International Journal of Secondary Metabolite
2024, Vol. 11, No. 1, 121–133
https://doi.org/10.21448/ijsm.1372709
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Antioxidant activity, phytochemical screening and GC-MS profile of *Abies marocana* Trab.

Malak Zirari[1,*], **Marouane Aouji**[2], **Meryem Zouarhi**[1], **Ahmed Dermaj**[1], **Hamid Erramli**[1], **Driss Hmouni**[2], **Nouredine El Mejdoub**[1]

[1]Laboratory of Organic Chemistry Catalysis and Environment, Department of Chemistry, Faculty of Sciences, Ibn Tofail University Kénitra, BP 133, Kenitra 14000, Morocco
[2]Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, BP 133, Kenitra 14000, Morocco

ARTICLE HISTORY

Received: Oct. 07, 2023
Accepted: Dec. 19, 2023

KEYWORDS

Abies marocana,
Biochemical characterization,
Antioxidant activity,
Terpenoid,
Phenol.

Abstract: The aim of this research was to explore the chemical composition and antioxidant activities of etheric extracts of *Abies marocana*. A Soxhlet apparatus was used to extract bioactive molecules from the various parts of the plant. Furthermore, the levels of antioxidant compounds were quantified, while the Gas chromatography was utilized to determine the chemical constituents of the extracted molecules. The extracts were evaluated for their antioxidant properties using the DPPH radical scavenging method and the total antioxidant capacity test. The levels of polyphenols varied across different parts of the plant, ranging from 2.474 ± 0.029 mg.g^{-1} DM in needles to 4.207 ± 0.008 mg.g^{-1} DM in twigs. Flavonoids were most abundant in needles 0.140 ± 0.001 mg.g^{-1} DM and least abundant in cones 0.069 ± 0.007 mg.g^{-1} DM. Tannins had the highest concentration in twigs 2.608 ± 0.114 mg.g^{-1} DM, followed by cones 1.948 ± 0.037 mg.g^{-1} DM and needles 1.512 ± 0.09 mg.g^{-1} DM. A chromatographic analysis revealed that 56 components were in the samples, with terpene compounds being the most abundant in the different organs. In terms of antioxidant activity, the extract derived from twigs exhibited the strongest antioxidant capacity 49.377 ± 0.371 mg EAA.g^{-1} DM, followed by cones 35.129 ± 0.084 mg EAA.g^{-1} DM and needles 13.663 ± 0.084 mg EAA.g^{-1} DM. Alternatively, the IC_{50} values for the three organs were found to be in the range of 3844 to 5047.67 µg.mL^{-1}. The results highlight the potential phytopharmaceutical value of *A. marocana* due to the presence of diverse phyto-components.

1. INTRODUCTION

The word "fir" pertains to a type of coniferous plant belonging to the Pinaceae family, specifically the genus *Abies*. Firs are easily distinguishable from other members of the Pinaceae family based on their botanical features such as their shape, bark, needles, and fragrance. In general, firs tend to grow in areas with partial shade, and they grow best in damp, nutrient-rich forest soils. They can also do well in moist sandy or clay soils, but they don't thrive in soils that are high in limestone (Mokaddem-Daroui *et al*., 2021). The genus *Abies* "fir" (Pinaceae) includes 51 species, with the majority of them distributed in the Northern Hemisphere's

*CONTACT: Malak Zirari malak.zirari@uit.ac.ma Ibn Tofail University, Faculty of Sciences, Department of chemistry, Kenitra, Morocco

e-ISSN: 2148-6905 / © IJSM 2024

temperate and boreal zones, especially in mountainous areas such as North Africa, the Himalayas, and Türkiye (Farjon *et al*., 1989; Zheng *et al*., 1978).

Abies marocana is an indigenous tree species found in the Rif area at elevations ranging from 1400 to 2000 meters. It contributes significantly to the creation of magnificent forest ecosystems, particularly at the summit of Moroccan city Chefchaouen (Uçar & Uçar, 2014; Flora of China, 1978). *A. marocana* can be located in the bioclimatic zone of the Mediterranean that is characterized by its high levels of humidity. This particular species thrives in an environment where the average annual rainfall reaches 1500 mm, and as one ascends to an altitude of 1700 mm, the precipitation increases to 1900 mm. The stands of *A. marocana* are predominantly observed on the slopes of mountain ridges that face north and are composed of dolomitic limestone. These specific geological conditions provide an ideal habitat for the growth and development of *A. marocana* (Benabid, 1983).

Previous studies have identified various bioactive compounds, including lignans, flavonoids (Tiwari *et al*., 1980), sesquiterpenoids (Xia *et al*., 2012), triterpenoids, and tetraterpenoids (Lavoie *et al*., 2013), from *Abies* species that possess diverse biological activities (Yang *et al*., 2008; Baydar, 2006; Raldugin & Shevtsov, 1990). They have potential as active pharmaceutical ingredients in the treatment of various diseases (Gupta & Kumar, 2017; Li *et al*., 2015). The essential oils that are acquired from the seeds of this particular species are widely utilized in the practice of traditional Moroccan medicine as a means of effectively addressing and alleviating various respiratory ailments (Hmamouchi, 1999). Furthermore, it has been recently revealed that the oil derived from this species contains a significant and notable concentration of limonene, which has garnered substantial interest and intrigue from the perfume industry (Bazdi *et al*., 2006). The concentration of phytochemicals found in plants can differ based on factors such as species, age, climate, and environmental conditions. To extract these compounds, a variety of solvent systems can be utilized (Gupta & Kumar, 2017). Recently, there has been an increasing interest in natural antioxidants derived from plants, as they are believed to have the potential to improve health and prevent illnesses. These plant-based antioxidants are widely accepted by consumers and are generally considered safe for consumption (Gorinstein *et al*., 2003).

