Send Orders for Reprints to reprints@benthamscience.ae

Current Bioactive Compounds **2015**, *11*, 23-30

Antimicrobial and Repellent Activity of the Essential Oils of Two Lamiaceae Cultivated in Western Himalaya

Iris Stappen[*,a], Abbas Ali[b], Nurhayat Tabanca[b], Ikhlas A. Khan[b], Jürgen Wanner[c], Velizar K. Gochev[d], Virendra Singh[e], Brij Lal[f], Vikas Jaitak[f,g], Vijay K. Kaul[h], Erich Schmidt[a] and Leopold Jirovetz[a]

[a]*Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria*; [b]*National Center for Natural Products Research, The University of Mississippi, University, USA*; [c]*Kurt Kitzing Co., Wallerstein, Germany*; [d]*Department of Biochemistry and Micr*obiology, "Paisii Hilendarski"-*University of Plovdiv, Plovdiv, Bulgaria;* [e]*Institute of Himalayan Bioresource Technology (CSIR), Palampur, India*; [f]*Biodiversity Division, Institute of Himalayan Bioresource Technology, Palampur, India*; [g]*Centre for Chemical and Pharmaceutical Sciences, Central University of Punjab, Bathinda, India*; [h]*Ex-Scientist of the Institute of Himalayan Bioresource Technology (CSIR), Palampur, India*

Iris Stappen

Abstract: The essential oils of two Lamiaceae cultivated in Western Himalaya were examined on their antimicrobial, biting deterrent as well as larvicidal activity. Additionally their odors are described and their chemical compositions analyzed by GC-MS are given. The main component of *Nepeta cataria* oil was 4aα,7α,7aα-nepetalactone (85%), whereas camphor (27%) and 1,8-cineol (27%) were dominant in the oil of *Rosmarinus officinalis.* The studied essential oils demonstrated high to moderately antimicrobial activity against reference strains, clinical and food spoilage isolates of *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, *Salmonella abony* and *Candida albicans* (MIC 160-640 µg/ml) and indicated low activity against *Pseudomonas aeruginosa* and *P. fluorescens.* Both oils showed biting deterrent activity above solvent control but lower than DEET. *Nepeta catarica* essential oil exhibited high toxicity with LD_{50} value of 20.2 whereas *R. officinalis* oil showed only 50% mortality at the highest tested dose of 125 ppm against 1-day old *Aedes aegypti* larvae at 24-hour post treatment.

Keywords: Antimicrobial, biting deterrent, essential oil, larvicidal, monoterpenes, *Nepeta cataria*, *Rosmarinus officinalis*.

1. INTRODUCTION

In some South American, African and Asian countries the use of insect repellents plays an important role in the prevention of insect-borne illnesses in humans such as malaria, dengue or yellow fever [1, 2]. Although several synthetic repellent substances, like *N*,*N*-diethyl-3-methylbenzamide (DEET), are available on the market, the search for effective natural alternatives, that at best are active against a great diversity of insect species, is still going on [3]. Also the demand for new antibiotics has grown, due to the increased resistance of bacteria against common antibiotics [4]. Essential oils (EO) verifiably exhibit antimicrobial activity and generate an enormous pool for promising natural antibiotic alternatives [5]. Especially for the poor population in abovementioned countries herbs are a major source as drugs and spices. In the Himalayan region a great percentage of the population lives apart from big cities with their medicinal facilities. These people are even more dependent on the rich variety of herbs growing in this special climate [6].

Most species of the Lamiaceae family are in worldwide use for medicinal purposes [7], and some of their representatives can be found in the Himalaya [8]. One of which is *Nepeta cataria* L. (catnip or catmint), a herbaceous plant native in the temperate regions of Western Himalaya [9]. The aerial parts are used as a remedy against respiratory and gastrointestinal disorders [10]. Its EO has long been known to exhibit an antimicrobial effect against a great number of plant pathogens [11] as well as bacteria responsible for infections of the respiratory tract and skin [12, 13]. Anyway, the oil has most commonly been used as an alternative insect repellent and formulated by a number of companies [14, 15]. The great amount of monoterpene nepetalactone and its diastereomers are attributed to this effect [16]. *Rosmarinus officinalis* L. is native in the Mediterranean area but also grows wild in the Himalayan region [17]. The plant and its EO are, besides other effects, known for their high antibacterial and antioxidant activity [18]. Rosemary appears in three main chemotypes: the 1,8-cineol, the camphor-borneol and the α-pinene chemotype [17].

Climate and altitude of plant origin play an important role in differences of EO compositions [19, 20]. To guarantee standardized EO quality, plants need to be cultivated under well-defined ecological conditions (e.g. glass houses) [21]. In our previous investigations we screened EOs of wild growing plants collected from Western Himalaya – medically used by the local publication – for their biological impact [8, 22, 23]. In the present study the EOs from two culti-

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, Faculty of Life Sciences, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria; Tel: +43-1-4277-55552; Fax: +43-1-4277-9551; E-mail: iris.stappen@univie.ac.at

1875-6646/15 $58.00+.00

vated medicinal important Lamiaceae are evaluated for their antimicrobial and repellent as well as larvicidal activity.

2. MATERIALS AND METHODS

2.1. Plant Material

Nepeta cataria and *R. officinalis* were cultivated in the institute farm of the Institute of Himalayan Bioresource Technology located at an altitude of 1400 m. Aerial parts of both herbs were harvested during June and July 2010. One kg of plant material, respectively, was air-dried separately in the shade at 25°C and hydrodistilled in a Clevenger-type apparatus of 5 kg capacity. Needles and aerial parts of *R. officinalis* yielded colorless oil (0.5% fresh wt basis), whereas aerial parts of *N. cataria* yielded a light-yellow colored oil (0.4% fresh weight basis). The two oil samples were dried over anhydrous sodium sulphate, filtered and stored at 4°C until analysis.

