

# **ORIGINAL ARTICLE**

# GC/MS Artemisia herba alba Asso (Asteraceae) Phytochemical Screening

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Methanolic (Meth) and ethanolic (Eth) Artemisia herba-alba Asso aerial parts extract including buds (AB), leaves (AL) and flowers (AF) were phytochemically analyzed using gas chromatography-mass spectrometry (GC/MS) analysis. A. herba-alba GC/MS chromatogram showed 16 and 39 compounds were occurred in Meth and Eth A. herba-alba AB extract, respectively, revealing that the Thujone (37.026% and 49.022%), 9-Octadecanamide, (Z)-(15.471% and 11.479%) and Eucalyptol (10.057% and 10.083%) were presented as a major compounds for Meth and Eth A. herba-alba AB extract, respectively. Whereas, 24 and 20 compounds were occurred in Meth and Eth A. herba-alba AL extract, respectively; where, 9-Octadecanamide, (Z)- (28.687%), Phytol (12.611%) and Palmitoleamide (12.304%) were presented as a major compounds for Meth A. herba-alba AL extract. Whereas, they were 9-Octadecanamide, (Z)- (25.687%), Dodecanamide (16.142%) and Camphor (14.494%) presented as a major compounds for Eth A. herba-alba AL extract. As for AF, 28 and 14 compounds were occurred in Meth and Eth A. herba-alba AF extract, respectively; where, 9-Octadecanamide, (Z)- (25.623%), Eucalyptol (11.879%) and Hexadecanamide (10.771%) were presented as a major compounds for Meth A. herba-alba AF extract. Whereas, they were 9-Octadecanamide, (Z)- (23.295%), Hexadecanamide (16.452%) and Thujone (13.144%) presented as a major compounds for Eth A. herba-alba AF extract. The current study highlights different bioactive compounds make this species as a good candidate to be used as a cheap natural source in pharmacology and medicine applications. The current study highlights for the first time A. herba-alba phytochemical analysis in Syria.

Key words: Artemisia herba-alba, gas chromatography–mass spectrometry (GC/MS), Phytochemical analysis, 9-Octadecanamide, (Z)-, Hexadecanamide *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, of which *Artemisia herba-alba* species wild grown in Syria (Mouterde 1983).

Artemisia herba-alba Asso, known as desert wormwood and as shīeḥ in Arabic. It is a perennial shrub commonly grows 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 (EOs) 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). More recently, Kshirsagar and Rao (2021) reviewed application of *Artemisia* sp. in medicine and pharmacology as antiviral and anti-inflammatory agents.

Of which, *A. herba-alba* herb exhibited many medicinal properties *e.g.* as antidiabetic, antimicrobial, antioxidant, antiradical, insectidal, 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.*, 2015; Riffi *et al.*, 2020).

Moufid and Eddouks (2012) reported that *A. herba alba* biological activity could mainly related to its content of many bioactive compounds *e.g.* herbalbin, cischryanthenyl 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 (FTIR) (Hameed et al., 2016); high-performance liquid chromatography (HPLC) (Bourgou et al., 2015); ultra-performance liquid chromatography (UPLC) coupled to photodiode array detection (PDA) and mass spectrometry (MS) (UPLC-PDA-MS) (Dane et al. 2016); gas chromatographymass spectrometry (GC/MS) (Vernin et al., 1995; Bourgou et al., 2015; Janaćković et al., 2015; Parameswari and Devika, 2017; Nasser and Arnold-Apostolides, 2018; Riffi et al., 2020) and liquid chromatography (LC) coupled to mass spectrometry (MS) (LC/MS) (Mamatova et al., 2019).

Little is known about phytochemical screening of *A. herba-alba* species in Syria. Thereby, the presented study focused on its phytochemical analysis during different development stages using GC/MS analysis for the first time.

#### MATERIAL AND METHODS

#### **Plant materials**

Buds (AB), leaves (AL) and flowers (AF) *Artemisia herba-alba* Asso aerial parts (10 plants/sample) were collected separately from wild *A. herba-alba* species grown in its natural habitat from rural Damascus regions-Syria (altitude of 950 m and annual rainfall of 240 mm). Samples were shade dried for two weeks, powdered by special electric mill and stored separately in glass bowls until extracts preparation.

#### **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  $\mu$ m)], 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. Each extract component was identified by comparing retention time values of gas chromatography on polar columns and by comparing mass spectrum and NIST-17 library databases.

