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Original Research

Preliminary investigation of Haloxylon persicum and Heliotropium bacciferum plants from the United Arab Emirates for possible development of new drugs

Mughair Abdul Aziz, Kandhan Karthishwaran, Chythra Somanathan Nair, Ramya Manoharan, Drishya Nishanth, Radhakrishnan Subramanian, Khaled Masmoudi, Mohammed Alyafei, Abdul Jaleel

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Abstract

Natural antioxidants from plants have proved to be effective against various free radicals that contribute to human diseases. In this study, we aimed to explore the phytochemicals, mineral composition, antioxidant and free radical scavenging activities of methanolic extracts derived from Haloxylon and Heliotropium species, specifically Haloxylon persicum and Heliotropium bacciferum. These two plants are native to the arid regions. Phytochemical screening and macro- and micro-elemental analysis of the leaf extracts were done. Four different in vitro antioxidant assays (ABTS, DPPH, superoxide anions, hydroxyl radicals) were performed to investigate the scavenging properties of these plant extracts. Both H. persicum and H. bacciferum extracts contained flavonoids, tannins, phenols, and cardiac glycosides. H. persicum contained steroids while H. bacciferum contained alkaloids, saponins, and terpenoids. Results of the mineral analysis revealed significant levels of macro- and micro-elements in the studied Haloxylon and Helitropium species. While H. persicum exhibited high amounts of Mg (12.16 mg/kg), H. bacciferum had elevated levels of Ca (38.45 mg/kg) and S (6.73 mg/kg). Micronutrient content was particularly high in H. persicum, with varied composition of Cr (8.46 mg/kg), Ni (8.06 mg/kg), Pb (3.55 mg/kg), and Zn (60.73 mg/kg). Antioxidant activity analysis demonstrated that the methanolic extract of H. bacciferum displayed efficient scavenging activity against ABTS, DPPH, and hydroxyl radicals at a concentration of 160 µg/ml, inhibiting them by 81.23%, 83%, and 83.46%, respectively, with IC_{50} values of 5.9 µg/ml, 26.16 µg/ml, and 25.82 µg/ml, respectively. Meanwhile, the methanolic extract of H. persicum exhibited the highest superoxide anion scavenging activity, at 160 µg/ml, with an inhibition of 80.06% and an IC50 value of 26.03 µg/ml. Methanolic extracts from the leaves of H. persicum and H. bacciferum are promising sources of natural antioxidants and has the potential to serve as effective free radical inhibitors in plant-based pharmaceutical products.

Keywords: Haloxylon persicum, Haloxylon bacciferum, phytochemicals, free radicals, antioxidant activities, drug development

Mughair Abdul Aziz. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Kandhan Karthishwaran. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Chythra Somanathan Nair. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Ramya Manoharan. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Drishya Nishanth. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Radhakrishnan Subramanian. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Khaled Masmoudi. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Mohammed Alyafei. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates.
Abdul Jaleel*. Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, United Arab Emirates. abdul.jaleel@uaeu.ac.ae

INTRODUCTION

Oxidative stress is a detrimental process that often leads to several diseases due to an imbalance in the generation of antioxidants and free radicals. It affects the health and functioning of cells. Oxidative stress thus alters the cell structure and function by inducing reactive molecules to target proteins, lipids and nucleic acids[1]. However, certain phytochemicals such as vitamins, polyphenols, carotenoids, and flavonoids are recognized for their ability to scavenge free radicals. Acting as natural antioxidants, these compounds neutralize peroxyl and hydroxyl radicals by providing an electron; in this process, they become free radicals with reduced damaging effects[2]. Currently, there is an increased demand for natural antioxidants for use as nutraceuticals and biopharmaceuticals.

Traditional medicines and medicinal plants have the potential to be antioxidant therapeutic agents[3]. There is also an increase in the scientific interest in medicinal plants. This is primarily driven by the increased effectiveness of plant-based novel drugs, growing concerns regarding the potential side effects of modern medicine, and the emergence of resistance to existing drugs[4]. Therefore, it is important to explore novel molecular structures from plants as templates for drug development.

Medicinal plants form the backbone of traditional medicine in various cultures and have been widely investigated for their pharmacological properties. These investigations have helped in revealing numerous essential phytochemicals in these plants. These phytochemicals have proven to have the potential to treat and manage various diseases. Popular examples include the discovery of paclitaxel (Taxol), an anticancer drug derived from the bark of the Himalayan yew tree[5], berberine isoquinoline alkaloid, an antineoplastic and antidiabetic drug found in plants of the Berberidaceae family[6], and artemisinin, an antimalarial drug derived from Artemisia annua[7]. Previous study showed that bioactive phytochemicals derived from plants exhibit activity against oxidative stress, indicating their implications in chronic, degenerative, and infectious diseases[8]. For example, catalpol, extracted from the roots of Rehmannia glutinosa, inhibits the production of reactive oxygen species (ROS), DNA damage, and telomere shortening[8] The activity of plant-derived extracts is associated with the presence of various phytocompounds and minerals.

Phenolic and mineral compounds are commonly present in both edible and non-edible herbs[9]. But scientific information on the antioxidant properties of plants in arid regions is scarce. This scarcity is due to the limited availability of arid plants, which are restricted to specific regions and are primarily known by local populations[10]. Consequently, the evaluation of such antioxidant properties remains a compelling and valuable task, especially in studies aimed at identifying promising sources of natural antioxidants from these plants.

Haloxylons and *Heliotropium* spp. are commonly found in arid environments. *Haloxylon persicum* is an endangered white saxaul shrub used for multiple purposes. This small desert-dwelling tree belongs to the Amaranthaceae family and is distributed across non-saline sandy deserts in regions such as Central Asia, the Middle East, Afghanistan, northwestern China, and the Near Eastern deserts[11]. Another well-known species, *Heliotropium bacciferum*, belongs to the Boraginaceae family and is a perennial herb with a woody rootstock[12]. It is widely distributed in the eastern province of Saudi Arabia, the UAE and in semi-desert areas with saline soils. Both *H. persicum* and *H. bacciferum* are used for various purposes, including antiseptic, anti-diabetic, and anti-inflammatory applications in folklore medicine. Thus, the ethnomedicinal uses of Haloxylon and Heliotropium species can inhibit the formation of free radicals under conditions of oxidative stress. However, there is currently little information available regarding the antioxidant potential of leaf extracts of *H. persicum* and *H. bacciferum*.

Because of the significance of natural plants and their practical attributes, this study focused on the investigation of *H. persicum* and *H. bacciferum*, which are native plants in arid regions. This study aimed to analyze the phytochemical composition, mineral content, and antioxidant properties of the methanolic extracts derived from these plants. In vitro methods were employed to assess the free-scavenging activity of the methanolic extracts against various free radicals, including 2,2 azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS), 1,1-diphenyl-2-picryl hydrazyl (DPPH), superoxide, and hydroxyl radicals.

MATERIALS AND METHODS

Collection and preparation of *H. persicum* and *H. bacciferum* plant materials

The aerial parts of *H. persicum* and *H. bacciferum* specimens were collected from the Al-Foah Experimental Station, College of Agriculture and Veterinary Medicine, United Arab Emirates University (UAEU), Experimental Nursery of Terrestrial and Marine Biodiversity Sector, Wildlife Assessment and Conservation, Environment Agency-Abu Dhabi (EAD), and United Arab Emirates (UAE). Identification and authentication of plants were performed at UAEU and EAD.

Preparation of *H. persicum* and *H. bacciferum* methanolic extracts

From the freshly collected aerial parts of plants, 600 g was cut into small pieces for extraction. Extracts were prepared from the cut pieces of the leaf samples which were dried and ground into a powder for 30 s using a mini mill (Retsch, Germany) for passage of entire material through a mesh with a maximum opening size of 0.5 mm. Powdered plant materials (500 g) were immersed in 1.5 l of methanol (Sigma-Aldrich, USA) for a day, and the process was repeated twice. The Soxhlet extraction was performed in methanol for 72 h. The resulting extract was concentrated to dryness under vacuum using a rotary evaporator at 40°C and was stored at 4°C until further use.

Phytochemical analysis of the plant extracts

A portion of the dried extract was used for preliminary phytochemical screening using the method outlined by Harborne (1998). The screening was aimed at detecting the presence of flavonoids, tannins, alkaloids, steroids, proteins, terpenoids, saponins, phenols, cardiac glycosides, and anthraquinone in the samples.

Flavonoids test

To assess for the presence of flavonoid compounds, a few drops of 1% ammonia solution were added to the methanolic extract of the plant sample in a test tube. Yellow indicated the presence of flavonoids.

