Volatile Organic Compounds and Essential Oil Composition of Selected Organs of Nepeta curviflora Collected from Two Regions in Jordan

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Abstract

The composition of volatile organic compounds and essential oils obtained by Solid Phase Micro Extraction (SPME) and hydro-distillation of fresh selected organs of *Nepeta curviflora* collected from two regions in Jordan (Salt and Irbid) were investigated by Gas Chromatography-Mass Spectrometry (GC-MS). The analysis of the SPME extracted volatile organic compounds (VOCs) revealed a variation of aroma composition emitted from the different plant organs collected from both locations. Among the different classes of compounds detected, oxygenated monoterpenes had the main contribution to the emitted aroma of the roots (20.83%) and petals (51.42%) collected from Salt and Irbid, respectively. On the other hand, the petals of the plant collected from Salt region had an emission profile dominated by monoterpenes hydrocarbons (67.30%). Other organs had an emission profile characterized by a high content of sesquiterpene hydrocarbons, their content ranged between 46.22 and 94.70%. Investigation of the composition of the hydro-distilled essential oils extracted from fresh leaves and flowers at the pre-flowering stage as well as the full flowering stage revealed that the oxygenated monoterpenes are the main components in the oils obtained from the three different organs, especially in the pre-flowering buds (Salt: 90.87%, Irbid: 92.59%). In all cases, the oxygenated monoterpene 4aα,7α,7aα-nepetalactone was the main component detected in the oils of the different organs from both locations. The current investigation revealed qualitative and quantitative differences in the composition using the two different extraction methods. The hydro-distillation method is more efficient in extracting less volatile compounds as compared to the SPME extraction method, which in general was dominated by a high hydrocarbon terpenoidal content.

Keywords: *Nepeta curviflora; SPME; GC-MS; volatile organic compounds; essential oil.*

Introduction

Nepeta is one of the largest genera belonging to the *Lamiaceae* family. It comprises about 250 species growing wild in different regions of the world, especially Europe, Asia, and Africa. Plants belonging to this genus are recognized as annual

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herbs.[1,2] Several *Nepeta* species are used as folk remedies due to their antimicrobial, antioxidant, antitumor, antihypertensive, antispasmodic and anti-asthmatic properties.[3]

Nepeta curviflora Boiss is one of six *Nepeta* species that are reported to grow wild in Jordan. Other species include *Nepeta italica, Nepeta trachonitica, Nepeta glomerata, Nepeta involucrata* and *Nepeta Cilicia*.[4]

N. curviflora (Figure 1), commonly known in Arabic as na'na' elher (Syrian catnip), is a perennial herbaceous mountain herb that is 30-40 cm high. This aromatic plant is characterized by its small leaves and numerous upright spikes of brilliant violet-blue flowers. It is reported in several Levant countries including Palestine, Jordan, Syria and Lebanon.[5]

Figure 1: *Nepeta curviflora* from wild populations in Jordan (Salt).

Literature survey revealed that several *Nepeta* species were investigated for their essential oil composition and secondary metabolites content.[6, 7] In most cases, whole aerial parts were used to extract the essential oil by hydro-distillation. The composition of the hydro-distilled oil obtained from the aerial parts of *N. curviflora* growing wild in Lebanon has been reported.[8, 9] Recently, Al-Qudah reported the chemical composition and the *in-vitro* antioxidant activity of the essential oils extracted from fresh and air-dried aerial parts of *N. curviflora* collected from Northern Jordan.[10] The effect of the extraction method on the composition of the volatile constituents extracted from different organs of *N. curviflora* has never been described before.

Thus, in our current investigation, Solid Phase Micro Extraction (SPME) method was used to extract the spontaneously emitted volatile organic compounds (VOCs) from different fresh aerial and terrestrial organs of *N. curviflora* collected from two different geographical regions in Jordan; Salt and Irbid. Moreover, the study was also designed to compare the chemical composition of hydro-distilled oils and SPME extracted VOCs of *N. curviflora* collected from wild populations in these two geographical regions, extracted from selected aerial parts including leaves and the flowers at the pre-and full flowering stages.

