Research Article

Soufiane Drioua, Otman El-Guourrami, Amine Assouguem*, Mouna Ameggouz, Mohammed Kara, Riaz Ullah, Ahmed Bari, Ahmed Zahidi, Azra Skender, Hanane Benzeid, Anass Doukkali

Phytochemical study, antioxidant activity, and dermoprotective activity of *Chenopodium ambrosioides* (L.)

https://doi.org/10.1515/chem-2023-0194
received October 23, 2023; accepted January 16, 2024

Abstract: *Chenopodium ambrosioides*, a member of the Chenopodiaceae family, is renowned for its toxic properties. Despite its toxicity, it has been traditionally utilized in various communities, particularly in pediatric contexts, for its vermifuge, antispasmodic, and antipyretic attributes. This study aims to unravel the phytochemical composition present in organic fractions and aqueous extracts obtained from the aerial components of *C. ambrosioides*. Furthermore, our objective is to evaluate the antioxidant activity of these extracts and fractions, coupled with a comprehensive examination of their toxicological effects. Polyphenols were quantified using the Folin–Ciocalteu reagent, flavonoids via the aluminum trichloride reagent $AlCl_3$, and tannins using the vanillin method. Identification of bioactive compounds within the plant specimen was accomplished through GC-MS spectrophotometric analysis. The assessment of antioxidant activity employed DPPH, ferric (Fe^{3+}) ion antioxidant reducing power (FRAP), ABTS, and TAC methods, with quercetin, catechin, and ascorbic acid serving as standards. Dermoprotective activity was studied using the ultraviolet absorption test. The GC-MS analysis conducted on the aqueous extracts (EAI and EAM) and assorted fractions (FCH, FE, FB, and FA) revealed the presence of diverse chemical families encompassing alcohols, acids, terpenes, steroids, and phenolic compounds. The components identified in the investigated samples, including *trans*-ascaridol glycol, palmitic acid, phenol, octadecadienoic acid, isoascaridol, eicosanoic acid, 2-methoxy-4-vinyl phenol, mexiletine, and thymol, are postulated as potential contributors to the observed antioxidant activity inherent in the plant extracts and fractions. Our findings highlight the remarkable antioxidant potential of *Chenopodium ambrosioides*, with the ethyl acetate fraction exhibiting the highest activity (IC_{50} = 0.54 mg/ml) in the DPPH test. In the FRAP and ABTS tests, the *n*-butanolic and ethyl acetate fractions demonstrated superior activity (IC_{50} = 4.43 mg/ml, 12.9 mg/ml and IC_{50} = 1.6 mg/ml, 4.54 mg/ml, respectively). Conversely, the TAC test revealed that the macerated aqueous extract displayed the highest activity (316.33 mg Eq AG/g), followed closely by the *n*-butanolic fraction (250.67 mg Eq AG/g). These outcomes can be attributed to the abundant presence of phenolic compounds in the *n*-butanolic and ethyl acetate

*** Corresponding author: Amine Assouguem,** Laboratory of Functional Ecology and Environment, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, PO Box 2202 Imouzzer Street, Fez 30000, Morocco; Laboratory of Applied Organic Chemistry, Faculty of Sciences and Techniques, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco, e-mail: assougam@gmail.com
Soufiane Drioua: Laboratory of Analytical Chemistry, Department of Drug Sciences, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat, Morocco, e-mail: Driouasoufiane92@gmail.com
Otman El-Guourrami: Laboratory of Analytical Chemistry, Department of Drug Sciences, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat, Morocco, e-mail: elgourrami29@gmail.com
Mouna Ameggouz: Laboratory of Analytical Chemistry, Department of Drug Sciences, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat, Morocco, e-mail: Mouna.ameggouz6@gmail.com
Mohammed Kara: Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVNR), Faculty of Sciences Dhar El Mehraz, Sidi Mohamed Ben Abdallah University, BP 1796 Atlas, Fez 30000, Morocco
Riaz Ullah: Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, e-mail: rullah@ksu.edu.sa
Ahmed Bari: Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Riyadh Province 11451, Saudi Arabia, e-mail: abari@ksu.edu.sa
Ahmed Zahidi: Laboratory of Medicinal Chemistry, Department of Drug Sciences, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco, e-mail: a.zahidi@um5r.ac.ma
Azra Skender: Department of Agriculture, Biotechnical Faculty, University of Bihac, 77000 Bihać, Bosnia and Herzegovina
Hanane Benzeid: Laboratory of Analytical Chemistry, Department of Drug Sciences, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat, Morocco, e-mail: benzeid_hanane@yahoo.fr
Anass Doukkali: Laboratory of Analytical Chemistry, Department of Drug Sciences, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat, Morocco, e-mail: doukkali73@gmail.com

fractions, as well as the macerated aqueous extract, playing a pivotal role in the observed antioxidant activity. Additionally, our investigation of the dermoprotective activity demonstrated robust efficacy in the ethyl acetate fraction (FE) and the *n*-butanolic fraction (FB) compared to the standard agents employed (ZnO and methyl salicylate). Overall, our comprehensive studies affirm that the extracts and fractions derived from *C. ambrosioides* manifest moderate antioxidant activities alongside significant dermoprotective potential, elucidated by the presence of phenolic compounds in moderate quantities within the plant.

Keywords: *Chenopodium ambrosioides* (L.), phytochemical analysis, antioxidant, dermoprotective activity

1 Introduction

Herbal medicine has been gaining popularity in developed countries as a complementary therapy to conventional medical treatments. Many people are looking for more natural and holistic alternatives to pharmaceutical drugs, and medicinal plants offer a unique way to achieve this. Herbal remedies have been found to be effective for treating a variety of conditions such as anxiety, depression, chronic pain, and inflammation.

Moreover, numerous ongoing research endeavors are dedicated to assessing the safety and efficacy of medicinal plants, with a plethora of scientific publications reinforcing the utilization of traditional medicine. Beyond being an alternative approach to disease treatment, the use of medicinal plants presents a promising avenue for the discovery of novel drugs.

For centuries, medicinal plants have served as a cornerstone in the treatment of a diverse array of illnesses and conditions, maintaining a pivotal role in healthcare practices worldwide. The World Health Organization (WHO) reports that approximately 80% of the population in Asian and African nations relies on traditional herbal medicine as their primary healthcare modality. This reliance stems not only from the accessibility and affordability of herbal remedies but also from the demonstrated effectiveness and safety associated with many plant-based therapeutic interventions.

There is a burgeoning interest in natural antioxidants, particularly in compounds such as flavonoids and other polyphenols, including tannins, abundantly present in plants. These bioactive constituents are being explored as promising reservoirs for natural skincare agents. Notably, these compounds exhibit robust antioxidant properties, offering a protective shield against skin damage induced by free radicals. Moreover, their association with a diminished risk of chronic diseases, including cancer, cardiovascular disease, and diabetes, underscores their multifaceted potential beyond skincare applications [1,2].

The dermoprotective properties of *Chenopodium ambrosioides* have been the focus of recent research, revealing that extracts from the plant possess anti-inflammatory, antioxidant, and antibacterial properties that are beneficial for the skin. A study by Ouadja et al. [3] found that the oil extract of the plant has anti-inflammatory activity by reducing the production of pro-inflammatory molecules such as prostaglandins and leukotrienes in skin cells. Santiago et al. [4] discovered that the essential oil of *C. ambrosioides* has antimicrobial properties, and Lahlou et al. [5] found that the methanolic leaf extract of *C. ambrosioides* demonstrated antioxidant activity. These findings indicate that *C. ambrosioides* may have potential benefits for the skin, and further research is needed to fully understand its dermoprotective properties and the active compounds responsible for its therapeutic benefits.