Tannins test

Approximately 0.5 g of the plant methanolic extract was boiled in 10 ml of water in a test tube, followed by filtration. In addition, a few drops of 0.1% ferric chloride were introduced, and the mixture was observed to see whether it developed a brownish dark green coloration.

Alkaloids test

A total of 5 milliliters of the plant extract was added to 2 ml of 2% HCl. Dragendroff's reagent (1 ml) was then added to the acidic medium. The immediate formation of an orange-red precipitate indicated the presence of alkaloids.

Steroids test

Acetic anhydride (2 ml of acetic anhydride was added to the plant extract (0.5 g) and mixed with 2 ml of H_2SO_4. The emergence of a greenish hue indicated the presence of steroids.

Proteins test

A small quantity of methanolic extract was combined with 5–6 drops of Millon's reagent. The presence of proteins was indicated by the presence of a white precipitate that changed into red colour upon heating.

Terpenoids test

Approximately 0.8 g of plant extract was blended with 2 ml of chloroform ($CHCl_3$), and then 3 ml of concentrated H_2SO_4 was cautiously introduced to create a layered structure. The development of a reddish-brown coloration indicates the presence of terpenoids.

Saponins test

Plant extracts (0.2 g) were agitated with 5 ml of distilled water, followed by heating until boiling. The presence of saponins were indicated by frothing, characterized by the appearance of a creamy mixture of small bubbles.

Cardiac glycosides test

To detect cardiac glycosides, 5 ml of the methanolic extract was treated with 2 ml of glacial acetic acid containing a single drop of ferric chloride solution. The mixture was then stratified using 1 ml of concentrated H_2SO_4. The presence of a brown ring at the interface indicated the presence of deoxysugar, which is characteristic of cardenolides. In the presence of cardiac glycosides in the sample, a violet ring may have appeared beneath the brown ring and a gradually spreading greenish ring may have appeared in the acetic acid layer.

Anthraquinones test

Plant powder (0.5 g) was added to 10 ml of benzene and filtered. Following filtration, 0.5 ml of ammonia solution was added to the filtrate and shaken thoroughly. The appearance of a violet color in the layered phase indicated the presence of anthraquinones.

Determination of macroelements and microelements in *H. persicum* and *H. bacciferum* leaves

Sample preparation involved weighing the plant material (0.5 g) in a PFA digestion vessel. To this solution, 10 ml of concentrated HNO_3 and 2 ml of HCl were added following Method 3015A, as outlined by the US Environmental Protection Agency (2008). The capped vessels were placed in a microwave digestion system. The percentage of each element in the sample was determined by plotting the absorbance of each element against its concentration using standard AR grade solutions of the measured elements. Elemental analysis focused on the aerial parts of the plant species and was conducted using inductively coupled plasma-optical emission spectrometry (ICP-OES; Agilent Technologies, model 710).

Analysis of methanolic extract scavenging activity using ABTS assay

The spectrophotometric assessment of total antioxidant activity was done . A refined technique was followed and it involves the production of a blue or green ABTS chromophore directly through the interaction between ABTS and potassium persulfate. A solution was prepared in water at a concentration of 7 mM and then mixed with 2.5 mM potassium persulfate. This mixture was left in the dark at room temperature for 12–16 h to allow for incomplete oxidation of ABTS. The resulting radical was stable in its form for over two days when stored in the dark, at room temperature[13].

For the assay, an incubation mixture with a total volume of 5 ml was prepared. This mixture consisted of 0.54 ml of ABTS, 0.5 ml of phosphate buffer, and varying concentrations of plant extract (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, and 160 µg/ml). The blank reference contained water in lieu of the plant extract. Absorbance was measured at 734 nm using a spectrophotometer and compared with that of a standard solution of gallic acid. IC_{50} values were determined using a dose–response curve graph.

Analysis of methanolic extract scavenging activity using DPPH assay

The radical scavenging activity of the plant extracts against DPPH was evaluated spectrophotometrically in a dark environment following the methodology outlined by[14]. DPPH is a stable free radical that accepts an electron or a hydrogen radical and transforms it into a stable diamagnetic molecule. In the presence of an antioxidant compound capable of donating hydrogen, DPPH undergoes reduction. The nature and amount of the radical scavenger is determined by the intensity of the yellow colour.

For the DPPH assay, a reaction mixture with a total volume of 3 ml was prepared. This mixture consisted of 1 ml of DPPH and various concentrations of plant extract (10, 20, 40, 80, and 160 µg/ml), and was made up to 3 ml with water. A blue chromophore was obtained as a result of incubation of the tubes at 10 min at 37°C.The absorbance of this chromophore was measured at 517 nm, with gallic acid serving as the standard for comparison. The IC_{50} value was determined using a dose–response curve graph.

Analysis of methanolic extract scavenging activity using superoxide anion assay

The method described by[15] was modified and followed to determine the superoxide anion scavenging activity of the plant extracts. The assay depends on the generation of superoxide anions from dissolved oxygen through the phenazine methosulphate-reduced nicotinamide adenine dinucleotide (PMS–NADH) coupling reaction and subsequent reduction of nitroblue tetrazolium (NBT).

Here, NADH is oxidized by PMS to liberate PMSred, which converts the oxidized NBToxi to its reduced form (NBTred). For the assay, 1 ml of NBT, 1 ml of NADH solution, and various concentrations of plant extract (10, 20, 40, 80, and 160 µg/ml) were combined and mixed thoroughly. The addition of 100 µL phenazine methosulfate (PMS) helped to initiate the reaction. The incubtion of the reactive mixture done at 30°C for 15 min, resulting in the formation of a violet-colored complex. This indicated the generation of superoxide anions. The absorbance was read at 560 nm using a spectrophotometer. Gallic acid was used as the standard and a decrease in the absorbance of the reaction mixture indicated an increase in superoxide anion scavenging activity. The dose-response curve graph was used to obtain the IC_{50} values.

Analysis of methanolic extract scavenging activity using hydroxyl assay

The hydroxyl radical scavenging activity of the plant extracts was done by following the method outlined by[16]. Here, hydroxyl radicals are generated by the reduction of H_2O_2 by a transition metal ion in the presence of ascorbic acid. The ability of hydroxyl radicals to degrade deoxyribose helps in their detection. This results in the formation of products that have a pink-colored chromogen upon heating with thiobarbituric acid (TBA).

For the assay, the incubation mixture in a total volume of 1 ml comprised 0.1 ml of buffer, varying concentrations of plant extract (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, and 160 µg/ml), 0.2 ml of ferric chloride, 0.1 ml of ascorbic acid, 0.1 ml of EDTA, 0.1 ml of H_2O_2, and 0.2 ml of 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. Subsequently, 1 ml of TBA and 1 ml of TCA were added, and the tubes were then placed in a boiling water bath for 30 min. Gallic acid was used as the positive control for comparison. A reagent blank containing only water was used to read the absorbance of the supernatant at 535 nm, using a spectrophotometer. A decrease in the absorbance of the reaction mixture indicated an increase in hydroxyl radical scavenging activity, and the IC_{50} value was measured using a dose-response curve graph.

Statistical analysis

The average and standard deviation for the nutrient content and antioxidant activities of the plant extracts were calculated using five and three samples, respectively. Differences in mineral content between plant samples were analyzed with a Student's t-test. Antioxidant activity data were examined using a one-way ANOVA in SPSS (version 10.0), followed by Tukey's test to compare each group with the control. Correlation between the samples and antioxidant activities was determined with Pearson's test in R. Results were reported with 95% confidence, and differences were considered significant if $p<0.05$.

RESULTS

Phytochemical constituents of *H. periscum* and *H. bacciferum*

The phytochemical components of *H. persicum* and *H. bacciferum* were assessed using aqueous extracts. These tests helped to reveal the presence of various phytochemicals such as flavonoids, tannins, proteins, phenols, and cardiac glycosides though at low to moderate concentrations (Table 1). In addition, alkaloids, saponins, terpenoids, and anthraquinones were absent in the *H. persicum* plant extracts. Conversely, *H. bacciferum* extract contained terpenoids and saponins at low concentrations and lacked the steroids that were present in the *H. persicum* extract (Table 1).

