Journal of Stress Physiology & Biochemistry, Vol. 20, No. 4, 2024, pp. 52-62 ISSN 1997-0838

ORIGINAL ARTICLE

OPEN ACCESS

Phytochemical Analysis of *Artemisia herba alba* Asso (Asteraceae) Species

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Received May 24, 2024

Phytochemical analysis of *Artemisia herba-alba* Asso (Asteraceae) species has been carried out using fourier-transform infrared spectroscopy (FT-IR) technique and gas chromatography–mass spectrometry (GC-MS) analyses. FT-IR spectra of the aerial parts (buds AB, leaves AL and flowers AF) of *A. herba-alba* powder revealed the presence of 12 peaks, of which 11 common peaks characteristics of the three *A. herba-alba* studied aerial parts. Whereas, the peak of 1632 cm^{-1} [(assigned to Alkenyl C=C stretch-Olefinic (alkene) group)] was observed in AB and AF aerial parts and not in AL. As for GC-MS analysis, data revealed 12 & 10 chemical compounds classes in *A. herba-alba* buds extracts of which, Bicyclic monoterpenoids (37.026 & 49.022%) was presented as a major compound in methanolic and ethanolic buds extracts, respectively. Whereas, 17 & 14 chemical compounds classes were detected in *A. herba-alba* leaves extracts, of which, Fatty acid amides (28.687 & 25.687%) was presented as a major compound in methanolic and ethanolic leaves extracts, respectively. While, 16 & 11 chemical compounds classes were detected in *A. herba-alba* flowers extracts, of which Fatty acid amides (25.623 & 23.295%) was presented as a major compound in methanolic and ethanolic flowers extracts, respectively. These bioactive materials make this species as a good candidates for different pharmaceutical and medicine academic researches and applications.

Key words: Artemisia herba-alba, FT-IR, GC-MS, Phytochemical analysis

Artemisia is a genus belongs to Asteraceae family, and includes approximately 300 species of small herbs and shrubs (Dob and Benabdelkader, 2006). In Syrian Flora, *Artemisia* genus is represented with about 5 species (Mouterde, 1983), of which *Artemisia herba-alba* species wild grown in Syria.

Artemisia herba-alba Asso, known as desert wormwood and as shīeḥ in Arabic. It is a perennial shrub grows commonly on the dry steppes of the Mediterranean regions in Northern Africa (Saharan Maghreb), Western Asia (Arabian Peninsula) and Southwestern Europe (USDA, 2010).

Abou EL-Hamd *et al.* (2010) reported that the sesquiterpene lactones, flavonoids, phenolic compounds & waxes and essential oils were isolated and identified as the main secondary metabolites from *A. herba-alba* and other *Artemisia* species.

It has been reported that the *Artemisia* genus has an important role in folk medicine by many cultures since ancient times (European medicine, North Africa and Arabic traditional medicine) (Moufid and Eddouks, 2012). Of which, *A. herba-alba* herb exhibited many medicinal properties *e.g*. as anti-diabetic, antimicrobial, antioxidant, antiradical, antispasmodic, antihypertensive, antimalarial, anthelmintic, antileishmanial, nematicidal, neurological pesticidal, allelopathic and cytoprotective activities (Abou EL-Hamd *et al.*, 2010; Moufid and Eddouks, 2012; Janaćković *et al.*, 2016; Younsi *et al.*, 2016; Ouchelli *et al.*, 2022; Kadri *et al.*, 2022; Houti *et al.*, 2023).

Moufid and Eddouks (2012) reported that *A. herba alba* biological activity could mainly related to its content of many bioactive compounds e.g. herbalbin, cis-chryanthenyl acetate, flavonoids (hispidulin and cirsilineol), monoterpenes and sesquiterpene.

Phytochemical screening of natural products presented in plants species is requested for any pharmaceutical and medicine researches and applications. In this regards, many different analytical methods have been employed to determine *Artemisia* phytochemical constituents; *e.g.* fourier-transform infrared spectroscopy (FT-IR) (Hameed *et al.*, 2016) and ultra-performance liquid chromatography (UPLC) coupled to photodiode array detection (PDA) and mass spectrometry (MS) (UPLC-PDA-MS) (Dane *et al.*, 2015).

