

Phytochemical Analysis of Two *Achillea* (Asteraceae) Species Using GC/MS Technique

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Wild *Achillea aleppica* DC and *Achillea arabica* Kotschy flowering, aromatic and perennial herbs, grown in the Middle-Southern regions of Syria were assessed for their ethanolic and acetonetic aerial parts extracts phytochemical analysis based on gas chromatography-mass spectrometry (GC/MS) analysis. Overall, GC/MS chromatogram revealed that the 9-Octadecenamide, (Z)- (41.656% and 61.097%) and Hexadecanamide (36.542% and 20.238%) were the most abundant compounds for ethanolic and acetonetic *A. aleppica* aerial parts extracts, respectively. Whereas, 9-Octadecenamide, (Z)- (41.280% and 53.990%) and Hexadecanamide (30.828% and 14.445%) were the most abundant compounds for ethanolic and acetonetic *A. Arabica* aerial parts extracts, respectively. This study could consider as the first report highlights *A. aleppica* and *A. arabica* extracts phytochemical analysis.

Key words: *Achillea aleppica*; *Achillea arabica* (*Achillea biebersteinii*); Gas chromatography-mass spectrometry (GC/MS); Phytochemical analysis; 9-Octadecenamide, (Z)-

Achillea genus belongs to Asteraceae family the largest angiosperms's family, comprise approximately 1500 genera and 23000 species. They spilled in three subfamilies and seventeen tribes. This genus involved 115 species of perennial herbs; all of them are native to temperate regions of the northern hemisphere (Moradkhani *et al.* 2012).

Achillea species exhibited wide range in medicine and pharmaceutical applications; e.g. as antimicrobial (Stojanović *et al.* 2005; Toncer *et al.* 2010; Tabanca *et al.* 2011; Albayrak and Silahtarlıoğlu 2019); antioxidant (Toncer *et al.* 2010; Manayi *et al.* 2012; Polatoglu *et al.* 2013; Albayrak and Silahtarlıoğlu 2019); insecticidal (Toncer *et al.* 2010; Tabanca *et al.* 2011; Polatoglu *et al.* 2013); herbicidal (Toncer *et al.* 2010; Polatoglu *et al.* 2013); cytotoxic (Albayrak and Silahtarlıoğlu 2019); antinociceptive and anti-inflammatory (Toncer *et al.* 2010) properties. Moreover, they used in traditional remedies against rheumatic pain and digestive complaints, fever, common cold, pneumonia and hemorrhage (Manayi *et al.* 2012).

The genus *Achillea* is represented in Syrian Flora with about 9 species (Mouterde 1983), of which *A. aleppica* DC and *A. arabica* Kotschy (Synonyms. *Achillea biebersteinii* Afanasiev) were wild grown in Syria. *A. aleppica* DC has antimicrobial, anti-inflammatory and antinociceptive properties (Toncer *et al.* 2010; Tabanca *et al.* 2011). Whereas, *A. arabica* Kotschy has hepatoprotective, antioxidant, herbicidal and insecticidal properties (Toncer *et al.* 2010; Tabanca *et al.* 2011; Başer 2016; Al-Said *et al.* 2016).

Different analytical methods allowed for long time identifying chemical compounds occurred in plants essential oils (EOs) and extracts. Of which, GC/MS analysis has been extensively employed worldwide for this purpose; e.g. in *A. millefolium*, *A. lingulata*, *A. holosericea* and *A. clavennae* species EOs (Boskovic *et al.* 2005); *A. biebersteinii*, *A. millefolium* and *A. wilhelmsii* EOs (Dehghan and Elmi 2014); *A. biebersteinii* EOs (Al-Said *et al.* 2016; Sevindiki *et al.* 2018); *A. fragrantissima* EOs (Hatem *et al.* 2018); *A. coarctata* EOs (Albayrak and Silahtarlıoğlu 2019) and

aqueous ethanolic (40 % v/v) vegetative parts *A. micrantha* extract (Astafyeva *et al.* 2018).

To our knowledge, the majority of researches on *A. aleppica* DC and *A. arabica* Kotschy species phytochemical analysis have been focused on their essential oils composition. However, little is known about their extracts phytochemical analysis. Thereby, the current investigation focused on their ethanolic and acetonetic aerial parts extracts phytochemical analysis using GC/MS analysis.