Methods and Materials

General

N. curviflora Boiss was collected during the flowering stage from Salt region, northwest of the capital Amman, from early to late May 2017 and from Bayt-Yafa, Irbid, Northern Jordan, from late May to early June 2017. The plant was identified by Prof. Barakat E. Abu-Irmaileh, Department of Plant Protection, School of Agriculture, the University of Jordan. A voucher specimen (No: BAU/2017/LNc) has been kept in the Department of Physics and Basic Sciences, Faculty of Engineering Technology, Al-Balqa Applied University, Amman, Jordan.

Solid Phase Micro Extraction experiments were performed using an SPME fiber assembly (Polydimethylsilocane/Divenylbenzene, PDMS/DVB; df 65 mm partially crossed-linked phase, fiber length 1 cm) and assemblies for manual sampling (Supelco, Bellefonte, PA, USA). GC-MS analysis was performed utilizing a Varian Chrompack CP-3800 GCMS-MS-200 (Saturn, Netherlands) system equipped with a DB-5 capillary column (5% diphenyl, 95% dimethyl polysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thicknesses). Quantitative analysis was performed using a Hewlett-Packard HP-8590 gas chromatograph equipped with a split-splitless injector (split ratio 1:50), an optima-5 fused silica capillary column (5% diphenyl, 95% dimethyl polysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness) and an FID detector.

The components of the volatile organic compounds and hydro-distilled essential oils were identified using the built-in libraries (NIST Co. and Wiley Co., USA) and by comparison of their calculated retention indices relative to (C_8–C_{20}) *n*-alkanes with literature values measured with columns of identical polarity or with authentic samples using the same temperature program profiles employed.[11] Authentic compounds including α- and β-pinenes, *p*-cymene, limonene, linalool and thujone (Sigma-Aldrich, Buchs, Switzerland) were used as reference substances in the GC-MS analysis. GC-grade hexane and analytical reagent grade anhydrous Na_2SO_4 were purchased from Scharlau (Barcelona, Spain) and Analar (UK), respectively.

Extraction of essential oils and volatile organic compounds (VOCs)

The hydro-distillation and SPME experiments were performed according to the procedure described in literature.[12-18] The extractions and analytical experiments were performed twice.

A 150 g sample each of leaves, pre-flowering buds, fully expanded flowers from a freshly herb was collected from the two geographical regions. The different organs were coarsely powdered and then hydro-distillated using Clevenger apparatus for 3 h. The extraction was repeated twice and the oils obtained were pooled separately, dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4°C in amber glass vials until analysis.

In the SPME procedure, about 10 mg of fresh plant selected organ (roots, stems, leaves, pre-flowering buds, fully expanded flowers, brackets and petals) was introduced separately into 4.0 mL amber glass vials, tightly capped with PTFE-coated septa, and SPME extraction was performed for 2.0 min at room temperature. Desorption of the analytes was carried out at 240 °C for 60 s.

GC-MS and GC-FID analysis

About 1 µL aliquot of each oil sample, appropriately diluted in GC-grade *n*-hexane, was subjected to GC-MS analysis. The actual temperature of the MS source reached approximately 180 °C. The ionization voltage was 70 eV. An isothermal column temperature (60 °C) was employed for 1 min and then raised to 246 °C at a rate of 3 °C/min. The temperature was then kept isothermally for another 3 min after which it was held constant at 246 °C for another 3 min. (isothermal). The flow rate of Helium as a carrier gas was 0.9 mL/min.

Results and Discussion

The current investigation was designed to evaluate the variation in the composition of the volatile organic compounds (VOCs) spontaneously emitted from the different organs of *N. curviflora* collected from two different regions of Jordan. These VOCs were extracted from the different organs by SPME and then analyzed by GC-MS. Table 1 lists the volatile organic compounds along with their retention indices (literature and experimental) and percentage composition.