As far as we know, there haven't been any studies conducted on the physiological impacts of *Abies marocana.* Hence, this research aims to investigate the antioxidant capacity of etheric extracts derived from the aerial parts of *A. marocana* and to identify and quantify the secondary metabolites present in each organ. The study seeks to shed light on the potential health benefits and pharmaceutical applications of this plant by exploring its antioxidative properties and characterizing its chemical composition.

2. MATERIAL and METHODS

2.1. Plant Collection and Authentication

The aerial parts of *Abies marocana* were harvested in Chefchaouen, a city located in the northern region of Morocco. The harvesting took place in June at an altitude of 1785 meters on Chouihate mountain (35°11'05.6" N 5°13'47.9" W). The plant was identified at the Scientific Research Center in Rabat. To prevent the samples from deteriorating during storage, they were dried in the laboratory for three days at a temperature of 45 ± 2 °C. Once dried, they were ground using a mill and stored in opaque glass containers until needed.

2.2. Extraction Method

The technique utilized was based on the process detailed by Kuluvar *et al*. (2009), with a few adjustments. Each section of *Abies marocana*, weighing 25 g, was positioned in the Soxhlet apparatus and subjected to extraction using 250 mL of petroleum ether, with a temperature of 40 °C maintained during the 8-hour process.

Following extraction, the filtered substances were concentrated at 35 °C, and the resulting extracts were dried and stored in a refrigerator at 4 °C until they were ready for analysis. In addition, the yield of the crude extracts was calculated using the method described in (NM ISO 734: 2020), Eq. (1).

$$w = \frac{m_1}{m_0} x100\% \quad (1)$$

Where, m_o is the mass of the sample powder and m_1 is the mass of the extract.

2.3. Phytochemical Screening

Phytochemical screening was performed to identify some major groups of secondary metabolites contained in our extracts and 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 triterpenes (Trease & Evans, 1989).

2.4. Determination of The Amount of Antioxidant Compounds

2.4.1. *Determination of total phenolic content (TPC)*

To determine the total polyphenol levels of the extracts, the Folin-Ciocalteu method (Cheok *et al*., 2013) was utilized. Initially, 0.5 mL of each extract was combined with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 in methanol) in a test tube. After 5 minutes at room temperature, 1 mL of a 7.5% aqueous sodium carbonate solution was added, and the mixture was thoroughly mixed and kept in the dark for 30 minutes. The resulting solution's absorbance at 765 nm was measured against a blank using a spectrophotometer. Additionally, a calibration curve was produced using Gallic acid as a standard under comparable conditions (Hatami *et al*., 2014).

2.4.2. *Determination of total flavonoid Content (TFC)*

The conventional method for measuring flavonoids, as described by Lamaison and Carnat, (1990), was used. To each sample, 1 mL (appropriately diluted) was added to 1 mL of $AlCl_3$ solution (2% in methanol), and the resulting mixture was left to process for 10 minutes. The blanks were prepared by substituting the extract with methanol. Afterward, the absorbance was measured at 430 nm and used to determine the flavonoid concentration using quercetin as a reference.

2.4.3. *Determination of total tannin content (TTC)*

The Vanillic acid method (Hagerma, 2002) was employed to determine the tannin content in the sample. In this process, 0.5 mL of the extract was mixed with 2.5 mL of Vanillin reagent, which is a combination of an equivalent mixture of 8% HCl in methanol. Blanks were also created by substituting the reagents with a 4% acid-methanol mixture. The test tubes were incubated at 30 °C for 20 minutes before the absorbance at 500 nm was measured. Catechin was utilized as a reference standard for comparison.

2.5. Estimation of Chemical Constituents by GC-MS

Individual extracts were characterized using an MS workstation system equipped with a BR-5ns FS capillary column (60 m x 0.32 mm ID x 0.25 µm) coupled to mass spectrometry. The carrier gas used was helium with a high purity at a rate of 1.7 $mL.min^{-1}$. The injectate temperature was 250 °C and the oven temperature was initially 40 °C and gradually elevated to 260 °C at a rate of 8 $°C.min^{-1}$. A syringe was used to withdraw extracts, which were then injected into the injector at a 40:1 split ratio. Full-scan mass spectra ranging from 40-550 AMU were collected to generate all of them. The temperature of the ion source was adjusted to 230 °C, while the quadrupole was maintained at a temperature of 150 °C. The voltage of the electron multiplier was maintained at 1100 V above self-tuning, with a solvent delay of 3 min.

The identification and characterization of these compounds in different crude extracts was based on their gas chromatographic retention times. Mass spectra were compared with Mass Spectral Library (NIST) standards. Results were expressed as percent of peak area.

2.6. Antioxidant Activity

2.6.1. *Total antioxidant capacity (TAC)*

The extract's total antioxidant capacity (TAC) was measured using the method described by Aouji *et al*. (2023). A 0.1 mL sample of extracts was combined with 1 mL of the reagent solution in a tube (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were closed and maintained for 90 minutes in a water bath at 95 °C. The absorbance of the solutions was measured at 695 nm in comparison to a blank. The contents are expressed as milligrams of ascorbic acid equivalent per gram of dry matter (mg EAA.g^{-1} DM).

2.6.2. *DPPH free radical scavenging activity*

Because of its stable free radical form and ease of analysis, DPPH (2,2-diphenyl-1-picrylhydrazyl) is the most commonly utilized substrate for evaluating antioxidant activity in a quick and direct way (McCune & johns, 2002). Subhashini *et al*. (2011) described the experimental methods utilized to investigate DPPH's radical scavenging capability.

