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Essential oils of *Atriplex canescens* and *Grayia spinosa*, two members of the Amaranthaceae growing in the Snake River canyon near Swan Falls, Idaho

Kathy Swor, Ambika Poudel, Prabodh Satyal and William N Setzer

Abstract
With the exception of *Dysphania ambrosioides*, the Amaranthaceae has been relatively understudied in terms of volatile characterization of members of the family. The purpose of this work was to examine the essential oils of two species, *Atriplex canescens* and *Grayia spinosa* growing wild in southwestern Idaho. The essential oils were obtained by hydrodistillation and analyzed by gas chromatography (GC-MS and GC-FID). Essential oil yields were generally low (0.006-0.151% for *A. canescens*, 0.064-0.104% for *G. spinosa*). The major components in the essential oil of male *A. canescens* were 4-vinylguaiacol (16.8%), α-cadinol (13.1%), *neo*-intermedeol (6.9%), δ-cadinene (5.1%), α-elemol (4.7%), and bornyl acetate (4.6%). Female *A. canescens*, on the other hand, showed δ-cadinene (11.7%), *epi*-cubebol (6.8%), α-elemol (6.7%), 1-*epi*-cubenol (6.4%), cubenol (6.2%), cubebol (5.4%), and α-cadinol (5.2%) as major components. *Grayia spinosa* essential oils (all female) showed wide variation in compositions. However, green-leaf volatiles were generally abundant (10.5-37.5%) as was 4-vinylguaiacol (4.2-17.5%). Alkanes were abundant in one sample (33.1%), while dihydroedulans were abundant (14.5%) in another. There seems to be much variation in essential oil compositions both within species and between species of the Amaranthaceae.

Keywords: Four-winged saltbush, spiny hopsage, Great Basin, 4-vinylguaiacol, dihydroedulan, green-leaf volatiles

1. Introduction

Atriplex canescens (Pursh) Nutt. (four-winged saltbush) is currently placed in the Amaranthaceae [1], but was formerly considered to be a member of the Chenopodiaceae [2]. The plant is a shrub, growing up to 2 m tall [3]. The plant naturally ranges in western North America, from British Columbia south to Mexico [4], but it has been introduced to arid zones of West Asia and North Africa, including Tunisia, Algeria, Libya, Syria, Iran, Iraq, and Pakistan as animal fodder and for land rehabilitation [5–9]. The plant was used in traditional medicine by several Native North American tribes. The Havasupai used a soapy lather from the leaves to treat itches or rashes; the Jemez, the Navajo, and the Zuni use a poultice of crushed leaves as a remedy for ant bites [10].

The plant is usually dioecious, but there may be as many as 10-20% monoecious [11,12]. Furthermore, environmental stress can induce the plants to alter from female to male [13]. Male flowers are small and borne in dense spikes at the ends of stems; female plants produce clusters of four-winged utricles (fruits) (Figure 1) [14].

The plant is salt-tolerant [15], and known to sequester metal ions such as Cd(II), Cr(III), Cr(VI) [16], Pb(II), Cu(II), and Zn(II) [17], resulting in accumulation of polyphenolics and flavonoids [18].

Grayia spinosa (Hook.) Moq. (Syn. *Atriplex grayi* Collotzi ex W.A. Weber, *Atriplex spinosa* Collotzi ex C.L. Hitchc., spiny hopsage, Amaranthaceae) occurs in the western United States (eastern Washington, eastern Oregon, southern and central Idaho, southern Montana, Nevada, Utah, western Wyoming, western Colorado, eastern and southern California, and northern Arizona) [19]. The shrub can be monoecious or dioecious, about 0.3-1.2 m in height. Leaves are fleshy, 5-30 mm long and 2-13 mm wide (Figure 2A). Male flowers consist of 4-5 lobed perianth and 4-5 stamens. Female flowers develop in dense bracteate spikes with one or more flowers in the axil of each bract; each pistil is enclosed in two cordate bracteoles [19] (Figure 2B).

Fig 1: Photographs of *Atriplex canescens*. A: Male *A. canescens*. B: Female *A. canescens*. (Photographs by K. Swor).

