Research Article

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Phytochemical analysis, *in vitro* antioxidant and antifungal activities of extracts and essential oil derived from *Artemisia herba-alba* Asso

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Abstract: *Artemisia herba-alba* Asso is an endemic plant from North-East Morocco offering a diverse range of pharmacological, cosmetic, and agro-ecological uses. However, *Artemisia herba-alba* has not been well exploited in the field of plant protection (post-harvest diseases). This is why the main objective of the present study is to evaluate the antifungal and antioxidant activities of extracts or the essential oil of this plant. Antifungal activity was assessed against *Botrytis cinerea*, *Penicillium expansum,* and *Penicillium digitatum* using the agar dilution method for the aqueous extracts and fumigation for the essential oil. Antioxidant activity was assessed using the DPPH scavenging test and the FRAP ferric ion reduction capacity. The chemical composition of the extracts was determined by HPLC and that of the essential oil by GC-MS/MS. In addition, the polyphenol, flavonoid, and tannin content was determined using colorimetric methods. The results of this study showed that the total content of polyphenols (217.60 mg GAE/g E), flavonoids (43.59 mg QE/g E), and tannins (32.58 mg GAE/g E) was significantly higher ($p \leq 0.05$) in the ethanolic extract than in the aqueous and hexanoic extracts. Moreover, the HPLC analysis of the aqueous extract revealed the presence of six compounds, namely, catechin, gallic acid, hydroxybenzoic acid, syringic acid, caffeic acid, and rutin. In addition, the ethanolic extract was found to contain seven phenolic compounds. On the one hand, 35 compounds were identified in the essential oil of *Artemisia herba-alba*, representing 99.7% of the total. According to this study, the dominant compounds in the essential oil of *Artemisia herba-alba* Asso are camphor (46.57%), endo-borneol (5.65%), eucalyptol (5.64%), and thymol (3.85%). Furthermore, the biological evaluation showed that the extracts and essential oil of *Artemisia herba-alba* have significant antioxidant and antibacterial activities. The findings show that *Artemisia herba-alba* is a plant that can be used as a source of antifungal chemicals to prevent putrefaction of foodstuffs and, more specifically, postharvest diseases.

Keywords: HPLC, GC-MS analysis, *Artemisia herba-alba*, essential oil, antioxidant activities, and antifungal activities

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List of Abbreviations

A. herba-alba	*Artemisia herba-alba Asso*
FC	Folin-Ciocalteu
UV	ultraviolet
Na_2CO_3	Sodium carbonate
$AlCl_3$	Aluminum chloride
HPLC	High-Performance Liquid Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Free Radical Scavenging Activity
PDA	Potato Dextrose Agar
EO	Essential Oil
TPC	Total Phenol Content
TFC	Total Flavonoids
TTC	Total Tannins Content
NI	Not Identified
NQ	Not Quantified
IR	Index of Retention
IC_{50}	Median inhibitory concentration
P. expansum	*Penicillium expansum*
B. cinerea	*Botrytis cinereal*
P. digitatum	*Penicillium digitatum*
PPO	polyphenol oxidase

1 Introduction

Fungal deterioration of fruits and vegetables during postharvest storage has had a serious impact on their economic quality for several years. Apples and oranges occupy an important place in the global fruit market, and with a cultivated area of almost 5 million hectares and a production of 81 million tons [1], apples represent one of the world's most outstanding fruit industries. Recently, biological protection against postharvest rotting of fruit and vegetables has become an attractive alternative to synthetic fungicides [2,3], as fungicide treatments are of great concern, in terms of both fungal residues remaining in foodstuffs and the development of fungicide resistance in pathogens, enabling researchers to find solutions beneficial to public health.

To prevent and limit the development of diseases on fruit destined for storage, many conventional control approaches are adopted, starting with cultural practices. These practices focus on controlling the factors that affect the physiological state of the fruit and the formation of mechanical damage. In several research studies, physical processes such as ultraviolet C radiation (UV-C), thermotherapy, as well as ozone treatment have been applied to minimize postharvest fruit disorders [4].

Recently, many scientists have discovered that natural substances from plants have interesting biochemical and biological properties [5], including antifungal, antioxidant, antidiabetic, anticancer, and anti-inflammatory activities [6].

Consequently, there is a need to find genuine biological solutions, including natural antifungal substances that are safe and beneficial for the environment [7]. Natural plant substances have been particularly noted as the main synthetic control solutions for postharvest fruit pathologies [4]. These are plant products derived mainly from medicinal plants. Their low toxicity and antifungal activity, as well as their low durability in the environment, make them interesting to use to control postharvest affections [8,9].

Morocco boasts a very rich and diverse flora [10]. Indeed, many of these plants (aromatic or medicinal) have biologically interesting properties and are used for a variety of purposes, including medicine, pharmacy, cosmetics, and agriculture [11]. Note that among the medicinal plant species that make up the plant heritage, the species *Artemisia herba-alba*, which is a perennial herbaceous plant, is very abundant in Morocco [12,13]. Moreover, this plant, which grows in stony soils and pastures, has a wide geographical distribution in Morocco (East, East-Rif, Middle Atlas, and High Atlas) [14,15]. In Arabic, *A. herba-alba* is commonly known as "chih" [16]. It is renowned for its therapeutic and medicinal virtues. Previous research into the medicinal characteristics of *A. herba-alba* has revealed multiple biological and pharmaceutical interests, including antifungal [17,18], antioxidant [19], and natural antibacterial activities [20]. Indeed, *A. herba-alba* essential oil is highly prized for its high commercial value, medicinal uses, and therapeutic applications. According to numerous studies carried out on the species *A. herba-alba*, the oil of this plant is characterized by its abundance of numerous substances such as camphor, α-thujone, β-thujone, eucalyptol, and chrysanthenone [19,20].

In this sense, the present study aims to evaluate the antioxidant and antifungal potential of the aqueous extract and essential oil of *A. herba-alba* against fungal infections that attack fruit in the postharvest period, resulting in reduced fruit quality and a direct impact on the agricultural economy.

2 Materials and methods

2.1 Plant material

A. herba-alba is a medicinal plant collected in the province of Taza, Gueldaman region of Morocco (34°11′10.7″N 3°55′ 26.5″W) in June 2022. This plant has been identified by Professor Ghizlane Echchgadda in the Department of Plant Protection and Environment (Plant Unit), National School of Agriculture, Meknes (Morocco), and the voucher specimen has been deposited in the department. Several plant organs were used (stem, leaves, flowers, fruits, and seeds). Tests were carried out using plant powder. The plants were cut before being dried at room temperature in the shade. The aerial part of each plant was ground using an electric grinder.