Different quantities of the plant's etheric extracts (1 mL) were combined with 1 mL of a solution containing DPPH radicals, with the final DPPH concentration being 0.025 g.L^{-1}. After being vigorously stirred, the mixture was left undisturbed for 30 minutes before its absorbance was measured at 517 nm. Ascorbic acid was employed as the reference. The experiment was carried out three times. The percentage inhibition of DPPH radical activity was determined using Eq. (2) (McCune & johns, 2002) as shown below:

$$PI\% = \frac{A_0 - A_1}{A_0} x100 \quad (2)$$

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

2.7. Statistical Analysis

To determine the statistical significance of the findings, a one-factor analysis of variance (ANOVA) test was conducted, followed by a Tukey-test at $\alpha = 5\%$. The results were reported as mean ± standard deviation, and the significance level was defined accordingly.

3. RESULTS and DISCUSSION

3.1. The Yield of The Extracts

The acquired findings are shown in Table 1. The comparing of the yields of *A. marocana* revealed that the extraction yield for the twig and needle extracts varied from 4.212 ± 0.001% to 5.736 ± 0.001%, respectively. The cone extract, on the other hand, yielded an intermediate yield of 5.013 ± 0.001%. These results outperform those achieved by Natalia *et al*. (2020) for *Picea Abies*. The extraction yield increases with sample particle size, extraction temperature, and solvent-to-sample extraction ratio (Herode *et al*., 2023).

Table 1. Amount and percentage yield of etheric extracts from *A. marocana*.

Samples	Amount (g)	Yield (%)
Twig	1.053 ± 0.032	4.212 ± 0.001 [a]
Needle	1.434 ± 0.022	5.736 ± 0.001 [b]
Cone	1.296 ± 0.027	5.013 ± 0.001 [c]

The significant difference ($p < 0.05$) is illustrated by the letters a, b, and c.

3.2. Phytochemical Screening

The outcomes of the phytochemical screening are displayed in Table 2. The results revealed that this plant includes phenolic chemicals, terpenes, essential oils, and sterols in various organs. The needles and cones contain more flavonoids than the twigs. Free tannins, on the other side, are more abundant in the cones compared to the other aerial parts. These findings are comparable to those of Rajalakshmi *et al*. (2016), who discovered steroids, phenols, flavonoids, tannins, and saponins in *A. webbiana*. Reducing compounds, proteins, and cardiac glycosides, on the contrary, are found in modest concentrations in all parts. While alkaloids and anthocyanins are absent in the different parts of *Abies marocana*.

Table 2. Identification of bioactive compounds in etheric extracts from *A. marocana.*

Compounds group	Twig	Needle	Cone
Polyphenols	+++	+++	+++
Flavonoids	++	+++	+++
Free tannins	++	++	+++
Sterols and terpenes	+++	+++	+++
Alkaloids	-	-	-
Essential oils	+++	+++	+++
Reducing compounds	+	+	+
Proteins	+	+	+
Cardiac glycosides	++	++	++
Anthocyanins	-	-	-

(-) Absent; (+) Present; (++) Abundant; (+++) So abundant

3.3. Determination of The Amount of Antioxidant Compounds

In accordance with Nunes *et al*. (2012), it is possible to predict the antioxidant capacity of a matrix by determining the total concentration of polyphenols. The results of the analyses conducted are presented in Table 3. The total phenolic content (TPC) of *A. marocana*'s etheric extracts was determined in terms of Gallic acid equivalent. The TPC was found to differ significantly, ranging from 2.475 ± 0.029 mg GAE.g^{-1} DM in the needle extract to 4.207 ± 0.008 mg GAE.g^{-1} DM in the twig extract. The cone extract had an estimated intermediate concentration of 3.661 ± 0.032 mg GAE.g^{-1} DM.

Table 3. Total phenolic, flavonoid and tannin content of *A. marocana.*

Samples	Twig	Needle	Cone
TPC (mg GAE.g^{-1} DM)	4.207 ± 0.008 ^{a}	2.475 ± 0.029 ^{b}	3.661 ± 0.032 ^{c}
TFC (mg EQ.g^{-1} DM)	0.088 ± 0.003 ^{a}	0.140 ± 0.001 ^{b}	0.068 ± 0.007 ^{c}
TTC (mg EC.g^{-1}DM)	2.608 ± 0.114 ^{a}	1.511 ± 0.094 ^{b}	1.948 ± 0.037 ^{c}

The significant difference ($p < 0.05$) is illustrated by the letters a, b, and c

The total flavonoid content (TFC) was measured in terms of Quercetin equivalent. The TFC varied from 0.068 ± 0.007 to 0.140 ± 0.001 mg EQ.g^{-1} DM. The flavonoid content found in needles is consistent with their protective role against the harmful effects of solar radiation and the prolonged exposure that they receive (Gehin *et al*., 2006). The results revealed a significant variation in the TTC, with the twig extract containing the highest amount 2.608 ± 0.114 mg EC. g-1 DM, followed by the cone 1.948 ± 0.037 mg EC.g^{-1} DM and needle extracts having lower

content 1.511 ± 0.094 mg EC.g^{-1} DM. These results are similar to those obtained for *Picea Abies* (TPC = 3.21 mg GAE.g^{-1}, TFC = 0.62 mg EQ.g^{-1}, and TTC = 0.84 mg TAE.g^{-1}) (Zeppetzauer *et al*., 2021).

Various factors, including geographical and climatic conditions, plant maturity, and shelf life, may significantly influence phenolic compound concentrations (El Hazzat *et al*., 2015; Merouane *et al*., 2014; Bouzid *et al*., 2010).

