Original Article

Chemical Composition and Antimicrobial Activities of Essential Oil of *Nepeta Cataria* L. Against Common Causes of Oral Infections

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Abstract

Objectives: Over the past two decades, there has been a growing trend in using oral hygienic products from natural resources such as essential oils and plant extracts. *Nepeta cataria* L. is a member of the mint family (Labiatae) with several medicinal properties. The objective of this study was to determine the chemical composition and antimicrobial activities of essential oils (EOs) from *N. cataria* leaves against pathogens causing oral infections.

Materials and Methods: The chemical composition of EOs from *N. cataria* was analyzed by gas chromatography/mass spectrometry (GC/MS). The antimicrobial activity of the essential oil was evaluated by broth micro-dilution in 96 well plates as recommended by the Clinical and Laboratory Standards Institute (CLSI) methods. The plates were incubated at 30°C for 24-48 h (fungi) or at 37°C for 24 h (bacteria).

Results: The analysis of the EOs indicated that 4a-α, 7-α, 7a-β-nepetalactone (55-58%), and 4a-α, 7-β, 7a-α-nepetalactone (30-31.2%) were the major compounds of the EOs at all developmental stages. The tested EOs exhibited antimicrobial activities against the tested bacteria at concentrations of 0.125-4 µL/mL. Moreover, the oils entirely inhibited the growth of *Candida* species at a concentration less than 1 µL/mL.

Conclusion: Based on these results, the EO of *N. cataria* can possibly be used as an antimicrobial agent in the treatment and control of oral pathogens.

Key Words: Nepeta Cataria; Volatile Oil; Anti-infective Agents; Mouth; Candida; Staphylococcus; Enterococcus; Streptococcus

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INTRODUCTION

For thousands of years, aromatic plants have been used for flavoring and medicinal properties [1,2]. These plants represent a renewable source of flavoring substances and are commonly used in food, cosmetics, and

pharmaceutical products [3,4]. Many of these plants and their aromatic products have potential antimicrobial activities [5-7]. In the recent decades, there is a great tendency towards using natural products and phytochemicals in medicine and industry to overcome antibiotic resistance and to reduce the toxicity of the synthetic drugs [7]. The previous studies demonstrated the successful usage of essential oil (EO)-based mouthwashes in preventing and controlling the formation of plaque and gingivitis as well as reducing bad breath and odor-causing bacteria [8]. In addition, many of these EOs have been used effectively in *in-vitro* and *in vivo* studies in the treatment of the causative agents of oral infections [8].

The family *Nepeta* (Lamiaceae) with the common Persian name "puneh" includes a large number of volatile oil plants that are wildly distributed in Europe, Asia, North America, and the mountains of tropical Africa [9]. About 67 species of this family are grown endemically in Iran. *Nepeta cataria* (Catnip), a tropical aromatic plant belonging to this family, is native to Asia and Southeast Europe. Its leaves resemble mint in appearance and the flowers are white and finely spotted with purple with a strong odor [10]. In Iran and some other countries, fresh or dried leaves and flowers of *N. cataria* are used in making sauce, soup and cheese [11].

In traditional medicine, this plant has been used for antispasmodic, carminative, stimulant and tonic properties [11-14]. Moreover, traditionally, the tea made of its leaves is known as sedative and soporific, also used to relieve gastrointestinal and respiratory disorders such as colic, diarrhea, cough, asthma and bronchitis [11,12,14]. It has been shown that many medical properties of *Nepeta* species are the characteristic of its essential oil (EO) and flavonoids. The EO of *N. cataria* is rich in nepetalactones [15-20] and has been reported to have antimicrobial [15,17,19], insecticidal [21,22] and antioxidant activities [15]. To the best of our knowledge, only a few published reports have been concerned with the antimicrobial effects of the *N. cataria* EOs, especially against oral pathogens. In the present study, the chemical constituents of three different phenological stages (vegetative, floral budding and full flowering) of *N. cataria* were determined and the antimicrobial effects of these EOs were evaluated against common causes of oral infections.