The investigation of the volatile principles emitted from the different organs of *N. curviflora* from Salt region resulted in the identification of 74 compounds. The emission profile of the roots sample was mainly rich in total with oxygenated monoterpenes (20.83%) and sesquiterpenes hydrocarbons (20.31%). These two classes were represented by isobornyl isobutanoate (5.73%) and γ–muurolene (7.93%). Notably, the oxygenated sesquiterpene cubebol was the main component detected in the aroma emitted by the roots accounting for 18.60% of the total emission profile followed by the aromatic compound *p*-cymene (9.30%).

Investigation of the composition of the VOCs emitted by the stems revealed *p*-cymene as the major component, accounting for 21.77% of the total emission profile. However, the total emission profile was again characterized by a high content of sesquiterpenes hydrocarbons that amounted to 67.52% of the total composition. *Allo*-aromadendrene (13.35%) and β-bisabolene (11.26%) were the main constituents in the class.

The leaves emission profile was also rich in sesquiterpene hydrocarbons that were detected at much higher concentration levels as compared to the emission profiles of other organs of the plant from Salt region (81.33%). This class was represented by *trans*-caryophyllene (24.65%), germacrene-D (12.68%) and α-humulene (10.09%).

Table 1: Percentage composition of the spontaneously emitted VOCs of *N. curviflora* collected from two regions and extracted by SPME.

	Lit RI	Calc RI	Name	Salt							Irbid						
				Root	Stem	Leaves	Pre-flowering Buds	Flower	Bracket	Petal	Root	Stem	Leaves	Pre-flower Buds	Flower	Bracket	Petal
1	901	903	Ethyl pentanoate	-	-	-	-	-	0.33	-	-	0.11	-	0.17	-	0.10	-
2	939	934	α-Pinene	-	3.21	-	-	-	1.58	-	-	1.47	-	0.18	-	0.14	0.98
3	975	979	Sabinene	-	0.98	-	-	-	0.06	-	3.75	2.06	1.47	0.54	0.87	0.10	-
4	979	979	β-Pinene	-	-	-	-	-	-	-	-	-	-	0.43	-	-	-
5	979	985	Octen-3-ol	1.25	-	-	-	-	-	-	-	-	-	-	0.52	-	-
6	991	994	Myrcene	2.58	-	-	-	-	-	-	1.05	-	-	0.34	0.52	-	-
7	991	999	3-Octanol	1.65	-	-	-	-	-	-	-	-	-	-	0.56	0.17	-
8	995	1004	2-Octanol	-	-	-	-	-	-	-	-	-	-	-	0.41	-	-
9	1002	1004	2-Carene	1.56	-	-	-	-	0.60	1.22		0.14	-	-	-	-	-
10	1025	1024	*p*-Cymene	9.30	21.77	0.44	0.38	1.24	16.13	-	3.57	-	-	-	-	1.43	-
11	1026	1026	*o*-Cymene	4.00	-	-	-	-	-	-		4.32	-	-	-	-	-
12	1029	1029	Limonene	-	-	-	0.67	-	-	-	10.95	-	-	-	-	-	-
13	1037	1035	*Z*-β-Ocimene	4.59	-	3.95	1.70	5.45	-	17.72	-	-	0.47	0.69	1.24	-	-
14	1050	1045	*E*-β-Ocimene	-	-	8.02	4.30	11.84	-	45.20	-	-	0.50	1.39	2.38	0.18	-
15	1060	1058	γ-Terpinene	3.70	-	-	-	-	0.48	-	-	0.38	0.41	0.07	-	0.06	-
16	1089	1086	Terpinolene	1.09	-	-	-	-	-	-	1.09	0.10	-	-	-	-	-
17	1091	1093	Methyl benzoate	-	-	0.59	-	-	0.49	-	-	-	-	-	-	0.04	-
18	1097	1100	Linalool	0.75	-	-	-	3.56	-	-	1.45	-	-	0.09	0.56	-	-
19	1108	1110	*cis*-Rose oxide	0.73	-	-	-	-	-	-	-	-	-	-	-	-	-
20	1114	1118	β-thujone	2.67	-	-	-	-	-	-	-	-	-	-	-	-	-
21	1122	1135	2-Ethylhexanoic acid	-	-	0.66	-	-	-	-	-	-	-	-	-	-	-
22	1132	1130	*allo*-Ocimene	-	-	-	-	0.95	-	2.34	1.05	-	-	0.08	-	-	-
23	1144	1140	*neo-allo*-Ocimene	4.76	-	0.59	-	-	-	0.82	-	-	-	-	-	-	-
24	1146	1147	Camphor	0.85	-	-	-	-	-	-	-	-	-	-	-	-	-
25	1153	1165	Menthone	0.71	-	-	-	-	-	-	-	-	-	-	-	-	-
26	1192	1193	Methyl salicylate	-	-	-	-	0.73	-	-	-	-	-	-	-	0.33	-
27	1202	1203	*n*-Decanal	-	-	0.94	-	3.33	-	-	-	0.12	1.44	-	-	-	-
28	1226	1229	Citronellol	-	-	-	0.31	0.90	-	-	-	-	-	-	-	0.25	-
29	1250	1254	Heptyl isobutanoate	-	-	-	-	-	-	-	-	-	-	0.12	0.67	-	-
30	1287	1285	Pregeijerene	-	-	-	-	1.95	-	-	-	-	-	-	-	-	-
31	1298	1298	2-Ethy-oxo-Fenchol	1.73	-	-	-	-	-	-	-	-	-	0.15	-	0.07	-
32	1299	1300	Carvacrol	1.63	-	-	-	1.33	-	-	-	-	-	-	-	-	-
33	1318	1317	*cis*-Dihydro-α-terpinyl acetate	0.70	-	-	-	-	-	-	-	-	-	0.19	-	-	-
34	1333	1323	*cis*-3-Hexenyl tiglate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1338	1333	δ-Elemene	-	-	-	0.27	-	-	-	-	0.98	1.02	1.84	1.47	1.05	-
36	1351	1344	α-Cubebene	-	-	0.65	-	-	-	-	-	0.48	0.56	0.40	-	-	-