2.2. Essential Oil Analyses

GC-MS-FID analyses were performed on a Finnigan ThermoQuest Trace GC with a FID detector at 250°C and two split/splitless injectors at 230°C, one injector connected to a 50 m x 0.25 mm x 1.0 µm SE-54 column (CS Chromatographie Service, Germany), the other injector connected to a 60 m x 0.25 mm x 0.25 µm Carbowax 20M column (Agilent and J & W, Germany); carrier gas He 5.0 at 170/180kPa, temperature program 40°C 1 min at 3°C/min to 230°C then 5 min isothermal. Both columns were united with a quartz Y-splitter, and the combined effluents were split via a self-made MS-FID splitter and interfaced with a short restriction column (0.1 mm ID) to a Finnigan ThermoQuest Automass Solo quadrupole mass spectrometer (Conditions of analysis: interface at 250°C, ion source at 150°C, mass range 40-500 amu, scan rate 0.75/sec) and to a FID detector. Identification of compounds was carried out according to [22].

2.3. Olfactory Evaluation

For olfactory evaluation, one droplet of each EO sample was applied onto commercially available paper blotters. Each sample was examined by two aroma-chemists and a professional perfumer over 90 min to control for odor progression.

2.4. Antimicrobial Testing

The antimicrobial effects of both EOs were tested against Gram-positive bacteria *Bacillus cereus* (ATCC 11778) and two strains of *Staphylococcus aureus* (ATCC 6538 and one food spoilage isolate), as well as the following Gram-negative bacteria: two strains of *Escherichia coli* (ATCC 25922 and one food spoilage isolate), two strains of *Salmonella abony* (ATCC 6017 and one clinical isolate), two strains of *Pseudomonas aeruginosa* (ATCC 27853 and one clinical isolate) and a food spoilage isolate of *Pseudomonas fluorescens* (sources given in Table **3**). Additionally antimicrobial testing against two strains of *Candida albicans* (ATCC 10231 and one clinical isolate) was performed. All strains were deposited in the Microbial Culture Collection of the Department of Biochemistry and Microbiology (University of Plovdiv, Bulgaria). The bacterial strains were stored on Nutritional Agar (NA, HiMedia Laboratories Ltd., India) and the yeasts strains were stored on Sabouraud Dextrose Agar with chloramphenicol (SDA, HiMedia Laboratories Ltd.).

Stock solutions of the EOs for antimicrobial testing were prepared by dispersing the respective EO sample in 2% DMSO (Sigma-Aldrich Co.). Stock solutions were added to the RPMI1640 broth medium buffered to pH 7.0 with 0.165 mol L^{-1} MOPS buffer (3-N-morpholinopropanesulfonic acid, Sigma-Aldrich, Co) to reach dilutions with final sample concentrations, after inoculation with microbial test suspension, between 5120 µg/mL and 80 µg/mL. Controls consisting of inoculated medium without EO sample and without DMSO, as well as with DMSO were also prepared. The DMSO concentration in the broth dilution assay was low to keep the effect on microbial growth to a minimum.

Antibacterial activity of EOs was performed according to Clinical Laboratory Standard Institute (CLSI) M2-A9 reference method for antimicrobial disk susceptibility tests [24] and CLSI M7-A7 reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically [25]. Anticandidal activity of both EOs was performed according to CLSI M44-A2 reference method for antifungal disk diffusion susceptibility testing of yeasts [26] and CSLI M27-A3 reference method for broth dilution antifungal susceptibility testing of yeasts [27].

Antimicrobial activity of EOs determined by disc diffusion tests was expressed as inhibitory zone (IZ) diameter in mm. IZ diameters were measured to the nearest millimeter by antibiotic zone scale (HiMedia Laboratories Ltd., India). Antimicrobial activity of EOs determined by broth microdilution tests was expressed as Minimal Inhibitory Concentration (MIC) in µg/mL. MIC was defined as the lowest concentration of the EO at which total inhibition of microbial growth was detected.

Antimicrobial activities of ciprofloxacin (CPH 5µg/disc, HiMedia Laboratories Ltd.) and fluconazole (FLC 25µg/disc, HiMedia Laboratories Ltd., India) were also determined as positive controls. MICs of ciprofloxacin (CPH MIC strip 0.016 - 256 µg/mL, HiMedia Laboratories Ltd.) and fluconazole (FLC MIC strip, 0.016 - 256 µg/mL, HiMedia Laboratories Ltd.) were also determined as positive controls by Hi-Comb™ MIC Test (HiMedia, Laboratories Ltd., India), according to manufacturer's instructions.

2.5. Mosquito Testing

Mosquitoes: *Aedes aegypti* used were from a laboratory colony and were reared as described in [8].

Mosquito Biting Bioassays: Experiments were conducted by using a six-celled *in vitro* Klun and Debboun (K&D) module bioassay system for quantitative evaluation of biting deterrent properties of the EOs of *N. cataria* and *R. officinalis*. Details of this bioassay are described in [28]. Treatments were replicated ten times.

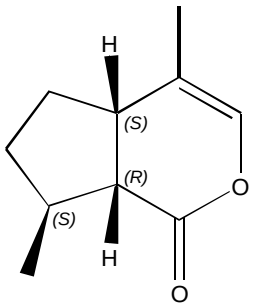

Fig. (1). Chemical structure of 4aα,7α,7aα-nepetalactone (4aS,7S,7aR)-4,7-dimethyl-5,6,7,7a-tetrahydrocyclo-penta[c]pyran-1(4aH)-one.

