J. Ecol. Field Biol. 29 (4): 381~387, 2006

The Composition of Essential Oil from *Nepeta cataria* and Its Effect on Microorganism

Kim, Jong Hee*, Dae Ho Jung and Hyun Kyung Park
Department of Biological Sciences, Kyungnam University, Masan 631-701, Korea

ABSTRACT: We analyzed the total yields and composition of essential oils in leaf extracts of *Nepeta cataria* by Gas Chromatography Mass Spectrometry (GC-MS). Thirty-six compounds representing 97.0% of total oil were detected. The major constituents of essential oils in *Nepeta cataria* were nepetalactone (90.9%), unidentified compound (Retention time 17.35; 1.82%), 1,8-cineol (1.49%), β-caryophyllene (1.12%), and β-pinene (1.078%). The volatile compounds in leaf extracts of *N. cataria* concentrated to nepetalactone (88.83~93.33%) remarkably. In the essential oil of *N. cataria cis,trans*-nepetalactone (30.2~37.8%) and *cis,cis*-nepetalactone (31.5~37.0%) were found as the main constituents. The effects of essential oil of *N. cataria* on the growth of six microorganisms (*Bacillus cereus*, *B. subtilis*, *B. amyloliquefaciens, Escherichia coli*, *Staphylococcus aureus* subsp. *aureus,* and *Pseudomonas aeruginosa*) were investigated. The essential oil of *N. cataria* had strong inhibitory effect on the growth of three fungal species (*Bacillus cereus*, *B. subtilis*, and *B. amyloliquefaciens*). The essential oil from *N. cataria* was found to have a low antimicrobial activity against *Staphylococcus aureus* subsp. *aureus,* while no activity were found against *Escherichia coli* and *Pseudomonas aeruginosa*. Results indicate the significant antimicrobial effect, which may be depended on the yield of nepetalactone.

Key words: Essential oil, GC-MS, Inhibitory effect, *Nepeta cataria*, Nepetalactone, Retention time

INTRODUCTION

Nepeta cataria is a perennial herb belonging to Lamiaceae family. It consists of about 300 species distributed in southwest Asia and Europe (Sonboli et al. 2005). The antispasmodic, carminative, sedative, stimulant and tonic properties of this species seem to account for its use in folk medicine; it is noteworthy plants. It is also a repellent and dried leaves and flowering tops are used to prepare a sedative 'tea'. The properties of these essential oils are mainly monoterpenoids and sesquiterpenoids, which both characterize aroma and belong to a structurally diverse group of natural products known as isoprenes. Mixtures of volatile essential oils lend a characteristic odour to plant foliage. Plant terpenes have been widely used in taxonomic (Skoula et al. 1999, Gross et al. 2002), phylogenetic (Thompson et al. 2003), microbial (Cosentino et al. 1999, Vokou et al. 2002) and ecological (Zygadlo et al. 1996, Palá-Paúl et al. 2001) studies. Large variations in the concentrations of constituent compounds were found due to plant parts, season, location, and individual differences (Perry et al. 1999, Ahn et al. 2003). The yield and composition of essential oil will be affected by crop maturity at harvest, environmental conditions and distillation practice.

Plants of the genus *Nepeta* (catmints) are also members of the produce an essential oil, which is a minor item of commerce. This oil is a very rich monoterpenoid compound known as iridoids (Inouye 1991), more specifically the cyclopetanoid monoterpenoid, nepetalactones (Clark et al. 1997) and derivatives. Several researchers have reported essential oil composition of *N. cataria*. Regnier et al. (1967) found the main constituents of *N. cataria* were camphor (0.8 %), caryophyllene (2.8%), humulene (0.3%), nepetalactone (77.6%), epinepetalactone (15.0%), and dihydronepetalactone (0.3 %). Tittel et al. (1982) determined that the essential oil of catmint (*N. cataria* var. *citriodora*) consists of citronellol (15.6%), elemol (11.9%), geraniol (9.5%), β-elemene (7.5%), β-caryophyllene oxide (4.5%), α-cadinol (5.0%), and nerol (3.7%). Baranauskiene et al. (2003) reported that the percentages of essential oil of *N. cataria* were geranyl acetate (54.8%), citronellyl acetate (13.4%), citronellol (6.9%), and geraniol (5.5%), germacrene D (1.9%), caryophyllen oxide (1.8%), and spathulenol (1.1%). However, many publications (Regnier 1967, Bourrel et al. 1993, Handjieva et al. 1996) on catmint volatile oil demonstrated that the main consistent are nepetalactone or nepetalactone derivatives as the dominating oil compounds.