Gas chromatography–mass spectrometry (GC-MS) analysis has been extensively used for phytochemical screening of *A. herba-alba* species worldwide ((Bellili *et al.*, 2016; Janaćković *et al.*, 2016; Younsi *et al.*, 2016; Amkiss *et al.*, 2021; Ouguirti *et al.*, 2021; Kadri *et al.*, 2022; Ouchelli *et al.*, 2022; Houti *et al.*, 2023).

Little is known about phytochemical screening of *A. herba-alba* species in Syria. Thereby, the presented study focused on its phytochemical analysis during different growth stages using FT-IR and GC-MS analyses.

MATERIALS AND METHODS

Plant materials and samples preparation

Artemisia herba-alba Asso aerial parts (10 plants/part) including buds (AB), leaves (AL) and flowers (AF) were collected separately from wild *A. herba-alba* species grown in their natural habitat from rural Damascus regions-Syria (altitude of 950 m and annual rainfall of 240 mm). Samples were shade dried for two weeks, and were milled to fine powder by special electric mill and stored separately in glass bowls until FT-IR and GC-MS analyses.

FT-IR analysis

The fine powder was used as template for FT-IR analysis in the wavenumber range of 3500-500 cm^{-1}. IR measurement has been performed using NXR FTIR (Thermo, USA) instrument for FT-IR analysis.

Plants extracts preparation

The fine powder for each sample was extracted with methanol and ethanol solvents, separately as flowing: 1 g of fine powder was extracted with 10 mL solvent overnight, filtrated with filter papers (Whatman no.1). Then, all extracts were kept in tightly fitting stopper bottles and stored at 4 °C. The final obtained extracts were then analyzed using GC-MS analysis.

GC-MS analysis

GC Chromatec-Crystal 5000 system, supported with Chromatec Crystal Mass Spectrometry Detector (Chromatec, Russia) has been employed to investigate

phytochemical methanolic and ethanolic *A. herba-alba* aerial parts extracts analysis. GC-MS analysis has been performed according to the following conditions: The range scan was 42-850 MU, the column [(BP-5-MS (30 m × 0.25 mm × 0.25 µm)], carrier gas (0.695 ml/min flow of Helium gas). Oven temperature was programmed initially at 35 °C for 1 min, then an increase by 10°C /1 min till 220 °C, then increase to 230 °C by 1°C /1 min followed by 10 °C /1 min increasing till 255 °C (hold for 5 min). Injector temperature was 275 °C and detector temperature was 280 °C and ionization energy was 70 ev.

RESULTS AND DISCUSSION

FT-IR spectra wavelength of the aerial parts (buds AB, leaves AL and flowers AF) of *A. herba-alba* powder was presented in Figure 1. FT-IR analysis revealed the presence of 12 peaks, of which 11 common peaks characteristics of the three *A. herba-alba* studied aerial parts (Table 1). These common peaks were: 876 cm^{-1} (assigned to =C-H oop bend-Aromatics group); 1055 cm^{-1} [(assigned to Methyne (CH–) Cyclohexane ring vibrations-Saturated aliphatic (alkane/alkyl) group)]; 1160, 1364 and 1733 cm^{-1} (assigned to C–O secondary alcohol stretch C–O stretch-Ethers group); 1265 cm^{-1} (assigned to C–O stretch-Carboxylic acid group); 1445 & 1515 cm^{-1} (assigned to C=C stretch aromatic-Aromatics group); 2850 and 2924 cm^{-1} (assigned to C–H stretch-Alkanes group) and 3425 cm^{-1} (assigned to Hydroxy group, H-bonded OH stretch-Alcohol and hydroxyl group). Whereas, the peak of 1632 cm^{-1} [(assigned to Alkenyl C=C stretch-Olefinic (alkene) group)] was observed in AB and AF aerial parts and not in AL.

Artemisia herba-alba aerial parts (buds AB, leaves AL and flowers AF) grown in rural Damascus regions, were phytochemically analyzed using FT-IR technique. Overall, FTIR spectra showed Aromatics (3 groups), Ethers (3 groups), Alkanes (2 groups), Saturated aliphatic (alkane/alkyl) (1 group), Carboxylic acids (1 group) and Alcohol & hydroxy (1 group) as common functional groups. Whereas, Olefinic (alkene) group was observed in AB and AF aerial parts and not in AL.

