

RESEARCH ARTICLE

Volatile components and antimicrobial activity of the *n*-hexane extracts of *Neomuretia pisidica* (Kit Tan) Kljuykov, Degtjareva & Zakharova

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Abstract

The fruits, aerial parts and roots of *Neomuretia pisidica* (Kit Tan) Kljuykov, Degtjareva & Zakharova were extracted with *n*-hexane. Total of 18 compounds were characterised by GC analyses of the *n*-hexane extracts. Main volatile components of the *n*-hexane extract of aerial parts were characterized as 1,8-cineole (23.4%), camphor (21.4%), 2-ethyl hexanol (14.6%), α -pinene (7.2%), and verbenone (6.4%). Methyl linoleate (19.3%), 1,8-cineole (16.5%), camphor (13.2%), α -pinene (6.1%) and 2-ethyl hexanol (4.9%) were found in the *n*-hexane extract of roots. Whereas, 1,8-cineole (23.3%), camphor (20.3%), 2-ethyl hexanol (14.2%), α -pinene (9.9%), and limonene (4.1%) were the major components of the *n*-hexane extract of fruits. Antimicrobial activity were identified using a microdilution assay against selected human pathogenic strains. The most potent inhibitor activities with 156 μ g/mL concentrations were detected against *S. aureus* and *E. faecalis*.

Keywords: *Neomuretia pisidica*, Apiaceae, volatile compounds, antimicrobial activity

Introduction

Neomuretia (Apiaceae) is a new genus of geophytic plants that represented by two species distributed in the Mediterranean region of Turkey and Northern Iraq. *Neomuretia pisidica* (Kit Tan) Kljuykov, Degtjareva & Zakharova (syn. *Hellenocarum pisidicum*) is an endemic species growing in the Karaman province of Turkey (Zakharova et al., 2016). Apiaceae species are among the richest in essential oils (Baser&Kirimer, 2014; Oroojelian et al., 2010; Sarebkhar&Iranshahi, 2010; Tabanca et al., 2006). Akalın et al., 2009 and Kljuykov et al., 2020 published recent botanical reviews on the family. According to our interviews with local people, basal leaves of this species are used as food and for the treatment of toothaches.

The current study was aimed to investigate the volatile components and antimicrobial activity of the *n*-hexane extracts of *N. pisidica*.

Materials and Methods

Plant material

The roots, aerial parts, and fruits of *N. pisidica* were collected from the northern slopes of Göksu river valley near Akçaalan village, Karaman Province in 2017, and identified by one of us (ÖÇ). Voucher specimens were deposited at the GAZI Herbarium (Herbarium No: 2986).

Extraction of plant materials

The air-dried plant materials (fruit, root, and aerial parts; each 200 g) were separately powdered and extracted with *n*-hexane (3x 200 mL) at room temperature and filtered. The *n*-hexane was removed in a rotary evaporator *in vacuo*.

GC-MS analysis

The GC-MS analysis was carried out using an Agilent 5975 GC-MSD system. The analysis conditions were as described in our previous publication (Karaca et.al., 2020).

GC analysis

The analyzes were carried out as described in previous publications (Karaca et.al., 2020). GC analysis results are given in Table 1. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0) (1,2) and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils.

Antibacterial activity

The antibacterial activity was studied using broth microdilution assay following the methods described by the CLSI, Clinical and Laboratory Standards Institute Standards (CLSI, 2006). The potential minimum inhibitory concentrations (MIC) were calculated against the selected human pathogenic; *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538, and *Escherichia coli* NRLL B-3008. The activity was studied as described in previous publications (Karadağ et.al., 2019). The antibacterial evaluations were in triplicates and reported as mean in Table 2.

Results and Discussion

The volatile constituents of the *n*-hexane extracts of *N. pisidica* fruits, roots, and aerial parts were analyzed using GC-FID and GC-MS which led to the identification of eighteen compounds. The main components of the *n*-hexane extract of the aerial parts were characterized as 1,8-cineole (23.4%), camphor (21.4%), 2-ethyl hexanol (14.6%), α -pinene (7.2%), and verbenone (6.4%). Methyl linoleate (19.3%), 1,8-cineole (16.5%), camphor (13.2%), α -pinene (6.1%) and 2-ethyl hexanol (4.9%) were identified in the *n*-hexane extract of roots. Whereas, 1,8-cineole (23.3%), camphor (20.3%), 2-ethyl hexanol (14.2%), α -pinene (9.9%), and limonene (4.1%) were the major components of the *n*-hexane extract of fruits.