3.4. Estimation of Chemical Constituents by GC-MS

The chemical components, including their respective retention time (RT), and concentration (%), are outlined in Table 4. 28 components were identified in the etheric extract of twigs representing 92.98% of the total chemical composition. Its main components are (12Z)-abienol (21.35%), Abietic acid (3.90%) and Octacosanol (3.54%). Fatty acids and their derivatives (51.32%) and terpene compounds (43.35%) were the most abundant fraction of this extract. Vitamin A derivatives were also present in considerable quantities, namely retinol and retinol acetate (1.50 and 0.89% respectively).

However, the GC-MS analysis of the needle extract identified six primary components, which were Dotriacontan-1-ol (24.92%), delta-Cadinene (15.47%), caryophyllene (7.48%), D-Limonene (7.01%), Neophytadiene (4.07%), and alpha-pinene (3.70%). Sesquiterpene hydrocarbons made up the majority of the components, accounting for 48.65%. These findings are consistent with those of Alejandro *et al*. (1992), who had previously identified a group of natural diterpenoids, in the hydrocarbon fraction of a hexanoic extract of *A. marocana* needles. The GC-MS analysis also detected Phytol, which has been linked to beneficial properties (Sun *et al*., 2020; Banjare *et al*., 2017). In addition to its antimicrobial properties, squalene also possesses antioxidant characteristics. (Bhattacharya *et al*., 2021; Ugoeze *et al*., 2020).

The GC-MS analysis of cone extract revealed that it contains 26.52% monoterpenes, with the primary compounds being D-Limonene (21.55%), 1-Dotriacontanol (18.97%), Caryophyllene (7.11%), Methyl dehydroabietate (6.79%), Retinol (6.01%), and α-Humulene (3.09%). Wajs *et al*. (2015) investigated the volatile composition of *Abies alba* Mill cone scales and found that α-pinene was the primary constituent of the cone scale oil.

These results suggest that *A. marocana* has potential applications in various pharmaceutical industries.

Table 4. Phytocomponents identified in the etheric extracts of *A. marocana* by GC-MS.

Name of the compounds	Twig		Needle		Cone	
	RT	%	RT	%	RT	%
α-pinene	11.551	14.95	11.220	3.70	11.234	2.00
Tricosane	-	-	12.970	0.12	-	-
(-)-β-Pinene	13.718	5.69	-	-	-	-
Mesitylene	-	-	14.493	2.62	-	-
β-Myrcene	14.525	0.71	-	-	-	-
3-Carene	-	-	15.197	2.55	15.209	0.90
D-Limonene	16.561	15.05	16.268	7.01	16.583	21.55
Verbenol	-	-	-	-	19.755	1.22
α-Copaene	-	-	28.505	1.35	28.530	1.09
Behenic acid	-	-	-	-	28.774	0.36
(-)-β-Copaene	-	-	-	-	29.436	0.65
caryophyllene	30.810	0.60	30.857	7.48	30.952	7.11
(-)-Germacrene D	32.752	0.56	31.137	1.41	31.170	1.95
α-Humulene	-	-	31.950	3.35	32.020	3.09
cis-Muurola-4(15),5-diene	-	-	33.061	1.77	32.186	0.26
γ-Muurolene	-	-	32.602	1.04	32.651	1.16

Table 4. *Continues*.

α-Muurolene	-	-	33.344	2.51	33.372	1.12
δ-Cadinene	-	-	34.026	15.47	-	-
Cubenene	-	-	-	-	34.380	0.48
D-Guaiene	-	-	35.892	0.48	-	-
α-Selinene	-	-	-	-	36.985	1.07
τ-Muurolol	-	-	37.588	5.70	-	-
τ-Cadinol	-	-	-	-	37.577	2.26
α-Cadinol	-	-	37.918	2.97	37.955	1.40
Ylangenol	-	-	38.484	0.34	-	-
Neophytadiene	-	-	42.713	4.07	-	-
Phytol	-	-	42.837	0.33	-	-
Bicyclo[9.3.1]pentadeca-4,14 diene	46.232	0.40	-	-	-	-
Palmitic acid	46.949	1.62	45.090	1.85	46.608	2.42
Margaric acid	48.233	0.88	-	-	-	-
(12Z)-abienol	50.373	18.71	-	-	-	-
Oleic acid	50.917	6.04	51.020	0.99	51.193	2.57
Stearic acid	52.282	0.99	52.906	0.96	51.492	1.81
Methyl dehydroabietate	53.927	5.25	-	-	52.603	6.79
Methyl abietate	54.927	2.25	-	-	-	-
Squalene	55.968	0.71	52.422	1.36	-	-
Linoleic acid	-	-	51.447	0.65	-	-
dehydroabietic ester	-	-	55.570	1.76	-	-
Abietic acid	57.076	3.41	-	-	57.094	8.38
Tridecenoic acid	57.319	0.20	-	-	-	-
Retinol	57.692	1.32	-	-	58.022	6.01
7-oxo-dehydroabietic acid, methyl ester	58.378	0.95	-	-	-	-
Retinol, acetate	58.552	0.78	-	-	58.709	0.14
Tetracosamethyl-cyclododecasiloxane	59.054	1.07	-	-	-	-
Eicosanal	-	-	59.451	0.64	-	-
1-Heptacosanol	60.708	1.59	-	-	-	-
1,4-Benzenedicarboxylic acid dimethyl ester	61.347	0.81	-	-	-	-
cis-9-Octadecenal	62.638	3.10	66.143	1.81	-	-
Dotriacontan-1-ol	-	-	69.820	24.92	69.534	18.97
Not identified	**-**	**12.36**	**-**	**0.79**	**-**	**5.24**

3.5. Total Antioxidant Capacity

The total antioxidant capacities were expressed as mg ascorbic acid equivalent per g of dry extract (mg EAA.g^{-1} DM). Figure 1 shows that the total antioxidant capacity of plant extracts varies significantly depending on the plant part used. The extracts obtained from twigs showed a higher antioxidant capacity (49.377 ± 0.371 mg EAA.g^{-1} DM) compared to cones and needles (35.129 ± 0.084 and 13.663 ± 0.084 mg EAA.g^{-1} DM, respectively). Thus, the high antioxidant capacity of *Abies marocana* extracts suggests the abundant presence of bioactive antioxidant compounds. Several studies have suggested that flavonoid and polyphenolic compounds are the primary contributors to the phosphomolybdate scavenging activity of medicinal plants (Oueslati *et al*., 2012; Khan *et al*., 2012; Sharififar *et al*., 2009; Negro *et al*., 2003).

