frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 01 March 2024
DOI 10.3389/fsufs.2024.1348141

OPEN ACCESS

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RECEIVED 01 December 2023
ACCEPTED 15 February 2024
PUBLISHED 01 March 2024

CITATION
Zirari M, Aouji M, Imtara H, Hmouni D, Tarayrah M, Noman OM and El Mejdoub N (2024) Nutritional composition, phytochemicals, and antioxidant activities of *Abies marocana* Trab. needles. *Front. Sustain. Food Syst.* 8:1348141. doi: 10.3389/fsufs.2024.1348141

Nutritional composition, phytochemicals, and antioxidant activities of *Abies marocana* Trab. needles

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Introduction: Finding natural sources of bioactive compounds is turning into a cutting- edge task for the scientific community and industry alike. *Abies marocana*, Moroccan fir, holds great importance due to its ecological, economic, social, and cultural significance.

Material and Methods: The current study aimed to evaluate the needles of *Abies marocana* in terms of its nutritional and anti-nutrient content, bioactive components, and antioxidant capacity. The AOAC technique was used to determine the composition of the needles. Mineral content was analyzed by inductively coupled plasma optical emission spectrometry. Phytochemical screening of methanol extract was performed using standard procedures, and multiple assays evaluated antioxidant activity. The extract's volatile profile was elucidated using GC-MS method.

Results and Discussion: The presence of various components in the needles was discovered through proximate analysis, including carbohydrates, crude protein, crude fiber, crude fat, ash, and moisture. Anti-nutrients such as chlorophyll, carotenoids, and vitamin A were confirmed. Needles are a rich source of mineral elements and contain significant levels of phenols, flavonoids, tannins, and phytosterols. Palmitic acid, 17-octadecynoic acid, and (Z)-18- octadec-9-enolide were the main components identified by GC-MS analysis. The methanolic extract exhibited substantial antioxidant capacity. The DPPH scavenging activity displayed a remarkable percentage inhibition. The integration of *Abies marocana* needles into sustainable diets can contribute to the well- being of humans and the planet, since the nutrient and bioactive compounds present have potential applications in the pharmaceutical and food industries.

KEYWORDS

***Abies marocana*, proximate analysis, mineral analysis, nutritional value, phytochemical content, antioxidant activity**

Introduction

Medicinal plants contain essential nutritional components, including carbohydrates, proteins and lipids. These components play a crucial role in the human body and are used for various physiological, metabolic and morphological processes (Radha et al., 2021). To develop the medicinal potential of these plants, it is imperative to identify their chemical and biological constituents and assess their therapeutic efficacy (Shahar et al., 2023). A considerable number of these phytochemicals have the advantageous characteristic of being antioxidants, which effectively protect cells against damage caused by reactive oxygen species (Priora et al., 2016). The exploration of plants endowed with an abundance of phenols, flavonoids, tannins, vitamins and many other phytochemical compounds has been undertaken as part of ethnomedicines research and is at the origin of a multitude of medicinal activities (Rice-Evans et al., 1995). *Abies* was initially documented by Miller (1691–1771) in 1754. This second-largest genus within the *Pinaceae* family is deemed to be more intricate than other genera within said family, and all its species are dispersed across the Northern Hemisphere (Nikolic et al., 2021). The *Abies* genus is estimated to include 52 recognized species, along with an additional 58 unidentified species yet to be included (WFO, 2023). *A. marocana*, a species indigenous to Morocco, is found exclusively in the Rif region, at elevations ranging from 1,400 to 2000 meters (Alaoui et al., 2011). *A. marocana* needles are not commonly utilized as a source of sustenance. Nonetheless, certain customary practices involve the utilization of specific constituents of this tree for medicinal or flavor-enhancing objectives (Rhattas et al., 2016). Nevertheless, it is important to exercise caution when consuming any portion of this tree, as *A. marocana* may contain compounds that can be hazardous in large amounts. In a broader context, *A. marocana* needles encompass certain constituents, such as terpenes, which, when present in substantial quantities, can be toxic or irritating. Indeed, Zirari et al. (2024) discovered that the etheric fraction of *Abies marocana* needles is abundant in terpene compounds. Extracts derived from conifer needles are abundant in α-pinene, myrcene, and terpinene compounds, which are aromatic hydrocarbons and exhibit antioxidant activities considerably greater than those of vitamin E (Kim et al., 2017). The different components of *A. marocana* (needles, twigs, and cones) have garnered attention in the field of pharmaceuticals because of the presence of active compounds (Zirari et al., 2024). Given their content of essential nutrients like vitamins, minerals, and phytochemicals, their integration into diets could serve as a sustainable means of nourishment, particularly in regions where traditional food sources are scarce or limited. It has been widely documented in various sources that the essential oils derived from certain species of coniferous trees possess considerable commercial importance and hold promise as potential antibiotics, anticarcinogens, sedatives, and even nutritional agents in the field of medicine (Sahin and Yalcin, 2017). In a more recent development, a newly discovered extract from the bark of the silver fir tree (*Abies alba*), which has already demonstrated its antioxidant properties in previous studies (Benković et al., 2014), has now been introduced into the market under the brand names Abigenol® and AlbiPhenol® (Leone et al., 2022). This innovative product holds great potential for various applications in the medicinal industry, owing to its proven beneficial effects and unique composition. To the best of our knowledge, there have been no investigations conducted concerning the nutritional implications that arise from the utilization of *Abies marocana* needles. In accordance with this, the present research endeavors to explore the nutritional worth, phytochemical composition, and antioxidant capacity of *A. marocana* needles. Furthermore, a comprehensive analysis of the bioactive elements of the needles was performed using gas chromatography–mass spectrometry (GC–MS), and the potential health advantages associated with these constituents were discussed. The purpose of this study is to provide insight into the potential health benefits and medicinal applications that can be derived from this particular plant.