Larvicidal Bioassays: Bioassays were conducted to test essential oils of *N. cataria* and *R. officinalis* for their larvicidal activity against *Ae. aegypti* by using the bioassay system described by Pridgeon *et al*. [29]. Details of this bioassay are described in [30].

Statistical Analyses: Proportion not biting (PNB) was calculated as described by Ali *et al*. [30]. Data on the PNB values were analyzed using the ANOVA procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC), and means were separated using the Ryan-Einot-Gabriel-Welsch Multiple Range Test.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition

Analyzed by simultaneous GC-FID and GC-MS using two different columns a total of 34 compounds were identified for *N. cataria* EO, representing about 96% of the total oil. The quantitative and qualitative composition of the EO is listed in Table **1**. It possessed the highest amount of the iridoid 4aα,7α,7aα-nepetalactone (84.0-85.7%; (*Z*,*E*)-nepetalactone) (Fig. **1**), accompanied by a much lesser amount of the sesquiterpene hydrocarbon (E)-caryophyllene (2.3-2.4%). Additionally two derivatives of nepetalactones (MW 184) were found, which might be identified as a substance formed through hydrolytic cleavage of the lactone ring (isomer 1: 0.9-1.4%; isomer 2: 2.8-4.8%).

A study on the investigation of catnip EO composition depending on the plants' developmental stages was carried out in 2011 in Iran [31]. The cultivated plants were harvested in 1800 m above sea level once a month from April to July. Although EO compositions differed in quality and quantity depending on growth stage, (*Z*,*E*)- (55-59%), (*E*,*Z*)-nepetalactones (30-31%) and α-pinene (3-5%) were the major oil constituents in all oil samples. In another investigation a seasonal variation between both nepetalactones in catnip were found [32], with the (*Z*,*E*)-isomer being more present in the very young plant (week one). The samples were collected from wild within nine weeks on the Iowa University campus (USA). The mean overall ratio was 1.73. In *N. cataria* EO from Pakistan, Gilani and coworkers did not find any nepetalactones but α-pinene (10.3%), 1,8-cineol (21.0%) and α-humulene (14.4%) as main constituents [33].

Table 1. Chemical composition of *N. cataria* EO.

compound	RI[#]	%Area$_{FID}$	RI[##]	%Area$_{FID}$
methyl 2-methyl-butanoate	773	tr.		
(*E*)-2-hexenal	849	tr.	1198	tr.
(*Z*)-3-hexenol	852	tr.	1342	tr.
styrene	894	tr.		
α-pinene	941	tr.	1013	tr.
1-octen-3-ol	976	tr.	1412	tr.
sabinene	979	0.1	1105	0.1
3-octanone	983	tr.		
6-methyl-5-hepten-2-one			1307	tr.
β-pinene	985	0.2	1093	0.2
myrcene			1137	tr.
octanal	1000	tr.	1264	tr.
limonene	1035	tr.	1178	tr.
(*Z*)-β-ocimene	1036	0.2	1208	0.1
(*E*)-β-ocimene	1048	0.6	1223	0.5
linalool	1100	tr.	1508	0.1
nonanal	1102	tr.		
citronellal	1153	tr.	1443	tr.
camphor	1155	0.1		
methyl salicylate			1717	tr.
neral	1242	0.2	1643	0.2
geranial	1270	0.3	1692	0.3
4aα,7α,7aα-nepetalactone	1381	85.7	1937	84.0
cis-3-hexenyl-*cis*-3-hexenoate			1678	tr.
4aα,7α,7aβ-nepetalactone	1404	0.4	1985	0.4
4aα,7β,7aα-nepetalactone	1408	0.4	2004	0.4
dihydro nepetalactone	1432	0.2	2078	0.2
(*E*)-caryophyllene	1441	2.4	1559	2.3
(*E*)-β-farnesene	1458	0.2	1629	0.2
α-humulene	1475	0.2		
nepetalactone derivative 1 *	1488	1.4	2735	0.9
nepetalactone derivative 2 *	1525	2.8	2742	4.8
caryophyllene oxide	1608	0.6	1944	0.7
humulene epoxide II	1634	tr.		
sum		95.8		95.3

[#] 50 m x 0.25 mm x 1.0 µm SE-54; [##] 60 m x 0.25 mm x 0.25 µm CW20M; tr. = trace (<0.05%); * MW 184 = nepetalactone (MW 166) + H_2O

The fact that in the present investigated catnip EO mainly (*Z*,*E*)-nepetalactone and only 0.4% of (*E*,*Z*)-nepetalactone were found, which is contractionary to other literature, might again be due to differences plant origins and physiological states.

For *R. officinalis* EO 86 compounds (97.4-97.6%), mainly monoterpenes, were found (Table **2**). The main components were 1,8-cineole (21.7-26.6%) and camphor (26.5-26.6%) followed by α-pinene (8.1-8.4%), verbenone (7.8-8.0%) camphene (3.7-3.8%), limonene (2.8-3.1%), α-terpineol (2.9%) and borneol (2.7-2.8%). Sesquiterpenes were all below 0.5%. These data are in accordance with other literature investigating Himalayan rosemary EO [17].

3.2. Odor Description

The odor of *N. cataria* EO is described as warm-spicy, dry-floral and aromatic with a balsamic note. Olfactory evaluation of the EO of *R. officinalis* revealed a fresh-herbaceous, slightly green-aromatic headnote with a terpeny eucalyptus touch, due to the high content of 1,8-cineole. Later the odor impression shifts to somewhat dusty-balsamic.