Table 1. The Volatile Composition of *Neomuretia pisidica* *n*-hexane extracts

RRI	Compounds	Aerial part %	Fruit %	Root %
1032	α -Pinene	7.2	9.9	6.1
1076	Camphene	1.8	3.2	1.6
1093	Hexanal	-	1.3	-
1174	Myrcene	1.5	2.5	1.0
1194	Heptanal	-	1.3	-
1203	Limonene	3.1	4.1	2.3
1213	1,8-Cineole	23.4	23.3	16.5
1280	<i>p</i> -Cymene	4.5	2.7	1.5
1496	2-Ethyl hexanol	14.6	14.2	4.9
1532	Camphor	21.4	20.3	13.2

1536	Pinocamphone	3.2	3.0	1.6
1553	Linalool	3.5	2.5	1.1
1706	α -Terpineol	1.3	0.8	0.5
1719	Borneol	5.0	2.8	2.2
1725	Verbenone	6.4	3.8	2.8
2242	Methyl hexadecanoate	-	-	4.3
2509	Methyl linoleate	-	-	19.3
2583	Methyl linolenate	-	-	4.1
Total		96.9	95.7	83.0

RRI: Relative retention indices calculated against *n*-alkanes. %: Calculated from FID data

Table 2. Antimicrobial activities of the *n*-hexane extracts of *N. pisidica* (MICs in mg/mL)

Sample	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>E. faecalis</i>
Fruit extract	2.5	2.5	0.625	0.156
Root extract	2.5	0.156	0.625	0.156
Aerial part extract	1.25	0.156	1.25	0.312
Tetracycline	16	0.25	>16	0.025

Antimicrobial activities of the *n*-hexane extracts of *N. pisidica* against bacterial strains were listed, in Table 2. The results revealed that the tested extracts are effective on *S. aureus* and *E. faecalis* at between 312-156 μ g/mL concentration. In previous studies, the antimicrobial activities of 1,8-cineole (Hendry et al., 2009; Kifer et al., 2016; Vuuren et al., 2007) and camphor (Jirovetz et al., 2005) are studied and demonstrated that camphor and 1,8-cineole have remarkable antimicrobial capacity. Based on this, it can be thought that the antimicrobial effect of *N. pisidica* essential oils is caused by camphor and 1,8-cineole. Furthermore, the antinociceptive and antiinflammatory activities of 1,8-cineole, camphor and essential oils that contain large proportional amounts of 1,8-cineole and camphor were proven (Lenardão et al., 2016; Chandrakanthan et al., 2020; Santos et al., 2000; Barkin, 2013). Thus, antinociceptive and antiinflammatory activities may explain the folkloric usage of *N. piscidica* for toothache.

Essential oils are known for their antimicrobial effects, and the different volatile components they contain may be responsible for this effect. In studies conducted with *n*-hexane extracts rich in volatile components, it has the potential of antibacterial effect as much as essential oils.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

- Akalın, E., Özhatay, N., Özhatay, E., & Ünlü, S. (2009). Rare and endemic taxa of Apiaceae in Turkey and their conservation significance. *İstanbul Üniversitesi Eczacılık Fakültesi Dergisi*, 40, 1-10.
- Barkin, R. L. (2013). The pharmacology of topical analgesics. *Postgraduate Medicine*, 125(sup1), 7-18.
- Baser, K. H. C., & Kirimer, N. (2014). Essential oils of Anatolian Apiaceae-A profile. *Natural Volatiles and Essential Oils*, 1(1), 1-50.