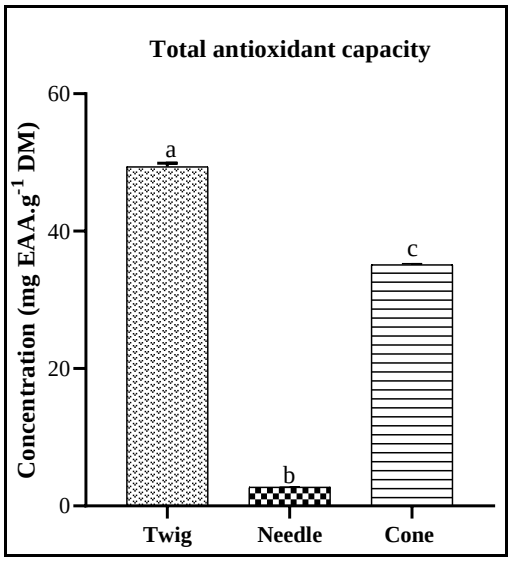

Figure 1. Variation in total antioxidant capacity in etheric extracts of *A. marocana*. The significant difference ($p < 0.05$) is illustrated by the letters a, b, and c.

3.6. DPPH Free Radical Scavenging Activity

Because of its ease and speed, the DPPH free radical scavenging activity is the preferred method for evaluating the antioxidant potential of natural and synthetic compounds (Angeli *et al*., 2021). Figure 2 illustrates the results obtained from the DPPH assay of the different extracts and ascorbic acid.

Extracts that were analyzed were compared based on their ability to scavenge DPPH free radicals, using the IC_{50} values, which serve as a quantifiable measure to determine the concentration level necessary to inhibit the activity of 50% of the free radicals. These free radicals, in themselves, serve as a reliable and trustworthy indicator of the overall effectiveness and efficiency of the antioxidants present within the extracts being evaluated.

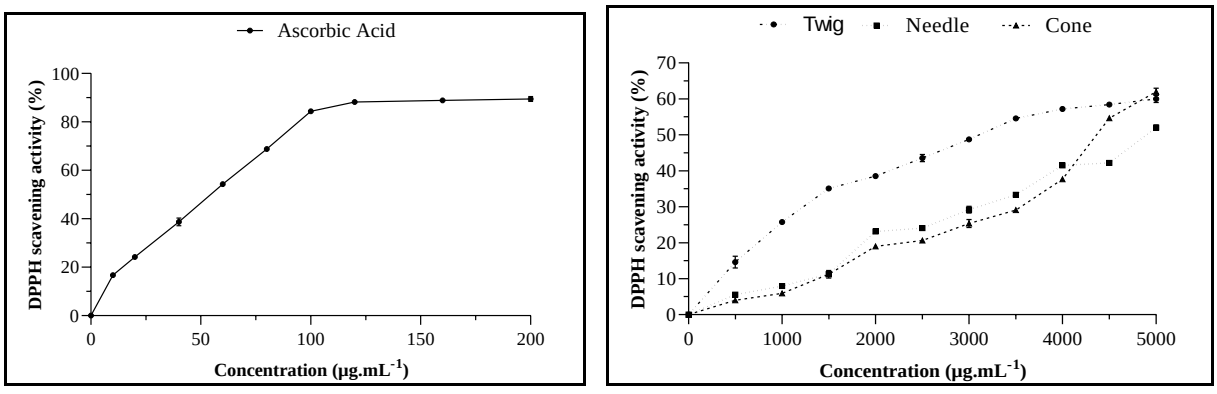

Figure 2. DPPH free radical scavenging activity of etheric extracts of *Abies marocana* and ascorbic acid.

Both ascorbic acid and the extracts were evaluated against each other, and it was found that the twig and cone extracts (3844 ± 55.95 and 4496 ± 31.00 µg. mL^{-1} respectively) had higher anti-radical activity than the needle (5047.67 ± 83.69 µg.mL^{-1}). Ascorbic acid, with the lowest IC_{50} value of 41.86 ± 0.49 µg.mL^{-1}, demonstrated the most significant free radical scavenging activity compared to the other extracts. This implies that the extracts derived from *A. marocana* possess remarkable capabilities in safeguarding cells from the detrimental effects caused by free radicals. This inhibition of DPPH activity may be due to the transfer of electrons from the

plant's phytoconstituents (Chukwuma *et al*., 2020). Therefore, the extracts' high DPPH scavenging activity could be attributed to the transfer of electrons from the polyphenols' various phenolic rings present in the extracts (Batool *et al*., 2019; Ishola *et al*., 2018).

Several studies have established that DPPH reduction is closely associated with the amount of phenolic compounds present (Boadi *et al*., 2021; Aryal *et al*., 2019; Sethi *et al*., 2020). This finding indicates the antioxidant ability of the three extracts.

