

## Phytochemical Characterization and Biological Activities of *Nepeta cadmea* Boiss.

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**Abstract:** The genus *Nepeta* (Lamiaceae) is represented by 39 species, in total 50 taxa, 19 of which are endemic, in Turkey. *Nepeta* species are known as “kedinanesi” in Turkish and has many traditional usages such as antispasmodic, diuretic, antiseptic, antitussive, and antiasthmatic activities. The aerial parts of *N. cadmea* were hydrodistilled for 3 hours by using Clevenger apparatus to gather essential oil. To determine its chemical characterization, the essential oil was analyzed by GC-FID and GC-MS, simultaneously. 4 $\alpha$ ,7 $\alpha$ ,7 $\beta$ -Nepetalactone (74.0%), 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (4.5%) and caryophyllene oxide (2.5%) were found as major components for essential oil. The potential *in vitro* antibacterial activity of the essential oil was evaluated using the broth microdilution assay. A panel of human pathogenic strains *Escherichia coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL B-3711 and *Streptococcus sanguinis* ATCC 10556 were used. Minimal Inhibitory Concentrations (MIC) of the samples were determined, where in ciprofloxacin was used as a positive control in the assay. MIC values were found 2500, 1000, 600, 600, 600  $\mu$ g/mL against *E. coli*, *P. aeruginosa*, *S. typhimurium*, *B. cereus* and *S. sanguinis*, respectively. Compared the literature, it was seen that the essential oil had lower effective against these strains.

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## 1. INTRODUCTION

The genus *Nepeta* L. (Lamiaceae) comprises by nearly 250 species throughout the world [1]. *Nepeta* species are generally distributed in Europe, South-West and Central Asia, North America, North Africa and the Mediterranean regions [2]. According to the recent researches, the genus contains 39 species, in total 50 taxa, 19 of which are endemic, in Turkey [3-5]. The plant is distributed the West Anatolia, South Anatolia, and South west Anatolian regions of Turkey [6]. As in most of the members of Lamiaceae, many *Nepeta* species have traditional usages against the common colds stomachache and as stimulants [7,8]. In addition, several *Nepeta* species have traditional use as diuretic, diaphoretic, antitussive, antispasmodic, antiasthmatic, febrifuge, soothing, nervous disorders, depression, spices and herbal tea [9-11].

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Some of the *Nepeta* species are good nectar sources for bees [12]. The endemic species *N. cadmea* Boiss., known as "honaz pisikotu" in Turkish [5], is herbaceous, perennial, often aromatic, rich in essential oils content, stems erect and several branched 30-120 cm, densely glandular hairs. Leaves triangular-ovate 3-6 x 1-3 cm, green, inflorescence clearly distant, many flowered verticillaster, calyx tubular, 9-12 mm, corolla with a yellow hood and white lower lip, 12-15 mm [3]. The essential oils of *N. cadmea* species collected from two different regions in Turkey were exhibited antimicrobial activity against some gram (+) and gram (-) bacteria by using disc diffusion method [13]. In addition, recent antimicrobial activity studies on *Nepeta* species have been reported to be a natural preservative and a strong antimicrobial agent on food products. [14,15]. In this work, we aimed to investigate chemical composition of essential oil and *in vitro* biological activities of endemic *N. cadmea* species in Turkey.

## 2. MATERIAL AND METHODS

### 2.1. Plant Material and Isolation of Essential Oil

Aerial parts of *N. cadmea* were collected from natural habitat in Denizli province and identified by Mehmet Çiçek. The voucher specimen has been deposited in the herbarium of the Faculty of Pharmacy, Ankara University in Ankara, Turkey (AEF). Collection locality: C2 Denizli: above Çamlık forest, old Kızılcabölük road, oak areas above second fountain, 880 m, 14.06.2017, M. Çiçek 2017-4-16 (Voucher number: AEF 28879). The aerial parts of *N. cadmea* were hydrodistilled for three hours using Clevenger-type apparatus to obtain essential oil.

### 2.2. GC-FID and GC-MS Analyzes

The essential oil was analyzed by GC using a Hewlett Packard 6890 system (SEM Ltd, Istanbul, Turkey) and an HP Innowax fused silica capillary column (FSC) (60 m x 0.25 mm  $\phi$ , with 0.25  $\mu$ m film thickness) was used with nitrogen at 1 mL/min. Initial oven temperature was 60°C for 10 min, and increased at 4°C/min to 220°C, then kept constant at 220°C for 10 min and increased at 1°C/min to 240°C. Injector temperature was set at 250°C. Percentage compositions of the individual components were obtained from electronic integration using flame ionization detection (FID, 250°C) Relative percentages of the separated compounds were calculated from FID chromatograms. GC-MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd, Istanbul, Turkey) and Innowax FSC column (60 m x 0.25 mm, 0.25  $\mu$ m film thickness) was used with Helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425 as previously reported [16]. Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial [17,18] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [19] was used for the identification as also previously reported in detail [16].