MATERIALS AND METHODS

Plant materials and preparation of extracts

Aerial parts of *A. aleppica* and *A. arabica* (10 plants for each species) were collected and bulked as representative for each *Achillea* sp. Samples have been collected during blooming stage from two wild *Achillea* species grown in their natural habitat from Middle-Southern regions in Syria. *Achillea aleppica* DC was collected from rural Damascus regions - Syria; whereas, *Achillea arabica* Kotschy was collected from rural Homs regions - Syria (Table 1).

Samples were shade dried for two weeks, and were milled to fine powder by special electric mill and stored separately in glass bowls for ethanolic and acetonetic extracts preparation.

The fine powder for each sample was extracted with ethanol and acetone 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 subjected to 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 ethanolic and acetonetic *A. aleppica* and *A. arabica* 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. 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

In the current study, GC/MS chromatogram revealed 11 and 23 compounds in ethanolic and acetic A. *aleppica* aerial parts extracts, respectively (Tables 2 & 3). Of which, 9-Octadecenamide, (Z)- (41.656%), Hexadecanamide (36.542%) and Tetradecanamide (9.965%) were the most abundant compounds in ethanolic A. *aleppica* aerial parts extracts (Table 2). Whereas, 9-Octadecenamide, (Z)- (61.097%), Hexadecanamide (20.238%) and Nonadecanamide (5.601%) were the most abundant compounds in acetic A. *aleppica* aerial parts extracts (Table 3). As for A. *Arabica*, they were 8 and 34 compounds in ethanolic and acetic A. *aleppica* aerial parts extracts, respectively (Tables 4 & 5). Of which, 9-Octadecenamide, (Z)- (41.280%), Hexadecanamide (30.828%) and Dodecanamide (8.940%) were the most abundant compounds in ethanolic A. *Arabica* aerial parts extracts (Table 4). Whereas, 9-Octadecenamide, (Z)- (53.990%), Hexadecanamide (14.445%) and 13-Docosanamide, (Z)- (7.829%) were the most abundant compounds in acetic A. *Arabica* aerial parts extracts (Table 5).

Overall, GC/MS chromatogram revealed that the 9-Octadecenamide, (Z)- (41.656% and 61.097%) and Hexadecanamide (36.542% and 20.238%) were the most abundant compounds for ethanolic and acetic A. *aleppica* aerial parts extracts, respectively. Whereas, 9-Octadecenamide, (Z)- (41.280% and 53.990%) and Hexadecanamide (30.828% and 14.445%) were the most abundant compounds for ethanolic and acetic A. *Arabica* aerial parts extracts, respectively.

It has been reported the presence of camphor (9-30%), 1,8-cineole (9-42%), p-cymene (5-27%) and piperitone (3-50%) as a main components in A.

biebersteinii EOs (Toncer *et al.* 2010; Tabanca *et al.* 2011). Whereas, α -terpinen (41.42%), 2-carene (13.96%), m-cymene (13.41%) and 1,8-cineole (8.91%) were mainly presented in A. *biebersteinii* EOs using GC/MS analysis (Dehghan and Elmi 2014). While, Al-Said *et al.* (2016) reported 44 compounds in A. *biebersteinii* EOs; of which α -Terpinene (29.2%), p-Cymene (22.9%), Terpinen-4-ol (4.7%) and 1,8-Cineole (4.3%) were mainly detected in their EOs using GC/MS analysis. Moreover, Sevindik *et al.* (2018) reported 29 components in A. *biebersteinii* EOs, of which 1,8-cineole (20.36%), cyclohexanone (8.39%), 2-cyclohexen-1-one (5.38%) and spathulenol (4.19%) were presented as a main components using GC/MS analysis.

Whereas, camphor (33-34%), 1,8-cineole (20-26%), p-cymene (14%), α -pinene (4%), α -terpineol (9%), α -bisabolol oxide (4%), T-cadinol (4%), caryophyllene oxide (3%) and spathulenol (3%) as a main components in A. *aleppica* EOs (Toncer *et al.* 2010).