4. CONCLUSION

In this study, *Abies marocana* was investigated, and its three parts were analyzed for bioactive compounds using phytochemical and GC-MS techniques. Terpenoids, polyphenols, fatty acids, and vitamin A derivatives were among the identified compounds and could be studied for their anticancer, anti-inflammatory and other potential therapeutic properties.

The highest antioxidant activity was found in the etheric extract of the twigs, which was attributed to the presence of retinol, phenolic compounds, and unsaturated fatty acids. The findings indicate that *Abies marocana* is rich in biologically active substances and should be recognized as a significant plant for phytopharmaceutical purposes.

This finding deserves to be elucidated by further in-depth studies on clinical trials to investigate the efficacy and safety of *Abies marocana* extracts in the treatment of specific diseases, and to develop new methods for extracting and purifying bioactive compounds from *Abies marocana*.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Malak Zirari: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. **Marouane Aouji**: Methodology, Analysis and interpretation. **Meryem Zouarhi**: Design, Visualization. **Ahmed Dermaj**: Materials, Resources. **Hamid Erramli**: Materials, Resources. **Driss Hmouni**: Methodology, Supervision, and Validation. **Nouredine El Mejdoub**: visualization, editing the original draft.

Orcid

Malak Zirari https://orcid.org/0009-0006-8685-4796
Marouane Aouji https://orcid.org/0000-0002-5884-0016
Meryem Zouarhi https://orcid.org/0000-0002-2123-0476
Ahmed Dermaj https://orcid.org/0009-0002-9537-8809
Hamid Erramli https://orcid.org/0000-0001-5367-4787
Driss Hmouni https://orcid.org/0000-0001-7598-6204
Nouredine El Mejdoub https://orcid.org/0000-0003-1225-5900

REFERENCES

Angeli, L., Imperiale, S., Ding, Y., Scampicchio, M., & Morozova, K. (2021). A Novel Stoichio- Kinetic Model for the DPPH Assay: The Importance of the Side Reaction and Application to Complex Mixtures. *Antioxidants*, *10*(07), 1019-1031. https://doi.org/10.3390/antiox10071019

Aouji, M., Imtara, H., Rkhaila, A., Bouhaddioui, B., Alahdab, A., Parvez, M. K., Saleh Alzahrani, M., Aicha Lrhorfi, L., & Bengueddour, R. (2023). Nutritional Composition, Fatty Acids Profile, Mineral Content, Antioxidant Activity and Acute Toxicity of the Flesh of *Helix aspersa* Müller. *Molecules*, *28*(17), 6323. https://doi.org/10.3390/molecules28176323

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content, and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, *8*, 96-107. https://doi.org/10.3390/plants8040096

Banjare, J., Salunke, M., Indapurkar, K., Ghate, U., & Bhalerao, S. (2017). Estimation of serum malondialdehyde as a marker of lipid peroxidation in medical students undergoing examination-induced psychological stress. *Journal of the Scientific Society*, *44*, 137-139. https://www.jscisociety.com/text.asp?2017/44/3/137/225497

Barrero, F.A., Sanchez, F.J., Alvarez-Manzaneda, J.E., Muñoz, M., & Haïdour, A. (1992). Diterpenoids and cyclolanostanolides from *Abies marocana*. *Phytochemistry*, *31*(2), 615-620. https://doi.org/10.1016/0031-9422(92)90046-S

Batool, R., Khan, M.R., Sajid, M., Ali, S., & Zahra, Z. (2019). Estimation of phytochemical constituents and in vitro antioxidant potencies of *Brachychiton populneus* (Schott & Endl) R. Br. *BMC Chem.*, *13*, 32-46. https://doi.org/10.1186/s13065-019-0549-z

Baydar, S.N. (2006). Encyclopedia of Medicinal Plants, Palme Publishing, Istanbul, Turkey.

Bazdi, B., Oller-López, J.L., Cuerva, J.M., Oltra, J.E., & Mansour, A.I. (2006). Composition of the Essential Oil from the Seeds of *Abies marocana*. *Journal of Essential Oil Research, 18*(2), 160-161. https://doi.org/10.1080/10412905.2006.9699053

Benabid, A. (1983). Études biogéographique et dynamique des peuplements forestiers du Rif (Maroc).

Bhattacharya, T., Dutta, S., Akter, R., Rahman, M.H., Karthika, C., Nagaswarupa, H.P., Murthy, H.C.A., Fratila, O., Brata, R., & Bungau, S. (2021). Role of Phytonutrients in Nutrigenetics and Nutrigenomics Perspective in Curing Breast Cancer. *Biomolecules*, *11*, 1176. https://doi.org/10.3390/biom11081176

BNS EN ISO 734-1:2020. *Oilseed meals - Determination of oil content* - Part 1: Extraction method with hexane (or light petroleum) (ISO 734-1:2020).

Boadi, N.O., Badu, M., Kortei, N.K., Saah, S.A., Annor, B., Mensah, M.B., Okyere, H., & Fiebor, A. (2021). Nutritional composition and antioxidant properties of three varieties of carrot (*Daucus carota*). *Scientific African*, *12*, e00801. https://doi.org/10.1016/j.sciaf.2021.e00801

Bouzid, W., Yahia, M., Abdeddaim, M., Aberkane, M.C., & Ayachi, A. (2010). Evaluation of the antioxidant and antimicrobial activity of extracts from hawthorn monogyne. *Lebanese Science Journal*, *12*(1), 59-69.

