



**Chemical characterization and nematicidal activity of the essential oil of *Nepeta nuda* L. ssp. *pubescens* and *Nepeta curviflora* Boiss. from Lebanon**

Journal:	<i>Journal of Essential Oil Bearing Plants</i>
Manuscript ID	TEOP-2017-0247.R1
Manuscript Type:	Original Article
Keywords:	Lamiaceae, <i>Panagrolaimus rigidus</i> , terpenes, biopesticides, anthelmintics

SCHOLARONE™  
Manuscripts

ew Only

1  
2 **Regular articles**

3  
4 **Chemical characterization and nematicidal activity of the essential oil of *Nepeta nuda* L. ssp.**  
5  
6 ***pubescens* and *Nepeta curviflora* Boiss. from Lebanon**  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review Only

**Abstract**

The chemical characterization and the nematicidal activity of the essential oil from *Nepeta nuda* L. ssp. *pubescens* growing wild in Lebanon are reported. A comparative study with the essential oil of *Nepeta curviflora* Boiss. growing in Lebanon as well, was carried out. The most abundant (> 5%) components of *N. nuda* ssp. *pubescens* essential oil were pinane (12.89%), 1-ethyl-1H-pyrrole (12.67 %), 1-cycloethyl-1-(2-methylenecyclohexyl)ethanol (10.37%), 3-methyl-2-cyclohexen-1-one (9.17%) and 2,3-dimethyl-3-hexanol (5.88%). Among oxygenated monoterpenes, two nepetalactones were identified, i.e. (*E,Z*)-nepetalactone (2.24%) and (*Z,E*)-nepetalactone (0.31%). The major constituents (> 5 %) of *N. curviflora* essential oil were 2-isopropyl-5-methyl-3-cyclohexen-1-one (12.51%), (-)-spathulenol (11.73%), *cis-Z*- $\alpha$ -bisabolene epoxide (8.07%), widdrol (7.0 %), (*E,Z*)-5,7-dodecadiene (6.93%), dihydronepetalactone (5.57%) and 4-propyl-cyclohexene (5.43%). The essential oil of *N. curviflora* was more active than the *N. nuda* ssp. *pubescens* one against the nematode *Panagrolaimus rigidus*. According to the motility assay, LD<sub>50</sub> was 0.5 mg/mL and 2.5 mg/mL 24 h after treatment with *N. curviflora* and *N. nuda* ssp. *pubescens* essential oil, respectively. To the best of our knowledge, *N. nuda* ssp. *pubescens* has not been investigated to date.

**Keywords:** *Lamiaceae*, *Panagrolaimus rigidus*, terpenes, nepetalactones, biopesticides, anthelmintics.

## Introduction

The genus *Nepeta* (Lamiaceae) consists of around 300 herbaceous, perennial, rarely annual species. This genus is distributed over a wide area including Europe, Asia, Africa and the Middle East (Formisano et al., 2011). Some of these species are widely used in traditional medicine and are endowed with a number of pharmacological properties, usually attributed to their essential oils (Sharma et al. 2013). The main bioactive compounds are a variety of secondary metabolites, including monoterpenes (iridoids and their glycosides), diterpenes, triterpenes as well as phenols. *Nepeta nuda* L. is one of the most common species of the genus *Nepeta*. It is widespread from central and southeast Europe to Russia and towards southwest and central Asia. The essential oil composition of *N. nuda* was studied in a number of investigations (De Pooter et al. 1987; Alim et al. 2009; Turkey et al., 2011; Bozari et al., 2013; Gormez et al., 2013).

As part of a continuing study on the essential oils of some *Nepeta* species growing in Lebanon, we were interested in studying the chemical composition and the nematicidal activity of the essential oil of *N. nuda* ssp. *pubescens*. To the best of our knowledge, this plant has not been investigated to date.

Nematodes or roundworms are parasites of humans, vertebrates, insects and plants. Plant-parasitic nematodes are soil-inhabiting organisms mostly 0.5 to 1 mm long. These soil invertebrates have a hollow spear, called stylet, that penetrates plant cells and withdraws the cytoplasmic contents, thus damaging root tissues, with typical root and foliar symptoms. Nematodes can be grouped by feeding habit as endoparasitic (entire body inside the root), ectoparasitic (entire body outside the root) and semi-endoparasitic (part of body inside the root), whereas, by movement when feeding, they are divided into sedentary (mostly immobile during their life cycle) and migratory (mobile for all their life). In addition, nematodes can be vectors of plant viruses. Currently, plant diseases caused by nematodes threaten the world agriculture because of their severity and economic burden: in the developing countries, crop production losses attributable to nematodes are estimated at around 15%, compared with around 9% in developed countries. Not least, chemical control of

1  
2 nematodes is troublesome after the ban of soil fumigants such as methyl bromide, and, in addition,  
3  
4 non-volatile nematicides, organophosphates and carbamates, can represent environmental and  
5  
6 human threats due to their toxicological and ecotoxicological risks (Perry et al., 2006; Thompson et  
7  
8 al., 2005; De Waele et al., 2007; Lewis et al., 2009; Tritten et al., 2012). Therefore, environmentally  
9  
10 friendly and low-cost alternatives to chemical control measures for phytoparasitic nematodes are  
11  
12 urgently needed. In this context, essential oils from plants could be attractive nematicide compound  
13  
14 sources, possibly safe for non-target organisms.

15  
16  
17 In this paper, we report, for the first time, the chemical characterization and the nematicidal activity  
18  
19 of the essential oil from *N. nuda* ssp. *pubescens* growing in Lebanon. A comparative study with the  
20  
21 essential oil of *Nepeta curviflora* Boiss. (Senatore et al., 2005; Mancini et al., 2009; Al-Qudah  
22  
23 2016), growing in Lebanon as well, was carried out.

## 24 25 26 27 28 **Materials and methods**

29  
30 **Plant material and essential oil production.** The dried parts of *Nepeta nuda* ssp. *pubescens*  
31  
32 (flowering tops, seeds and leaves) were collected between 2005 and 2009 in a mountain area (1500  
33  
34 m above sea level) near to Tannourine, Lebanon. The dried parts of *Nepeta curviflora* (flowering  
35  
36 tops, seeds, and leaves) were collected in 2005 in Lebanon, near to the St. Antonio of Qozhaya  
37  
38 convent, Ehden region at 664 m above sea level.

39  
40  
41 Essential oils were obtained by steam distillation (250 mL water) from a sample (5 g) of air-dried  
42  
43 aerial parts of the plant. The collected aqueous phase (150 mL) containing the essential oil was  
44  
45 extracted with diethyl ether (40 mL × 5). The organic layer was dried over anhydrous sodium  
46  
47 sulfate, filtered and evaporated to obtain 12 mg (0.2%) of a light yellow oil from *N. nuda* and 19  
48  
49 mg (0.4%) from *N. curviflora*. The extracted EO samples were stored in sealed vials at low  
50  
51 temperature (4°C) before gas chromatography coupled with mass spectrometry (GC-MS) analysis.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 **Gas chromatography-mass spectrometry (GC-MS) analysis.** Oil compositions were analysed by  
3  
4 SPME/GC-MS, as reported by Scaglia et al. (2011) with some modifications. A manual SPME  
5  
6 device and divinylbenzene (DVB)/Carboxen/polydimethylsiloxane (PDMS) 50-30  $\mu\text{m}$  fiber -  
7  
8 Supelco, Bellefonte, PA, USA) was used. The compounds were adsorbed from the gas-phases by  
9  
10 exposing the fiber, preconditioned for 3 h at 250°C as suggested by the Supplier, to the oils on 20  
11  
12 ml vials. The fibers were incubated at 40°C for 60 min under agitation condition (speed of 500 rpm  
13  
14 with on time of 5 seconds and off time of 2 seconds).

15  
16  
17 Oils analysis was performed using an Agilent 5975C Series GC/MSD and FID as detector.  
18  
19 Volatiles were separated using a capillary column Zebron-WAX (Zebron, Phenomenex, USA) of 30  
20  
21 m x 0.25 mm (ID) and a film thickness of 0.25  $\mu\text{m}$ . Carrier gas was helium at a flow rate of 1 ml  
22  
23  $\text{min}^{-1}$ . Molecules were desorbed exposing the SPME in the GC injection port in splitless condition  
24  
25 for 600 seconds at 250 °C. The temperature program was isothermal for 3 min at 40 °C, then three  
26  
27 different temperature ramps were done to raise 115°C, 150°C and 220°C at a rate of 3 °C  $\text{min}^{-1}$ , 5°C  
28  
29  $\text{min}^{-1}$  and 7°C  $\text{min}^{-1}$  respectively. Finally an isothermal at 220°C for 15 min was done. The transfer  
30  
31 line to the mass spectrometer was maintained at 150 °C. The mass spectra were obtained by  
32  
33 electronic impact at 70 eV, a multiplier voltage of 1294 V and collecting data at a m/z range of 45–  
34  
35 300. Compounds were tentatively identified by comparing their mass spectra with those contained  
36  
37 in the NIST08 library (USA). Results were expressed as spectrum % and the molecules were  
38  
39 successively classified in main chemical groups.