According to the essential oil composition, *Nepeta* species can be divided into two groups nepetalactone-containing and nepetalactone-less. Nepetalactone-containing species contain 4aα-7α-7aα-nepetalactone and 4aα-7α-7aβ-nepetalactone as the main nepetalactone isomers. Nepetalactone-less species contain caryophyl-

* Corresponding author; Phone: +82-55-249-2242, e-mail: biokim@kyungnam.ac.kr

lene oxide or 1,8-cineole/linalool as the main constituent in their essential oils (Baser et al. 2000). Baranauskiene et al. (2003) noted that there are two main chemotypes of catmint: one with nepetalactones as the dominating oil compounds and another with citral derivatives as the major components. Different *Nepeta* species had previously been shown to produce several nepetalactone stereoisomers, *N. cataria* containing *trans,cis*-isomer with some *cis,cis*-isomer, *N. racemosa* containing *cis,cis*-isomer (Clark et al. 1997), *N. elliptica* and *N. nuda* containg the *trans,trans*-isomer (Bottini et al. 1987, De Pooter et al. 1987). Since there are different ways of naming nepetalactones and the corresponding lactols, clarifying schemes, using the IUPAC nomenclature, are shown in Fig. 1. Therefore, it has been proposed that the study of essential oil profile of catmint cultivated in Korea is interested.

Iridoid monoterpenoids have long been known to be effective repellents to the pet species (Dawson et al. 1990). It is widely known that catmint is a potent behavior altering drug and/or hallucinogen in domestic cats and other wild animals (Hallahan et al. 1998). Many researches (Cruz et al. 1993, Hilli et al. 1997, Maoz and Neeman 1998, Hammer et al. 1999, Burt and Reinders 2003) reported that essential oils isolated from plants have antimicrobial activity, although the mechanism of action of essential oils or their components is unclear. Recently, the effect of essential oils from *Nepeta* species on microorganisms has been studied. Metabolites of *N. leucophulla* and *N. clarkei* have been screened for antifungal activities (*Aspergillus flavus, A. ochraceus, Penicillium citrinum,* and *P. viridicatum*) (Saxena and Mathela 1996). The major constituents of *N. cataria* appear to be the nepetalactones, cyclopentanoid monoterpenes with antimicrobial activities (Bourrel et al. 1993, Nostro et al. 2001). Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is very important. The aim of this study was to investigate the composition of essential oil from *N. cataria* grown in Korea and its antimicrobial activity.

MATERIALS AND METHODS

Plant Extract Preparation

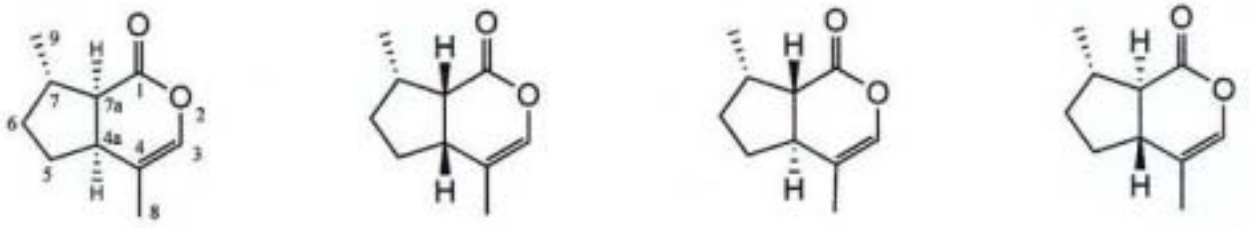
Nepeta cataria leaves were collected from three sites, not environmentally different, at abandoned area of Mt. Muhak (in 35° 11' 15" N and 128° 32' 30" E) during their maturing periods (May and June) in 2005. Sampling was carried out on five plants in a similar development stage at each site. Samples for young leaves, old leaves, and petals were harvested at the mature flowering stage of development. Young leaves (approximately less than 5 cm long) obtained from top of the individual plant. Samples were sealed in plastic bags and transported to the laboratory. All materials were removed from each plant, and immediately three-gram samples were ground with pure sand and extracted with n-pentane and 1 mL of 1% tetradecane as an internal standard. Plant extracts were filtered with sodium sulphate and concentrated by evaporation with a gentle stream of nitrogen gas (Kim and Langenheim 1994).

