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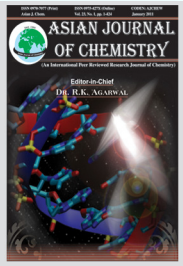

# Antimicrobial Effects and Composition of Essential Oils of *Nepeta glomerata* Montbret et Aucher ex Bentham (Lamiaceae) from Turkey

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In this study, antimicrobial activities and composition of essential oils of *Nepeta glomerata* Montbret et Aucher ex Bentham. were studied. The antimicrobial activity of this plant was evaluated by using disk diffusion method with *Micrococcus luteus* LA 2971, *Listeria monocytogenes* SCOTT A, *Bacillus cereus* EÜ, *Enterococcus faecalis* ATCC 15753, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium smegmatis* RUT, *Escherichia coli* DM, *Aeromonas hydrophila* ATCC 7966, *Yersinia enterocolitica* AÜ 19, *Bacillus brevis* FMC 3, *Corynebacterium xerosis* UC 9165, *Klebsiella oxytocica* DC 113, *Rhodotorula rubra* DC 86, *Saccharomyces cerevisiae* WET 136 and *Candida albicans* DC 146. The results indicated that antimicrobial and antifungal activities were found in essential oils of *Nepeta glomerata*. The essential oil was analyzed by GC and GC/MS. Fifty components were identified representing 92.6 % of the oil. The main component of the *Nepeta glomerata* Montbret et Aucher ex Bentham was carvacrol (16.4 %).

**Key Words: Essential oils, *Nepeta glomerata* Montbret et Aucher ex Bentham, Antibacterial activity, Antifungal activity, Composition.**

## INTRODUCTION

Turkey is a large peninsula, rectangular in shape and situated at the southwestern corner of Asia meeting Europe across the Aegean and Marmara seas to the west with land in Europe, named Thrace. Turkey is under the influence of three different climates, namely, Mediterranean, continental and oceanic. Turkey is situated at the junction of three important phytogeographic regions, namely Mediterranean, Irano-Turanian and Euro-Siberian[1].

The genus Nepeta, also called Glecho ma and Cataria, is named after the ancient Italian city of Nepi[2]. This genus which belongs to Stachyoideae-Nepeteae tribe, Lamiaceae family, consists of about 250 species distributed in the central and southern parts of Europe, Asia and Middle East[3].

Many reports on phytochemical analysis of this genus, including essential oil analysis, are found in the literature[4-6]. Previous chemical investigations on several Nepeta species have shown the presence of nepetalactones in relatively high concentrations[7,8], spathulenol and caryophyllene[4,9], germacrene D[4,9], 1,8-cineole, β-pinene and geranyl acetate[6].

Nepeta species are still used in the traditional medicine of many countries as diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge, emmenagogue and sedative agents[5]. The Turkish flora comprises 34 species of Nepeta and one of the them is *Nepeta glomerata*[10].

*Nepeta glomerata* is perennial; stems often branched from below, 24-100 cm, shortly pilose to villous, gradular-papillose. Leaves ovate, 1.0-3.8 cm × 0.7-3.5 cm, ± cordate, crenate, glabrescent to pilose; petiole 0-2.5 mm. Inflorescence usually branched, verticillate, lower cymes pedunculate, upper ± sessile, flowers congested[11].

Numerous plants in the flora of Turkey have been used as traditional medicines. However, most of them have not been evaluated scientifically. The aim of this study is to investigate the antimicrobial activity and the chemical composition of the hydrodistilled oil of *Nepeta glomerata* Montbret et Aucher ex Bentham.

## EXPERIMENTAL

*Nepeta glomerata* Montbret et Aucher ex Bentham was used in this study. The plants were collected from the region Ahir Mountain (Kahramanmaras-Turkey). The collected species were authenticated according to the conventional method[12] and were deposited in Kahramanmaras Sütçü Imam University Biology Department. Plants were broken intopieces using blender. The plants were distilled for 3 h, using a Clevenger-type apparatus according to the European Pharmacopoeia (1975).