2.2 Extracts preparation

2.2.1 Solvent extracts

The extracts are prepared using the following method: Initially, 10 g of powdered aerial parts from the dried *A. herba-alba* plant are dissolved in 100 mL of ethanol, water, and hexane, and the mixture is macerated at room temperature. The samples were then filtered and concentrated using a Büchi Rotavapor R-210 with different evaporation temperatures (40°C for the evaporation of the extract of hexane and 50°C for ethanolic extract) to obtain a dry extract, while the aqueous extract was obtained using a freeze-dryer. Finally, extracts were stored at 4–8°C until use.

2.2.2 Hydrodistillation of essential oil

A. herba-alba essential oil is extracted by hydrodistilling 100 g of fresh aerial plant parts in a Clevenger-type apparatus (Clevenger, 1928) for 3 h. The essential oil was stored at 4°C for the time of use.

2.3 Phytochemical analysis

2.3.1 Phytochemical screening

Phytochemical screening of *A. herba-alba* was carried out according to the standard modalities [21] to determine the following categories: flavonoids, alkaloids, tannins, terpenoids, free quinones, saponosides, and glucosides. Results are read by visual observation of color change or precipitate formation after the addition of specific reagents.

2.4 Quantification of total phenolic, flavonoid content, and condensed tannins: Phenolic content

Total phenolic content was determined using the Folin–Ciocalteu (FC) technique [6]. Briefly, an overall volume of 100 µL of extract was mixed with 500 µL of FC reagent and 400 µL of 7.5% (*w*/*v*) Na_2CO_3. The mixture was then stirred and incubated for 10 min in the dark at room temperature. A UV spectrophotometer (Perkin Elmer) was used to measure absorbance at 760 nm. Results are expressed in milligrams of gallic acid equivalent/grams of dry plant matter, using the gallic acid calibration curve as a reference.

2.5 Flavonoids content

The analysis of flavonoid content was conducted following the method described in Dehpeur et al. [22]. This involved combining 500 µL of each extract with 1,500 µL of 95% methanol, 100 µL of 10% (*w*/*v*) $AlCl_3$, 2.8 mL of distilled water, and 100 µL of sodium acetate (1 M). The mixture was then shaken, and it necessitated storage in the dark for 30 min.

For the preparation of the control, the extract is replaced by 500 µL of methanol, and the absorbance value is determined with an ultraviolet spectrophotometer at 415 nm (Perkin Elmer). The calibration curve of quercetin is used as a reference, and the values of the results are expressed in milligrams of quercetin equivalent to/grams of dry matter of the plant.

2.6 Condensed tannins

Condensed tannin values were measured using a colorimetric approach [6]. Succinctly, a quantity of (50 µL) of each diluted extract was mixed with 1.5 mL of 4% vanillin, followed by the addition of 750 µL of concentrated hydrochloric acid. The preparation was shaken and incubated at room temperature in the dark for 20 min. Absorbance values were determined at 500 nm. A standard curve was established using catechin at a concentration of 50–500 µg/mL. All results were expressed in milligrams of catechin equivalents per gram extract (mg CE/g extract).

2.7 Chemical composition HPLC analysis

Chromatographic analysis and quantification for the phenolic composition of both *A. herba-alba* extracts were carried out with an EC NUCLEOSIL column (5 µm, C18, 100-5, 250 × 4.6 mm; Macherey-Nagel, Germany), following the conditions described in this protocol [23] with some modifications. The mobile phase consisted of two solvents: water-formic acid (0.5%) (A) and acetonitrile (B), starting with 10% B and using a gradient to obtain 20% B at 35 min, 25% B at 65 min, 35% at 80 min, and 100% at 100 min. UV detection was performed at 280 nm. The injection volume was 20 µL, and the flow rate was 0.7 mL/min. The HPLC system is controlled by the HPLC software Chrom NAV 2.0 – JASCO. Identification was performed by comparing the retention time of each picture with the corresponding standard.

2.8 Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis method was carried out for the plant *A. herba-alba* out according to the program and protocol previously described [24] with a few modifications.

The temperature program was as follows: injector temperature 250°C, initial oven temperature 50°C, programmed rate 2°C/min to final temperature 200°C for 5 min, interface line temperature 250°C, and detector temperature 250°C. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. To carry out the GC-MS analysis protocol, the samples were dissolved in cyclohexane at a dilution ratio of 10:100. Subsequently, a volume of 1 µL was injected automatically (Autosampler Model TriPlus RSH). Finally, ionization was performed with an electron ionization energy of 70 eV.

The mass range was set in the range 50–550 *m/z*, and the MS spectra of the separated components were identified on NIST libraries.

2.9 Determination of antioxidant activity

To evaluate the antioxidant power of *A. herba-alba* extracts and essential oil, the DPPH and FRAP tests were performed.

2.9.1 DPPH radical assay

The free radical neutralizing powers of aqueous extract and essential oil were determined on a radical basis by adding 2,2-diphenyl-1-picrylhydrazyl (DPPH) as previously described [25]. Antioxidant substances react with the stable DPPH free radical (deep violet color) and transform it into 1,1-diphenyl-2-picrylhydrazine, causing color degradation. Analyzed samples were combined with a methanolic solution of DPPH (0.02 mM) and incubated in the dark for 30 min at room temperature. The absorbances of the samples were then measured at 517 nm, and the DPPH trapping power of the extracts was determined using the following formula:

$$\text{DPPH activity (\%)} = [(A_0 - A_s)/A_0] \times 100,$$

where A_0 is the absorbance value of the negative control and A_s is the absorbance of the sample tested at 30 min.

The positive control was ascorbic acid, and the scavenging activity of the samples was expressed in terms of the IC_{50}, which represents the highest value of the 50% scavenging activity of the DPPH scavenging effect.

2.9.2 Reducing power of ferric ions by FRAP

The reducing properties of the extracts was measured using FRAP test [26], and 1 mL phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL) were mixed. Once the mixture had been incubated for 20 min at 50°C (water bath), and 2.5 mL trichloroacetic acid (10%) was added to the contents. The solution was then centrifuged at 3,000 rpm for 10 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm. Ascorbic acid (50–450 µg/mL) is used as a standard. The reducing potential is represented by the equivalence in milligrams of ascorbic acid per gram of extract (mg AAE/g Ex).

2.10 Antifungal activity

2.10.1 Fungal pathogen culture

The present evaluation was carried out for the following fungi: *Botrytis cinerea*, *Penicillium expansum*, and *Penicillium digitatum* previously isolated from infected fruit (apples and oranges). For the *in vitro* test [27], culture samples were stored at 4°C on glass or plastic supports on a PDA medium (Potato Dextrose Agar, Merck, Darmstadt, Germany). For the *in vivo* test, all strains were first isolated from deteriorating fruit and tested for virulence by introducing them into artificially wounded fruit. These strains were placed in slants at 4°C on a PDA medium enriched with 50 mg/L streptomycin (Merck, Darmstadt, Germany) [28].