Samples were analyzed by gas chromatography-mass spectrometry (GC-MS, Hewlett Packard 5890) using a 30 m long HP5 (id. 0.25 mm, a flame ionization detector) capillary column. Helium was used as the carrier gas. The temperature program for terpene was initially 37℃ for five minutes, increased to 180℃ at a rate of 5℃ min^{-1}, then by 20℃ per minute until a final temperature of 320℃ was reached. One μL of the resulting extract was used for GC-MS analysis.

The individual terpenes were identified by comparison with the spectral data of the internal spectral library of the instrument (Wiley library) and retention times, based on references. The concentrations of peaks at selected retention times were estimated from peak area using the internal standard curve of tetradecane.

Organisms and Media

The essential oil used for the antimicrobial activity was obtained commercially (Botanix). The main component of essential oil pur-



1
4a*S*,7*S*,7a*R*
cis-trans
4aα,7α,7aα

2
4a*R*,7*S*,7a*S*
cis-cis
4aβ,7α,7aβ

3
4a*S*,7*S*,7a*S*
trans-cis
4aα,7α,7aβ

4
4a*R*,7*S*,7a*R*
trans-trans
4aβ,7α,7aα

Fig. 1. All nepetalactone isomers (Referred from Liblikas et al. 2005).

chased from Botanix is nepetalactone. The test organisms used in this study were as follows: 3711 *Bacillus cereus* Frankland and Frankland 1887, 3729 *B. subtilis*, 3002 *B. amyloliquefaciens*, 1116 *Escherichia coli,* 1927 *Staphylococcus aureus* subsp. *aures*, and 2695 *Pseudomonas aeruginosa*. The strains were maintained and tested on nutrient agar (beef extract 3.0 g, peptone 5 g, agar 15 g, and distilled water 1L). For the antimicrobial tests, cells were grown overnight in nutrient agar medium. Strains of *E. coli*, *S. aureus*, and *P. aeruginosa* were grown for 24 h on chamber at 37℃. 3711 *Bacillus cereus* Frankland and Frankland 1887 and 3729 *B. subtilis* and *B. amyloliquefaciens* cultivated on agar for 24 h at 30℃ incubator. All strains were purchased from KCCM (Seoul, Korea).

For antimicrobial testing (Disc diffusion test), a 20% of dimethylsulfoxide (DMSO) was prepared as stock solution as followed by Nostro et al. (2000). Overnight cultures, adjusted to yield approximately 1.0×10^8 cells mL^{-1} for bacteria and 1.0×10^7 cells mL^{-1} for fungi, were streaked with a calibrated loop on plates containing appropriate solid medium. Filter paper discs (8 mm diameter) were placed on the inoculated agar surfaces in the center of each plate and impregnated with 20 and 30 μL of essential oil in DMSO stock solutions (1:1). Pure DMSO (20 μL) was used as a negative control. The plates were observed after 24 and 48 h at 37℃ for bacteria and at 30℃ for fungi, respectively. All tests were performed at least in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the essential oil. To compare the inhibitory effect on control, *t*-testing was performed.

RESULTS AND DISCUSSION

Thirty-six compounds representing 97.0% of total oil were detected from leaf extracts of *N. cataria* (Table 1). Of these, 29 were identified as monoterpenoids and 7 components as sesquiterpenoids. Nepetalactone (90.9%) was present in the greatest amount, followed by with the relative yields of monoterpenoids being very high. The total amounts of essential oils composed by monoterpenoids and sesquiterpenoids in the leaf of *N. cataria* were 0.5～2.3 mg/g leaf fresh weight (data not shown). The total amounts of monoterpenoids were 2.5 to 5.3 times greater than those of sesquiterpenoid, and highly correlated with the level of nepetalactone. In addition to nepetalactone, unidentified compound (Retention time 17.35; 1.82 %), 1,8-cineol (1.49%), β-caryophyllene (1.12%), β-pinene (1.078 %), benzenepropanoic acid, α-humulene, germacrene-D, and unidentified compound (R.T. 12.41 and R.T. 25.15) were detected. According to the essential oil composition, *Nepeta* species can be divided into two groups nepetalactone-containing and nepetalactone-less as