Materials and methods

Chemicals and reagents

All chemicals used in this study were purchased from Sigma Aldrich, with a high degree of purity ≥98%.

Plant material and sample preparation

Fresh needles of *Abies marocana* were collected from the Chouihate Mountain, situated in Chefchaouen (35°11′05.6″ N 5°13′47.9″ W), a city located in the northern region of Morocco, at an elevation of 1,785 m. A total of 2,000 young needles were gathered from a single *A. marocana* genotype. Subsequently, they were subjected to an extensive washing process using water and then dried for a duration of 3 days at a controlled temperature of (45 ± 2°C). The desiccated needles underwent the process of pulverization, utilizing an electric grinding machine, and were then conserved in a refrigerator for subsequent analysis.

Nutritional characteristics

Proximate analysis

The determination of moisture, ash, and crude fibers (based on a dry measurement) was carried out successfully by following the conventional techniques specified by the Association of Analytical Chemists (AOAC) (AOAC, 2016). The protein content was evaluated by measuring the nitrogen using the micro-Kjeldahl method. To calculate the protein value, the nitrogen measurement was multiplied by a factor of 6.257 (Kjeldahl, 1983). The fat content of the samples was ascertained using a direct solvent extraction method of the Soxhlet type (AOAC, 1995). The carbohydrate content was determined by employing the difference method (Hussain et al., 2009). The assessment of the entire energy composition was carried out by multiplying the numerical values that represent the quantities of crude protein, crude fat, and total carbohydrates using the Atwater factors. Subsequently, these products were combined to yield a summation. The obtained summation was then expressed as kilocalories per 100 g of the given sample (Merrill and Watt, 1973; Shad et al., 2013).

Determination of mineral content

The resultant residue of white ash, as indicated in the previous section, was dissolved in a concentrated solution of nitric acid HNO_3 (25%) and subsequently filtered. The resulting solution was then subjected to an analysis to determine the composition of specific major minerals. The concentration of various ions in the needles was assessed by employing the technique of inductively coupled plasma

optical emission spectrometry (ICP-OES) (Skujins, 1998). The experimental parameters employed for the ICP-OES were: the instrument used was the Perkin Elmer Optima 8,000 model, with an RF power of 1,500 watts, a plasma gas flow rate (Ar) of 8 L min^{-1}, and an auxiliary gas flow rate (Ar) of 0.2 L min^{-1}. Additional parameters included the axial view size, the copying and play-back time of 45 min, and a copying time of 15 min.

Anti-nutrients and vitamin

Chlorophylls a, b and total

The method outlined by Santiago-Saenz et al. (2018) was employed to determine the chlorophyll content. An 80% (v/v) acetone solution was utilized for the preparation of the samples. Following filtration, the resulting solution was transferred into tubes and subjected to centrifugation. Subsequently, the absorbance of the samples was assessed spectrophotometrically at 645 nm and 663 nm (ZUZI spectrophotometer model 4201/20). The obtained data is presented in terms of milligrams of chlorophyll per gram of dry weight (mg g^{-1} DW).

Total carotenoid content

The carotenoids were quantified using the methodology delineated by Sass-Kiss et al. (2005). This method involved the combination of 1 g of needle powder with a solvent mixture comprising hexane, acetone, and ethanol (in a ratio of 1:2:2 respectively) for a duration of 15 min. Afterward, the resulting mixture underwent centrifugation at a rate of 4,500 rpm at a temperature of 4°C for 15 min. The upper layer containing hexane and the associated pigment was separated, and the absorbance was measured at 430 nm. The total carotenoid content was expressed as milligrams of β-carotene equivalent per 100 grams by extrapolating a standard curve that was constructed using β-carotene.

Vitamin A (retinol)

The needle powder, weighing 1 g, was macerated in 20 mL of petroleum ether for 1 h. The resulting mixture underwent filtration and evaporation until it completely dried. Thereafter, the remaining residue was treated with a 0.2 mL solution of chloroform-acetic anhydride and 2 mL of a 30% solution of trichloroacetic acid in chloroform, and the absorbance at 620 nm was measured using spectrophotometry, with retinol as the standard and a calibration curve established under identical experimental conditions (Oladayo Amed et al., 2019).

Extraction of bioactive compounds

Powdered needles (25 g) were delipidated using petroleum ether and extracted in a Soxhlet extractor with methanol (Kuluvar et al., 2009). The extract was then concentrated and preserved at a temperature of 4°C until further analysis. In addition, the crude extract yield was determined using the method described in NM ISO 734:2020, Eq. 1.

$$w = \frac{m_1}{m_0} \times 100\% \quad (1)$$

where, m_0 is the mass of the sample powder and m_1 is the mass of the extract.

Evaluation of phytochemical content

Phytochemical screening

Phytochemical screening was performed to identify the major groups of secondary metabolites contained in our extract that were responsible for the possible activities. The following chemical groups were identified using conventional characterization reagents: alkaloids, reducing compounds, flavonoids, polyphenols, tannins, anthocyanins, proteins, essential oils, cardiac glycosides, sterols, and terpenes (Trease and Evans, 1989; Sofowora, 1993; Harborne, 1998).

Phenolic compounds content

The Folin-Ciocalteu method (Cheok et al., 2013) was used to determine the total polyphenol levels of the methanolic extract. The method involved combining the extract with Folin–Ciocalteu reagent and a sodium carbonate solution. The mixture was left in the dark and the absorbance was measured at 765 nm. Gallic acid was used as a standard and a calibration curve was created. The flavonoid content was evaluated using a method developed by Lamaison and Carnat (Lamaison and Carnat, 1990). Within this approach, the sample was mixed with a 2% $AlCl_3$ solution in methanol and allowed to incubate for a duration of 10 min. The absorbance of the resulting mixture was then measured at 430 nm, and the concentration of flavonoids was determined using quercetin as a reference. Blank samples were prepared using methanol. The measurement of tannin content was accomplished through the application of the vanillic acid method (Hagerma, 2002). The extract was mixed with vanillin reagent and incubated at 30°C for 20 min. The absorbance at 500 nm was measured, and catechin was used as a reference standard. Blanks were made with a 4% acid-methanol mixture.