As for GC-MS analysis, chromatogram of the aerial parts of methanolic buds (A), ethanolic buds (B), methanolic leaves (C), ethanolic leaves (D), methanolic flowers (E) and ethanolic flowers (F) *A. herba-alba* extracts using GC-MS analysis has been presented in Figure 2.

It worth noting that the chemical compounds classes presented in scare amounts (≤ 1%) did not recorded. GC-MS data revealed 12 & 10 chemical compounds classes in *A. herba-alba* buds extracts of which, Bicyclic monoterpenoids (37.026 & 49.022%) was presented as a major compound in methanolic and ethanolic buds extracts, respectively (Table 2). Otherwise, 7 common chemical compounds classes were detected in *A. herba-alba* buds extracts: Bicyclic monoterpenoids (37.026 & 49.022%), Bicyclic ether (10.057 & 10.083%), Monoterpenoids (1.876 & 2.708%), Long-chain fatty acids (1.255 & 2.061%), Fatty acid amides (15.471 & 11.283%), Carboximidic acids (11.851 & 5.929%) and Sesquiterpenoids (2.155 & 6.188%) in methanolic and ethanolic buds extracts, respectively (Table 2).

Whereas, 17 & 14 chemical compounds classes were detected in *A. herba-alba* leaves extracts, of which, Fatty acid amides (28.687 & 25.687%) was presented as a major compound in methanolic and ethanolic leaves extracts, respectively (Table 3). Otherwise, 5 common chemical compounds classes were detected in *A. herba-alba* leaves extracts: Fatty acid amides (28.687 & 25.687%), Bicyclic ether (5.392 & 8.789%), Bicyclic monoterpenoids (2.325 & 2.581%), Fatty amides (21.275 & 19.683%) and ClassyFire Class: Fatty acyls (4.659 & 7.920%) in methanolic and ethanolic leaves extracts, respectively (Table 3).

While, 16 & 11 chemical compounds classes were detected in *A. herba-alba* flowers extracts, of which Fatty acid amides (25.623 & 23.295%) was presented as a major compound in methanolic and ethanolic flowers extracts, respectively (Table 4). Otherwise, 9 common chemical compounds classes were detected in *A. herba-alba* flowers extracts: Fatty acid amides (25.623 & 23.295%), Bicyclic ether (11.879 & 9.408%), Bicyclic monoterpenoids (4.000 & 13.144%), Stereoisomers (3.139 & 9.479%), Monoterpene ketone (2.815 & 6.729%), Long-chain fatty acids (24.808 & 1.123%), Fatty amides (2.649 & 5.560%), Terpenoids (3.366 & 3.639%) and Eudesmane

sesquiterpenoid (7.145 & 6.462%) in methanolic and ethanolic flowers extracts, respectively (Table 4).

GC-MS analysis has been extensively employed for *A. herba-alba* essential oils (EOs) phytochemical analysis worldwide. In this regards, Abou El-Hamd *et al.* (2010) reported that 1,8-Cineole (50%), Thujone (27%), Terpinen-4-ol (3.3%), Borneol (3%) and Camphor (3%) were major compounds in EOs *A. herba-alba* from Egypt. Whereas, Tilaoui *et al.* (2015) reported the difference in phytochemicals presented in EOs *A. herba-alba* from Morocco, according to the plant parts used. In this respect, β-thujone was found to be 1.24, 7.00 and 6.14 % and Verbenol was found to be 2.16, 5.99 and 21.83% in leaves, capitulum and aerial parts, respectively. Whereas, Eucalyptol (1,8-Cineole) was found to be 20.37, 7.71, 1.49 and 2.27% in leaves, stems, capitulum and aerial parts, respectively. Moreover, Fekhar *et al.* (2017) reported that the major compounds recorded in Algerian *A. herba-Alba* EOs were Camphor (32.98%), α-Thujone (18.43%), β-Thujone (16.62%) and p-Cymene (13.19%). While, Mohammadhosseini (2017) reported that Camphor (0.2-40.3%), 1,8-Cineole (0.1-19.3%), β-Pinene (0.7-23.6%), Sabinene (0.2-18.6%), Camphene (0.2-24.2%) and α-Pinene (1.1-13.9%) were the major compounds recorded in *A. herba alba* species according to the collection sites in Iran.