### 2.3. Antimicrobial Activity

The standard antibiotic ciprofloxacin was supplied from commercial sources like Sigma-Aldrich (St. Louis, USA) was evaluated for its antibacterial properties, which were in pharmaceutical grade or highest possible purity. Microorganisms strains used for the evaluation of antimicrobial activity were obtained from the American Type Culture Collection (ATCC) and Agricultural Research Service Culture Collection (NRRL) in lyophilized form. The microorganisms were stored at -85°C in glycerol until inoculation and purity testing.

The potential *in vitro* antimicrobial activity of the essential oil was evaluated using the broth microdilution assay. A panel of human pathogenic strains *Escherichia coli* NRRL B-

3008, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL B-3711 and *Streptococcus sanguinis* ATCC 10556 were used. Minimal Inhibitory Concentrations (MIC) of the samples were determined where in ciprofloxacin was used as a positive control in the experiments.

Antimicrobial activity of the essential oil was evaluated by broth microdilution assay according to a modified Clinical and Laboratory Standards Institute (CLSI) method. *E. coli* NRRL B-3008, *P. aeruginosa* ATCC 27853, *S. typhimurium* ATCC 13311, *B. cereus* NRRL B-3711 and *S. sanguinis* ATCC 10556 were used as test microorganisms. The essential oil (20-0.019 mg/mL) was dissolved in sterile dimethyl sulfoxide (DMSO) for the initial stock solution. 100  $\mu$ L of essential oil was applied to 96-well microplates and 2 fold serial dilutions were performed. After the dilutions, 50  $\mu$ L aliquots of turbidometrically adjusted microorganisms were inoculated (105-106 CFU/mL) on to the plates. After incubation at 37°C for 24 h the first well was treated with 20  $\mu$ L of resazurin, which insured on all microplates the MIC, where the lowest concentration of the samples prevented visible growth. The standard antibiotic ciprofloxacin (128-0.25  $\mu$ g/mL) were used as standard controls. Solvent and microbial controls were also added to the assay plate. Antibacterial assays were repeated at least three times for all the test samples [20].

### 3. RESULTS and DISCUSSION

#### 3.1. GC-FID and GC-MS analyzes

The oil yield was 0.25%. The essential oil was analyzed by using GC-FID and GC-MS, simultaneously to determined the chemical characterization of its. 4 $\alpha$ ,7 $\alpha$ ,7 $\beta$ -Nepetalactone (74.0%), 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (4.5%) and caryophyllene oxide (2.5%) were found as main components. Other components were given in Table 1. According a previous work, 84 compounds were characterized represent 94% of the essential oils for two different specimens from Muğla and Antalya and 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (44.51% and 74.96%) were found as the main constituents [21]. In a study of Başer et al. [22] *Nepeta* species were divided into two main groups as nepetalactone-containing and nepetalactone-lesscontaining. According to this study, 4 $\alpha$ -7 $\alpha$ -7 $\alpha$ -Nepetalactone was found the most frequently encountered nepetalactone in *Nepeta* essential oils. Four *Nepeta* species containe 4 $\alpha$ -7 $\alpha$ -7 $\alpha$ -nepetalactone as the main constituent while in a species the main constituent is 4 $\alpha$ -7 $\alpha$ -7 $\beta$ -nepetalactone. Çelik et al. [23] have defined the 97.91% of *N. cadmea* species essential oil profile, which contains 13 compounds in total. The major essential oils in the *N. cadmea* were nepetalactone with the percentages of 81.6%, caryophyllene (3.71%), and germacrene D (3.25%), respectively [23]. Öz et al. [24] identified eighteen components in *N. cadmea* (94.48%). Caryophyllene oxide (22.96%), viridiflorol (12.23%), cis-calamenene (10.67%), cis-14-nor-muurool-5-en-4-one (7.53%),  $\alpha$ -cadinol (6.92%) and caryophylla-4(12),8(13)-dien-5- $\beta$ -ol (6.11%) were identified as the main components.

#### 3.2. Antimicrobial activity

The *in vitro* antimicrobial activity of the essential oil of *N. cadmea* was evaluated against two gram-positive bacteria namely *B. cereus* and *S. sanguinis*, and also three gram-negative bacteria namely *E. coli*, *Pseudomonas aeruginosa* and *S. typhimurium* by broth microdilution method.