#### **RESULTS AND DISCUSSION**

*A. herba-alba* GC/MS analysis revealed 16 and 39 compounds were occurred in methanolic and ethanolic *A. herba-alba* buds extract, respectively; of which, 8 compounds were common for the two both buds extract. It has been found that the Thujone (37.026% and 49.022%), 9-Octadecanamide, (Z)- (15.471% and 11.479%) and Eucalyptol (10.057% and 10.083%) were presented as a major compounds for methanolic and ethanolic *A. herba-alba* buds extract, respectively (Tables 1 & 2).

Whereas, 24 and 20 compounds were occurred in methanolic and ethanolic *A. herba-alba* leaves extract, respectively; of which, 9 compounds were common for the two both leaves extract. It has been found that the 9-Octadecanamide, (*Z*)- (28.687%), Phytol (12.611%) and Palmitoleamide (12.304%) were presented as a major compounds for methanolic *A. herba-alba* leaves extract (Table 3). Whereas, they were 9-Octadecanamide, (*Z*)- (25.687%), Dodecanamide (16.142%) and Camphor (14.494%) presented as a major compounds for ethanolic *A. herba-alba* leaves extract (Table 3).

As for flowers parts, 28 and 14 compounds were occurred in methanolic and ethanolic *A. herba-alba* flowers extract, respectively; of which, 9 compounds were common for the two flowers extract. It has been found that the 9-Octadecanamide, (Z)- (25.623%), Eucalyptol (11.879%) and Hexadecanamide (10.771%)

were presented as a major compounds for methanolic *A. herba-alba* flowers extract (Table 5). Whereas, they were 9-Octadecanamide, (Z)- (23.295%), Hexadecanamide (16.452%) and Thujone (13.144%) presented as a major compounds for ethanolic *A. herba-alba* flowers extract (Table 6).

Extracts of wild *A. herba-alba* aerial parts (buds AB, leaves AL and flowers AF) grown in rural Damascus regions-Syria, were phytochemically analyzed using GC/MS technique.

It worth noting that the 9-Octadecatrienoic acid (Z), tetradecyl ester and Agaricic acid compounds presented in ethanolic *A. herba-alba* buds extract in the current study were presented in methanolic *A. nilagirica* leaves extract using GC/MS analysis (Parameswari and Devika 2017).

Vernin et al. (1995) reported that the camphor (19-48%), 1,8-cineole (5–20%), chrysanthenone (5–22.5%),  $\alpha$ -thujone (1.0–26.7%),  $\beta$ -thujone (1.65–9.3%) and camphene (1.7-7.9%) were mainly presented in Algerian A. herba alba EOs using GC/MS analysis. Whereas, Zouari et al. (2010) reported that cischrysantenyl acetate (10.60%), sabinyl acetate (9.13%) and  $\alpha$ -thujone (8.73%) were the major compounds presented in leaves and flowers Tunisian A. herba-alba EOs. Moreover, Abou-Darwish et al. (2015) reported β-Thujones (25.1%),  $\alpha$ -Thujones (22.9%), that Eucalyptol (20.1%) and Camphre (10%) were the major compounds presented in Jordanian A. herba-alba EOs. Indeed, El-Seedi et al. (2017) reported that Piperitone (26.5%), ethyl cinnamate (9.5%), camphor (7.7%) and hexadecanoic acid (6.9%) were the major compounds recorded in Egyptian A. herba-Alba leaves EOs. Similarly, Bourgou et al. (2015) reported that Camphor (0.64- 31.51 %), α-Thujone (11.62- 13.93%), Fenchol (7.51-13.85%) and Nordavanone (1.26-9.44%) were the major compounds presented in Tunisian A. herba-Alba EOs using GC/MS analysis. Whereas, p-Coumaric acid (6.19-23.34%), Naringenin (3.36-20.19%) and Caffeic acid (1.32-14.04%) were presented in methanolic A. herba-Alba extract using HPLC analysis.

Janaćković *et al.* (2015) reported that the Camphor (24.7%), Chamazulene (20.9%), Isomer C14H18 (6.3%) and Bornyl acetate (4.9%) were mainly presented in *A.* 