Table 1. Percentage composition of major essential oils in leaf extracts of *Nepeta catria*. Values in bold type represent constituents of > 1.00%

	Retention time	Compound	Percentage (%)
1	5.35	2-Hexenal	0.057
2	5.50	3-Hexen-1-ol	0.013
3	6.03	1-Octen-3-ol	0.008
4	6.70	Styrene	0.130
5	7.59	U.I.	0.005
6	8.15	α-Thujene	0.031
7	8.34	δ - 3-Carene	0.127
8	8.45	U.I.	0.033
9	9.86	β-Pinene	1.077
10	10.26	1-Octen-3-ol	0.023
11	10.37	U.I.	0.030
12	10.48	3-Octanone	0.050
13	10.65	β-Myrcene	0.047
14	10.90	β-Pinene	0.027
15	11.97	1,8-Cineole	1.487
16	12.30	1,3,6-Octatriene	0.140
17	12.41	U.I.	0.373
18	12.85	γ-Terpinene	0.037
19	13.07	*trans*-Sabinene	0.020
20	13.87	U.I.	0.037
21	14.28	L-Linalool	0.157
22	15.43	Cyclopenetane	0.083
23	16.20	δ-Terpineol	0.030
24	16.51	3-Cyclohexen-1-ol	0.063
25	16.95	Naphtalene	0.143
26	17.35	U.I.	1.820
27	18.42	Citral	0.107
28	19.41	Benzenepropanoic acid	0.650
29	21.56	Nepetalactone	90.900
	22.40	Tetradecane (Standard)	0.423
30	23.09	β-Caryophyllene	1.120
31	23.29	Undeca-5,7-dien-1-ol	0.123
32	23.93	α-Humulene	0.223
33	23.98	Isopulegol	0.053
34	24.15	*trans*- β-Farnesene	0.079
35	24.63	Germacrene-D	0.137
36	25.25	U.I.	0.572

U.I. : unidentified compound.

the first main compound (Baser et al. 2000). Tittel et al. (1982), Osinska and Suchorska (1990), and Baranauskiene et al. (2003) described the main constituent of essential oil of catmint as geranyl acetate (54.8%). Baser et al. (2000) reported that the essential oil of seven species (*N. betonicifolia, N. cilicia* Boiss., *N. fissa, N. nuda, N. concolor, N. conferta Hedge,* and *N. isaurica* Boiss.) contain caryophyllene oxide as the main constituent. Many researchers (Regnier 1967, Bourrel et al. 1993, Handjieva et al. 1996) reported the main constituent of essential oils of *N. cataria* as nepetalactone. Our results suggested that nepetalactone is the major constituent (88.83～93.33%) of catmint essential oils as was previously reported by Regnier et. al. (1967), although their percentages were quite different as compared with published results. It seems to be considered that large variations in the concentrations of constituent compounds were found due to plant parts, season, location, and individual differences. The yield and composition of essential oil will be affected by crop maturity at harvest, environmental conditions and distillation practice (Perry et al. 1999, Ahn et al. 2003).

Fig. 2 shows the total ion chromatograms from combined gas chromatogram spectrometry analysis of an extracted nepetalactone enriched fraction from available catmint oil. Our result was observed that the principal nepetalactone stereoisomer accumulated are *cis,trans*-nepetalactone (30.2～37.8%) and *cis,cis*-nepetalactone (31.5～37.0%) in *N. cataria*. However, Regnier et al. (1967) reported that the principal isomer present in *N. cataria* is *cis,trans*-nepetalactone with some *cis,cis*-nepetalactone. Other stereoisomers (*cis, cis*-nepetalactone) is present in trace amounts in this species. Nepetalactone-containing species is characterized by nepetalactone isomers (Sonboli et al. 2005), 4a α ,7 α ,7a α -nepetalactone is the most frequently encountered nepetalactone in *Nepeta* species (*N. caesarea* Boiss., *N. cataria*, and *N. cadmea* Boiss.) and 4a α ,7 α , 7a β -nepetalactone as the main constituent in one species (*N. racemosa*) (Baser et al. 2000). It seems to be considered that the stereochemically differences of nepetalactone occurred from biosynthesis pathway of precursors, although they have a same chemotypes.