The tested microorganisms in this study were provided from the culture collections of the Microbiology Laboratory of the Science and Art Faculty of the University of

Kahramanmaras Sütçü Imam, in Kahramanmaras Turkey. *Micrococcus luteus* LA 2971, *Listeria monocytogenes* Scoot A, *Bacillus cereus* EÜ, *Enterococcus faecalis* ATCC 15753, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium smegmatis* RUT, *Escherichia coli* DM, *Aeromonas hydrophila* ATCC 7966, *Yersinia enterocolitica* AÜ 19, *Bacillus brevis* FMC3, *Corynebacterium xerosis* UC 9165, *Klebsiella oxytocica* DC 113 bacteria and *Rhodotorula rubra* DC 86, *Saccharomyces cerevisiae* WET 136 and *Candida albicans* DC 146 fungi were used.

**Isolation of the essential oils:** The collected species was distilled using a Clevenger-type apparatus. The essential oils thus obtained were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Shüll No:2668, Germany)[13,14]. In addition, reference antibiotic discs such as ampicillin, streptomycin, nystatin were used for comparision (Oxoid).

**Preparation of microorganism cultures:** The bacteria were first incubated at 37 ± 0.1 ºC for 24 h in Nutrient Broth (Difco) and the yeasts were incubated in Sabouraud Dextrose Broth (Difco) at 25 ± 0.1 ºC for 24 h. After injecting cultures of the bacteria and yeast (prepared as above) into petri dishes (9 cm) in the amount of 0.01 mL ($10^6$/mL for the bacteria and $10^5$/mL for the fungi), 15 mL of Mueller Hinton Agar (MHA, Oxoid) and Sabouraud Dextrose Agar (SDA) (sterilized in a flask and cooled to 45-50 ºC) were homogenously distributed onto the sterilized petri dishes[13].

Sterilized blank paper discs 6 mm in diameter were saturated with 1, 2, 4 and 6 µL of essential oil per disc, then placed onto the agar plates which had previously been inoculated with the above organisms. The petri dishes were left at 4 ºC for 2 h and then the injected plates with bacteria were incubated at 37 ± 0.1 ºC for 24 h, plates inoculated with fungi were incubated at 25 ± 0.1 ºC for 48 h[13-15]. At the end of the period, inhibition zones were measured in millimeters (mm). These studies were performed in triplicate.

**Gas chromatographic (GC) analysis:** The essential oil was analyzed using HP 6890 GC equipped with and FID detector and an HP-5 MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm) capillary column was used. The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

**Gas chromatography/mass spectrometry (GC-MS) analysis:** The oil was analyzed by GC-MS, using a Hewlett Packard system. HP-Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Research Laboratory, Firat University. An HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 µm) was used with helium as the carrier gas. Injector temperature was 250 ºC, split flow was 1 mL/min. The GC oven temperature was kept at 70 ºC for 2 min and programmed to 150 ºC at a rate of 10 ºC/min and then kept constant at 150 ºC for 15 min to 240 ºC at a rate of 5 ºC/min. *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). MS were taken at 70 eV and a mass range of 35-425. Component identification was carried out using spectrometric electronic libraries (WILEY, NIST). The identified constituents of the essential oils are listed in Table-2.

## RESULTS AND DISCUSSION

The antibacterial and antifungal activities of essential oil of *Nepeta glomerata* is provided in Table-1. In addition, the inhibition zones formed by standard antibiotic discs are listed in Table-1. The result of the analysis of *Nepeta glomerata* essential oil is present in Table-2.