MINERAL CONTENT OF THE *H. PERSICUM* AND *H. BACCIFERUM*

Macronutrient content

There is a variation in the macronutrient content at the leaf level in *H. persicum* and *H. bacciferum*. Several macronutrients, including Ca, K, Mg, Na, P, and S, were measured (Figures 1A–F). Specifically, the analysis of Ca levels indicated a highly significant difference ($p < 0.001$) between *H. persicum* and *H. bacciferum* extracts, with an increased concentration of 38.45 mg/kg in *H. bacciferum* (Figure 1A). However, a slight yet significant difference ($p < 0.05$) was observed in the K and Mg content ($p < 0.01$) of *H. persicum* and *H. bacciferum* plants (Figures 1B and C). *H. bacciferum* had a higher K level, 15.78 mg/kg, whereas *H. persicum* had a higher Mg level (12.16 mg/kg). Furthermore, there was a significant difference ($p < 0.001$) in the Na + content of both plant extracts, and *H. persicum* had a higher content (13.2 mg/kg. Moreover, there were highly significant differences ($p < 0.05$, $p < 0.001$) in the P and S levels in the leaf compositions of *H. persicum* and *H. bacciferum* (Figure 1D,E, and F). Particularly, *H. bacciferum* showed elevated levels of P and S, 0.97 mg/ kg and 6.73 mg/kg of each, respectively.

Micronutrient content

Micronutrients, including Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, was assessed in the fresh leaves of *H. persicum* and *H. bacciferum* (Figures 2A-I). A significant difference ($p < 0.01$) was observed in the Cd content of *H. persicum* and highest concentration found at 1.21 mg/kg in *H. bacciferum*. Conversely, *H. persicum* had higher Co, Cr, Ni, and Zn content, 0.61 mg/kg,

Table 1: Preliminary phytochemical constituents of *H. persicum* and *H. bacciferum* plant species

Plant species	Flavonoid	Tannin	Alkaloid	Steroid	Protein	Terpenoid	Saponin	Phenol	Cardiac glycoside	Anthra-quinone
Haloxylon persicum	+	++	-	+	+	-	-	+	+	-
Heliotropium bacciferum	+	+	++	-	+	+	+	++	+	-

+ = low concentration, ++ = moderate concentration, +++ = high concentration, − = absent

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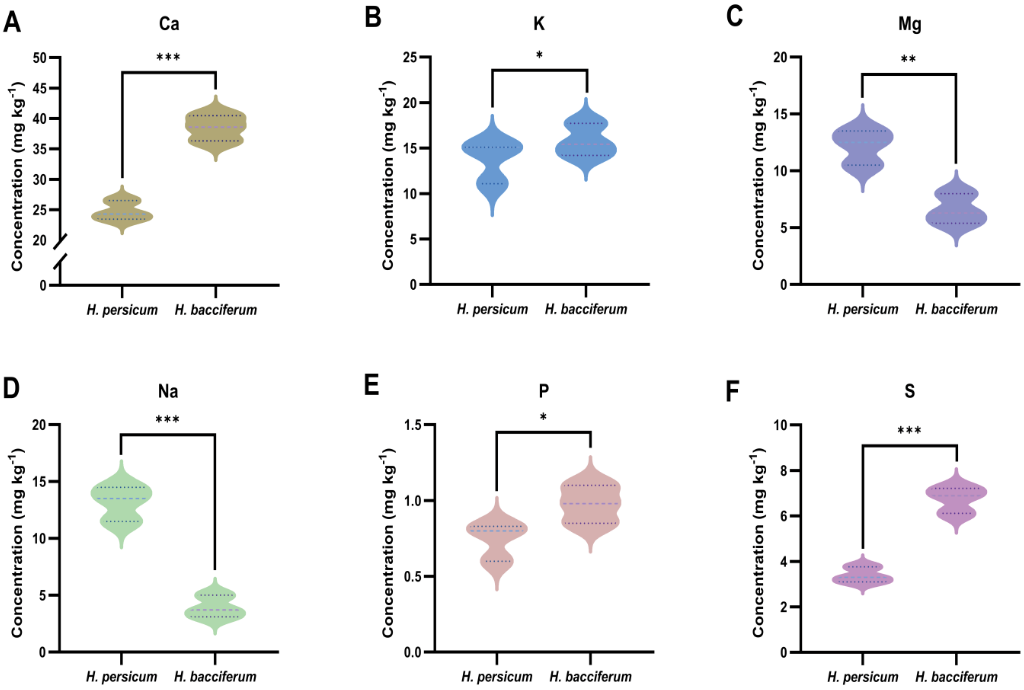

Figure 1. Macronutrients analysis of fresh H. persicum and H. bacciferum leaves. (A) Mineral element concentration of Ca (B) Mineral element concentration of K (C) Mineral element concentration of Mg (D) Mineral element concentration of Na (E) Mineral element concentration of P (F) Mineral element concentration of S. Values are the mean ± SD, n = 5. Statistically significant differences between groups were tested by the Student's t test. ***: P ≤ 0.001, **: P ≤ 0.01, *: P ≤ 0.05.

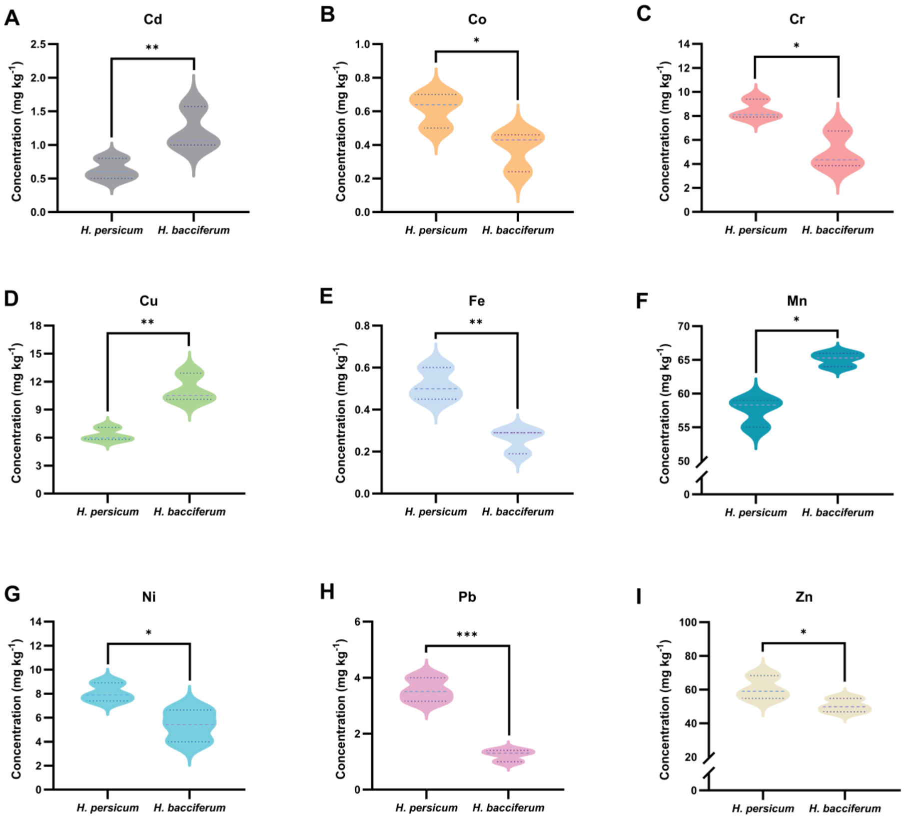

Figure 2. Micronutrients analysis of H. persicum and H. baccifeurm fresh leaves. (A) Mineral element concentration of Cd (B) Mineral element concentration of Co (C) Mineral element concentration of Cr (D) Mineral element concentrations of Cu (E) Mineral element concentrations of Fe (F) Mineral element concentrations of Mn. (G) Mineral element concentrations of Ni. (H) Mineral element concentrations of Pb. (I) Mineral element concentrations of Zn. Values are the mean ± SD, n = 5. Statistically significant differences between groups were tested by the Student's t test. ***: P ≤ 0.001, **: P ≤ 0.01, *: P ≤ 0.05.