40  
41  
42  
43 **Motility assay.** *Panagrolaimus rigidus* adults were suspended in M199 medium and, then,  
44  
45 approximately 100 larvae in 100  $\mu\text{L}$  of suspension were added to each well of a 96-well microplate,  
46  
47 followed by immediate addition of 100  $\mu\text{L}$  of the essential oil dilutions. Microplates were stored in  
48  
49 incubator at 20°C. After 1, 3, 5, 12 and 24 h, nematodes were removed from each well and mobile  
50  
51 and immobile roundworms were counted using an optical microscope at 40x magnification.  
52  
53 Nematodes were considered paralyzed or died when presenting with straight body and absence of  
54  
55  
56  
57  
58  
59  
60

1  
2 any motility after manipulation stimulus. M199 solution with 0.05% DMSO, and 50, 25, 5, 0.5,  
3  
4 0.05 and 0.005% methanol were used as controls. Experiments were carried out in triplicate for  
5  
6 each essential oil concentration and controls, and results are expressed as LD<sub>50</sub> (mg/mL).  
7  
8  
9

## 10 **Results and Discussion**

11  
12 Analysis by GC-MS of the essential oil obtained by hydrodistillation of the aerial parts of *Nepeta*  
13  
14 *nuda* ssp. *pubescens* allowed the identification of 49 compounds, representing 100% of the total oil  
15  
16 composition. The most represented chemical classes included monoterpene hydrocarbons (18.58%),  
17  
18 aldehydes/ketones (13.86%) and oxygenated monoterpenes (9.83%) (Tab. 1). The essential oil was  
19  
20 obtained in a yield of 0.2% (w/w), based on the dry weight of the sample. The most abundant (>  
21  
22 5%) components were pinane (12.89%), 1-ethyl-1H-pyrrole (12.67%), 1-cycloethyl-1-(2-  
23  
24 methylenecyclohexyl)ethanol (10.37%), 3-methyl-2-cyclohexen-1-one (9.17%) and 2,3-dimethyl-3-  
25  
26 hexanol (5.88%) (Table 1). Among oxygenated monoterpenes, two nepetalactones were identified,  
27  
28 *i.e.* (*E,Z*)-nepetalactone (2.24%) and (*Z,E*)-nepetalactone (0.31%) (Tab. 1).  
29  
30  
31

32  
33 The essential oil composition of *N. nuda* ssp. *pubescens* investigated in this study is different from  
34  
35 that reported for other subspecies of *N. nuda*. In fact, the essential oil from *N. nuda* L. ssp. *albiflora*  
36  
37 (Boiss.) Gams. growing wild in Lebanon was characterized by higher contents of three  
38  
39 nepetalactones: (*Z,E*)-nepetalactone (0.8-3.6%), (*E,Z*)-nepetalactone (4.4-8.0%) and (*Z,Z*-  
40  
41 nepetalactone (0.5-2.9%) (Mancini et al. 2009). However, these compounds were not detected in  
42  
43 the essential oil from *N. nuda* ssp. *albiflora* collected in Turkey, characterized by higher amount of  
44  
45 spathulenol (7.35%) compared with our *N. nuda* ssp. *pubescens* samples (2.92%) (Alim et al.,  
46  
47 2009). Similarly, nepetalctones were not found in *N. nuda* L. ssp. *nuda* essential oil from Turkey,  
48  
49 where higher levels of caryophyllene oxide (21.8%), spathulenol (13.8%) and *allo*-aromadendrene  
50  
51 (9.0%) were measured (Kökdil et al., 1998).  
52  
53  
54

55 The steam distillation of *N. curviflora* aerial parts yielded 0.4% (w/w on dry weight basis) essential  
56  
57 oil. GC-MS analysis identified 41 constituents, representing 100% of the total oil composition (Tab.  
58  
59  
60

2). The most represented chemical classes were oxygenated sesquiterpenes (41.65%) and oxygenated monoterpenes (24.73%) (Tab. 2). The major components (> 5%) were 2-isopropyl-5-methyl-3-cyclohexen-1-one (12.51%), (-)-spathulenol (11.73%), *cis-Z*- $\alpha$ -bisabolene epoxide (8.07%), widdrol (7.0%), (*E,Z*)-5,7-dodecadiene (6.93%), dihydronepetalactone (5.57%) and 4-propyl-cyclohexene (5.43%) (Tab. 2).

In a previous report, the most abundant compounds in *N. curviflora* essential oil were  $\beta$ -caryophyllene (41.6%), caryophyllene oxide (9.5%), (*E*)- $\beta$ -farnesene (6.2%) and (*Z*)- $\beta$ -farnesene (4.8%) (Mancini et al., 2009). Noteworthy, lower amounts of dihydronepetalactone were previously reported in essential oils from *Nepeta persica* Boiss. (3.5%), *N. nuda* ssp. *albiflora* (0.2%) and *Nepeta cataria* L. (0.08%) (Kökdil et al., 1996; Javidnia et al., 2002; Dmitrović et al., 2015). Therefore, the composition of essential oil of *N. curviflora* is quite different from the ones previously reported in the literature (Senatore et al., 2005; Mancini et al., 2009; Al-Qudah 2016). However, it should be noted that chemical polymorphism is characteristic of this species, and the oil composition depends on variety, growing site, climatic conditions, balsamic period, time of collection and analytical method (Formisano et al., 2011).

A number of biological properties have been ascribed to *Nepeta* species. In particular, phytotoxic, antibacterial, antiviral and antioxidant activities have been reported in *N. nuda* ssp. *albiflora*, *N. nuda* ssp. *nuda* and *N. curviflora*. (Alim et al., 2009; Mancini et al., 2009; Gormez et al., 2013; Todorov et al., 2015). As regards nematocidal activity of *Nepeta* species, it was only reported for *Nepeta cataria* L. Both solvent (methanol, ethanol and water) extracts and essential oil from aerial parts of this plant species exhibited anthelmintic effects on *Meloidogyne chitwoodi*, *Meloidogyne incognita* and *Haemonchus contortus* (Bandh et al., 2011; Pavaraj et al., 2012; Alam et al., 2015; Faria et al., 2016). Our results demonstrated that the essential oils of *N. curviflora* and *N. nuda* ssp. *pubescens* are endowed with nematocidal activity as well. In particular, the essential oil of *N. curviflora* was more active than the *N. nuda* ssp. *pubescens* one against *Panagrolaimus rigidus*. According to the motility assay, LD<sub>50</sub> was 0.5 mg/mL and 2.5 mg/mL 24 h after treatment with *N.*



1  
2 *curviflora* and *N. nuda* ssp. *pubescens* essential oil, respectively (Fig. 1). *P. rigidus*, a free-living  
3 bacteriovorus nematode, represents a model organism for research on novel anthelmintics. Indeed,  
4 this nematode has been widely used in basic research on anthelmintic pharmacology of human and  
5 agricultural parasites, as well as at understanding the mechanisms of resistance to anthelmintics.  
6 Several characteristics make *P. rigidus* a reliable experimental model, such as rapid life cycle, easy  
7 growth and manipulation in laboratory conditions, knowledge of its genome and phylogenetic  
8 proximity to other nematodes.  
9

10 The different *in vitro* nematicidal activity of the two *Nepeta* species can be due to the diverse  
11 essential oil composition. In particular, a number of reports highlighted the anthelmintic potential of  
12 terpenes. The nematicidal activity of these compounds against *Meloidogyne incognita* was found to  
13 decrease in the order carvone > pulegone > *trans*-anethole > geraniol > eugenol > carvacrol >  
14 thymol > terpinen-4-ol, with EC<sub>50</sub> values (24 h) calculated in the range of 115-392 µg/mL (Ntalli et  
15 al., 2010). Similar results and EC<sub>50</sub> values were reported against *M. javanica* (Oka et al., 2000). The  
16 highest mortality (100%) on this nematode species was induced by carvacrol, geraniol and thymol  
17 (0.5 mg/mL, 72 h incubation) (Andrés et al., 2012). Carvacrol, thymol, nerolidol,  $\alpha$ -terpinene,  
18 geraniol, citronellol, farnesol, limonene, pseudoionone and eugenol also caused 50% mortality at 50  
19 µg/mL on the model nematode *Caenorhabditis elegans* (Abdel-Rahman et al., 2013). Noteworthy,  
20 the contribution of each terpene constituent to the overall activity of an essential oil seems to be due  
21 to a rather complicated pattern of interactions, since they may act together synergistically or  
22 antagonistically. Some terpene pairs exhibiting synergistic activity on *M. incognita* paralysis in  
23 decreasing order were: *trans*-anethole/geraniol > *trans*-anethole/eugenol > carvacrol/eugenol >  
24 geraniol/carvacrol (Ntalli et al., 2011). Therefore, it is very important to understand the synergy and  
25 antagonism interactions among individual components of a nematicidal essential oil. In this view,  
26 the presence of nematicidal terpenes in the *N. curviflora* essential oil, *i.e.* pulegone, carvacrol,  
27 eugenol and thymol, could contribute to explain its higher activity against *P. rigidus* motility,  
28 compared with *N. nuda* ssp. *pubescens* essential oil.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 In conclusion, the present study revealed that essential oil of *N. curviflora* exhibits promising  
3  
4 nematicidal activity, in addition to the previously reported phytotoxic effects. On the whole, these  
5  
6 results highlight the possibility of using this plant product as biopesticide in soil treatment to  
7  
8 control phytopathogenic nematodes and weeds.  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## References