Other phytochemical researches have been carried out in other *Artemisia* species using different analytical methods. In this regards, Kumar and Kumud (2010) reported the occurrence of phytosterol, saponins, carbohydrate, tannin, flavonoids, phenolic compounds, amino acid and proteins in hexane and methanolic *A. vulgaris* aerial parts extracts. Whereas, Ruwali *et al.* (2015) reported the occurrence of reducing sugars, carbohydrates, tri-terpenoids, sterols, glycosides, phenolics and flavonoids in methanol, ethanol and hydro-methanol *A. indica* aerial parts extracts. Moreover, Dane *et al.* (2015) reported the occurrence of flavonoid glycosides, flavonoid aglycones and phenolic acids in methanolic *A. absinthium* extracts using UPLC-PDA-MS analysis. Indeed, Enas *et al.* (2015) reported the presence of flavonoids, tannins, saponin, alkaloids, phenols, steroids and glycosides in acetonic *A. annua* extracts. Whereas, Hameed *et al.* (2016) reported the occurrence of C-H Alkenes, C-F stretch Aliphatic fluoro compounds, C-O Alcohols, Ethers, Carboxlic acids, Esters and H-O H-bonded H-X group in methanolic *A. annua* extracts using FT-IR analysis.

Table 1: Observed functional groups in aerial parts of *A. herba-alba* using FT-IR analysis.

Peak N°	IR frequency (cm^{-1})	Observed IR (cm^{-1})	Bond	Functional groups
1	900-690	876	=C-H oop bend	Aromatics
2	1055-1000	1055	Methyne (CH–) Cyclohexane ring vibrations	Saturated aliphatic (alkane/alkyl)
3	2000-1000	1160	C–O secondary alcohol stretch C–O stretch	Ethers
4	1300-1200	1265	C–O stretch	Carboxylic acids
5	2000-1000	1364	C–O secondary alcohol stretch C–O stretch	Ethers
6	1600-1400	1445	C=C stretch aromatic	Aromatics
7	1600-1400	1515	C=C stretch aromatic	Aromatics
8	1680-1620	1632	Alkenyl C=C stretch	Olefinic (alkene)
9	2000-1000	1733	C–O secondary alcohol stretch C–O stretch	Ethers
10	2970-2850	2850	C–H stretch	Alkanes
11	2970-2850	2924	C–H stretch	Alkanes
12	3570-3200	3425	Hydroxy group, H-bonded OH stretch	Alcohol and hydroxy

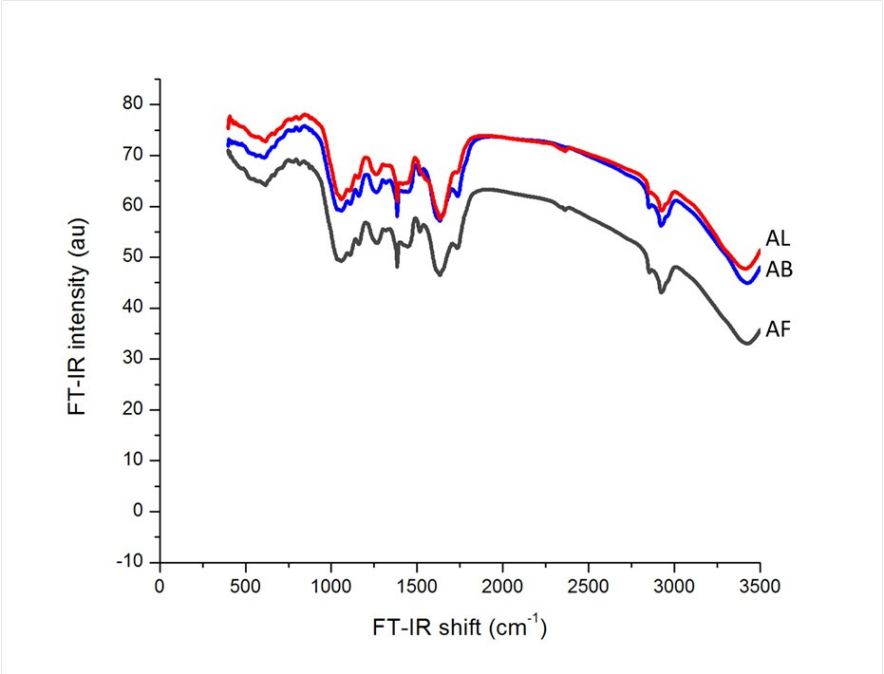

Figure 1. FT-IR spectra wavelength of the aerial parts (buds AB, leaves AL and flowers AF) of *A. herba-alba*.