The botanical specimen, *C. ambrosioides*, colloquially referred to as “Mkhinza,” is a ubiquitous annual herb classified within the Amaranthaceae family. Indigenous to Central and South America, this plant has proliferated and can now be observed in tropical and subtropical regions across the globe. Throughout history, the leaves and stems of *C. ambrosioides* have been harnessed for medicinal applications, addressing conditions such as headaches, abdominal pain, joint pain, and respiratory ailments. Additionally, the leaves exhibit efficacy in the treatment of lice and warts.

Scientific investigations have unveiled the anti-inflammatory and analgesic attributes inherent in *C. ambrosioides*, attributable to the presence of phytochemical compounds like flavonoids and triterpenes. Furthermore, its pharmacological repertoire encompasses antiparasitic properties against lice and tapeworms, coupled with antioxidant capabilities. However, a more comprehensive understanding of the pharmacological properties of *C. ambrosioides*, and the specific compounds responsible for its therapeutic benefits, necessitates further dedicated research efforts [6].

The objectives of our research are to contribute to a phytochemical and pharmacodynamic study of the plant *C. ambrosioides* by conducting phytochemical screening, dosage of some active families, antioxidant, and dermoprotective activity of this plant.

2 Results and discussion

2.1 Phytochemical screening

The outcomes of the phytochemical screening are delineated in Tables 1 and 2. The assessment of secondary

Table 1: Results of phytochemical screening of *C. ambrosioides* (Chenopodiaceae)

Extract fractions metabolites secondary		BF	AF	IAE	MAE	CHF	EAF
Polyphenols		++	+	+++	+++	–	+++
Flavonoids		++	+	++	++	–	+++
Tannins		+	+	+++	++	–	++
Alkaloids	**Mayer**	++	+	+++	++	–	–
	Dragendorff	++	+	+++	+++	–	–
Terpenoids		+	+	++	+++	–	–
Saponosides		+	+++	+++	+++	–	–
Quinone		++	++	++	++	–	+

The presence of chemical compounds is: (+++) significant, (++) moderate, (+) slight, and (–) absent.

metabolites within the aerial parts of *C. ambrosioides* reveals the presence of various families of these compounds. Concerning the aqueous extracts (EAI, EAM) and the remaining *n*-butanolic and aqueous fractions (EB, FA), alkaloids are present as indicated by the positive test using Dragendorff's reagent (formation of a red precipitate), which is confirmed by Mayer's reagent test (formation of a white precipitate). However, the test using Dragendorff's and Mayer's reagents are negative for the cyclohexane and ethyl acetate fractions.

Polyphenols are present in high quantities in the aqueous extracts (EAI, EAM) and in moderate to low quantities in the remaining *n*-butanol, ethyl acetate, and aqueous fractions (EB, EAE, FA), respectively, as indicated by the test performed (formation of an intense black-green precipitate). However, they are absent in the cyclohexane fraction (ECH). The flavonoid detection test confirms the presence of polyphenols in the aqueous extracts (EAI, EAM) and fractions (EB, EAE, FA) (change in color to orange) and the total absence of flavonoids in the ECH fraction.

The tannin test confirms the presence of tannins (blue-black coloration) in the aqueous extracts (EAI, EAM) and the remaining *n*-butanol, ethyl acetate, and aqueous fractions (EB, EAE, FA), and their absence in the cyclohexane fraction (ECH). The tests for triterpenes and free quinones are positive for the aqueous extracts (EAI, EAM) and the remaining *n*-butanolic and aqueous fractions (EB, FA). The ethyl acetate fraction (EAE) contains only free quinones, while the cyclohexane fraction contains only triterpenes. The aqueous extracts (EAI, EAM) and the remaining *n*-butanolic and aqueous fractions (EB, FA) have higher content of saponosides than the other fractions. The saponoside test for the cyclohexane and ethyl acetate fractions is negative.

Table 2: Quantification of polyphenols, flavonoids, and condensed tannins in extracts derived from the aerial parts of *C. ambrosioides*

Plant extracts	TPC (mg EGA/g extract)	TFC-1 (mg EQ/g extract)	TTC (mg EC/g extract)
IAE	12.64 ± 0.46^{b}	7.58 ± 0.48^{b}	5.49 ± 0.26^{b}
MAE	13.12 ± 0.1^{c}	$7.00 \pm 0.8^{a,\ b}$	10.68 ± 0.15^{c}
CHF	$13.53 \pm 0.24^{d,\ c}$	$28.68 \pm 1.64^{d,\ e}$	38.52 ± 1.36^{f}
EAF	94.62 ± 3.44^{e}	45.78 ± 0.07^{f}	14.85 ± 0.22^{e}
BF	119.68 ± 3.37^{f}	28.72 ± 0.09^{e}	$13.2 \pm 0.029^{d,e}$
AF	5.58 ± 0.21^{a}	15.02 ± 0.05^{c}	3.87 ± 0.08^{a}

The data presented herein depict mean ± standard deviation of three distinct experiments. Significance among values in the same column is denoted by differing superscript letters, indicating statistical differences (p-value < 0.05). The abbreviations used are as follows: MAE, aqueous macerated extract; IAE, aqueous infused extract; CHF, cyclohexanoic fraction; EAF, ethyl acetate fraction; BF *n*-butanolic fraction; AF, remaining aqueous fraction; TPC, total phenolic content; TFC-1, total flavonoid content; and TTC, total tannin content.

2.2 Quantitative analysis

2.2.1 Total polyphenols

The findings depicted in Figure 1 indicate that the *n*-butanolic and ethyl acetate fractions of the plant exhibit noteworthy polyphenol contents, measuring 119.68 ± 3.37 mg Eq GA/g and 94.62 ± 3.44 mg Eq GA/g, respectively. These values demonstrate a statistically significant difference ($p < 0.05$) when compared to the polyphenol yields of the cyclohexane fraction, as well as the macerated and infused aqueous extracts, and the remaining aqueous fraction (13.53 ± 0.24 mg GAE/g, 13.12 ± 0.1 mg GAE/g, 12.64 ± 0.46 mg GAE/g, and 5.58 ± 0.21 mg GAE/g, respectively).

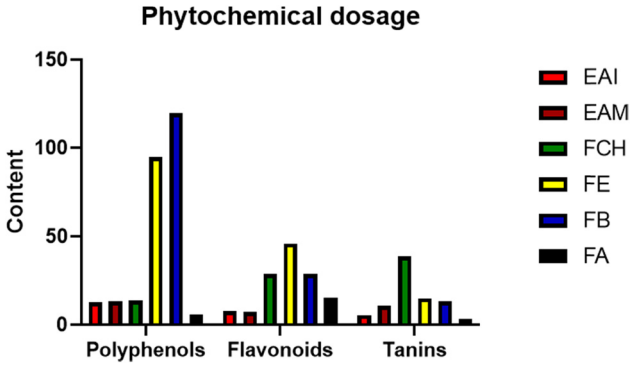

Figure 1: Total polyphenol, flavonoid, and tannin content.

2.2.2 Flavonoids

From Figure 1, it can be seen that the ethyl acetate fraction of the plant contained the highest amount of flavonoids, with a concentration of 45.78 ± 0.07 mg EQ/g. These concentrations are significantly ($p < 0.05$) higher than the *n*-butanolic and cyclohexanolic fractions, which also contain significant quantities of flavonoids, with concentrations of 28.72 ± 0.09 mg EEQ/g and 28.68 ± 1.64 mg EEQ/g, respectively. The remaining aqueous fraction has a moderate content of flavonoids compared to the ethyl acetate fraction, with a concentration of 15.02 ± 0.05 mg QE/g. However, the infused and macerated aqueous extracts have the lowest flavonoid content compared to the other fractions and extracts, with concentrations of 7.58 ± 0.48 mg QE/g and 7.00 ± 0.8 mg QE/g, respectively. These results confirm the findings of the phytochemical screening.