arborescens EOs. Whereas, Chrysanthenone (20.5%) and cis-Chrysanthenyl acetate (17.7%) were mainly presented in A. herba-alba EOs. While, Piperitone (30.2%), cis-Chrysanthenol (9.1%) and Davana ether (7.9%) were mainly presented in A. judaica EOs using GC/MS analysis. While, Parameswari and Devika (2017) reported that Ergosta-5, 7, 22-trien- 3- o1, acetate, (3a, 22E), Agaricic acid, Bufa- 20, 22-dienolide, 3, 14dihydroxy- (3a, 5a) and 9-Octadecenoic acid (Z)tetradecyl ester, were the majors constituents presented in methanolic A. nilagirica leaves extract using GC/MS analysis. Whereas, Mamatova et al. (2019) reported the occurrence of flavonoids: apigenin, luteolin, rutin, two Omethylated flavonols (isorhamnetin & rhamnazine), coumarin compounds (umbelliferone, scopoletin and scopolin (scopoletin 7-glucoside), 3-hydroxycoumarin and 4-hydroxycoumarin), chlorogenic acid and two dicaffeoylquinic acid isomers in ethanolic and chloroform A. gmelinii extracts using LC/MS analysis. Moreover, Siddiqui et al. (2018) reported the occurrence of alkaloids, flavonoids, saponin, tannins, steroids. glycosides and phenols in the twelve different solvents extract of A. annua.

Nasser and Arnold-Apostolides (2018) reported that the  $\alpha$ -pinene (45.89%), borneol (11.3%) and 1.8-cineole (10.8%) were the most abundant compounds in the A. herba-alba EOs; whereas, camphene (15.71%), myrtenal (6.47%) and *m*-cymene (5.97%) were the most abundant compounds in its ethanolic extract; while camphor (32.91%), 1,8-cineole (9.98%) and borneol (6.78%) were the most abundant compounds in its acetonic extract, using GC/MS analysis. Recently, Riffi et al. (2020) reported that the Camphor (96.15%), Caryophyllene oxide (29,45%), Santoline alcohol (22.56%), 10,12-Octadecadienoic acid (20.68%) and Chrysanthyl acetate (16.82%) were the major compounds presented in the 4 fractions (F1, F2, F3 and F4) of A. herba alba EOs using GC/MS analysis.

It worth noting that in the current study, Eucalyptol content ranged between 5.392-11.879%; whereas, this

compound was recorded to be 20.1% in Jordanian A. herba-alba EOs (Abou-Darwish et al., 2015). Otherwise, Camphor content ranged between 0.315-14.494% in the current study, whereas, this compound was ranged between 19-48% in Algerian A. herba alba EOs (Vernin et al., 1995); 7.7% in Egyptian A. herba-Alba leaves EOs (El-Seedi et al., 2017); between 0.64- 31.51 % in Tunisian A. herba-Alba EOs (Bourgou et al., 2015); 24.7, 1.8 and 0.3% in Libyan A. arborescens, A. herbaalba and A. judaica EOs, respectively (Janaćković et al., 2015); 32.91% in Lebanon acetonic A. herba-alba extract (Nasser and Arnold-Apostolides, 2018) and 96.15% in Moroccan A. herba-Alba EOs (Riffi et al., 2020). Moreover, Camphene content in the current study was ranged between 0.184-1.028%, whereas, this compound was ranged between 1.7-7.9% in Algerian A. herba alba EOs (Vernin et al., 1995); 1.6, 0.7 and 0% in Libyan A. arborescens, A. herba-alba and A. judaica EOs, respectively (Janaćković et al., 2015) and 15.71% in Lebanon ethanolic A. herba-alba extract (Nasser and Arnold-Apostolides, 2018). Indeed, p-Cymene in the current study was recorded to be 0.422%, whereas it was recorded to be 0.5, 0.5 and 1.7% in Libyan A. arborescens, A. herba-alba and A. judaica EOs, respectively (Janaćković et al., 2015) and 5.97% in Lebanon ethanolic A. herba-alba extract (Nasser and Arnold-Apostolides, 2018). Moreover, Caryophyllene oxide in the current study was ranged between 1.181-3.639%, whereas, it was recorded to be 0.2 % in Libyan A. arborescens EOs along with its absence in Libyan A. herba-alba and A. judaica EOs (Janaćković et al., 2015) and 29,45% in Moroccan A. herba-Alba EOs (Riffi et al., 2020).

These differences in compounds content could be attributed to many factors like *e.g.* studied *Artemisia* species and substrate type, where in the current study, extracts have been prepared with solvents whereas, for the other studies they were EOs. Moreover, as known geographical distribution play an important role as a main factor affecting phytochemical composition (Zhang *et al.*, 2017).