	Lit RI	Calc RI	Name	Salt							Irbid						
				Root	Stem	Leaves	Pre-flowering Buds	Flower	Bracket	Petal	Root	Stem	Leaves	Pre-flower Buds	Flower	Bracket	Petal
37	1360	1361	4aα,7α,7aα-Nepetalactone	-	1.51	1.87	21.05	12.74	2.26	5.33	25.99	-	0.74	7.22	18.38	-	48.34
38	1377	1374	α-Copaene	-	1.19	0.89	0.25	-	-	-	-	1.23	1.19	0.14	13.37	-	-
39	1387	1392	4aα,7α,7aβ-Nepetalactone	1.20	-	0.57	-	-	0.57	-	1.68	-	-	-	0.44	0.44	-
40	1388	1383	β-Bourbonene	-	11.04	2.64	1.86	1.65	0.95	0.96	-	1.34	0.91	0.68	19.45	4.88	-
41	1391	1390	β-Elemene	2.10	-	1.40	0.46	-	-	-	-	1.00	0.93	0.60	1.58	-	-
42	1392	1393	4aα,7β,7aα-Nepetalactone	4.12	-	-	0.53	-	-	-	-	-	-	7.21	2.72	5.83	3.08
43	1408	1407	Longifolene	-	-	-	-	-	-	-	-	-	-	0.17	-	-	-
44	1409	1410	*cis*-Caryophyllene	-	-	-	48.12	-	-	0.69	-	-	-	0.09	-	-	-
45	1410	1406	α-Gurjunene	-	0.66	-	-	-	0.79	0.68	-	0.97	1.62	0.57	1.14	-	-
46	1419	1420	*trans*-Caryophyllene	3.53	3.60	24.65	0.27	26.50	42.74	17.75	4.33	37.81	46.39	23.34	1.73	40.03	12.98
47	1432	1427	β-Copaene	-	-	1.14	0.43	-	-	-	-	-	-	0.39	-	-	-
48	1434	1431	β-Gurjunene	-	-	0.58	-	-	-	-	-	-	-	0.46	-	-	-
49	1434	1435	Isobornyl isobutanoate	5.73	-	-	-	-	-	-	-	-	-	-	-	-	-
50	1435	1430	*trans*-α-Bergamotene	-	4.35	-	-	3.46	2.00	-	15.77	-	-	-	-	-	-
51	1437	1440	γ-Elemene	1.06	-	-	-	-	-	-	1.22	-	-	0.46	-	-	-
52	1441	1435	Aromadendrene	-	-	0.60	0.48	-	-	-	1.05	3.78	3.88	3.76	2.57	6.10	-
53	1450	1442	*cis*-Muurola-3,5-diene	-	-	0.48	-	-	-	-	-	-	0.27	-	-	-	-
54	1451	1454	α-Himchalene	-	-	-	5.22	2.58	-	-	2.61	-	-	12.27	6.09	-	-
55	1455	1451	α-Humulene	-	-	10.09	-	-	0.55	0.61	-	-	0.78	-	-	1.59	-
56	1460	1456	*allo*-Aromadendrene	0.82	13.35	0.76	0.38	1.33	5.79	0.45	1.22	8.60	-	0.34	2.04	13.76	9.24
57	1466	1457	9-epi-*E*-Caryophyllene	-	-	-	-	-	-	-	-	-	5.15	1.77	-	-	-
58	1467	1459	*cis*-Muurola4(14),5-diene	-	-	0.71	-	-	-	-	-	-	-	0.16	-	-	-
59	1477	1467	γ-Gurjunene	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-
60	1480	1476	γ-Muurolene	7.93	-	3.02	0.87	1.07	1.01	-	1.33	-	-	0.78	-	0.18	-
61	1485	1477	Germacrene-D	-	3.89	12.68	5.03	1.55	2.86	0.76	2.95	9.03	7.37	3.31	2.08	0.45	0.96
62	1485	1488	α-amorphene	-	-	1.68	-	-	-	-	-	-	-	0.14	-	-	-
63	1493	1480	*cis*-β-Guaiene	-	-	-	-	-	-	-	1.25	-	-	-	0.33	-	-
64	1496	1486	Valencene	-	6.38	-	2.35	-	2.79	-	2.01	-	-	0.48	1.32	-	-
65	1497	1489	Viridiflorene	-	3.47	0.73	-	-	-	0.71	-	9.60	-	2.25	-	9.55	-
66	1498	1505	α-Selinene	0.84	-	-	-	-	-	-	-	-	-	-	-	-	-
67	1500	1491	Bicyclogermacrene	-	-	1.88	1.67	0.86	-	-	1.99	-	6.41	8.04	7.09	-	8.56
68	1500	1495	α-Muurolene	-	-	0.98	0.29	-	-	-	-	-	-	0.19	-	-	-
69	1506	1505	β-Bisabolene	-	11.26	5.21	-	4.07	8.52	-	4.09	6.90	9.12	6.52	-	-	9.10
70	1512	1498	δ-Amorphene	-	-	1.95	-	-	-	-	-	-	-	-	3.73	7.44	-
71	1514	1508	γ-Cadinene	-	-	3.67	0.56	-	-	0.92	1.02	-	-	0.31	0.50	-	-
72	1515	1520	Cubebol	18.60	-	-	-	-	-	-	-	-	-	-	-	-	-
73	1523	1514	δ-Cadinene	-	8.33	-	1.05	-	-	1.06	1.75	8.35	9.38	0.87	-	5.34	-

