicity and anti-cancer efficacy against HepG2 cells. Furthermore, in animal models, freeze-dried P. oceanica leaves showed antioxidant and anti-diabetic benefits [5]. Furthermore, Staphylococcus aureus development is inhibited by the chemical linoleic acid that is derived from seagrasses. Boseidonisol, a substance that was isolated from P. oceanica, has demonstrated antibacterial properties. Additionally, P. oceanica-derived peptides showed anti-biofilm efficacy against Staphylococcus aureus [5]. According to European legislation, the Mediterranean Sea's indigenous seagrass Posidonia oceanica is a priority habitat for environmental conservation [6]. Although it offers substantial environmental benefits, this plant is vulnerable to human disruptions [7]. Research shows that P. oceanica accumulates contaminants, including heavy metals, and it has been suggested that it could be used as a biological indicator of pollution [8]. P. oceanica changes the content of phenolic chemicals, which are thought to be appropriate biomarkers, in response to stress brought on by pollution and natural stressors [9]. These substances have advantageous biological characteristics, such as antioxidant capacity [10].
Studies on the phenolic components in P. oceanica have identified a number of important chemicals, including caffeic acid and ferulic acid [11]. However, the roots have received little attention in research, which has frequently focused on leaf tissues [12]. The majority of the research that is currently available on the roots has focused on either the lignin content or total phenolics [13]. In this study, we examine algae in Libya's coastal Sabratha region, which has received little attention in our nation. In particular, this study aims to P. oceanica's phytochemicals, focusing on its phenolic and flavonoid content.

Methods

Study area

A selection of seaweeds was gathered at Libya's Sabratha Beach. Sabratha Beach is a seaweed-rich eco-area with great biodiversity, which makes it the perfect place to carry out this kind of research (Figure 1).

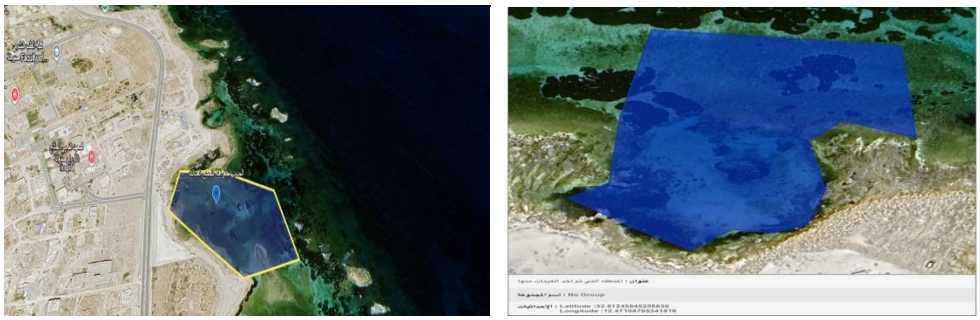

Figure 1. Geographical boundaries of samples collected from the beach of Sabratha city.

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Preparation of P. Oceanica Extract

A predetermined procedure was followed in order to extract the hydrophilic components from P. oceanica Delile. In August 2024, dried leaves were gathered, dried, then chopped, and suspended for three hours at 65 °C in 10 milliliters of 70% ethanol per gram of leaves. Centrifugation was used to separate the ethanol extract from the debris, and the supernatant was combined 1:1. Hydrophilic chemicals were kept in the pure extract, which was aliquoted in 1 mL increments, whereas hydrophobic compounds were eliminated by shaking. Before being used, the extracts were dissolved in 0.5 mL of 70% ethanol in sterile water after being concentrated using a UnivapoTM vacuum-spin concentrator**.**

Qualitative phytochemical screening

Detection of Alkaloids

A series of experiments on separately dissolved extracts in diluted hydrochloric acid were used to identify alkaloids, and the solutions were then filtered.

Mayer's Test

The filtrates were subjected to Mayer's reagent, which is made up of 5 g of potassium iodide dissolved in 10 ml of water, 1.358 g of mercuric chloride dissolved in 60 ml of water, and water diluted to 100 ml. The presence of alkaloids is indicated by the production of a yellow cream precipitate.

Wagner's Test

Wagner's reagent, which consists of 1.2 g of iodine and 2 g of potassium iodide dissolved in 5 ml of water and then diluted to 100 ml with distilled water, was also applied to the filtrates. Alkaloids are indicated by the presence of a brown or reddish-brown precipitate.

Detection of Flavonoids

Lead Acetate Test

A few drops of lead acetate solution were added to the extracts. The presence of flavonoids is shown by the production of a yellow precipitate.

H_2SO_4 Test

Sulfuric acid (H_2SO_4) was added in little drops to the extracts. Flavonoids are indicated by the appearance of an orange tint.

Detection of Steroids

The Liebermann-Burchard test was used to determine whether steroids were present. In this process, 0.5 g of the extract was mixed with 2 ml of acetic anhydride, and then 2 ml of sulfuric acid (H_2SO_4) was added. In certain samples, the presence of steroids is indicated by a color shift from violet to blue or green.

