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ORIGINAL ARTICLE / ÖZGÜN MAKALE

TOTAL PHENOLIC AND FLAVONOIDS QUANTIFICATION AND ANTIOXIDANT ACTIVITY OF BIOACTIVE EXTRACTS FROM THE LEAVES OF *ATRIPLEX HALIMUS*

ATRIPLEX HALIMUS YAPRAKLARINDAN ELDE EDİLEN BİYOAKTİF EKSTRAKTLARIN TOPLAM FENOLİK VE FLAVONOİD ÖLÇÜMÜ VE ANTİOKSİDAN AKTİVİTESİ

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ABSTRACT

Objective: *This study sought to identify potential sources for upcoming novel antioxidants in food and pharmaceutical formulations by screening various solvent extracts from the leaves of Atriplex halimus Lin. for their ability to exhibit strong antioxidant activity in vitro, as well as their total phenolic and flavonoid contents.*
Material and Method: *To determine the total amount of polyphenols and flavonoids in Atriplex halimus extracts, including ethyl ether, ethyl acetate, and n-butanol extracts, as well as their corresponding impact on this plant's antioxidant activity, were carried out using the conventional procedures.*
Result and Discussion: *In the current investigation, total phenolic and flavonoid contents in butanolic extract were found to be 68.20 mg gallic acid equivalent (GAE)/g dry extract) and 439 mg quercetin equivalent (QE)/g dry extract. The hydro-alcoholic extract was extracted by liquid/liquid partition with solvents of increasing polarity: ethyl ether, ethyl acetate and n-butanol) by the free radical DPPH removing garbage and HPTLC as well as their reduction kinetics. It was found that the extract of butanol and ethyl acetate had powerful uplifting power garbage DPPH with IC_{50} values of 2.1959 and 2.4234 mg/ml, respectively.*
Keywords: *Antioxidant activity, Atriplex halimus, bioactive extract, DPPH, phytochemical, quercetin*

ÖZ

Amaç: *Bu çalışmada, Atriplex halimus bitkisinin tamamının çeşitli solvent ekstrelerini, in vitro güçlü antioksidan aktivite sergileme yetenekleri ve ayrıca toplam fenolik ve flavonoid içerikleri açısından tarayarak, gıda ve farmasötik formülasyonlarda gelecek yeni antioksidanlar için potansiyel kaynakları belirleme amaçlandı.*
Gereç ve Yöntem: *Etil eter, etil asetat ve n-butanol ekstreleri dahil olmak üzere Atripleks halimus ekstrelerindeki polifenollerin ve flavonoidlerin toplam miktarının yanı sıra bunların bu bitkinin antioksidan aktivitesi üzerindeki karşılık gelen etkilerini belirlemek için geleneksel prosedürler kullanılarak gerçekleştirildi.*
Sonuç ve Tartışma: *Mevcut araştırmada, bütanolik ekstredeki toplam fenolik ve flavonoid içeriğinin 68.20 mg gallik asit eşdeğeri (GAE)/g kuru ekstrakt) ve 439 mg kersetin eşdeğeri (QE)/g kuru ekstre olduğu*

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bulunmuştur. Hidro-alkolik ekstresi, artan polariteye sahip solventler (etil eter, etil asetat ve n-butanol) ile sıvı/sıvı partitisyona tabi tutulmasıyla, serbest radikal DPPH'nin atıkları ve HPTLC'yi ve bunların indirgeme kinetiklerini ortadan kaldırmasıyla ekstre edildi. Butanol ve etil asetat ekstreleri sırasıyla 2.1959 ve 2.4234 mg/ml IC_{50} değerleriyle DPPH radikali süpürücü aktivitesine sahip olduğu bulundu.
Anahtar Kelimeler: *Antioksidan aktivite, Atriplex halimus, biyoaktif ekstre, DPPH, fitokimyasal, quercetin*

INTRODUCTION

Numerous plants, including aromatic, medicinal, and other types, have intriguing biological qualities that are used in a variety of contexts, including cosmetics, pharmacy, and medicine. However, assessing the antibacterial and antioxidant qualities of plant protection remains a highly intriguing issue when using whole particles for uncommon, or unknown plants in traditional medicine [1]. *Atriplex halimus* is a shrubby, succulent halophyte that is commonly found in semi-arid Mediterranean regions, particularly on high plateaus and along the littoral regions, where favorable conditions are regrouped with an intra- and interindividual polymorphism for a number of floral morphological characters, such as styles, ovule types and radicle orientation according to salinity [2,3]. *A. halimus* has up to 10% sodium chloride, according to a study of its chemical composition, and it also contains secondary metabolites such tannins, flavonoids, saponins, alkaloids, and resins [4,5].