	Lit RI	Calc RI	Name	Salt							Irbid						
				Root	Stem	Leaves	Pre-flowering Buds	Flower	Bracket	Petal	Root	Stem	Leaves	Pre-flower Buds	Flower	Bracket	Petal
74	1523	1521	β-Sesquiphellandrene	-	-	4.25	1.50	2.80	7.92	-	3.62	-	-	4.92	2.20	-	5.64
75	1539	1532	α-Cadinene	-	-	0.70	-	-	-	-	-	-	-	0.19	-	-	-
76	1561	1556	Germacrene-B	4.03	-	-	-	-	-	-	-	-	-	0.07	-	0.06	-
77	1564	1569	Longicamphenylone	1.20	-	-	-	-	-	-	-	-	-	-	-	-	-
78	1568	1565	z-Isoeugenol acetate	1.19	-	-	-	-	-	-	-	-	-	-	-	-	-
79	1578	1572	Spathulenol	-	-	-	-	-	-	-	-	-	-	0.08	-	-	-
80	1583	1577	Caryophyllene oxide	-	2.90	0.55	-	0.83	-	-	-	0.11	-	0.07	-	-	-
81	1601	1599	Cedrol	-	-	-	-	-	0.28	-		-	-	-	-	-	-
82	1624	1627	10-epi-γ-Eudsemol	-	-	-	-	2.54	-	-		-	-	-	-	-	-
83	1675	1678	8-Hydroxy-isobornyl isobutanoate	-	-	-	-	0.81	-	-	-	-	-	-	-	-	0.77
			Aliphatic hydrocarbons	**2.90**	**-**	**1.61**	**-**	**3.33**	**0.33**	**-**	**-**	**0.23**	**1.44**	**0.29**	**2.17**	**0.27**	**-**
			Aromatic hydrocarbons	**14.49**	**21.77**	**1.04**	**0.38**	**1.97**	**16.62**	**-**	**3.57**	**4.32**	**-**	**-**	**-**	**1.81**	**-**
			Monoterpenes hydrocarbons	**18.27**	**4.20**	**12.56**	**6.67**	**18.25**	**2.73**	**67.30**	**17.89**	**4.15**	**2.85**	**3.72**	**5.01**	**0.48**	**0.98**
			Oxygenated monoterpenes	**20.83**	**1.51**	**2.44**	**21.89**	**18.53**	**2.83**	**5.33**	**29.12**	**-**	**0.74**	**14.86**	**22.11**	**6.59**	**51.42**
			Sesquiterpene hydrocarbons	**20.31**	**67.52**	**81.33**	**70.80**	**47.83**	**75.94**	**24.60**	**46.22**	**90.08**	**94.70**	**75.63**	**66.69**	**90.44**	**46.47**
			Oxygenated sesquiterpenes	**19.81**	**2.90**	**0.55**	**-**	**4.18**	**0.28**	**-**	**-**	**0.11**	**-**	**0.15**	**-**	**-**	**0.77**
			Total identified	**96.60**	**97.9**	**99.53**	**99.74**	**94.09**	**98.73**	**97.23**	**96.80**	**98.89**	**99.73**	**94.65**	**95.98**	**99.59**	**99.64**