Table 2: Chemical compounds class observed in methanolic and ethanolic *A. herba-alba* buds extracts using GC-MS analysis.

		Methanolic buds extracts	
Peak No	RT (min)	Compound class	Peak area (%)
1	9.505	Bicyclic ether	10.057
2	10.695	Bicyclic monoterpenoids	37.026
3	10.865	Stereoisomers	4.631
4	11.344	Monoterpenoids	1.876
5	23.558	Long-chain fatty acids	1.255
6	24.252	Endogenous fatty acid amide	4.402
7	25.074	Carboximidic acids	8.527
8	26.776	Cannabinoids (CBs) ligands	1.868
9	29.513	Fatty acid amides	15.471
10	30.159	Carboximidic acids	3.324
11	30.446	Sesquiterpenoids	2.155
12	32.826	Eudesmane sesquiterpenoid	6.500
		Ethanolic buds extracts	
Peak No	RT (min)	Compound class	Peak area (%)
1	9.501	Bicyclic ether	10.083
2	10.704	Bicyclic monoterpenoids	49.022
3	11.342	Monoterpenoids	2.708
4	25.047	Carboximidic acids	5.929
5	26.745	Furopyrans	1.431
6	29.480	Fatty acid amides	11.283
7	30.138	Long-chain fatty acids	2.061
8	30.404	Esquiterpene	1.837
9	31.956	ClassyFire Class: Fatty acyls	1.031
10	32.786	Sesquiterpenoid	6.188

