International Journal of Life Science and Engineering
Vol. 1, No. 3, 2015, pp. 93-96
http://www.aiscience.org/journal/ijlse

First Report of *Nepeta grandiflora* Grown in Egypt

Hussein A. H. Said-Al Ahl[1, *], Ahmed S. H. Gendy[2], Ali S. Sabra[1], Elsayed A. Omer[1], Kirill G. Tkachenko[3]

[1]Medicinal and Aromatic Plants Research Department, National Research Centre, Dokki, Giza, Egypt
[2]Horticulture Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
[3]V. L. Komarov Botanical Institute of the Russian Academy of Sciences, Saint Petersburg, Russia

Abstract

Nepeta grandiflora (Lamiaceae) is a perennial plant. It contains essential oils, steroids, tannins, flavonoids. It is used in folk medicine as a general, emollient, during anemia as a substitute for tea. This work reports the essential oil content and its composition of *Nepeta grandiflora* grown in pots under the natural conditions of the greenhouse of the National Research Center, Egypt, during 2013 and 2014 season and determines their active constituents. The volatile oil content has been analyzed by GC/MS. The GC/MS revealed the presence of p-cymene (43.46%); ç-terpinene (18.58%); carvacrol (12.95%) and o-cymene (10.99%) which represents the main compounds of volatile oil extracted from *Nepeta grandiflora* flowering herb. Other important compounds were trans-caryophyllene (2.04%), α-thujene(1.69%), α-bisabolene (1.57%) and borneol (1.52%).

Keywords

Nepeta grandiflora, Essential Oil, P-Cymene, Ç-Terpinene, Carvacrol, O-Cymene

Received: April 14, 2015 / Accepted: April 29, 2015 / Published online: June 5, 2015

1. Introduction

Aromatic plants are at present widely studied for their large therapeutic potential and benefits. These benefits depend largely on essential oils, which, in general terms, occur in many herbs. The essential oils that are the essence of the plant's fragrance are also called ethereal oils or volatile oils because they evaporate quickly when exposed to the air at ordinary temperatures (De Almeida et al., 2011). In general, the essential oils consist of chemical mixtures involving several tens to hundreds of different types of molecules. Only a few have a high percentage of a single component. Essential oils are used to give flavor to foods and drinks and as fragrances in the food and cosmetics industries, where numerous herbal plant and spice ingredients are components in the manufacture of skin creams, lip balms, shampoos, soaps and perfumes (De Almeida et al., 2011). One of the largest genera of the Lamiaceae family, genus Nepeta, which comprises about 300 herbaceous perennial, rarely annual species (Formisano et al., 2011). This genus has beautiful flowers with a pleasant odor (Cantino et al., 1992). Several Nepeta spp. are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue and sedative agents, and for antiseptic and astringent properties as a topical remedy in children with cutaneous eruptions, and for snake and scorpion bites (Formisano et al., 2011). Most Nepeta species are rich in essential oils, and various biologically active; antibacterial, antifungal and antiviral activities (Tucker and Tucker, 1988).

Lamiaceae is a large widely distributed family with about 220 genera nearly 4000 species. *Nepeta grandiflora* Bieb. (Lamiaceae) is aperennial plant with stem erect, branched, glabrous to minutely pubescent, leaves ovate, cordate at the base, crenulate (Griffiths, 1994), afraquent casual in E. and E.C. Europe and locally naturalized (Turner, 1972). It is widespread in Central Europe and Central Asia. In the literature, its growth in Ukraine, Dnipro district, the Crimea, in the vicinity. Okhtirka Sumy region, *Nepeta grandiflora*,

* Corresponding author
E-mail address: hussein_saidalahl@yahoo.com (H. A. H. Said-Al Ahl)

collected in 1916 P.V.Kryzhevskym Ai-Petryn- vivYayla. According to "Flora USSR" this type occurs in many regions Ukraine as wild, and introduced in botanical gardens and has the ability to naturalization. It was cited for several authors in the flora of Romania Moldavia and Bucovina (Weryszko-Chmielewska and Chwil, 2002). It was probably cultivated in gardens for ornament and occasionally naturalized (Padru, 2003). It is a good honey plant; decorative features. Elevated part *N. grandiflora* contains essential oils, steroids, tannins, flavonoids. It is used in folk medicine as a general, emollient, during anemia as a substitute for tea (Kovtun, 2004).