TABLE-1
ANTIMICROBIAL ACTIVITIES OF *Nepeta glomerata* ESSENTIAL OILS

| Microorganisms | Inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Essential oil (µL/disc) | | | | Standard antibiotic discs (µL/disc) | | |
| | 1 | 2 | 4 | 6 | 1 | 2 | 3 |
| *Micrococcus luteus* | – | – | 12 | 15 | 33 | – | NT |
| *Listeria monocytogenes* | – | – | 10 | 15 | 38 | 18 | NT |
| *Bacillus cereus* | – | – | 9 | 13 | 12 | 16 | NT |
| *Enterococcus faecalis* | – | – | 12 | 16 | 16 | 17 | NT |
| *Pseudomonas aeruginosa* | – | – | 14 | 16 | 10 | 13 | NT |
| *Mycobacterium smegmatis* | – | – | 13 | 15 | 19 | 15 | NT |
| *Escherichia coli* | – | – | 9 | 11 | 11 | - | NT |
| *Aeromonas hydrophila* | – | – | 11 | 12 | 13 | 14 | NT |
| *Yersinia enterocolytica* | – | – | 10 | 12 | 13 | 17 | NT |
| *Bacillus brevis* | – | – | 12 | 15 | 14 | 16 | NT |
| *Corynebacterium xerosis* | – | – | 11 | 13 | 12 | 10 | NT |
| *Klebsiella oxytoca* | – | – | 10 | 12 | 15 | 14 | NT |
| *Rhodotorula rubra* | – | – | 10 | 14 | NT | NT | 14 |
| *Saccharomyces cerevisiae* | – | – | 9 | 11 | NT | NT | 18 |
| *Candida albicans* | – | – | 11 | 13 | NT | NT | 18 |

1: Ampicillin (10 µg/disc), 2: Streptomycin (10 µg/disc), 3: Nystatin (30 µg/disc), NT: Not tested. *Includes diameter of disc (6 mm), (–) indicates that no inhibition zone was determined.

As can clearly be seen from Table-1, in this study, undiluted essential oils of *Nepeta glomerata* have antibacterial and antifungal activity against all tested bacteria and fungi at 4-6 µL concentrations. But, 1-2 µL concentrations of essential oils of *Nepeta glomerata* have no antibacterial and antifungal activity against all tested bacteria and fungi.

Saxena and Mathela[16] reported that the antifungal activity of new compounds from *Nepeta leucophylla* and *Nepeta clarkei*. According to Sonboli *et al.*[8], the results of the antimicrobial activity bioassays of the essential oil of *Nepeta crispa* Willd. showed the interesting antimicrobial activity, in which the gram-positive, *Bacillus subtilis* and *Staphylococcus aureus*, were the most sensitive to the oil. Also, the oil exhibited a remarkable antifungal activity against all the tested fungi.

In another study, the *in vitro* activities of *Nepeta camphorate* oil and *Nepeta argolica* ssp. *dirphya* oil and the three isolated compounds of them, against 25 clinically isolated and commercial strains of *Helicobacter pylori* was investigated and antimicrobial activity was found[7]. Our findings concur results with those studies.