GC/MS analysis has been extensively also used to investigate of other *Achillea* species for their EOs composition. In this regards, Boskovic *et al.* (2005) reported that β -pinene in A. *millefolium*, τ -cadinol in A. *lingulata*, 1,8-cineole in A. *holosericea* and camphor in A. *clavennae* were the main constituents in their EOs. Moreover, Astafyeva *et al.* (2018) reported that aldehydes (41.93%), alcohols (21.24%), hydrocarbons (14.45%), aromatic hydrocarbons (7.78%), esters (3.21%) and ketones (2.37%) functional groups, were mainly recorded in the aqueous ethanolic (40 % v/v) vegetative parts A. *micrantha* extract. Whereas, Hatem *et al.* (2018) reported 51 compounds of which artemisia ketone (29.97%), α -thujone (13.34%), germacrene (11.5%) followed by α -cubebene (6.25%), spathulenol (3.63%), β -sesquiphellandrene (3.52%) and γ -muurolene (3.27%) were mainly components in the fresh aerial parts of A. *fragrantissima* EOs. Moreover, Albayrak and Silahtarlioğlu (2019) reported 45 compounds were presented in A. *coarctata* EOs; of which Camphor (29.44%), 1,8-cineole (19.87%), borneole (8.25%), β -eudesmol (7.65%) and caryophyllene oxide (7.29%) were mainly occurred using GC/MS analysis. Whereas, other compounds were presented in minor amounts (0.17%–2.91%).

Whereas, Dehghan and Elmi (2014) reported 20 compounds in *A. millefolium* EOs of which 1,8-cineole (28.0%), camphor (19.2%), borneol (98.8%) and β -pinene (6.3%) were mainly presented. While, 23 compounds in *A. wilhelmsii* EOs of which carvacrol (29.2%), linalool (10.3%), 1,8-cineole (11.0%), (E)-nerolidol (8.4%) and borneol (5.04%) were mainly presented in their EOs. Whereas, Farajpour et al. (2017) reported that the 1,8-Cineole, 1.2–19.8%; β -thujone, 0.4–55.3%; camphor, 0.6–25.5%; germacrene-D, 2–20.6%; trans-nerolidol, 0.4–48.1%; isospathulenol, 0.5–36%; and cubenol, 0.1–42.9% were mainly detected in the *A. millefolium* EOs. Recently, Yener et al. (2020) reported that α -terpinene, β -eudesmol, piperitone, endo-borneol, artemisia ketone, verbenol, eucalyptol and camphor were the main constituents in *Achillea* species EOs.

In the current study, caryophyllene oxide was

recorded to be 0.264% in acetonic *A. aleppica* aerial parts extract; whereas, it was recorded to be 1.01% in *A. biebersteinii* EOs (Baris et al. 2006); 3% in *A. aleppica* EOs (Toncer et al. 2010); 2% in *A. tenuifolia* EOs (Manayi et al. 2014); 0.52% in *A. fragrantissima* EOs (Choucry 2017) and ranged between 0.76–11.9% in *A. millefolium* EOs (Farajpour et al. 2017). Otherwise, in the current study, oleic acid was recorded to be 41.656% and 61.097% for ethanolic and acetonic *A. aleppica* aerial parts extracts, respectively and to be 41.280% and 53.990% for ethanolic and acetonic *A. Arabica* aerial parts extract's, respectively. Whereas, it was 9.7% in *A. tenuifolia* EOs (Manayi et al. 2014). These differences could be attributed to the fact that geographical distribution, species, plant phynological stages and extraction type (solvents or EOs) affect *Achillea* sp. chemical composition (Dehghan and Elmi 2014; Al-Said et al. 2016; Farajpour et al. 2017).

Table 1. Collection sites of *A. aleppica* and *A.arabica* species.

Species	Collection site	Code	Altitude (m)	Annual rainfall (mm)
<i>A. aleppica</i>	Damascus	A.A	950	260
<i>A. arabica</i>	Homs	A.R	265	400

Table 2. GC/MS analysis of ethanolic *A. aleppica* aerial parts extracts.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	9.482	Eucalyptol	1.002
2	11.745	endo-Borneol	1.523
3	20.611	Hexadecanenitrile	0.748
4	22.259	Pentadecanal	2.027
5	24.126	Dodecanoic acid, 3-hydroxy-	3.314
6	24.982	Hexadecanamide	36.542
7	29.404	9-Octadecenamide, (Z)-	41.656
8	30.084	Tetradecanamide	9.965
9	32.232	9-Octadecenenitrile, (Z)-	0.803
10	33.743	13-Docosenamide, (Z)-	1.431
11	33.881	9-Octadecenoic acid (Z)-, tetradecyl ester	0.988