2.2.3 Determination of tannins

The outcomes from the tannin content analysis reveal that the cyclohexane fraction registers the highest values, measuring 38.52 ± 1.36 mg EC/g. These values are significantly elevated ($p < 0.05$) when compared to the tannin content in the ethyl acetate fraction, the butanolic extract, and the macerated aqueous extract, which present the next highest concentrations at 14.85 ± 0.22, 13.2 ± 0.029, and 10.68 ± 0.15 mg EC/g, respectively. Conversely, the infused aqueous extract and the remaining aqueous fraction exhibit the lowest tannin contents, quantified at 5.49 ± 0.26 and 3.87 ± 0.08 mg CE/g, respectively. These results are supported by previous studies [3,7], which found that the hydroethanolic and ethanolic extracts of *C. ambrosioides* leaves contain high levels of phenolic compounds and flavonoids. The divergence in polarities among the extraction solvents serves as a plausible explanation for the observed fluctuations in the phenolic content within the plant extracts and fractions. Furthermore, the insolubility of numerous phenolic compounds in water is a pertinent factor influenced by parameters, such as the molecular weight, the abundance of hydroxyl groups, and chain length. The introduction of water into organic solvents has been shown to enhance the solubility of polyphenols, as illustrated by Sripad et al. [8].

2.3 GC-MS analysis

The analysis of plant extracts using GC-MS is a pivotal method employed in identifying and quantifying compounds present in natural sources. In this investigation, various extracts and fractions of *C. ambrosioides* were subjected to this analytical approach, revealing a rich diversity of compounds across different samples.

Starting with the aqueous extract (IAE), the chromatographic analysis showed an intriguing array of compounds. Notably, compounds like 2,2-dichloroethyl propyl carbonate, benzofuran, 2,3-dihydro-, *trans*-ascaridol glycol, and others were prominently identified. Each compound carries its distinct chemical signature, potentially contributing to the plant's pharmacological properties or biological activities.

Moving on to the aqueous extract MAE, a different spectrum of compounds emerged. Phenol, acetyl chloride, dichloro-, 1*H*-pyrazol-3-amine, and more were among the identified compounds. This variation in chemical composition among extracts underscores the complexity and diversity of the plant's constituents, likely influenced by the extraction methods or specific components of the plant material.

Further analysis of the CHF fraction revealed a unique set of compounds, showcasing a distinctive chemical profile. Isoascaridol, thymol, neophytadiene, and several fatty acids such as palmitic acid, stearic acid, and others were prominent among the identified compounds. The presence of fatty acids, alongside other bioactive molecules, suggests potential implications for the plant's use in traditional medicine or as a source of natural compounds for pharmaceutical purposes.

The EAF fraction exhibited its own set of compounds, including hexadecanoic acid, ethyl ester, (*E*)-palmitoleic acid, and more. The identification of these compounds points to the diversity and complexity of lipid-derived molecules present in this fraction, potentially contributing to the plant's biological activities and therapeutic potential.

Finally, the AF fraction showcased compounds like 2,3-dihydrobenzofuran, benzene, 1-chloro-2-diethoxymethyl, phenol, 2,6-dichloro-4-(1-methylpropyl)-, among others. The presence of such compounds with varying chemical structures and properties hints at the multifaceted nature of *C. ambrosioides* and its potential applications in diverse fields, such as medicine, agriculture, or even in the synthesis of novel compounds.

This comprehensive analysis demonstrates the diverse chemical composition present in different extracts and fractions of *C. ambrosioides*. The plant exhibits a rich repertoire of compounds spanning various chemical families and structures, from simple phenols to complex fatty acids and nitrogenous compounds. Such diversity within the plant's chemical makeup suggests a reservoir of bioactive molecules, offering potential avenues for further exploration and application in pharmaceutical or medicinal realms.

The significance of these findings lies not only in understanding the chemical composition of *C. ambrosioides* but

also in exploring its potential therapeutic or industrial applications. Further research could focus on isolating and characterizing specific compounds, investigating their individual biological activities, and exploring synergistic effects within the plant's chemical milieu. This in-depth understanding of the plant's chemistry can pave the way for the development of novel drugs, nutraceuticals, or agricultural products, harnessing the diverse array of compounds present in *C. ambrosioides* for the benefit of human health and well-being.

2.4 Evaluation of antioxidant activity

To demonstrate the impact of concentration on inhibition rates, we determined the IC_{50} values of the different extracts and fractions of the plant in the four antioxidant activity tests performed (DPPH, ferric (Fe^{3+}) ion antioxidant reducing power [FRAP], ABTS, and TAC).

2.4.1 Determination of antioxidant activity by DPPH free radical scavenging

The IC_{50} value, which represents the concentration of the sample required to inhibit the DPPH radical by 50%, was determined for different extracts and fractions of the aerial parts of the plant to compare their free radical scavenging capacity. The antioxidant activity of the tested fractions was evaluated by determining the IC_{50} values, with a smaller IC_{50} value indicating higher antioxidant activity.

It is a well-established method to evaluate antioxidant activity of different compounds. DPPH is a synthetic stable free radical and the reduction of its color from purple to yellow indicates the scavenging of the radical by the antioxidant compound; the lower the IC_{50} value, the higher the antioxidant activity.

Based on the IC_{50} values obtained, it can be concluded that the ethyl acetate fraction of the plant exhibits the highest antioxidant activity, as evidenced by its lowest IC_{50} value of 25.17 µg/ml ± 0.18/ml. These are significantly higher ($p < 0.05$) than those of the *n*-butanolic fraction, the cyclohexane fraction, the aqueous fraction, and the macerated and infused aqueous extracts, which have lower antioxidant activity than the ethyl acetate fraction, as evidenced by their higher IC_{50} values of 522.92 ± 0.44, 604.06 ± 0.006, 641.20 ± 0.23, 705.24 ± 0.32, and 826.91 ± 0.028 µg/ml, respectively.

The variations observed in the antioxidant activity across diverse extracts and fractions derived from the aerial components of the plant can be ascribed to the distinct concentrations of phenolic compounds within each fraction. Phenolic compounds, extensively studied for their antioxidant attributes, constitute the primary entities responsible for antiradical activity. Consequently, the increased antioxidant efficacy exhibited by the ethyl acetate and *n*-butanolic fractions can be elucidated by their pronounced richness in phenolic compounds relative to the other fractions.

The scientific literature firmly establishes that phenolic compounds, including flavonoids and tannins, possess inherent antioxidant properties, enabling them to effectively scavenge free radicals. Notably, these compounds tend to exhibit a more polar nature, and their extraction is often facilitated using polar solvents such as ethyl acetate and *n*-butanol.

2.4.2 FRAP test

The outcomes derived from the FRAP assay reveal that the *n*-butanolic and ethyl acetate fractions of the plant showcase the most pronounced antiradical activity, demonstrated by their low IC_{50} values of 234.58 ± 0.01 and 577.9 ± 0.03 µg/ml, respectively. These findings align consistently with the results obtained from the DPPH assay, further indicating that the *n*-butanolic and ethyl acetate fractions manifest the highest levels of antioxidant activity.

2.4.3 ABTS free radical scavenging

The outcomes from the ABTS assay reveal that the *n*-butanolic fraction of the plant demonstrates the highest antioxidant activity, evident by its low IC_{50} value of 28.19 ± 0.006 µg/ml. Notably, these values are markedly superior ($p < 0.05$) to those of the ethyl acetate fraction, which also displays substantial antioxidant efficacy with an IC_{50} value of 64.41 ± 0.04 µg/ml. This aligns consistently with the results obtained from the DPPH assay. In contrast, the cyclohexanolic fraction, the aqueous fraction, and the macerated and infused aqueous extracts exhibit comparatively lower antioxidant activity when juxtaposed with the *n*-butanolic and ethyl acetate fractions.