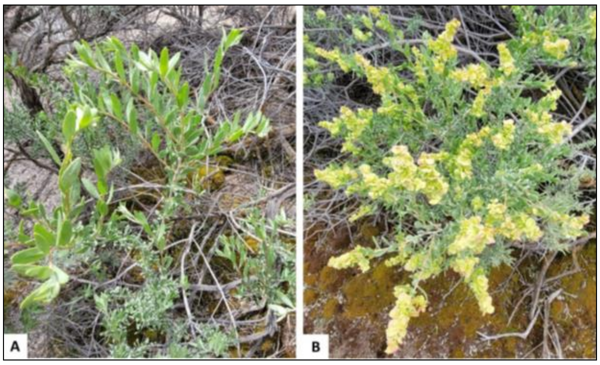

Fig 2: Photographs of *Grayia spinosa*. A: Leaves of *G. spinosa*. B. Female inflorescences of *G. spinosa*. (Photographs by W.N. Setzer).

As far as we are aware, the volatile phytochemistry of these two plants have not been investigated. As an extension of our studies on aromatic and medicinal plants from the Great Basin, we have investigated the essential oils of *A. canescens* and *G. spinosa* growing in southwestern Idaho.

2. Materials and Methods

2.1 Plant Material

The above-ground parts of male and female *A. canescens* were collected from the Swan Falls area of the Snake River in southwestern Idaho (43°14′47″ N, 116°22′47″ W, 704 m elevation and 43°14′45″ N, 116°22′40″ W, 707 m elevation, respectively) on 28 July 2021. The plants were identified in the field by K. Swor and W.N. Setzer using the field guide [3] and verified by comparison with specimens from the C. V. Starr Virtual Herbarium of the New York Botanical Garden (http://sweetgum.nybg.org/science/vh/specimen-list/?SummaryData=Atriplex%20canescens, accessed on 28 May 2022). Voucher specimens (WNS-ACM-4626 and WNS-ACF-4621, respectively) have been deposited in the University of Alabama in Huntsville herbarium. The fresh aerial parts of male *A. canescens* (75.51 g) were hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with dichloromethane for 3 h to give a pale-yellow essential oil (113.7 mg). Hydrodistillation of the aerial parts of female *A. canescens* (95.03 g) was carried out as above to give 5.9 mg of a colorless essential oil.

The aerial parts of female *G. spinosa* (three plants) were collected from the Swan Falls area of the Snake River in southwestern Idaho on 3 May 2022. The plants were identified in the field by K. Swor and W.N. Setzer and verified by comparison with specimens from the C.V. Starr Virtual Herbarium of the New York Botanical Garden (https://sweetgum.nybg.org/science/vh/specimen-list/?SummaryData=Grayia%20spinosa) (accessed on 28 May 2022). A voucher specimen (WNS-GSF-5670) has been deposited in the University of Alabama in Huntsville herbarium. The fresh samples were stored frozen (–20 °C) until distilled. The samples were hydro distilled using a Likens-Nickerson apparatus with continuous extraction with dichloromethane for 3 h to give pale-yellow essential oils (Table 1).

Table 1: Collection and hydrodistillation of female *Grayia spinosa* aerial parts from Swan Falls, Snake River, Idaho.

Sample	Collection site	Mass (g)	Essential oil yield (mg)
#1	43°14′47″N, 116°22′48″W, 704 m asl	87.33	91.2
#2	43°14′47″N, 116°22′48″W, 704 m asl	72.18	46.5
#3	43°14′48″N, 116°22′48″W, 707 m asl	144.38	97.8

2.2 Gas Chromatographic Analysis

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID) were carried out as previously described [20].

3. Results and Discussion

3.1 *Atriplex canescens*: Hydrodistillation of the aerial parts of male *A. canescens* gave an essential oil in 0.151% yield, while the essential oil yield of female *A. canescens* was much lower (0.0062% yield). The essential oil compositions from the aerial parts of *A. canescens* are summarized in Table 2.

Table 2: Essential oil compositions of *Atriplex canescens* collected in southwestern Idaho.