The aim of this study was to establish the chemical composition of the essential oil of *Nepeta grandiflora* as a new growing in Egypt.

2. Materials and Methods

2.1. Plant Material

Seeds of *Nepeta grandiflora* were obtained from the Komarov Botanical Institute, Saint Petersburg, Russia. Seeds were sown in the nursery on 25th November, 2013. On February 10, 2014, uniform seedlings were transplanted into the experimental farm of the Faculty of Pharmacy, Cairo University, Giza, Egypt, which represents clay-loamy soil. The fresh herb was collected at the end of July.

2.2. Gas Chromatography/Mass Spectrometry (GC/MS)

Extraction of essential oil: Flowering fresh herb (100 g) was extracted by water distillation using Clevenger apparatus for 2 h according to Guenther (1961).GC/MS Analytical Condition: The volatile oil analysis was carried out using gas chromatography-mass spectrometry instrument stands at the Central Laboratories, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp.,USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer).The GC/MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 ^{o}C for 1 min; rising at 4.0 ^{o}C/minto 160 ^{o}C and held for 6 min; rising at 6 ^{o}C/min to 210 ^{o}C and held for1min. The injector and detector were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: relative retention time and mass spectra (authentic chemicals, Wileyspectral library collection and NSIT library).

3. Results and Discussions

The essential oil of *Nepeta grandiflora* growing in Egypt was subjected todetailed GC/MS analysis. Exactly14 compounds were identified, representing 95.20 % of the total oil (Table 1). The major compounds were p-cymene (43.46%), ç-terpinene (18.58 %), carvacrol (12.95%) and o-cymene (10.99%). Other important compounds were trans-caryophyllene (2.04%), α-thujene (1.69%), α-bisabolene (1.57%) and borneol (1.52%).

p-Cymene (p-isopropyltoluene, 1) is a mono-terpene biological precursor of carvacrol and one of the main constituents of the essential oil from *Nepeta grandiflora*, with more than 40% of this species, Lamiaceae, rich in p-cymene, show antinociceptive activity in rodents (Oliveira et al., 2005; Otuki et al., 2001; Bispo et al., 2001; Ramezani et al., 2004). There are several reports on the chemical composition of the essential oils from the members of the family lamiaceae which the composition of their essential oils contains the compounds found in *Nepeta grandiflora* under study, Reda et al. (2015) found that the major terpenoids constituents of the leaf oil extracted by hydro distillation of *Thymus vulgaris* L, family, Lamiaceae were thymol (31.977%), o-cymene (29.992%), carvacrol (14.541%), ç-terpinene (9.079%), linalylanthranilate (4.762%), α-terpinene (1.756%) and 4-terpineol (1.464%). Nezhadali et al. (2014) reported that the major components in the leaf oils were: thymol (38.23–63.01%), o-cymene (5.56–15.47%), γ-terpinene (4.43–7.17%), borneol (1.72–6.65%), 4-terpineol(1.24–5.16%) and 1,8-cineole (0.09–1.54%).These variations of the active principles present in the essential oils may be attributed to the different environmental and climatic conditions of different regions (Hoerster, 1974; Krussmann, 1991). Kraujalyte et al. (2011) reported that γ-terpinene (14.6%), 4-terpineol (11.7%), α-terpinene (11.5%), β-phellandrene (11.1%), sabinene (11.0%) and α-thujene (7.0%) were major constituents among 22 compounds identified in dry marjoram headspace. Rather et al. (2012) reported that the major components were (Z)-β-farnesene (49.2%), δ-3-carene (12.3%), α-bisabolene (9.4%) and germacrene-D-4-ol (5. 8%). In plants, the biosynthesis of p-cymene is considered to be an aromatization reaction of c -terpinene (Poulose and Croteau 1978). The transient formation of α-thujene and c-terpinene during sabinene consumption suggests a similar pathway involving an alylic rearrangement of sabinene to α-thujene (Harder and Fob, 1999).