Bukhanko, N., Attard, T., Arshadi, M., Eriksson, D., Budarin, V., Hunt, A.J., Geladi, P., Bergsten, U., & Clark, J. (2020). Extraction of cones, branches, needles and bark from Norway spruce (*Picea abies*) by supercritical carbon dioxide and Soxhlet extractions techniques. *Industrial Crops and Products*, *145*, 112096. https://doi.org/10.1016/j.indcrop.2020.112096

Cheok, C., Chin, N., Yusof, Y., Talib, R., & Law, C. (2013). Optimization of total monomeric anthocyanin (TMA) and total phenolic content (TPC) extractions from mangosteen (*Garcinia mangostana* Linn.) hull using ultrasonic treatments. *Industrial crops and Products*, *50*, 1-7. https://doi.org/10.1016/j.indcrop.2013.07.024

Chukwuma, F.I., Nkwocha, C.C., Ezeanyika, L.U.S., & Ogugua, V.N. (2020). Phytochemical Investigation and In vitro Antioxidant Potency of Root Bark of *Brenania brieyi* Fractions. *Tropical Journal of Natural Product Research*, *4*(11), 970-975. https://doi.org/10.26538/tjnpr/v4i11.21

El Hazzat, N., Iraqi, R., & Bouseta, A. (2015). GC-MS and GCFID-O identification of volatile compounds in green olives of the Moroccan variety & Picholine" effect of geographical origin. *International Journal of Biological and Chemical Sciences*, *9*(4), 2219-2233. https://doi.org/10.4314/ijbcs. v9i4.40

Farjon, A., & Rushforth, K.D. (1989). *A classification of Abies* Miller (Pinaceae). Notes from the Royal Botanic Garden, Edinburgh., 46(1), 59-79.

Gehin, A., Guyon, C., & Nicod, L. Glyphosate induced antioxydant imbalance in HacaT: The protective effect of vitamin C and E. *Environmental Toxicology and Pharmacology*, *22*, 27-34. https://doi.org/10.1016/j.etap.2005.11.003

Gorinstein, S., Yamamoto, K., Katrich, E., Leontowicz, H., Lojek, A., Leontowicz, M., Ciz, M., Goshev, I., Shalev, U., & Trakhtenberg S. (2003). Antioxidative properties of Jaffa sweeties and grapefruit and their influence on lipid metabolism and plasma antioxidative potential in rats. *Bioscience Biotechnology Biochemical*, *67*, 907-910. https://doi.org/10.1271/bbb.67.907

Gupta, D., & Kumar, M. (2017). Evaluation of in vitro antimicrobial potential and GC–MS analysis of *Camellia sinensis* and *Terminalia arjuna*. *Biotechnology Reports*, *13*, 19-25. https://doi.org/10.1016/j.btre.2016.11.002

Hagerma, A.E. (2002). Tannin Handbook. 2éme edition. Miami University. Oxford, USA, 116.

Hatami, T., Emami, S.A., Miraghaee, S.S., & Mojarrab, M. (2014). Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of *Artemisia biennis* Willd. *Iranian Journal of Pharmaceutical Research*, *13*(2), 551. https://doi.org/10.22037/ijpr.2014.1518

Herode, S.S., Hadolin, M., Škerget, M., & Knez, Z. (2003). Solvent extraction study of antioxidants from balm (*Melissa officinalis* I.) leaves. *Food Chemistry*, *80*, 275-282. https://doi.org/10.1016/S0308-8146(02)00382-5

Hmamouchi, M. (1999). Les plantes médicinales et aromatiques marocaines: utilisation, biologie, écologie, chimie, pharmacologie, toxicologie, lexiques. *Bibliographie du patrimoine culturel immatériel*.

Ishola, I.O, Ikuomola, B.O., & Adeyemi, O.O. (2018). Protective role of Spondias mombin leaf and *Cola acuminata* seed extracts against scopolamine-induced cognitive dysfunction. *Alexandria J Med., 54*, 27-39. https://doi.org/10.1016/j.ajme.2016.08.001

Khan, R.A., Khan, M.R., & Sahreen, S. (2012). Assessment of flavonoids contents and in vitro antioxidant activity of *Launaea procumbens*. *Chem Central J., 6*, 43.

Kuluvar., G., Mahmood, R., Mohamed, R., Ahamed, K., Babu, P., & Venkatarangaiah, K. (2009). Wound healing activity of *Clerodendrum infortunatum* Linn. *Root extracts, Int J of Biom and Pharm Sci., 3*(1), 21-25.

Lamaison, J.L., & Carnat, A. (1990). Teneur en principaux flavonoides des fleurs et des feuilles de Crataegus monogyna Jacq. et de *Crataegus laevigata* (Poiret) DC. (Rosacea). *Pharmaceutica Acta Helvetia*, *65*(11), 315-320.

Lavoie, S., Gauthier, C., Legault, J., Mercier, S., Mshvildadze, V., & Pichette, A. (2013). Lanostane- and Cycloartane-Type Triterpenoids from *Abies balsamea* Oleoresin. *Beilstein J. Org. Chem., 9*, 1333-1339. https://doi.org/10.3762/bjoc.9.150

Li, Y.L., Gao, Y.X., Jin, H.Z., Shan, L., Chang, W.L., Yang, X.W., Zeng, H.W., Wang, N., Steinmetz, A., & Zhang, W.D. (2015). Chemical Constituents of *Abies fabri*. *Phytochemistry*, *117*, 135-143. https://doi.org/10.1016/j.phytochem.2015.06.012

McCune, L.M., & Johns, T. (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by indigenous peoples of North American boreal forest. *J. Ethnopharmacology*, *82*, 197-205. https://doi.org/10.1016/s0378-8741(02)00180-0

Merouane, A., Noui, A., Medjahed, H., Nedjari Benhadj Ali, K., & Saadi, A. (2014). Antioxidant activity of phenolic compounds in olive oil extracted by traditional method. *Int. J. Biol. Chem. Sci., 8*(4), 1865-1870. https://doi.org/10.4314/ijbcs. V8i4.45