3.3. Antimicrobial Activity

Antimicrobial activities of the EOs of *N. cataria* and *R. officinalis* were determined by disc diffusion and broth dilution methods. Both methods were applied, because usually the diffusion method is used for preliminary testing of inhibitory activity of natural compounds, but it is characterized by some disadvantages such as limited diffusion of the oil into solid medium. On the other hand the diameter of inhibitory zones strongly depends on external factors including type and thickness of nutritive medium into Petri dish, temperature, etc. To overcome these disadvantages broth dilution method is strongly recommended as more reliable and reproducible. As shown in Table **3** both EOs demonstrated antimicrobial activity against all test microorganisms. Gram-positive bacteria were more susceptible (MIC values starting from 160 µg/ml) to antimicrobial effect of EOs in comparison to Gram-negative bacteria (MIC values from 640 µg/ml to 5120 µg/ml). Usually the different susceptibility of both bacterial types is explained by the presence of so-called outer membrane on the cell walls of Gram-negative bacteria, which to some extent prevent diffusion of the oil into the cells [34, 35]. Among the tested Gram-negative bacteria both species from genera *Pseudomonas*, *P. aeruginosa* and *P. fluorescens*, were more resistible to *N. cataria* and *R. officinalis* EOs. In our previous investigations [36] we also detected an increased resistance of psychrotrophic food spoilage *Pseudomonas* spp. against pimento, thyme, clove and oregano oils. Fluorescent strains were determined as highly resisting in comparison with non-fluorescent strains. This might be explained by the production of fluorescent pigments by the strains which possibly inactivate the EOs and defend the cells by still unknown mechanisms (e.g. active efflux mechanism [37]). Of course such speculation needs additional experiments to be proved. Both yeast strains of *C. albicans* are characterized by susceptibility equal to Gram-positive bacteria (MIC 160 µg/ml).

Table 2. Chemical composition of *R. officinalis* EO.

compound	RI[#]	%Area$_{FID}$	RI[##]	%Area$_{FID}$
cis-3-hexenol	846	0.1	1351	0.1
tricyclene	924	0.1	1004	0.1
α-thujene	925	0.2		
α-pinene	935	8.1	1015	8.4
α-fenchene	948	tr.	1043	tr.
camphene	951	3.7	1051	3.8
thuja-2,4(10)-diene	954	0.1	1106	0.2
1-octen-3-ol	969	0.3	1422	0.4
sabinene	972	0.1		
3-octanone	976	0.2	1235	0.2
β-pinene	978	2.5	1096	2.5
3-octanol			1372	0.1
myrcene	984	0.9	1138	0.8
dehydro-1,8-cineole	988	tr.	1172	tr.
octanal	993	tr.		
cis-3-hexenyl acetate	995	tr.	1288	tr.
α-phellandrene	1003	0.2	1144	0.2
δ-3-carene	1015	0.1	1130	tr.
α-terpinene			1158	0.1
p-cymene	1022	2.4	1244	2.4
limonene	1029	3.1	1181	2.8
1,8-cineole	1033	26.6	1202	27.3
γ-terpinene	1056	tr.	1224	0.1
cis-sabinene hydrate	1065	0.2	1444	0.2
trans-linalool oxide	1069	tr.	1418	tr.
cis-linalool oxide	1084	tr.		
terpinolene	1087	0.2	1259	0.1
p-cymenene			1408	0.1
linalool	1093	0.8	1522	1.0
trans-sabinene hydrate	1097	0.2	1529	0.2
a-pinene oxide	1100	tr.		
endo-fenchol	1116	0.1		
cis-p-menth-2-en-1-ol	1121	0.1	1540	0.2
chrysanthenone	1124	0.3	1477	0.2
trans-p-menth-2-en-1-ol	1140	0.1	1604	0.1
trans-sabinol	1144	0.1	1774	tr.

Table (2) contd….

compound	RI[#]	%Area$_{FID}$	RI[##]	%Area$_{FID}$
isopulegol			1547	0.2
camphor	1150	26.5	1491	26.6
trans-pinocarveol			1620	0.1
cis-verbenol			1622	tr.
camphene hydrate	1154	0.1	1569	tr.
trans-verbenol			1649	0.2
iso-isopulegol	1159	0.1	1535	0.1
trans-pinocamphone	1163	0.1		
pinocarvone	1164	0.2	1538	0.2
δ-terpineol	1167	0.5	1645	0.6
borneol	1169	2.8	1668	2.7
terpinen-4-ol	1179	1.5	1577	1.3
p-cymen-8-ol	1182	0.2	1815	0.2
cryptone	1186	0.1		
α-terpineol	1191	2.9	1674	2.9
myrtenal			1597	tr.
myrtenol	1198	0.3	1759	0.2
α-campholenol	1201	0.1	1764	0.1
trans-piperitol	1207	0.1	1720	tr.
verbenone	1212	7.8	1682	8.0
citronellol	1219	0.4	1741	0.4
trans-carveol			1807	0.1
isobornyl formate	1231	tr.		
geraniol	1246	0.2	1819	0.1
piperitone	1255	0.1		
isopiperitenone	1271	0.1		
bornyl acetate	1286	0.4	1550	0.4
thymol	1290	0.1	2125	tr.
carvacrol	1296	tr.	2168	0.1
4-hydroxy cryptone	1317	tr.		
piperitenone	1344	tr.	1896	tr.
eugenol	1355	0.3	2118	0.4
α-ylangene	1365	0.1	1469	tr.
α-copaene	1381	0.1		
methyl eugenol	1394	0.2	1981	0.2
(E)-caryophyllene	1434	0.5	1572	0.3
β-copaene	1441	0.1		
geranyl acetone	1445	0.1	1830	0.1
α-humulene	1468	0.2	1632	tr.
γ-muurolene	1485	0.1		
β-bisabolene	1509	tr.		
γ-cadinene	1525	tr.		
δ-cadinene	1530	0.1	1730	tr.
calacorene	1554	tr.		
caryophyllene oxide	1601	0.3	1959	0.3
humulene epoxide I	1615	tr.		
humulene epoxide II	1627	0.2	2016	0.1
cubenol	1647	0.1		
τ-muurol	1667	tr.		
sum		97.1		97.0