Detection of Terpenoids

Salkowski's assay was utilized to identify terpenoids. This process involved combining 0.2 g of the extract with 2 ml of chloroform, then carefully adding 3 ml of strong sulfuric acid (H_2SO_4) to create a separate layer. The presence of terpenoids is indicated by the development of a reddish-brown coloring at the inner face.

Detection of Anthraquinones

Borntrager's assay was used to identify anthraquinones. In a water bath, 0.2 g of the extract was heated for a few minutes with 10% hydrochloric acid (HCl), filtered, and then allowed to cool. After adding an equal amount of chloroform ($CHCl_3$) to the filtrate, a few drops of 10% ammonia (NH_3) were added, and the mixture was heated. Anthraquinones can be detected by the production of a pink tint.

Detection of Phenols

Ferric Chloride Test

A few drops of a 5% ferric chloride solution were added to the extracts, giving them a bluish-black hue that denotes the presence of phenols.

Lead Acetate Test

A few drops of lead acetate solution were added to the extracts. There are phenols present when a yellow precipitate forms.

Froth Test

Five milliliters of distilled water were mixed with around 0.2 grams of the extract. There are saponins present when a steady froth with tiny bubbles forms.

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Detection of Tannins
Ferric Chloride Test
In a water bath, a tiny amount of extract was combined with water and boiled. After filtering the mixture, the filtrate was treated with 0.1% ferric chloride. Tannins are indicated by the development of a dark green tint.

Fehling's Solution A
A final volume of 500 milliliters is achieved by dissolving 34.66 grams of copper sulfate in distilled water.

Total phenolic content assay
With some adjustments, the Folin-Ciocalteu reagent method was used to quantify total phenolics. Either water or ethanol extracts of the studied algae were used to evaluate their phenolic content. In particular, 1 mL of a 10% diluted Folin-Ciocalteu reagent was combined with 0.2 mL of the extract (alcoholic or aqueous). After that, the combination was left to react for four minutes in the dark. After 30 minutes, the total volume was adjusted to 10 mL using solvent after adding 0.8 mL of 7.5% sodium carbonate. A wavelength of 765 nm was used to measure the solution's absorbance. Gallic acid was utilized as a reference compound, and the average total phenolic content was calculated using different amounts of each product. using gallic acid as a reference substance. The concentrations 10, 20, 28, 30, 40, 50, and 60 mg/L were used to create the calibration curve.

Flavonoid content
Using particular reference materials, the chlorogenic acid technique was used to quantify total flavonoids. The standard calibration curve was created utilizing concentrations of 1, 5, 10, 20, 40, and 60 mg/L in order to calculate the total flavonoid content equal to rutin. Furthermore, the number of flavonoids in the algae's ethanolic and aqueous extracts was measured. 0.3 mL of sodium nitrite ($NaNO_2$), 4 mL of distilled water, and 1 mL of the extract (alcoholic or aqueous) were mixed together. 0.3 mL of aluminum chloride and 6 mL of distilled water were added after 5 minutes. After 10 minutes, the volume was increased to 10 mL with distilled water after adding 2 mL of 1 M sodium hydroxide. At 510 nm, the absorbance of the resultant solution was measured.

DPPH Free Radical Scavenging Assay
Following published protocols, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical assay was used to assess the extract of P. oceanica's capacity to scavenge free radicals (13). To create a 50 mL diluted solution, 25 mg of dry crude extracts were dissolved in distilled methanol. This solution was used as a foundation for test concentrations of 50 µg/mL, 100 µg/mL, and 150 µg/mL. As a standard reference, ascorbic acid was used. In the experimental setup, 1 mL of a 0.001 M DPPH solution was added to 5 mL of each produced solution in a test tube. For half an hour, all solutions were incubated in the dark. One milliliter of DPPH solution was mixed with five milliliters of methanol extract to create a control solution.
After the incubation period, an Optima UV-Visible spectrophotometer set to 517 nm was used to measure the mixtures' antioxidant activity. For dependability, every experiment was carried out three times. Using the proper formula, the % inhibition of DPPH was determined.
%I=Ab-As/Ab×100
Whereas:
%I = Percentage of inhibition rate
Ab = Absorbency of DPPH solution without extract
As = Absorbency of DPPH solution after adding the extract.

Results and Discussion
Table 1 displays the results of the qualitative phytochemical analysis of the ethanol extract of Tamarix conducted for this study. Alkaloids, coumarin, anthraquinone, terpene, flavonoids, tannins, alkaloids, and phenols.