All monoterpenes are derived from the ubiquitous precursor geranyl pyrophosphate (GPP), produced by the isoprenoid pathway (McGarvey and Croteau, 1995). Perphaps the best known example of iridoids are the nepetalactones, present in the essential oil of catmints (*Nepeta* spp.). Different *Nepeta* species had previously been shown to produce several nepetalactone stereoisomers, *N. racemosa* containing predominantly the *cis,cis*-isomer((4a*R*, 7*S*, 7a*S*)-nepalactone) (Regnier et al. 1967), *N. elliptica* and *N. nuda* containing the *trans,trans*-isomer (Bottini et al. 1987, De pooter et al. 1987) (4a*R*, 7*S*, 7a*R*). It would appear from these analyses that distinct chemotypes of catmint plants by a single nepetalatone stereoisomer constitutes (Baranauskiene et al. 2003). However, the oil of *N. cataria* from Bulgarian origins was constituted mainly of 4a β ,7 α , 7a α -nepetalactone and 4a α ,7 α ,7a β -nepetalactone, the oil of catmint grown in France consisted mainly of geraniol and nerol, and the main constituents of *N. cataria* grown in Cordoba were nepetalactone. Early studies of nepetalactone biosynthesis in *N. cataria* indicated (3*S*)-citronellol was a more efficient precursor (Bellesia et al. 1984). However, further studies on the biosynthesis of *cis,trans*-nepetalactone, the principal isomer present in *N. cataria*, established its origin from mevalonate, the biosynthesis of methylpentanoids in other plant species was shown to be derived from mevalonate via geraniol or isomer nerol (Inouye 1991). Recently, because glandular trichomes have been shown to be the site of accumulation of nepetalactonee in *N. cataria*, the presence of nepetalactol oxidoreductase activity within the trichomes suggests that nepetalactol may be an intermediate in the biosynthesis of nepe-

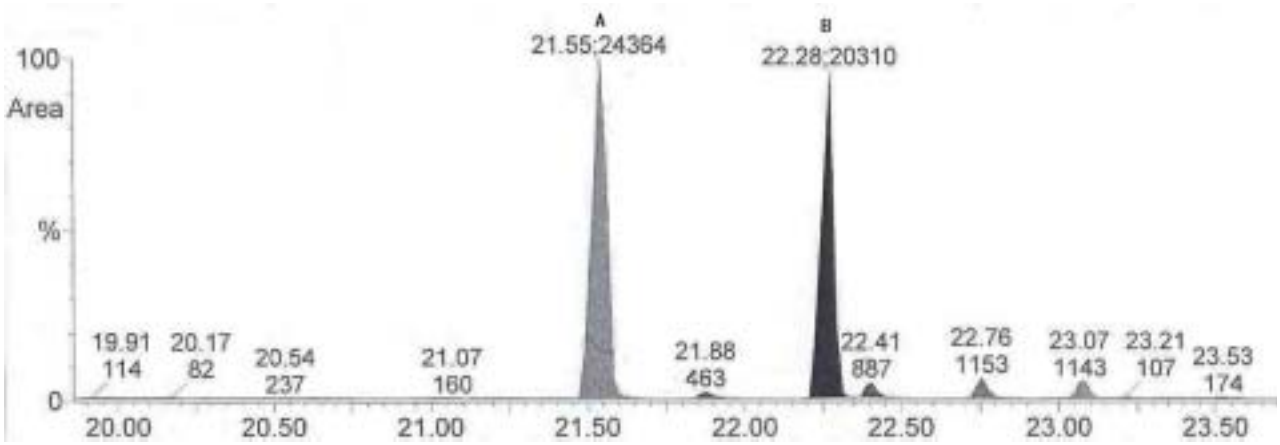


Fig. 2. GC-MS total ion current chromatogram of essential oil from *N. cataria* grown in Korea. Peak A: *cis,trans*-nepetalactone (RT21.50), Peak B: *cis,cis*-nepetalactone (RT 22.28).

talactone (Hallahan et al. 1998). In conclusion, our results indicate that the main constituent of essential oils of *N. cataria* grown in Korea is nepetalatone, which are shown to be derived from genetic factor, while their different percentages depend on environmental conditions such as growing site and climatic conditions. The different proportions of nepetalactone stereoisomer might be occurred from biosynthesis pathway, which also might be depends on environmental conditions.