The different flowering organs including pre-flowering buds, fully expanded flowers and brackets were characterized by a high sesquiterpene hydrocarbons content (70.80%, 47.83% and 75.94%, respectively). *cis*-Caryophyllene was the main sesquiterpene hydrocarbon detected in the pre-flowering buds (48.12%) while *trans*-caryophyllene had the highest contribution to this class in the fully expanded flowers (26.50%) as well as in brackets (42.74%). Oxygenated monoterpenoids was the second main class of terpenoids detected in the emitted VOCs of pre-flowering buds (21.89%) and fully expanded flowers (18.53%), represented mainly by 4aα,7α,7aα-nepetalactone (pre-flowering: 21.05%; full flowering: 12.74%). Interestingly, petals had a different emission pattern in comparison with the other flowering organs, characterized by a high content of monoterpene hydrocarbons (67.30%). This class contained mainly *E*-β-ocimene (45.20%) and *Z*-β-ocimene (17.72%).

The analysis of the SPME extracted VOCs from plant samples collected from Northern Jordan region (West of Irbid) resulted in the identification of a total of 68 compounds emitted from the different organs of *N. curviflora*. In general, sesquiterpene hydrocarbons dominated the emission profile of the different organs, but detected at much higher concentration levels as compared to the Salt samples. The content of this particular class of terpenoids amounted to 46.22% of the emission profile of the roots, 90.08% of the stems, 94.70% of the leaves, 75.63% in the pre-flowering buds, 66.69% of the full flowering stage and 90.44% in the brackets. The *N. curviflora* root samples from northern Jordan were also characterized by a high content of 4aα,7α,7aα-nepetalactone (25.99%) which was completely absent in the emission profile of the samples collected from Salt region. Again, *trans*-caryophyllene was the main sesquiterpene hydrocarbon detected in the spontaneously emitted VOCs of the stems (37.81%), leaves (46.39%), closed flowering buds at the pre-flowering stage (23.34%) and flower brackets (40.03%) during the full flowering stage. However, the main sesquiterpene hydrocarbons detected in the emission profiles of the flowers during the full flowering stage included β-bourbonene (19.45%), α-copaene (13.37%) and bicyclogermacrene (7.09%).

The petals sample from Northern Jordan showed an emission profile pattern characterized by a high content of oxygenated monoterpenes (51.42%) that was represented mostly by nepetalactone isomers including 4aα,7α,7aα-nepetalactone (48.34%) and 4aα,7β,7aα-nepetalactone (3.08%).