RI_{calc}	RI_{db}	Compound	Percent composition	
			Male *A. canescens*	Female *A. canescens*
803	801	Hexanal	---	4.9
826	825	Furfural	---	1.1
850	850	(2*E*)-Hexenal	---	3.8
852	853	(3*Z*)-Hexenol	---	0.5
862	864	(2*E*)-Hexenol	---	0.2
866	867	Hexanol	---	0.7
889	889	2-Heptanone	---	1.7
904	906	Heptanal	---	0.4
934	933	α-Pinene	---	1.7
956	956	(2*E*)-Heptenal	---	0.7
962	960	Benzaldehyde	---	0.4

973	971	Sabinene	---	0.4
979	978	β-Pinene	---	0.5
979	978	1-Octen-3-ol	---	0.3
985	982	6-Methyl-5-hepten-2-one	---	0.5
991	991	2-Pentylfuran	---	1.0
1005	1006	Octanal	---	0.3
1044	1045	Phenylacetaldehyde	---	0.5
1059	1059	(2*E*)-Octenal	---	0.4
1067	1067	(2*E*)-Octen-1-ol	---	0.3
1071	1069	1-Octanol	---	0.3
1101	1101	Linalool	0.9	0.2
1103	1102	6-Methyl-3,5-heptadien-2-one	0.7	---
1106	1107	Nonanal	1.4	2.3
1161	1163	(2*E*)-Nonenal	---	0.5
1182	1184	Terpinen-4-ol	---	0.3
1188	1186	*p*-Cymen-8-ol	0.3	---
1196	1195	α-Terpineol	0.6	0.2
1200	1201	Safranal	1.3	---
1208	1208	Decanal	0.4	0.3
1216	1217	Coumaran	0.7	0.4
1263	1263	(2*E*)-Decenal	0.3	0.4
1264	---	Unidentified [a]	1.3	---
1273	---	Unidentified [b]	2.7	0.3
1285	1285	Bornyl acetate	4.6	2.0
1309	1309	4-Vinylguaiacol	16.8	1.7
1319	1318	(2*E*,4*E*)-Decadienal	0.4	0.7
1348	1348	α-Cubebene	---	0.4
1365	1365	(2*E*)-Undecenal	0.4	---
1378	---	Unidentified [c]	1.4	---
1388	1387	β-Cubebene	---	0.4
1409	1410	Dodecanal	0.8	---
1420	1417	(*E*)-β-Caryophyllene	1.2	---
1447	1447	Geranyl acetone	0.9	---
1449	1448	*cis*-Muurola-3,5-diene	---	0.7
1464	1463	γ-Decalactone	0.7	---
1475	1475	γ-Muurolene	---	0.2
1478	1481	(*E*)-β-Ionone	---	0.1
1481	1480	Germacrene D	---	0.4
1484	1487	β-Selinene	---	0.3
1492	1492	*trans*-Muurola-4(14),5-diene	0.7	2.2
1496	1497	*epi*-Cubebol	2.6	6.8
1498	1497	α-Muurolene	0.6	0.5
1513	1512	γ-Cadinene	0.7	0.2
1515	1515	Cubebol	1.5	5.4
1518	1518	δ-Cadinene	5.1	11.7
1521	1519	*trans*-Calamenene	---	0.7
1523	1521	Zonarene	---	0.6
1533	1533	*trans*-Cadina-1,4-diene	0.4	1.3
1548	1549	α-Elemol	4.7	6.7
1561	1561	(*E*)-Nerolidol	2.8	0.5
1570	1571	(3*Z*)-Hexenyl benzoate	0.6	---
1577	1576	Spathulenol	0.3	---
1582	1587	Caryophyllene oxide	1.1	---
1586	1584	Gleenol	---	0.5
1596	1596	Fokienol	1.4	---
1603	1607	β-Oplopenone	0.9	0.5
1623	1624	Selina-6-en-4β-ol	0.6	1.3
1628	1628	1-*epi*-Cubenol	2.8	6.4
1632	1632	γ-Eudesmol	0.6	0.9
1643	1643	Cubenol	2.7	6.2
1644	1645	τ-Muurolol	3.2	---
1646	1644	δ-Cadinol (= α-Muurolol)	1.2	1.1
1655	1655	α-Cadinol	13.1	5.2
1665	1665	*neo*-Intermedeol	6.9	3.0
1687	1688	α-Bisabolol	0.7	---
1688	1686	Shyobunol	---	0.6
1741	1742	(6*S*,7*R*)-Bisabolone	1.1	---
1777	1775	8α-Acetoxyelemol	3.4	3.0
1793	1793	α-Phellandrene dimer A	0.6	0.6