2.11 *In vitro* effect of plant extracts against mycelial growth

2.11.1 Agar dilution technique

Sample preparation of aqueous extract was carried out according to the method described in the previous studies [29,30]. Briefly, 10 g of *A. herba-alba* powder was infused in 100 mL of sterile distilled water for 10 min on a hot plate. To achieve a final concentration of 10% (*v/v*), the extracts were then filtered using Whatman no. 1 filter paper.

Using the agar dilution technique, the potential ability of the aqueous extract of the *A. herba-alba* plant to inhibit the mycelial growth of fungal strains was evaluated. Plant extracts were analyzed at different concentrations, including 1.25, 2.5, 5, and 10% (*w/v*) for aqueous extract. Agar plugs 5 mm in diameter were taken from previously prepared cultures, mycelium down, and used to inoculate them into plates of the fungal strains. To assess mycelial development, Petri dishes were incubated for 7 days at 25°C.

The percentage of mycelial growth inhibition of the extracts was determined by the following formula (1), described [31] as follows:

$$[(\text{diameter of control} - \text{diameter of aqueous extract}) / \text{diameter of control}] \times 100 = \%\ \text{mycelial growth inhibition (MGI)} \quad (1)$$

For each treatment, three boxes were deployed as replicates, and the test was repeated twice [7].

2.12 Essential oil fumigation assay

Fumigation of the bioassays was carried out according to the given instructions. The methodology of fumigation assay [32] was used but with some modifications for unique reasons. Rather than the traditional approach, a 6 mm fungal disk was seeded in the center of a 15 mL/90 mm Petri dish. A disk of sterilized filter paper, with a diameter of 9 mm, was placed in the center of the lid and sprayed with varying volumes (12, 5, 25, 50 µL) of EO. The methodology was repeated for the controls but with distilled water. To maintain consistency, all assays were performed in triplicate and plates were immediately covered with parafilm and incubated at 25°C for 5–10 days. Formula (1) was implemented to calculate the growth inhibition percentage.

2.13 Statistical analysis

Each experiment was performed in triplicate, and all values obtained are expressed as mean ± standard deviation (SD) and are subjected to an analysis of variance (ANOVA). GraphPad Prism version 6.00 was used for these statistical analyses. We considered the differences between the groups to be statistically significant at $p < 0.05$.

3 Results and discussion

3.1 Phytochemical screening

Qualitative phytochemical analysis was carried out using standard techniques to determine the active metabolites of *A. herba-alba*. The results of detection of the presence and absence of secondary metabolites in the aqueous extract of *A. herba-alba* Asso. are presented in Table 1.

Analysis of aqueous extracts of *A. herba-alba* plant showed that when a deep yellow color appears, it indicates the presence of flavonoids, while a yellow precipitate indicates the presence of alkaloids. Gallic tannins are blue-black. The presence of saponins is indicated by the appearance of foam, and the presence of steroids is indicated by a color change from purple to blue to green. However, the aqueous extract does not contain quinones. These results are similar to those of the present study [33].

3.2 Assessment of total phenolic, flavonoid, and tannin contents

This study shows that the various extracts tested are rich in phenolic compounds, flavonoids, and tannins. Table 2 and

Table 1: Quantitative analysis to detect the main groups of secondary metabolites in the aqueous extract of *A. herba-alba* (present and absent)

	Flavonoids	Alkaloids	Tannins	Saponins	Steroids	Quinones
Artemisia herba alba Asso	***	***	**	**	*	—

Table 2: Total phenolic, flavonoid, and tannins contents in extracts

Artemisia herba alba Asso.	Total polyphenols (mg GAE/L)	Flavonoids (mg QE/L)	Tannins (mg CE/L)
Ethanolic extract	217.60 ± 0.58[a]	43.59 ± 0.06[a]	20.99 ± 0.20[b]
Aqueous extract	198.70 ± 0.04[b]	35.94 ± 0.05[b]	31.58 ± 0.21[a]
Extract of hexane	27.65 ± 0.08[c]	16.80 ± 0.86[c]	14.03 ± 1.33[c]

In each column, the values with different letters (a–c) are significantly different according to the Tukey test ($p < 0.05$). SD values represent the means of three independent repetitions ± standard deviation.

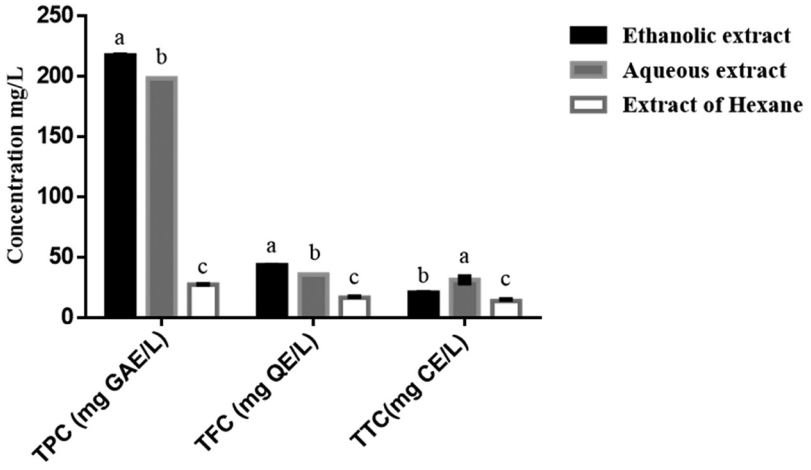

Figure 1: The total content of polyphenols, flavonoids, and tannins. TPC: total phenol content, TFC: total flavonoids content, TTC: total tannins content.

Figure 1 show the results obtained from secondary metabolite analysis.

The total content of polyphenols (217.60 mg GAE/g E), flavonoids (43.59 mg QE/g E), and tannins (32.58 mg GAE/g E) is significantly higher ($p \leq 0.05$) in the ethanolic extract than in the aqueous and hexanolic extracts. We also note that the most remarkable tannin compound content is presented in the aqueous extract with a value of 31.58 mg GAE/L.

Indeed, the results of the present work are in agreement with previous studies [34,35], which evaluated the total polyphenol, flavonoid, and tannin contents, of three extracts of *A. herba-alba*. In contrast to these results, another study [36] evaluated the total content of bioactive compounds in four extracts of *A. herba-alba*. They revealed that the level of phenols, flavonoids, tannins, and proanthocyanidins is highest in the ethyl acetate extract, followed by the butanoic extract, the water extract, and the chloroform extract. As pointed out by a previous study [5], multiple research studies indicate that the aerial part of the *A. herba-alba* plant is highly concentrated in bioactive compounds such as polyphenols, flavonoids, and tannins. Compared with the present study, the differences in results could be linked to the origin of the plant, the nature of the solvents used, the extraction methods and times, as well as the temperature and quantity of plant used for extraction [37,38]. It is worth noting that the richness of *A. herba-alba* aqueous extract in secondary metabolites such as tannins, flavonoids, and other phenolic compounds plays a very important role in its antifungal activity [33].