The chemical composition of the essential oils extracted by hydro-distillation of the fresh leaves, pre-flowering buds and flowers during the full flowering stage was performed in parallel with the samples collected from both locations. The percentage yield of the hydro-distilled oils in both locations was 0.53% (fresh leaves), 0.30% (pre-flowering buds) and 0.57% (fully flowering buds). A total of 32 compounds were identified from the fresh organs of the plant from the two locations. Results for the GC-MS analysis of the hydro-distilled oils are summarized in Table 2.

Table 2: Percent composition for essential oil from *N.curviflora* obtained by hydro-distillation.

	Lit RI	Calc RI	Name	Salt			Irbid		
				Leaves	Pre-flowering Buds	Flower	Leaves	Pre-flowering Buds	Flower
1	900	906	Nonane	1.24	0.54	2.45	-	-	-
2	939	940	β-Pinene	-	-	-	-	-	1.54
3	1037	1034	*Z*-β-Ocimene	0.54	-	-	0.57	-	-
4	1050	1044	*E*-β-Ocimene	2.08	0.10	-	1.58	-	-
5	1226	1229	Citronellol	-	0.05	-	0.14	-	-
6	1333	1323	*cis*-3-Hexenyl tiglate	0.17	0.05	-	0.05	-	-
7	1338	1330	δ-Elemene	-	0.09	-	-	0.71	0.47
8	1360	1361	4a-α,7-α,7a-α-Nepetalactone	66.60	89.95	71.46	75.76	85.74	83.59
9	1388	1381	β-Bourbonene	0.72	0.23	0.68	0.35	-	-
10	1387	1392	4a-α,7-α,7a-β-Nepetalactone	0.43	0.18	0.29	0.38	0.52	-
11	1391	1387	β-Elemene	0.34	-	-	-	0.06	-
12	1392	1393	4a-α,7-β,7a-α-Nepetalactone	0.19	0.65	1.00	0.82	6.33	5.69
13	1410	1403	α-Gurjunene	-	-	-	0.04	-	-
14	1419	1416	*trans*-Caryophyllene	6.92	4.05	10.10	1.57	1.93	2.74
15	1432	1426	β-Copaene	-	-	-	0.06	-	-
16	1441	1457	Aromadendrene	0.13	-	-	0.05	-	-
17	1457	1451	*E*-β-Farnesene	2.12	0.99	3.64	1.79	0.71	-
18	1460	1455	*allo*-Aromadendrene	-	-	-	0.35	0.10	1.04
19	1485	1477	Germacrene-D	10.59	1.12	1.65	7.33	0.51	0.18
20	1500	1491	Bicyclogermacrene	1.32	0.32	1.17	3.40	1.30	1.70
21	1506	1505	β-bisabolene	1.70	0.43	2.41	1.91	0.68	0.90
22	1514	1514	γ-Cadinene	-	-	-	0.05	-	-
23	1523	1521	β- Sesquiphellandrene	1.24	0.35	1.39	1.06	0.48	0.58
24	1550	1547	α-Agarofuran	0.17	-	-	0.06	-	-
25	1564	1569	Longicamphenylone	-	-	0.13	0.10	-	-
26	1578	1572	Spathulenol	0.53	0.12	0.24	1.02	0.33	-
27	1583	1577	Caryophyllene oxide	2.65	0.38	1.87	0.86	0.30	0.66
28	1601	1599	Cedrol	-	-	-	0.05	-	-
29	1604	1603	Khusimone	-	-	-	0.13	-	-
30	1619	1611	1,10-diepi-Cubenol	-	0.05	0.14	0.25	0.05	-
31	1624	1627	10-epi-γ-Eudsemol	-	0.08	0.15	0.10	0.06	-