Total phytosterol content

The phytosterol content was determined using a method established by Shahar et al. (2023). Powder (1 g) was mixed with ethanol: acetone (1:1) solution and centrifuged at 2,000 rpm for 20 min. The supernatant was then dried, chloroform (10 mL) was added, and the mixture was shaken. Sample extract (1 mL) was combined with Liebermann reagent (3 mL) and incubated in the dark for 15 min. The resulting olive-green color was measured at 680 nm.

Estimation of chemical constituents by GC–MS

Individual samples were analyzed using a Gas Chromatography–Mass Spectrometry (BRUKER 456-GC EVOQ) system equipped with a BR-5 ns FS capillary column (60 m × 0.32 mm ID × 0.25 μm). The carrier gas employed was high-purity helium at a flow rate of 1.7 mL min^{-1}. The temperature of the injectate was set at 250°C, while the oven temperature started at 40°C and gradually increased to 260°C at a rate of 8°C min^{-1}. The extracts were withdrawn using a syringe and injected into the injector with a split ratio of 40:1. Full-scan mass spectra ranging from 40–550 AMU were collected to obtain comprehensive data. The ion source temperature was adjusted to 230°C, and the quadrupole temperature was maintained at 150°C. The electron multiplier voltage was kept at 1,100 V above self-tuning, with a solvent delay of 3 min. The identification and characterization of the compounds in the different crude extracts were based on their retention times in gas chromatography. The obtained mass spectra were compared with the standards in the Mass Spectral Library (NISTII). The results were reported as a percentage of the peak area.

Evaluation of antioxidant activities

Total antioxidant capacity

The method employed by Aouji et al. (2023) was utilized to determine the total antioxidant capacity (TAC) of the extract. To carry out this measurement, a 0.1 mL sample of the extracts was mixed with a reagent solution consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate in a tube. These tubes were sealed and subjected to a temperature of 95°C for a duration of 90 min in a water bath. The absorbance of the resulting solutions was then assessed at 695 nm relative to a blank. The findings were expressed as milligrams of ascorbic acid equivalent per gram of dry weight (mg EAA g^{-1} DW).

Determination of the antiradical activity by the DPPH assay

Due to its inherent stability in the form of a free radical and its ease of analysis, DPPH (2,2-diphenyl-1-picrylhydrazyl) is widely employed as a substrate to assess antioxidant activity in a rapid and straightforward manner (McCune and Johns, 2002). Subhashini et al. (2011) presented the experimental methodologies employed to investigate the radical scavenging capability of DPPH. Distinct volumes of the plant's etheric extracts (1 mL) were combined with 1 mL of a solution containing DPPH radicals, resulting in a final DPPH concentration of 0.025 g L^{-1}. Following vigorous stirring, the mixture was allowed to settle undisturbed for 30 min, after which its absorbance was measured at 517 nm. Ascorbic acid was utilized as the reference compound. The percentage inhibition of DPPH radical activity was calculated using Eq. 2 (McCune and Johns, 2002) as depicted below.

$$PI\% = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

where, A_0 and A_1 are the absorbance of the control and extract.

Results and discussion

Nutritional characteristics

Proximate analysis

The proximate composition of *A. marocana* needles, including moisture, protein, ash, fiber, fat, and carbohydrate, is illustrated in Figure 1 on a dry weight basis (DW). The moisture content of the needles was recorded as 7.709 ± 0.232 g.100 g^{-1}. Comparative analysis revealed that the moisture content of the needles was similar to that reported for *A. pindrow* (Kumar and Kumar, 2017), but slightly higher than that reported for *A. webbiana* (6.90 g.100 g^{-1} DW) (Rajalakshmi et al., 2016). This low moisture content is advantageous as it contributes to the extended shelf life of the sample (Akinsola et al., 2021). The crude protein content of *A. marocana* needles was determined to be 2.556 ± 0.066 g.100 g^{-1} DW. According to Asibey-Berko and Tayie (1999), plants with a caloric value in protein exceeding 12% are considered valuable protein sources. The ash content in the needles was relatively low at 3.288 ± 0.677 g.100 g^{-1} DW, indicating a minimal presence of inorganic nutrients in the sample (Marzuki et al., 2018). *A. webbiana*, on the other hand, exhibited higher ash content (5.23 g.100 g^{-1}) when compared to the findings of this study. The nutritional content of plants grown in different geographical conditions is primarily influenced by environmental factors such as soil fertility, moisture content, and growth temperature. Crude fiber in food or plants serves as an indicator of non-digestible carbohydrates and lignin (Karoly, 2011). The crude fiber content obtained for *A. marocana* needles was determined to be 2.323 ± 0.212 g.100 g^{-1} DW. In the context of nutrition, the presence of fiber at this level may play a beneficial role in supporting the health of the intestines and colon, in addition to contributing to other regulatory and healing functions within the field of human nutrition (Karoly, 2011). The fat content of the needles highlights their potential as a dietary supplement with promising nutritional properties (Romes et al., 2019). This claim is supported by the high energy value of 389.934 ± 4.589 kcal.100 g^{-1} and the carbohydrate content of 80.538 ± 0.904 g.100 g^{-1} DW. These results align with those reported by Jyske et al. (2020) for *Picea abies* needles. Carbohydrate-rich foods play a significant role in maintaining a well-balanced diet. The primary purpose of carbohydrates is to supply the body with the necessary fuel for physiological functions. This fuel is essential for carrying out vital bodily processes such as respiration, thermoregulation, as well as the contraction and relaxation of the heart and muscles. Moreover, energy is also required for engaging in physical activities. Glucose is the exclusive source of energy utilized by the brain, nerve cells, and developing red blood cells. Nevertheless, the excessive consumption of refined, uncomplicated, and inferior carbohydrates directly impacts both the physical and mental pathophysiology (Clemente-Suárez et al., 2022). In conclusion, the findings presented here emphasize that *A. marocana* needles could serve as a valuable source of essential nutrients and energy, making them suitable for enhancing the dietary and overall health of individuals.

Determination of mineral content

Minerals are widely acknowledged to play a crucial role in human nutrition, contributing to the overall well-being of both the body and mind. Indeed, a variety of necessary macro and micro elements, also known as trace elements, are essential for numerous bodily functions (Shahar et al., 2023). In *Abies marocana* needles, a significant quantity of both essential macro (K, Na, Ca, Mg, and P) and micro-mineral (Fe, Zn, and Mn) elements were found on a dry weight basis (Figure 2). The most abundant macro element identified in the needles is Ca (11529.897 ± 40.732 mg kg^{-1}), followed by K (3550.142 ± 35.204 mg kg^{-1}), Mg (1946.290 ± 57.841 mg kg^{-1}), P (493.33 ± 15.275 mg kg^{-1}) and Na (210.112 ± 6.326 mg kg^{-1}). Potassium (K) and sodium (Na) are crucial minerals for preventing cardiovascular and oncogenic diseases as they play a significant role in regulating osmotic pressure (Kumari et al., 2017). Additionally, the non-essential element Al was also detected (173.591 ± 3.352 mg kg^{-1}). Among the micro-minerals, Fe (91,020 ± 2.706 mg kg^{-1}) is the primary element, followed by Zn (37.951 ± 0.590 mg kg^{-1}) and Mn (19,383 ± 0.818 mg kg^{-1}). Iron and Calcium are essential for the survival of an organism due to their involvement in a multitude of metabolic processes (Kumari et al., 2017). These findings align with those reported by Parzych et al. (2018) who examined the nutrient and heavy metal content in needles of *Picea abies* and *Picea omorika*. Their analysis confirmed the presence of calcium, potassium, magnesium, and other minerals in the needles of both

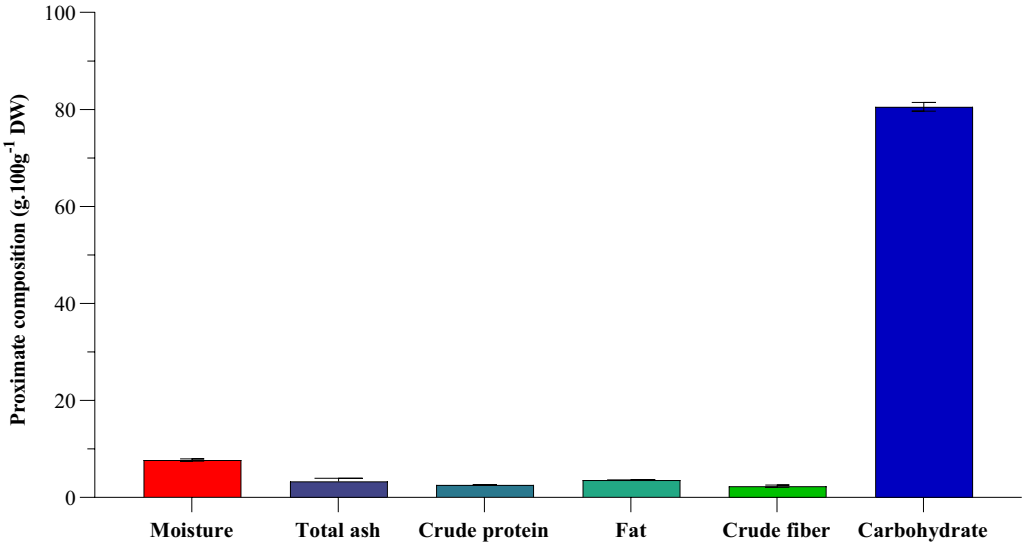

FIGURE 1
Nutritional composition of *Abies marocana* needles (mean ± SD, *n* = 3).

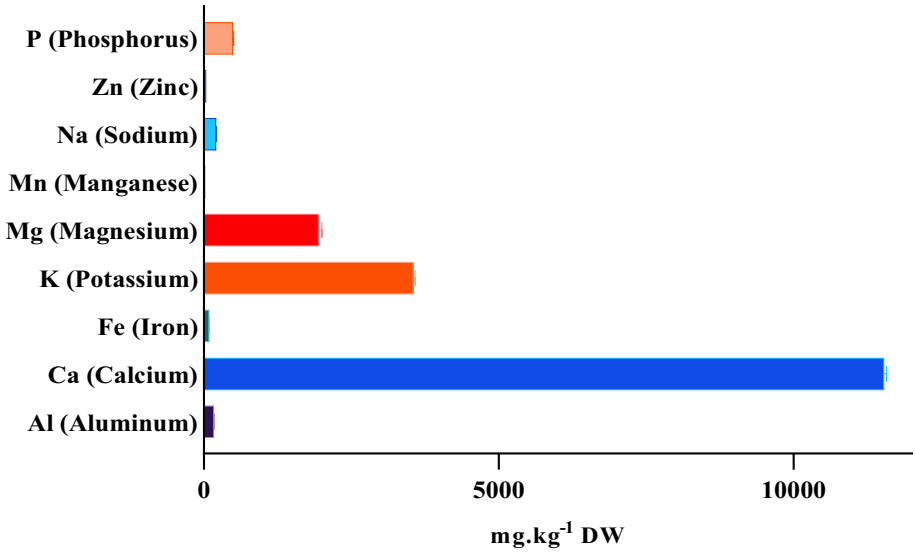

FIGURE 2
Mineral composition of the needles of *A. marocana* in mg Kg^{-1} DW (mean ± SD, *n* = 3).

species. Minerals play crucial roles in our body to carry out essential functions, ranging from fortifying our bones to facilitating the transmission of nerve impulses, all in the pursuit of a healthy and prolonged lifespan. The presence of a variety of minerals not only enables the production of different hormones but also serves as a means of regulating a regular heartbeat. Certain macro- and micro-elements can be found in the composition of teeth, specifically calcium and phosphorus, as well as in bones, such as calcium, magnesium, manganese and phosphorus. In addition, a majority of micro-elements, including copper, iron, manganese, magnesium and zinc, serve as vital components within numerous enzymes, fulfilling a pivotal role in their structural composition (Gharibzahedi and Jafari, 2017).