**Table 1.** Chemical composition of *Nepeta cadmea* Boiss. essential oil.

RRI	Component	%
1132	Sabinene	tr
1174	Myrcene	tr
1203	Limonene	tr
1213	1,8-Cineol	0.3
1255	$\gamma$ -Terpinene	tr
1266	( <i>E</i> )- $\beta$ -Ocimene	tr
1280	<i>p</i> -Cymene	0.1
1437	$\alpha$ -Thujone	0.4
1450	trans-Linalool oxide ( <i>furonoid</i> )	tr
1451	$\beta$ -Thujone	0.2
1499	$\alpha$ -Campholene aldehyde	tr
1532	Camphor	1.0
1553	Linalool	1.0
1590	Bornyl acetate	tr
1611	Terpinen-4-ol	0.3
1612	$\beta$ -Caryophyllene	2.0
1687	$\alpha$ -Humulene	0.2
1694	Neral	0.6
1704	$\gamma$ -Muurolen	0.5
1706	$\alpha$ -Terpineol	0.1
1719	Borneol	0.2
1726	Germacrene D	0.4
1740	Geranial	0.8
1740	$\alpha$ -Muurolene	0.1
1765	Geranyl acetate	0.1
1849	Calamenene	1.3
1918	$\beta$ -Calacorene	0.2
2016	4 $\alpha$ -7 $\alpha$ -7 $\alpha$ -Nepetalactone	4.5
2008	Caryophyllene oxide	2.5
2069	4 $\alpha$ -7 $\alpha$ -7 $\beta$ -Nepetalactone	74.0
2080	1,10-di-epi-Cubenol	0.3
2088	4 $\beta$ -7 $\alpha$ -7 $\beta$ -Nepetalactone	0.3
2104	Viridiflorol	0.6
2187	T-Cadinol	tr
2198	Thymol	0.6
2255	$\alpha$ -Cadinol	0.7
2256	Cadalene	0.3
2264	4,7-Dimethyl-1-tetralone	0.2
2316	Caryophylledienol-I	0.3
2389	Caryophyllenol-I	0.4
2373	Dehydronepatalactone	0.1
2392	Caryophyllenol-II	0.5
	Total	95.1

tr: trace

As a result, it was found that essential oil of *N. cadmea* have different antimicrobial activity. Antibacterial activity values of the essential oil of *N. cadmea* were found between 2500-600 µg/ml. MIC values were found as 2500, 1000, 600, 600, 600 µg/mL against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus cereus* and *Streptococcus sanguinis*, respectively. The results were given in Table 2.

Literature survey showed to be found the study of antimicrobial activity on a few of *Nepeta* species. In a study conducted by Saraç and Uğur [13], an antimicrobial activity test was performed on the essential oil of *N. cadmea* species by using disc diffusion method. As a result, some gram-positive and gram-negative bacteria were found to exhibit activity against. Zomorodian et al. [14] studied on antimicrobial activity of the essential oil of *N. cataria* using different microorganisms with the broth microdilution method. They concluded that the essential oil of *N. cataria* can be used as a natural protective agent on food products. Yavuz et al. [15] reported that the methanol extract of *N. nuda* can be evaluated as a potent good antimicrobial agent on 5 bacteria. Çelik et al. [23] have reported that the essential oil of *N. cadmea* showed a strong antimicrobial activity against *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *Cowan liyofilii*, *Morganella morgana*, *Proteus vulgaris* RSKK 96026, *B. cereus* RSKK863, *E. coli* ATCC 218, *Klebsiella pneumoniae* ATCC 27736, *S. enteritidis* RSKK 171, *Yersinia enterocolitica* ATCC 1501, *E. coli* ATCC 25922 and *Micrococcus luteus* MRL B-4375 by using the disc diffusion method. To the best of our knowledge, the study presented herein is the first report on the antimicrobial activity of the essential oil of *N. cadmea* by using broth microdilution method.

**Table 2.** Minimal Inhibitory Concentrations (MIC) of *Nepeta cadmea* essential oil (µg/mL).

Bacteria	Essential oil	Ciprofloxacin
<i>Escherichia coli</i> NRRL B-3008	2500	30
<i>Pseudomonas aeruginosa</i> ATCC 27853	1000	30
<i>Salmonella typhimurium</i> ATCC 13311	600	10
<i>Bacillus cereus</i> NRRL B-3711	600	10
<i>Streptococcus sanguinis</i> ATCC 10556	600	10

#### 4. CONCLUSION

In the present study, we concluded that the essential oil of *N. cadmea* was exhibited different activity against *E. coli* NRRL B-3008, *P. aeruginosa* ATCC 27853, *S. typhimurium* ATCC 13311, *B. cereus* NRRL B-3711 and *S. sanguinis* ATCC 10556. To the best of our knowledge, this is the first report on the antimicrobial activity by microdilution. However, it will be useful to investigate different antibacterial and antifungal tests in later studies. We also plan to compare different *Nepeta* species and compare them. In the future, we suggest that essential oils obtained from plants can be used as antimicrobial agents in search of infectious diseases, treatment and new drugs.

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#### Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

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