[#] 50 m x 0.25 mm x 1.0 μm SE-54; [##] 60 m x 0.25 mm x 0.25 μm CW20M; tr. = trace (<0.05%).

The obtained antimicrobial results of *N. cataria* EO from Western Himalaya are comparable with those of the EO from the same botanical species from Iran [38]. Antimicrobial activity of the studied catnip oil sample was higher compared to catnip oils with lower content of nepetalactone isomers [13], indicating that inhibitory activity of EOs from *N. cataria* could be attributed to these compounds. Additional effects of constituents such as pinene and thymol might be assumed [39]. Antimicrobial activity of EO of *R. officinalis* from Western Himalaya can be attributes to compounds such as camphor, 1,8-cineole and α-pinene, which is proven by the results of Celiktas *et al*. [40].

3.4. Repellent and Larvicidal Activity

In *in vitro* biting deterrent bioassays, both EO were more active than solvent control whereas biting activity in both oils was significantly lower than DEET (Fig. **2**). It has been reported that topical application of catnip essential oil can effectively prevent biting by various mosquito species, and there is an evidence of spatial repellency [41, 42]. Chauhan *et al*. showed high activity of nepetalactones from catnip oil against *Ae. aegypti* in *in vitro* K & D bioassays, although efficacy of these compounds in *in vivo* cloth patch bioassays was significantly lower than DEET. In their study, catnip oil has been reported as insect repellent including mosquitoes and has also been marketed as an alternative repellent for biting arthropods. However, catnip oil and nepetalactone isomers were found less effective than DEET in deterring biting of *Ae. aegypti* by using *in vitro* and *in vivo* K& D bioassays [14]. Bernier *et al*. [41] reported lower activity of

Table 3. Antimicrobial activity of *N. cataria* and *R. officinalis* EOs and reference substances.

Test microorganism	*Nepeta cataria*		*Rosmarinus officinalis*		Ciprofloxacin		Fluconazole	
	IZ, mm	MIC, µg/ml	IZ, mm	MIC, µg/ml	IZ, mm	MIC, µg/ml	IZ, mm	MIC, µg/ml
B. cereus ATCC 11778	24.2	160	23.8	160	28.0	0.5	-	-
S. aureus ATCC 6538	26.3	320	25.2	320	30.0	0.5	-	-
S. aureus food isolate from spoiled minced meat	28.2	160	26.4	160	30.0	0.25	-	-
E. coli ATCC 8739	12.3	640	14.2	1280	21.5	0.5	-	-
E. coli food isolate from spoiled pork fillet	18.2	640	18.4	640	22.5	0.5	-	-
S. abony ATCC 6017	11.2	1280	10.4	2560	18.5	0.5	-	-
S. abony clinical isolate	10.3	1280	10.2	2560	12.5	0.5	-	-
P. aeruginosa ATCC 9027	nd	2560	nd	2560	15.0	1.0	-	-
P. aeruginosa clinical isolate	nd	5120	nd	5120	12.0	2.0	-	-
P. fluorescens food spoilage isolate from spoiled pork fillet	nd	2560	9.3	2560	16.5	1.0	-	-
C. albicans ATCC 10231	19.2	160	18.2	160	-	-	21.0	1.0
C. albicans clinical isolate	19.8	160	18.6	160	-	-	16	16

broth dilution and disc diffusion assays; nd = not detected

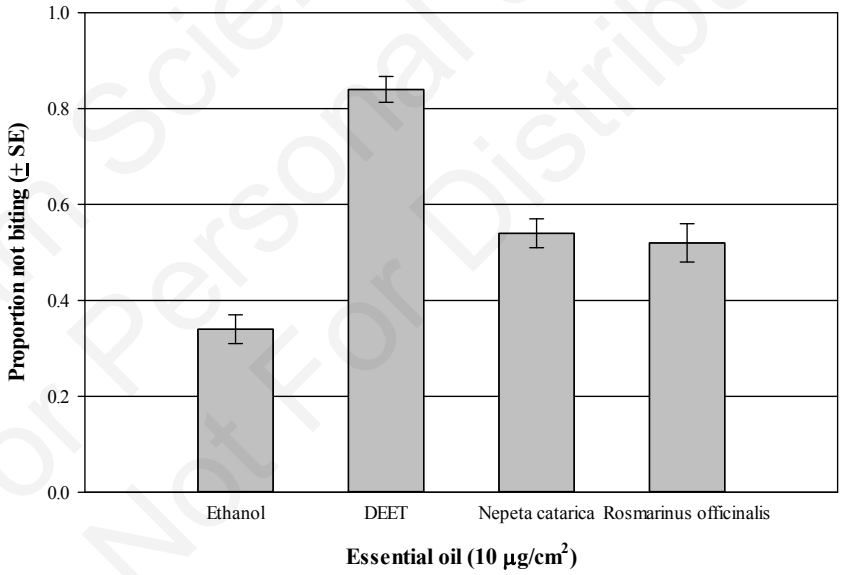

Fig. (2). Proportion not biting values of *N. cataria* and *R. officinalis* EOs against female *Ae. aegypti*. DEET, at 4.8 µg/cm^2, was used as positive control. Ethanol was used as solvent control.

catnip EO than DEET in human based cloth patch assay against *Ae. aegypti, Anopheles albimanus* and *An. quadrimaculatus*. Although the contents of nepetalactones were extremely high in these oil samples, biting deterrent activity was significantly lower than DEET in our bioassays. In an investigation of Schultz *et al*., (*E,Z*)-nepetalactone was found to be more repellent than the (*Z,E*)-isomer, even at a lower concentration [32].