MATHERIALS AND METHODS

Plant material:

This study was carried out in the research field station of the Faculty of Agriculture, Shiraz University, Iran. The station is located 1810 m above the mean sea level, with the latitude of 29º 36´ north and altitude of 52º 32´ east. The minimum and maximum temperatures of the field in the recent ten years were -10ºC and 38ºC, respectively. The daily climatic data during this study were obtained from the agro-meteorological station of Irrigation Department located in a state farm about 500 m far from the experimental site. Catnip seeds (obtained from Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Iran) were sown in January 1010 in a sandy-loam textured soil with pH=7.5, EC=1.8 dS m^{-1}, 0.97% organic matter, 0.094% N, 24 ppm P, 250 ppm K, 4.5 ppm Fe, 0.42 ppm Zn, 20 ppm Mn and 0.94 ppm Cu. The plant samples were harvested at vegetative, floral budding and full flowering stages. The plant species was identified and authenticated by A.R. Khosravi, a plant taxonomist at Shiraz University Herbarium Shiraz, Iran. Voucher specimen (no. 24995) has been deposited in the herbarium.

Essential oil extraction:

The aerial parts of the plants were harvested at vegetative, floral budding and full flowering stages, and then air dried. The samples (30 g, three replicates for each stage) were hydro-distillated for 3 hours using an all

glass Clevenger-type apparatus to extract EOs according to the method recommended by the European Pharmacopoeia [23]. The extracted EO samples were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature (4°C) before gas chromatography (GC) and gas chromatography/mass spectrometric (GC/MS) analysis.

GC and GC/MS analysis:

The analysis of EOs was carried out using a Thermoquest- Finnigan Trace GC-MS instrument equipped with a DB-5 fused silica capillary column (60m×0.25mm i.d., film thickness 0.25 mm). The oven temperature was programmed to increase from 60 to 250ºC at a rate of 4ºC min $^{-1}$ and finally held for 10 min. The transfer line temperature was 250ºC. Helium was used as the carrier gas at a flow rate of 1.1mL min^{-1} with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 35-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 mA.

Chromatography Flame Ionisation Detector (GC/FID) analyses:

The GC/FID analysis of the oils was conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60m×0.25mm i.d., film thickness 0.25 mm). Nitrogen was used as the carrier gas at the constant flow rate of 1.1mLmin^{-1}; the split ratio was the same as that used for GC/MS. The oven temperature was raised from 60 to 250ºC at a rate of 4ºC min^{-1} and held for 10 min. The injector and detector (FID) temperatures were kept at 250ºC and 280ºC, respectively. Semi-quantitative data were obtained from FID area percentages without the use of correction factors.

Identification of essential oil components:

Retention indices (RI) were calculated using retention times of n-alkanes (C6-C24) injected after the oil at similar temperature and conditions. The compounds were identified by comparison of their RI with those reported in the literature, and their mass spectrum was compared with the Wiley Library [24].

Determination of antimicrobial activities

Microorganisms

The antimicrobial activities of the EOs against some oral pathogens including twelve standard species of *Streptococcus mutans* (ATCC 35668), *S. sanguis* (ATCC 10556), *S. salivarius* (ATCC 9222), *S. sobrinus* (ATCC 27607), *Enterococcus faecalis* (ATCC11700), *Staphylococcus aureus* (ATCC 29213 and ATCC 700698), *Candida albicans* (ATCC 10261), *C. dubliniensis* (CBS 8501), *C. tropicalis* (ATCC 750), *C. krusei* (ATCC 6258) and *C. glabrata* (ATCC 90030), and clinical isolates of *S. aureus* and *S. mutans* were determined in this study.

Determination of minimum inhibitory concentration (MIC)

MICs were determined using broth microdilution method recommended by the CLSI with some modifications [25,26]. Briefly, for determination of antifungal activities against yeasts and filamentous fungi, serial dilutions of the EOs (0.031 to 16.0 µl/ml) were prepared in 96-well microtitre plates using RPMI-1640 media (Sigma, St. Louis, USA) buffered with MOPS (Sigma, St. Louis, USA). To determine the antibacterial activities, serial dilutions of the EOs (0.125 to 128.0 µl/ml) were prepared in Muller-Hinton Broth media (Merck, Darmstadt, Germany). Test fungi or bacteria strains were suspended in the media and the cell densities were adjusted to 0.5 McFarland standards at 530 nm wavelength using a spectrophotometric method (this yields stock suspension of 1-5 × 10^6 cells/ml for yeast and 1-1.5 × 10^8 cells/ml for bacteria).

0.1 ml of the working inoculums was added to the micotiter plates and the plates (treated wells and untreated controls) were incubated in a humid atmosphere at 30°C for 24-48 h (fungi) or at 37°C for 24 h (bacteria). 200 µl of the uninoculated medium was included as a sterility control (blank). In addition, growth controls (medium with inoculums, but without essential oil) were also included. The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of the essential oil producing no visible growth. Each experiment was performed in triplicate. In addition, media from wells with fungi showing no visible growth were further cultured on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) and from wells with bacteria showing no visible growth on Muller-Hinton agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). MBCs and MFCs were determined as the lowest concentration yielding no more than 4 colonies that corresponds to a mortality of 99.9% of the microbes in the initial inoculums.