- 1  
2  
3  
4 **Abdel-Rahman, F. H., Alaniz, N. M., Saleh, M. A. (2013).** Nematicidal activity of terpenoids. J.  
5  
6 Environ. Sci. Health B. 48: 16-22.  
7  
8 **Alam, S., Sarkar, T. K., Shah, I. A., Reshi, I., Shah, M. A.(2015).** In-vitro anthelmintic efficacy  
9  
10 of some Kashmiri medicinal plants against *Haemonchus contortus*. Int. J. Livest. Res. 5: 73-79.  
11  
12 **Alim, A., Goze, I., Cetin, A., Atas, A. D., Cetinus, S. A., Vural, N. (2009).** Chemical composition  
13  
14 and in vitro antimicrobial and antioxidant activities of the essential oil of *Nepeta nuda* L. subsp.  
15  
16 *albiflora* (Boiss.) gams. Afr. J. Microbiol. Res. 3: 463-467.  
17  
18 **Al-Qudah, M. A. (2016).** Antioxidant Acitivity and Chemical Composition of Essential Oils of  
19  
20 Fresh and Air-dried Jordanian *Nepeta curviflora* Boiss. J. Biologically Active Products from  
21  
22 Nature. 6: 101-111.  
23  
24 **Andrés, M. F., González-Coloma, A., Sanz, J., Burillo, J., Sainz, P. (2012).** Nematicidal activity  
25  
26 of essential oils: a review. Phytochem. Rev. 11: 371-390.  
27  
28 **Bandh, S. A., Lone, B. A., Chishti, M. Z., Kamili, A. N., Ganai, B. A., Salem S. (2011).**  
29  
30 Evaluation of anthelmintic and antimicrobial activity of the methanolic extracts of *Nepeta cataria*.  
31  
32 New York Science Journal. 4: 129-135.  
33  
34 **Bozari, S; Agar, G; Aksakal, O; Erturk, F. A.; Yanmis, D. (2013).** Determination of chemical  
35  
36 composition and genotoxic effects of essential oil obtained from *Nepeta nuda* on *Zea mays*  
37  
38 seedlings. Toxicol. Ind. Health. 29: 339-348.  
39  
40 **De Pooter, H. L., Nicolai, B., De Buyck, L. F., Goethebeur, P., Schamp, N. M. (1987).** The  
41  
42 essential oil of *Nepeta nuda*. Identification of a new nepetalactone diastereoisomer. Phytochemistry.  
43  
44 26: 2311-2314.  
45  
46 **De Waele, D., Elsen, A. (2007).** Challenges in tropical plant nematology. Annu Rev Phytopathol.  
47  
48 45: 457-485.  
49  
50 **Dmitrović, S., Perišić, M., Stojić, A., Živković, S., Boljević, J., Živković, J. N., Aničić, N.,**  
51  
52 **Ristić, M., Mišić, D. (2015).** Essential oils of two *Nepeta* species inhibit growth and induce  
53  
54  
55  
56  
57  
58  
59  
60

1 oxidative stress in ragweed (*Ambrosia artemisiifolia* L.) shoots in vitro. *Acta Physiol. Plant.* 37: 1-  
2  
3  
4 15.

5  
6 **Faria, J. M. S., Sena, I., Ribeiro, B., Rodrigues, A. M., Maleita, C. M. N., Abrantes, I., Bennet,**  
7  
8 **R., Mota, M., da Silva Figueiredo, A. C. (2016).** First report on *Meloidogyne chitwoodi* hatching  
9  
10 inhibition activity of essential oils and essential oils fractions. *J. Pest. Sci.*89: 207-217.

11  
12 **Formisano, C., Rigano, D., Senatore, F. (2011).** Chemical constituents and biological activities of  
13  
14 *Nepeta* species. *Chem. Biodivers.* 8: 1783-1818.

15  
16  
17 **Gormez, A; Bozari, S; Yanmis, D; Gulluce, M; Agar, G; Sahin, F. (2013).** Antibacterial activity  
18  
19 and chemical composition of essential oil obtained from *Nepeta nuda* against phytopathogenic  
20  
21 bacteria. *J. Essent. Oil Res.* 25: 149-153.

22  
23  
24 **Gormez, A., Bozari, S., Yanmis, D., Gulluce, M., Sahin, F., Agar, G. (2015).** Chemical  
25  
26 composition and antibacterial activity of essential oils of two species of Lamiaceae against  
27  
28 phytopathogenic bacteria. *Pol. J. Microbiol.* 64: 121-127.

29  
30  
31 **Javidnia, K., Miri, R., Safavi, F., Azarpira, A., Shafiee, A. (2002).** Composition of the essential  
32  
33 oil of *Nepeta persica* Boiss from Iran. *Flavour Fragr. J.* 17: 20-22.

34  
35 **Kökdil, G., Kurucu, S., Topçu, G. (1996).** Composition of the essential oil of *Nepeta nuda* L. ssp.  
36  
37 *albiflora* (Boiss.) Gams. *Flavour Fragr. J.* 11: 167-169.

38  
39  
40 **Kökdil, G., Kurucu, S., Yıldız, A. (1998).** Essential oil composition of *Nepeta nuda* L. ssp. *nuda*. .  
41  
42 *Flavour Fragr. J.* 13: 233-234.

43  
44 **Lewis, S. C., Dyal, L. A., Hilburn, C. F., Weitz, S., Liau, W. S., LaMunyon, C. W., Denver, D.**  
45  
46 **R. (2009).** Molecular evolution in *Panagrolaimus* nematodes: origins of parthenogenesis,  
47  
48 hermaphroditism and the Antarctic species *P. davidi*. *BMC Evol. Biol.* 9:15.

49  
50  
51 **Mancini E., Apostolides Arnold, N., De Feo, V., Formisano, C., Rigano, D., Piozzi, F.,**  
52  
53 **Senatore, F. (2009).** Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta*  
54  
55 *nuda* L. subsp. *albiflora* growing wild in Lebanon. *J. Plant Interact.* 4: 253-259.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Ntalli, N. G., Ferrari, F., Giannakou, I., Menkissoglu-Spiroudi, U. (2010).** Phytochemistry and nematicidal activity of the essential oils from 8 Greek Lamiaceae aromatic plants and 13 terpene components. *J. Agric. Food Chem.* 58: 7856-7863.

**Ntalli, N. G., Ferrari, F., Giannakou, I., Menkissoglu-Spiroudi U. (2011).** Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece. *Pest Manag. Sci.* 67: 341-351.

**Oka, Y., Nacar, S., Putievsky, E., Ravid, U., Yaniv, Z., Spiegel, Y. (2000).** Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology*, 90, 710-715.

**Pavaraj, M., Bakavathiappan, G., Baskaran, S. (2012).** Evaluation of some plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita*. *J. Biopesticides.* 5: 106-110.

**Perry, R.N. and M. Moens. (2006).** *Plant Nematology.* CABI: Wallingford.

**Senatore, F.; Apostolides Arnold, N.; Piozzi, F. (2005).** Composition of the Essential Oil of *Nepeta curviflora* Boiss. (Lamiaceae) from Lebanon. *J. Essent. Oil Res.* 17: 268-270.

**Sharma, A., Cannoo, D. S. (2013).** Phytochemical composition of essential oils isolated from different species of genus *Nepeta* of Labiatae family: a review. *Pharmacophore.* 4: 181-211.

**Scaglia, B., Orzi, V., Artola, A., Font, X., Davoli, E., Sanchez, A., Adani, F. (2011).** Odours and volatile organic compounds emitted from municipal solid waste at different stage of decomposition and relationship with biological stability. *Bioresour. Technol.* 102: 4638-4645.

**Thompson, G., De Pomerai, D. I. (2005).** Toxicity of short-chain alcohols to the nematode *Caenorhabditis elegans*: A comparison of endpoints. *J. Biochem. Mol. Toxicol.* 19: 87-95.

**Todorov, D., Shishkova, K., Dragolova, D., Hinkov, A., Kapchina-Toteva, V., Shishkov, S. (2015).** Antiviral activity of medicinal plant *Nepeta nuda*. *Biotechnol. Biotechnol. Equip.* 29: S39-S43.

1  
2 **Tritten, L., Braissant, O., Keiser, J. (2012).** Comparison of novel and existing tools for studying  
3  
4 drug sensitivity against the hookworm *Ancylostoma ceylanicum* in vitro. *Parasitology*. 139: 348-  
5  
6 357.  
7

8 **Turkey K., O.; Hayta, S.; Bagci, E. (2011).** Chemical Composition of Essential Oil of *Nepeta*  
9  
10 *nuda* L. subsp. *nuda* (Lamiaceae) from Turkey. *Asian J. Chem.* 23: 2788-2790.  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1.** Essential oils of *Nepeta nuda* L. ssp. *pubescens*

RT (min)	%	Compound
14.50	0.15	1-Octen-3-one
16.67	0.11	Cytronellol hydrate
20.18	0.58	Linalool oxide <i>trans</i>
20.96	1.48	Furfural
23.18	1.32	Benzaldehyde
24.72	0.42	Linalool
25.23	0.11	Cyclopentanol 1-(methylenecyclopropyl)-
25.59	0.26	2-Cyclopentene-1,4-dione
28.66	1.57	2-Methoxy-5-methylphenol
30.9	1.74	Azulene
32.09	0.50	Methyl salicylate
32.65	0.11	Phorone
33.52	0.43	$\beta$ -Damascenone
33.95	0.91	Fenchone
34.14	1.84	Hexanoic acid
34.78	2.11	Benzyl alcohol
35.27	0.71	Cyclohexane ethenyl-
35.62	3.02	Phenylethyl alcohol
35.72	2.51	Bicyclo[10,1,0]tridec-1-ene
36.69	1.39	Methyl ethyl cyclopentene
36.85	1.16	Ethanone 1-(1H-pyrrol-2-yl)-
37.02	0.31	( <i>Z,E</i> )-Nepetalactone
37.54	0.49	(+)-3-Carene 10-(acetylmethyl)-