In this work, we use a DPPH radical scavenging and reducing power test to examine the polyphenol content and antioxidant capacity in *A. halimus* leaves in methanolic extract. (The aqueous residue was then partitioned sequentially with ethyl ether, ethyl acetate and n-butanol) [6].

MATERIAL AND METHOD

Plant Material

Atriplix halimus was collected in march 2019 from Boukais (South Western Algeria) Algeria. It was identified by several herborists, a voucher specimen was deposited at the herbarium of the Chemistry and Science Environment Laboratory, South West of Algeria, University of Béchar.

Extraction

Using a soxhlet apparatus, 100 g of dried *Atriplex halimus* plant leaves were extracted with 400 ml of 80% MeOH; reflux was carried out for four hours.

The residue was evaporated in a vacuum device, and the natural product present in the bioactive extract was identified using the working principles of chemical screening [7-8]. The resulting product can be dissolved in 100 ml of distilled water to produce a brown-colored aqueous solution. This aqueous residue was divided using n-butanol, ethyl ether, and ethyl acetate in that order [9-10].

Total Phenolic Quantification

Standard process designed the procedure. For the quantification of total polyphenols, this method has been used. Each sample extract was transferred to a 25 ml volumetric flask containing 2.5 ml of 3.54 $g.l^{-1}$ Iron(III) chloridehexahydrate ($FeCl_3.6H_2O$) solution. The sample solution was then placed in a volumetric flask and kept at 80°C in a water bath for 20 min. Following that, 2.5 ml of acetate buffer (CH_3COOH/CH_3COOK) solution (pH 4.6), 5.0 ml of 3.28 $g.l^{-1}$ 1,10-phenanthrolinehydrate (1,10-phen), and 2.5 ml of 3.72 $g.l^{-1}$ Ethylene diaminetetraaceticaciddihydrate (EDTA) solutions were added, in that order. Finally, each flask was filled with distilled water to the specified level, chilled, and absorbance measurements were taken at 511 nm [11].

Total Flavonoid Quantification

The total flavonoid content of the plant extracts was determined by producing different aliquots of the extracts. 0.1 ml 10 percent aluminum chloride and 0.1 ml potassium acetate (1 M) were added to this method, and the final volume was increased to 3 ml by adding distilled water. The samples were then incubated at room temperature for 30 minutes.

The calibration curve was created by reading the absorbance at 415 nm and using quercetin as a reference. The total flavonoid content was quantified using the standard curve of quercetin and the results were represented in milligrams of quercetin equivalents (QE) per gram of dry extract (mg QE/g of dry extract) [12].

Determination of Free Radical Scavenging Activity by DPPH Method

The scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was used to determine the antioxidant potential of the crude extracts of n-butanol, ethyl ether, and ethyl acetate. In summary, 1.9 ml of a DPPH (0.004%) methanol solution has been mixed with 100 µl of different extract concentrations in methanol. The mixture was first given a good shake before being let to stand at room temperature for half an hour in the dark. A double-beam UV–vis Camspec M550 spectrophotometer was used to test the mixture's absorbance at 517 nm. A mixture of 100 µl of methanol and 1.9 ml of DPPH is used as the control. Using the following formula, the scavenging activity on the DPPH radical was expressed as an inhibition percentage [13]:

$$\%\text{Inhibition} = [(A_B - A_S)/A_B] \times 100$$

Where A_S is the absorbance of the test compound and A_B is the absorbance of the control reaction, which is made up of all the reagents except the test compound. Antioxidant ascorbic acid has been utilized as a positive control or for comparison. There were three copies of each test run. The graph of the inhibition percentage plotted against the extract concentration (0.5; 0.25; 0.125; 0.0625; 0.0312; 0.0156; 0.0078 mg/ml) was used to determine the extract concentration producing 50% inhibition (IC_{50}). Quercetin was used as a standard to determine the calibration curve after the absorbance was measured at 415 nm. To measure the total flavonoid content using the quercetin standard curve, each test was run three times, and the findings were represented in milligrams of quercetin equivalents (QE) per gram of dry extract (mg QE/g of dry extract).