Table 3. GC/MS analysis of acetonic *A. aleppica* aerial parts extracts.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	7.038	Styrene	0.142
2	9.491	Eucalyptol	0.777
3	10.087	p-Menth-8-en-1-ol, stereoisomer	0.260
4	10.599	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-,cis	0.383
5	10.674	Thujone	0.428
6	11.749	endo-Borneol	0.689
7	17.412	Caryophyllene oxide	0.264
8	18.430	Eicosane	0.325
9	18.510	Nonadecane	0.832
10	18.596	Heptanoic acid, heptyl ester	0.263
11	19.693	Tetradecanal	0.608
12	20.199	Benzaldehyde, 4-(dimethylamino)-	0.183
13	20.622	Octadecanal	1.170
14	20.885	Dodecyl nonyl ether	0.803
15	21.381	10-Octadecenal	0.833
16	22.270	Pentadecanal	1.676
17	23.211	Z,Z-4,16-Octadecadien-1-ol acetate	1.016
18	23.621	9-Hexadecenoic acid	0.549
19	24.994	Hexadecanamide	20.238
20	25.500	Palmitoleic acid	0.627
21	29.434	9-Octadecenamide, (Z)-	61.097
22	30.101	Nonadecanamide	5.601
23	32.240	9-Octadecenenitrile, (Z)	1.238

Table 4. GC/MS analysis of ethanolic *A.arabica* aerial parts extracts.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	13.654	4-Hydroxy- α -Thujone	5.002
2	14.894	4-Hydroxy- β -Thujone	5.913
3	20.618	Dodecanoic acid, 3-hydroxy-	0.925
4	24.124	Palmitoleamide	5.608
5	24.984	Hexadecanamide	30.828
6	27.163	Humulenol-II	1.505
7	29.405	9-Octadecenamide, (Z)-	41.280
8	30.088	Dodecanamide	8.940

Table 5. GC/MS analysis of acetonic *A.arabica* aerial parts extracts.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	7.038	Styrene	0.375
2	9.322	Benzoic acid, 2,4-dimethyl-, (2,4-dimethylphenyl)methyl ester	0.145
3	11.866	Octanoic acid, 7-oxo-	0.358
4	12.724	Isopulegol acetate	0.599
5	13.430	1,4-dihydroxy-p-menth-2-ene	0.269
6	14.415	Dimethylmuconic acid	0.082
7	14.512	Ricinoleic acid	0.170
8	14.712	Arginine	0.160
9	14.896	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	1.526
10	17.201	1-Eicosanol	0.080
11	18.508	Tetradecane	0.559
12	18.836	Benzeneacetic acid, 4-tetradecyl ester	0.191
13	19.185	Cyclobuta[a]dibenzo[c,f]cycloheptadiene, 7-oxo-	0.847
14	19.677	Tetradecanal	0.426
15	20.197	Didodecyl phthalate	0.222
16	20.620	Hexadecanenitrile	0.744
17	20.887	Nonadecane	0.580
18	21.373	trans-2-Hexadecenoic acid	0.835
19	22.265	Tridecanal	1.007
20	23.200	9-Octadecenenitrile, (Z)	0.691
21	23.604	Hexadecanal	0.350
22	24.162	13-Docosenamide, (Z)-	7.829
23	24.582	9-Hexadecenoic acid	0.680
24	24.992	Hexadecanamide	14.445
25	25.502	Octadecanenitrile	0.606
26	27.156	Phthaloylaspartic acid	0.506
27	27.648	α -Amyrin	0.327
28	29.427	9-Octadecenamide, (Z)-	53.990
29	30.095	Deoxyspergualin	5.003
30	31.556	4-((2-Amino-phenylthio)-1-benzyl-6-methylpiperidin-2-thione	0.933
31	32.238	Palmitoleonitrile	1.011
32	33.344	Carnegine	1.276
33	33.628	Benzen,1,1-[2-methyl-2-(phenylthio)cyclopropylidene]bis-	1.505
34	33.748	Cis-11-Eicosenamide	1.668

CONCLUSION

Ethanol and acetonic *A. aleppica* and *A. arabica* aerial parts extracts have been investigated for their chemical composition using GC/MS analysis. Among different compounds detected in GC/MS ethanol and acetonic *A. Arabica* and *A. aleppica* aerial parts extracts,

9-Octadecenamide, (Z)- and Hexadecanamide were the most abundant compounds. The two studied *Achillea* species showed some differences in their chemical composition of their extracts. The observed differences in their extracts composition could be attributed to the tested solvents, studied *Achillea* species and their geographical distribution.

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CONFLICTS OF INTEREST

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

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