Carvacrol, a monoterpene phenol, present as an active constituent of the volatile oil has been shown to have anti-proliferative effects on various human cancer cell lines, such as breast cancer, hepatoma, and cervical cancer. Also effect of carvacrol on angiogenesis and metastasis has never been

studied (Yax, 2013). Carvacrol is known to have potent anti-inflammatory and anti-oxidant effects and to inhibit platelet aggregation and the growth of a variety of cancer cells. Its potential hepatoprotective and neuroprotective activities (Suganthi and Manpal, 2013).

Table 1. Principal constituents of *Nepeta grandiflora* essential oil.

Compound	%
α-thujene	1.69
p-cymene	43.46
o-cymene	10.99
ç-terpinene	18.58
borneol	1.52
carvacrol	12.95
carvacrol acetate	0.39
trans-caryophyllene	2.04
α-bisabolene	1.57
Ë-cadinene	0.40
caryophyllene oxide	0.33
γ-cadinene	0.20
(cis)-isokalafungin	0.53
α-levantenolide	0.55

The terpenes, found in essential oils of different plants, are micro-constituents most commonly used as flavor additives in food, toiletries, and perfumes [1]. Recent studies from different groups around the World have shown that terpenes and terpenoids exert a plethora of pharmacological effects (Leal-Cardoso and Fonteles, 1999). Trans-Caryophyllene is an important constituent of the essential oil of several species of plants. It is the major chemical constituent (20.6%) of the essential oil of Pterodonpolygalaeflorus (EOPp). EOPp blocks the electromechanical excitation-contraction coupling without affecting the pharmacomechanical coupling (Evangelista et al., 2007). The blocking effect caused by EOPp was suggested to result from inhibition of dihidropyridine-sensitive Ca2+ channels, but there were no evidence to support that suggestion (Evangelista et al., 2007). Trans-Caryophyllene has been reported to possess many pharmacological effects. For example, it displays antimicrobial (Astani et al., 2011) and analgesic activity (Chavan et al., 2010). It activates the endocannabinoid system (Gertsch, 2008). Trans-Caryophyllene also has a well-documented anti-inflammatory activity (Fernandes et al., 2007; Medeiros et al., 2007). Additionally, trans-caryophyllene is effective on intestinal smooth muscle, blocking the electromechanical and pharmacomechanical excitation-contraction coupling (Leonhardt et al., 2010). Those activities allow it to be considered as a potential anti-spasmodic agent in tracheal smooth muscle.

Publications on Nepeta volatile oils clearly demonstrate that chemical polymorphism is characteristic of this species, and the composition of essential oils depends on variety, growing site, climatic and ecological conditions plant organ and vegetative cycle stage and analysis method (Formisano et al., 2011). Thus, it is of utmost importance to characterize the essential oils composition as well as the influence of the referred parameters on its quality, in order to obtain essential oils of constant composition. According to Guedes et al. (2012), this could only be possible if essential oils are extracted under the same conditions from the same organ of the plant, which has been growing on the same soil, under the same climate and has been picked in the same season.

4. Conclusion

It can be concluded that the major constituents of *Nepeta grandiflora* essential oil grown in Egypt from the aerial parts were p-cymene, ç-terpinene, carvacroland o-cymene.

References

[1] Astani, A.; Reichling, J.; Schnitzler, P. (2011). Screening for Antiviral Activities of Isolated Compoundsfrom Essential Oils. Evid. Based Complement Alternat. Med. doi:10.1093/ecam/nep187.

[2] Bispo, M. D., Mourão, R. H. V., Franzotti, E. M., Bomfim, K. B. R., Arrigoni-Blank, M. F., Moreno, M. P. N., Marchioro, M. and A. R. Antoniolli (2001). Antinociceptive and antiedematogenic effects of the aqueous extract of *Hyptispectinata* leaves in experimental animals. J. Etnopharmacol., 76: 81-86.

[3] Cantino, P.D., Harley R.M. and S.J. Wagstaff (1992). Genera of Labiatae: Status and classification. In: Advances in Labiate Science, 1st edn. Edits.,R.M. Harley and T. Reynolds, pp. 511–522, Royal Botanic Gardens, Kew.