8.46 mg/kg, 8.06 mg/kg, and 60.73 mg/kg, respectively, with a significant difference ($p < 0.05$) compared to *H. bacciferrum*. Moreover, significant differences ($p < 0.01$ and $p < 0.05$) were found in the levels of Cu and Mn, with higher concentrations in *H. bracciferum*, at 11.16 mg/kg and 65.1 mg/kg, respectively. The micronutrients Fe and Pb exhibited highly significant differences ($p < 0.001$) between the leaves of *H. persicum* and *H. braciferrum*. Specifically, *H. persicum* displayed higher levels of Fe and Pb, 0.51 mg/kg, and 3.55 mg/kg, respectively, compared to *H. bacciferrum.*

ABTS radical scavenging activity of *H. persicum* and *H. bacciferum* methanolic extracts

The radical scavenging activity against ABTS was assessed for both control which is gallic acid and the samples at various concentrations (Figures 3A and B). A significant difference ($p < 0.05$) was observed between the radical scavenging activity of the plant extracts and gallic acid treatments (Figure 3A). Tukey's test revealed no difference between *H. persicum* extracts and gallic acid treatment, but a significant difference was observed between these treatments and H. bacciferum extract at 10 µg/ml and 160 µg/ml. The highest scavenging activities at these concentrations were 44.1% and 81.23% for H. bacciferm, respectively (Figure 3A). At concentration 20, 40, and 80 µg/ml treatments a significant difference ($p < 0.05$) was observed in the ABTS radical scavenging activities of the (Figure 3A). Tukey's test indicated that differences were present among the three treatments, with the highest recorded scavenging activities of 55.13%, 61.3%, 66.53% evident for H. bacciferum (Figure 3A). H. bacciferum extracts at various concentration exhibited the highest ABTS radical scavenging activity. The IC50 values of the plant extracts were significantly different ($p < 0.05$) for ABTS radical scavenging activity (Figure 3B). The plant extracts' IC_{50} values for ABTS radical scavenging activity differed significantly from those of the gallic acid treatment. The highest IC50 value (7.71 µg/ml) was obtained for gallic acid, followed by 6.67 µg/ml of *H. persicum.*

DPPH radical scavenging activity of *H. persicum* and *H. bacciferum* methanolic extract

The DPPH activity of the methanolic plant extracts was assessed at various concentrations, ranging from 10 to 160 µg/ml (Figures 4A–B). A significant difference ($p < 0.05$) was observed among the control treatment, gallic acid, and plant extracts at the different concentrations tested. At 10 µg/ml, according to Tukey's test, no significant difference was found between the control, gallic acid, and *H. bacciferum* extracts, which differed significantly from the DPPH activity of the *H. persicum* extract. The gallic acid treatment exhibited the highest scavenging activity, 38.06 % (Figure 4A). Similarly, plant extract at 20 **µg**/ml demonstrated significant scavenging activity ($p < 0.05$). However, the *H. bacciferum* extract showed similar activity to that of the control treatment. The highest scavenging activity at this concentration was 48.26%, which was observed for *H. bacciferum* (Figure 4A).

Furthermore, at 40 **µg**/ml, the *H. persicum* and control displayed no significant differences based on the Tukey's test; however, they differed from *H. bacciferum*, which showed the highest scavenging activity (63.6%) at 40 µg/ml. At 80 µg/ml and160 µg/ml, no significant difference was observed between the DPPH scavenging activities of the plant extracts; however, they differed significantly from gallic acid, based on Tukey's test. The highest scavenging activities (80 µg/ml and 160 µg/ml) were 75.1% and 83% for the *H. bacciferum* extract (Figure 4A). At most of the measured concentrations, *H. bacciferum* extract showed higher DPPH radical scavenging activity. The IC_{50} values displayed a slightly significant difference ($p < 0.05$) between the control and plant extract treatments for the DPPH radical scavenging activity (Figure 4B). The highest IC_{50} value for DPPH was recorded for gallic acid at a concentration of 31.6 µg/ml, while the IC_{50} values for *H. persicum* and *H. bacciferum* were 29 µg/ml and 26.16 µg/ml, respectively.

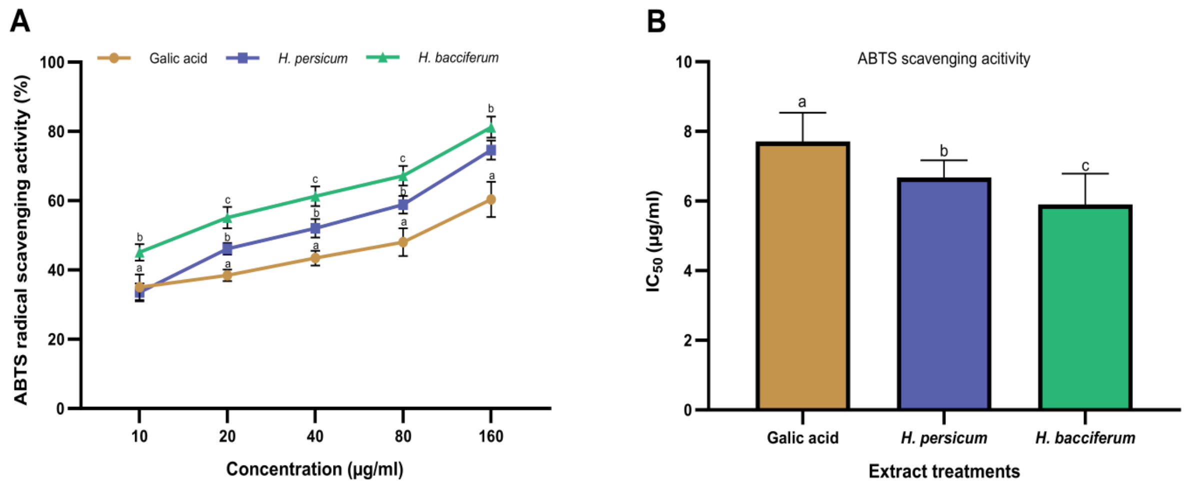

Figure 3. Determination of the ABTS radical scavenging activity of methanolic extracts of H. persicum and H. bacciferum. (A) Variation in ABTS scavenging activity of plant extracts at different concentrations compared to the galic acid. (B) IC50 values of methanolic extractives of plants and control galic acid. Values are the mean ± SD, n = 3. Different letters indicate statistically significant difference between groups analysed using Tukey's test.

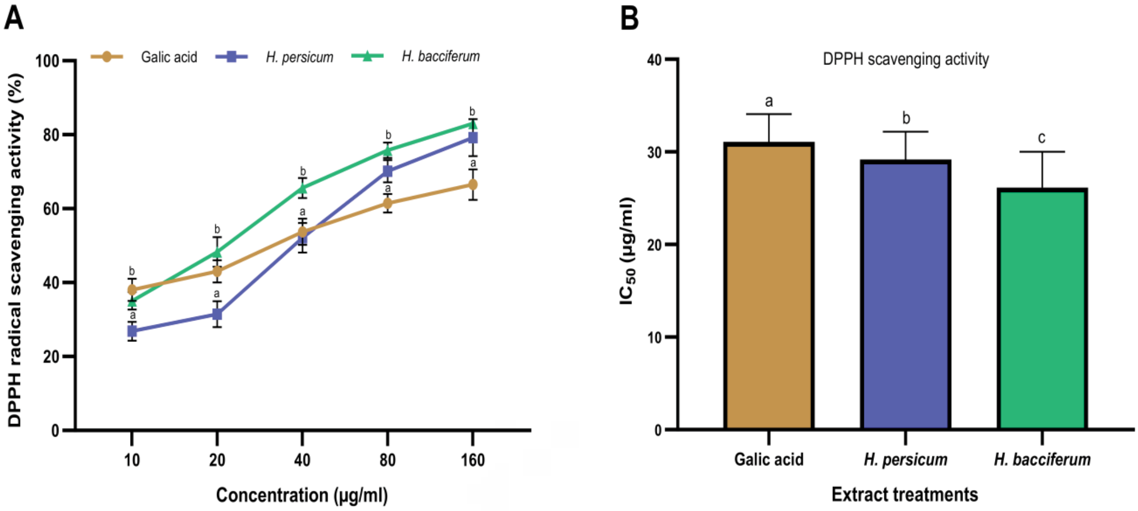

Figure 4. Determination of the DPPH radical scavenging activity of methanolic extracts of H. persicum and H. bacciferum. (A) Variation in DPPH scavenging activity of plant extracts at different concentrations compared to the galic acid. (B) IC50 values of methanolic extractives of plants and control galic acid. Values are the mean ± SD, n = 3. Different letters indicate statistically significant differences between groups analysed using Tukey's test.

Superoxide radical scavenging activity of *H. persicum* and *H. bacciferum* methanolic extracts

In the measurement of superoxide radical scavenging activity, there was no significant difference ($p > 0.05$) between the treatments with plant extracts and gallic acid at a concentration of 10 µg/ml (Figure 5A). Nevertheless, as the concentration increased, a significant difference ($p < 0.05$) was observed in the superoxide radical scavenging activity among the treatments. Tukey's test showed that, at concentrations of 20 µg/ml and 40 µg/ml, the superoxide scavenging activity of the plant extract treatments was similar, but differed significantly from that of gallic acid. The maximum superoxide scavenging activity recorded was 57.66% at 20 µg/ml and 63.06% at 40 µg/ml for *H. bacciferum* and *H. persicum*, respectively (Figure 5A). At 80 µg/ml and 160 µg/ml, the superoxide radical scavenging activity differed significantly ($p < 0.05$) among the three treatments, with H. persicum extract displaying the highest activity, 76% and 80.06 %, respectively, based on Tukey's test. Moreover, *H. persicum* extract consistently demonstrated higher superoxide scavenging activity at various concentrations than *H.bacciferum*. Among the methanolic extrcats of *H. persicum, H. bacciferum*, and gallic acid as a control, IC_{50} values for superoxide radical scavenging activity were significantly different ($p < 0.05$) between the plant extracts and control treatments. The highest IC_{50} value of (33.3 µg/ml) was exhibited by control which is gallic acid, followed by 27.44 µg/ml and 26.03 µg/ml for *H. bacciferum and H. persicum*, respectively.