TABLE-2
CHEMICAL COMPOSITION OF *Nepeta glomerata* MONTBRET ET AUCHER EX BENTHAM

| No. | Compounds | RRI | Percentage |
|---|---|---|---|
| 1 | Heptane | 897 | 6.3 |
| 2 | Toluene | 910 | 0.4 |
| 3 | α-Pinene | 973 | 2.7 |
| 4 | Benzene 1-methyl-2 | 1035 | 0.5 |
| 5 | 1,8-Cineole | 1041 | 1.4 |
| 6 | γ-Terpinene | 1061 | 0.3 |
| 7 | Linalool | 1097 | 1.6 |
| 8 | *cis*-Verbeneol | 1136 | 0.2 |
| 9 | *trans*-Verbeneol | 1140 | 0.6 |
| 10 | Cyclohexen-1-ol | 1173 | 0.3 |
| 11 | *p*-Cymene-8-ol | 1180 | 0.9 |
| 12 | α-Terpineol | 1188 | 0.3 |
| 13 | Bicyclo[3.3.1]hept-3-en-2-one | 1230 | 0.1 |
| 14 | Carvacrol methyl ether | 1229 | 1.2 |
| 15 | Geraniol | 1241 | 0.7 |
| 16 | 2-Methyl phenol | 1282 | 1.5 |
| 17 | Carvacrol | 1293 | 16.4 |
| 18 | Carvacrol acetate | 1354 | 0.1 |
| 19 | (+)-Cyclosativene | 1357 | 0.2 |
| 20 | α-Cubebene | 1364 | 0.5 |
| 21 | β-Damascanone | 1367 | 0.6 |
| 22 | β-Bourbonene | 1371 | 0.9 |
| 23 | β-Elemene | 1378 | 0.7 |
| 24 | 1*H*-3a, 7-methanoazulene | 1402 | 0.2 |
| 25 | β-Caryophyllene | 1406 | 4.6 |
| 26 | (+)-Epi-bicyclosesquiphellandrene | 1416 | 0.5 |
| 27 | Thujopsene | 1420 | 0.2 |
| 28 | β-Sesquiphelladrene | 1427 | 0.1 |
| 29 | β-Farnesene | 1439 | 0.9 |
| 30 | γ-Cadinene | 1459 | 0.2 |
| 31 | γ-Curcumene | 1462 | 2.9 |
| 32 | Germacrene D | 1466 | 14.2 |
| 33 | 1,3-Cyclohexadiene | 1478 | 3.8 |
| 34 | α-Muurolene | 1481 | 0.2 |
| 35 | α-Farnesene | 1487 | 0.3 |
| 36 | 1-Methyl-4-cyclohexene | 1490 | 0.4 |
| 37 | β-Curcumene | 1491 | 1.1 |
| 38 | Naphthalene | 1499 | 1.3 |
| 39 | *cis*-Calamenene | 1509 | 0.3 |
| 40 | 3-Hexen-1-ol | 1547 | 0.8 |
| 41 | 1,4-Methano-1*H*-indiene | 1580 | 1.3 |
| 42 | δ-Cadinene | 1609 | 2.0 |
| 43 | α-Cadinol | 1621 | 2.0 |
| 44 | α-Bisabolol | 1650 | 10.5 |
| 45 | Benzyl benzoate | 1713 | 0.1 |
| 46 | Nonadecane | 1821 | 0.1 |
| 47 | Hexadecanoic acid | 1867 | 0.2 |
| 48 | Manolyloxide | 1903 | 1.6 |
| 49 | Kaur-16-ene | 1925 | 2.1 |
| 50 | Phytol | 1972 | 2.3 |
| | Total | | 92.6 |

A number of factors hamper the evaluation of the antimicrobial activity of essential oils, namely, their volatility at room temperature, their water insolubility and their complexity[17].

In case of *Nepeta glomerata*, 50 compounds were identified representing 92.6 % of the oils. Carvacrol was determined to be present at high percentage (16.4 %). The presence of germacrene D (14.2 %), α-bisabolol (10.5 %), heptane (6.3 %), β-caryophyllene (4.6 %), 1,3-cyclohexadiene (3.8 %), γ-curcumene (2.9 %), phytol (2.3 %), kaur-16-ene (2.1 %) are also important for the oil profile.

Although the presence of nepetalactones in several *Nepeta* species in relatively high concentrations has been reported[7,8], no nepetalactones were found in *Nepeta macrosiphon* Boiss oil[5]. Antibacterial, fungicidal, antiviral and opioid analgesic activities have been attributed to nepetalactones[18]. The predominance of spathulenol and caryophyllene oxide has been found in essential oils of two Turkish Nepeta species[4,9]. These compounds and germacrene D are typical in most Nepeta species[5]. Barazandeh[6], reported that GC and GC/MS analyses of *Nepeta menthoides* Boiss. et Bushe oil revealed presence of 18 compounds, among which 1,8-cineole (57.3%), β-pinene (8.8 %) and geranyl acetate (8.1 %) were the major constituents[6]. It is not surprising that there are differences in the composition of *Nepeta glomerata*, due to phytochemical properties.

## Conclusion

For the evaluation of plants that grow naturally in Turkey and are potential useful resources, additional studies will be beneficial from medicinal and economic standpoints.

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