Nat. Volatiles & Essent. Oils, 2016; 3(2): 1-8

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Chemical composition and antimicrobial activity of *Tanacetum tomentellum* (Boiss.) Grierson essential oil from Turkey

Nurhayat Tabanca[1, 2, 3*], K. Hüsnü Can Başer[4], Zeki Aytaç[5], Melissa R. Jacob[1], Shabana I. Khan[1] and Betül Demirci[6*]

[1] National Center for Natural Products Research, the University of Mississippi, University, MS 38677, USA

[2] Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL 32610, USA

[3] USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608, USA

[4] Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Nicosia, N. Cyprus

[5]Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, Turkey

[6] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, 26470, TURKEY

*Corresponding author. Email: ntabanca@ufl.edu; nurhayat.tabanca@ars.usda.gov; betuldemirci@gmail.com

Abstract

The chemical composition of essential oil obtained by hydrodistillation from the dried aerial parts of *Tanacetum tomentellum* (Boiss.) Grierson (Asteraceae) was analysed by GC-FID and GC-MS. Eighty-eight compounds, constituting about 84.1% of the total oil, were identified. The main constituents were camphor (9.4%), linalool (7.6%), *α*-terpineol (7.1%), *trans*-pinocarveol (5.3%) and *trans*-verbenol (4.5%). The oil was evaluated for antimicrobial and antimalarial activity. The oil showed antifungal activity against *Cryptococcus neoformans* with an IC_{50} value of 45 μg/mL, while it showed no antimicrobial activity against other tested microorganisms (*Candida albicans*, *Aspergillus fumigatus, Staphylococcus aureus* methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa* and *Mycobacterium intracellulare*) up to a concentration of 200 μg/mL. No antimalarial activity was observed against chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum* up to 15.9 μg/mL. We report for the first time the essential oil composition and biological activity of *T. tomentellum*.

Keywords: *Tanacetum tomentellum*, antimicrobial, antimalarial, GC-FID, GC-MS

Introduction

Emerging infectious diseases are substantially threat to global human health. Increasing population, poor sanitation, ecological changes, travel and threats could increase the spread of infections (Bueno, 2015; Coker et al., 2011; Haines et al., 2006; Daszak, Cunningham & Hyatt, 2000; Martinez, 2000). The emergence of multiple drug resistance in *Staphylococcus aureus,* methicillin resistant *S. aureus* (MRSA), *Streptococcus pneumoniae*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi, Enterococcus faecalis*, *Klebsiella pneumoniae* and *Plasmodium falciparum* is of growing a major concern (Fauci & Morens, 2012; Lepelletier, Andremont & Grandbastien, 2011; Tängdén et al., 2010; McManus & Keylley, 2005; Byarugaba, 2004; White, 2004). The threat of emerging vector-borne diseases can also cause severe human morbidity and mortality (Benelli & Mehlhorn, 2016). The recent outbreak of Zika virus has issued alarms worldwide (Benelli & Mehlhorn, 2016; Samarasekera U & Triunfol, 2016). Discovery of potent, safe and new antimicrobial agents from plant extracts are urged to be encouraged (Ud-Daula et al., 2016; Tabanca et al., 2015; Demirci et al., 2015; Sadgrove, Greatrex & Jones, 2015; Krist et al., 2015; Stappen et al., 2015a; 2015b; 2015c; Kaczmarczyk et al., 2015; Stappen et al., 2014; Ghosh et al., 2014; Al-Rehaily et al., 2014; Iscan et al., 2012; Ozek et al., 2010; Kurkcuoglu et al., 2010). Furthermore, the effective and safe vector control tools and strategies are

also required by new research activities (Benelli&Mehlhorn, 2016; Samarasekera U. & Triunfol, 2016; Hemingway et al., 2006). To contribute to these studies, we investigated the chemical composition and antimicrobial and antimalarial activity of essential oil of *Tanacetum tomentellum* (Boiss.) Grierson (Asteraceae) from Turkey.