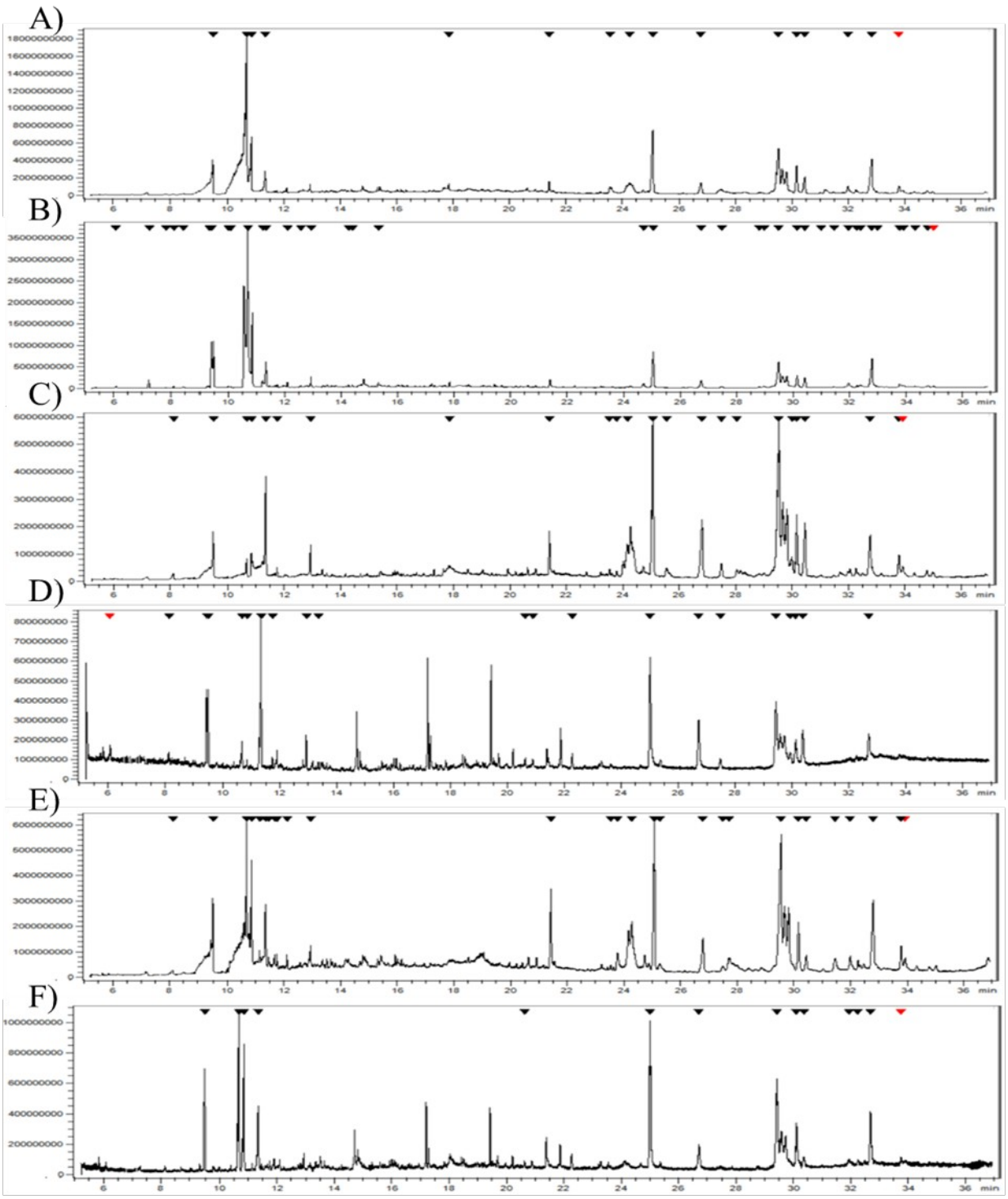

Figure 2. Chromatogram of the aerial parts of methanolic buds (A), ethanolic buds (B), methanolic leaves (C), ethanolic leaves (D), methanolic flowers (E) and ethanolic flowers (F) *A. herba-alba* extracts using GC-MS analysis.

Table 3: Chemical compounds class observed in methanolic and ethanolic *A. herba-alba* leaves extracts using GC-MS analysis.

		Methanolic leaves extracts	
Peak No	RT (min)	Compound class	Peak area (%)
1	9.505	Bicyclic ether	5.392
2	10.854	Stereoisomers	1.417
3	11.356	Monoterpenoids	4.660
4	12.941	Bicyclic monoterpenoids	1.168
5	21.404	Carboxylic ester	1.905
6	24.269	Diterpenoid	12.611
7	25.063	Fatty amides	12.304
8	25.549	Fatty amides	1.274
9	26.799	Terpenoid	6.218
10	27.483	Bicyclic monoterpenoids	1.157
11	28.037	Fatty amides	2.137
12	29.530	Fatty acid amides	28.687
13	29.980	Terpenoids	1.181
14	30.163	Fatty amides	4.504
15	30.435	Sesquiterpenoids	4.462
16	32.756	ClassyFire Class: Fatty acyls	4.659
17	33.773	Fatty amides	1.356
		Ethanolic leaves extracts	
Peak No	RT (min)	Compound class	Peak area (%)
1	8.159	Monoterpenes	1.028
2	9.493	Bicyclic ether	8.789
3	10.681	Bicyclic monoterpenoids	2.581
4	11.348	Monoterpene ketone	14.494
5	12.937	Menthane monoterpenoids	2.176
6	22.278	Fatty aldehydes	1.427
7	25.003	Fatty amides	16.142
8	26.713	ClassyFire Class: Fatty acyls	7.920
9	27.479	Eremophilane	1.428
10	29.434	Fatty acid amides	25.687
11	29.933	Triterpene	1.397
12	30.117	Fatty amides	3.541
13	30.368	Polysaccharide	5.325
14	32.687	Ester	3.651

Table 4: Chemical compounds class observed in methanolic and ethanolic *A. herba-alba* flowers extracts using GC-MS analysis.