1			
2	37.59	1.20	(-)-Isosativene
3			
4	37.83	0.17	$\alpha$ -Santalol
5			
6	38.06	3.95	2,6 octadiene 2,6-dimethyl-
7			
8	38.43	2.24	( <i>E,Z</i> )-Nepetalactone
9			
10	38.56	1.25	Ethanone 1-(2-methyl-1-cyclopenten-1-yl)-
11			
12	38.64	0.61	Octanoic acid
13			
14	38.76	1.04	6-Methyl-3,5-heptadiene-2-one
15			
16	38.95	9.17	2-Cyclohexen-1-one 3-methyl-
17			
18	39.25	5.88	3-Hexanol 2,3-dimethyl-
19			
20	39.52	10.37	1-Cyclohexyl-1-(2-methylenecyclohexyl)ethanol
21			
22	39.86	2.92	Spathulenol
23			
24	39.95	1.54	Cyclopentanecarboxylic acid 3-methylene-1,7,7-
25			
26			
27			
28			
29			
30			
31	40.2	12.67	1H-Pyrrole 1-ethyl-
32			
33	40.37	2.03	Isopulegol
34			
35	40.53	1.03	2,3-Dehydro-1,8-cineole
36			
37	40.85	12.89	Pinane
38			
39	41.10	0.27	Thymol
40			
41	41.55	1.26	Artemiseole
42			
43	41.65	3.69	Eicosanoic acid
44			
45	41.99	0.18	1,7-Octadien-3-one 2-methyl-6-methylene-
46			
47	42.15	0.23	Cariophyllene oxide
48			
49	42.32	0.79	$\alpha$ -Caryophylladienol
50			
51	43.39	0.52	Alloaromadendrene oxide-(1)
52			
53	43.93	0.13	1-Cyclohexene-1-butanal $\alpha$ -2,6,6-tetramethyl-
54			
55			
56			
57			
58			
59			
60			



---

44.10	0.49	Indole
44.76	0.28	Benzoic acid 2,6-dimethoxy- methyl ester

**Grouped compound** 

Monoterpene	
hydrocarbons	18.58
Oxygenated	
monoterpenes	9.83
Sesquiterpene	
hydrocarbons	1.37
Oxygenated	
sesquiterpenes	4.46
Aliphatic hydrocarbons	4.61
Aldehydes/ketones	13.86
Fatty acids and esters	8.46
Others	38.8
Total	100

---

**Table 2.** Essential oils of *Nepeta curviflora* Boiss. 

RT (min)	%	Compound
15.14	0.28	2-Methoxy-5-methylphenol
29.91	0.39	Cyclohexanecarboxylic acid 3-phenylpropyl ester
33.14	1.77	Pulegone
34.14	1.04	Hexanoic acid
35.60	0.90	Phenylethyl alcohol
36.70	5.43	Cyclohexene 4-propyl-
36.74	0.70	Bicyclo[2,2,1]heptane 2-methyl-
36.78	6.93	5,7-Dodecadiene, (E,Z)-
36.97	0.98	Cyclohexene, 3-(2-methylpropyl)-
37.34	1.68	Caryophyllene oxide
37.79	2.72	Germacrene
38.56	0.58	Ethanone 1-(1,3-dimethyl-3-cyclohexen-1-yl)-
38.64	1.86	Octanoic acid
38.76	1.73	Ledol
38.97	12.51	3-Cyclohexen-1-one 2-isopropyl-5-methyl-
39.43	1.44	2-nonenic acid methyl ester
39.54	5.57	Dihydronepetalactone -
39.73	0.68	Dehydrocurdione
39.85	11.73	(-)-Spathulenol
40.21	3.15	<u><math>\gamma</math>-Cadinene</u>
40.34	1.20	Eugenol
40.68	2.76	Carvacrol
41.09	0.93	p-Thymol

41.40	0.54	Isoaromadendrene epoxide
41.54	7.00	Widdrol
41.77	0.48	3-Octyne
42.25	0.37	Alloaromadendrene oxide
42.34	4.84	caryophylla-3(4),8-dien-5-ol
42.55	1.01	Phenol 2,4-bis(1,1-dimethylethyl)-
42.87	8.07	<i>cis-Z-<math>\alpha</math></i> -Bisabolene epoxide
43.01	3.50	$\alpha$ -Selinene
43.40	3.59	<i>trans-Z-<math>\alpha</math></i> -Bisabolene epoxide
43.61	0.54	$\alpha$ -pinene oxide
43.93	0.65	L-Fenchone
44.25	0.27	Patchoulane
44.76	0.38	N-(1-Cyclohexen-1-yl)piperidine
45.05	0.43	Isolongifolol
46.39	0.22	Thujopsene
46.55	0.63	(-)-Neoclovene-(I) dihydro-
48.17	0.31	2,6-Dimethylbicyclo[3,2,1]octane
49.17	0.23	Caryophyllene-(II)

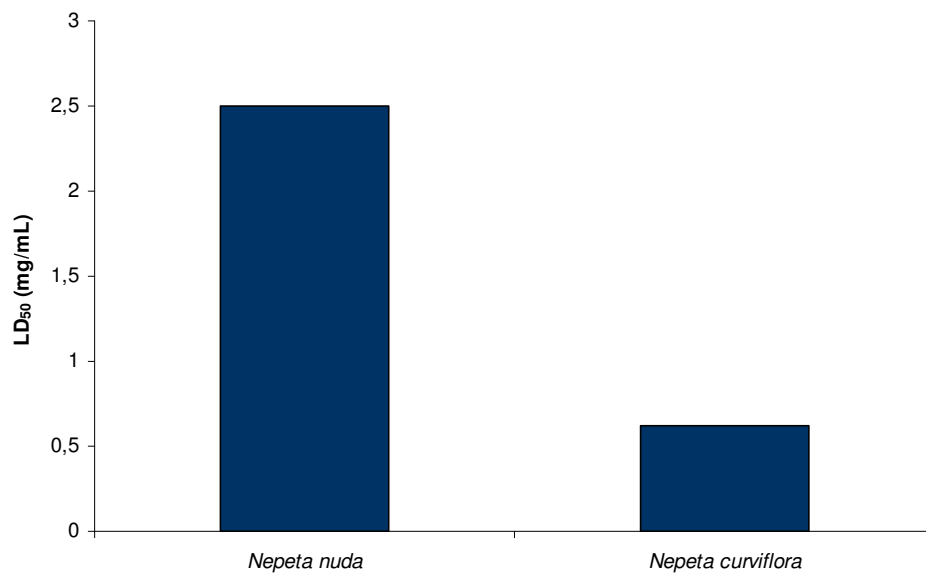
#### Grouped compounds

Monoterpene hydrocarbons	0.31
Oxygenated monoterpenes	24.73
Sesquiterpene hydrocarbons	9.73
Oxygenated sesquiterpenes	41.65
Aliphatic hydrocarbons	14.52

1		
2	Fatty acids and esters	4.73
3		
4	Others	4.35
5		
6	Total	100
7		
8	<hr/>	
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		
48		
49		
50		
51		
52		
53		
54		
55		
56		
57		
58		
59		
60		

For Peer Review Only

Figure 1



**Figure 1.** *In vitro* nematocidal activity of essential oils obtained from *Nepeta nuda* L. ssp. *pubescens* and *Nepeta curviflora* Boiss on *Panagrolaimus rigidus* adults.

1  
2 **Regular articles**

3  
4 **Chemical characterization and nematocidal activity of the essential oil of *Nepeta nuda* L. ssp.**  
5  
6 ***pubescens* and *Nepeta curviflora* Boiss. from Lebanon**  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review Only

**Abstract**

The chemical characterization and the nematicidal activity of the essential oils from *Nepeta nuda* L. ssp. *pubescens* and *Nepeta curviflora* Boiss. growing wild in Lebanon are reported. A comparative study was carried out as, to the best of our knowledge, no information is available on *Nepeta nuda* L. ssp. *pubescens*. In addition, both *Nepeta* species were collected in the same geographical area in order to rule out the environmental factors influencing essential oil composition and bioactivity.

The most abundant (> 5%) components of *N. nuda* ssp. *pubescens* essential oil were pinane (12.89%), 1-ethyl-1H-pyrrole (12.67%), 1-cycloethyl-1-(2-methylenecyclohexyl)ethanol (10.37%), 3-methyl-2-cyclohexen-1-one (9.17%) and 2,3-dimethyl-3-hexanol (5.88%). Among oxygenated monoterpenes, two nepetalactones were identified, i.e. (*E,Z*)-nepetalactone (2.24%) and (*Z,E*)-nepetalactone (0.31%). The major constituents (> 5 %) of *N. curviflora* essential oil were 2-isopropyl-5-methyl-3-cyclohexen-1-one (12.51%), (-)-spathulenol (11.73%), *cis-Z-α*-bisabolene epoxide (8.07%), widdrol (7.0 %), (*E,Z*)-5,7-dodecadiene (6.93%), dihydronepetalactone (5.57%) and 4-propyl-cyclohexene (5.43%). The essential oil of *N. curviflora* was more active than the *N. nuda* ssp. *pubescens* one against the nematode *Panagrolaimus rigidus*. According to the motility assay, LD<sub>50</sub> was 0.5 mg/mL and 2.5 mg/mL 24 h after treatment with *N. curviflora* and *N. nuda* ssp. *pubescens* essential oil, respectively.

**Keywords:** aromatic plants, nematodes, isoprenoids, biopesticides, anthelmintics, Lebanese flora.

## Introduction

The genus *Nepeta* (Lamiaceae) consists of around 300 herbaceous, perennial, rarely annual species. This genus is distributed over a wide area including Europe, Asia, Africa and the Middle East (Formisano et al., 2011). Some of these species are widely used in traditional medicine and are endowed with a number of pharmacological properties, usually attributed to their essential oils (Sharma et al. 2013). The main bioactive compounds are a variety of secondary metabolites, including monoterpenes (iridoids and their glycosides), diterpenes, triterpenes as well as phenols. *Nepeta nuda* L. is one of the most common species of the genus *Nepeta*. It is widespread from central and southeast Europe to Russia and towards southwest and central Asia. The essential oil composition of *N. nuda* was studied in a number of investigations (De Pooter et al. 1987; Alim et al. 2009; Turkey et al., 2011; Bozari et al., 2013; Gormez et al., 2013).