Materials and Methods

Plant Material

The aerial parts of *T. tomentellum* were collected during flowering from Sirnak: Senova-Hakkari, southeast of Turkey at an altitude of 1550 m in July. The voucher specimen has been deposited at the Herbarium in the Gazi University, Faculty of Science, Ankara, Turkey (Voucher specimen no: ZA8188).

Isolation of the Essential Oil

The air dried plant materials (flowers, leaves, and stems) were hydrodistilled for 3 hours using a Clevenger-type apparatus. The resulting oil was stored at 4 °C until the analysis. The oil yield was calculated as 0.38%, v/w on dry weight basis.

Gas Chromatography Analysis Conditions

Essential oil was analysed by GC using a Hewlett Packard 6890 system (SEM Ltd, Istanbul, Turkey) and an HP Innowax FSC column (60 m x 0.25 mm Ø, with 0.25 µm film thickness) was used with nitrogen at 1 mL/min. Initial oven temperature was 60 °C for 10 min, and increased at 4 °C/min to 220 °C, then kept constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage compositions of the individual components were obtained from electronic integration using flame ionization detection (FID, 250 °C). Relative percentages of the separated compounds were calculated from FID chromatograms as cited in Table 1.

Gas Chromatography-Mass Spectrometry Analysis Conditions

GC-MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd, Istanbul, Turkey) and Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with Helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 425.

Identification of Components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes (Adams, 2011; Curves et al., 1985; Wang & Sun, 1987). The fragmentation patterns of the mass spectra were compared with the Wiley (McLafferty & Stauffer, 1989), MassFinder 3 (König, Joulain, & Hochmuth, 2004), in-house “Baser Library of Essential Oil Constituents” and as well as MS literature data (Jennings & Shibamoto, 1980; Joulain & König, 1998; ESO 2000, 1999).

Antimicrobial Activity

The modified Clinical and Laboratory Standards Institute (NCCLS) methods as described earlier (Tabanca et al., 2003; Tabanca et al., 2005) were followed for this study. Ciprofloxacin (ICN Biomedicals; ≥ 98%) for bacteria and amphotericin B (ICN Biomedicals; ≥ 98%) for fungi were used as positive controls. The tested organisms were from the American Type Culture Collection (ATCC): *Candida albicans* (ATCC 90028), *Aspergillus fumigatus* (ATCC 90906), *Cryptococcus neoformans* (ATCC 90113), *Staphylococcus aureus* (ATCC

29213), methicillin-resistant *S. aureus* (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 27853) and *Mycobacterium intracellulare* (ATCC 23068).

Antimalarial Activity

The antimalarial activity against two *Plasmodium falciparum* strains, D6 (chloroquine sensitive) and W2 (chloroquine resistant), was determined using parasitic LDH assay as described earlier (Tabanca et al., 2003; Tabanca et al., 2005). Chloroquine (Sigma-Aldrich; ≥ 98%) and artemisinin (Sigma-Aldrich; ≥ 98%) were used as positive controls.

Results and Discussion

Essential oil was obtained by hydrodistillation from air dried aerial parts of *T. tomentellum.* The oil was subsequently analyzed by GC and GC-MS and the individual identified components with their relative percentages are given in Table 1. Camphor (9.4%), linalool (7.6%), *α*-terpineol (7.1%), *trans*-pinocarveol (5.3%) and *trans*-verbenol (4.5%) were identified as the main components of *T. tomentellum*. The essential oil of this species was also found to be rich in oxygenated monoterpenes, and total 88 components were characterized with a sum of 84.1%.