Hydroxyl radical scavenging activity of *H. persicum* and *H. bacciferum* methanolic extracts

Hydroxyl radical scavenging activity was assessed for both plant extracts and gallic acid at concentrations ranging from 10

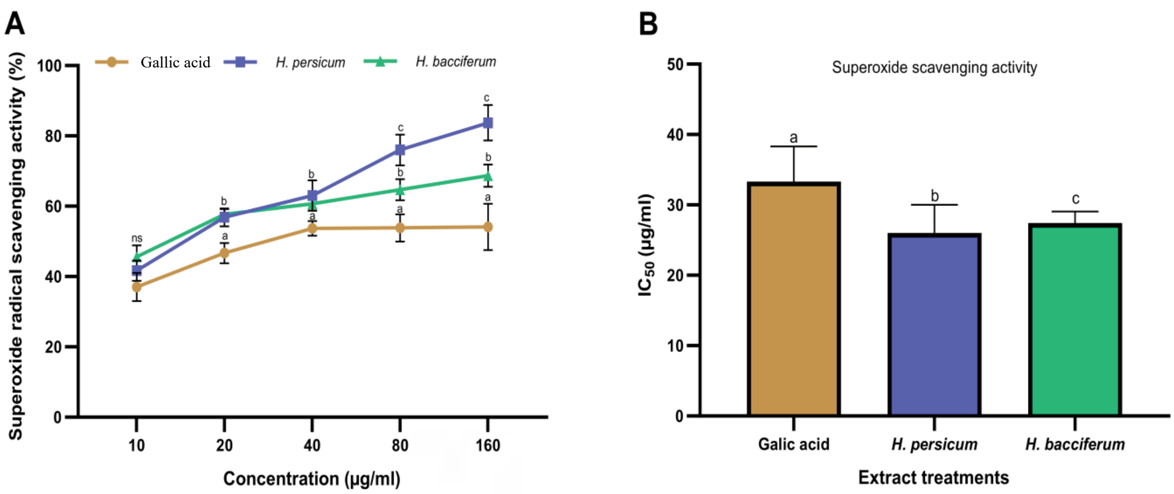

Figure 5. Determination of the superoxide anion radical scavenging activity of methanolic extracts of H. persicum and H. bacciferum. (A) Variation in superoxide scavenging activity of plant extracts at different concentrations compared to the galic acid. (B) IC50 values of the methanolic extractives of plants and control galic acid. Values are the mean ± SD, n = 3. Different letters indicate statistically significant differences between groups analysed using Tukey's test.

to 160 µg/ml (Figures 6A and B). At the lower concentration of 10 µg/ml, no significant difference ($p > 0.05$) was observed in the hydroxyl radical scavenging activity of the plant extracts and gallic acid treatment. However, a significant difference ($p < 0.05$) was observed in the radical scavenging activity of the 20 µg/ml treatments. Tukey's test indicated a significant difference between the radical scavenging activity of the plant extracts and gallic acid at 20 and 160 µg/ml; however, the plant extracts exhibited a similar scavenging effect. *H. bacciferum* showed the highest hydroxyl radical scavenging activities: 55% at 20 µg/ml and 83.46% at 160 µg/ml. At the concentrations of 40 µg/ml and 80 µg/ml, the hydroxyl radical scavenging activity differed significantly ($p < 0.05$) among the three treatments, with *H. bacciferum* exhibiting the highest activities (66.1% and 74.33%, respectively).

A significant difference ($p < 0.05$) was observed in the IC_{50} values of the hydroxyl radical scavenging activity of the gallic acid and plant extract treatments. Tukey's test indicated a significant difference between the three treatments. Gallic acid treatment displayed the highest IC_{50} value (36.26 µg/ml), whereas *H. persicum* and *H. bacciferum* had an IC_{50} value of 29.96 µg/ml and *H. bacciferum* recorded an IC_{50} value of 25.82 µg/ml.

Association between plant extracts and free radical scavenging activities

The association between plant extracts and radical scavenging activity was assessed at different concentrations (10–160 µg/ml). The *H. persicum* and *H. bacciferum* extracts demonstrated an augmentation in free radical scavenging activity, and correlational analyses between the extracts and free radical scavenging assays revealed a positive association (Figures 7A and B).

Specifically, the concentration of *H. persicum* exhibited a linear positive correlation with ABTS ($r = 0.91$, $p < 0.05$), DPPH ($r = 0.91$, $p < 0.05$), superoxide ($r = 0.93$, $p < 0.05$), and hydroxyl ($r = 0.98$, $p < 0.01$) free radicals (Figure 7A). Similarly, *H. bacciferum* methanolic extracts of *H. bacciferum* exhibited a positive linear correlation with ABTS ($r = 0.95$, $p < 0.05$), DPPH ($r = 0.89$, $p < 0.05$), superoxide ($r = 0.83$, $p < 0.05$), and hydroxyl ($r = 0.96$, $p < 0.01$) free radicals (Figure 7B). These findings suggest that the scavenging activity improved with increasing concentrations of plant extracts. Furthermore, in both plant radical scavenging assays, significant positive correlations were identified between ABTS, DPPH, superoxide, and hydroxyl scavenging activities ($r > 0.9$, $p < 0.05$).

DISCUSSION

Phytochemical analysis of the extracts revealed the presence of various classes of compound. The presence of flavonoids, tannins, steroids, proteins, phenols, and cardiac glycosides have been identified in *H. persicum*. Similar phytochemicals have also been found in the aqueous extracts of *Combretum molle* leaves[4] and *Tricosanthes dioica*[17]. Additionally, *Albizia coriaria* stem bark organic extracts exhibit similarities in the presence of alkaloids, steroids, proteins, saponins, phenols, and cardiac glycosides[4]. Qualitative phytochemical screening of *H. bacciferum* aligned with the *Corchorus oliterius* extract confirmed the presence of saponins, alkaloids, phenols, and cardiac glycosides[18]. Phytochemical contents present in the studied plant samples supports the traditional medicinal use of these species, suggesting its potential benefits in wound healing, antidiabetic activity, and reduction of cholesterol levels, in similar to the health-promoting properties commonly associated with phytochemicals found in fruit and vegetables[19].

Several phytochemicals have been found to exhibit different

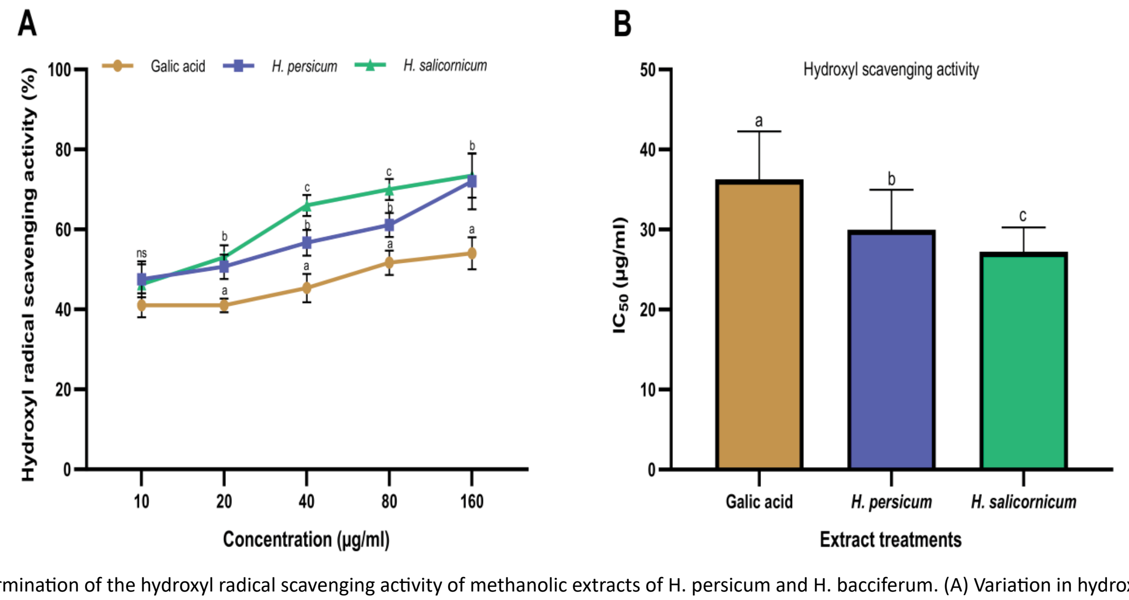

Figure 6. Determination of the hydroxyl radical scavenging activity of methanolic extracts of H. persicum and H. bacciferum. (A) Variation in hydroxyl scavenging activity of plant extracts at different concentrations compared to the galic acid. (B) IC50 values of the methanolic extractives of plants and control galic acid. Values are the mean ± SD, n = 3. Different letters indicate statistically significant differences between groups analysed using Tukey's test.