It is pertinent to underscore that the ABTS assay gauges antioxidant activity through the reduction of ABTS radical cations by the antioxidant compound. Consequently, a lower IC_{50} value is indicative of increased antioxidant activity, as elucidated by these results.

2.4.4 Total antioxidant capacity

The results obtained from the TAC assay indicate that the macerated aqueous extract and the *n*-butanolic fraction

exhibit the highest antioxidant activity, as indicated by their values of 478.46 ± 1.69 and 246.78 ± 1.10 mg Eq AG/g, respectively. These results are higher than those obtained for the other fractions (EAF, CHF, and AF), and the infused aqueous extract, which have values of 87.64 ± 0.58 mg, 56.85 ± 0.02, 46.13 ± 1,12B, and 64.59 ± 1.10 mg Eq AG/g, respectively. This finding confirms the high antioxidant activity of the *n*-butanolic fraction, as determined by the other antioxidant activity assays (DPPH, FRAP, and ABTS).

It is well established that TAC assay measures the total antioxidant capacity of the sample by the reduction of the ferric ion to ferrous ion, thus higher TAC value indicates higher antioxidant activity. It is important to note that the values obtained from TAC assay should be compared with the values obtained from other assays, as well as with reference standards, to confirm the antioxidant activity of the different extracts and fractions of the plant.

Additionally, the values obtained from the TAC test should be compared with the values obtained from other antioxidant assays, such as DPPH, FRAP, and ABTS, in order to confirm the antioxidant activity of the different extracts and fractions of the plant and to get a more comprehensive understanding of their antioxidant properties.

The results of the antioxidant assays (DPPH, FRAP, ABTS, and TAC) indicate that the *n*-butanolic fraction and the ethyl acetate fraction of the plant exhibit the highest antioxidant activity compared to the other fractions and extracts studied. Comparing the IC_{50} values of the plant extracts and fractions tested with those of the standards (quercetin, catechin and ascorbic acid) (4.18, 7.03 µg/ml) for the four tests performed under the same conditions, it can be seen that the IC_{50} values of the plant extracts and fractions tested are relatively high, indicating moderate antioxidant capacity.

These results can be attributed to the presence of phenolic compounds, which are known to exhibit antioxidant properties, in moderate quantities in the plant. These compounds are considered to be the primary agents responsible for the antioxidant activity of the *n*-butanolic and ethyl acetate fractions.

Previous studies have also reported that the *n*-butanolic and ethyl acetate fractions of *C. ambrosioides* exhibit potent antioxidant activity. For example, Ghareeb et al. [9] found that the *n*-butanol and ethyl acetate extract of *C. ambrosioides* grown in Egypt had IC_{50} values of 2.98 and 16.48 mg/ml, respectively, for the DPPH assay. In addition, in the TAC assay, these extracts were also found to be the most potent with TAC values of 554.54 ± 2.27 and 418.93 ± 2.62 AA Eq/g, respectively. Skender et al. [10] also reported that the hexane fraction of *C. ambrosioides* grown in Nigeria had the most potent antioxidant activity, as determined by the DPPH assay, with an IC_{50} value of 0.02 ± 0.00 mg/ml, compared to the *n*-butanol fraction, which had an IC_{50} value of 1.74 ± 0.26 mg/ml.

The results obtained from the antioxidant assays indicate that the *n*-butanolic and ethyl acetate fractions of the plant exhibit potent antioxidant activity. These results serve as an important indicator of the antioxidant potential of these fractions and justify further chromatographic isolation to identify the specific compounds responsible for their biological and pharmacological activities.

The observed antioxidant activity can be attributed to the various mechanisms exhibited by the different polyphenols, such as flavonoids, as well as to the synergistic effects of different chemical compounds known as secondary metabolites, which are responsible for maintaining cell integrity and fluidity [11]. The increasing knowledge in the field of biology about free radicals and reactive oxygen species (ROS) has led to a significant advancement in the medical field. Oxygen, which is essential for life, can under certain conditions lead to the formation of ROS, which can cause damage to nucleic acids, proteins, and lipids of cell membranes, as well as plasma lipoproteins, and can contribute to certain cancers, cardiovascular diseases, age-related degenerative diseases, and accelerate aging. The importance of consuming fruits and vegetables as a part of a healthy diet is well-established, and it is believed that one of the potential reasons for their health benefits is the presence of antioxidants such as vitamins C and D, carotene, selenium, and phenolic compounds, including flavonoids [12].

It is important to note that the antioxidant activity of a plant extract or fraction is influenced by various factors, such as the type and concentration of the antioxidant compounds present, the method of extraction, and the conditions of the assay. Additionally, it is important to note that the antioxidant activity of a plant extract or fraction can be influenced by other factors such as pH.

2.5 Dermoprotective activity

The relationship between sunlight and health has been recognized by ancient civilizations and is still an important topic in modern times. A tanned complexion is often viewed as a sign of good health; however, recent research has shown that excessive exposure to the sun can be harmful and can increase the risk of skin cancer. In order to evaluate the potential protective properties of extracts and fractions of the *C. ambrosioides* plant against UV radiation, UV absorption was analyzed using a spectrophotometer. Methyl salicylate (100 µg/ml) was used as a positive control for UVB absorption, and ZnO (5%) was used as a positive control for UVA absorption.

Table 3: Compounds identified in extracts and fractions of *Chenopodium ambrosioides* by GC-MS