Nematodes are parasites of humans, vertebrates, insects and plants. In particular, plant diseases caused by nematodes threaten the world agriculture because of their severity and economic burden.

In the developing countries, crop production losses attributable to nematodes are estimated around 15%, compared with around 9% in developed countries. Not least, chemical control of nematodes is troublesome after the ban of soil fumigants such as methyl bromide, and, in addition, non-volatile nematicides, organophosphates and carbamates, can represent environmental and human threats due to their toxicological and ecotoxicological risks. Therefore, environmentally friendly and low-cost alternatives to chemical control measures for phytoparasitic nematodes are urgently needed. In this context, essential oils from plants could be attractive nematicide compound sources, possibly safe for non-target organisms.

As part of a continuing investigation on essential oils of *Nepeta* species growing in Lebanon, in the present study, we report, for the first time, the chemical characterization and the nematicidal activity of the essential oil from *N. nuda* ssp. *pubescens*. As, to the best of our knowledge, no information is available on this plant species, we used *Nepeta curviflora* Boiss. as reference species (Senatore et al., 2005; Mancini et al., 2009; Al-Qudah 2016), in order to compare these two *Nepeta*



species growing wild in Lebanon. In this comparative study, both *Nepeta* species were collected in the same geographical area, in order to rule out the environmental factors influencing essential oil composition and bioactivity.

### Materials and methods

**Plant material.** The dried parts of *Nepeta nuda* ssp. *pubescens* (flowering tops, seeds and leaves) were collected in April 2005 in a mountain area (Laklouk, Mount Lebanon, 1780 m above sea level) near to Tannourine, Lebanon (average temperature  $8.4 \pm 0.8$  °C and 1105 mm of rain in 2005; GPS coordinates: 34°8'19"N 35°52'20"E). The dried parts of *Nepeta curviflora* (flowering tops, seeds, and leaves) were collected in April 2005 in Lebanon, near to the St. Antonio of Qozhaya convent, Ehden region at 664 m above sea level (average temperature  $13.07 \pm 1.3$  °C and 940 mm of rain in 2005; GPS coordinates 34°16'59"N 35°56'50"E).

**Essential oil production.** Essential oils were obtained by steam distillation (250 mL water) from a sample (5 g) of air-dried aerial parts of the plant. The collected aqueous phase (150 mL) containing the essential oil was extracted with diethyl ether (40 mL  $\times$  5). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to obtain 12 mg (0.2%) of a light yellow oil from *N. nuda* and 19 mg (0.4%) from *N. curviflora*. The extracted EO samples were stored in sealed vials at low temperature (4 °C) before gas chromatography coupled with mass spectrometry (GC-MS) analysis.

**Gas chromatography-mass spectrometry (GC-MS) analysis.** Oil compositions were analysed by SPME/GC-MS, as reported by Scaglia et al. (2011) with some modifications. A manual SPME device and divinylbenzene (DVB)/Carboxen/polydimethylsiloxane (PDMS) 50-30  $\mu$ m fiber - Supelco, Bellefonte, PA, USA) was used. The compounds were adsorbed from the gas-phases by exposing the fiber, preconditioned for 3 h at 250 °C as suggested by the Supplier, to the oils on 20 ml vials. The fibers were incubated at 40 °C for 60 min under agitation condition (speed of 500 rpm with on time of 5 s and off time of 2 s).

Oils analysis was performed using an Agilent 5975C Series GC/MSD and FID as detector. Volatiles were separated using a polar capillary column Zebron-WAX (Zebron, Phenomenex, USA) of 30 m x 0.25 mm (ID) and a film thickness of 0.25  $\mu\text{m}$ . Carrier gas was helium at a flow rate of 1 mL  $\text{min}^{-1}$ . Molecules were desorbed exposing the SPME in the GC injection port in splitless condition for 600 seconds at 250  $^{\circ}\text{C}$ . The temperature program was isothermal for 3 min at 40  $^{\circ}\text{C}$ , then three different temperature ramps were done to raise 115  $^{\circ}\text{C}$ , 150  $^{\circ}\text{C}$  and 220  $^{\circ}\text{C}$  at a rate of 3  $^{\circ}\text{C min}^{-1}$ , 5  $^{\circ}\text{C min}^{-1}$  and 7  $^{\circ}\text{C min}^{-1}$  respectively. Finally an isothermal at 220  $^{\circ}\text{C}$  for 15 min was done. The transfer line to the mass spectrometer was maintained at 150  $^{\circ}\text{C}$ . The mass spectra were obtained by electronic impact at 70 eV, a multiplier voltage of 1294 V and collecting data at a  $m/z$  range of 45–300. The retention indices were determined in relation to a homologous series of *n*-alkanes (C7-C30) under the same operation conditions. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST08 library (USA) and peak retention indices (RI) relative to *n*-alkanes. From the raw spectra (Table SI-1 and Table S-2), the typical constituents of the essential oils (Babushok et al., 2001; Dobravalskyte et al., 2013; Zebib et al., 2015) have been selected and considered. Therefore, concentrations of these typical components have been recalculated as percent of the sum of raw spectra of essential oil constituents.

**Motility assay.** *Panagrolaimus rigidus* adults were suspended in M199 medium and, then, approximately 100 larvae in 100  $\mu\text{L}$  of suspension were added to each well of a 96-well microplate, followed by immediate addition of 100  $\mu\text{L}$  of the essential oil dilutions. Microplates were stored in incubator at 20 $^{\circ}\text{C}$ . After 1, 3, 5, 12 and 24 h, nematodes were removed from each well and mobile and immobile roundworms were counted using an optical microscope at 40x magnification. Nematodes were considered paralyzed or died when presenting with straight body and absence of any motility after manipulation stimulus. M199 solution with 0.05% DMSO, and 50, 25, 5, 0.5, 0.05 and 0.005% methanol were used as controls. Experiments were carried out in triplicate for each essential oil concentration and controls, and results are expressed as LD<sub>50</sub> (mg/mL).

**Statistical analysis.** Statistical analysis was performed using OriginPro<sup>®</sup> software (version 8.0). All experiments were carried out in triplicate and results are expressed as mean  $\pm$  standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) and comparison among means was determined according to Fisher's least significant difference (LSD) test. Differences were considered significant at  $P < 0.05$  and represented by different letters.

## Results and Discussion

### Essential oil composition of *N. nuda* ssp. *pubescens*

The essential oil was obtained in a yield of 0.2% (w/w), based on the dry weight of the sample. Among the molecules recognised with the GC MS analysis (Table SI 1), 49 have been identified as typical of essential oils and considered for the successive discussion (Table 1) (Babushok et al., 2001; Dobravalskyte et al., 2013; Zebib et al., 2015; Yermakov et al., 2010)

The most represented chemical classes included monoterpene hydrocarbons ( $18.58 \pm 0.70\%$  oil composition), aldehydes/ketones ( $13.86 \pm 0.20\%$  oil composition) and oxygenated monoterpenes ( $9.83 \pm 0.20\%$  oil composition) (Tab. 1). The most abundant ( $> 5\%$ ) components were pinene ( $12.89 \pm 0.80\%$ ), 1-ethyl-1H-pyrrole ( $12.67 \pm 0.40\%$ ), 1-cycloethyl-1-(2-methylenecyclohexyl)ethanol ( $10.37 \pm 0.50\%$ ), 3-methyl-2-cyclohexen-1-one ( $9.17 \pm 0.60\%$ ) and 2,3-dimethyl-3-hexanol ( $5.88 \pm 0.50\%$ ) (Table 1). Among oxygenated monoterpenes, two nepetalactones were identified, *i.e.* (*E,Z*)-nepetalactone ( $2.24 \pm 0.20\%$ ) and (*Z,E*)-nepetalactone ( $0.31 \pm 0.00\%$ ) (Tab. 1).

The essential oil composition of *N. nuda* ssp. *pubescens* investigated in this study is different from that reported for other subspecies of *N. nuda*. In fact, the essential oil from *N. nuda* L. ssp. *albiflora* (Boiss.) Gams. growing wild in Lebanon was characterized by higher contents of three nepetalactones: (*Z,E*)-nepetalactone (0.8-3.6%), (*E,Z*)-nepetalactone (4.4-8.0%) and (*Z,Z*)-nepetalactone (0.5-2.9%) (Mancini et al. 2009). However, these compounds were not detected in the essential oil from *N. nuda* ssp. *albiflora* collected in Turkey, characterized by higher amount of

1 spathulenol (7.35%) compared with our *N. nuda* ssp. *pubescens* samples (2.92%) (Alim et al.,  
2 2009). Similarly, nepetalctones were not found in *N. nuda* L. ssp. *nuda* essential oil from Turkey,  
3 where higher levels of caryophyllene oxide (21.8%), spathulenol (13.8%) and *allo*-aromadendrene  
4 (9.0%) were measured (Kökdil et al., 1998).  
5  
6  
7  
8  
9