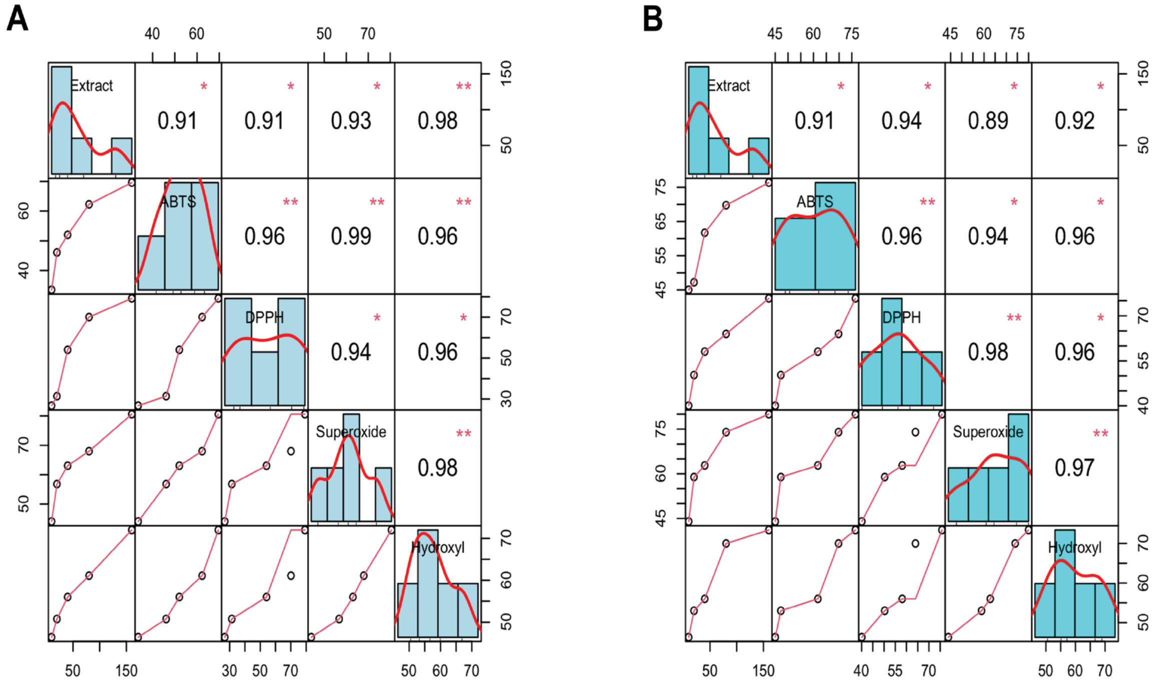

Figure 7. Correlation between plant methanolic extracts concentration and free radicals scavenging activity. (A) H. persicum methanolic extracts antioxidant potential. (B) H. baccifeurm methanolic extracts antioxidant potential. Correlation between assays determined with Pearson's coefficient (r), ***: P ≤ 0.001, **: P ≤ 0.01, *: P ≤ 0.05.

activities in plants in various studies[20,21] For example, alkaloids sourced from *Solanum sodomaeum* berries, *Zanthoxylum leprieurii* stem bark, and the cyanobacterium *Fischerella ambigua* have demonstrated anti-mycobacterial effects[22,23,24,25]. In addition, saponin present in *Colubrina retusa* and tannins in *C. molle* stem bark exhibits anti-mycobacterial activity[26,27]. Furthermore, flavonoids from various plant species have been identified for their anti-mycobacterial properties[28,29]. Some research studies have shown steroids isolated from the twigs and leaves of *Radermachera boniana* have been observed to possess antitubercular properties[30]).

Studies on macro- and micro-elements in Haloxylon and Heliotropium species, revealed that macronutrients, Mg and Na were found in large amount in *H. persicum* , whereas *H. bacciferum* contained only substantial amounts of Ca, K, P, and S. These minerals play crucial roles in enzyme activities related to carbohydrate metabolism and glucose oxidation[31]. The macronutrient values in Haloxylon species were lower than those in dry ginger roots[32] and powders of Annona muricata, Camellia sinensis, and Zingiber officinale as previously examined[33]. In contrast, micronutrient levels were higher in *H. persicum* than in *H. bacciferum*. Mn is an antioxidant nutrient that is high in *H. bacciferum*, and is essential for fat and cholesterol breakdown[34]. Other micronutrients found to be high in Haloxylon species play vital roles in human development[35] for instance, Zn is a component of several enzymes that contribute to wound healing[36]. These mineral rich herbal plants, are associated with various health benefits, aligning with the principles of herbal therapy, in which a high mineral content is considered essential for profound therapeutic effects[38,10].

In the present study, the ABTS radical scavenging activity of the plant species extracts were concentration-dependent. The methanolic extracts of *H. bacciferum* exhibited the highest ABTS radical scavenging activity at various concentrations. Previous research has shown some polyphenols such as catechin, rutin, and their derivatives were effective scavengers of ABTS radicals[40]. The phytochemical results of the present study indicated a high phenolic content in both H. persicum and H. bacciferum species, which requires further investigation for the isolation of various polyphenolic compounds present in the extract. The ABTS radical scavenging activity was higher than that of the ethyl-acetate and dichloromethane extracts of Solanum sisymbriifolium, while it was comparable to their ethanolic extracts' ABTS inhibition at high concentrations[40]. This suggests a higher accumulation of polyphenolics in the methanolic extracts of Haloxylon and Heliotropium species. H.persicum showed highest radical scavenging activity of 50%. However, the IC_{50} values of these plant extracts were lower than the ABTS scavenging activity of *Prangos ferulacea* extract[41]. Similarly, the IC_{50} value for the free radical scavenging activity of *P. ferulacea* in terms of ABTS was higher than that of *H. persicum* and *H. bacciferum*[42]. Moreover, in the present study the hexane solvent extract of Thymus satureioides had higher IC_{50} values for ABTS radical scavenging than the Haloxylon species[43].

Natural products which has the ability to donate electrons that can be assessed using the DPPH method, which involves disappearence of a purple-colored DPPH solution. The intensity of color change is directly proportional to the concentration and effectiveness of antioxidants. In the present study, the methanolic extracts of *H. persicum* and *H. bacciferym* exhibited comparable inhibition percentages in the DPPH assay. However, the IC_{50} value for DPPH scavenging was slightly higher for the methanolic extracts of *H. persicum* plants. The DPPH scavenging activity of the examined plants in the present study was higher than that of fresh guava leaves, as determined by the DPPH radical scavenging activity method[44]. Nevertheless, the DPPH inhibition of both *H. persicum* and H. bacciferum extracts was comparable to the DPPH scavenging activity of the ethanolic extract of S. sisymbriifolium at concentrations ranging from 31.25 to 250 µg/ml. Additionally, the IC_{50} values for DPPH radical scavenging of both plants were lower than those of red fruits of pomegranate extracts[45], but higher than the IC_{50} values of *Schima wallichii* extract in scavenging DPPH free radicals[46]. Moreover, in contrast to the findings of the present study[43] reported a high IC_{50} value for DPPH radical scavenging activity of T. satureioides extract obtained from hexane. Similarly, the IC_{50} value for the free radical scavenging power of *P. ferulacea* essential oil in terms of DPPH was higher than those of *H. persicum* and *H. bacciferum* methanolic extracts[42].