Table 1. The Composition of *T. tomentellum* Essential Oil

RRI[a]	Compound	%[b]
1032	α-Pinene	0.1
1076	Camphene	<0.01
1118	β-Pinene	0.1
1132	Sabinene	0.1
1176	α-Phellandrene	0.2
1203	Limonene	1.0
1213	1,8-Cineole	2.1
1255	γ-Terpinene	0.1
1280	*p*-Cymene	0.6
1285	Isoamyl isovalerate	0.3
1286	2-Methyl butyl 2-methyl butyrate	0.1
1290	Terpinolene	<0.01
1300	Tridecane	0.2
1348	6-Methyl-5-hepten-2-one	0.1
1439	γ-Campholene aldehyde	0.2
1450	*trans*-Linalool oxide (Furanoid)	<0.01
1452	α,*p*-Dimethylstyrene	0.1
1452	1-Octen-3-ol	0.2
1467	6-Methyl-5-hepten-2-ol	0.4
1468	*trans*-1,2-Limonene epoxide	0.2
1474	*trans*-Sabinene hydrate	0.8
1480	Nerol oxide	0.2
1487	Isoneroloxide-I	0.5
1497	α-Copaene	0.2
1499	α-Campholene aldehyde	1.3
1532	Camphor	9.4
1535	β-Bourbonene	0.1
1553	Linalool	7.6
1556	*cis*-Sabinene hydrate	0.6
1562	Isopinocamphone	0.1
1571	*trans*-*p*-Menth-2-en-1-ol	0.2

1574	Menthyl acetate	0.4
1586	Pinocarvone	2.8
1591	Bornyl acetate	0.1
1600	β-Elemene	0.3
1611	Terpinen-4-ol	1.0
1612	β-Caryophyllene	0.2
1639	*trans*-*p*-Mentha-2,8-dien-1-ol	3.3
1648	Myrtenal	0.5
1663	*cis*-Verbenol	0.5
1670	*trans*-Pinocarveol	5.3
1678	*cis*-*p*-Mentha-2,8-dien-1-ol	1.2
1682	γ-Terpineol	0.1
1683	*trans*-Verbenol	4.5
1700	*p*-Mentha-1,8-dien-4-ol (=Limonen-4-ol)	0.3
1706	α-Terpineol	7.1
1719	Borneol	0.8
1725	Verbenone	0.6
1726	Germacrene D	1.2
1738	*p*-mentha-1,5-dien-8-ol	0.3
1742	β-Selinene	0.4
1744	α-Selinene	0.3
1751	Carvone	1.2
1755	Bicyclogermacrene	0.4
1758	*cis*-Piperitol	0.4
1773	δ-Cadinene	0.8
1797	*p*-Methyl acetophenone	0.2
1802	Cumin aldehyde	0.3
1804	Myrtenol	0.9
1811	*trans*-*p*-Mentha-1(7),8-dien-2-ol	3.0
1823	*p*-Mentha-1(7),5-dien-2-ol	0.3
1845	*trans*-Carveol	2.2
1864	*p*-Cymen-8-ol	0.6
1871	Neryl isovalerate	0.3
1882	*cis*-Carveol	0.4
1896	*cis*-*p*-Mentha-1(7),8-diene-2-ol	3.1
1900	*epi*-Cubebol	0.1
2007	*p*-Mentha-1,8-dien-10-ol	0.2
2008	Caryophyllene oxide	0.3
2029	Perilla alcohol	0.3
2030	Methyl eugenol	0.1
2069	Germacrene D-4β-ol	0.4
2092	β-Oplopenone	0.2
2100	Heneicosane	0.3
2144	Spathulenol	3.0
2187	T-Cadinol	0.5
2209	T-Muurolol	0.6
2219	δ-Cadinol	0.2
2239	Carvacrol	0.4
2245	Elemicine	0.2
2247	*trans*-α-Bergamotol	0.2
2255	α-Cadinol	1.1
2300	Tricosane	0.7
2384	Hexadecanol	0.6

2500	Pentacosane	1.0
2607	1-Octadecanol	0.7
2622	Phytol	0.1
2700	Heptacosane	0.5
	Total	**84.1**

[a]RRI: Relative retention indices calculated against *n*-alkanes, % calculated from FID data

T. tomentellum essential oil was evaluated for antimicrobial activity against human pathogenic bacteria, filamentous fungi, and yeasts in addition to antimalarial activity. No antimicrobial activity was observed at the highest test concentration of 200 μg/mL against *Candida albicans*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRS), *Mycobacterium intracellulare*, *Pseudomonas aeruginosa* and *Aspergillus fumigatus*. The oil only demonstrated mild activity against *Cryptococcus neoformans* with an IC_{50} value of 45 μg/mL where the positive control, amphotericin B, exhibited an IC_{50} of 0.70 μg/mL (Table 2). *T. tomentellum* essential oil showed no antimalarial activity against *P. falciparum* D6 or W2 clone up to 15.9 μg/mL.