Mokaddem-Daroui, H., Mostefa, M.B., Aydogmus-Ozturk, F., Erol, E., Ozturk, M., Ertaş, A., Emin Duru, M., Kabouche, A., & Kabouche Z. (2021). Antioxidant, Anticholinesterase Activities and Polyphenolic Constituents of Cones of Algerian Fir (*Abies numidica*) By Lc-

Esi-Ms/Ms with Chemometric Approach. *J. Res. Pharm*, *25*(3), 230-237. http://dx.doi.org/10.29228/jrp.13

Negro, C., Tommasi, L., & Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extract. *Bioresource Technology.*, *87*, 41-4. https://doi.org/10.1016/S0960-8524(02)00202-X

Nunes, P.X., Silva, S.F., Guedes, R.J., & Almeida, S. (2011). *Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health*. London: InTech. https://doi.org/10.5772/26956

Oueslati, S., Ksouri, R., Falleh, H., Pichette, A., Abdelly, C., & Legault, J. (2012). Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticose* Forssk. *Food Chemistry, 132*(2), 15, 943-947. https://doi.org/10.1016/j.foodchem.2011.11.072

Rajalakshmi, P., Vadivel, V., Ravichandran, N., Sudha, V., & Brindha, P. (2016). Pharmacognostic Evaluation of *Abies webbiana* Leaf: A Siddha Herbal Ingredient. *Asian Journal of Pharmaceutical and Clinical Research.*, *9*(4), 1-7.

Raldugin, V.A., & Shevtsov, S.A. (1990). Triterpenoids of Plant of the Genus Abies Hill. *Chemistry of Natural Compounds, 26*, 373-382. https://doi.org/10.1007/BF00598986

Sethi, S., Joshi, A., Arora, B., Bhowmik, A., Sharma, R.R., & Kumar, P. (2020). Significance of FRAP, DPPH, and CUPRAC assays for antioxidant activity determination in apple fruit extracts. *European Food Research and Technology, 246*, 591-598. https://doi.org/10.1007/s00217-020-03432-z

Sharififar, F., Dehghn-Nudeh, G., & Mirtajaldini, M. (2009). Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chemistry, 112*, 885-888.

Subhashini, N., Nagarajan, G., & Kavimani, S. (2011). In Vitro Antioxidant and Anticholinesterase Activities of *Garcinia Cambogia*. *International Journal of Pharmacy and Pharmaceutical Sciences, 3*, 129-132.

Sun, C., Zhao, C., Guven, E.C., Paoli, P., Simal-Gandara, J., Ramkumar, K.M., Wang, S., Buleu, F., Pah, A., Turi, V., Damian, G., Dragan, S., Tomas, M., Khan, W., Wang, M., Delmas, D., Portillo, M.P., Dar, P., Chen, L., & Xiao, J. (2020). Dietary polyphenols as anti-diabetic agents: Advances and opportunities. *Food Frontiers, 1*, 18-44. https://doi.org/10.1002/fft2.15

The Editorial Board of Flora of China. (1978). *Flora of China*. Beijing: Science Press, 7, 59-94 (in Chinese).

Tiwari, K.P, Minocha, P.K., & Chalcone, A. (1980). Glycoside from *Abies pindrow*. *Phytochemistry, 19*(11), 2501-2503. https://doi.org/10.1016/S0031-9422(00)91072-0

Trease, G.E., & Evans, W.C. (1989). *Textbook of Pharmacology*. 13th Ed. Macmillan Publishers Limited, London, 343-383.

Uçar, G., & Uçar, M.B. (2014). Geographical Variation in the Composition of *Abies bornmuelleriana* Mattf, Needle Oils. *Records of Natural Products, 8*(1), 56-60.

Ugoeze, K.C., Oluigbo, K.E., & Chinko, B.C. (2020). Phytomedicinal and Nutraceutical Benefits of the GC-FID Quantified Phytocomponents of the Aqueous Extract of *Azadirachta indica* leaves. *Journal of Pharmacy and Pharmacology Research, 4*, 149-163. https://doi.org/10.26502/fjppr.039

Wajs-Bonikowska, A., Sienkiewicz, M., Stobiecka, A., Maciąg, A., Szoka, Ł., & Karna, E. (2015). Chemical composition and biological activity of *Abies alba* and *A. koreana* seed and cone essential oils and characterization of their seed hydrolates. *Chem Biodivers, 12*(3), 407-18. https://doi.org/10.1002/cbdv.201400167

Xia, J.H., Zhang, SD, Li, Y.L., Wu, L., Zhu, Z.J., Yang, X.W., Zeng, H.W., Li, H.L., Wang, N., Steinmetz, A., & Zhang, W.D. (2012). Sesquiterpenoids and Triterpenoids from *Abies*

holophylla and Their Bioactivities. *Phytochemistry*, *74*, 178-184. https://doi.org/10.1016/j.phytochem.2011.11.011

Yang, X.W., Li, S.M., Shen, Y.H., & Zhang, W.D. (2008). Phytochemical and Biological Studies of *Abies* species. *Chemistry & Biodiversity*, *5*(1), 56-81. https://doi.org/10.1002/cbdv.200890015

Zeppetzauer, F., Nadányi, R., Kamm, B., Putz, R., Lisý, A., & Šurina, I. (2021). Investigation of Selective Extraction of Phenolic Compounds and of Saccharides from *Picea abies* Bark Using Organosolv Solvents. *Preprints*, 2021100095. https://doi.org/10.20944/preprints202110.0095.v1

Zheng, W.J., & Fu, L.G. (1978). *Flora reipublicae popularis sinicae*. Gymnospermae; Science Press: Beijing, China, 7, 55.