Superoxides are a major source of ROS. Although the superoxide anion is a weak oxidant, it can lead to the generation of potent and hazardous hydroxyl radicals and singlet oxygen, both contributing to oxidative stress[20]. The present study revealed that the *H. persicum* extract demonstrated an effective capacity for scavenging superoxide radicals. Reported that flavonoids are effective antioxidants because of their ability to scavenge superoxide anions, which could be associated with the total flavonoid content in *H. persicum* and *H. bacciferum*. Similarities were observed in the superoxide free radical scavenging assay between *H. persicum* and the ethanolic extract of *S. sisymbriifolium*[47]. *H. persicum* exhibits higher activity compared to the ethyl acetate (EAA) and dichloromethane (DCM) extracts of *S. sisymbriifolium*[40]. The IC_{50} values for superoxide scavenging of both *H. persicum* and H. bacciferum were higher than those found in the methanolic extract of Spondias pinnata stem bark[48] but lower than the IC_{50} values of Kyllinga nemoralis methanolic extracts[49].

The hydroxyl radical is a strong ROS in biological systems that can react with polyunsaturated fatty acid moieties phospholipids present in the cell membrane, leading to cell damage[50]. With increasing methanolic concentration of *H. bacciferum,* the assessment of hydroxyl radical scavenging activity was enhanced. Various free radical-scavenging organic compounds, such as flavonoids and phenols were present in the plant extract. They play a significant role in quenching ROS. The free radical scavenging activity of *H. bacciferum* might be due to the presence of phenolic groups in its extracts, which are capable of donating electrons to hydrogen peroxide, thereby neutralizing it in water. This is similar with the dose-dependent antioxidant activity of Torilis leptophylla, which increases at concentration ranging from 25 to 500 µg/ml[51]. Additionally, the hydroxyl radical-scavenging activity of *Bauhinia vahlii* methanolic extracts was similar to that of *H. persicum* and *H. bacciferum*[52]. Reports revealed that the *P. ferulacea* extracts for hydroxyl radical scavenging activity showed higher IC_{50} values compared to *H. persicum* and *H. bacciferum* extracts[41]. However, the IC_{50} values for *Spondias pinnata* stem bark extract[48] and *K. nemoralis*[49]. Moreover, the IC_{50} values obtained for hydroxyl scavenging were lower for *H. persicum* and *H. bacciferum* extracts than *P. ferulacea* extracts[41]. Analysis of correlation revealed a significant association between the concentrations of saxauls and heliotropium plant extracts and their free radical-scavenging activity. In addition, correlations were identified and extensively reported in the literature between ABTS, DPPH, superoxide, and hydroxyl free radical-scavenging activities[53,54]. The observed correlations in antioxidant values can be attributed to the shared electron transfer antioxidant mechanism underlying these assays[55]. Similar correlations between assays have been reported for the free radical-scavenging activities of Thai vegetal extracts[56].

CONCLUSION

The present study highlights that Haloxylon species, specifically *H. persicum* and *H. bacciferum,* contains a significant number of phytochemicals, macro- and micro-minerals, making them viable option with antioxidant potential. With increased extract concentrations, the methanolic extracts from these plants demonstrated concentration-dependent inhibition of free radicals. *H. persicum* and *H. bacciferum* expressed the antioxidant potential which may be due to the presence of various phytochemicals that can help prevent free radical-induced disorders. However, the specific compounds responsible for that antioxidant potential remains unclear. Further research is required in isolation and identification of these antioxidant compounds present in the extracts. *In vivo* studies are necessary to better understand and validate antioxidant activities in a physiological context. These results and the long history of their use in traditional medicine indicate that *H. persicum* and *H. bacciferum* extracts are potent alternatives to conventional antioxidant agents.

AUTHOR CONTRIBUTIONS

Conceptualization: A.J., Formal analysis: K.K., R.S., Funding acquisition: A.J., Investigation: K.K. C.S.N, R.M, D.N., Methodology: A.J., K.K., Project administration: A.J., Supervision: A.J., Validation: A.J., K.K., Writing - original draft: M.A.A., C.S.N, R.M., D.N., Writing - review & editing: R.S., A.J., K.M., M.A. All authors have read and agreed to the submitted version of the manuscript.

COMPETING INTERESTS

No competing interests.

References

1. Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., ... & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in chemistry, 11, 1158198.
2. Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Archives of toxicology, 97(10), 2499-2574
3. Abdelmola, A. O., Bahri, A., Abuallut, I., Refaei, B. A., Hakami, W. K., Abutaleb, A. K., ... & Aldarbi, K. F. (2021). Prevalence, knowledge, and perception about the use of herbal medicines jazan-Saudi Arabia. Journal of Family Medicine and Primary Care, 10(6), 2386-2393.
4. Oloya, B., Namukobe, J., Ssengooba, W., Afayoa, M., & Byamukama, R. (2022). Phytochemical screening, antimycobacterial activity and acute toxicity of crude extracts of selected medicinal plant species used locally in the treatment of tuberculosis in Uganda. Tropical medicine and health, 50(1), 16.
5. Nadeem, M., Rikhari, H. C., Kumar, A., Palni, L. M. S., & Nandi, S. K. (2002). Taxol content in the bark of Himalayan Yew in relation to tree age and sex. Phytochemistry, 60(6), 627-631
6. Singh, N., & Sharma, B. (2018). Toxicological effects of berberine and sanguinarine. Frontiers in molecular biosciences, 5, 21.
7. Czechowski, T., Rinaldi, M. A., Famodimu, M. T., Van Veelen, M., Larson, T. R., Winzer, T., ... & Graham, I. A. (2019). Flavonoid versus artemisinin anti-malarial activity in Artemisia annua whole-leaf extracts. Frontiers in plant science, 10, 984.
8. Mattioli, R., Mosca, L., Sánchez-Lamar, A., Tempera, I., & Hausmann, R. (2018). Natural bioactive compounds acting against oxidative stress in chronic, degenerative, and infectious diseases. Oxidative medicine and cellular longevity, 3894381.
9. Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. Molecules, 28(4), 1845.
10. Masmoudi, K., Aziz, M. A., Shamim, A., Sabeem, M., Hazzouri, K. M., & Amiri, K. M. (2021). Metagenomics of beneficial microbes in abiotic stress tolerance of date palm. In The Date Palm Genome, Vol. 2: Omics and Molecular Breeding (pp. 203-214). Cham: Springer International Publishing.
11. Al-Khalifah, N. S., & Shanavaskhan, A. E. (2007). On the distribution, status and phenology of Ghada (Haloxylon, persicum Bunge) in the Arabian Peninsula. Tropical Ecology, 48(1), 51-60.
12. Aïssaoui, H., Mencherini, T., Esposito, T., De Tommasi, N., Gazzerro, P., Benayache, S., ... & Mekkiou, R. (2019). Heliotropium bacciferum Forssk.(Boraginaceae) extracts: chemical constituents, antioxidant activity and cytotoxic effect in human cancer cell lines. Natural product research, 33(12), 1813-1818.
13. Wolfenden, B. S., & Willson, R. L. (1982). Radical-cations as reference chromogens in kinetic studies of ono-electron transfer reactions: pulse radiolysis studies of 2, 2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate). Journal of the Chemical Society, Perkin Transactions 2, (7), 805-812.
14. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30.
15. Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications, 46(2), 849-854.ytobiotic plants from west Cameroon. Journal of Agriculture and Food Research, 3, 100105.
16. Halliwell, B., Gutteridge, J. M., & Aruoma, O. I. (1987). The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Analytical biochemistry, 165(1), 215-219.
17. Sharmila, B. G., Kumar, G., & Rajasekara, P. M. (2007). Cholesterol lowering activity of the aqueous fruit extract of Trichosanthes dioica Roxb. in normal and streptozotocin diabetic rats. Journal of Clinical and Diagnostic Research, 1(6), 561-569.
18. Sadat, A. B. D. U. L., Hore, M., Chakraborty, K. A. U. S. H. I. K., & Roy, S. U. B. H. R. A. J. Y. O. T. I. (2017). Phytochemical analysis and antioxidant activity of methanolic extract of leaves of Corchorus olitorius. International Journal of Current Pharmaceutical Research, 9(5), 59-63.
19. Webb, D. (2013). Phytochemicals' role in good health. Today's Dietitian, 15(9),70.
20. Abdul Aziz, M., & Masmoudi, K. (2023). Insights into the transcriptomics of crop wild relatives to unravel the salinity stress adaptive mechanisms. International journal of molecular sciences, 24(12), 9813.
21. Tize T, Ngatsi PZ, Lontsi Dida SL, Njome Toka A, Songwe Atindo T, Ndongo EB'a, Sale Essome C, Ndongo B (2024) Bio-effectiveness of Balanites aegyptiaca (L.) Del. seed extracts against Colletotrichum capsici (Syd.) Butler & Bisby causal agent of anthracnose in cowpeas (Vigna unguiculata (L.) Walps.) under natural conditions. Innovations in Agriculture 7: 1-9. https://doi.org/10.3897/ia.2024.131803
22. Oloya, B., Namukobe, J., Heydenreich, M., Ssengooba, W., Schmidt, B., & Byamukama, R. (2021). Antimycobacterial Activity of the Extract and Isolated Compounds From the Stem Bark of Zanthoxylum leprieurii Guill. and Perr. Natural Product Communications, 16(8), 1934578X211035851.
23. Bunalema, L., Fotso, G. W., Waako, P., Tabuti, J., & Yeboah, S. O. (2017). Potential of Zanthoxylum leprieurii as a source of active compounds against drug resistant Mycobacterium tuberculosis. BMC Complementary and Alternative Medicine, 17, 1-6.