RESULTS

The hydro-distillation of 30 g of the aerial parts of *N. cataria* at the vegetative, floral budding, and full flowering stages yielded 0.3, 0.5 and 0.9%(w/w) essential oil, respectively.

The composition of EOs at different growth stages is shown in Table 1 in the order of their elution from a DB-5 column.

A total of 13, 12 and 14 compounds representing 93.6, 99.6 and 99% of the total was detected at vegetative, floral budding and full flowering stages, respectively. The nepetalactones including 4a-α, 7-α, 7a-β-nepetalactone (55-58%) and 4a-α, 7-β, 7a-α-nepetalactone (30-31.2%) were the major oil constituents of all growth stages.

Table 1. EO Composition (%) of Catnip (*N. cataria*) at Three Stages of Harvest

Component	RI*	Vegetative (%)	Floral budding (%)	Full flowering (%)
α-Pinene	936	2.7	3.1	4.6
Sabinene	957	0.0	0.0	0.15
β-Pinene	978	0.8	1.13	1.64
1-Cyclohexen-1-yl-methyl ketone	980	0.5	0.6	0.7
Triplal	1023	0.1	0.4	0.4
Thymol	1294	0.4	0.4	0.57
4a-α,7-α,7a-β-Nepetalactone	1332	55	58	55.03
4a-α,7-β,7a-α-Nepetalactone	1342	30.06	31.1	31.2
trans Caryophyllene	1430	1.1	2.7	2.1
α-Humulene	1446	0.82	0.92	0.87
11-Dodecenol	1500	0.84	0.69	1.1
Spathulenol	1580	0.6	0.4	0.3
Caryophyllene oxide	1569	0.5	0.2	0.12
6,10-dimethyl-2-undecane	1907	0.2	0.0	0.25

* Retention Index

The highest amount of components such as α-pinene (4.6%), β-pinene (1.64%), and 4a-α,7-β,7a-α-nepetalactone (31.2%) was detected at full flowering stage. The antibacterial activities of the EOs of *N. cataria* against the common causes of oral infections are shown in Table 2. The EOs inhibited the growth of the examined bacteria at concentrations of 0.125-2 µL/mL. Furthermore, the EOs exhibited the bactericidal activity (MBC) for all of the above-mentioned gram-positive bacteria at concentrations ranging from 0.5 to 32 µL/mL. For the clinical and standard yeasts tested, the MICs for the EOs were 0.125-0.5 µL/mL. All the tested *Candida* spp. were killed by the EOs at the same or twice concentration of their corresponding MICs.

DISCUSSION

It has been reported that EOs are capable of inhibiting the growth of microorganisms as well as the formation of biofilms [5-7,17]. In various cases, the potency of chlorohexidine was found to be even lower than that of the EOs [27]. On the other hand, the composition of the EOs might be affected by the developmental stages and geographical region of the plant [5-7,28]. Similar to the previous reports [15-20], we identified nepetalactone isomers as the major constituent of Catnip EOs in all stages of growth that reached its maximum level at the floral budding stage. However, some studies have detected no nepetalactones in their examined EOs and reported 1,8-cineol [29] and alpha-citral [30] as the most abundant compounds of the catnip oil.

The pinene (α and β) was detected as the third main component of the EO in the present study, increasing gradually following the maturation of the plant. As expected from the results of GC/MS analysis, no significant differences in MICs were found between the EOs distilled from different growth stages.

Oral pathogens accumulated on the mucosal and dental surfaces of the oral cavity were composed of native oral flora. About twenty-five species of *streptococci* live in the oral cavity. Some of these oral *streptococci* such as *Streptococcus mutans* and *S. sobrinus* are associated with tooth decay [31,32], while others such as *S. sanguinis* and *S. salivarius* are harmless and considered as normal inhabitant sof the oral cavity. It has also been shown that the extract of *N. cateria* has an inhibitory activity on growth, enzyme production and adhesion of some bacteria [15,33]. Similar to the previous report,[15] growth of the standard and clinical isolates of the studied *streptococci* was inhibited by EOs at concentrations of 1 to 4 µL/mL, respectively.