3.3 Chemical composition HPLC analysis

Analysis of ethanol and aqueous extracts of the studied *A. herba-alba* plant using RP-HPLC-UV reveals the presence of phenolic acids and flavonoids (Figure 2 and Table 3). The aqueous extract contains six compounds: gallic acid, catechin, hydroxybenzoic acid, syringic acid, caffeic acid, and rutine. While that ethanolic extract contains seven phenolic compounds: gallic acid, catechin, hydroxybenzoic acid, syringic acid, *p*-coumaric acid, rutin, and quercetin.

The quantification of the identified polyphenols showed that the main compound of the aqueous extract is hydroxybenzoic acid with a concentration of 9.79 µg/mg of crude extract, followed by rutin (3.18 µg/mg of crude extract), gallic acid (1.15 µg/mg of crude extract), and caffeic acid (0.39 µg/mg of crude extract), and it is impossible to quantify the

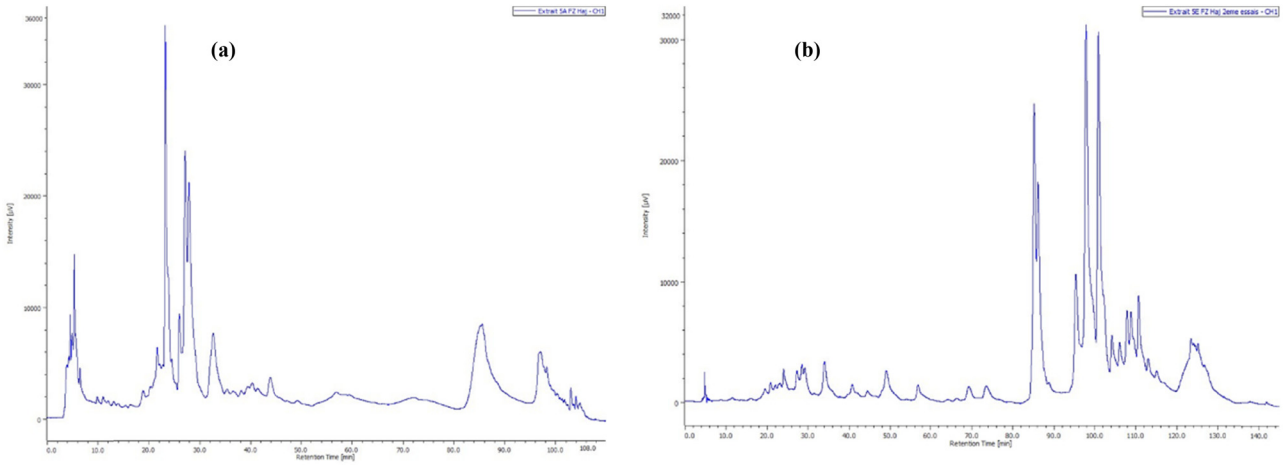

Figure 2: HPLC chromatogram of aqueous extract (a) and ethanolic extract (b).

other molecules because of their low concentration. In the case of the ethanolic extract, only three polyphenols could be quantified, the main compound being quercetin with a concentration of 21.85 µg/mg of crude extract, followed by rutin (3.23 µg/mg of crude extract) and then hydroxybenzoic acid (0.62 µg/mg of crude extract).

Other studies have shown that *A. herba-alba* extract contains high concentrations [39] of 3-O-methylquercetin (31.88 mg/g), eupatiline (29.64 mg/g), acetin (22.04), and nepetin (15.47 mg/g). In a study by Khennouf et al. using HPLC analysis, they estimated the phenolic compounds in three different extracts, and they found that the ethyl acetate phase contains significant amounts of flavonoids (apigenin and luteolin) and phenolic acids (protocatechic acid, caffeic acid, and ferulic acid). In the same study, flavonoid aglycones, such as apigenin and luteolin, and phenolic acids, such as protocatechic acid, vanillic acid, caffeic acid, and ferulic acid, were the major compounds in the chloroform phase.

In addition, Carvalho et al. [40] found that six species of *Artemisia*. *Artemesia* spp. leaves contained 80 µg/g dry matter of catechins and 430 µg/g dry matter of hydroxybenzoic acids through the HPLC analysis. In the same study, they found that the five hydroxycinnamic acids identified were cinnamic acid, chlorogenic acid, conjugates of caffeic acid and ferulic acid, sinapic acid, and *p*-coumaric acid. In all *Artemisia* leaves, the conjugates of caffeic acid and ferulic acid are the major hydroxycinnamic acids. This is largely in line with our study, as the main molecules activated are gallic acid, catechin, hydroxybenzoic acid, syringic acid, *p*-coumaric acid, rutin, and quercetin. According to the present study and previous studies, it can be suggested that phenolic compounds contained in medicinal plants, such as flavonoids, *p*-coumaric acid, rutin, and quercetin, may be present in the plant *A. herba-alba*, as well as bioactive secondary metabolites, have several biological activities such as antioxidant, anti-inflammatory, antibacterial, and antifungal activities, which can be determined by performing biological and biochemical tests [41].

Table 3: List of compounds identified of *A. herba-alba* of ethanolic and aqueous extracts using RP-HPLC-UV

Standards	Retention time (min)	Concentrations (µg/mg) of crude extracts	
		Aqueous extract	Ethanolic extract
Gallic acid	6.408	1.157829	NQ
Catechin	21.583	NQ	NQ
Hydroxybenzoic acid	23.175	9.79812	0.625886
Syringic acid	24.433	NQ	NQ
Caffeic acid	25.950	0.398803	NI
p-coumaric acid	33.950	NI	NQ
Rutin	43.742	3.181247	3.236523
Quercetin	86.0.25	NI	21.85023

NI: not identified; NQ: not quantified.

3.4 GC-MS identification

The volatile compounds of the *A. herba-alba* plant studied are separated by gas chromatography (GC) (Figure 3), followed by an analysis using mass spectroscopy (MS). The results of this analysis are summarized in Figure 3 and Table 4.

The hydrodistillation analysis of *A. herba-alba* plant material dried in the dark produced a yield of 1.05 ± 0.02%. The volatile compounds of the plant studied were

separated by gas chromatography (GC) (Figure 3) and identified by mass spectrometry (MS). Table 4 summarizes all the results obtained from the GC-MS analysis of *A. herba-alba* essential oil. In brief, 35 compounds were identified in the essential oil of *A. herba-alba*, representing 99.7% of the total. According to the present study, the dominant compounds in *A. herba-alba* essential oil are as follows: camphor (46.57%), endo-borneol (5.65%), eucalyptol (5.64%), thymol (3.85%), Verbenyl angelate, cis- (3.24%), (−)-Spathulenol (3.06 %), pinocarvone (2.42%), and ledol (2.06%). In terms of previous studies conducted on the chemical composition of Moroccan essential oil *A. herba-alba*, the major components of this plant from Errachidia are camphor (17.8%), α-thujone (17.3%), cis-chrysanthenyl acetate (10.9%), β-thujone (9. 9%), davanone (6.7%), chrysanthenone (6.4%), and eucalyptol (5%), while β-thuyone (46.8%), α-thuyone (21.2%), and camphor (8.4%) are the main components identified for the same plant from the oujda region [42]. Furthermore, in 2023, Ez-Zoubi et al. [43] identified α-Tujone (65.0%) as the most abundant compound, with three other main constituents, β-thujone (14.4%), camphor (6.0%), and α-phellandrene (2.3%), while Houti et al. [10] reported three main groups such as cis chrysanthenyl acetate, β-thujone, and camphor from the Middle Atlas region of Morocco. In other studies [19,44,45], camphor was classified as one of the most common compounds found in *A. herba-alba* essential oil, which is in agreement with the results of the present work.

The study of the chemical composition of medicinal plants is carried out by several researchers at various universities around the world (Table 5) due to its importance and value in many fields such as agriculture, health, and cosmetics. In Jordan, it was observed that the most important components are α- and β-thujones (27.7%), and the other major components identified are sabinyl acetate (5.4%), germacrene D (4.6%), α-eudesmol (4.2%), and caryophyllene [53]. In Tunisia, a study of *A. herba-alba* essential oil [48] revealed that the main components are α-thujone (35.23%), norboran-2-one (25.67%), chrysanthenone (7.66%), and 1,8-cineole (5.80%). The dominant components of oil originating in Algeria are Thujone (10.555%), Borneol (5.978%), and eucalyptol (1.628%) [52]. In Spain, some studies have revealed that camphor, 1,8-cineole, *p*-cymene, and davanone are the main components observed [55]. On the other hand, α-thujene, acenaphthylene, and 3-methyl-2-chloro-benzo (b) thiophene-1-oxide are the majority compounds of the plant *A. herba-alba* originating from Russia but which is cultivated in Egypt, and from the results obtained, it can be noticed that there is a difference at the level of the majority compounds compared to the same plant originating from Egypt [50]. Generally, we all know that essential oils vary in composition depending on the ecological niche they cover.

Broadly speaking, the variability of the chemical composition of *A. herba-alba* can be attributed to the techniques used for extraction and to exogenous factors: sunshine and the nature and composition of the soil. This variability of chemical composition relative to each plant is affected by geographical location, and climatic changes observed between certain countries or certain areas of the same country [56]. According to previous research, the difference observed in the chemical composition of the plant studied in Morocco is linked to the plant's adaptation to abiotic factors such as the climate and soil of each region.

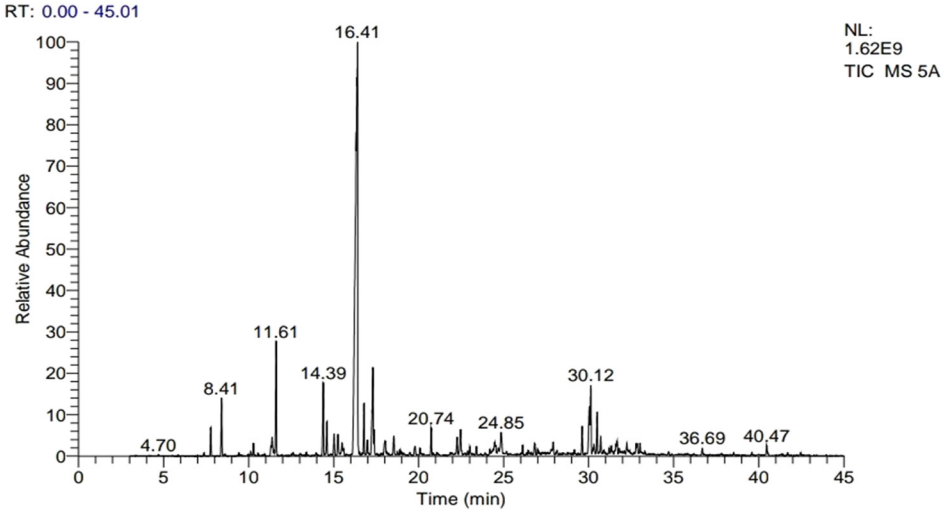

Figure 3: GC analysis of *A. herba-alba* essential oil.

Table 4: Composition (%) of *A. herba-alba* essential oil isolated by hydrodistillation