RESULT AND DISCUSSION

Using the Folin-Ciocalteu technique, the total phelolic content of all examined extracts was determined. The butanolic extract was shown to be the most active, with a total concentration of 68.20 ± 0.03 GAE mg/g in dry extract. However, ethyl acetate had 38.80 ± 0.11 mg GAE/g, but diethyl ether extract contained 26.40 ± 4.73 GAE mg/g, dry extract (Table 1).

The total flavonoid content of butanolic extract was 439 ± 2.77 mg QE/g of dry extract, indicating the presence of the most polyphenols in *Atriplix halimus*, followed by ethyl acetate extract with 411 ± 5.69 mg QE/g of dry extract (Table 1).

Phenolics compounds were extracted by Soxhlet method and analyzed by the Folin–Ciocalteu colorimetric method, while flavonoids were determined by aluminum trichloride assay. All tested extracts contain phenolic compounds, however the most significant amount of total phenolic and flavonoid contents was presented in butanolic extract (68.20 mg GAE/g, dry extract and 439 mg QE/g, dry extract) respectively.

Table 1. Total phenolic and flavonoid contents (mg/g) of the *Atriplex halimus*

Extraction Solvents	Total Polyphenol Content (mg GAE/g dry extract)	Flavonoids Content (mg QE/g dry extract)
Ethyl ether	26.40 ± 4.73	212 ± 4.15
Ethyl acetate	38.80 ± 0.11	411 ± 5.69
Butanol	68.20 ± 0.03	439 ± 2.77

Due to its additional electron, DPPH produces a potent absorption band in visual spectroscopy at 517 nm [14].

Using the thin layer chromatography (TLC) bioautography technique, we noted on the TLC plate the appearance of zones of antiradicalaire activity of pale yellow hue on purple bottom for the under-researched extracts as well as for the ascorbic acid [15].

Table 2. IC_{50} concentrations of DPPH scavenging capacity from bioactive extracts of *Atriplex halimus*

Bioactive Extracts	**IC_{50} (mg/ml)**
Ethyl ether	2.9382
Ethyl acetate	2.4234
Butanol	2.1959
Ascorbic acid (positive control)	0.0331

Table 2 shows that the scavenging effects of samples on DPPH radical and were in the following order: *n*-butanol extract > ethyl acetate extract > ethyl ether extract. The IC_{50} values of scavenging DPPH radicals for the n-butanol and ethyl acetate extracts were 2.1959 and 2.4234 mg/ml respectively. Previous findings have demonstrated a substantial correlation between the phenolic content and the antioxidant ability of fig leaves [16]. Researchers in the fields of food science, health, and medicine have recently shown a growing interest in antioxidant properties. A popular technique for assessing a sample's capacity to scavenge free radicals is the scavenging of the stable DPPH radical, which can be applied to plant extracts as well. This method was applied in this study to investigate the extracts of the Algerian species *Atriplex halimus* for their strong antioxidant content. Based on the findings, and by comparing the IC_{50} values of each extract to ascorbic acid, which is a genuine simple IC_{50} of 0.0331 mg/ml [17], the results showed that the Butanolic extract of *Atriplex halimus* had the activity with IC_{50} value of 2.1959 mg/ml. Generally, the antioxidant activity of polyphenol is related to their major compounds.

Conclusion

One theory is that *A. halimus*, like all halophyte plants, produces bioactive compounds like polyphenols that may have therapeutic use as well as serve as a natural food preserver. The distribution of these molecules was unequal in different parts of the plant; the leaves showed a higher phenolic content in comparison with the previous studies; however, the flavonoids in ethyl acetate and butanolic fractions possess potential antioxidant activity which explains the relation structure-activity; further isolation and identification of potential bioactive compounds, particularly flavonoids responsible for antioxidant activity, are needed. The results of the study showed that n-butanol and ethyl acetate extracts have significant antioxidant activity; these two fractions' high levels of observed antiradical capabilities may be related to the presence of phenolic chemicals, which include phenolic hydroxyls.

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AUTHOR CONTRIBUTIONS

Concept: L.Z., A.B.; Design: L.Z., A.B.; Control: L.Z., A.B.; Sources: L.Z., A.B.; Materials: L.Z., A.B.; Data Collection and/or Processing: L.Z., A.B.; Analysis and/or Interpretation: L.Z., A.B.; Literature Review: L.Z., A.B.; Manuscript Writing: L.Z.; Critical Review: A.B.; Other: L.Z., A.B.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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