- Chandrakanthan, M., Handunnetti, S. M., Premakumara, G. S. A. and Kathirgamanathar, S. (2020). Topical anti-inflammatory activity of essential oils of *Alpinia calcarata* Rosc., its main constituents, and possible mechanism of action. *Evidence-Based Complementary and Alternative Medicine*, 2020, 2035671.
- Clinical and Laboratory Standards Institute M7-A7, 2006. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Seventh Edition, CLSI document A. Wayne, Pa. USA. 26(2).
- Hendry, E. R., Worthington, T., Conway, B. R., & Lambert, P. A. (2009). Antimicrobial efficacy of eucalyptus oil and 1, 8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. *Journal of Antimicrobial Chemotherapy*, 64(6), 1219-1225.
- Jirovetz, L., Buchbauer, G., Denkova, Z., Stoyanova, A., Murgov, I., Schmidt, E., & Geissler, M. (2005). Antimicrobial testinas and gas chromatographic analysis of pure oxygenated monoterpenes 1,8-cineole, α -terpineol, terpinen-4-ol and camphor as well as target compounds in essential oils of pine (*Pinus pinaster*), rosemary (*Rosmarinus officinalis*), tea tree (*Melaleuca alternifolia*). *Scientia Pharmaceutica*, 73(1), 27-39.
- Karaca, N., Şener, G., Demirci, B., & Demirci, F. (2020). Synergistic antibacterial combination of *Lavandula latifolia* Medik. essential oil with camphor. *Zeitschrift für Naturforschung C*, 1(ahead-of-print).
- Karadağ, A. E., Demirci, B., Çaşkurlu, A., Demirci, F., Okur, M. E., Orak, D., Başer, K. H. C. (2019). In vitro antibacterial, antioxidant, anti-inflammatory and analgesic evaluation of *Rosmarinus officinalis* L. flower extract fractions. *South African Journal of Botany*, 125, 214-220.
- Kifer, D., Mužinić, V., & Klarić, M. Š. (2016). Antimicrobial potency of single and combined mupirocin and monoterpenes, thymol, menthol and 1, 8-cineole against *Staphylococcus aureus* planktonic and biofilm growth. *The Journal of Antibiotics*, 69(9), 689-696.
- Kljuykov, E. V., Petrova, S. E., Degtjareva, G. V., Zakharova, E. A., Samigullin, T. H., & Tilney, P. M. (2020). A taxonomic survey of monocotylar Apiaceae and the implications of their morphological diversity for their systematics and evolution. *Botanical Journal of the Linnean Society*, 192(3), 449-473.
- Lenardão, E. J. Savegnago, L., Jacob, R. G., Victoria, F. N. and Martinez, D. M. (2016). Antinociceptive effect of essential oils and their constituents: an update review. *Journal of the Brazilian Chemical Society*, 27(3), 435-474.
- Oroojalian, F., Kusra-Kermanshahi, R., Azizi, M., & Bassami, M. R. (2010). Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. *Food chemistry*, 120(3), 765-770.
- Santos, F.A. and Rao, V.S.N. (2000). Antiinflammatory and antinociceptive effects of 1,8-Cineole a terpenoid oxide present in many plant essential oils. *Phytotherapy Research*, 14, 240-244.
- Sahebkar, A., & Iranshahi, M. (2010). Biological activities of essential oils from the genus *Ferula* (Apiaceae). *Asian Biomedicine*, 4(6), 835-847.
- Tabanca, N., Demirci, B., Ozek, T., Kirimer, N., Baser, K. H. C., Bedir, E., Wedge, D. E. (2006). Gas chromatographic–mass spectrometric analysis of essential oils from *Pimpinella* species gathered from Central and Northern Turkey. *Journal of Chromatography A*, 1117(2), 194-205.
- Vuuren, S. V., & Viljoen, A. M. (2007). Antimicrobial activity of limonene enantiomers and 1, 8-cineole alone and in combination. *Flavour and Fragrance Journal*, 22(6), 540-544.
- Zakharova, E.A., Kljuykov, E.V., Degtjareva, G.V., Samigullin, T., Ukrainskaya, U.A., & Downie, S.R. (2016). A taxonomic study of the genus *Hellenocarum* H. Wolff (Umbelliferae-Apioideae) based on morphology, fruit anatomy, and molecular data. *Turkish Journal of Botany*, 40(2), 176-193.