Table 2. Antimicrobial activity of *T. tomentellum* essential oil

	IC_{50} (μg/mL)[1]						
Sample	***C. albicans***	***C. neoformans***	***S. aureus***	**MRSA**	***P. aeruginosa***	***M. intracellulare***	***A. fumigatus***
T. tomentellum oil	-	45	-	-	-		-
Ciprofloxacin	NT	NT	0.06	0.06	0.04	0.15	NT
Amphotericin B	0.40	0.70	NT	NT	NT		NT

"-"= inactive at the highest dose of 200 μg/mL; [1]IC_{50}= The concentration (μg/mL) that affords 50% inhibition of growth; NT = Not tested; Ciprofloxacin and Amphotericin B= positive controls

In our study, *T. tomentellum* essential oil showed very poor antimicrobial activity and no antimalarial activity. It appears that the major compounds may not be responsible for the activity or the quantity of these compounds may not be enough to generate the biological activity. In a previous study, (+)-camphor and (-)-camphor were reported to demonstrate insignificant antimicrobial activity on *C. albicans*, however, combination of 1,8-cineole and (-)-camphor produced greater inhibitory effect against *C. albicans* (Viljoen et al., 2003). To the best of our knowledge, this is the first published report describing the chemical composition and biological activity of *T. tomentellum* essential oil. In conclusion, since medicinal and aromatic plants are gaining much interest as antimicrobial agents, the research into the chemistry of plant extracts and responsible active compounds should be encouraged.

ACKNOWLEDGMENT

The authors thank Ms. Marsha A. Wright (antimicrobial) and Mr. John M. Trott (antimalarial) for their contributions in performing bioassays. This work was supported in part by the NIH, NIAID, Division of AIDS, Grant No. AI 27094 (antifungal) and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-1-603 (antibacterial).

REFERENCES

Adams, R. P. (2001). Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation: Carol Stream, IL, USA.

Al-Rehaily, A. J., Alqasoumi, S. I., Yusufoglu, H. S., Al-Yahya, M. A., Demirci, B., Tabanca, N., Wedge, D. E., Demirci, F., Bernier, U. R., Becnel, J. J., Temel, H. E. & Baser, K. H. C. (2014). Chemical Composition and biological activity of *Haplophyllum tuberculatum* Juss. essential oil. *Journal of Essential Oil Bearing Plants*, *17*, 452-459.

Benelli, G. & Mehlhorn, H. (2016). Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitology Research*, *115*, 1747-1754.

Bueno, J. (2015). Models of evaluation of antimicrobial activity of essential oils in vapour phase: a promising use in healthcare decontamination. *Natural Volatiles & Essential Oils*, *2*(2), 16-29.

Byarugaba, D. K. (2004). Antimicrobial resistance in developing countries and responsible risk factors. International *Journal of Antimicrobial Agents*, *24*, 105-110.

Coker, R. J., Hunter, B. M., Rudge, J. W., Liverani, M. & Hanvoravongchai, P. (2011). Emerging infectious diseases in Southeast Asia: regional challenges to control. *The Lancet*, *377*, 599-609.

Curvers, J., Rijks, J., Cramers, C., Knauss, K. & Larson, P. (1985). Temperature programmed retention indexes: calculation from isothermal data. Part 1: Theory. *Journal of High Resolution Chromatography*, *8*, 607-610.

Daszak, P., Cunningham, A. A. & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science*, *287*, 443-449.

Demirci, F., Bayramic, P., