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Moroccan Endemic *Artemisia herba-alba* Essential Oil: GC-MS Analysis and Antibacterial and Antifungal Investigation

Habiba Houti [1], Mohamed Ghanmi [2], Badr Satrani [2], Fouad El Mansouri [3,*], Francesco Cacciola [4,*], Moulay Sadiki [5] and Abdellatif Boukir [1,*]

[1] Laboratory of Microbial Biotechnology and Bioactive Molecules LB2MB, Faculty of Sciences and Techniques of Fez, University Sidi Mohammed Ben Abdellah, Imouzzer Road, P.O. Box 2202, Fez 30007, Morocco
[2] Chemistry and Microbiology Laboratories, Forest Research Center, P.O. Box 763, Rabat Agdal 10050, Morocco
[3] Research Team: Materials, Environment and Sustainable Development (MEDD), Faculty of Sciences and Techniques of Tangier, Abdelmalek Essaâdi University, P.O. Box 416, Tangier 93000, Morocco
[4] Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, 98125 Messina, Italy
[5] Geo-Bio-Environment and Innovation Engineering Laboratory, Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Agadir 80000, Morocco
* Correspondence: fouad.elmansouri@etu.uae.ac.ma (F.E.M.); cacciolaf@unime.it (F.C.); abdellatif.boukir@usmba.ac.ma (A.B.); Tel.: +212-662-102-847 (F.E.M.); +39-090-676-6570 (F.C.); +212-668-495-147 (A.B.)

Abstract: In Morocco, the endemic *Artemisia herba-alba* is well known by its traditional uses and health benefits. The search for natural, safe, and effective antibacterial and antifungal agents from plants is in high demand due to microbial and fungal resistance to conventional synthetic antibiotics and antifungal drugs. In this study, the *A. herba-alba* was collected from the region of Fez-Boulemane during the periods of March, June, and September. Essential oils (EOs) were extracted from the aerial part of the plant by the hydrodistillation method. The chemical constituents were determined using GC-MS as analytical tools. The antimicrobial activities of different oils were tested using the macrodilution method. The results showed the difference in the yields between the three EOs (0.49, 1.74, 1.30% (mL/100 g)), respectively, as well as in their corresponding chemical compositions. The main constituents revealed by GC-MS are higher contents of oxygenated monoterpenes (84.7, 84.4, 81%), such as cis chrysanthenyl acetate (30, 26.7, 27.6%), β-thujone (23.2, 12.9, 15.4%), camphor (9.76, 14.3, 15.8%), chrysanthenone (2.4, 1, 14%), 1,8-cineole (1.5, 11.7, 11.8%), trans β-dihydroterpineol (7.8, 7.2, 6.9%), α-thujone (4.8, 3, 5.4%), and sesquiterpenic davanone (3.9, 1.5, 1.4%), respectively. The three EOs biological activities' results showed significant antimicrobial effects against four bacteria tested (*E. coli, B. subtilis, S. aureus*, *M. luteus*), with the MIC values ranging from 0.1 to 0.03% (*v*/*v*), as well as interesting antifungal effects on both wood rot fungi against four fungi examined (*G. trabeum*, *P. placenta*, *C. puteana*, *C. versicolor*) and molds against three microorganisms tested (*A. niger, P. digitatum*, *P. expansum*), with MIC values ranging from 0.2 to 0.03% (*v*/*v*) and 0.4 to 0.03% (*v*/*v*), respectively. The June and September EO samples showed more potent activities than those collected during March. Our research findings showed quantitative variability in both EO contents and chemical compositions, which could be due to the phenological stages, climatic conditions of growth, and harvesting periods. The potent results of the antimicrobial/antifungal activities were provided by the EOs of June and September and might be correlated to the contribution and synergism effect of all oxygenated monoterpenes. These results support the possible application of *A. herba-alba* EOs as natural and safe antibacterial agents, and an effective alternative to synthetic drugs, enabling the prevention and treatment of certain pathogenic infections in food and health, and the preservation of wood alteration against fungi.

Keywords: *Artemisia herba-alba*; GC-MS analysis; essential oil; antimicrobial activities; antifungal activities

Citation: Houti, H.; Ghanmi, M.; Satrani, B.; Mansouri, F.E.; Cacciola, F.; Sadiki, M.; Boukir, A. Moroccan Endemic *Artemisia herba-alba* Essential Oil: GC-MS Analysis and Antibacterial and Antifungal Investigation. *Separations* **2023**, *10*, 59. https://doi.org/10.3390/separations10010059

Academic Editors: Paraskevas D. Tzanavaras

Received: 18 November 2022
Revised: 21 December 2022
Accepted: 12 January 2023
Published: 16 January 2023

1. Introduction

Morocco has a vegetal flora that is rich and diversified. Among the medicinal plants that constitute vegetal cover, the genus *Artemisia* is a greenish-silver perennial, biennial, annual herb dwarf and evergreen shrub, growing widely in arid and semiarid climates as well as in cold and subhumid regions, warm climates, and muddy areas [1–3]. *Artemisia herba-alba* Asso, called by the common name wormwood, is widespread throughout the northern half of the world, found in the steppes and deserts of North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt) [4,5], the Middle East (Turkey, desert of Sinai, Jordan, and Saudi Arabia), Asia and the Northwestern Himalayas, and extending into Europe (Spain) and North America [6–8]. This perennial herb is abundant in Morocco, where it inhabits clay steppes, meadows, rocky earth plateaus, and low mountains in dry and piedmont regions. It grows in stony lands and pastures, and spans a wide geographical distribution in Morocco (Est, Est-Rif, Middle-Atlas, High-Atlas, Saharan-Atlas) under a hot Saharan bioclimate [1,6,8].

Artemisia herba-alba Asso is commonly known as "chih" in Arabic or "Ifsi" in Berber. It is known for its therapeutic and medicinal properties and has been widely used in traditional Arab-Muslim culture as a source of green nanoinsecticides against mosquito vectors [9], and in modern medicine [10] to treat bronchitis, hypertension, neuralgia, and stomach disorders. It can be consumed as a carminative and presents a wormer effect during diseases [11].

In addition, different types of *Artemisia* species represent a source of economic support for local (traditional activities) or nonlocal (recent activities) populations in several fields: cosmetics, food, medicine, and folk medicine, ethnopharmacology (as an analgesic and hemostatic agent), and pharmaceuticals [7,10]. Referring to previous studies concerning the medicinal properties of *Artemisia herba-alba* essential oil, numerous biological and pharmacological activities were reported, such as antioxidant [12,13], antifungal [14], and antispasmodic activity [15]; being a natural and alternative antibacterial agent [16]; neuroprotective effects; anti-inflammatory effects with high potency against COX-1; and a reduction of the effects of ischemia [17]. In addition, the essential oil extracted from seeds was known for therapeutic virtues, such as being antiangiogenic [18], anticancer, and antifungal, and presenting an insecticidal effect [19]. Moreover, other beneficial effects well known in folk medicine have been manifested against diseases, such as the treatment of eczema, pimples and sores [13,20], hepatitis [21], hair loss, cough, fever [22], poisoning, and vomiting, as well as the healing of external wounds [23] and use as a relaxation agent [6,8,18].

Recently, Younsi et al. 2018 [24] reported on the relationship between the interpopulation chemotype variation and genetic diversity of *Artemisia herba-alba* growing in Tunisia, using for discrimination the PCA (principal component analysis) method. Concerning the chemical composition, numerous studies in the literature have focused their investigation on the secondary metabolite constituents in both essential oils and extracts containing bioactive compounds. Phytochemical studies have shown their richness with new biologically active compounds that are described as promising for the treatment of cancer [25]. Among the constituents of oxygenated monoterpenes EO (75%) which present different chemical classes (alcohol, ether, ketone, ester), Al-Shuneigat et al. (2015) [26] indicated the presence of the three predominant components (cis-chrysanthenol, 1,8-cineole; α-terpinenol), accounting for 33.6%, while the remaining 41.4% is attributed to the 33 compounds that are in minority (γ-terpinenol; terpinen-4-ol acetate, trans-thujone, carvenone, cis-chrysanthenyl acetate, isobornyl acetate, iso-menthyl acetate, Z-jasmone . . .). Based on a previous literature review [27–31], a wide variety of dominated and nondominated EO compounds are responsible for biological activities, and their mixture provides a noticeable inhibitory effect, which is more pronounced than those obtained by the main components [16]. This can be explained by the involvement of the synergistic effect between the oxygenated monoterpenoid molecules [16,17,32,33]. Other studies in the literature have reported that the lactonic sesquiterpenes (artemisinin, eudesmanolide, artemin, artemisolide) [24] as well

as flavones (eupatilin, jaceosidin, circilineol, 6-methoxytricin) and flavon-3-ols (genkwanin, kamatakenin, isorhamnetol, eupatorin, and myricetin 3, 3′,4′-trimethyl) are also responsible for biological activities [25].

Referring to recent studies [17] and references therein as well as the past works [34,35], the chemical composition of the EOs could be impacted by several biotic and abiotic factors, such as plant components; genetic diversity; geographical location, climate conditions (annual rainfall, arid and semiarid climate, hydric stress such as droughts or floods), especially during a plant's developmental stages; soil characteristics (soil salinity, silt, clay ...); growing conditions; collecting time; and season of harvesting. All the above-mentioned factors are responsible for the formation of many interpopulation chemotype variations and phytochemical polymorphisms (forming new molecules), presenting potent chemical bioactive constituents allowing for the treatment of numerous diseases.

According to previous scientific studies [36–39], the emergence and development of resistant bacterial strains towards synthetic antibiotics drugs has increased during the last few years, posing major problems to the public health and food industries concerning the effectiveness and the success of synthetic antibacterial treatments. This has prompted researchers to find other alternative pathways by exploring the new constituents of natural plants considered as safe, nontoxic, ecofriendly, and presenting crucial biological activity, allowing for the circumvention of synthetic drugs' resistance problems and the improvement of the EOs' antibacterial power.

In recent study conducted by Jaradat et al. (2022) [17], the mixture of *A. arborescens* EOs exhibited a remarkable bacterial inhibitory effect compared to Ampicillin and Ciprofloxacin synthetic antibiotics, which indicate a weak effect. The EOs tested showed more significant inhibition on the amplitude, desensitization, and deactivation mechanisms generated by the receptors. The noticeable activities could be correlated to the presence of oxygenated monoterpenoid components, which represent 90 to 96%. The presence of thujone as well as monoterpenic hydrocarbons in EOs could damage the cellular integrity, resulting in cell permeability alteration and the inhibition of respiration [40,41]. In the same trend, Marsoul et al. (2020) [36] indicated that phenolic compounds could affect the cell membrane structure by disrupting the lipid structures of the cells, altering the permeability of subcellular structures and causing the damage to the cell membranes as well as cell walls (reduction in intracellular ATP concentrations, cell membrane depolarization, leakage of cytoplasm membrane, decrease in bacterial protein content); it results an inhibition of bacterial and fungal growth.

Concerning the wood biomaterial, it is used in various applications such as buildings and public artworks, historical monuments (wooden museums) for cultural heritage. and wooden artifacts.

Several studies in the literature have reported the involvement of fungi in the biodeterioration of wood constituents, leading to the destruction of the chemical biopolymeric structures, and thus resulting in wood rots [37,42–45] as well as changes in the color of wood [46]. According to Magdalena (2022) [37] and Pandey (2003) [47], brown-rot fungi selectively decay structural carbohydrates with limited lignin degradation, while white rot fungi are not selective, depolymerizing all three major wood components (hemicellulose, cellulose, and lignin); however, some kinds, such as *Phellinus pini*, decay only hemicellulose and lignin, making the cellulose relatively unaltered. To overcome the problem of wood alteration, numerous scientific investigations have been reported using some synthetic antifungal preservative agents (Fluconazole, itranazole, ketonazole), or fungistatic chemical preventive agents such as creosote or pentachlorophenol (organochlorine family) [37]. Some organochlorines, such as pentachlorobenzene and hexachlorobenzene, are well known by their toxicity and pollutant character to the environment, appertaining to the persistent organic pollutants (POPs) family, and are already banned by the Stockholm Convention [48]. Referring to a recent scientific report [17], the action of synthetic Fluconazole revealed a weak antifungal effect when compared to the effect of a natural EO mixture deriving from *A. arborescens*.

Molds and blue stain fungi (sapstain) usually do not cause significant damage to the wood structure, but degrade the nutrient reserves of the wood (mainly extractives and water soluble components); however, *Aspergillus niger, A. versicolor, Penicillium brevicompactum,* and *Rhizopus spp.* can attack wood and wood products [37]. Molds impact the mechanical resistance of the cellulose, hemicellulose and lignin chains, causing enormous disruption and alteration to the biopolymeric chain structures [49–51]. In addition, the molds are also known to be causative agents of food rot, leading to certain forms of toxicity and pathogenicity (toxi-infections) in humans and animals that have consumed altered food products [52].

Accordingly, this study deals with essential oils of the endemic plant *Artemisia herba-alba*, originating from the mountainous area of the Middle-Atlas (Morocco), and collected during the three periods of March, June, and September.

It aims to:

- Investigate the effects of the phenological stage, climatic conditions of growth, and season of harvesting on both essential oil yields and variability of the chemical compositions, with updated literature data up to 2022.
- Evaluate the antibacterial effect against four pathogenic bacteria, aiming to support the potential uses of *A. herba-alba* essential oil as an alternative and safe antimicrobial agent in food and health.
- Determine the antifungal activity toward four types of wood rot fungi, known by their implication in the decay and biodegradation of wood biopolymeric chains and historical and cultural wooden monument structures, as well as the coloring change of rotten wood. The evaluation concerns antifungal activity toward three types of mold, selected on the basis of their involvement in the food alteration phenomenon (food rot), that could generate food infectious diseases to humans and animals. The main goal of this section of study is to support the application of *A. herba-alba* essential oil as an alternative ecofriendly and natural antifungal agent enabling the prevention and preservation of wood against decays, as well as the protection of food against alteration and infection in order to reduce food safety problems.

2. Materials and Methods

2.1. Plant Material

The samples of aerial parts of *Artemisia herba-alba* were collected from the Middle-Atlas area of Morocco (Fez-Boulemane region) in March, June, and September. The choice of sampling during the periods of March, June and September was based on the following considerations:

March is the active vegetation period of the plant where there are developing leaves (usually spring);
June is the period of appearance of young flower buds, accompanied by the presence of leaf density;
September is the stage of flowering and fruiting of the plant (generally autumn), considered as the date of maturity of the plant, and the flowers mainly develop towards the end of Summer.

2.2. Essential Oil Extraction

The dry aerial parts (leaves and stems) of plant material studied were subjected to hydrodistillation for 1 using a Clevenger-type apparatus. The repeatability of the hydrodistillation was carried out by respecting the recommendations of the French Agency of Safety Health and Health Products AFSSAPS, currently called ANSM [53,54]. The obtained essential oils were kept in the dark at 4 °C until further use.

2.3. Essential Oil Analysis: GC-FID and GC-MS

2.3.1. GC-FID Analysis

Analytical gas chromatography was carried out on a Perkin-Elmer Sigma-115 gas chromatograph (Perkin-Elmer, Waltham, MA, USA) equipped with an FID and a data

handling processor. The separation was achieved using an HP-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Column temperature 40 °C, with 5 min initial hold, and then to 270 °C at 2 °C/min, 270 °C (20 min); injection mode splitless (1 µL of a 1:1000 n-hexane solution). Injector and detector temperatures were 250 °C and 290 °C, respectively. The analysis was also run by using a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm i.d., 0.25 µm film thickness). In both cases, helium was used as the carrier gas (1.0 mL/min).

2.3.2. GC-MS Analysis

Analysis was performed on an Agilent 6850 Ser. II apparatus (Agilent, Roma, Italy), fitted with a fused-silica DB-5 capillary column (30 m × 0.25 mm × i.d. = 0.33 µm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973 (Agilent); ionization energy voltage 70 eV; electron multiplier voltage energy 2000 V. Mass spectra were scanned in the range of 40–500 amu, with a scan time of 5 scan/s. Gas chromatographic conditions were reported in the previous paragraph; transfer line temperature, 295 °C. The identification of the components was carried out based on their Kováts Indices (KI) and the literature data of gas chromatography coupled with the mass spectrometry GC/MS, as well as the comparison with spectral library NIST 98 [55].

2.4. Bacterial and Fungal Strains

The antimicrobial effect of *Artemisia herba-alba* essential oils was tested against the following eleven microbial strains distributed on three sets. The first one included four types of pathogenic bacterial strains (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, and *Micrococcus luteus*). The latest were provided by the American Type Culture Collection (ATCC) and maintained by subculture on nutrient agar favorable to their growth in darkness at 37 °C for 24 h. The second set contained four types of wood rot fungi (*Gloeophyllum trabeum* ATCC 11,539, *Poria placenta* ATCC 9891, *Coniophora puteana* ATCC 9351, and *Coriolus versicolor* ATCC 12,679).

The third includes three types of molds (*Aspergillus niger, Penicillium digitatum*, and *Penicillium expansum*). Both fungal and mold strains were maintained in medium-nutrient potato dextrose agar for seven days at 25 °C in the dark.

2.5. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) represents the lowest essential oil concentration that completely inhibits bacterial growth. It was determined using the agar dilution method according to Remmal et al. [56]. Briefly, the EOs were serially diluted using a sterile agar solution at 0.2% in distilled water. The dilutions were prepared at 10, 4, 2, 1, 0, 5, 0.33, and 0.2% (*v*/*v*). Subsequently, each test tube, containing 13.5 mL of trypto-casein-soy agar (TSA) for bacteria and potato dextrose agar (PDA) for fungi, was sterilized in an autoclave for 20 min at 121 °C and then cooled to 45 °C in a water bath; 1.5 mL of each dilution was aseptically added to obtain a final concentration of 1, 0.4, 0.2, 0.1, 0.05, 0.03, and 0.02% (*v*/*v*). Finally, the tubes were vortexed properly before being poured into Petri dishes.

Controls, containing the culture medium and the agar solution at 0.2% alone, were also prepared.

For bacteria and molds, the inoculation of each isolate per plate was performed by streaking using a calibrated platinum loop. The inoculum suspensions were adjusted to standard McFarland scale 0.5. For wood decay fungi, the inoculation was performed by placing fragments (1 cm in diameter) taken from the periphery of a mycelium cultured for seven days in PDA.

The incubation was performed at 37 °C for 24 h for bacteria and seven days at 25 °C for mold and wood rot fungi. Each test was performed in triplicate.

3. Results and Discussion

3.1. Statistical Analysis

The GC-MS results are illustrated in Table 1, and reported as the means of triplicate analysis. The data obtained were subjected to one-way analysis of variance (ANOVA) to assess the significance of quantitative changes in the variables as a result of the concentrations of the chemical constituents of the essential oils harvested during the three months of March, June, and September (Table 2). Statistical analysis was performed by the Statistical Package for Social Science (IBM SPSS Statistics 23.0, Chicago, IL, USA).

Table 1. Chemical composition of Artemisia herba-alba essential oils (March, June, and September).

N°	Kovats Indices	Components	(%) of Components		
			March	June	September
1	926	tricyclene	----	0.13	0.10
2	929	artemisia triene	0.75	0.73	**1.73**
3	931	α-thujene	----	0.06	0.16
4	943	α-pinene	**2.28**	**4.08**	1.56
5	953	camphene	0.09	0.14	0.16
6	968	sabinene	1.60	0.59	**3.05**
7	972	β-pinene	0.10	0.26	0.18
8	974	cis-pinane	----	0.19	0.08
9	986	myrcene	0.53	0.11	0.91
10	1005	α-phellandrene	----	----	0.08
11	1012	α-terpinene	0.09	0.13	0.18
12	1022	ortho-cymene	0.33	0.51	0.34
13	1031	limonene	0.19	0.11	0.17
14	1033	**1,8-cineole**	1.54	**11.71**	**11.77**
15	1050	E- β-ocimene	0.15	0.51	0.30
16	1062	γ-terpinene	0.11	0.08	0.06
17	1062	artemisia ketone	0.31	0.82	0.12
18	1095	α-pinene oxide	0.19	----	0.13
19	1098	sabinene trans hydrate	----	0.26	0.65
20	1101	**α-thujone**	**4.77**	**3.06**	**5.37**
21	1112	**β-thujone**	**23.24**	**12.85**	**15.36**
22	1118	trans-pinan-2-ol	0.45	0.29	0.28
23	1123	**chrysanthenone**	2.41	1.00	**13.98**
24	1134	terpinol	2.13	1.33	0.94
25	1140	**camphor**	**9.76**	**14.31**	**5.8**
26	1156	β-pinene oxide	0.17	0.12	0.08
27	1158	**trans β-dihydro terpineol**	**7.77**	**7.18**	**6.85**
28	1163	trans β-terpineol	1.54	1.72	0.47
29	1177	terpinen-4-ol	0.52	0.53	0.55
30	1181	thuj-3-en-10-al	0.11	0.06	0.09
31	1183	*p*-cymen-8-ol	----	0.11	0.04
32	1189	α-terpineol	0.22	0.33	0.13
33	1205	trans piperitol	0.20	0.29	0.07
34	1206	*p*-cymen-9-ol	----	0.10	0.22
35	1235	trans chrysanthenyl acetate	0.15	0.10	0.10
36	1252	piperitone	0.19	0.26	0.16
37	1258	**cis chrysanthenyl acetate**	**30.02**	**26.73**	**27.63**
38	1271	neo-3- thujyl acetate	----	----	0.13
39	1281	α-terpinen-7-al	0.18	0.32	----
40	1287	γ-terpinen-7-al	1.28	0.92	0.68
41	1347	α-terpinyl acetate	----	0.28	0.44
42	1391	β-elemene	0.31	0.13	0.16
43	1418	E-caryophyllene	----	0.10	----
44	1467	9-epi E–caryophyllene	0.63	0.60	0.45
45	1477	ô-muurolene	----	0.15	----

Table 1. *Cont.*

N°	Kovats Indices	Components	(%) of Components		
			March	June	September
46	1480	germacrene D	1.12	1.87	0.50
47	1499	α-muurolene	0.16	0.47	----
48	1524	ô-cadinene	----	0.15	----
49	1574	germacrene D-4-ol	----	0.21	----
50	1586	**davanone**	**3.87**	1.54	1.4
Hydrocarbon monoterpenes (%)			**6.17**	**7.89**	**9.72**
Oxygenated monoterpenes (%)			**84.66**	**84.43**	**80.92**
Total (%)			**96.96**	**97.53**	**93.02**
Sesquiterpenes			**6.09**	**5.17**	**2.51**
Aliphatic hydrocarbons			**8.44**	**11.23**	**10.17**
Ketones			**44.55**	**33.84**	**42.19**
Esters			**30.17**	**27.11**	**28.3**
Alcohols			**12.94**	**12.15**	**9.64**
Ethers			**1.90**	**11.83**	**11.98**
Aldehydes			**1.57**	**1.30**	**0.77**

Table 2. Statistical analysis of the means performed by one-way analysis of variance (ANOVA).

Compounds	Type of Analysis	Type of Sample	Mean (%)	Std. Error	Std. Deviation	95% Confidence Interval		ANOVA Test
						Lower Bound	Upper Bound	Sig.
tricyclene	GC-MS	AHEO	0.076	0.039	0.068	−0.092	0.245	0.002
artemisia triene	GC-MS	AHEO	1.070	0.330	0.571	−0.350	2.490	0.000
α-thujene	GC-MS	AHEO	0.073	0.046	0.080	−0.127	0.274	0.001
α-pinene	GC-MS	AHEO	2.640	0.749	1.298	−0.584	5.864	0.000
camphene	GC-MS	AHEO	0.130	0.020	0.036	0.040	0.219	0.001
sabinene	GC-MS	AHEO	1.746	0.713	1.236	−1.325	4.818	0.004
β-pinene	GC-MS	AHEO	0.180	0.046	0.080	−0.018	0.378	0.001
cis-pinane	GC-MS	AHEO	0.090	0.055	0.095	−0.147	0.327	0.011
myrcene	GC-MS	AHEO	0.516	0.231	0.400	−0.477	1.510	0.002
α-phellandrene	GC-MS	AHEO	0.026	0.026	0.046	−0.088	0.141	0.001
α-terpinene	GC-MS	AHEO	0.133	0.026	0.045	0.021	0.245	0.001
ortho-cymene	GC-MS	AHEO	0.393	0.058	0.101	0.142	0.644	0.002
limonene	GC-MS	AHEO	0.156	0.024	0.041	0.053	0.260	0.001
1.8-cineole	GC-MS	AHEO	8.340	3.400	5.889	−6.289	22.969	0.000
E- β-ocimene	GC-MS	AHEO	0.320	0.104	0.180	−0.129	0.769	0.000
γ-terpinene	GC-MS	AHEO	0.083	0.014	0.025	0.020	0.145	0.001
artemisia ketone	GC-MS	AHEO	0.416	0.208	0.361	−0.482	1.315	0.002
α-pinene oxide	GC-MS	AHEO	0.106	0.056	0.097	−0.134	0.347	0.002
sabinene trans hydrate	GC-MS	AHEO	0.303	0.188	0.327	−0.509	1.116	0.000
α-thujone	GC-MS	AHEO	4.400	0.692	1.198	1.422	7.377	0.001
β-thujone	GC-MS	AHEO	17.150	3.130	5.421	3.682	30.617	0.001
trans-pinan-2-ol	GC-MS	AHEO	0.340	0.055	0.095	0.103	0.577	0.002
chrysanthenone	GC-MS	AHEO	5.796	4.111	7.121	−11.895	23.488	0.000
terpinol	GC-MS	AHEO	1.466	0.350	0.606	−0.040	2.973	0.001
camphor	GC-MS	AHEO	9.956	2.458	4.258	−0.621	20.535	0.000
β-pinene oxide	GC-MS	AHEO	0.123	0.026	0.045	0.011	0.235	0.001
trans β-dihydro terpineol	GC-MS	AHEO	7.266	0.269	0.466	6.108	8.424	0.003
trans β-terpineol	GC-MS	AHEO	1.243	0.390	0.675	−0.435	2.922	0.001
terpinen-4-ol	GC-MS	AHEO	0.533	0.008	0.015	0.495	0.571	0.021
thuj-3-en-10-al	GC-MS	AHEO	0.086	0.014	0.025	0.024	0.149	0.002
p-cymen-8-ol	GC-MS	AHEO	0.050	0.032	0.055	−0.088	0.188	0.001
trans piperitol	GC-MS	AHEO	0.186	0.063	0.110	−0.088	0.461	0.002
p-cymen-9-ol	GC-MS	AHEO	0.106	0.063	0.110	−0.167	0.380	0.000
trans chrysanthenyl acetate	GC-MS	AHEO	0.116	0.016	0.028	0.045	0.188	0.001
piperitone	GC-MS	AHEO	0.203	0.029	0.051	0.075	0.330	0.000
cis chrysanthenyl acetate	GC-MS	AHEO	28.126	0.981	1.700	23.902	32.350	0.001
neo-3- thujyl acetate	GC-MS	AHEO	0.043	0.043	0.075	−0.143	0.229	0.004

Table 2. *Cont.*

Compounds	Type of Analysis	Type of Sample	Mean (%)	Std. Error	Std. Deviation	95% Confidence Interval		ANOVA Test
						Lower Bound	Upper Bound	Sig.
α-terpinen-7-al	GC-MS	AHEO	0.166	0.092	0.160	-0.231	0.565	0.001
γ-terpinen-7-al	GC-MS	AHEO	0.960	0.174	0.301	0.209	1.710	0.000
α-terpinyl acetate	GC-MS	AHEO	0.240	0.128	0.222	−0.313	0.793	0.001
β-elemene	GC-MS	AHEO	0.200	0.055	0.096	−0.039	0.439	0.002
E-caryophyllene	GC-MS	AHEO	0.03	0.033	0.057	−0.11	0.17	0.002
9-epi E–caryophyllene	GC-MS	AHEO	0.560	0.055	0.096	0.320	0.799	0.000
ô-muurolene	GC-MS	AHEO	0.050	0.050	0.086	−0.165	0.265	0.001
germacrene D	GC-MS	AHEO	1.163	0.396	0.686	−0.540	2.867	0.001
α-muurolene	GC-MS	AHEO	0.210	0.137	0.238	−0.383	0.803	0.002
ô-cadinene	GC-MS	AHEO	0.050	0.050	0.086	−0.165	0.265	0.000
germacrene D-4-ol	GC-MS	AHEO	0.070	0.070	0.121	−0.231	0.371	0.001
davanone	GC-MS	AHEO	2.270	0.801	1.387	−1.176	5.716	0.000
Monoterpene hydrocarbons (%)	GC-MS	AHEO	7.926	1.024	1.775	3.516	12.336	0.001
Oxygenated monoterpenes (%)	GC-MS	AHEO	83.336	1.210	2.096	78.129	88.543	0.000
Total (%)	GC-MS	AHEO	95.836	1.417	2.455	89.735	101.937	0.001
Sesquiterpenes	GC-MS	AHEO	4.590	1.073	1.859	−0.028	9.208	0.004
Aliphatic hydrocarbons	GC-MS	AHEO	9.946	0.813	1.408	6.448	13.445	0.001
Ketones	GC-MS	AHEO	40.193	3.248	5.627	26.214	54.172	0.011
Esters	GC-MS	AHEO	28.526	0.890	1.542	24.694	32.358	0.002
Alcohols	GC-MS	AHEO	11.576	0.994	1.723	7.296	15.857	0.001
Ethers	GC-MS	AHEO	8.570	3.335	5.776	−5.780	22.920	0.001
Aldehydes	GC-MS	AHEO	1.213	0.234	0.406	0.202	2.224	0.002

Values are averages ± standard deviation of triplicate analysis. Data obtained were subjected to one-way analysis of variance (ANOVA). S: significant ($p < 0.05$). AHEO: Artemisia herba-alba essential oils.

3.2. Essential Oil Yield

The yields obtained of the essential oils of *Artemisia herba-alba* harvested in March, June, and September are 0.49, 1.74, and 1.30% (mL/100 g), respectively. The difference shown could be explained by the influence of both important parameters during the harvest periods: vegetative stage and the effect of climatic conditions.

Our results corroborate those found in the essential oils of Algeria (0.62–1.19%) [57–60] and Tunisia (0.1–1.86%) [61–65]. However, those of Jordan (1.3–3%) [11,66] and Spain (0.41–2.30%) [67] present the highest percentage, while those originating from Egypt showed the lowest value, 0.11% [68].

3.3. Essential Oil Chemical Composition

GC-MS analysis of the three aforementioned essential oils allowed us to identify the presence of a total of compounds ranging between 37 (March) and 47 (September), accounting for 93% (September) and 97.5% (June) of the total essential oils (Table 1). The highest percentage of constituents was manifested during the two early harvest periods of March and June (approximately 97–97.5%) as compared to that of September (93%), except for monoterpene hydrocarbon components (9.7%) instead of (6.2–7.9%). Oxygenated monoterpenes represent the highest proportion of essential oils (81–84.7%), followed by monoterpene hydrocarbons (6.2–9.7%), oxygenated sesquiterpenes (1.4–3.9%), and in the last row, sesquiterpene hydrocarbons (1–3.7%) (Table 1).

According to the results shown in Table 1, the three essential oils of Moroccan *Artemisia herba alba* were found to be rich in six characteristic predominant oxygenated monoterpenes of various proportions: cis chrysanthenyl acetate (26.7–30%), β-thujone (12.9–23%), camphor (5.8–14.3%), chrysanthenone (1–14%), 1,8-cineole (1.5–11.8%), and trans β-dihydro terpineol (6.9–7.8%), as well as davanone (3.9%) as an oxygenated sesquiterpene. The mentioned major constituents are considered a fingerprint of the chemotype of the studied plant. Moreover, other irregular monoterpene hydrocarbons are present in small quantities, including α-pinene (4.1%) and sabinene (3.1%), as well as irregular sesquiterpene hydrocarbons, such as germacrene D (1.9%).

Great variability in the content of constituents was noticed, particularly during the harvest period of June as compared to that of September. The decrease in content was manifested in the case of the β-thujone compound (23 to 12.9%) during the early period of harvest from March to June, and the camphor compound (14.3 to 5.8%) from June to September. As for the two other compounds, 1,8-cineole (1.5 to 11.8%) and chrysanthenone (1 to 14%), they underwent an important increase during the early period from March to June and an extended period of harvest from June to September, respectively. The reduction in the content of camphor from 14.3% (June) to 5.8% (September) partially promoted the formation of chrysanthenone, which appeared in large quantities during the prolonged period of harvest (September) with 14% instead of 1% in June. However, some compounds underwent very few changes in their content, such as α-thujone (3.5 to 4%), trans β-dihydro-terpineol (7.8 to 6.9%), and cis chrysanthenyl acetate (30 to 26.7%). The chemical variability in the content of constituents might be explained by the following parameters: phenological stage; effect of the climatic conditions of growth (rainfall during the month of March (Spring), an increase in both temperature and hydric stress during June (Summer) and September (Autumn), etc.); and the season of harvesting [17,34,35,69].

Additionally, other constituents that are very appreciated by industry also underwent a rate of change in their constitution and deserve to be reported. The davanone component is often requested in the perfume industry and is in high demand, with its highest percentage (3.9%) represented during March; this is more important than α-terpineol (0.3%) used in large quantities by perfumers. Meanwhile, the 1,8-cineole (eucalyptol) component, which is sought in the pharmaceutical and food industries, appears unchanged, with significant amount of 11.8% during the two latest extended periods of June and September. The α- and β-thujones are frequently used for flavoring foods and beverages, and they represent 28% of the mixture (in March) and 21% during the prolonged period (June and September). However, numerous literature data have reported their in vitro neurotoxicity, genotoxicity, and carcinogenicity, with the α-thujone being three times more toxic than the β isomer [70].

The statistical analysis reported in Table 2 shows a significant difference ($p < 0.05$) between the means of concentrations of the chemical constituents of the essential oil detected by GC-MS during the three different harvesting months.

Referring to recent literature data conducted by the previous authors [70], the synthesis pathway of α- and β-thujones is based on four essential steps, starting from precursor geranyldiphosphate (GPP), then neryldiphosphate (NPP), and via the sabinene precursor, which leads to the formation of sabinone after undergoing an oxidation reaction on sabinol, followed by the reduction of its exocyclic double bond.

Based on the above-mentioned recent published work, in our case study, we could advance that the formation of the predominant compounds α- and β-thujone 23%, cis chrysanthenyl acetate 30%, and camphor 9.76% in March, and chresanthenone in September, is due mainly to the contribution of certain unstable and more reactive biyclic monoterpene skeletons, which are obtained via terpenyle cation intermediate. The latter undergoes biosynthetic structural transformations, partially and precociously favoring the formation of the main constituents. Thus, thujones are obtained via the thujane (thujene, sabinene) precursors, while cis chrysanthenyl acetate is obtained via the pinane (α-pinene) precursors and camphor is obtained via the camphane (camphenes that are obtained from α- and β-pinene) precursors. The contribution of the mentioned monoterpene hydrocarbons is justified by their resulting small quantities, not exceeding the sum of 4% (March). Concerning 1.8-cineole, the highest rate (11.8%) was manifested during prolonged times of harvest (June and September). Its related biosynthesis starts through GPP, followed by neryl cation, which promotes the formation of terpenyle cation as an intermediate, enabling the formation of α-terpeniol, which in turn cyclizes to obtain the eucalyptol heterocycle (1.8-cineole).

The produced changes in some proportion during harvest periods might be explained by the proposal biosynthesis pathways of camphor, as well as chrysanthenone via unstable bicyclic monoterpenes (pinene), as illustrated in Figures 1–3.

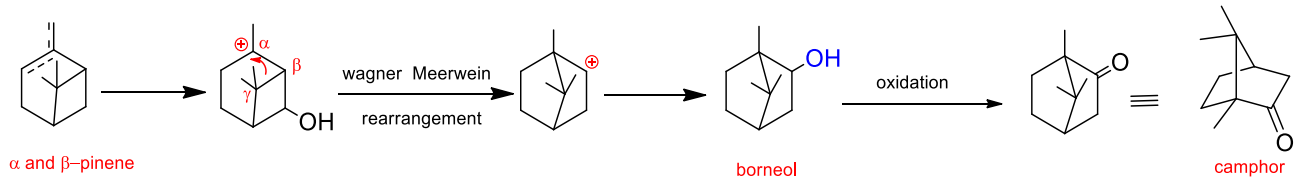

Figure 1. Hypothetic biosynthesis pathway explaining the formation of camphor content via unstable bicyclic monoterpenes (pinene).

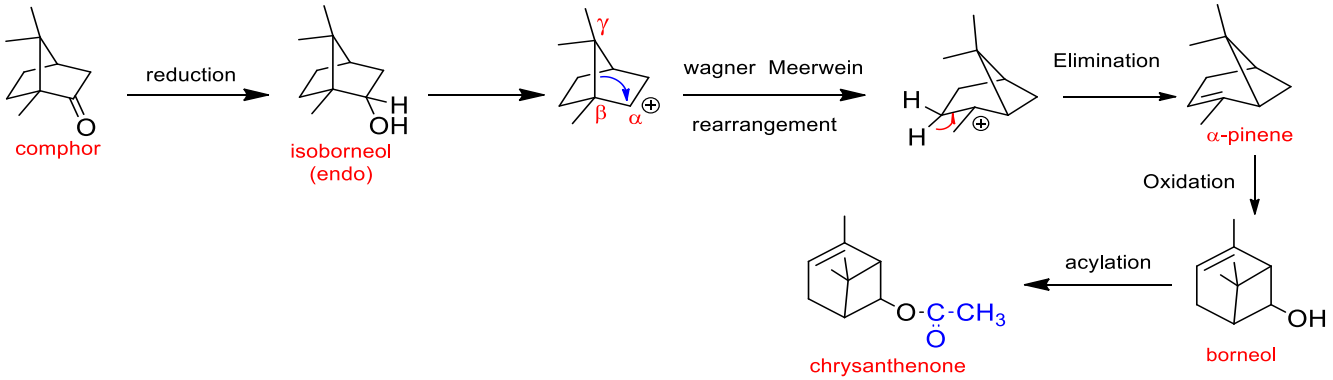

Figure 2. Proposed biosynthesis pathway showing the reduction in the content of camphor (June to September) and toward an increase in chrysanthenone fraction (June to September).

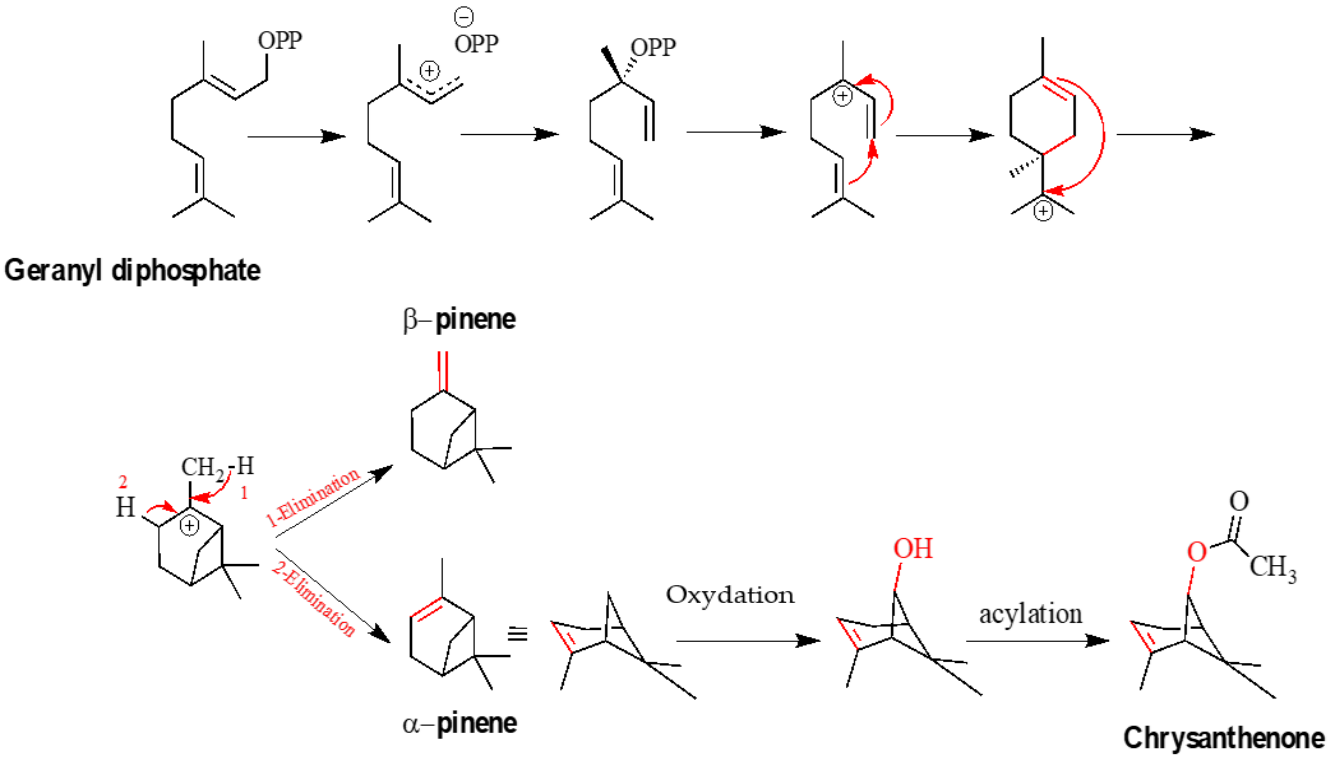

Figure 3. Hypothetic biosynthesis pathway explaining the reduction in the proportion of the pinene fraction toward an increase in chrysanthenone content.

The biosynthesis of chrysanthenyl acetate type compounds (pinene precursors skeleton) (Figure 3) starts from the general precursors geranyldiphosphate (GPP) [71] and neryl-diphosphate through the eight steps of the biosynthetic pathway [72].

The novelty of this work highlights the presence of three new chemotypes contained in the *A. herba-alba* EOs harvested during the following three periods (March, June, and September) and impacted by abiotic/biotic factors, as well as their chemical variability intraspecific. Then, we obtain an idea of the type of EO chemical constituents (mixture) involved in the improvement of the antibacterial and antifungal effects. The GC-MS analyses revealed that the chemotype of the *A. herba-alba* plant EO collected during the period of March is mainly dominated by the following six components: chrysanthenyl acetate (30%), β-thujone (23.2%), camphor (9.76%), trans β-dihydroterpineol (7.8%), α-thujone (4.8%) and sesquiterpenic davanone (3.9%). That of the period of June is characterized by the presence of five main components, with 1,8-cineole as new product: chrysanthenyl acetate (26.7%), β-thujone (12.9%), camphor (14.3%), 1,8-cineole (11.7%), and trans β-dihydroterpineol (7.2%). As for that of September, it is characterized by the presence of six majority compounds, of which chrysanthenone appears as a new former: chrysanthenyl acetate (27.6%), β-thujone (15.4%), camphor (15.8%), chrysanthenone (14%), 1,8-cineole (11.8%), trans β-dihydroterpineol (6.9%), and α-thujone (5.4%).

A comparison of our findings to numerous recent and updated literature data (from 2015 to 2022) of A. herba-alba essential oils (Table 2) reveals that the most predominant previously reported chemotypes are α/β-thujone, camphor, and chrysanthenone, which are present in our findings, with a surplus of another major compound, cis chrysanthenyl acetate. Sabinyl acetate (13%) and both α- and β-thujone (57.3%) have already been described as a new chemotype of A. herba-alba growing wild in South-East Morocco in the region of Ziz [19]. However, a too-significant difference has been recorded as compared to Saudi Arabian essential oils (EOs), indicating the presence of other, mainly unusual compounds such as piperitone (44.6%) and (Z)- and (E)-ethylcinnamate (14.7%, 4.6%) [73]. In the case of Northern African EOs, the change has not affected the preponderant constituents (α/β-thujone, camphor, chrysanthenone), but rather concerns those presenting a relative proportion, such as verbenone (8.3%), sabinol (7.5%), carvone (5.1%) [74], and filifolone (12.7%) [75]. In the case of Algerian EOs, the following components with different proportions were manifested: norbornan-2one (25.7%), enorborane (5.5%), borneol (3%) [76]; while in the case of Tunisian EOs, other unusual compounds were present, such as vanilyl alcohol (11.5%), nor-davanone (7.8%), cis threo-davanafuran (5.8%), isobornyl n-butyrate (4.9%), and trans arbusculone (4.5%) [77]. For Moroccan EOs, the presence of both cis carvyl acetate (2.8%) and borneol (2.6%) was recently reported [78]. In this finding, and according to numerous recent literature data of chemical compositions (Table 2), the Moroccan Middle-East-Atlas oils stand out from the others by the marked coexistence of cis chrysanthenyl acetate and chrysanthenone [19,79], similar to those originating from Libyan EOs [80]. Another factor of discrimination in our study is the appearance of other compounds presenting a moderate amount, such as 1,8-cineole (11.8%), trans β-dihydro terpineol (7.8%), and davanone (3.9%). As for Jordanian EOs, the manifested difference concerns verbenone (8.3%), sabinol (7.5%), carvone (5.1%), and a higher concentration of 1,8-cineole (20%) [11].

Some previous and updated literature results concerning the chemical composition of Artemisia herba-alba essential oils in Northern Africa (Morocco, Algeria, Tunisia, Libya, and Egypt) and the Middle East (Jordan and Saudi Arabia) are summarized and illustrated in Table 3.

The variability in chemical composition related to each plant is influenced by the geographical location, the change in climatic parameters between different countries or different regions in the same country, as well as other influencing factors which contribute to the ascription of the chemotype which is oil-dependent to the target plant [73,79].

The difference observed between the chemical composition of the Artemisia herba-alba essential oil of Morocco and those of the other countries (Table 3) could be explained by an adaptation of the plant to abiotic factors such as the specific climate of the Middle Eastern Atlas.

Table 3. Comparison of updated literature data concerning the main chemical composition and yield of Artemisia herba-alba essential oils.

Geographic Region	Main Compound (%) and Yield	References
Morocco		
Middle-East: Middle-Atlas	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%), germacrene D (0.5–1.9%). Yield: 0.49–1.74%.	[this work]
South-West: Essaouira	β-thujone (24.3%), camphor (22.2%), α-thujone (14.6%), 1,8-cineole (10.3%), camphene (7.8%), cis carvyl acetate (2.8%), borneol (2.6%). Yield: 0.99%.	[78]
Middle-West: Azzemmour	α-thujone (25.5%), β-thujone (17.7%), vanilyl alcohol (11.5%), nor-davanone (7.8%), cis threo-davanafuran (5.8%), isobornyl n-butyrate (4.9%), camphor (4.9%), cis chrysanthenyl acetate (4.7%), trans arbusculone (4.5%). Yield: 0.86%.	[77]
Sarghina and Oulad Ali Youssef,	Trans thujone (33.78%), camphor (18–46%), vetevinic acid (14.91%), dava ether (14.64%). Yield: 0.84–2.19%.	[34]
South-East:	Thujone (48.3%), sabinyl acetate (13%), β-thujone (9%), 1,8-cineole (2.2%), chrysanthenyl acetate (2.1%), chrysanthenone (1.2). Yield: 0.59%.	[19]
Ziz		
Algeria	α-thujone (24.6%), β-thujone (13.73%), verbenone (8.3%), sabinol (7.5%), carvone (5.1%), 1,8-cineole (4.8%). Yield: not given.	[74]
Djemorah		
Bouilef	Chrysanthenone (50.5%), filifolone (12.7%), α-thujone (10%), β-thujone (8.2%), p-cymene (8.2%), camphene (2.4%), camphor (2.3). Yield: 0.6%.	[75]
South Region	α-thujone (23-28%), camphor (17–28%), chrysanthenone (4–19%). Yield: 0.2–0.9%.	[79]
Tunisia	β-thujone (27.8%), camphor (22.7%), chrysanthenone (18%), α-thujone (13.6%). Yield: 2.16%.	[81]
Si Bouzid (Jelma)		
Zaghouan	α-thujone (35.2%), norbornan-2-one (25.7%), chrysanthenone (7.7%), 1,8-cineole (5.8%), 2.2-dimethyl-3-methlen enorborane (5.5%), germacrene D (3.1%), borneol (3%). Yield: 1.48%.	[76]
Kirchaou.	Thujones (11.5%), camphor (13%), sabinyl acetate (12%), ger-macrene D (4%), (E)-ethylcinnamate (2.8%).	[82]
Subarid to Saharan		
Libya	Chrysanthenone (20.8%), chrysanthenyl acetate (17.6%), α-thujone (13.6%), sabinyl acetate (13%), β-thujone (9%), 1,8-cineole (2.2%), trans pinocarveol (1%). Yield: 0.180%.	[80]
Zintan		
Jordan	β-thujone (25.1%), α-thujone (22.9%), 1,8-cineole (20%), verbenone (8.3%), sabinol (7.5%), carvone (5.1%), camphor (10.5%), terpinen-4-ol (2.8%). Yield: 3%.	[11]
Buseirah		
Southern Amman	α- and β-thujones (27.7%), santolina alcohol (13%), artemisia ketone (12.4%), trans-sabinyl acetate (5.4%), caryophyllene ace-tate (5.7%). Yield: 1.3%.	[66]
Saudi Arabia	Piperitone (44.6%), (E)-ethylcinnamate (14.7%), (Z)-ethylcinnamate (4.6%), thymol (3.4%), myrtenyl acetate (3.3%), spathulenol (3.3%), isophorone (1.9%). Yield: 0.051%.	[73]
Egypt	Piperitone (26.5%), ethyl cinnamate (9.5%), camphor (7.7%), hexadecanoic acid (6.9%). Yield: 0.14%.	[68]

In addition, the production of each component is managed by biogenetic mechanisms of formations. Indeed, most of the studies on Artemisia species have shown the effects of different plant growth regulators (PGRs) on secondary metabolism such as cytokinins and gibberellins, which induced the enhancement of secondary metabolites in Artemisia species. For example, cytokinins stimulated the production of phenolics, while gibberellin increased the production of terpenoids [83]. The existence of such communication depends on the presence and concentration of the endogenous precursors of the isoprenoids.

According to El-Amin et al. (2016) [4], chiral thujones and camphor were present in Artemisia herba-alba EOs as a single predominant enantiomeric isomer, characterizing the regional differences between essential oils. As for the mechanism of action of sesquiterpene lactones, it is still unclear; however, their activities are widely correlated to the α- and β-unsaturated carbonyl groups and α-methylene-γ-lactone groups, which react with proteins and other nucleophilic biomolecules [84].

It could be concluded that the phytochemical polymorphism shown could be attributed to the endogenous factors, including the genetic inheritance of the individuals, morphological and DNA polymorphisms, and varieties, as well as exogenous geographical and ecological factors, such as the altitude of the sun, the sun exposure temperature, the type of soil, the amount of precipitation, and the type of soil that directs biosynthesis towards the preferential formation of specific products. Indeed, light is the cornerstone of

the development process of growing plants and can trigger the biosynthetic pathways of phenols and terpenoids and of secondary metabolism. Likewise, environmental parameters, such as the phenological stage of the plant, biotic and abiotic stresses such as fungi and bacteria, or their metabolic products, can exert stress on cells, which in turn produce secondary metabolites as defense products [13,83]. All these data show that the chemical composition of Artemisia herba-alba essential oil is quite variable according to the period and place of harvest.

3.4. Minimal Inhibitory Concentrations (MIC)

The antimicrobial activities of three Artemisia herba-alba essential oils were evaluated against bacteria, molds, and wood rot fungi, and are illustrated in Tables 4 and 5. As can be noted in this finding, all tested essential oils present important antibacterial and antifungal effects. Indeed, the minimal inhibitory concentration (MIC) values ranged from 0.1 to 0.03% (*v*/*v*) against bacteria. The essential oil harvested in September exhibits the highest antibacterial effect against all bacterial strains studied, with an MIC value of 0.03% (*v*/*v*), except for *M. luteus*. Moreover, *S. aureus* and *E. coli* were the most sensitive bacterial strains for all essential oils tested.

Table 4. Determination of MIC values of Artemisia herba-alba essential oil against bacteria.

Concentration % (*v*/*v*)	**1**			**0.4**			**0.2**			**0.1**			**0.05**			**0.03**			**0.02**		
Harvest Period	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**
Bacteria																					
E. coli	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
B. subtilis	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	+	+	+
S. aureus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
M. luteus	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+

M: March; **J:** June; **S:** September; (−): inhibition; (+): growth; positive control: bacterial suspensions and culture medium supplemented with agar solution at 0.2%.

Table 5. Determination of MIC values of Artemisia herba-alba essential oil against molds and wood rot fungi.

Concentration % (*v*/*v*)	**1**			**0.4**			**0.2**			**0.1**			**0.05**			**0.03**			**0.02**		
Harvest Period	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**	**M**	**J**	**S**
Molds																					
A. niger	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	+	−	+	+	+	+
P. digitatum	−	−	−	−	−	−	+	−	−	+	+	−	+	+	−	+	+	−	+	+	+
P. expansum	−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Wood rot fungi																					
G. trabeum	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+
C. puteana	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+
C. versicolor	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
P. placenta	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+

M: March; **J:** June; **S:** September; (−): inhibition; (+): growth; positive control: fungal suspensions/mycelium fragment and culture medium supplemented with agar solution at 0.2% for molds and wood rot fungi, respectively.

Concerning the two molds (*P. digitatum* and *A. niger*), an interesting sensitivity has been shown toward the two essential oils collected in September and June, inhibited by MIC values of 0.03: 0.2% (*v*/*v*) and 0.1, 0.03% (*v*/*v*) respectively. Meanwhile, in the case of *P. expansum*, great resistance has been manifested toward two essential oils tested, except that from March, which recorded an MIC value of 0.1% (*v*/*v*).

Regarding the wood rot fungi, the essential oil harvest in June exhibited an interesting antifungal activity, followed by those of March and September. It could be noted that *C. versicolor* is the most sensitive to all essential oils tested, with the same MIC value of

0.03% (*v*/*v*), followed by *G. trabeum*, with MIC values ranging from 0.05 to 0.03% (*v*/*v*). Meanwhile, *P. placenta* and *C. puteana* were most resistant to the wood rot fungi studied.

Based on the results obtained, the Artemisia herba-alba essential oils of September and June are potentially active against most of the microorganisms tested compared to that of March. In fact, this potent antimicrobial activity could be correlated to their chemical compositions, characterized by the high content of oxygenated hydrocarbons, including cis chrysanthenyl acetate (27.63, 26.73%), β-thujone (15.36, 12.85%), chrysanthenone (13.98%), 1,8-cineole (11.71, 11.77%), trans β-dihydro terpineol (6.85, 7.18%), and camphor (5.8, 14.31%) during the periods of September and June, respectively, and which are known for their signifcant antimicrobial power [16,17,70,85,86].

Artemisia oils rich in camphor, 1,8-cineole, and terpinen-4-ol have previously been reported to inhibit the growth of bacteria and fungi. It has been suggested that diterpenoids derive their activity from their ability to cross or damage bacterial cell membranes, and a variety of acids could inhibit the growth of bacterial and fungal cells [83,84,87]. According to the above-mentioned studies and those carried out by Karabegovic et al. [88], the various biological activities found are correlated to the presence and effect of polyphenols.

The biological activities of the A. herba-alba plant collected during different periods showed a significant variation in bioactivities, which could be attributed to the variability in the content of active molecules according to the seasons [18]. Thereby, the antimicrobial activity difference between the three essential oils tested in this study could be assigned to the variability of change in their qualitative and quantitative chemical composition, which depend on many factors, such as growth stage and extraction conditions, enabling the determination of the plant's chemotaxonomy [89].

The essential oil isolated from the seeds of *Artemisia campestris* L. growing in the wild in eastern Morocco indicated that good antibacterial activity was due mainly to the monoterpene and sesquiterpene oxygen compounds [90,91]. Indeed, sesquiterpene lactones are one of the main defense mechanisms of plants against microbial attacks. They work by disrupting the cell membrane of a microbe—an effect attributable to polar groups on these antimicrobial compounds disrupting the phospholipid membrane [92]. In addition, oils from different species of the genus Artemisia have shown strong antimicrobial activity against phytopathogenic agents and insecticidal activity against harmful insects [7].

In general, the plant does not contain an immune system, so it synthesizes bioactive organic compounds, antifungal proteins, and peptides to defend against pathogens such as fungi [93]. According to the references cited, it is clear that the compounds, such as 1,8-cineole, cis-chrysantenyl acetate, camphor, terpinen-4-ol, and thujone, were known by their interesting antimicrobial activity, [17,70,85,86]. Thus, the interesting biological activity revealed in our findings could be correlated to the mentioned molecules. However, this activity is probably due to the synergistic effect of other components present in the analyzed oil. Thus, it is difficult to correlate the activity of a complex mixture to a single or particular constituent, as the presence of a majority or minority (trace) in the content of compounds could give rise or contribute to the identified antimicrobial activity. In addition, when studying the biological activity in the essential oil matrix, the probable synergistic and antagonistic effects between compounds could take place in microbial inhibition and should also be taken into account. Therefore, it would be interesting to separate the mixture and purify the isolated product, and then test it for its ability to inhibit microbial growth.

Finally, and based on literature data, the richness of Artemisia herba-alba in oxygenated monoterpenes and sesquiterpene (cis chrysanthenyl acetate, α- and β-thujone, camphor, 1,8-cineole, trans β-dihydroterpineol, chrysanthenone, and davanone, known for their antimicrobial, antifungal, and insecticidal activities as well as their antioxidant properties, could support the use of this plant in many industrial applications, including the perfumery and cosmetics domain, food and beverages, biological defenses (biopesticide), and pharmaceutical uses [16,17,19,33,77,85,94].

4. Conclusions

The lowest essential oil yield (0.49%) was correlated to the month of March (flowering period), with major contents of β-thujone (23.24%), cis chrysanthenyl acetate (30%), and davanone (3.87%). In the case of both the harvesting periods of June and September, the yields undergo an increase, reaching 1.74% and 1.3%, respectively, resulting in a variation in chemical composition, consisting mainly of 1,8-cineole (11.7%) and chrysanthenone (13.98%), accompanied by a decrease in the content of β-thujone (15.36%), camphor (5.8%), and davanone (1.4%). This polymorphism could be mainly due to the effect of the vegetative stage and harvest period, deeply influencing both essential oil yields and chemical composition.

In addition, the biological activities also align with this effect. Thus, the results of the antimicrobial activities corresponding to the two periods of June and September revealed to be the most active against microorganisms. Finally, the three tested essential oils presented very interesting antibacterial and antifungal effects, with MIC values ranging from 0.1 to 0.03% (v/v) and from 0.1 to 0.03% (v/v), respectively. This suggests their exploration as a potent and ecofriendly antimicrobial agent for food safety and medicinal uses, as well as a preservative against wood rot.

Author Contributions: Conceptualization, H.H. and M.G.; methodology, A.B.; software, F.E.M., H.H. and M.G.; validation, A.B.; formal analysis, H.H. and B.S.; investigation, H.H., M.S. and B.S.; resources, A.B.; writing—original draft preparation, H.H., M.G. and A.B.; writing—review and editing, A.B., F.C. and F.E.M.; visualization, A.B.; supervision, A.B. and F.C.; project administration, A.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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