Peak No	RT (min)	Name of Compound	Peak area (%)
1	9.505	Eucalyptol	10.057
2	10.695	Thujone	37.026
3	10.865	Bicyclo[3.1.0]hexan-3-one,4-methyl-1-(1-methylethyl)-	4.631
4	11.344	p-Mentha-1,8-dien-7-ol	1.876
5	17.851	Jasmonic acid	0.348
6	21.400	n-Hexadecanoic acid	0.821
7	23.558	Hexadecanamide	1.255
8	24.252	Palmitoleamide	4.402
9	25.074	Octadecanamide	8.527
10	26.776	Caryophyllene oxide	1.868
11	29.513	9-Octadecanamide, (Z)-	15.471
12	30.159	Octadecanamide	3.324
13	30.446	ß-Guaiene	2.155
14	31.987	1-Heptatriacotanol	0.991
15	32.826	Corymbolone	6.500
16	33.773	13-Docosenamide, (Z)-	0.746

 Table 1: GC/MS spectrum of methanolic A. herba-alba Asso buds extract.

 Table 2: GC/MS spectrum of ethanolic A. herba-alba Asso buds extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	6.068	Ethylene glycol diglycidyl ether	0.180
2	7.257	α-Pinene	0.989
3	7.845	trans-ß-Ocimene	0.077
4	8.127	Camphene	0.184
5	8.458	3-Carene	0.211
6	9.263	p-Cymene	0.422
7	9.501	Eucalyptol	10.083
8	10.012	R-Limonene	0.144
9	10.101	p-Menth-8-en-1-ol, steroisomer	0.125
10	10.704	Thujone	49.022
11	11.229	Camphor	0.558
12	11.342	p-Mentha-1,8-dien-7-ol	2.708
13	12.109	4-Hydoxy-α-thujone	0.419
14	12.579	Methyl 10,11-tetradecadienoate	0.133
15	12.939	cis-p-Mentha-2,8-dien-1-ol	0.689
16	14.271	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	0.194
17	14.504	9,12-Octadecadienoyl chloride, (Z,Z)-	0.166
18	15.327	Caryophyllane,4,8-ß-epoxy	0.397
19	24.700	Hexadecanamide	0.691
20	25.047	Octadecanamide	5.929
21	26.745	Picrotoxinin	1.431
22	27.474	Olean-12-ene-3,28-diol, (3ß)-	0.205
23	28.804	9-Octadecatrienoic acid (Z), tetradecyl ester	0.151
24	28.976	cis-11-Eicosenamide	0.186

25	29.480	9-Octadecanamide, (Z)-	11.283
26	30.138	Hexadecanamide	2.061
27	30.404	Xanthumin	1.837
28	30.987	9-Hexadecanoic acid, eicosyl ester, (Z)-	0.120
29	31.445	Ergosta-5,22-dien-3-ol,acetate, (3ß,22E)-	0.131
30	31.956	1-Heptatriacotanol	1.031
31	32.250	9-Octadecanenitrile, (Z)-	0.196
32	32.388	ß-Santanol acetate	0.276
33	32.786	Corymbolone	6.188
34	32.986	Ethyl iso-allocholate	0.114
35	33.762	Agaricic acid	0.479
36	33.902	cis-9,10-Epoxyoctadecanamide	0.213
37	34.314	Deoxyspergualin	0.161
38	34.756	Triaziquone	0.378
39	34.958	7-Heptadecene, 17-chloro-	0.239

#### Table 3: GC/MS spectrum of methanolic A. herba-alba Asso leaves extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	8.130	Camphene	0.427
2	9.505	Eucalyptol	5.392
3	10.685	Bicyclo	0.645
4	10.854	Bicyclo[3.1.0]hexan-3-one,4-methyl-1-(1-methylethyl)-	1.417
5	11.356	p-Mentha-1,8-dien-7-ol	4.660
6	11.753	Camphor	0.315
7	12.941	Isoborneol	1.168
8	17.843	(+)-cis-Verbenol, acetate	2.281
9	21.404	Butanoic acid, octyl ester	1.905
10	23.533	n-Hexadecanoic acid	0.326
11	23.784	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	0.307
12	24.269	Phytol	12.611
13	25.063	Palmitoleamide	12.304
14	25.549	Dodecanamide	1.274
15	26.799	Corymbolone	6.218
16	27.483	ß-Neoclovene	1.157
17	28.037	13-Docosenamide, (Z)-	2.137
18	29.530	9-Octadecanamide, (Z)-	28.687
19	29.980	Caryophyllene oxide	1.181
20	30.163	Hexadecanamide	4.504
21	30.435	ß-Guaiene	4.462
22	32.756	1-Heptatriacotanol	4.659
23	33.773	13-Docosenamide, (Z)-	1.356
24	33.905	Palmitoleamide	0.607

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Peak No	RT (min)	Name of Compound	Peak area (%)
1	6.063	3-Nitropropanoic acid	1.270
2	8.159	Camphene	1.028
3	9.493	Eucalyptol	8.789
4	10.681	Thujone	2.581
5	10.858	Bicyclo[3.1.0]hexan-3-one,4-methyl-1-(1-methylethyl)-	0.459
6	11.348	Camphor	14.494
7	11.745	Isoborneol	0.710
8	12.937	Carveol	2.176
9	13.359	Isobornyl acetate	0.484
10	20.628	Hexadecanenitrile	0.739
11	20.894	2(3H)-Furanone, 5-dodecyldihydro-	0.750
12	22.278	Pentadecanal-	1.427
13	25.003	Dodecanamide	16.142
14	26.713	1-Heptatriacotanol	7.920
15	27.479	Nootkatone	1.428
16	29.434	9-Octadecanamide, (Z)-	25.687
17	29.933	Urs-12-ene	1.397
18	30.117	Palmitoleamide	3.541
19	30.368	Xanthumin	5.325
20	32.687	9,12,15-Octadecatrienoic acid, 2,3-dihyroxypropyl ester, (Z,Z,Z)-	3.651

Table 4: GC/MS spectrum of ethanolic A. herba-alba Asso leaves extract.

Table 5: GC/MS spectrum of methanolic A. herba-alba Asso flowers extract .

Peak No	RT (min)	Name of Compound	Peak area (%)
1	8.125	Camphene	0.323
2	9.506	Eucalyptol	11.879
3	10.691	Thujone	4.000
4	10.867	Bicyclo[3.1.0]hexan-3-one,4-methyl-1-(1-methylethyl)-	3.139
5	11.157	Bornyl chloride	0.362
6	11.356	Camphor	2.815
7	11.479	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	0.398
8	11.680	3-Buten-2-one, 4-(3-cyclohexane-1-yl)-	0.461
9	11.756	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	0.482
10	12.115	4-Hydroxy-ß-Thujone	0.403
11	12.950	p-Mentha-1(7),8(10)-dien-7-ol	0.537
12	21.423	n-Hexadecanoic acid	4.232
13	23.533	9-Octadecanoic acid (Z)-, methyl ester	0.197
14	23.775	Dodecanamide	1.366
15	24.281	17-Octadecynoic acid	9.805
16	25.070	Hexadecanamide	10.771
17	25.256	Picrotoxin	0.631
18	26.791	Caryophyllene oxide	3.366
19	27.495	Aromandendrene	0.513
20	27.729	Palmitoleamide	2.649

21	29.538	9-Octadecenamide, (Z)-	25.623
21	29.550	9-Octavecenaniue, ( $Z$ )-	25.025
22	30.157	Octadecenamide	3.661
23	30.429	ß-Guaiene	1.280
24	31.452	1-Heptatriacotanol	1.196
25	31.981	Cholestan-3-ol, 2-methylene-, (3ß,5α)-	0.772
26	32.784	Corymbolone	7.145
27	33.766	9-Hexadecanoic acid	1.456
28	33.917	13-Docosenamide, (Z)-	0.538

Table 6: GC/MS spectrum of ethanolic A. herba-alba Asso flowers extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	9.493	Eucalyptol	9.408
2	10.687	Thujone	13.144
3	10.861	Bicyclo[3.1.0]hexan-3-one,4-methyl-1-(1-methylethyl)-	9.479
4	11.358	Camphor	6.729
5	20.628	Hexadecanenitrile	0.608
6	24.998	Hexadecanamide	16.452
7	26.701	Caryophyllene oxide	3.639
8	29.419	9-Octadecenamide, (Z)-	23.295
9	30.088	Palmitoleamide	5.560
10	30.360	Ethyl iso-allocholate	1.473
11	31.916	Cucurbitacin b, 25-desacetoxy-	1.730
12	32.234	Oleic acid	1.123
13	32.677	Corymbolone	6.462
14	33.735	Deoxyspergualin	0.898

### CONCLUSION

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GC/MS *A. herba-alba* aerial parts extracts chromatogram revealed that the 9-Octadecanamide, (Z)was presented as a common and major compound in all studied parts extracts regardless tested solvent. The different bioactive compounds mainly occurred in *A. herba-alba* aerial parts extracts like 9-Octadecanamide, (Z)-, Thujone, Eucalyptol, Palmitoleamide, Hexadecanamide and others, make them as potential natural sources to be used in different pharmacology and medicine applications with low cost.

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## **CONFLICTS OF INTEREST**

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

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