We recently investigated the single compounds camphor [43] and 1,8-cineole [44] which are major compounds of *R. officinalis* oil in the mosquito biting-deterrent activity.

Based on the biting deterrence index (BDI) values, (+)-camphor (0.62), (-)-camphor (0.64) and 1,8-cineole (0.34) were active but the activity was significantly lower than DEET at the same concentration of 25 nm/cm^2 in our previous study [44, 45].

In larvicidal screening bioassays, *N. catarica* showed high activity whereas the *R. officinalis* showed 50% mortality only at the highest tested dose of 125 ppm. LD_{50} value of *N. catarica* was 20.2 (17.6 -23.2 ppm) against 1-d-old *Ae. aegypti* at 24-h post treatment and there was no statistical increase in mortality at 28-h post treatment.

CONCLUSION

The EOs of two Lamiaceae, *N. cataria* and *R. officinalis*, obtained by steam distillation from cultivated herbs in Western Himalaya, were mainly composed of monoterpenes. Our results indicated high to moderate antimicrobial activity of both EOs against Gram-positive bacteria and *C. albicans*. They also showed moderate repellent activity, but *N. cataria* EO revealed high larvicidal activity against *Ae. aegypti* larvae. There are three optically active centers in nepetalactone which account to eight stereoisomers and four diastereomers. Thermodynamically the (*Z*,*E*)/(*E*,*Z*)-isomers should be the most stable and therefore are present in higher amounts throughout the literature. In our investigated catnip oil only one isomer was found, probably due to environmental factors, which play a role in plant growth and EO production, resulting in significant discrepancies in biological properties. It has been claimed that character impact compounds of EOs are mainly responsible for their bioactivity, but there is much of evidence that a combination of two or more components is more effective than a single compound [39, 45, 46, 47, 48]. Therefore it can be assumed that compounds that have not been identified by two-dimensional analytical methods can also have a strong impact on bioactivity of EOs.

Based on the obtained results, Himalayan catnip EO and nepetalactones are promising targets to investigate for antimicrobial and larvicidal activity. Given the urgent need for environmentally safe chemicals, volatile constitutes from catnip EO warrant further search in mosquito control program and antimicrobial agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported in part by USDA/ARS grant No. 56-6402-1-612, Deployed War-Fighter Protection research program grant funded by the U.S. Department of Defense through the Armed Forces Pest Management Board. We thank Ms. J.L. Robertson, Ms. R. Pace for performing bioassays, Dr. James J. Becnel, Mosquito and Fly Research Unit, Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, for supplying *Ae. aegypti* eggs. Authors Vijay K Kaul, Virendra Singh and Vikas Jaitak are thankful to the Director IHBT Palampur for providing necessary facilities for cultivating the plant materials and for carrying out hydrodistillations and analytical work.

REFERENCES

[1] Lawrence, K.L.; Achee, N.L.; Bernier, U.R.; Mundal, K.D.; Benante, J.P. Field evolutions of tropical arthropod repellents in north, central and south America. *J. Med. Entomol.*, **2014**, *51*, 980 - 988.

[2] Debboun, M.; Strickman, D. Insect repellents and associated personal protection for a reduction in human disease. *Med. Vet. Entomol.*, **2013**, *27*, 1-9.

[3] Pohlit, A.M.; Lopes, N.P.; Gama, R.A.; Tadei, W.P.; de Andrade Neto, V.F. Patent literature on mosquito repellent inventions which contain plant essential oils – a review. *Planta Med.*, **2011**, *77*, 598-617.

[4] Bozin, B.; Mimica-Dukic, N.; Simin, N.; Anackov, G. Characterization of the volatile composition of essential oils of some Lamiaceae species and the antimicrobial and antioxidant activities of the entire oils. *J. Agric. Food Chem.*, **2006**, *54*, 1822-1828.

[5] Edris, A.E. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. *Phytother. Res.*, **2007**, *21*, 308-323.

[6] Jaryan, V.; Uniyal, S.K.; Gupta R.C., Singh, R.D. Alien flora of Indian Himalayan state of Himachal Pradesh. *Environ. Monit. Assess.*, **2013**, *185*, 6129-6153.

[7] De Martino, L.; De Feo, V.; Nazzaro, F. Chemical composition and *in vitro* antimicrobial and mutagenic activities of seven Lamiaceae essential oils. *Molecules*, **2009**, *14*, 4213-4230.

[8] Stappen, I.; Wanner, J.; Tabanca, N.; Wedge, D.E.; Ali, A.; Kaul, V.K.; Lal, J.; Jaitak, V.; Gochev, V.K.; Schmidt, E.; Jirovetz, L. Chemical composition and biological activity of essential oils of Dracocephalum heterophyllum and Hyssopus officinalis from Western Himalaya. *NPC*, **2015**, *10*, 133-138.