No. Library/ID	RT/min	Area	Pct.%	Molecular weight	Molecular formula
Compounds identified in the aqueous extract IAE of the *C. ambrosioides* plant					
1	2,2-Dichloroethyl propyl carbonate	13.053	4.30	166.60	$C_6H_{11}ClO_3$
2	Benzofuran, 2,3-dihydro-	13.110	10.39	120.15	C_8H_8O
3	*trans*-Ascaridol glycol	13.988	6.26	170.24	$C_{10}H_{18}O_2$
4	1,4-Dihydroxy-*p*-menth-2-ene	14.259	11.91	170.25	$C_{10}H_{18}O_2$
5	Indolizine	14.360	1.67	117.15	C8H7N
6	Guanidineacetic acid	15.521	4.36	117.11	$C_3H_7N_3O_2$
7	2-Methoxy-4-vinylphenol	14.687	1.96	150.17	$C_9H_{10}O_2$
8	*E*-9-Methyl-8-tridecen-2-ol, acetate	16.231	8.16	254.40	$C_{16}H_{30}O_2$
9	Benzene, 1-chloro-2-diethoxymethyl	15.870	8.74	214.69	$C_{11}H_{15}ClO_2$
10	Phenol, 2,6-dichloro-4-(1-methylpropyl)-	16.772	27.17	219.108	$C_{10}H_{12}Cl_2O$
Compounds identified in the aqueous extract MAE of the *C. ambrosioides* plant					
1	Phenol	12.884	4.77	94.11	C_6H_6O
2	Acetyl chloride, dichloro-	13.109	1.12	147.38	$Cl_2CHCOCl$
3	1*H*-Pyrazol-3-amine	13.402	1.11	83.09	$C_3H_5N_3$
4	Propanoic acid, 2-chloro-, ethyl ester	13.729	0.13	136.577	$C_5H_9ClO_2$
5	Benzofuran, 2,3-dihydro-	14.304	0.99	120.15	C_8H_8O
6	1*H*-Pyrazole-4-carboxylic acid, 3-amino-	14.958	0.72	127.10	$C_4H_5N_3O_2$
7	3-Hexyne-2,5-diol, 2,5-dimethyl-	15.149	0.99	142.20	$C_8H_{14}O_2$
8	Indole	15.239	0.31	117.15	C_8H_7N
9	2-Methoxy-4-vinylphenol	15.465	0.19	150.17	$C_9H_{10}O_2$
10	Phenol, 2,6-dimethoxy-	15.949	0.13	154.16	$C_8H_{10}O_3$
11	Ethylam	16.073	0.22	45.08	$C_2H_5NH_2$
12	Propanoic acid, 2-chloro-, ethyl ester	15.758	0.14	136.57	$C_5H_9ClO_2$
13	Ethanol, 2-bromo-1-(5-tetrazolyl)-	16.129	0.28	192.96	$C_3H_6BrON_4$
14	2,4-Pentanedione, 3-(2-propenyl)-	16.682	1.30	140.18	$C_8H_{12}O_2$
15	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	17.493	0.22	142.20	$C_7H_{14}N_2O$
Compounds identified in the CHF fraction of the *C. ambrosioides* plant					
1	Isoascaridol	6.206	11.49	168.23	$C_{10}H_{16}O_2$
2	*trans*-Ascaridol glycol	6.510	2.32	170.24	$C_{10}H_{18}O_2$
3	Thymol	6.792	4.27	150.22	$C_{10}H_{14}O$
4	1*H*-Pyrazole-1-carboxaldehyde, 4-ethyl-4,5-dihydro-5-propyl	9.169	1.55	168.24	$C_9H_{16}N_2O$
5	*E*-15-Heptadecenal	20.731	0.66	252.4	$C_{17}H_{32}O$
6	Neophytadiene	17.790	1.42	278.5	$C_{20}H_{38}$
7	Palmitic acid	21.610	14.40	256.42	$C_{16}H_{32}O_2$
8	Phytol	22.872	0.53	296.5	$C_{20}H_{40}O$
9	3,4-Dihydroxymandelic acid	23.559	0.46	184.15	$C_8H_8O_5$
10	9,12-Octadecadienoic acid (*Z*,*Z*)-	24.337	16.64	280.4	$C_{18}H_{32}O_2$
11	Stearic acid	24.664	2.03	284.5	$C_{18}H_{36}O_2$
12	Eicosanoic acid	27.503	2.33	312.5	$C_{20}H_{40}O_2$
13	Octadecane	28.980	0.31	254.5	$C_{18}H_{38}$
14	Docosanoic acid	30.309	0.65	340.6	$C_{22}H_{44}O_2$
15	17-Pentatriacontene	31.673	0.26	490.9	$C_{35}H_{70}$
16	Tetrapentacontane, 1,54-dibromo	31.797	0.47	917.2	$C_{54}H_{108}Br_2$
17	Acide lignocérique	33.081	1.22	368.63	$C_{24}H_{48}O_2$
18	1*H*-Indene, 1-ethyl-2,3-dihydro-1-methyl	33.228	0.37	160.2554	$C_{12}H_{16}$
19	Vitamin E	36.834	0.27	430.70	$C_{29}H_{50}O_2$
20	Ethyl iso-allocholate	38.085	0.12	436.6	$C_{26}H_{44}O_5$
21	25,26-Dihydroelasterol	38.321	5.33	412.7	$C_{29}H_{48}O$
22	Scottenol	38.941	2.18	414.7	$C_{29}H_{50}O$
23	9,19-Cyclo-9β-lanostan-3β-ol	39.617	0.41	438.74	$C_{31}H_{50}$
24	Phytyl palmitate	42.040	0.63	534.93	$C_{36}H_{70}O_2$
25	Hexadecanoic acid, octadecyl ester	42.209	0.47	508.90	$C_{34}H_{68}O_2$
26	Tris(2,4-di-tert-butylphenyl) phosphate	42.468	1.60	662.9	$C_{42}H_{63}O_4P$
27	Pregna-4,6-diene-3,20-dione, 2.alpha. 17-dihydroxy-6-methyl-, diacetate,	43.865	1.06	358.5	$C_{22}H_{30}O_4$

(Continued)

Table 3: *Continued*

No. Library/ID	RT/min	Area	Pct.%	Molecular weight	Molecular formula
28	Eicosanoic acid, octadecyl ester	46.243	1.10	565	$C_{38}H_{76}O_2$
29	6-Octadecenoic acid	47.967	0.45	282.5	$C_{18}H_{34}O_2$
30	3-(2-Naphthyl)-5-(1-naphthyloxymethyl)-2-oxazolidone	49.928	0.54	369.4	$C_{24}H_{19}NO_3$
31	Fumaric acid, pent-4-en-2-yl tridecyl ester	51.652	0.33	366.5	$C_{22}H_{38}O_4$
32	Benz[*c*]acridine, 5,10-dimethyl	53.320	0.41	257.3	$C_{19}H_{15}N$
Compounds identified in the EAF fraction of the *C. ambrosioides* plant					
1	Hexadecanoic acid, ethyl ester	20.889	18.85	284.5	$C_{18}H_{36}O_2$
2	9,17-Octadecadienal, (*Z*)-	23.605	9.09	264.4	$C_{18}H_{32}O$
3	(*R*)-(−)-14-Methyl-8-hexadecyn-1-ol	23.729	8.27	252.4	$C_{17}H_{32}O$
4	(*E*)-Palmitoleic acid	23.898	30.59	254.41	$C_{16}H_{30}O_2$
5	4-Cyclohexene-1,2-dicarboximide, *N*-butyl-, cis	54.774	11.15	207.27	$C_{12}H_{17}NO_2$
Compounds identified in the BF fraction of the *C. ambrosioides* plant					
1	*N*,*N*-Dimethylaminoethanol	2.366	0.69	89.13	$C_4H_{11}NO$
2	Ethyl oxamate	3.335	0.70	117.10	$C_4H_7NO_3$
3	Cyclopropyl carbinol	3.527	0.60	72.10	C_4H_8O
4	2-Cyclopenten-1-one, 2-hydroxy-	4.406	1.54	98.09	$C_5H_6O_2$
5	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-4.901	0.26	153.14	$C_6H_7N_3O_2$	
6	Cyclobutanol	5.285	0.24	72.11	C_4H_8O
7	Phenol	5.442	1.93	94.11	C_6H_6O
8	2(3*H*)-Furanone, dihydro-4-hydroxy-	6.163	0.89	102.09	$C_4H_6O_3$
9	3-Methylcyclopentane-1,2-dione	6.456	2.65	112.13	$C_6H_8O_2$
10	1-[alpha-(1-Adamantyl)benzylidene	6.569	1.27	222.36	$C_{17}H_{18}$
11	3-Chloro-1,2-propanediol	7.313	2.37	110.54	$C_3H_7ClO_2$
12	Phenol, 2-methoxy-	7.876	1.34	124.13	$C_7H_8O_2$
13	Mexiletine	8.485	0.86	179.26	$C_{11}H_{17}NO$
14	2-Cyclopenten-1-one, 3-ethyl-2-hyd	8.676	1.62	126.15	$C_7H_{10}O_2$
15	Cycloserine	9.634	0.41	102.09	$C_3H_6N_2O_2$
16	Benzofuran, 2,3-dihydro-	11.257	4.16	120.15	C_8H_8O
17	2-methoxy-4-vinylphenol	13.668	2.97	150.17	$C_9H_{10}O_2$
18	Norpseudoephedrine	16.812	0.31	151.21	$C_9H_{13}NO$
19	Tetraacetyl-D-xylonic nitrile	17.387	0.32	343.29	$C_{14}H_{17}NO_9$
20	Hexanohydroxamic acid	19.032	0.70	131.17	$C_6H_{13}NO_2$
21	1*H*-[1,2,3]Triazole-4-carboxylic ac	21.489	0.32	113.08	$C_3H_3N_3O_2$
22	*N*-methyl-*N*-nitrosourée	21.827	1.12	103.08	$C_2H_5N_3O_2$
23	2*H*-Azepin-2-one, hexahydro-1-methyl	22.086	1.02	127.18	$C_7H_{13}NO$
24	2-Methylaminomethyl-1,3-dioxolane	22.266	0.93	117.15	$C_5H_{11}NO_2$
25	*N*,*N*′-Diacetylethylenediamine	22.526	2.49	144.17	$C_6H_{12}N_2O_2$
26	(*E*)-2,6-Dimethoxy-4-(prop-1-en-1-yl) phenol	22.909	0.39	194.22	$C_{11}H_{14}O_3$
27	*n*-Hexadecanoic acid	28.227	0.37	256.42	$C_{16}H_{32}O_2$
28	β-Carboline	28.374	0.49	168.19	$C_{11}H_8N_2$
29	*trans*-Sinapyl alcohol	28.825	0.78	210.23	$C_{11}H_{14}O_4$
30	3-(Ethyl-hydrazono)-butan-2-one	7.786	4.12	128.17	$C_6H_{12}N_{2O}$
31	Phenol, 2,6-dimethoxy-	14.649	2.89	154.16	$C_8H_{10}O_3$
Compounds identified in the AF fraction of the *C. ambrosioides* plant					
1	2,3-Dihydrobenzofuran	13.357	7.95	120.15	C_8H_8O
2	2-Methoxy-4-vinylphenol	14.856	2.67	150.17	$C_9H_{10}O_2$
3	(4-Carbamoyl-2-nitrophenyl)acetic acid	15.487	2.79	254.20	$C_{10}H_{10}N_2O_6$
4	Benzene, 1-chloro-2-diethoxymethyl	15.972	13.28	214.69	$C_{11}H_{15}ClO_2$
5	Phenol, 2,6-dichloro-4-(1-methylpropyl)-	16.839	36.42	219.10	$C_{10}H_{12}Cl_2O$

The results of the UV absorption analysis of different extracts and fractions of the *C. ambrosioides* plant revealed that the FB fraction and the EAM and EAI extracts dissolved in distilled water exhibited strong UVA and UVB absorption properties, comparable to those of a chemical sunscreen (as can be seen in Table 3). The UVA absorption values of FB,

Table 4: Assessment of antioxidant activity in aqueous extracts, organic fractions, and standards of *C. ambrosioides* (aerial parts) through DPPH, ABTS, FRAP, and TAC assays

Plant extracts	DPPH IC_{50} (µg/ml)	ABTS IC_{50} (µg/ml)	FRAP EC_{50} (µg/ml)	TAC mg EAA/g of extract
IAE	705.24 ± 0.32 [f]	409.59 ± 0.006[e]	2,412 ± 0.03 [g]	64.59 ± 1.10
MAE	641.20 ± 0.23[d]	533.74 ± 0.003 [g]	1,761 ± 0.03 [f]	478.46 ± 1.69
CHF	604.06 ± 0.006[c]	396.18 ± 0.01[d]	1,025 ± 0.05[d]	56.85 ± 0.02
EAF	25.17 ± 0.18[b]	64.41 ± 0.04[c]	577.9 ± 0.03[c]	87.64 ± 0.58
BF	666.32 ± 0.44[e]	28.19 ± 0.006[b]	234.58 ± 0.01[b]	246.78 ± 1.10
AF	826.91 ± 0.028 [g]	492.8 ± 0.0002 [f]	1,671 ± 0.06[e]	, 46.13 ± b
Quercetin	5.49 ±0.02[a]	—	—	—
Ascorbic acid	—	2.52 ± 0.02[a]	—	—
Catechin	—	—	13.90 ± 0.03[a]	—

The presented data depict mean ± standard deviation of three independent experiments. Significant differences among values in the same column are denoted by distinct superscript letters (a to h), indicating the significance at the 5% level (p-value < 0.05).
MAE: aqueous macerated extract; IAE: aqueous infused extract; CHF: cyclohexanoic fraction; EAF: ethyl acetate fraction BF: *n*-butanolic fraction AF: remaining aqueous fraction; IC_{50}: 50 % inhibitory concentration; EC_{50}: effective concentration that transforms 50% of Fe^{3+} into Fe^{2+}.

EAM, and EAI were greater than 0.8, with a value of 2.33 for EAM, and the UVB absorption values were greater than 0.79, with a value of 2.2 for EAM. Among the extracts and fractions of the *C. ambrosioides* plant tested, EAM, FB, and EAI were found to have the highest UVA and UVB absorption properties.

It is important to note that the protective effects of plant extracts and fractions against UV radiation may vary depending on the method of extraction, the conditions of the assay, and the presence of other compounds in the samples (Table 4).

Table 5: Absorbance of different extracts and fractions of the *C. ambrosioides* plant for UVA and UVB absorption

Extracts and fractions	Absorption	
	UVA	UVB
EAM	0.797	0.711
EAI	0.538	0.543
FCH	0.326	0.302
FE	2.22	2.337
FB	1.483	1.200
FA	0.647	0.893
Methyl salicylate	0.40	3.05
ZnO	2.55	3.59

3 Materials and methods

3.1 Plant materials

The entire *C. ambrosioides* plant, belonging to the Chenopodiaceae family, was systematically collected during the period spanning May to July 2021 from the Rabat region, Morocco (Geographical coordinates: 33° 58′ 06″; N6° 49′ 04″W).

Table 6: Colorimetric reactions for phytochemical screening

Chemical families		Reagents (compositions)	Positive results
Polyphenols		$FeCl_3$ 2%	Blackish blue or green coloration, more or less dark
Flavonoids		Concentrated HCl and magnesium chips	An orange to red coloration
Tanins		$FeCl_3$ (10%)	Blue-black or blue-green color
Alkaloids	Mayer	Mayer (potassium iodide + mercury chloride)	A yellowish-white precipitate
	Dragendorff	Dragendorff (iodobismuthate reagent)	An orange precipitate
Terpenoids		Chloroform and concentrated H_2SO_4	A brownish-red or purple color at the contact area
Saponosides		Distilled water	The formation of a stable foam (height greater than 1 cm), persistent for 15 minutes
Quinone		NaOH (10 %)	The color turns yellow, red, or purple

Botanical authentication was conducted by the floristics team at the Rabat Scientific Institute, and the identified species was cataloged in the herbarium of the Scientific Institute in Rabat, under the specimen number RAB113708. Subsequent to collection, the samples were subjected to drying at room temperature within the laboratory before undergoing the extraction process. The dried plant material was further pulverized using a Binatone Moulinex blender.

3.2 Preparation of plant extracts

We macerated and infused 50 g of the aerial part of *C. ambrosioides* in 500 ml of distilled water. The macerate (EM) and infusion (EI) were filtered using Watmann paper No.1, and then the solvent of the filtrates was evaporated at 45°C under reduced pressure using a rotary evaporator.

For the preparation of the fractions, the dried aerial part (50 g) of *C. ambrosioides* was subjected to Soxhlet extraction with cyclohexane, and after dry evaporation, the cyclohexane fraction (FCH) was obtained. The pomace obtained was dried in the oven for 24 h and subjected to hydroalcoholic maceration (ethanol/water: 50/50). After filtration of the mixture and evaporation of ethanol, successive liquid–liquid separations were carried out on the aqueous phase by solvents of increasing polarity (ethyl acetate and *n*-butanol), in three replicates for each solvent (100 ml). After dry evaporation, ethyl acetate (FAE), *n*-butanol fractions (FB), and the remaining aqueous fraction (FA) were obtained.

The extraction rate was calculated by the following formula:

$$R = (Mi/M) \times 100,$$

where Mi is the mass of the extract, and M is the mass of the starting plant material.