24. Mo, S., Krunic, A., Chlipala, G., & Orjala, J. (2009). Antimicrobial ambiguine isonitriles from the cyanobacterium Fischerella ambigua. Journal of natural products, 72(5), 894-899.
25. El Sayed, K. A., Hamann, M. T., Abd El-Rahman, H. A., & Zaghloul, A. M. (1998). New pyrrole alkaloids from Solanum sodomaeum. Journal of Natural Products, 61(6), 848-850.
26. ElSohly, H. N., Danner, S., Li, X. C., Nimrod, A. C., & Clark, A. M. (1999). New antimycobacterial saponin from Colubrina retusa. Journal of Natural Products, 62(9), 1341-1342.
27. Asres, K., Bucar, F., Edelsbrunner, S., Kartnig, T., Höger, G., & Thiel, W. (2001). Investigations on antimycobacterial activity of some Ethiopian medicinal plants. Phytotherapy Research, 15(4), 323-326.
28. Begum, S., Wahab, A., & Siddiqui, B. S. (2008). Antimycobacterial activity of flavonoids from Lantana camara Linn. Natural Product Research, 22(6), 467-470.
29. Koysomboon, S., Van Altena, I., Kato, S., & Chantrapromma, K. (2006). Antimycobacterial flavonoids from Derris indica. Phytochemistry, 67(10), 1034-1040.
30. Truong, N. B., Pham, C. V., Doan, H. T., Nguyen, H. V., Nguyen, C. M., Nguyen, H. T., ... & Chau, M. V. (2011). Antituberculosis cycloartane triterpenoids from Radermachera boniana. Journal of natural products, 74(5), 1318-1322.
31. Serdar, M. A., Bakir, F., Haşimi, A., Çelik, T., Akin, O., Kenar, L., ... & Yildirimkaya, M. (2009). Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose. International journal of diabetes in developing countries, 29(1), 35.
32. Ogbuewu, I. P., Jiwuba, P. D., Ezeokeke, C. T., Uchegbu, M. C., Okoli, I. C., & Iloeje, M. U. (2014). Evaluation of phytochemical and nutritional composition of ginger rhizome powder. International Journal of Agriculture and Rural Development, 17(1), 1663-1670.
33. Ndomou, S. C. H., Djikeng, F. T., Teboukeu, G. B., Doungue, H. T., Foffe, H. A. K., Tiwo, C. T., & Womeni, H. M. (2021). Nutritional value, phytochemical content, and antioxidant activity of three ph
34. Chaturvedi, U. C., Shrivastava, R., & Upreti, R. K. (2004). Viral infections and trace elements: a complex interaction. Current science, 1536-1554.
35. Hotz, C., and Brown, K. H. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull,25:194–195
36. Annan, K., Kojo, A. I., Cindy, A., Samuel, A. N., & Tunkumgnen, B. M. (2010). Profile of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations. Pharmacognosy Research, 2(1), 41.
37. Agomuo, E. N. (2011). Proximate, phytochemical and mineral element analysis of the sclerotium of Pleurotus tuber-regium. Int. Sci. Res. J, 3, 104-107.
38. Agomuo, E. N. (2011). Proximate, phytochemical and mineral element analysis of the sclerotium of Pleurotus tuber-regium. Int. Sci. Res. J, 3, 104-107.
39. Rahimtula, A. D., Béréziat, J. C., Bussacchini-Griot, V., & Bartsch, H. (1988). Lipid peroxidation as a possible cause of ochratoxin A toxicity. *Biochemical pharmacology*, *37*(23), 4469-4477.
40. More, G. K., & Makola, R. T. (2020). In-vitro analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by Solanum sisymbriifolium extracts. Scientific reports, 10(1), 6493.
41. Jalil Sarghaleh, S., Alizadeh Behbahani, B., Hojjati, M., Vasiee, A., & Noshad, M. (2023). Evaluation of the constituent compounds, antioxidant, anticancer, and antimicrobial potential of Prangos ferulacea plant extract and its effect on Listeria monocytogenes virulence gene expression. Frontiers in microbiology, 14, 1202228
42. Bruno, M., Ilardi, V., Lupidi, G., Quassinti, L., Bramucci, M., Fiorini, D., ... & Maggi, F. (2021). Composition and biological activities of the essential oil from a Sicilian accession of Prangos ferulacea (L.) Lindl. Natural Product Research, 35(5), 733-743.
43. Labiad, M. H., Harhar, H., Ghanimi, A., & Tabyaoui, M. (2017). Phytochemical screening and antioxidant activity of Moroccan Thymus satureioïdes extracts. Journal of Materials and Environmental Sciences, 8(6), 2132-2139.
44. Akhila, B., Vijayalakshmi, R., Hemalatha, G., & Arunkumar, R. (2018). Development and evaluation of functional property of guava leaf based herbal tea. Journal of Pharmacognosy and Phytochemistry, 7(3), 3036-3039.
45. Kaur, R., Aslam, L., Kapoor, N., & Mahajan, R. (2018). Phytochemical analysis and antioxidant activity of wild pomegranate collected from patnitop, Jammu and Kashmir. Biosciences biotechnology research asia, 15(2), 335-341.
46. Lalhminghlui, K., & Jagetia, G. C. (2018). Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth in vitro. Future science OA, 4(2), FSO272.
47. Robak, J., & Gryglewski, R. J. (1988). Flavonoids are scavengers of superoxide anions. Biochemical pharmacology, 37(5), 837-841.
48. Hazra, B., Biswas, S., & Mandal, N. (2008). Antioxidant and free radical scavenging activity of Spondias pinnata. BMC complementary and Alternative Medicine, 8, 1-10.
49. Sindhu, T., Rajamanikandan, S., & Srinivasan, P. (2014). In vitro antioxidant and antibacterial activities of methanol extract of Kyllinga nemoralis. Indian journal of pharmaceutical sciences, 76(2), 170.
50. Abdul Aziz, M., Sabeem, M., Mullath, S. K., Brini, F., & Masmoudi, K. (2021). Plant group II LEA proteins: intrinsically disordered structure for multiple functions in response to environmental stresses. Biomolecules, 11(11), 1662.
51. Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant

extracts Torilis leptophylla L. BMC complementary and alternative medicine, 12, 1-12.
52. Sowndhararajan, K., & Kang, S. C. (2013). Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight & Arn. Saudi journal of biological sciences, 20(4), 319-325
53. Surveswaran, S., Cai, Y. Z., Corke, H., & Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food chemistry, 102(3), 938-953.
54. Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food chemistry, 105(3), 940-949.
55. Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life sciences, 74(17), 2157-2184.
56. Prommajak, T., Kim, S. M., Pan, C. H., Kim, S. M., Surawang, S., & Rattanapanone, N. (2015). Prediction of antioxidant capacity of Thai vegetable extracts by infrared spectroscopy. Chiang Mai J. Sci, 42(3), 657-668.
57. Abdul Aziz, M., Sabeem, M., Mullath, S. K., Brini, F., & Masmoudi, K. (2021). Plant group II LEA proteins: intrinsically disordered structure for multiple functions in response to environmental stresses. Biomolecules, 11(11), 1662.
58. Aziz, M. A., & Masmoudi, K. (2023). Multifaceted roles of versatile LEA-II proteins in plants. In Multiple Abiotic Stress Tolerances in Higher Plants (pp. 143-161). CRC Press.
59. Harborne, J. B. (1998). Textbook of phytochemical methods. A guide to modern techniques of plant analysis. 5th Edition, Chapman and Hall Ltd, London, pp. 21–72.
60. Sabeem, M., Abdul Aziz, M., Mullath, S. K., Brini, F., Rouached, H., & Masmoudi, K. (2022). Enhancing growth and salinity stress tolerance of date palm using Piriformospora indica. Frontiers in Plant Science, 13, 1037273.