[4] Chavan, M. J.; Wakte, P. S.; Shinde, D. B. (2010). Analgesic and anti-inflammatory activity of Caryophyllene oxide from Annonasquamosa L. bark. Phytomedicine 17, 149–151.

[5] Craveiro, A. A.; Fernandes, A. G.; Andrade, C. H. S.; Matos, F. J. A.; Alencar, J.W.; Machado, M.I.L. (1981). ÓleosEssenciais de Plantas do Nordeste; UFC: Fortaleza, Brazil.

[6] De Almeida, R.N., Agra, M.F.,MaiorF.N.S.and D.P. de Sousa (2011). Essential oils and their constituents: Anticonvulsant activity. Molecules, 16: 2726–2742.

[7] Evangelista, G.L.; Coelho-de-Souza, A.N.; Santos, C.F.; Leal-Cardoso, J.H.; Lopes, E.A.;dos Santos, M.V.; Lahlou, S.; Magalhães, P.J. (2007). Essential oil of Pterodonpoly galaeflorus inhibits electromechanical coupling on rat isolated trachea. J. Ethnopharmacol., 109: 515–522.

[8] Fernandes, E.S.; Passos, G.F.; Medeiros, R.; da Cunha, F.M.; Ferreira, J.; Campos, M.M.; Pianowski, L.F., Calixto, J.B. (2007). Anti-inflammatory effects of compounds alpha-humulene and (−-)-trans-caryophyllene isolated from the essential oil of Cordiaverbenacea. Eur. J. Pharmacol. 569, 228–236.

[9] Formisano, C., Rigano,D. and F. Senatore (2011). Chemical constituents and biological activities of Nepeta species. Chem. Biodivers, 8: 1783–1818.

[10] Gertsch, J. (2008). Anti-inflammatory cannabinoids in diet: Towards a better understanding of CB (2)receptor action? Commun. Integr. Biol. 1, 26–28.

[11] Griffiths, M. (1994). Index of garden plants, the new dicitionary, Royal Horticult. Society, 779-780.

[12] Guedes, A.P., Gregory F. and M. Fernandes-Ferreira (2012). Hypericum sp.: Essential oil composition and biological activities. Phytochem. Rev., 11: 127–152.

[13] Gunther, G. (1961). The Essential Oils. Nastrand Press, New York, USA.

[14] Harder, J. and S. Fob (1999). Anaerobic Formation of the Aromatic Hydrocarbonp-Cymene from Monoterpenes by MethanogenicEnrichment Cultures.Geomicrobiology Journal, 16:295–305.

[15] Hoerster, V.H. (1974). Variability of the oils of *Juniperuscommunis* and the composition of the oils in fruits of different age. Planta Med., 25: 73-79.

[16] Kovtun,S.M. (2004). Research the biology seeds of *Nepetagrandiflora*. Bieb.UDC, 633.88:581:96-100.

[17] Kraujalyte,V., Andjelkovic, M., Verhé, R., and P. R. Venskutonis (2011). Determination of Volatiles, Tocopherols and Colour Changes in Aromatised Oils with Marjoram (*Origanummajorana* L.). FOODBALT, 84-89.

[18] Krussmann, G. (1991). Manual of cultivated conifers (English translation by Michael E.Epp) Timber Press, Portland, 103-105.

[19] Leal-Cardoso, J.H.; Fonteles, M.C. (1999). Pharmacological effects of essential oils of plants of thenortheast of Brazil. An. Acad. Bras. Cienc. 71, 207–213.

[20] Leonhardt, V.; Leal-Cardoso, J.H.; Lahlou, S.; Albuquerque, A.A.; Porto, R.S.; Celedônio, N.R.; Oliveira, A.C.; Pereira, R.F.; Silva, L.P.; Garcia-Teófilo, T.M.; (2010). Antispasmodic effects of essential oil of Pterodonpolygalaeflorus and its main constituent beta-caryophyllene on rat isolated ileum. Fundam. Clin. Pharm. 24, 749–758.

[21] Medeiros, R.; Passos, G.F.; Vitor, C.E.; Koepp, J.; Mazzuco, T.L.; Pianowski, L.F.; Campos, M.M.; Calixto, J.B. (2007). Effect of two active compounds obtained from the essential oil of Cordiaverbenacea on the acute inflammatory responses elicited by LPS in the rat paw. Br. J. Pharmacol. 151, 618–627.