		Methanolic flowers extracts	
Peak No	RT (min)	Compound class	Peak area (%)
1	9.506	Bicyclic ether	11.879
2	10.691	Bicyclic monoterpenoids	4.000
3	10.867	Stereoisomers	3.139
4	11.356	Monoterpene ketone	2.815
5	21.423	Long-chain fatty acids.	4.232
6	23.775	Fatty amides	1.366
7	24.281	Long-chain fatty acids	9.805
8	25.070	Long-chain fatty acids	10.771
9	26.791	Terpenoids	3.366
10	27.729	Fatty amides	2.649
11	29.538	Fatty acid amides	25.623
12	30.157	Carboximidic acids	3.661
13	30.429	Sesquiterpenoids	1.280
14	31.452	ClassyFire Class: Fatty acyls	1.196
15	32.784	Eudesmane sesquiterpenoid	7.145
16	33.766	Saturated long-chain fatty acid	1.456
		Methanolic flowers extracts	
Peak No	RT (min)	Compound class	Peak area (%)
1	9.493	Bicyclic ether	9.408
2	10.687	Bicyclic monoterpenoids	13.144
3	10.861	Stereoisomers	9.479
4	11.358	Monoterpene ketone	6.729
5	24.998	Fatty amides	16.452
6	26.701	Terpenoids	3.639
7	29.419	Fatty acid amides	23.295
8	30.088	Fatty amides	5.560
9	31.916	Triterpenoids	1.730
10	32.234	Long-chain fatty acids	1.123
11	32.677	Eudesmane sesquiterpenoid	6.462

In other researches, different number of phytochemical compounds in essential oils (EOs) or/and extracts of *A. herba-alba* aerial parts using GC-MS analysis was detected; *e.g*. 152 chemical compounds were observed in Tunisian *A. herba-alba* EOs (Bellili *et al.*, 2016); 75, 74 and 45 chemical compounds were observed in Libyan *A. judaica* L., *A. herba alba* Asso. and *A. arborescens* L. (cultivated) EOs, respectively (Janaćković *et al.*, 2016); 23 chemical compounds were observed in Tunisian *A. herba-alba* EOs (Younsi *et al.*, 2016); 28 chemical compounds were observed in Algerian *A. herba-alba* EOs (Ouguirti *et al.*, 2021); 21 chemical compounds were observed in Moroccan *A. herba-alba* ethanolic extract (Amkiss *et al.*, 2021); 79 chemical compounds were observed in Algerian *A. herba-alba* EOs (Ouchelli *et al.*, 2022); 39 chemical compounds were observed in Algerian *A. herba-alba*

EOs (Kadri *et al.*, 2022) and 50 chemical compounds were observed in Moroccan *A. herba-alba* EOs (Houti *et al.*, 2023).

In the current study, the observed functional groups in *A. herba alba* using FT-IR have been reported for their biological activity in different researches; *e.g*. carboxylic acid served as anti-inflammatory drugs (NSAIDs), antibiotics, anticoagulants, and cholesterol-lowering (Saleh, 2020) or as anticancer and antifertility (Hameed *et al.*, 2016); Aromatic group as isonicotinamide, antimicrobial and anti-inflammatory agents (Saleh, 2020) and the Ether group as antifungal and antimicrobial agents (Hameed *et al.*, 2016).

CONCLUSION

In conclusion, *A. herba-alba* aerial parts (buds AB, leaves AL and flowers AF) grown in rural Damascus regions, were phytochemically analyzed using FT-IR and GC-MS techniques. Overall, FT-IR spectra showed Aromatics (3 groups), Ethers (3 groups), Alkanes (2 groups), Saturated aliphatic (alkane/alkyl) (1 group), Carboxylic acids (1 group) and Alcohol & hydroxy (1 group) as common functional groups. Whereas, Olefinic (alkene) group was observed in AB and AF aerial parts and not in AL. As for GC-MS analysis, data revealed 12 & 10 chemical compounds classes in *A. herba-alba* buds extracts of which, Bicyclic monoterpenoids (37.026 & 49.022%) was presented as a major compound in methanolic and ethanolic buds extracts, respectively. Whereas, 17 & 14 chemical compounds classes were detected in *A. herba-alba* leaves extracts, of which, Fatty acid amides (28.687 & 25.687%) was presented as a major compound in methanolic and ethanolic leaves extracts, respectively. While, 16 & 11 chemical compounds classes were detected in *A. herba-alba* flowers extracts, of which Fatty acid amides (25.623 & 23.295%) was presented as a major compound in methanolic and ethanolic flowers extracts, respectively, some of these bioactive compounds worldwide exhibited a known potential biological role. However, for the other ones, more future performance experiments for determine their unknown potential activities are requested in pharmaceutical aspect.

ACKNOWLEDGEMENTS

I thank Dr. I. Othman (Director General of AECS) and Dr. A. Almariri (Head of Molecular Biology and Biotechnology Department in AECS) for their support, and also the Plant Biotechnology group for technical assistance.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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