1841	1841	Phytone	1.1	0.4
1923	1921	Methyl palmitate	0.7	---
1958	1958	Palmitic acid	---	0.5
		Monoterpene hydrocarbons	0.0	2.7
		Oxygenated monoterpenoids	7.6	2.7
		Sesquiterpene hydrocarbons	8.7	19.8
		Oxygenated sesquiterpenoids	50.2	48.1
		di-*nor*-Sesquiterpenoids	0.9	0.1
		Diterpenoids	0.6	0.6
		Benzenoid aromatics	18.2	3.0
		Green-leaf volatiles	0.0	10.1
		Fatty acids	0.7	0.5
		Others	7.7	11.7
		Total identified	94.7	99.4

RI_{calc} = Retention index calculated with respect to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Retention index obtained from the databases [21–24].

[a] MS(EI): 194(7%), 151(41%), 126(100%), 123(15%), 111(19%), 95(35%), 85(13%), 70(24%), 67(14%), 55(10%), 43(7%), 41(8%).

[b] MS(EI): 194(3%), 151(21%), 126(100%), 123(10%), 111(21%), 95(24%), 81(10%), 70(31%), 67(12%), 55(17%), 43(12%), 41(14%).

[c] MS(EI): 195(3%), 154(17%), 125(57%), 121(12%), 110(18%), 109(15%), 101(27%), 95(100%), 69(48%), 55(14%), 43(12%), 41(21%).

Sesquiterpenoids made up a large proportion of the composition of the male *A. canescens* essential oil (58.9%) along with benzenoid aromatic compounds (18.2%). The major components in the male *A. canescens* were 4-vinylguaiacol (16.8%), α-cadinol (13.1%), *neo*-intermedeol (6.9%), δ-cadinene (5.1%), α-elemol (4.7%), and bornyl acetate (4.6%). The essential oil of the female *A. canescens* was also rich in sesquiterpenoids (19.8% sesquiterpene hydrocarbons and 48.1% oxygenated sesquiterpenoids). Green leaf compounds and other fatty acid derivatives were also important. The major components in the female *A. canescens* were δ-cadinene (11.7%), *epi*-cubebol (6.8%), α-elemol (6.7%), 1-*epi*-cubenol (6.4%), cubenol (6.2%), cubebol (5.4%), and α-cadinol (5.2%).

3.2 *Grayia spinosa*

The pale-yellow essential oils of *G. spinosa* were obtained in relatively low yields (0.064-0.104%). Gas chromatographic (GC-MS and GC-FID) showed the essential oils to be very different, both qualitatively and quantitatively (Table 3).

Table 3: Essential oil compositions of *Grayia spinosa* collected in southwestern Idaho.

RI_{calc}	RI_{db}	Compound	Percent composition		
			#1	#2	#3
798	797	(3*Z*)-Hexenal	0.7	-	-
799	801	Hexanal	5.6	6.2	0.7
849	849	(2*E*)-Hexenal	19.7	30.0	9.3
851	853	(3*Z*)-Hexenol	2.9	-	-
862	864	(2*E*)-Hexenol	0.7	1.3	0.6
932	932	α-Pinene	1.1	1.9	1.0
962	959	Benzaldehyde	-	1.2	1.4
972	972	Sabinene	-	-	0.4
972	971	Artemiseole	-	2.0	-
989	989	2-Pentylfuran	0.6	1.2	1.1
994	996	Yomogi alcohol	-	1.8	0.6
1000	1000	Decane	-	-	1.4
1004	1004	Octanal	-	-	0.3
1024	1025	*p*-Cymene	0.6	0.3	0.6
1028	1030	Limonene	-	1.6	1.3
1030	1031	β-Phellandrene	-	1.1	1.0
1032	1032	1,8-Cineole	-	2.1	1.5
1034	1034	(*Z*)-β-Ocimene	-	2.7	0.9
1043	1043	Phenylacetaldehyde	1.3	2.8	3.8
1057	1056	Artemisia ketone	-	2.3	-
1057	1059	(2*E*)-Octenal	1.7	-	0.9
1070	1069	*cis*-Linalool oxide (furanoid)	-	-	0.8
1093	1094	Methyl benzoate	-	-	0.7
1099	1101	Linalool	-	-	7.6
1103	1104	Hotrienol	-	-	1.0
1105	1107	Nonanal	0.7	1.2	2.1
1106	1105	α-Thujone	-	-	0.6
1113	1113	(*E*)-4,8-Dimethylnona-1,3,7-triene	0.5	2.9	1.8
1120	1120	Isophorone	-	-	2.7
1132	-	Unidentified [a]	-	2.3	-
1146	1145	Camphor	-	4.1	2.4
1159	1163	(2*E*)-Nonenal	-	-	0.5
1162	1164	β-Artemisyl acetate	-	4.3	-
1171	1170	Borneol	-	-	0.8