[9] Sarkar, M.; Rashmi, R.; Vikramaditya, P.; Varma, P.N. Pharmacognosy of Nepeta cataria. *Anc Sci Life*, **1995**, *14*(4), 225-234.

[10] Gilani, A.H.; Shah, A.J.; Zubair, A.; Khalid, S.; Kiani, J.; Ahmed, A.; Rasheed, M.; Ahmed, V.U. Chemical composition and mechanisms underlying the spasmolytic and bronchodilatatory properties of the essential oil of Nepeta cataria L. *J. Ethnopharm.*, **2009**, *121*, 405-411.

[11] Kokoskova, B.; Pouvova, D.; Pavela, R. Effectiveness of plant essential oils against Erwinia amylovory, Pseudomonas syringae pv. syringae and associated saprophytic bacteria on/in host plants. *J. Plant Pathol.*, **2011**, *93*(1), 133-139.

[12] Suschke, U.; Sporer, F.; Schneele, J.; Geiss, H.K.; Reichling J. Antibacterial and cytotoxic activity of Nepeta cataria L., N. cataria var. citriodora (Beck.) Balb. and Melissa officinalis L. essential oils. *NPC*, **2007**, *2*(12), 1277-1286.

[13] Adiguzel, A.; Ozer, H.; Sokmen, M.; Gulluce, M.; Sokmen, A.; Kilic, H.; Sahin, F.; Baris, O. Antimicrobial and antioxidant activity of the essential oil and methanol extract of Nepeta cataria. *Polish J. Microbiol.*, **2009**, *58*(1), 69-76.

[14] Chauhan, K.R.; Klun, J.A.; Debboun, M.; Kramer, M. Feeding deterrent effects of catnip oil components compared witz two synthetic amides against Aedes aegypti. *J. Med. Entomol.*, **2005**, *42*(4), 643-646.

[15] Spero, N.; Gonzalez, Y.I.; Scialdone, M.A.; Hallahan, D.L. Repellency of hydrogenated catmint oil formulations to black flies and mosquitoes in the field. *J. Med. Entomol.*, **2008**, *45*(6), 1080-1086.

[16] Birkett, M.A.; Hassanali, A.; Hoglund, S.; Pettersson, J.; Pickett, J.A. Pepellent activity of catmint, Nepeta cataria, and iridoid nepetalactone isomers against Afro-tropical mosquitoes, ixodid ticks and red poultry mites. *Phytochem.*, **2011**, *72*, 109-114.

[17] Verma, R.S.; Rahman, L.; Mishra, S.; Verma, R.K.; Singh, A.; Chauhan, A.; Yadav, A.K. *J. Chil. Chem. Soc.*, **2012**, *57*, 1066-1068.

[18] Wang, W.; Wu, N.; Zu, Y.G.; Fu, Y.J. Antioxidative activity of Rosmarinus officinalis L. essential oil compared to its main components. *Food Chem.*, **2008**, *108*, 1019-1022.

[19] Haider, F.; Kumar, N.; Naqvi, A.; Das Bagchi, G. Oil constituents of Artemisia nilagirica var. septentrionalis growing at different altitudes. *NPC*, **2010**, *5*, 1959-1960.

[20] Adams, R.P. Geographic variation in leaf essential oils and RAPDs of Juniperus polycarpos K. Koch in central Asia. *Biochem. System. Ecol.*, **2001**, *29*, 609-619.

[21] Nemeth, E.; Bernáth, J.; Tarján, G. Quantitative and qualitative studies of essential oils of Hungarian Achillea populations. *JHSMP* **2007**, *13*, 57-69.

[22] Stappen, I.; Wanner, J.; Tabanca, N.; Wedge, D.E.; Ali, A.;, Khan, I.A.; Kaul, V.K.; Jaitak, V.; Gochev, V.K.; Girova, T.; Stovanova. A.; Schmidt, E.; Jirovetz, L. Chemical composition and biological effects of Artemisia maritima and A. nilagirica essential oils from wild plants of western Himalaya. *Planta Med.*, **2014**, *13*, 1079-1087.

[23] Stappen, I.; Tabanca, N.; Ali, A.; Wedge, D.E.; Wanner, J.; Kaul, V.K.; Lal, B.; Jaitak, V.; Gochev, V.K.; Schmidt, E.; Jirovetz, L. Chemical composition and biological activity of essential oils from wild growing aromatic plant species of Skimmia laureola and Juniperus macropoda from Western Himalaya. *NPC*, **2015**, *16*(6), 1071-1074.

[24] Clinical Laboratory Standard Institute, Performance standards for antimicrobial disk susceptibility tests, Approved Standard M2-A9, 9th Ed, CLSI, Wayene, PA, USA, **2006**.

[25] Clinical Laboratory Standard Institute, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard M7-A7, 7th Ed, CLSI, Wayene, PA, USA, **2006**.

[26] Clinical Laboratory Standard Institute, Reference Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline M44-A2, 2nd Ed, CLSI, Wayene, PA, USA, **2009**.

[27] Clinical Laboratory Standard Institute, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A3, 3rd Ed, CLSI, Wayene, PA, USA, **2008**.

[28] Klun, J. A., M. Kramer, and M. Debboun. A new *in vitro* bioassay system for discovery of novel human-use mosquito repellents. *J. Am. Mosq. Control Assoc.*, **2005**, *21*, 64-70.

[29] Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. A high-throughput screening method to identify potential pesticides for mosquito control. *J Med. Entomol.,* **2009**, *46*, 335-341.

[30] Ali A, Murphy CC, Demirci B, Wedge DW, Sampson BJ, Khan IA, Baser KHC, Tabanca N. Insecticidal and biting activity of rose-scented geranium (*Pelargonium* ssp) individual compounds against *Stephanitis pyrioides* and *Aedes aegypti. Pest Man. Sci.,* **2013**, *69*, 1385-1392.

[31] Mohamadi, S.; Saharkhiz, M.J. Changes in essential oil content and composition of catnip (Nepeta cataria L.) during different develoomental stages. *Jeobp*, **2011**, *14*(4), 396-400.

[32] Schultz, G.; Simbro, E.; Belden, J.; Zuh, J.; Coates, J. Catnip, Nepeta cataria (lamiales: Lamiaceae) - A closer look: Seasonal occurrence of nepetalactones isomers and comparative repellenca or three terpenoids to insects. *Chem. Ecol.*, **2004**, *33*(6), 1562-1569.

[33] Gilani, A.H.; Shah, A.J.; Zubair, A.; Khalid, S.; Kiani, J.; Ahmed, A.; Rasheed, A.; Ahmad, V.A. Chemical composition and mechanisms underlyint the spasmolytic and bronchodilatory properties of the essential oil of Nepeta cataria L. *J. Ethnopharm*., **2009**, *121*, 405-411.

[34] Dorman, H.; Deans, S. Antimicrobial agents from plants: antibacterial activity of plant volatile oils, *J. Appl. Microbiol.,* **2000**, *88*, 308-316.

[35] Sivropoulou, A.; Kokkini, S.; Lanaras, T.; Arsenakis, M. Antimicrobial activity of mint essential oils. *J. Agr. Food Chem.*, **1995**, *43*, 2384-2388.

[36] Girova, T.; Gochev, V.; Jirovetz, L.; Buchbauer, G.; Stoyanova, A. Antimicrobial activity from spices against psychotropic food spoilage microorganisms, *Biotech. Biotechnol. Equipment*, **2010**, *24*: suppl.1, 547-552.

[37] Cox, S.D.; Markham, J.L. Susceptibility and intrinsic tolerance of Pseudomonas aeruginosa to select plant volatile compounds. *J. Appl. Microbiol.*, **2007**, *103*, 930-936.

[38] Zomorodian, K.; Saharkhiz, MJ.; Rahimi, MJ.; Shariatifard, S.; Pakshir, K.; Khashei, R. Chemical composition and antimicrobial activities of essential oil of Nepeta cataria L. against common causes of oral infections. *J. Dentistry*, Tehran University of Medical Sciences, **2013**, *10*(4), 329-337.

[39] Klein, G.; Rueben, C.; Upmann, M. Antimicrobial activity of essential oil compounds against potential food spoilage microorganisms. *Curr. Microbiol.*, **2013**, *67*, 200-208.

[40] Celiktas, O.Y.; Kocabas, E.E.H.; Bedir, E.; Sukan, F.V.; Ozek, T.; Baser K.H.C. Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. *Food Chem.*, **2007**, *100*, 553-559.

[41] Bernier, U.R.; Furman, K.D.; Kline, D.L.; Allan, S.A.; Barnard, D.R. Comparison of contact and spatial repellency of catnip oil and N,N-diethyl-3-methylbenzamide (deet) against mosquitoes. *J. Med. Entomol.,* **2005**, *42*, 306-311.

[42] Trongtokit, Y.; Rongsriyam, Y.; Komalamisraand, N.; Apiwathnsorn, C. Comparative repellency of 38 essential oils against mosquito bites. *Phytother. Res.*, **2005**, *19*, 303-309.

[43] Ali, A.; Tabanca, N.; Kurkcuoglu, M.; Duran, A.; Blyhthe, E.K.; Khan, I.A.; Baser, K.H.C. Chemical composition, larvicidal and biting deterrent activity of essential oils of two subspecies of *Tanacetum argenteum* (Lam.) Willd and individual constituents against *Aedes aegypti* (L) (Diptera: Culicidae). *J. Med. Entomol.,* **2014**, *51*, 1-7.

[44] Tabanca, N.; Avonto, C.; Wang, M.; Parcher, J.F.; Ali, A.; Demirci, B.; Raman, V.; Khan, I.A. Comparative investigation of *Umbellularia californica* and *Laurus nobilis* leaf essential oils and identification of constituents active against *Aedes aegypti*. *J. Agric. Food Chem.,* **2013**, *61*, 12283-12291.

[45] Gupta, N.; Saxena, G.; Kalra, S.S. Antimicrobial activity pattern of certain terpenoids. *Int. J. Pharm. Bio Sci.*, **2011**, *2*(1), 87-91.

[46] Filipowicz, N.; Kaminsk, M.; Durlenda, J.; Asztemborska, M.; Ochocka, J.R. Antibacterial and antifungal activity of juniper berry oil and its selected components. *Phytother. Res.*, **2003**, *17*, 227-231.

[47] Aridogan, B.D.; Baydar. H.; Kaya, S.; Demiric, M.; Özbasar, D.; Mumcu, E. Antimicrobial activity and chemical composition of some essential oils. *Arch. Pharm. Res.*, **2002**, *25*(6), 860-864.

[48] Papadopoulos, C.; Carson, C.F.; Chang, B.J.; Riley, T.V. Role of the MexAB-OprM dfflus pump of *Pseudomonas aeruginosa* in tolerance to tea tree (*Melaleuca alternifolia*) oil and its monoterpene components terpinen-4-ol, 1,8-cineol, and α-terpineol. *Applied and Envir. Microbiol.*, **2008**, *74*(6), 1932-1935.

Received: March 30, 2015 Revised: April 28, 2015 Accepted: May 6, 2015