3.3 Phytochemical screening

The aqueous extracts and fractions obtained were used for a preliminary phytochemical screening, which is a set of methods and techniques used to detect secondary metabolites present in a plant. This is a qualitative analysis based on color and/or precipitation reactions to identify the main chemical groups. For this purpose, several types of reagents were used, and the analytical techniques described in the studies of Gul et al. [13] and Deyab et al. [14] and Pandey and Tripathi [15] were utilized.

3.4 Quantitative analysis

3.4.1 Total polyphenols

The quantification of total phenolic content in various extracts and fractions utilized the spectrophotometric approach delineated by Poh-Hwa et al. [16], employing the Folin–Ciocalteu reagent. This method relies on the reduction of a mixture of phosphotungstic acid ($H_3P(W_3O_{10})_4$) and phosphomolybdic acid ($H_3PMo_{12}O_4$) in a basic medium through the oxidizable groups present in the phenolic compounds within the sample. The resultant reduction products, tungsten metal oxide (W_8O_{23}) and molybdenum metal oxide (Mo_8O_3), exhibit a characteristic blue color with maximum absorption at approximately 750 nm. The intensity of this absorption is directly proportional to the concentration of phenolic compounds in the sample (Table 5).

In the experimental procedure, a 200 µl aliquot of the extract, fraction, or standard was combined with 1,000 µl of 10% Folin–Ciocalteu reagent. Subsequently, 800 µl of a 7.5% sodium carbonate solution (Na_2CO_3) was introduced to the reaction medium. Following a 30-min incubation in darkness, absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Lambda 35 UV-Visible spectrophotometer with a 1 cm-thick quartz cell). The spectrophotometer was equipped with a deuterium discharge lamp for UV measurements and a tungsten–halogen lamp for visible measurements. Measurements were made against a blank containing the solvent in which the extract was solubilized, with each sample undergoing three repetitions (Table 6).

Quantification of the phenolic compound content was achieved by applying the regression equation derived from the calibration range, established using gallic acid under identical assay conditions. The final results are expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

3.4.2 Flavonoids

The quantification of flavonoids followed the procedure outlined by Ordonez et al. [17] utilizing the aluminum trichloride reagent $AlCl_3$. A yellow complex is formed between aluminum trichloride and flavonoids, with absorption occurring in the visible spectrum at 420 nm. For each extract and fraction, 0.5 ml was combined with 0.5 ml of 2% aluminum chloride ($AlCl_3$). The resulting mixture was incubated at room temperature, and shielded from light for 1 h. Absorbance was promptly measured at 420 nm against a control. The total flavonoid content in each extract was determined

by referencing a calibration curve derived from a quercetin calibration range, previously established under identical conditions. The calculation involved the regression equation of the calibration curve and was expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

3.4.3 Determination of tannins

To determine tannins in the extracts and fractions, the method outlined by Price et al. [18] was employed, utilizing vanillin. Specifically, 50 µl of each extract and fraction were combined with 1.5 ml of a 4% methanolic solution of vanillin and 750 µl of concentrated hydrochloric acid. The resulting mixture underwent a 20 min incubation in darkness at room temperature, and absorbance was measured at 500 nm against a blank. The total tannin content was quantified as milligrams of catechin equivalents per gram of each extract (mg CE/g extract), determined based on the catechin calibration curve.

3.5 GC-MS analysis

The analysis of aqueous extracts and fractions obtained from *C. ambrosioides*, a medicinal plant, involved gas chromatography-mass spectrometry (GC-MS) using the Agilent 7890A Series at the Moroccan Foundation for Advanced Science, Innovation, and Research (MAScIR) Institute. Employing a GC-MS system equipped with a multi-mode injector and a 123-BD11 column (15 m × 320 µm × 0.1 µm), the extracts and fractions were introduced into the column through a split 1/4 mode, with helium as the carrier gas at a flow rate of 2 ml/min.

For compositional analysis, peak areas were meticulously examined and expressed as a percentage of the total compounds detected in the sample (XLXR electron multiplier detector). The analysis operated in full scan mode over a mass range of 30–1,000 *m/z*, utilizing a gain factor of 5 and electron impact ionization. Operational temperatures were configured at 230°C for the ion source and 150°C for the quadrupole, while the oven temperature program spanned from 30 to 360°C. Compound identification was achieved through consultation of the NIST 2017 MS Library [19].

3.6 Antioxidant activity

3.6.1 Determination of antioxidant activity by DPPH free radical scavenging

The capacity of the samples to neutralize DPPH (2,2-diphenylpicrylhydrazyl) free radicals was assessed following the methodology detailed by Şahin et al. [20]. In this procedure, 50 µl of extracts at varying concentrations were blended with 2 ml of a methanolic solution containing DPPH (60 µM DPPH, dissolved in methanol). After a 20-min incubation in the dark at room temperature, the absorbance was measured at 517 nm using a UV/Vis spectrophotometer.

This approach relies on the reduction in absorbance at 517 nm attributable to the stable DPPH free radical in the presence of a hydrogen radical ($H^{\cdot}$) donor. For comparative purposes, a methanolic solution of quercetin was introduced to 2 ml of the DPPH solution as a positive control, while a negative control was established using the methanolic DPPH solution alone.

The attenuation in absorbance is converted into the percentage of free radical scavenging activity, calculated using the following equation:

$$\text{Antiradical activity (\%)} = ((A \text{ control} - A \text{ sample}) / A \text{ control}) \times 100,$$

where A control is the absorbance of the control and A sample is the absorbance of the tested samples.

The anti-free radical activity was evaluated as the percentage of free radical scavenging and µg of quercetin equivalents per ml (µg QE/ml).

3.6.2 FRAP test

The presence of reducing agents in the samples induces the reduction of iron Fe^{3+}/ferricyanide complex to the ferrous form Fe^{2+} as a result of a single electron transfer [21].

Each extract (0.5 ml) at various concentrations was combined with 2.5 ml of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 ml of a 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution. The mixture was thoroughly blended and then subjected to incubation in a water bath at 50°C for 20 min. Subsequently, 2.5 ml of 10% trichloroacetic acid was introduced to halt the reaction. Following centrifugation at 3,000 rpm for 10 min, 2.5 ml of the supernatant from each concentration was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% $FeCl_3$ aqueous solution. The resulting reaction medium was measured at 700 nm.

Catechin served as the reference compound in the assay, conducted in triplicate, with IC_{50} values reported as mean ± SD of the triplicates. An increase in the absorbance of the reaction mixture signifies an augmentation in the reduction capacity.

3.6.3 Free radical scavenging by ABTS

The ABTS test, following the methodology outlined by Pukalskas et al. [22], involved the generation of the $ABTS^{\cdot}$ + radical by

dissolving 37.7 mg of ABTS and 6.48 mg of potassium persulfate in 9.802 ml of water. After homogenization and a 16-h incubation in darkness, the solution was diluted with ethanol to achieve an absorbance of approximately 0.70 ± 0.02 at 734 nm. The absorbance demonstrated a linear relationship with the $ABTS^{\cdot}$ + radical concentration up to an absorbance of 2 at this wavelength.

In the assay, 100 µl of each extract or fraction, along with methanol (used as a control blank), was added to 2 ml of the prepared ABTS solution. The mixture was incubated at room temperature for 1 min. The reduction in absorbance for both the controls and the extracts/fractions was then recorded at 734 nm. The quantity of trapped $ABTS^{\cdot}$ + radicals was determined using the standard curve for ascorbic acid. The results were expressed as micrograms of ascorbic acid equivalents per milligram of extract (µg EAA/mg extract).