### 10 **Essential oil composition of *N. curviflora***

11 The steam distillation of *N. curviflora* aerial parts yielded (0.4% w/w) essential oil. GC-MS  
12 analysis identified 41 constituents (Tab. 1). The most represented chemical classes were oxygenated  
13 sesquiterpenes ( $41.65 \pm 0.70\%$ ) and oxygenated monoterpenes ( $24.73 \pm 0.30\%$ ) (Tab. 1). The major  
14 components ( $> 5\%$ ) were 2-isopropyl-5-methyl-3-cyclohexen-1-one ( $12.51 \pm 0.60\%$ ), (-)-  
15 spathulenol ( $11.73 \pm 0.60\%$ ), *cis*-*Z*- $\alpha$ -bisabolene epoxide ( $8.07 \pm 0.20\%$ ), widdrol ( $7.0 \pm 0.30\%$ ),  
16 (*E,Z*)-5,7-dodecadiene ( $6.93 \pm 0.60\%$ ), dihydronepetalactone ( $5.57 \pm 0.50\%$ ) and 4-propyl-  
17 cyclohexene ( $5.43 \pm 0.20\%$ ) (Tab. 1).  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29

30 In a previous report, the most abundant compounds in *N. curviflora* essential oil were  $\beta$ -  
31 caryophyllene (41.6%), caryophyllene oxide (9.5%), (*E*)- $\beta$ -farnesene (6.2%) and (*Z*)- $\beta$ -farnesene  
32 (4.8%) (Mancini et al., 2009). Noteworthy, lower amounts of dihydronepetalactone were previously  
33 reported in essential oils from *Nepeta persica* Boiss. (3.5%), *N. nuda* ssp. *albiflora* (0.2%) and  
34 *Nepeta cataria* L. (0.08%) (Kökdil et al., 1996; Javidnia et al., 2002; Dmitrović et al., 2015).  
35 Therefore, the composition of essential oil of *N. curviflora* is quite different from the ones  
36 previously reported in the literature (Senatore et al., 2005; Mancini et al., 2009; Al-Qudah 2016).  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47

### 48 **Effects of geographical origin on essential oil composition**

49 A number of factors could affect essential oil composition, including genetic traits, soil, climatic  
50 conditions, environmental constraints, harvesting as well as extraction and analytical methods. Not  
51 least, a high level of chemical polymorphism is characteristic of *Nepeta* species (Formisano et al.,  
52 2011). Indeed, *N. nuda* ssp. *pubescens* and *N. curviflora* collected from two different areas in  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Lebanon showed different essential oil composition, probably due to diverse climates and environments. As previously introduced, due to the lack of information available on *N. nuda* ssp. *pubescens*, we compared our results with those reported on other subspecies of *N. nuda*. Essential oil from *N. nuda* ssp. *albiflora* harvested in Lebanon was devoid of monoterpene hydrocarbons and richer in sesquiterpenes, both hydrocarbons and oxygenated derivatives (Mancini et al., 2009), compared with *N. nuda* ssp. *pubescens* essential oil (Tab. 2). Similarly, higher levels of oxygenated sesquiterpenes were detected in essential oil from *N. nuda* ssp. *albiflora* collected in Turkey (Alim et al. 2009) than in *N. nuda* ssp. *pubescens* essential oil (Tab. 2). Finally, in *N. nuda* ssp. *nuda*, a much higher sesquiterpene fraction was measured (Tab. 2) (Kökdil et al., 1998), thus pointing out that geographical origin represents a relevant constraint in essential oil composition.

As regards *N. curviflora*, essential oil the same species harvested in Lebanon and Jordan was characterized by much higher levels of sesquiterpene hydrocarbons (Senatore et al., 2005; Mancini et al., 2009; Al-Qudah 2016), in comparison with our *N. curviflora* essential oil (Tab. 2).

### **Nematicidal activity**

A number of biological properties have been ascribed to *Nepeta* species. In particular, phytotoxic, antibacterial, antiviral and antioxidant activities have been reported in *N. nuda* ssp. *albiflora*, *N. nuda* ssp. *nuda* and *N. curviflora*. (Alim et al., 2009; Mancini et al., 2009; Gormez et al., 2013; Todorov et al., 2015). As regards nematicidal activity of *Nepeta* species, it was only reported for *Nepeta cataria* L. Both solvent (methanol, ethanol and water) extracts and essential oil from aerial parts of this plant species exhibited anthelmintic effects on *Meloidogyne chitwoodi*, *Meloidogyne incognita* and *Haemonchus contortus* (Bandh et al., 2011; Pavaraj et al., 2012; Alam et al., 2015; Faria et al., 2016). Our results demonstrated that the essential oils of *N. curviflora* and *N. nuda* ssp. *pubescens* are endowed with nematicidal activity as well. In particular, the essential oil of *N. curviflora* was more active than the *N. nuda* ssp. *pubescens* one against *Panagrolaimus rigidus*. According to the motility assay, LD<sub>50</sub> was  $0.6 \pm 0.1$  mg/mL and  $2.5 \pm 0.3$  mg/mL 24 h after

1 treatment with *N. curviflora* and *N. nuda* ssp. *pubescens* essential oil, respectively ( $P < 0.05$ ) (Fig.  
2  
3  
4 1). *P. rigidus*, a free-living bacterivorous nematode, represents a model organism for research on  
5  
6 novel anthelmintics. Indeed, this nematode has been widely used in basic research on anthelmintic  
7  
8 pharmacology of human and agricultural parasites, as well as at understanding the mechanisms of  
9  
10 resistance to anthelmintics. Several characteristics make *P. rigidus* a reliable experimental model,  
11  
12 such as rapid life cycle, easy growth and manipulation in laboratory conditions, knowledge of its  
13  
14 genome and phylogenetic proximity to other nematodes.

15  
16  
17 The different *in vitro* nematicidal activity of the two *Nepeta* species can be due to the diverse  
18  
19 essential oil composition. In particular, a number of reports highlighted the anthelmintic potential of  
20  
21 terpenes. The nematicidal activity of these compounds against *Meloidogyne incognita* was found to  
22  
23 decrease in the order carvone > pulegone > *trans*-anethole > geraniol > eugenol > carvacrol >  
24  
25 thymol > terpinen-4-ol, with EC<sub>50</sub> values (24 h) calculated in the range of 115-392 µg/mL (Ntalli et  
26  
27 al., 2010). Similar results and EC<sub>50</sub> values were reported against *M. javanica* (Oka et al., 2000). The  
28  
29 highest mortality (100%) on this nematode species was induced by carvacrol, geraniol and thymol  
30  
31 (0.5 mg/mL, 72 h incubation) (Andrés et al., 2012). Carvacrol, thymol, nerolidol,  $\alpha$ -terpinene,  
32  
33 geraniol, citronellol, farnesol, limonene, pseudoionone and eugenol also caused 50% mortality at 50  
34  
35 µg/mL on the model nematode *Caenorhabditis elegans* (Abdel-Rahman et al., 2013). Noteworthy,  
36  
37 the contribution of each terpene constituent to the overall activity of an essential oil seems to be due  
38  
39 to a rather complicated pattern of interactions, since they may act together synergistically or  
40  
41 antagonistically. Some terpene pairs exhibiting synergistic activity on *M. incognita* paralysis in  
42  
43 decreasing order were: *trans*-anethole/geraniol > *trans*-anethole/eugenol > carvacrol/eugenol >  
44  
45 geraniol/carvacrol (Ntalli et al., 2011). Therefore, it is very important to understand the synergy and  
46  
47 antagonism interactions among individual components of a nematicidal essential oil. In this view,  
48  
49 the presence of nematicidal terpenes in *N. curviflora* essential oil, *i.e.* pulegone, carvacrol, eugenol  
50  
51 and thymol, could contribute to explain its higher activity against *P. rigidus* motility, compared  
52  
53 with *N. nuda* ssp. *pubescens* essential oil.  
54  
55  
56  
57  
58  
59  
60

## Conclusion

The present study confirmed that geographical origin of plants represents a major factor influencing essential oil composition, as well as environmental variables due to annual rainfall and temperature. Noteworthy, difference in nematocidal activity of the two essential oils could be attributed to their diverse chemical composition. The presence of nematocidal terpenes in the *N. curviflora* essential oil, *i.e.* pulegone, carvacrol, eugenol and thymol could contribute to explain the observed biological activity. Overall, these results revealed that essential oil of *N. curviflora* exhibits promising nematocidal activity, in addition to the previously reported phytotoxic effects, thus highlighting the possibility of using this plant product as biopesticide/bioherbicide in soil treatment to control phytopathogenic nematodes and weeds.



## References

- 1  
2  
3  
4 **Abdel-Rahman, F. H., Alaniz, N. M., Saleh, M. A. (2013).** Nematicidal activity of terpenoids. J.  
5  
6 Environ. Sci. Health B. 48: 16-22.  
7  
8 **Alam, S., Sarkar, T. K., Shah, I. A., Reshi, I., Shah, M. A. (2015).** In-vitro anthelmintic efficacy  
9  
10 of some Kashmiri medicinal plants against *Haemonchus contortus*. Int. J. Livest. Res. 5: 73-79.  
11  
12 **Alim, A., Goze, I., Cetin, A., Atas, A. D., Cetinus, S. A., Vural, N. (2009).** Chemical composition  
13  
14 and in vitro antimicrobial and antioxidant activities of the essential oil of *Nepeta nuda* L. subsp.  
15  
16 *albiflora* (Boiss.) gams. Afr. J. Microbiol. Res. 3: 463-467.  
17  
18 **Al-Qudah, M. A. (2016).** Antioxidant Acitivity and Chemical Composition of Essential Oils of  
19  
20 Fresh and Air-dried Jordanian *Nepeta curviflora* Boiss. J. Biologically Active Products from  
21  
22 Nature. 6: 101-111.  
23  
24 **Andrés, M. F., González-Coloma, A., Sanz, J., Burillo, J., Sainz, P. (2012).** Nematicidal activity  
25  
26 of essential oils: a review. Phytochem. Rev. 11: 371-390.  
27  
28 **Bandh, S. A., Lone, B. A., Chishti, M. Z., Kamili, A. N., Ganai, B. A., Salem S. (2011).**  
29  
30 Evaluation of anthelmintic and antimicrobial activity of the methanolic extracts of *Nepeta cataria*.  
31  
32 New York Science Journal. 4: 129-135  
33  
34 **V. I. Babushok, V.I., Linstrom, P.J., Zenkevich, I.G. (2011).** Retention Indices for Frequently  
35  
36 Reported Compounds of Plant Essential Oils. J. Phys. Chem. Ref. Data, 40: 1-47.  
37  
38 **Bozari, S; Agar, G; Aksakal, O; Erturk, F. A.; Yanmis, D. (2013).** Determination of chemical  
39  
40 composition and genotoxic effects of essential oil obtained from *Nepeta nuda* on *Zea mays*  
41  
42 seedlings. Toxicol. Ind. Health. 29: 339-348.  
43  
44 **De Pooter, H. L., Nicolai, B., De Buyck, L. F., Goethebeur, P., Schamp, N. M. (1987).** The  
45  
46 essential oil of *Nepeta nuda*. Identification of a new nepetalactone diastereoisomer. Phytochemistry.  
47  
48 26: 2311-2314.  
49  
50 **Dmitrović, S., Perišić, M., Stojić, A., Živković, S., Boljević, J., Živković, J. N., Aničić, N.,**  
51  
52 **Ristić, M., Mišić, D. (2015).** Essential oils of two *Nepeta* species inhibit growth and induce  
53  
54  
55  
56  
57  
58  
59  
60