Compounds	*IR	Concentration (% peak area)	Molecular weight (M.wt)	Molecular formula (M.F)	Chemical class
α-Pinene	949	1.32 ± 0.02	136.23 g/mol	$C_{10}H_{16}$	Monoterpene hydrocarbon
Camphene	958	2.69 ± 0.01	136.23 g/mol	$C_{10}H_{16}$	Monoterpene hydrocarbon
1,2,3-Trimethylbenzene	973	0.57 ± 0.02	120.19 g/mol	C_9H_{12}	Aromatic hydrocarbon
o-Cymene	980	1.02 ± 0.01	134.22 g/mol	$C_{10}H_{14}$	Monoterpene hydrocarbon
Eucalyptol	985	5.64 ± 0.01	154.25 g/mol	$C_{10}H_{18}O$	Oxygenated monoterpenes
Thymol	992	3.85 ± 0.01	150.22 g/mol	$C_{10}H_{14}O$	Oxygenated monoterpenes
α-Thujone	1,025	1.68 ± 0.02	152.23 g/mol	$C_{10}H_{16}O$	Oxygenated monoterpenes
β-Thujone	1,038	1.16 ± 0.02	152.23 g/mol	$C_{10}H_{16}O$	Oxygenated monoterpenes
Borneol	1,049	1.28 ± 0.02	154.25 g/mol	$C_{10}H_{18}O$	Oxygenated monoterpenes
Camphor	1,128	46.57 ± 0.04	152.23 g/mol	$C_{10}H_{16}O$	Oxygenated monoterpenes
Pinocarvone	1,137	2.42 ± 0.02	150.22 g/mol	$C_{10}H_{14}O$	Oxygenated monoterpenes.
trans-Verbenol	1,167	0.88 ± 0.02	152.23 g/mol	$C_{10}H_{16}O$	Oxygenated monoterpenes
endo-Borneol	1,189	5.65 ± 0.07	154.25 g/mol	$C_{10}H_{18}O$	Oxygenated monoterpenes.
Terpinen-4-ol	1,204	0.88 ± 0.09	154.25 g/mol	$C_{10}H_{18}O$	Oxygenated monoterpenes.
Terpineol	1,218	1.09 ± 0.02	154.25 g/mol	$C_{10}H_{18}O$	Oxygenated monoterpenes
Myrtenal	1,229	1.07 ± 0.02	150.22 g/mol	$C_{10}H_{14}O$	Oxygenated monoterpenes
D-Carvone	1,276	1.33 ± 0.01	150.22 g/mol	$C_{10}H_{14}O$	Oxygenated monoterpenes
3,5-Heptadien-2-ol, 2,6-dimethyl-	1,297	0.93 ± 0.03	140.23 g/mol	$C_9H_{16}O$	Cyclohexylacetone
1,6-Dimethylhepta-1,3,5-triene	1,309	1.70 ± 0.02	122.21 g/mol	C_9H_{14}	Alkyl
Caryophyllene	1,321	1.51 ± 0.02	204.35 g/mol	$C_{15}H_{24}$	Sesquiterpene hydrocarbons
Aromandendrene	1,359	0.52 ± 0.01	288.25 g/mol	$C_{15}H_{24}$	Sesquiterpene hydrocarbons
Germacrene D	1,398	0.80 ± 0.01	204.35 g/mol	$C_{15}H_{24}$	Sesquiterpene hydrocarbons
δ-Cadinene	1,427	0.82 ± 0.01	204.35 g/mol	$C_{15}H_{24}$	Sesquiterpene hydrocarbons
2,6-Octadiene,2,4-dimethyl-	1,442	1.42 ± 0.01	138.25 g/mol	$C_{10}H_{18}$	Monoterpene hydrocarbons
(−)-Spathulenol	1,457	3.06 ± 0.04	220.35 g/mol	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes
Verbenyl angelate, cis-	1,477	3.24 ± 0.01	234.33 g/mol	$C_{15}H_{22}O_2$	Oxygenated sesquiterpenes
Ledol	1,496	2.06 ± 0.02	222.37 g/mol	$C_{15}H_{26}O$	Oxygenated sesquiterpenes
Caryophyllene oxide	1,510	0.80 ± 0.02	220.35 g/mol	$C_{15}H_{24}O$	Oxygenated sesquiterpenes
Aromadendrene oxide-(2)	1,526	0.64 ± 0.02	220.35 g/mol	$C_{15}H_{24}O$	Oxygenated sesquiterpenes
α-Copaene	1,587	0.64 ± 0.02	204.35 g/mol	$C_{15}H_{24}$	Sesquiterpene hydrocarbons
davanone	1,600	0.45 ± 0.02	236.35 g/mol	$C_{15}H_{24}O_2$	Oxygenated sesquiterpenes
Total identified components (%)	97.69%				
Monoterpene hydrocarbons	6.45%				
Oxygenated monoterpenes	73.5%				
Sesquiterpene hydrocarbons	4.29 %				
Oxygenated sesquiterpenes	10.25%				
Others	3.2%				

3.5 Antioxidant Activity

Medicinal plants contain high concentrations of antioxidant compounds that can be isolated and reused as antioxidants to combat the damage caused by free radicals. This study aimed to use one of these chemical methods to assess the antioxidant power of phenolic compounds in this plant endemic to Morocco. Based on the results obtained, it is evident that each extract exhibits an anti-free radical effect that is reliant on the administered dose (Table 6). This research found that the ethanolic extract displayed the greatest antioxidant potency compared to the other extracts (IC_{50} = 27.26 ± 2.62 µg/mL), followed by the aqueous extract (IC_{50} = 33.21 ± 1.12 µg/mL) and finally,

Table 5: Comparative analysis of updated literature on the main chemical composition and yield of *A. herba-alba* essential oil

Geographical area	Principal compound (%)	Yield	References
Morocco			
Taza region	Camphor (46.57%), endo-borneol (5.65%), eucalyptol (5.64%), thymol (3.85%), Verbenyl angelate, cis- (3.24%), (−)-Spathulenol (**3.06** %), pinocarvone (2.42%), and ledol (2.06%)	1.05 ± 0.02%	The current study
Fez-Boulemane region	Cis chrysanthenyl acetate (26.7–30%), β-thujone (12.9–23.2%), camphor (5.8–14.3%), chrysanthenone (2.4–14%), 1,8-cineole (1.5–11.8%), trans β-dihydro terpineol (6.9–7.8%), α-thujone (3.1–5.4%), davanone (1.4–3.9%), α-pinene (2.3–4%), sabinene (1.6–3%), and germacrene D (0.5–1.9%).	0.49–1.74%	[10]
Rabat region	α-Tujone (65.0%), β-thujone (14.4%), camphor (6.0%), and α-phellandrene (2.3%).	Not given	[43]
Boulemane and Ifrane region	Trans thujone (33.78%), camphor (18–46%), vetevinic acid (14.91%), dava ether (14.64%).	0.84–2.19%	[46]
Agadir region	β-thujone (24.34%), camphor (22.23%), α-thujone (14.56%), and 1,8-cineole (10.3%).	0.99 ± 0.06%	[47]
Tunisia			
North Tunisia	α-thujone (35.23%), norboran-2-one (25.67%), chrysanthenone (7.66%), and 1,8-cineole (5.80%)	Not given	[48]
Southern Tunisia	Thujones (11.5%), camphor (13%), sabinyl acetate (12%), ger-macrene D (4%), and (E)-ethylcinnamate (2.8%).	0.68–1.93%	[49]
Egypt			
Cairo-Egypt	Piperitone (26.5%), ethyl cinnamate (9.5%), camphor (7.7%), and hexadecanoic acid (6.9%)	0.11%	[50]
Grown in Egypt	α-thujene, acenaphthylene, and 3-methyl-2-chloro-benzo (b) thiophene-1-oxide	Not given	[51]
Alger			
South-east region	Chrysanthenone (24.1%), camphor (16.2%), α-thujone (12.8%), 1,8-cineole (9.3%), and β-thujone (4.8%)	0.93 ± 0.06%	[19]
Northeast region	Thujone (10.555%), Borneol (5.978%), and eucalyptol (1.628%)	1.07 ± 0.0681%	[52]
Jordan			
Amman region	α- and β-thujones (27.7%), santolina alcohol (13%), artemisia ketone (12.4%), trans-sabinyl acetate (5.4%), caryophyllene acetate (5.7%).	1.3%	[53]
Spain	Davanone, 1,8-cineole, chrysanthenone, and cis-chrysanthenol.	Not given	[54]