[22] Nezhadali, A.,Nabavi, M.,Rajabian, M.,Akbarpour, M.,Pourali, P. and F. Amini(2014). Chemical variation of leaf essential oil at different stages of plant growth and *in vitro* antibacterial activity of *Thymus vulgaris*Lamiaceae, from Iran. Beni-Suef University Journal of Basic and Applied Sciences, 3(2): 87–92.

[23] Oliveira,F.A., Costa,C.L.S., Chaves,M.H., Almeida,F.R.C., Cavalcante, I.J.M., Lima, A.F., Lima Júnior, R.C.P., Silva, R.M., Campos, A.R., Santos, F.A. and V.S.N. Rao (2005). Attenuationof capsaicin-induced acute and visceral nociceptivepain by alpha- and beta-amyrin, a triterpene mixtureisolated from Protiumheptaphyllum resin in mice.Life Sci., 77: 2942-2952.

[24] Otuki,M.F., Lima,F.V., Malheiros, A., Filho,V.C., Monache, F.D.M., Yunes, R.A., J. Calixto(2001). Evaluation of theantinociceptive action caused by ether fraction and atriterpene isolated from resin of Protiumkleinii. LifeSci., 69: 2225-2236.

[25] Padru,I.M.(2003). Fruit morphology, anatomy and mixocarpy in *Nepetacataria* L. citriodora and *Nepeta grandiflora* Bieb. (Lamiaceae). Rev. Roum. Biol. Biol.Veget., Tome, 48 (1-2): 23-30.

[26] Pinho-da-Silva, L., Mendes-Maia,P. V., Teófilo,T.M.N. G., Barbosa,R., Ceccatto, V. M., Coelho-de-Souza,A. N., Cruz, J. S. and J. H. Leal-Cardoso (2012).trans-Caryophyllene, a Natural Sesquiterpene, Causes TrachealSmooth Muscle Relaxation through Blockade ofVoltage-Dependent Ca2+ Channels.Molecules, 17: 11965-11977.

[27] Poulose,A.J. andR.Croteau(1978). Biosynthesis of aromatic monoterpenes: conversion of c-terpinene top-cymene and thymol in *Thymus vulgaris* L. Arch.Biochem.Biophys., 187:307–314.

[28] Ramezani, M., Hosseinzadeh, H. and S. Samizadeh (2004). Antinociceptive effects of ZatariamultifloraBoiss fractions in mice. J. Ethonopharmacol., 91: 167-170.

[29] Rather, M. A., Hassan, T., Dar, B. A., Shaw,A. S., Qurishi M. A. and B.A. Ganai (2012). Essential Oil Composition of *Nepetaraphanorhiza*Benth growing in Kashmir valley. Rec. Nat. Prod., 6 (1):67-70

[30] Reda, T., Unnithan, C.R., TayeK. and L.Brhane(2015). Chemical composition and antibacterial activity of the leaf oil of *Thymus vulgaris* L.Inter. J. of Phytotherapy, 5 (1): 4-8.

[31] Suganthi, R.U. and S. Manpal (2013). Biological and pharmacological actions carvacrol and its effects on poultry: An updated review. J. Pharmacy and pharmaceutical Sciences, 2(5):3581-3595.

[32] Tucker, A.O. and S.S. Tucker (1988). Catnip and the catnip response. Econ. Bot., 42, 214–231

[33] Turner, C. (1972). Flora Europaea, In: TutinT.G. et al (Eds), Cambridge University Press, Cambridge, *Diapensiaceae* to *Myoporaceae*, 3:158-160.

[34] Weryszko-Chmielewska, E.andM. chwil(2002). Blooming and nectar secretion of *Nepeta grandiflora* Bieb. Under the condition of lead excess. Journal of Apicultural Science, 46(1):59-65.

[35] Yax, T. (2013). Carvacrol as an anti-cancer agent on human metastatic breast cancer cell line, MDA-MB-231. M.Sc., Dissertation/Thesis, Long Island University, The Brooklyn Center University location United States- New York.