3.6.4 Total antioxidant capacity

The assessment of the total antioxidant capacity in our extracts and fractions followed the established protocol by Prieto et al. [23]. In this procedure, 0.2 ml of each extract and fraction was introduced into a mixture containing sulfuric acid (0.6 M), ammonium molybdate (4 mM), and sodium phosphate (28 mM) after 90 min of incubation in a water bath at 95°C. The absorbance of our extracts and fractions was then measured at 695 nm against a blank. The outcomes, derived from triplicate testing, were expressed in milligrams of ascorbic acid equivalents per gram of extract (mg EAA/g extract).

3.7 Dermoprotective activity

3.7.1 Ultraviolet absorption test

The assessment of dermoprotective activity in extracts and fractions derived from the *C. ambrosioides* plant was conducted through an ultraviolet absorption test, following the methodology outlined by Lee et al. [2]. These extracts and fractions were dissolved in either DMSO or distilled water, reaching a final concentration of 10 mg/ml. The absorbance of the test solution was measured at 365 nm (UVA) and 300 nm (UVB). The test, performed in triplicate, included 5% zinc oxide (OXZ) and 100 µg/ml methyl salicylate (SAM) as positive controls.

4 Conclusion

This investigation delved into the chemical constituents and functional attributes of *C. ambrosioides* extracts and fractions. Through phytochemical screening, diverse metabolites like polyphenols, flavonoids, and alkaloids were identified, showcasing the rich chemical diversity of this plant species.

Quantitative analysis revealed distinct content variations among extracts, emphasizing their unique compositions and potential bioactivity. The GC-MS analysis uncovered several compounds, including *trans*-ascaridol glycol and phenol, contributing to the plant's pharmacological potential.

Evaluation of antioxidant activity demonstrated varying effectiveness across different extracts and fractions, suggesting their suitability for diverse antioxidant applications. Notably, the dermoprotective assessment revealed promising UV absorption properties in specific extracts (EAM, FB, and EAI), hinting at their potential for natural sun-protection formulations.

The discoveries made in this study present promising avenues for further exploration. Future research could delve deeper into isolating and characterizing specific compounds responsible for the observed bioactivities. Furthermore, investigating the mechanisms underlying their antioxidant and dermoprotective properties would enhance our understanding and pave the way for potential therapeutic applications.

Innovations arising from this study lie in the exploration of *C. ambrosioides* as a potential source of bioactive compounds with antioxidant and dermoprotective capabilities. The diverse chemical profile of its extracts opens doors for novel pharmaceutical or cosmetic developments harnessing its natural benefits.

In conclusion, this study not only sheds light on the diverse chemical makeup of *C. ambrosioides* but also lays the foundation for future research aiming to harness its bioactive compounds for therapeutic and skincare innovations.

Acknowledgments: The authors wish to thank Researchers Supporting Project Number (RSP2024R110) at King Saud University Riyadh Saudi Arabia for financial support.

Funding information: This research work is supported by researchers supporting project number (RSP2024R110) at King Saud University Riyadh Saudi Arabia.

Author contributions: Conceptualization: Soufiane Drioua, Otman El-Guourrami, formal analysis: Mohammed Kara,

Amine Assouguem Writing—original draft preparation. Soufiane Drioua, Otman El-Guourrami, Mouna Ameggouz, writing—review and editing, Mohammed Kara, Amine Assouguem, Riaz Ullah, Ahmed Bari, Ahmed Zahidi, Azra Skender, Hanane Benzeid, Anass Doukkali, Funding acquisition; Riaz Ullah, Ahmed Bari. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflicts of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The data presented in this study are available on request from the corresponding author.

References

[1] Cieślik E, Gręda A, Adamus W. Contents of polyphenols in fruit and vegetables. Food Chem. 2006;94(1):135–42.

[2] Lee C-J, Chen L-G, Chang T-L, Ke W-M, Lo Y-F, Wang C-C. The correlation between skincare effects and phytochemical contents in Lamiaceae plants. Food Chem. 2011;124(3):833–41.

[3] Ouadja B, Katawa G, Toudji GA, Layland L, Gbekley EH, Ritter M, et al. Anti-inflammatory, antibacterial and antioxidant activities of *Chenopodium ambrosioides* L. (Chenopodiaceae) extracts. J Appl Biosci. 2021;162(1):16764–94.

[4] Santiago JA, das Graças Cardoso M, Batista LR, de Castro EM, Teixeira ML, Pires MF. Essential oil from *Chenopodium ambrosioides* L.: Secretory structures, antibacterial and antioxidant activities. Acta Sci Biol Sci. 2016;38(2):139–47.

[5] Lahlou Y, Rhandour Z, Amraoui BE, Bamhaoud T. Screening of antioxidant activity and the total polyphenolic contents of six medicinal Moroccan's plants extracts. J Mater Env Sci. 2019;10(12):1332–48.

[6] Lohdip AM, Oyewale A. Studies of *Chenopodium ambrosioides* (Linn) for analgesic and antipyretic activities. J Sci Eng Technol. 2009;16(1):8690–8.

[7] Villalobos-Delgado LH, González-Mondragón EG, Govea AYS, Andrade JR, Santiago-Castro JT. Potential application of epazote (*Chenopodium ambrosioides* L.) as natural antioxidant in raw ground pork. LWT. 2017;84:306–13.

[8] Sripad G, Prakash V, Rao MSN. Extractability of polyphenols of sunflower seed in various solvents. J Biosci. 1982;4:145–52.

[9] Ghareeb MA, Saad AM, Abdou AM, Refahy LA-G, Ahmed WS. A new kaempferol glycoside with antioxidant activity from *Chenopodium ambrosioides* growing in Egypt. Orient J Chem. 2016;32(6):3054–61.

[10] Skender A, Hadžiabulić S, Ercisli S, Hasanbegović J, Dedić S, Almeer R, et al. Morphological and biochemical properties in fruits of naturally grown sustainability cornelian cherry (*Cornus mas* L.) Genotypes in Northwest Bosnia and Herzegovina. Sustainability. 2022;14:4579.

[11] Hii CL, Law CL, Suzannah S, Cloke M. Polyphenols in cocoa (*Theobroma cacao* L.). Asian J Food Agro-Ind. 2009;2(4):702–22.

[12] Gardès-Albert M, Bonnefont-Rousselot D, Abedinzadeh Z, Jore D. Espèces réactives de l'oxygène. L'actualité Chimique. 2003;91.

[13] Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. Sci World J. 2017;2017:5873648.

[14] Deyab M, Elkatony T, Ward F. Qualitative and quantitative analysis of phytochemical studies on brown seaweed, *Dictyota dichotoma*. Int J Eng Dev Res. 2016;4(2):674–8.

[15] Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014;2(5):115–9.

[16] Poh-Hwa T, Yoke-Kqueen C, Indu Bala J, Son R. Bioprotective properties of three Malaysia Phyllanthus species: An investigation of the antioxidant and antimicrobial activities. Int Food Res J. 2011;18(3):887.

[17] Ordonez AAL, Gomez JD, Vattuone MA. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 2006;97(3):452–8.

[18] Price ML, Van Scoyoc S, Butler LG. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J Agric Food Chem. 1978;26(5):1214–8.

[19] Ramdani C, El Fakhouri K, Sbaghi M, Bouharroud R, Boulamtat R, Aasfar A, et al. Chemical composition and insecticidal potential of six essential oils from morocco against *Dactylopius opuntiae* (Cockerell) under field and laboratory conditions. Insects. 2021;12(11):1007.

[20] Şahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, et al. Biological activities of the essential oils and methanol extract of Origanum vulgare ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control. 2004;15(7):549–57.

[21] Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986;44(6):307–15.

[22] Pukalskas A, van Beek TA, Venskutonis RP, Linssen JPH, van Veldhuizen A, de Groot Æ. Identification of radical scavengers in sweet grass (*Hierochloe odorata*). J Agric Food Chem. 2002;50(10):2914–9.

[23] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337–41.