1179	1180	Terpinen-4-ol	-	-	0.8
1190	1190	Methyl salicylate	-	-	1.7
1194	1195	α-Terpineol	-	-	1.7
1197	1201	Safranal	-	-	0.6
1200	1200	Dodecane	0.8	1.2	2.1
1206	1206	Decanal	0.5	-	1.5
1211	1210	(2*E*)-Octenol acetate	2.2	2.0	-
1217	1211	β-Cyclocitral	-	-	1.2
1259	-	Unidentified [b]	-	-	3.0
1262	1263	(2*E*)-Decenal	0.4	-	0.9
1278	-	Unidentified [c]	-	-	3.0
1281	1282	Bornyl acetate	1.2	-	-
1283	1287	*iso*-Bornyl acetate	2.6	-	-
1287	1287	Dihydroedulan IA	-	-	3.8
1295	1294	Dihydroedulan IIA	-	4.7	10.7
1299	1299	*cis*-Theaspirane	-	-	4.4
1300	1300	Tridecane	23.4	-	-
1310	1309	4-Vinylguaiacol	5.8	17.5	4.2
1315	1314	*trans*-Theaspirane	-	-	3.2
1390	1387	(6*E*)-6-Methyl-5-(1-methylethylidene)-6,8-nonadien-2-one	-	-	5.3
1438	1439	Isoamyl benzoate	-	-	3.0
1489	1487	β-Selinene	1.3	-	-
1519	1518	7-*epi*-α-Selinene	0.6	-	-
1571	1571	(3*Z*)-Hexenyl benzoate	-	-	1.5
1851	-	Unidentified [d]	-	1.4	-
1925	1921	Methyl palmitate	0.8	-	-
1958	1958	Palmitic acid	3.4	-	-
2126	2128	Linoleic acid	2.9	-	-
2135	2141	Oleic acid	7.7	-	-
2500	2500	Pentacosane	0.5	-	-
2800	2800	Octacosane	8.4	-	-
		Monoterpene hydrocarbons	1.7	7.5	5.2
		Oxygenated monoterpenoids	3.8	16.5	19.6
		Sesquiterpene hydrocarbons	1.9	0.0	0.0
		Oxygenated sesquiterpenoids	0.0	0.0	0.0
		di-*nor*-Sesquiterpenoids	0.0	4.7	27.3
		Benzenoid aromatics	7.2	21.5	16.3
		Alkanes	33.1	1.2	2.1
		Fatty acids	14.8	0.0	0.0
		Green-leaf volatiles	29.6	37.5	10.5
		Others	6.5	7.4	13.1
		Total identified	98.5	96.3	94.0

RI_{calc} = Retention index calculated with respect to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Retention index obtained from the databases [21–24].

[a] MS(EI): 221(8%), 167(13%), 123(12%), 105(11%), 95(100%), 93(25%), 86(16%), 78(13%), 67(50%), 55(15%), 53(18%), 41(17%).

[b] MS(EI): 207(5%), 192(54%), 177(27%), 159(9%), 150(9%), 149(11%), 136(88%), 121(97%), 107(100%), 93(65%), 91(44%), 79(32%), 77(22%), 55(18%), 43(56%), 41(26%).

[c] MS(EI): 207(3%), 192(57%), 177(43%), 163(11%)150(12%), 149(22%), 136(37%), 135(31%), 121(48%), 105(40%), 93(74%), 91(29%), 77(42%), 59(100%), 43(33%), 41(27%).

[d] MS(EI): 133(11%), 109(17%), 95(28%), 85(41%), 84(36%), 71(69%), 58(98%), 43(100%).