Anti-nutrient and vitamin

Chlorophylls a, b and total

According to the data presented in Table 1, the *Chl a*, *Chl b*, and total *chlorophyll* contents for the species *A. marocana* were determined to be 0.793 ± 0.005, 0.253 ± 0.026, and 1.046 ± 0.023 mg g^{-1} DW, respectively. In comparison to our findings, lower levels of chlorophyll have been observed in *Picia Abies* (Werk et al., 1993). It should be noted that in addition to factors such as needle age and season, the concentration of chlorophyll has been shown to be affected by irradiance (Lichtenthaler et al., 1981), temperature (Lewandowska, 1977), water availability (Linder and Rook, 1984), and nutrient supply

TABLE 1 Chemical composition of *A. marocana* needles (mean ± SD, $n = 3$).

Constituents	Value
Chl a (mg g^{-1} DW)	0.793 ± 0.005
Chl b (mg g^{-1} DW)	0.253 ± 0.026
Chl t (mg g^{-1} DW)	1.046 ± 0.023
Carotenoids (mg EβC g^{-1} DW)	0.082 ± 0.001
Vitamin A (μg retinol g^{-1} DW)	52.660 ± 0.247

Chl a, chlorophyll a; Chl b, chlorophyll b; Chl t, total chlorophyll.

TABLE 2 Summary of extraction yield, bioactive compounds and antioxidant activity of *A. marocana* needles (mean ± SD, $n = 3$).

Sample		Value
Extraction yield (%)	–	27.467 ± 0.005
Bioactive compounds	Polyphenols (mg GAE g^{-1} DW)	10.459 ± 0.365
	Flavonoids (mg EQ g^{-1} DW)	0.506 ± 0.001
	Tannins (mg EC g^{-1} DW)	2.359 ± 0.075
	Phytosterols (mg g^{-1} DW)	37.816 ± 0.961
Antioxidant activity	DPPH (μg mL^{-1})	82.594 ± 2.450
	TAC (mg EAA g^{-1} DW)	122.670 ± 2.203

(Hind and McCarty, 1973). These findings hold significance in terms of nutritional and antioxidant evaluation as research has indicated that *chlorophyll* presence in plants can reduce the levels of reactive oxygen species. Ferruzzi et al. (2002) demonstrated that conventional derivatives of *chl a* exhibited a greater capacity for antioxidation in comparison to derivatives of *chl b*. Besides incorporating *chlorophyll* into the diet during the early stages of life can help reduce weight gain, improve glucose tolerance and reduce inflammation, which may prevent obesity (Li et al., 2019). In addition, the effect of chlorophyll on rats with type 1 diabetes has been studied, and it has been confirmed that chlorophyll a can reduce the risk of diabetes (Wunderlich et al., 2020). Furthermore, all chlorophyll compounds exhibit similar anti-carcinogenic mechanisms, albeit with varying degrees of potency (Hsu et al., 2013).

Carotenoid content

Carotenoids, which are synthesized by plants and microorganisms, are pigments that are intensely colored and soluble in fat. Besides their association with vitamin A, they are recognized for their antioxidant properties (Carazo et al., 2021). Carotenoids are widely distributed throughout nature and serve numerous functions, ranging from light absorption and protection against photodamage in photosynthesis to safeguarding the eyes (El-agamey et al., 2004). The carotenoid content of *A. marocana* needles was determined to be 0.082 ± 0.001 mg EβC g^{-1} DW (Table 1). This outcome is inferior to the findings of Tomislav et al. (2003) for *Abies alba Mill* needles, which reported a content of 0.91 ± 0.15 mg EβC g^{-1} DW. Various environmental and developmental factors, such as nutrient deficiency, temperature, and needle age, can influence the levels of these carotenoids. In fact, research has demonstrated that the pigment content in the needles of *Picea abies* (Norway spruce) and other trees is strongly correlated with the age of the needle, with younger needles typically exhibiting significantly lower pigment levels (Young, 1993).

Vitamin A (retinol)

Vitamin A is a group of unsaturated monohydric alcohols that possess an alicyclic ring structure. Within this classification, retinol, retinal, and retinoic acid are all categorized as different forms of vitamin (Sommer, 2008). The quantification of the plant's fat-soluble vitamin A content was conducted using the retinol equivalent method. As presented in Table 1, the measured vitamin A content of the sample was determined to be 52.660 ± 0.247 μg retinol g^{-1} DW. *Picea abies (L.) Karst*, notably, exhibited a considerably higher concentration of vitamin A (Jyske et al., 2020). The antioxidant properties of vitamin A, specifically retinol, along with various pro-vitamin A compounds such as alpha and beta carotenes, have been scientifically demonstrated (Khan et al., 2023). Additionally, it is imperative to acknowledge that vitamin A plays a crucial role in maintaining normal vision, regulating gene expression, promoting growth, and enhancing immune function through its impact on the maintenance of epithelial cell functionality (Lukaski, 2004).