	Lit RI	Calc RI	Name	Salt			Irbid		
				Leaves	Pre-flowering Buds	Flower	Leaves	Pre-flowering Buds	Flower
32	1664	1664	7-epi-α-Eudesmol	-	-	-	0.06	-	0.21
		Aliphatic hydrocarbons		**1.24**	**0.54**	**2.45**	**-**	**-**	**-**
		Aromatic hydrocarbons		**-**	**-**	**-**	**-**	**-**	**-**
		Monoterpenes hydrocarbons		**2.62**	**0.10**	**-**	**2.15**	**-**	**1.54**
		Oxygenated monoterpenes		**67.39**	**90.87**	**72.74**	**77.15**	**92.59**	**89.28**
		Sesquiterpene hydrocarbons		**25.08**	**7.58**	**21.04**	**17.97**	**6.48**	**7.61**
		Oxygenated sesquiterpenes		**3.35**	**0.63**	**2.53**	**2.63**	**0.74**	**0.87**
		Total identified		**99.68**	**99.72**	**98.76**	**99.90**	**99.81**	**99.30**

Hydro-distilled essential oils of the fresh aerial organs including leaves, pre-flowering buds and fully expanded flowers were in general richer in oxygenated monoterpenes as compared to the SPME results, especially in oils obtained from the pre-flowering buds (Salt: 90.87%, Irbid: 92.59%). In both regions, 4aα,7α,7aα-nepetalactone was mostly the main component detected in the pre-flowering buds of both samples, amounting to 89.95% and 85.74% of the total content in Salt and Irbid samples, respectively.

Previously, Al-Qudah reported the chemical composition of the hydro-distilled essential oil of fresh and air-dried aerial parts of *N. curviflora* growing wild in Bayt-Yafa, Irbid (Northern Jordan).[10] Sesquiterpene hydrocarbons was the main class of compounds detected in the fresh samples (55.27%) while oxygenated monoterpenes had the highest contribution to the oil obtained from whole air dried samples (50.31%). As compared to our investigation, hydro-distillation of a selected organ during a certain growth stage clearly revealed the selective accumulation of different types of terpenoids. Accordingly, in the current study, high sesquiterpene content was observed in the essential oils of fresh leaves and flowers, detected at much higher concentration levels as compared to the results of Al-Qudah[10] and those reported for the essential oil composition of the air dried aerial parts of *N. curviflora* from Lebanon.[8,9] The results of both investigations[8,9] revealed higher concentration levels of sesquiterpene hydrocarbons (61.2%, 66.6%) indicating the effect of location and the collecting period among many other factors on the essential oil composition.

Conclusions

The current investigation reports the chemical composition of the hydro-distilled oils and the SPME extracted VOCs spontaneously emitted from the different terrestrial and aerial parts of *N. curviflora* collected from two different locations in Jordan. The results clearly indicated the accumulation of certain classes of terpenoids in selected organs. The results of the current study confirmed the effect of the extraction method, location, period of collection, organ being investigated, and climate on the composition of both, VOCs and hydro-distilled oils. Comparison between the two extraction methods showed that SPME has the advantages of being simple, fast, green and consuming small amount of solvents. The two extraction methods are complementary for full investigation of VOCs emitted from aromatic plants material.

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