Staphylococcus aureus is one of the causes of oral infections, often causing angular cheilitis [34], parotitis [35] and staphylococcal mucositis [35]. It can be isolated from the oral cavity of particular groups such as children [36] and the elderly [37]. The major concern about this species is the fast development of methicillin resistance. The MICs of *N. cataria* EOs against methicillin sensitive *S. aureus* and methicillin resistant *S. aureus* in this study were much lower than those reported by Zenasi *et al.*, who used the MTT method [30]. In contrast to the study conducted by Adiguzel et al. [15], the EOs successfully inhibited the growth of *E. faecalis* recognized as the commonly isolated bacteria from endodontic infections [38,39].

Candida spp. are one of the other residents of the oral cavity associated with oral candidiasis and biofilm formation [40]. Similar to the previous study [15], the EOs exhibited fungicidal activities against the standard species of *Candida* at concentrations ranging from 0.125-1 µL/mL.

Since the EOs exhibited a similar antimicrobial effect against the tested antibiotic-resistant and antibiotic-susceptible strains,

Table 2. Antimicrobial Activity (MIC and MBC) of Essential Oils Distilled from *N. Cataria*'s Stages Against Oral Pathogens

	Bacteria (Number of Strains)	Stage 1		Stage 2		Stage 3	
		MIC* (µl/ml) GM# (range)	MMC** (µl/ml) GM# (range)	MIC* (µl/ml) GM# (range)	MMC** (µl/ml) GM# (range)	MIC* (µl/ml) GM# (range)	MMC** (µl/ml) GM# (range)
Bacteria	***S. mutans* (5)**	2 (1-4)	3.48 (2-8)	1.32 (0.5-4)	2.64 (1-8)	1.15 (1-2)	3.48 (2-8)
	Methicillin resistant *S. aureus* (6)	0.22 (0.125-0.5)	1.12 (1-2)	0.17 (0.125-0.5)	1.41 (1-2)	0.28 (0.25-0.5)	1.41 (1-2)
	Methicillin sensitive *S. aureus* (6)	0.19 (0.125-0.5)	0.89 (0.5-2)	0.15 (0.125-0.25)	1.26 (1-2)	0.22 (0.125-0.5)	1.41 (1-4)
	***S. sanguis* ATCC10556**	1	2	1	2	1	2
	***S. salvarius* ATCC 9222**	1	2	1	2	1	2
	***S. sabrinus* ATCC 27607**	1	2	1	2	1	2
	***E. feacalis* ATCC 11700**	2	32	2	32	1	16
Fungi	***C.albicans* ATCC 10261**	0.125	0.125	0.125	0.25	0.125	0.125
	***C. dubliniensis* CBS 8501**	0.5	1	0.25	0.5	0.25	0.5
	***C. tropicalis* ATCC 750**	0.125	0.5	0.125	0.25	0.125	0.25
	***C. glabrata* ATCC 90030**	0.25	0.5	0.25	0.5	0.25	0.5
	***C. krusei* ATCC 6258**	0.5	1	0.25	0.25	0.25	0.25

* Minimum Inhibitory Concentration, ** Minimum Microbicidal Concentration, # Geometric mean

it could be assumed that the mechanism of action of the EOs is different from the above mentioned antibacterial and antifungal drugs. As nepetalactone isomers were detected as major compounds of catnip EO, the good antimicrobial properties of the EO found in this study and the other reports [15,17,19] might be the characteristic of nepetalactones that are bicyclic terpenoid and account for over 85% of essential oil at different growth stages. The similar antibacterial and antifungal activities were also reported from other *Nepeta* species containing nepetalactones as the main component such as *Nepeta persica* and *Nepeta crispa* [41,42]. One of the main characteristics of EOs, which enables their incorporation into the cell membrane, is their hydrophobicity [28]. It has also been reported that some EOs such as *H. italicum* EO contain substances which act as efflux pump inhibitors. These EOs are supposed to be active against the resistant microorganisms through inhibition of over-expression of efflux pumps [43]. These results support the idea of using EOs as an alternative to well-established drugs since they show high efficacy in inhibiting drug-resistant bacterium strains. In addition, the EOs could be used on their own, as well as in combination with synthetic active agents since synergy was observed by combining these substances.

CONCLUSION

Since there is a great demand to reduce the use of chemical preservatives in the oral hygienic products, EOs of *N. cataria* with active antimicrobial properties might be considered as a candidate for use in antimicrobial mouth rinses.

In addition, detectable taste and odor of EOs is an additional advantage to its antimicrobial activities. However, further studies, especially in animal models, are still required to determine the in-vivo antimicrobial activity of the EO and its ingredients.

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