Evaluation of phytochemical content

Extraction yield

The extraction step plays a vital role in the process of separating active compounds from plant materials and minimizing the presence of interfering substances (Do et al., 2014). By utilizing the Soxhlet extraction technique and employing methanol as the solvent for extraction, the bioactive compounds were successfully isolated from the needles of *Abies marocana*. Notably, the needles exhibited a favorable yield of 27.467 ± 0.005% of dry matter, as indicated in Table 2. This outcome surpasses the results reported by Vishnoi et al. (2007) in their study on *Abies webbiana Lindl* (*Pinaceae*). It is worth noting that the extraction yield is influenced by various factors, including the particle size of the sample, the temperature at which the extraction is conducted, and the ratio of solvent to sample employed during the extraction process (Herode et al., 2003).

Phytochemical screening

Phytochemical analysis demonstrated the presence of sterols, terpenes, reducing compounds, phenols, flavonoids, tannins, saponins, and proteins in the methanol extract obtained from *A. marocana* (Table 3). Conversely, the extract did not contain alkaloids, anthocyanins, quinones, coumarins, and cardiac glycosides. These biologically active constituents are recognized for their diverse mechanisms of action and their ability to exhibit antioxidant and antimicrobial properties (Ameen et al., 2021). The findings of this investigation correspond with previous phytochemical studies conducted on *Abies webbiana* needles (Rajalakshmi et al., 2016), differing only in the absence of quinones, which may be attributed to variances in geographical and ecological factors.

Phenolic compounds content

Plants serve as a significant reservoir of phenolic compounds with diverse origins and functionalities. A majority of these compounds are biologically potent substances derived from plants, exhibiting anti-cancer, antiviral, and antibacterial attributes (Kumari et al., 2017). The findings of the conducted analyses were presented in Table 2. The

quantification of the total phenolic content (TPC) in the methanolic extract was determined using Gallic acid equivalent, yielding a value of 10.459 ± 0.365 mg GAE g^{-1} DW. These findings align with the research conducted by Karapandzova et al. (2015) on the needles of *Pinus peuce* and other pine species from the Macedonian Flora. It should be noted that TPC values are influenced by factors such as the location of sample collection, plant growth stages, and plant age, as highlighted by Santiago-Saenz et al. (2018). The total flavonoid content (TFC) was assessed in terms of quercetin equivalent, resulting in an estimated value of 0.506 ± 0.001 mg EQ g^{-1} DW. This outcome is consistent with the findings reported by Zeppetzauer et al. (2021), who observed a diverse range of flavonoids in the bark of *Picea abies*. Flavonoids, while typically categorized as non-essential nutrients, play a crucial role in the human diet because of their significant antioxidant properties (Kumari et al., 2017). Conversely, the total tannin content (TTC) was determined through the utilization of a Catechin calibration curve, revealing a significant value of 2.359 ± 0.075 mg EC g^{-1} DW. This result is similar to the findings obtained for *Picea abies* (Zeppetzauer et al., 2021). As discussed earlier, the composition of phenolic compounds and flavonoids in plants is influenced by various environmental and physiological factors. According to Alves et al. (2017), the variation in TPC and TFC values can be attributed to genetic variability and edaphoclimatic conditions, both within and between species. Furthermore, it is important to highlight that the antioxidant properties of plants are directly associated with their phenolic content, as emphasized by Phuyal et al. (2020).

TABLE 3 Phytochemical profile of methanolic extract of *Abies marocana* needles.

Class of phytoconstituents	Extract
Polyphenols	+
Flavonoids	+
Tannins	+
Anthocyanins	–
Alkaloids	–
Terpenes	+
Sterols	+
Quinones	–
Reducing compounds	+
Proteins	+
Coumarins	–
Cardiac glycosides	–

+, Present; –, absent.

Total phytosterol content

Phytosterols possess qualities that are associated with the reduction of cholesterol levels, specifically triglycerides and low-density lipoprotein cholesterol. They have the ability to decrease cholesterol levels in human subjects by up to 15% and may also have a role in the prevention of cancer (Borah and Banik, 2020). The current study provides evidence that the total phytosterol content in the needles is present in a relatively high concentration (37.816 ± 0.961 mg g^{-1} DW) (Table 2). Wajs-Bonikowska et al. (2013) discovered that the average amount of phytosterols in the seed of *Abies koreana* is 414 ± 95.6 µg 100 g^{-1}, with the primary phytosterols being ergosta-8,24(28)-dien-3-ol and β-sitosterol, which were found at similar levels: 153 and 151 µg 100 g^{-1}, respectively. Phytosterols are utilized in the development of functional foods to enhance their cholesterol-lowering capabilities (Shahar et al., 2023). The needles of *A. marocana* can be regarded as a potential resource.