Table 1. Phytochemical screening of the Ethanol extract of Posidonia oceanica

Phytochemicals	Ethanol extract	Methanol extract	Acetone extract
Alkaloids Mayer's test Wagner's test	+++	+	_
Flavonoids Lead acetate test H_2SO_4 test	+++	+	+
Steroids Liebermann Burchard test	+++	++	+++
Terpenoids Salkowski test	_	_	_
Anthraquinone Borntrager's test	_	_	_
Phenols, Ferric chloride test, Lead acetate test	+	+	_
Saponin	_	_	+

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Tannin	++	++	+
Carbohydrates	+	+	+
Oil And Resin	_	_	+

The total phenolic content

Posidonia oceanica has a total phenolic content of 8.42 mg of gallic acid equivalents per gram for aqueous extracts and 27.5 mg of gallic acid equivalents per gram of dry weight for ethanolic extracts. In line with the results presented in the reference, this suggests that the alcoholic extract contains more phenols than the water extract.

The flavonoid content

The flavonoid content in the ethanol extracts is higher than that in the aqueous extracts, with a value of 48.3 mg equivalent to protein per gram of dried algae for the ethanol extract and 18.2 mg equivalent to protein per gram for the aqueous extract.

Antioxidant activity

An authorized method for assessing the antioxidant capacity of plant extracts is the DPPH-based scavenging of free radicals. This approach is frequently used to measure the antioxidant capacity of plant extracts because it takes less time to analyze. Because of their capacity to donate hydrogen, DPPH is regarded as a highly powerful antioxidant. Eliminating these free radicals is essential to preventing their harmful effects on a number of illnesses, including cancer (14,15). The acetone extract of Posidonia oceanica has higher quantities than vitamin C (ascorbic acid), while the methanol extract has lower levels, as in table 4.

Table 2. Illustrates the estimation of the antioxidant activity of Posidonia oceanica.

	Methanol	Ethanol	Acetone	VIT C
10	6.8712	20.613	49.325	78.2
20	7.9755	24.785	52.883	80.5
30	13.988	25.644	57.669	83.3
40	19.877	28.466	60	86.2

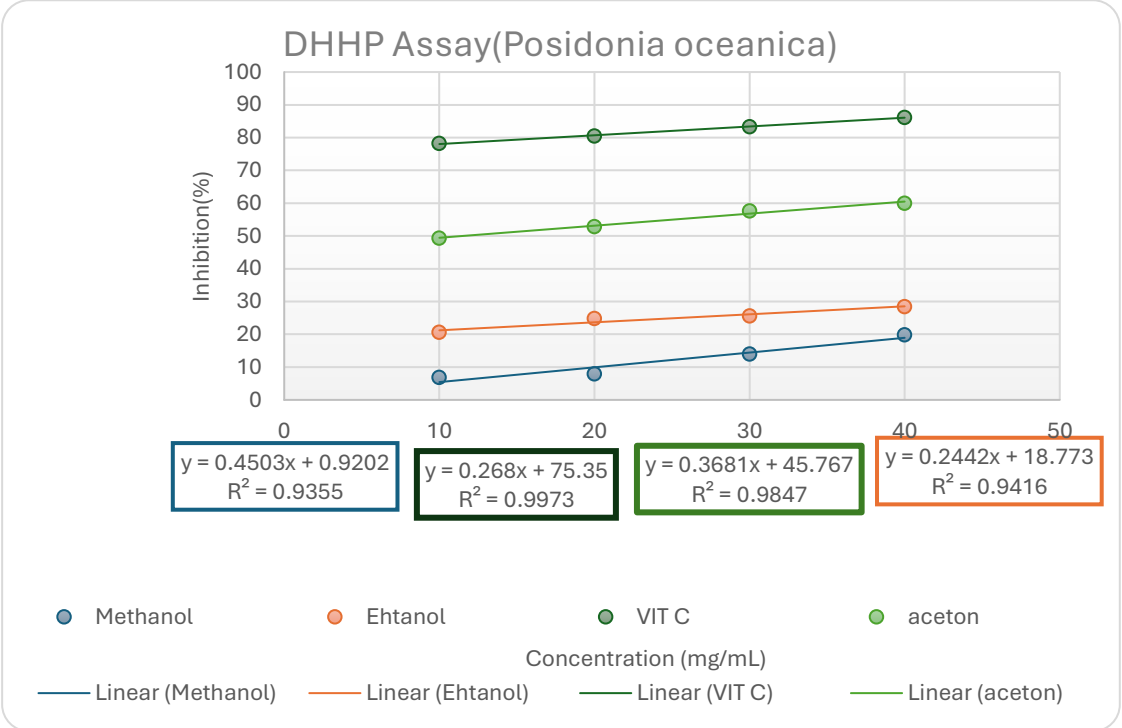

Figure-2 illustrates the estimation of the antioxidant activity of the findings of this study.

Conclusion

The findings of this study suggest that the utilization of seaweed extracts could enhance the potential for developing natural medicines. Further experimentation is required to optimize the extraction processes. Seaweeds, known for their therapeutic properties, exhibit rapid growth and possess a high protein content, along with a diverse profile of bioactive compounds. Specifically, phenolic compounds derived from the leaves of *P. oceanica* should be recognized as a significant source of phenolics that may serve as functional

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ingredients. In in vitro assays, the ethanolic extract demonstrated the highest antioxidant activity, indicating its potential application in health-related products.

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