In Table 2, the results of analyses of petals, young expanding leaves and matures leaves of *N. cataria*. The data show that the essential oil from leaves of this compound contains a higher proportion of nepetalactone than those of petals, but little difference was observed between young and older leaves ($p>0.001$).

The results of the disc diffusion testing of essential oil of *N. cataria* are listed in Table 3. The control (DMSO) showed no inhibiting effect. In this study we found that *N. cataria* oil inhibited the growth of some microorganisms. The effects of 20 μL of essential oil against *E. coli* and *P. aeruginosa* had no inhibition diameters, and although 30 μL of essential oil had ranged from 2 to 6 mm, there were no inhibition effect compared with control ($p>0.05$). While the effects of both of 20 and 30 μL of essential oil against the *B. subtilis*, *B. amyloliquefaciens*, *B. cereus* and *S. aureus* ($p<0.05$) were found to have a high antimicrobial activity (inhibition diameters to 10～24 mm). Cruz et al. (1993) reported that the activity was greater in the essential oils containing larger amounts of geraniol, however our results showed that there are no differences ($p>0.001$) between 20 μL and 30 μL of essential oil.

Table 2. The percentage of nepetalactone in plant parts from *N. cataria*

Organ	Nepetalactone (%)
Young leaf	86.11
Older leaf	87.21
Petal	79.5

Table 3. Inhibition effect of the essential oil of *N. cataria* on microorganisms

Organisms	Diameter (mm)	
	20 μL essential oil	30 μL essential oil
Escherichia coli	0	2
Pseudomonas aeruginosa	0	8
Bacillus subtilis	18	18
Bacillus amyloliquefaciens	20	24
Bacillus cereus	18	20
Staphylococcus aureus	10	12

Our results showed that the activity was more pronounced against fungal organism and Gram-positive than against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Nostro et al. (2001) also suggested that diethyl ether extract of this plant has been shown to have antimicrobial activity against fungi and Gram-positive bacteria. Smith-Palmer et al. (1998) and Shelef (1983) supported that the difference in sensitivity between Gram-positive and Gram-negative bacteria to inhibition by plant essential oils. The other hand, Hili et al. (1997) reported that the cinnamon oil had a strong inhibition effects against *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus*. Burt and Reinders (2003) studied that *Origanum vulgare* oil and *Thymus vulgaris* oil had strong inhibition effect against *E. coli* O157. These all researches are suggested that antimicrobial activity do not always give parallel results, it might depend on methodologies such as agar diffusion and serial dilution methods (Hili et al. 1997). Particularly reproducibility is difficult using the drop diffusing method, such as insolubility and rate of volatilization of the essential oils (Nostro et al. 2000). Moreover such differing results remarkably depend on the kinds of microorganisms, compounds and their concentrations.

The antifungal compounds of the plants assayed are not well known; however, the presence of flavonoids and terpenes and a certain degree of lipophilicity might determine toxicity by the interactions with the membrane constituents and their arrangement (Tomas-Barberan et al. 1990). The reason for the different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms, Gram-negative bacteria having an outer phospholipidic membrane carrying the structrual lipopolysaccharide components (Nikaido and Vaara 1985). In conclusion, *N. cataria* oil showed well-defined inhibition bands in correspondence with nepetalactone. Many previous studies (Bourrel et al. 1993, Hammer et al. 1999, Nostro et al. 2000, 2001) demonstrated the presence of nepetalactone in *N. cataria* and their bacteristatic and fungistatic properties. The toxin present in *N. cataria* is an essential oil, the nepetalactone. However, it is difficult to compare the data with the literature because several variables influence the results, such as the environmental and climatic conditions of the plant and the choice of the extraction method and antimicrobial test.

Analysis of each oil by gas chromatography showed that similar chemical constituents were found in some of these oils. However, the existence of a synergistic or antagonistic relationship between components may explain the differences in antimicrobial activity that arise for oils of the same species tested in different laboratories. In order to compare results of different studies the precise com-

position of oils must be known. The geographical origin of oils also needs to be considered in studies of antimicrobial activity.

Studies of the repellency of catmint oil (predominantly nepetalactone) showed that it was effective repellency towards a number of insect species on short exposure (Jefson et al. 1983). The results obtained from *N. cataria* might be considered sufficient for further studies aimed at isolating and identifying the single active principles and evaluating possible synergism of antimicrobial activity among these extracts. These aspects must be investigated if the use of essential oils as medicine and/or repellents is to become commercially viable.