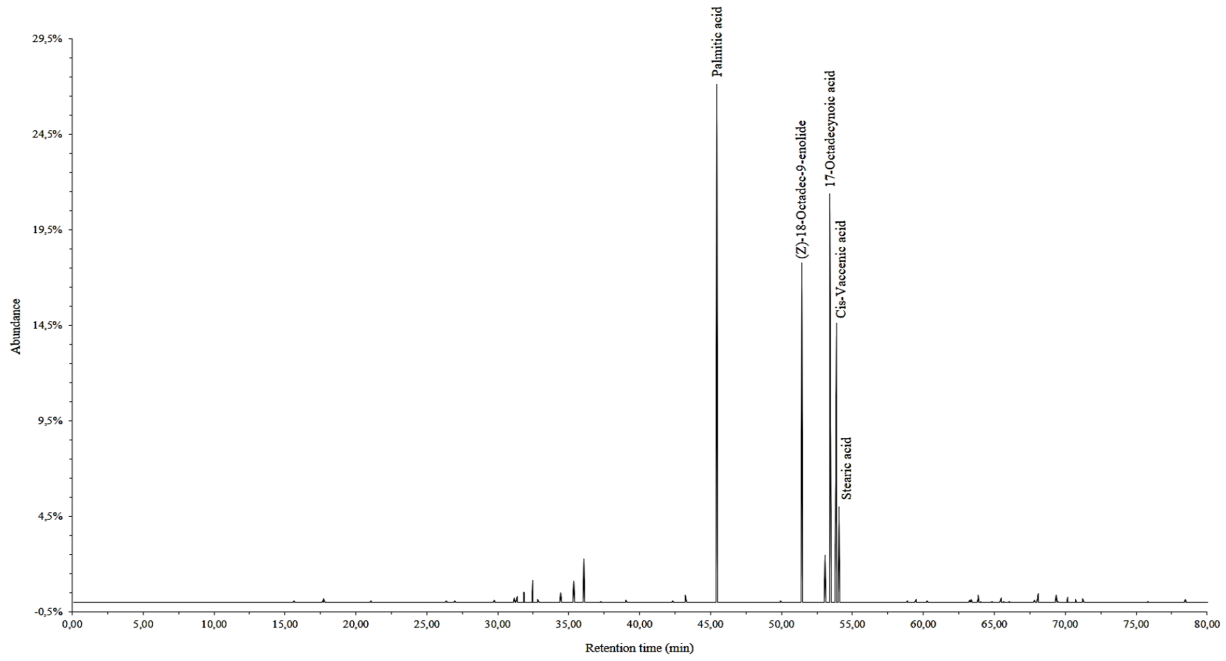

FIGURE 3
GC–MS chromatogram of methanolic extract of *A. marocana* needles.

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The GC–MS analysis was utilized to characterize the volatile compounds in the methanolic extract of the needles. A total of 37 compounds were identified (Figure 3). Table 4 provides the molecular formula (MF), molecular weight (MW), retention time (RT), and area percentage composition (quantity) of the bioactive compounds. The results indicate that the primary compounds in the extract are palmitic acid (27.127%), 17-Octadecynoic acid (21.375%), (Z)-18-Octadec-9-enolide (17.753%), cis-Vaccenic acid (14.629%), and stearic acid (5.013%). Fatty acids are widely recognized as necessary constituents for the constitution of cells, tissues, and organs, in addition to their involvement in the formation of biologically active compounds. It has been proposed by certain scholars that omega-3 fatty acids might exert a substantial influence on the deterrence of diverse forms of cancer (Fabian et al., 2015). The most abundant fractions of this extract are fatty acids and their derivatives (51.32%) and phenolic compounds (43.35%). Additionally, phytosterols, namely Cholesta-3,5-diene and Stigmasta-3,5-diene, are present in considerable quantities (0.469 and 0.400% respectively). These findings align with those of Rajalakshmi et al. (2016), who previously identified 29 compounds in the methanolic extract of *Abies webbiana* needles, with benzenepropanol, 4-hydroxy-methyl, 2-furancarboxaldehyde, and 5-hydroxymethyl furfural as the predominant components. Overall, the methanolic extract of *A. marocana* needles contains a range of compounds such as ketones, alcohols, saturated and unsaturated fatty acids, phenols, as well as glycerides and phytosterols.

TABLE 4 Phytochemicals identified in the methanolic extract of *A. marocana* needles by GC–MS.

RT	Peak area %	MF	MW	Name of compound
17.694	0.109	$C_6H_6O_3$	126	5-hydroxymethylfurfural
21.065	0.065	$C_{10}H_{12}O_2$	164	Eugenol
24.915	0.016	$C_{10}H_{18}O_2$	170	Lilac alcohol
26.349	0.080	$C_8H_{10}O_3$	154	Pyrogallol dimethyl ether
26.990	0.075	$C_{10}H_{14}O_2$	166	P-cresyl methyl ether
29.775	0.099	$C_9H_{10}O_3$	166	Acetovanillone
31.173	0.223	$C_{10}H_{12}O_2$	164	Rheosmin
31.385	0.324	$C_6H_6O_2$	110	Hydroquinone
31.810	0.552	$C_{10}H_{12}O_3$	180	Dihydroconiferyl aldehyde
31.879	0.449	$C_{10}H_{12}O_2$	164	Frambinone
32.461	1.162	$C_7H_8O_3$	140	P-Methoxycatechol
32.802	0.152	$C_6H_{12}O_6$	180	Inositol
34.419	0.496	$C_{10}H_{14}O_3$	182	Dihydroconiferol
35.301	0.200	$C_{20}H_{36}O_2$	308	11,14-Eicosadienoic acid
35.351	1.106	$C_{11}H_{16}O_2$	180	2-(tert-Butyl)-4-methoxyphenol
36.059	2.257	$C_9H_{10}O_3$	166	2-Hydroxy-6-methoxyacetophenone
37.287	0.031	$C_{16}H_{30}O_2$	254	Cyclopentaneundecanoic acid
39.007	0.126	$C_{14}H_{28}O_2$	228	Myristic acid
42.327	0.057	$C_{15}H_{30}O_2$	242	Pentadecylic acid
43.212	0.368	$C_{19}H_{38}O$	282	2-nonadecanone
45.452	27.127	$C_{16}H_{32}O_2$	256	Palmitic acid
49.925	0.076	$C_{21}H_{36}O_2$	320	8,11,14-Eicosatrienoic acid
51.445	17.753	$C_{18}H_{32}O_2$	280	(Z)-18-Octadec-9-enolide
53.065	2.450	$C_{18}H_{32}O_2$	280	Linoleic acid
53.405	21.375	$C_{18}H_{32}O_2$	280	17-Octadecynoic acid
53.899	14.629	$C_{18}H_{34}O_2$	282	Cis-Vaccenic acid
54.071	5.013	$C_{18}H_{36}O_2$	284	Stearic acid
63.882	0.373	$C_{21}H_{30}O_2$	314	Methyl dehydroabietate
65.481	0.235	$C_{27}H_{46}O$	386	Cholest-5-en-3-ol
67.826	0.111	$C_{15}H_{30}O_3$	258	2R-hydroxy-pentadecanoic acid
68.092	0.469	$C_{27}H_{44}$	368	Cholesta-3,5-diene
69.384	0.400	$C_{29}H_{48}$	396	Stigmasta-3,5-diene
70.176	0.257	$C_{28}H_{48}O$	400	Dihydrobrassicasterol-H_2O
70.705	0.170	$C_{29}H_{46}$	394	(22E)-4,4-Dimethylcholesta-6,22,24-triene
71.214	0.174	$C_{31}H_{52}O_2$	456	Clionasterol-H_2O
75.811	0.031	$C_{31}H_{62}O$	450	16-hentriacontanone
78.445	0.149	$C_{29}H_{48}O$	412	γ-Sitostenone
–	1.261	–	–	Not identified