1 oxidative stress in ragweed (*Ambrosia artemisiifolia* L.) shoots in vitro. Acta Physiol. Plant. 37: 1-  
2  
3  
4 15.

5  
6 **Dobravalskyte, D., Ventskutonis, P.R., Zebib, B., Merah, O., Talou, T. (2013).** Essential oil  
7  
8 composition of *Myrrhis odorata* (L.) Scop. Leaves grown in Lithuania and France. J. Essent. Oil  
9  
10 Res. 25: 44-48.

11  
12 **Faria, J. M. S., Sena, I., Ribeiro, B., Rodrigues, A. M., Maleita, C. M. N., Abrantes, I., Bennet,**  
13  
14 **R., Mota, M., da Silva Figueiredo, A. C. (2016).** First report on *Meloidogyne chitwoodi* hatching  
15  
16 inhibition activity of essential oils and essential oils fractions. J. Pest. Sci.89: 207-217.

17  
18 **Formisano, C., Rigano, D., Senatore, F. (2011).** Chemical constituents and biological activities of  
19  
20 *Nepeta* species. Chem. Biodivers. 8: 1783-1818.

21  
22 **Gormez, A; Bozari, S; Yanmis, D; Gulluce, M; Agar, G; Sahin, F. (2013).** Antibacterial activity  
23  
24 and chemical composition of essential oil obtained from *Nepeta nuda* against phytopathogenic  
25  
26 bacteria. J. Essent. Oil Res. 25: 149-153.

27  
28 **Gormez, A., Bozari, S., Yanmis, D., Gulluce, M., Sahin, F., Agar, G. (2015).** Chemical  
29  
30 composition and antibacterial activity of essential oils of two species of Lamiaceae against  
31  
32 phytopathogenic bacteria. Pol. J. Microbiol. 64: 121-127.

33  
34 **Javidnia, K., Miri, R., Safavi, F., Azarpira, A., Shafiee, A. (2002).** Composition of the essential  
35  
36 oil of *Nepeta persica* Boiss from Iran. Flavour Fragr. J. 17: 20-22.

37  
38 **Kökdil, G., Kurucu, S., Topçu, G. (1996).** Composition of the essential oil of *Nepeta nuda* L. ssp.  
39  
40 *albiflora* (Boiss.) Gams. Flavour Fragr. J. 11: 167-169.

41  
42 **Kökdil, G., Kurucu, S., Yıldız, A. (1998).** Essential oil composition of *Nepeta nuda* L. ssp. *nuda*.  
43  
44 Flavour Fragr. J. 13: 233-234.

45  
46 **Mancini E., Apostolides Arnold, N., De Feo, V., Formisano, C., Rigano, D., Piozzi, F.,**  
47  
48 **Senatore, F. (2009).** Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta*  
49  
50 *nuda* L. subsp. *albiflora* growing wild in Lebanon. J. Plant Interact. 4: 253-259.

1 Ntalli, N. G., Ferrari, F., Giannakou, I., Menkissoglu-Spiroudi, U. (2010). Phytochemistry and  
2 nematocidal activity of the essential oils from 8 Greek Lamiaceae aromatic plants and 13 terpene  
3 components. J. Agric. Food Chem. 58: 7856-7863.  
4

5  
6  
7  
8 Ntalli, N. G., Ferrari, F., Giannakou, I., Menkissoglu-Spiroudi U. (2011). Synergistic and  
9 antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematocidal activity of  
10 essential oils from seven plants indigenous to Greece. Pest Manag. Sci. 67: 341-351.  
11

12  
13 Oka, Y., Nacar, S., Putievsky, E., Ravid, U., Yaniv, Z., Spiegel, Y. (2000). Nematicidal activity  
14 of essential oils and their components against the root-knot nematode. Phytopathology, 90, 710-  
15 715.  
16

17  
18  
19  
20 Pavaraj, M., Bakavathiappan, G., Baskaran, S. (2012). Evaluation of some plant extracts for  
21 their nematocidal properties against root-knot nematode, *Meloidogyne incognita*. J. Biopesticides. 5:  
22 106-110.  
23

24  
25  
26  
27 Senatore, F.; Apostolides Arnold, N.; Piozzi, F. (2005). Composition of the Essential Oil of  
28 *Nepeta curviflora* Boiss. (Lamiaceae) from Lebanon. J. Essent. Oil Res. 17: 268-270.  
29

30  
31  
32  
33 Sharma, A., Cannoo, D. S. (2013). Phytochemical composition of essential oils isolated from  
34 different species of genus *Nepeta* of Labiatae family: a review. Pharmacophore. 4: 181-211.  
35

36  
37  
38  
39 Scaglia, B., Orzi, V., Artola, A., Font, X., Davoli, E., Sanchez, A., Adani, F. (2011). Odours and  
40 volatile organic compounds emitted from municipal solid waste at different stage of decomposition  
41 and relationship with biological stability. Bioresour. Technol.. 102: 4638-4645.  
42

43  
44  
45  
46 Todorov, D., Shishkova, K., Dragolova, D., Hinkov, A., Kapchina-Toteva, V., Shishkov, S.  
47 (2015). Antiviral activity of medicinal plant *Nepeta nuda*. Biotechnol. Biotechnol. Equip. 29: S39-  
48 S43.  
49

50  
51  
52  
53 Turkey K., O.; Hayta, S.; Bagci, E. (2011). Chemical Composition of Essential Oil of *Nepeta*  
54 *nuda* L. subsp. *nuda* (Lamiaceae) from Turkey. Asian J. Chem. 23: 2788-2790.

55  
56  
57  
58 Zebib, B., EL Beyrouthy, M., Sarfi, C., Merah, O. (2015). Chemical composition of the essential  
59 oil of *Satureja myrtifolia* (Boiss. & Hohen.) from Lebanon. TEOP 18: 248-254.  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review Only

**Table 1.** Essential oils of *Nepeta nuda* L. ssp. *pubescens* and *Nepeta curviflora* Boiss.

RI <sup>a</sup>	Compound	<i>Nepeta nuda</i> L.	<i>Nepeta</i> ssp. <i>curviflora</i> Boiss.
1224.2	1-Octen-3-one	0.15±0.10	-
1300.13	2-Methoxy-5-methylphenol	-	0.28±0.00
1300.9	Cytronellol hydrate	0.11±0.00	-
1400.5	Linalool oxide <i>trans</i>	0.58±0.00	-
1401.1	Furfural	1.48±0.10	-
1500.1	Benzaldehyde	1.32±0.20	-
1500.8	Linalool	0.42±0.00	-
1501.4	Cyclopentanol 1-(methylenecyclopropyl)-	0.11±0.00	-
1502.0	2-Cyclopentene-1,4-dione	0.26±0.10	-
1601.0	2-Methoxy-5-methylphenol	1.57±0.20	-
1605.42	Cyclohexanecarboxylic acid 3-phenylpropyl ester	-	0.39±0.20
1700.2	Azulene	1.74±0.10	-
1701.3	Methyl salicylate	0.50±0.10	-
1703.2	Phorone	0.11±0.00	-
1713.44	Pulegone	-	1.77±0.30
1800.1	β-Damascenone	0.43±0.10	-
1800.3	Fenchone	0.91±0.20	-
1800.5	Hexanoic acid	1.84±0.30a	1.04±0.10a
1801.4	Benzyl alcohol	2.11±0.20	-
1803.7	Cyclohexane ethenyl-	0.71±0.10	-
1812.9	Phenylethyl alcohol	-	0.9±0.00
1813.7	Phenylethyl alcohol	3.02±0.50	-
1835.5	Bicyclo[10,1,0]tridec-1-ene	2.51±0.20	-
1900.8	Methyl ethyl cyclopentene	1.39±0.10	-
1900.82	Cyclohexene 4-propyl-	-	5.43±0.20
1900.89	Bicyclo[2,2,1]heptane 2-methyl-	-	0.7±0.00
1900.96	5,7-Dodecadiene, (E,Z)-	-	6.93±0.60
1901.1	Ethanone 1-(1H-pyrrol-2-yl)-	1.16±0.20	-
1901.4	Cyclohexene, 3-(2-methylpropyl)-	-	0.98±0.20
1901.6	(Z,E)-Nepetalactone	0.31±0.00	-
1903.28	Caryophyllene oxide	-	1.68±0.10
1906.4	(+)-3-Carene 10-(acetylmethyl)-	0.49±0.00	-
1908.1	(-)-Isosativene	1.20±0.10	-
1987.09	Germacrene	-	2.72±0.20
2000.0	α-Santalol	0.17±0.00	-
2000.2	2,6 Octadiene 2,6-dimethyl-	3.95±0.00	-
2000.6	(E,Z)-Nepetalactone	2.24±0.20	-
200.76	Ethanone 1-(1,3-dimethyl-3-cyclohexen-1-yl)-	-	0.58±0.00
2000.8	Ethanone 1-(2-methyl-1-cyclopenten-1-yl)-	1.25±0.10	-
2000.9	Octanoic acid	0.61±0.00a	1.86±0.30b
2001.2	6-Methyl-3,5-heptadiene-2-one	1.04±0.20	-
2001.22	Ledol	-	1.73±0.10
2001.9	2-Cyclohexen-1-one 3-methyl-	9.17±0.60	-
2002.04	3-Cyclohexen-1-one 2-isopropyl-5-methyl-	-	12.51±0.60