Table 6: Extraction yield and antioxidant activity of plant extracts

	Extracts	Yield %	DPPH IC_{50} (µg/mL)	FRAPmg AAE/g
Artemisia herba alba Asso	Ethanol	5.45 ± 0.02	27.26 ± 2.62^{b}	55.7 ± 0.05^{a}
	Aqueous	10.69 ± 0.08	33.21 ± 1.12^{c}	20.26 ± 0.02^{b}
	Hexane	3.9 ± 0.14	59.38 ± 0.48^{d}	17.83 ± 0.02^{c}
	A. Herba-alba Asso EO	1.05 ± 0.02	54.27 ± 2.04^{d}	6.84 ± 0.5^{d}
	Ascorbic acid	Nd	1.84 ± 0.035^{a}	Nd

Data are reported to mean (n = 3) ± SD. FRAP in mg AAE/g of extract or essential oil. In each column, the values with different letters are significantly different according to the Tukey test ($p < 0.05$). Nd: Not detected.

the hexane-based extract, which demonstrated a DPPH reduction with an IC_{50} of 59.38 ± 0.48 µg/mL. The *A. herba-alba* plant's essential oil demonstrated low antifree radical activity (IC_{50} = 54.27 ± 2.04 µg/mL). Table 6 summarizes also the FRAP test results expressed in mg EAA/g. For *A. herba-alba*, the ethanolic extract showed a remarkable reducing power of 55.7 ± 0.05 mg EAA/g extract, followed by the aqueous extract with a reducing power of 20.26 ± 0.02 mg EAA/g extract, then the hexane-based extract with a reducing power of 17.83 ± 0.02 mg EAA/g extract. In contrast, the essential oil of this plant has a very low reducing power of 6.84 ± 0.5 mg EAA/g.

According to previous studies, the IC_{50} value of ethyl acetate and aqueous extract of *A. herba-alba*, measured by

the DPPH method, was 32.9 ± 0.036 and 154 ± 0.014 µg/mL, respectively [57]. In addition, Khlifi et al. [58] also revealed a high IC_{50} of 20.64 ± 0.84 mg/L for *A. herba-alba* in comparison with several plant extracts. The same study showed that the ethanolic extract of white wormwood had 50% antifree radical activity compared to the concentration of 0.8 mg/mL. This extract also showed a greater Fe^{3+} reducing power (0.838 ± 0.2 mg/mL) compared with ascorbic acid [59]. Concerning the antioxidant activity of the essential oil of *A. herba-alba* and according to Ouchelli et al. [19], this oil was unable to reach IC_{50} in the concentration range studied (300–1,000 µg/mL). Both DPPH and FRAP tests carried out to assess the level of antioxidant activity showed that *A. herba-alba* had moderate antioxidant activity [60].

It appears in the literature that the antioxidant activity is related to the content of phenolic compounds [61], and some studies have already shown that the antioxidant powers of some plants are due to the presence of low-molecular-weight phenolics, in particular flavonoids, which are effective antioxidants [62]. This survey shows a remarkable percentage of total polyphenols and flavonoids for both the ethanolic (presence of Quercetin obtained by HPLC analysis) and aqueous extracts, which explains the moderate results obtained by the DPPH test. Flavonoids and phenolic compounds give medicinal plant extracts outstanding antioxidant activity [63]. Furthermore, the low DPPH activity and FRAP test of this plant's oil can be explained by the absence of chemical compounds such as carvacrol and eugenol, which play a very important role in increasing the antioxidant activity [64]. To conclude, having a very significant antioxidant activity of essential oil, aqueous or organic extracts depend on the presence of minor or major chemical compounds as well as on the synergy between part or all of these molecules [41] extracted by sonication (aqueous or organic extracts) or hydrodistillation (essential oils).

3.6 *In vitro* effect of plant extract against mycelial growth

The present study showed that the mycelial inhibition of the pathogenic fungi tested, treated either with the aqueous extract of *A. herba-alba* or with the essential oil of the same plant at different concentrations, was significantly different compared with the control. For all incubation times, the essential oil of *A. herba-alba* had a major impact ($p < 0.05$) on the mycelial growth of *B. cinerea* and *P. expansum*, while the aqueous extract had a medium inhibitory effect on the mycelial growth of *P. expansum* (Figure 4).

This study found that the mycelial growth of *P. expansum* was significantly affected ($p < 0.05$) by the aqueous extract of *A. herba-alba*, irrespective of the length of

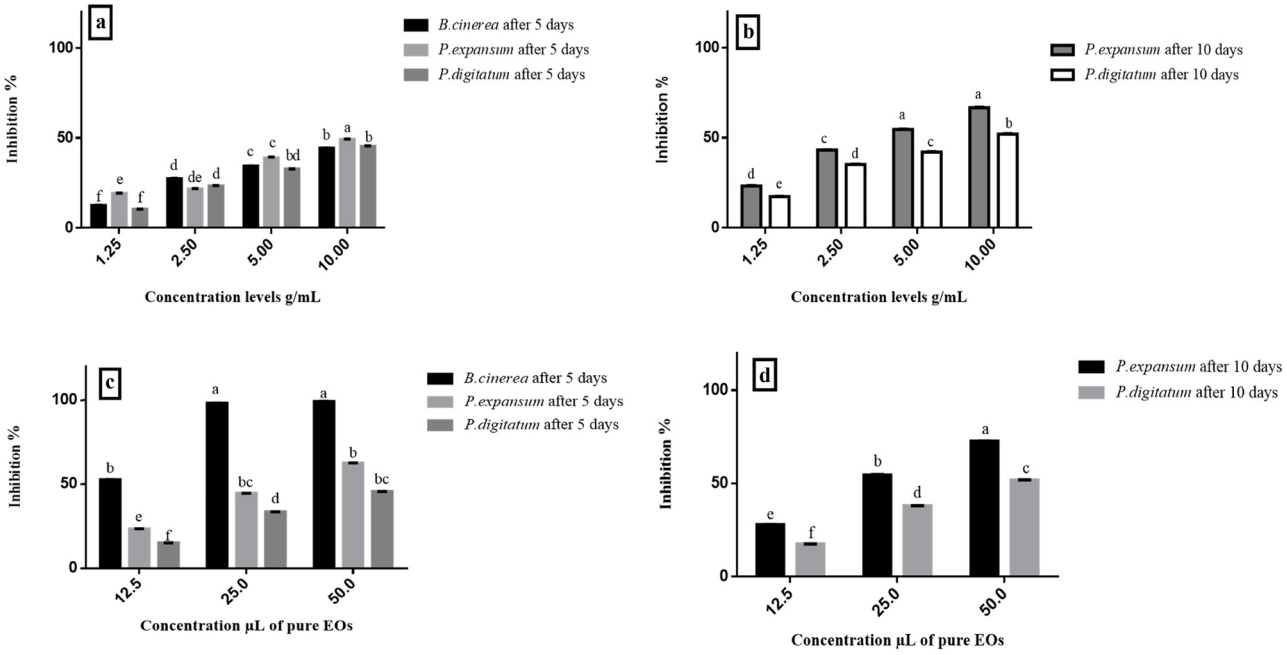

Figure 4: *In vitro* impact of the aqueous extract of *A. herba-alba* on *Penicillium expansum*, *Botrytis cinerea*, and *Penicillium digitatum* for 5 days (a) and on *P. expansum* after and *P. digitatum* for 10 days (b). Similarly, the *in vitro* effect of *A. herba-alba* essential oil on *P. expansum*, *B. cinerea*, and *P. digitatum* after 5 days (c) and on *P. expansum* and *P. digitatum* after 10 days (d). Nb: *B. cinerea* was only evaluated in 5 days due to its rapid growth. (a–f) Values represent means ($n = 3$) ± standard deviations. In each column, the values followed by the same letter are not significantly different according to the analysis of variance and Tukey test ($p < 0.05$).