ACKNOWLEDGEMENT

This work was supported by the Korea Research Foundation Grant funded by the Korean Government R04200400101210200 4.

LITERATURE CITED

Ahn JC, Kim SH, Kim MY, Kim OK, Kim KS, Hwang B. 2003. Seasonal variations in yields of Hwangchil Lacquer and major sesquiterpene compounds from selected superior individuals of *Dendropanax norbifera* Lév. J Plant Biol 46: 38-40.

Baranauskiene R, Venskutonis RP, Demyttenaere JCR. 2003. Sensory and instrumental evaluation of Catnip (*Nepeta cataria* L.) aroma. J Agric Food Chem 51: 3840-3848.

Baser KHC, Kirimer N, Kurkcuoglu M, Demirci B. 2000. Essential oils of *Nepeta* species growing in Turkey. Chem Nat Comp 36: 356-359.

Bellesia F, Grandi R, Pagnoni U, Pinetti A, Trave R. 1984. Biosynthesis of nepetalactone in *Nepeta cataria*. Phytochemistry 23: 83-87.

Bottini AT, Dev V, Garfagnoli DJ, Lohani H, Mathela CS, Pant AK. 1987. (7R)-*trans,trans*-nepetalactone from *Nepeta elliptica*. Phytochemistry 26: 1200-1202.

Bourrel C, Perineau F, Michel G, Bessiere Jm. 1993. Catnip (*Nepeta cataria* L.) essential oil: analysis of chemical constituents, bacteriostatic and fungistatic properties. J Essent Oil Res 5: 159-167.

Burt SA, Reinder RD. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett Appl Microbiol 36: 162-167.

Clark LJ, Hamilton JG, Chapman JV, Rhodes MJ, Hallahan DL. 1997. Analysis of monoterpenoids in glandular trichomes of the catmint *Nepeta racemosa*. Plant J 11: 1387-1393.

Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F. 1999. *In-vitro* antimicrobial activity and chemical composition of *Sandinian Thymus* essential oils. Lett Appl Microbiol 29: 130-135.

Cruz U, Cabo MM, Castillo MJ, Jimenez J, Ruiz C, Ramos-Cormenzana A. 1993. Chemical composition and antimicrobial activity of the essential oils of different samples of *Thymus baeticus* Boiss. Phytotheraphy Res 7: 92-94.

Dawson GW, Griffiths DC, Merritt LA, Mudd A, Pickett JA, Wadhams LJ, Woodcock CM. 1990. Aphid semiochemicals-a review, and recent advances on the sex pheromone. J Chem Ecol 26: 1481-1485.

De Pooter, Nicolai HL, De Buyck LF, Goetghebeur P, Schamp NM. 1987. The essential oil of *Nepeta nuda*. Identification of a new nepetalactone diasteroisomer. Phytochemistry 26: 2311-2314.

Gross M, Friedman J, Dudai N, Larkov O, Cohen Y, Bar E, Ravid U, Putievsky E, Lewinsohn E. 2002. Biosynthesis of estragole and *t*-anethole in bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*) chemotypes. Changes in SAM: phenylpropene O-methyltransferase activities during development. Plant Sci 163: 1047-1053.

Hallahan D, West JM, Smiley DWM, Pickett JA. 1998. Nepetalactol oxidoreductase in trichomes of the catmint *Nepeta racemosa*. Phytochemistry 48: 421-427.

Hammer KA, Carson CF, Riley TV. 1999. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 86: 985-990.

Handjieva NV, Popvov SS, Evstatieva LN. 1996. Constituents of essential oils from *Nepeta cataria* L., *N. grandiflora* M.B. and *N. nuda* L. J Essent Oil Res 8: 639-643.

Hilli P, Evans CS, Veness RG. 1997. Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Lett Appl Microbiol 24: 269-275.

Inouye H. 1991. Iridoids. Meths Plant Biochem 7: 99-143.

Jefson M, Jerrold M, Stephen N, Karen H, Thomas E. 1983. Chemical defense of a rove beetle (*Creophilus maxillosus*). J Chem Ecol 9:159-180.

Kim JH, Langenheim JH. 1994. The effects of *Pseudotsuga menziesii* monoterpenoids on nitrification. Korean J Ecol 17: 251-260.