Evaluation of antioxidant activities

Total antioxidant capacity

The fundamental principle of this technique is based on the reduction of molybdenum (IV) to molybdenum (V) by the botanical sample, resulting in the creation of a phosphate/molybdenum (V) complex under acidic conditions. This complex displays a distinct green coloration (Moonmun et al., 2017). Our investigation revealed that the extract derived from *A. marocana* needles possessed an antioxidant capacity of 122.670 ± 2.203 mg EAA g^{-1} DW (Table 2). This finding implies that the antioxidant potential of the needles may be attributed to the presence of phenolics and flavonoids in the methanolic extract of the plant (Mbinda and Musangi, 2019).

Determination of the antiradical activity by the DPPH assay

DPPH acts as the source of liberated radicals, characterized by the possession of an unpaired electron, which is responsible for the absorption at wavelengths ranging from 515 to 517 nm and also enhances the manifestation of a distinctively visible deep purple color (Hasan et al., 2009). When the sample extract is introduced into the DPPH solution, the purple color of the DPPH solution fades due to the existence of electron-donating substances or hydrogen-donating substances, such as phenolic compounds, within the extract. In the context of this investigation, the methanolic extract obtained from the needles of *A. marocana* demonstrated a higher level of radical scavenging activity, quantified as $IC_{50} = 82.594 \pm 2.450\ \mu g\ mL^{-1}$ (Table 2). According to a study conducted by Albanese et al. (2019), the DPPH activity of *A. alba needles* in an aqueous extract was reported to be $10.1 \pm 0.4\ \mu g\ mL^{-1}$, indicating a higher potential for antioxidation compared to the current study. The variation in antioxidant activity can be attributed to differences in the extraction methods used, the geographical locations, and the timing of the harvesting process (Palit and Mandal, 2021). The high concentration

TABLE 5 Correlation coefficient between phytochemicals and antioxidant assays.

	DPPH	TAC	Polyphenol	Flavonoid	Tannin	Phytosterol
DPPH	1					
TAC	−0.942	1				
Polyphenol	0.991	−0.979	1			
Flavonoid	−0.517	0.774	−0.628	1		
Tannin	−0.876	0.987	−0.933	0.866	1	
Phytosterol	−0.856	0.633	−0.778	0.00	0.500	1

of antioxidants and bioactive components in the needles of *Abies marocana*, along with their similar characteristics to other *Abies* species, make them a viable addition to sustainable diets. Notably, the bioactive properties of White fir needles (*Abies alba* Mill.) have garnered significant attention. To enhance the quality of whole wheat bread, researchers utilized the aqueous extract of *Abies alba* Mill., obtained via a controlled hydrodynamic cavitation (HC) process (Parenti et al., 2022).

Correlation analysis

A correlation analysis by SPSS version 27 (IBM Corp., Armonk, New York) was conducted on the chemical composition and antioxidant activity of *A. marocana* needles in order to examine their interaction (Table 5). The analysis focused on phenol, flavonoid, tannin, phytosterol, DPPH, and TAC, and the results revealed remarkably strong positive correlations between phenol and DPPH (0.991) as well as negative correlations between phenol and TAC (−0.979). These findings suggest that the presence of phenol in the plant is closely associated with potent antioxidant activity. Interestingly, no correlation was found between flavonoid and phytosterol. Additionally, a robust correlation of 0.987 was established between tannin and TAC, while a substantial negative correlation of −0.942 was observed between DPPH and TAC.

Conclusion

In the current investigation, a comprehensive and rigorous analysis was conducted to examine the antioxidative activity, nutritional composition, and bioactive compounds present in the needles of *A. marocana*. It was uncovered that the needles harbor substantial quantities of proximate composition and mineral contents, thereby indicating their potential significance in terms of nutrition. The phytochemical analysis revealed the existence of numerous secondary metabolites possessing medicinal properties. Moreover, the methanolic extract of the needles exhibited favorable characteristics as an antioxidant. Notably, the presence of chlorophyll, carotenoids, phenols, and vitamin A in the examined plant highlights their potential as viable sources of nutrients and bioactive compounds for human nourishment. Therefore, based on these results, it can be deduced that the needles of *A. marocana* can constitute beneficial sources of nutrition and antioxidants, thus playing a crucial role in solving malnutrition problems and mitigating various diseases due to human deficiencies, making them a valuable element of sustainable diets. Nevertheless, additional research is imperative to evaluate the safety, effectiveness, bioavailability, and interactions of the compounds found in the needles.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MZ: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. MA: Formal analysis, Methodology, Writing – review & editing. HI: Conceptualization, Data curation, Formal analysis, Resources, Writing – review & editing. DH: Formal analysis, Writing – review & editing. ON: Resources, Writing – review & editing. NM: Formal analysis, Supervision, Writing – review & editing. MT: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by the King Saud University, Researchers Supporting Project number (RSPD2024R1087), King Saud University, Riyadh, Saudi Arabia.

Acknowledgments

The authors extend their appreciation to the King Saud University, Researchers Supporting Project number (RSPD2024R1087), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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