incubation time (Figure 4). In the four concentrations examined, it was observed that the rate of mycelial growth was significantly reduced, ranging from 19.16 to 49.76% on the fifth day of incubation. It was also noted that the highest inhibition rate was observed with the aqueous extract at the 10% concentration, reaching 66.76%. In addition, the effects on mycelial inhibition were reduced in the *B. cinerea* species, ranging from 12.03 to 44.2 % after the fifth day of incubation, and in *P. digitatum* with an inhibition rate varying between 17.37 and 52% on the tenth day of incubation, also depending on the concentrations of the extract used. Fumigation bioassays with *A. herba-alba* EO showed that the EO of this plant exerted very significant ($p < 0.05$) antifungal activity against *B. cinerea* fungi with inhibition percentages ranging from 52.5, 98.1 and 99.0% (for concentrations of 12.5, 25 and 50 µL respectively). Subsequently, *P. expansum* also showed remarkable sensitivity ($p < 0.05$) to *A. herba-alba* EO, with percentages of inhibition ranging from 27.90, 54.54, and 72.70% for the same concentrations studied, during the 10-day incubation period, when the results reached their maximum. In the case of the *P. digitatum* fungus, moderate sensitivity was observed, reaching its maximum value at the 50 µL concentration, with an inhibition percentage of 51.8% after 10 days' incubation at an ambient temperature of 21°C.

Plants use some mechanisms to protect and defend themselves against external attacks from pests, animals, weeds, and, in particular, harmful micro-organisms. There are two main types of defense mechanisms: mechanical and chemical. For the first type, plants can use the secretions of tree gums, solid bark, or thorns. The second is based on the use of secondary metabolites (polyphenols, alkaloids, flavonoids, tannins, etc.), which in turn can help to make the organs unpalatable or even toxic [41]. According to previous studies, the aqueous extracts derived from the aerial parts of *A. herba-alba* showed antimicrobial activity against both *Fusarium graminearum* and *F. sporotrichioides* [65]. In addition, the presence of polyphenols affects biomembranes, as well as inhibiting enzymes and DNA alkylation. For their part, the presence of tannins can inactivate microbial adhesins, certain enzymes, and proteins that transport cell envelopes [66]. It can therefore be proposed that the inhibition of mycelial growth of *B. cinerea*, *P. expansum*, and *P. digitatum* may be due to the presence of polyphenols and tannins as well as bioactive molecules revealed by HPLC analyses, and this can be confirmed by the study carried out in 2007 [67], in which it is proposed that alkaloids and their derivatives have significant antifungal activity. In reality, there is not a great deal of documentation on how products extracted from nature prevent the proliferation of fungi, since the development of an antifungal agent is linked to the ultrastructure of fungi; however, in the majority of cases, it is researchers who have developed ideas based on the operating principles of synthetic molecules.

Regarding the literature, *A. herba-alba* has been reported among plants rich in bioactive molecules that play several roles at various domains. Unlike the aqueous extract, the essential oil of *A. herba-alba* showed significant inhibitory activity, and this may be due, according to the results of our GC-MS analysis, to the presence of camphor, thymol, eucalyptol, camphene, and pinocarvone. According to the research conducted in 2012 [68], thymol is the most virulent antifungal compound against the four fungi tested *Rhizoctonia solani*, *Fusarium oxysporum*, *P. expansum*, and *Aspergillus niger*. Also, he added that the antifungal activity of thymol can be compared to that of the fungicide carbendazim, also adding that (S)-limonene and 1,8-cineole have remarkable activity against the four fungi previously mentioned. This previous study also showed that thymol had a strong inhibitory-promoting effect on cellulase isolated from *P. digitatum* with IC_{50} = 6.08 mg/L and that this compound (thymol) had a strong inhibitory effect on polyphenol oxidase (PPO) isolated from *P. digitatum*. Mycelial inhibition can be affected by several factors, and according to a study carried out in Morocco [10], it was found that inhibition depends on the harvesting period of the *A. herba-alba* plant. For the two molds *P. digitatum* and *A. niger*, they showed significant sensitivity to EOs collected in September and June of the plant studied, while *P. expansum* showed resistance to these EOs except for the month of March. We can also add that mycelial inhibition can be affected depending on the species of the same genus of the plant studied. According to this previous study [69], *Artemisia campestris* L. exerts a significant inhibitory effect on the mycelial growth of two molds *B. cinerea* and *P. expansum* with 87.06 and 49.75% during 6 days of incubation and with a concentration of 20 µL, and these results are in agreement with the results obtained in our study.

In conclusion, based on the analyses carried out previously, it appears that the *A. herba-alba* plant is rich in interesting bioactive molecules such as polyphenols, tannins, and alkaloids for the extracts, as well as camphor, 1,8-cineole, α- and β-thujone, eucalyptol, and thymol for the essential oils. These compounds are used for the inhibition of several antifungal, and antioxidant activities, and insecticides. So this plant can be applied in various fields of cosmetics, pharmaceuticals, and also in agroecological applications [10].

4 Conclusion

The present study was carried out on the *A. herba-alba* plant from North-East Morocco to achieve one main

objective, which is to enhance the value of the plant to publicize its benefits for preserving harvests and, in particular, combating postharvest diseases that attack fruit and vegetables. The results of this study show that extracts (aqueous, ethanolic, and hexane-based) are rich in phenolic compounds such as flavonoids (43.59 mg QE/g E) and tannins (32.58 mg GAE/g E), as well as volatile compounds present at the EO level. So we can say that the *A. herba-alba* plant has significant antioxidant and antifungal activities thanks to these bioactive compounds (camphor, endo-borneol, eucalyptol, thymol, trans-verbenol, pinocarvone, and ledol). Nevertheless, further *in vivo* research is recommended to determine the biological characteristics of these substances, as well as their adverse effects, before proceeding with the use or distribution of these products.

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