Liblikas I, Santangelo EM, Sandell J, Baeckström P, Svensson M, Jacobsson U, Unelius CR. 2005. Simplified isolation procedure and interconversion of the diastereomers of nepetalactone and nepetalactol. J Nat Prod 68: 886-890.

Maoz M, Neeman I. 1998. Antimicrobial effects of aqueous plant extracts on the fungi *Microsporum canis* and *Trichophyton rubrum* and on three bacterial species. Lett Appl Microbiol 26: 61-63.

McGarvey DJ, Croteau R. 1995. Terpenoid metabolism. The Plant Cell 7: 1015-1026.

Nikaido H, Vaara M. 1985. Molecular basis of bacterial outer membrane permeability. Microbiol Rev 49: 1-32.

Nostro A, Cannatelli MA, Crisafi G, Alonzo V. 2001. The effect of *Nepeta cataria* extract on adherence and enzyme production of *Staphylococcus areus*. Int J Antimicrobial Agents 18: 583-58.

Nostro A, GermanòMP, D'Angelo V, Marino A, Cannatelli MA. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 30:

379-384.

Osinska E, Suchorska K. 1990. Developmental and chemical characteristics of three species of *Nepeta* genus. Ann Warsaw Agric Univ Hortic 19: 39-44.

Palá-Paúl J, Perez-Alonso MJ, Velasco-Negueruela A, Palá-Paúl R, Sang T, Conejero F. 2001. Seasonal variation in chemical constituents of *Santolina rosmarinifolia* L. ssp. *rosmarinifolia*. Biochem Syst Ecol 29: 663-672.

Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heaney AJ, McGimpsey JA, Smallfield BM. 1999. Essential oils from Dalmatian Sage (*Salvia officinalis* L.): Variations among individuals, plant parts, seasons, and sites. J Agric Food Chem 47: 2048-2054.

Regnier FE, Waller GR, Eisenbraun EJ. 1967. Studies on the composition of the essential oils of three *Nepeta* species. Phytochemistry 6: 1281-1289.

Saxena J, Mathela CS. 1996. Antifungal activity of new compounds from *Nepeta leucophylla* and *Nepeta clarkei*. Appl Environ Microbol 62: 702-704.

Shelef LA. 1983. Antimicrobial effects of spices. J Food Safety 6: 24-29.

Simth-Palmer A, Stewart J, Fyfe L. 1998. Antimicrobial properties of plant essential oils and essences against five important foodborne pathogens. Lett Appl Microbiol 26: 118-122.

Skoula M, Hilali IEL, Makris AM. 1999. Evaluation of the genetic diversity of *Salvia fruticosa* Mill. Clones using RAPP markers and comparison with the essential oil profiles. Biochem Syst Ecol 27: 559-568.

Sonboli A, Salehi P, Allahyari L. 2005. Essential oil composition of *Nepeta involucrate* from Iran. Chem Nat Comp 41: 683-685.

Thomson JD, Chalchar JC, Michet A, Linhart YB, Ehlers B. 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. J Chem Ecol 29: 859-880.

Tittel G, Wagner H, Bos R. 1982. Chemical composition of Melissa oil. Planta Med 46: 91-98.

Tomas-Barberan F, Iniesta-Sanmartin E, Tomas-Lorente F, Rumbero A. 1990. Antimicrobial phenolic compounds from three Spanish *Helichrysum* species. Phytochemistry 29: 1093-1095.

Venskutonis PR, Dapkevičius A, Baranauskiené M. 1995. Flavor composition of some lemon-like aroma herbs from Lithuania. In: Food Flavors: Generation, Analysis and Process Influence (Charalambous G, ed) Elsevier, Amsterdam, The Netherlands, pp 833-847.

Vokou D, Chalkos D, Karamanlidou G, Yiangou M. 2002. Activation of soil respiration and shift of the microbial population balance in soil as a response to *Lanvandula stoechas* essential oil. J Chem Ecol 28: 755-768.

Zygaldo JA, Maestri DM, Lamarque AL, Guzman CA, Velasco-Neturuela A, Perez-Alonso MJ, Garcia-Vallejos MC, Grosso NR. 1996. Essential oil variability of *Minthostachys verticillata.* Biochem Syst Ecol 24: 319-323.

(Received July 24, 2006; Accepted August 10, 2006)