Green-leaf volatiles [(3*Z*)-hexenal, hexanal, (2*E*)-hexenal, (3*Z*)-hexenol, and (2*E*)-hexenol] were relatively abundant in all three *G. spinosa* samples. However, *n*-alkanes and fatty acids were particularly abundant in *G. spinosa* #1, while oxygenated monoterpenoids and benzenoid aromatics were relatively abundant in samples #2 and #3. *Grayia spinosa* sample #3 had relatively large concentrations of the di-*nor*-sesquiterpenoids dihydroedulan IIA (10.7%), dihydroedulan IA (3.8%), *cis*-theaspirane (4.4%), and *trans*-theaspirane (3.2%).

3.3 The Amaranthaceae: Since these are the first investigations of the essential oils of *A. canescens* and *G. spinosa*, there are no previous reports to compare. There are, however, reports in the literature on essential oil compositions of other *Atriplex* species as well as other members of the Amaranthaceae (see Table 4). A perusal of Table 4 shows that *Atriplex semibaccata* was rich in 4-vinylguaiacol as was found in both *A. canescens* and *G. spinosa*. *n*-Alkanes are also important constituents of several Amaranthaceae essential oils, including *Atriplex lentiformis*, *Aerva javanica*, and *Alternanthera sessilis*.

Table 4: Major components of essential oils of several members of the Amaranthaceae from the literature.

Plant species	Collection site	Major components	Ref.
Atriplex cana Ledeb.	Urumqi, China	Dibutyl phthalate (21.8%),[a] 1,8-cineole (20.1%), myrtenyl acetate (15.6%), and camphor (5.1%).	[25]
Atriplex halimus L.	Mascara, Algeria	Viridiflorol (40.2%), phytol (18.2%), and germacrene D (6.9%).	[26]
Atriplex lentiformis (Torr.) S.Watson	Adrar, Algeria	Longiborneol (14.4%), caryophyllene oxide (10.5%), undecylenic acid (10.5%), linalool (9.8%), nonadecane (8.4%), menthol (8.2%), and phytol.[b]	[27]
Atriplex semibaccata R.Br.	Kettara region, Morocco	4-Vinylguaiacol (48.9%) and benzyl alcohol (6.3%)	[28]
Atriplex undulata (Moq.) D. Dietr.	Bahía Blanca, Argentina	*p*-Acetanisole (28.4%), β-damascenone (9.3%), (*E*)-β-ionone (5.1%), and viridiflorene (4.7%).	[29]
Aerva javanica Juss.	Mehendri-Jo-Par, Pakistan	Hentriacontane (21.5%), nonacosane (20.6%), heptacosane (19.8%), and pentacosane (5.6%).	[30]
Alternanthera brasiliana (L.) Kuntze	Franca, Brazil	2,6-di-*t*-Butyl-4-methylphenol[c] (42.2%), γ-eudesmol (10.6%), geraniol (9.2%), geranyl tiglate (8.2%), and aristolene (7.0%).	[31]
Alternanthera sessilis (L.) DC.	Lagos, Nigeria	Hexahydrofarnesyl acetone (35.6%), (*E*)-β-caryophyllene (16.1%), heptadecane (10.7%), caryophyllene oxide (6.4%), and hexadecane (6.4%).	[32]

[a] Dibutyl phthalate is a common plasticizer contaminant and not likely an essential oil component.
[b] This essential oil analysis cannot be considered reliable. There are inconsistencies in several of the RI values and there are halogenated compounds reported (Pentachloroethane, 1,2-dibromododecane, 2-bromooctadecanal), which should be rare in an essential oil. The RI values are not correct for longiborneol, caryophyllene oxide, dotriacontane (based on RI, probably nonadecane), menthol, and phytol.
[c] This is butylated hydroxytoluene (BHT), a common synthetic antioxidant, and is probably a contaminant and not an essential oil component.

4. Conclusions

This is the first report on the essential oil compositions of male and female *Atriplex canescens* as well as female *Grayia spinosa*. The essential oil yields as well as the volatile phytochemical makeup in male and female plants of *A. canescens* are very different. Female *Grayia spinosa* essential oils show wide compositional variation between the samples. Additional research on the *Atriplex* and *Grayia* genera as well as the Amaranthaceae family is needed to further characterize the phytochemistry of this family.

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6. Conflicts of Interest

The authors declare no conflicts of interest.

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