1				
2	2005.0	3-Hexanol 2,3-dimethyl-	5.88±0.50	-
3	2015.11	2-Nonenoic acid methyl ester	-	
4	2100	Dihydronepetalactone -	-	5.57±0.50
5	2100.14	Dehydrocurdione	-	0.68±0.10
6	2100.4	1-Cyclohexyl-1-(2-methylenecyclohexyl)ethanol	10.37±0.50a	1.44±0.00b
7	2100.3	(-)-Spathulenol	2.92±0.20a	11.73±0.60b
8		Cyclopentanecarboxylic acid 3-methylene-1,7,7-		
9	2100.4	trimethylbicyclo[2,2,1]hept-2-yl ester	1.54±0.20	-
10	2100.78	γ-Cadinene	-	3.15±0.30
11	2100.8	1H-Pyrrole 1-ethyl-	12.67±0.40	-
12	2101.2	Isopulegol	2.03±0.10	-
13	2101.1	Eugenol	-	1.2±0.00
14	2101.8	2,3-Dehydro-1,8-cineole	1.03±0.00	-
15	2102.93	Carvacrol	-	2.76±0.00
16	2106.0	Pinene	12.89±0.80	-
17	2200.0	Thymol	0.27±0.10a	0.93±0.2a
18	2200.3	Isoaromadendrene epoxide	-	0.54±0.00
19	2200.5	Artemiseole	1.26±0.10	-
20	2200.51	Widdrol	-	7.0±0.30
21	2200.7	Eicosanoic acid	3.69±0.20	-
22	2200.98	3-Octyne	-	0.48±0.10
23	2201.9	1,7-Octadien-3-one 2-methyl-6-methylene-	0.18±0.00	-
24	2203.2	Caryophyllene oxide	0.23±0.20	-
25	2205.1	Alloaromadendrene oxide	-	0.37±0.00
26	2207.7	α-Caryophylladienol	0.79±0.20	-
27	2208.88	Caryophylla-3(4),8-dien-5-ol	-	4.84±0.30
28	2300.05	Phenol 2,4-bis(1,1-dimethylethyl)-	-	1.01±0.00
29	2300.42	<i>cis</i> -Z-α-Bisabolene epoxide	-	8.07±0.20
30	2300.67	α-Selinene	-	3.5±0.10
31	2302.3	Alloaromadendrene oxide-(1)	0.52±0.10	-
32	2302.34	<i>trans</i> -Z-α-Bisabolene epoxide	-	3.59±0.3
33	2306.19	α-Pinene oxide	-	0.54±0.00
34	2400.06	L-Fenchone	-	0.65±0.20
35	2400.1	1-Cyclohexene-1-butanal α-2,6,6-tetramethyl-	0.13±0.00	-
36	2400.1	Indole	0.49±0.20	-
37	2400.22	Patchoulane	-	0.27±0.00
38	2400.6	Benzoic acid 2,6-dimethoxy- methyl ester	0.28±0.10	-
39	2400.61	N-(1-Cyclohexen-1-yl)piperidine	-	0.38±0.10
40	2400.96	Isolongifolol	-	0.43±0.00
41	2500.02	Thujopsene	-	0.22±0.20
42	2500.14	(-)-Neoclovene-(I) dihydro-	-	0.63±0.200
43	2600.11	2,6-Dimethylbicyclo[3,2,1]octane	-	0.31±0.10
44	2601.5	Caryophyllene-(II)	-	0.23±0.20
45				
46				
47				
48				
49				
50				
51	<b>Grouped</b>			
52	<b>compounds</b>			
53	Monoterpene			
54	hydrocarbons		18.58±0.70b	0.31±0.10a
55	Oxygenated			
56	monoterpenes		9.83±0.20a	24.73±0.30b
57	Sesquiterpene		1.37±0.20a	9.73±0.20b
58				
59				
60				

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

hydrocarbons		
Oxygenated		
sesquiterpenes	4.46±0.10a	41.65±0.70b
Aliphatic		
hydrocarbons	4.61±0.30a	14.52±0.50b
Aldehydes/ketones	13.86±0.20	-
Fatty acids and		
esters	8.46±0.30b	4.73±0.20a
Others	38.8±0.60b	4.35±0.30a
Total	100	100

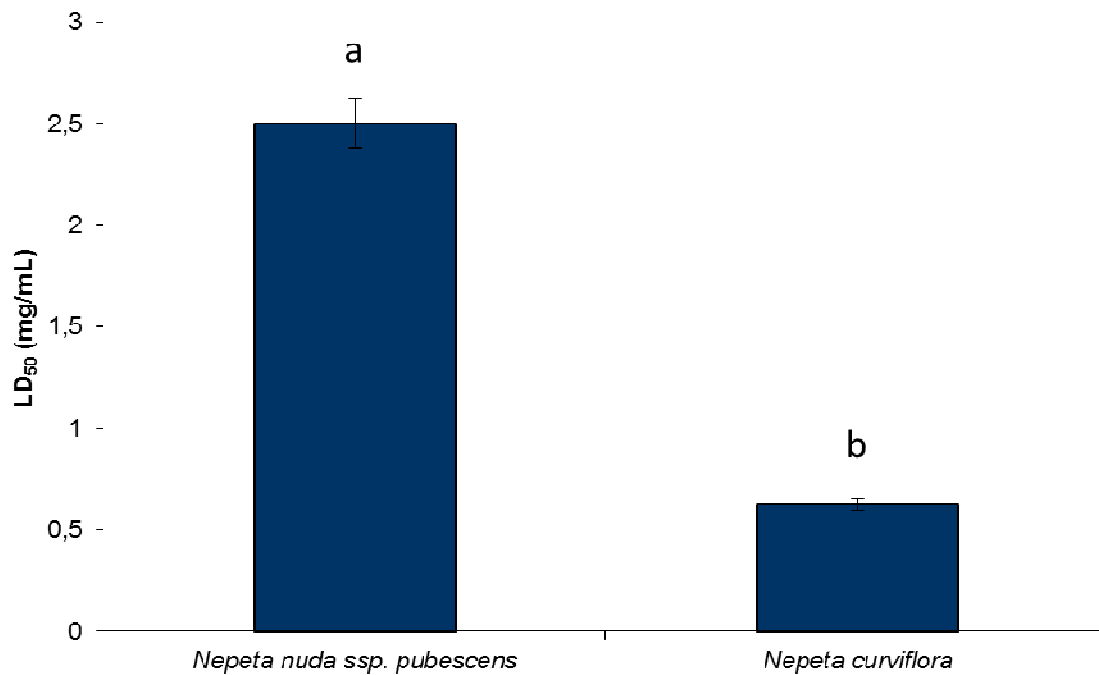
<sup>a</sup>RIs are retention indices calculated using a polar column (ZebronWax). Data are mean ± standard deviation of three replicates and values with different letters within the same line are statistically different ( $P < 0.05$ ).

For Peer Review Only

**Table 2.** Main constituents and geographical origin of essential oils from *Nepeta nuda* subspecies and *Nepeta curviflora*

<i>Nepeta</i> species	Geographical origin	Main essential oil constituents	References
<i>N. nuda</i> ssp. <i>albiflora</i>	Turkey	<i>trans</i> -Caryophyllene (23.9%), isopulegone (12.6%), <i>cis</i> -sabinol (10.1%), $\beta$ -pinene (10.0%)	Alim et al., 2009
<i>N. nuda</i> ssp. <i>albiflora</i>	Lebanon	Oxygenated monoterpenes (17.5-27.3 %) sesquiterpene hydrocarbons (22.7-38.4%) oxygenated sesquiterpenes (15.6-22.5%)	Mancini et al., 2009
<i>N. nuda</i> ssp. <i>nuda</i>	Turkey	Sesquiterpene fraction (81.9%)	Kökdil et al., 1998
<i>N. curviflora</i>	Lebanon	Sesquiterpene hydrocarbons (62.5%)	Senatore et al., 2005
<i>N. curviflora</i>	Lebanon	Sesquiterpene hydrocarbons (61.2%)	Mancini et al., 2009
<i>N. curviflora</i>	Jordan	Sesquiterpene hydrocarbons (55.27%)	Al-Qudah 2016

Figure 1



**Figure 1.** *In vitro* nematocidal activity of essential oils obtained from *Nepeta nuda* L. ssp. *pubescens* and *Nepeta curviflora* Boiss. on *Panagrolaimus rigidus* adults. Results are mean of three replicates; bars indicate standard deviation; bars carrying different letters are significantly different at  $P < 0.05$  (Fisher's LSD test).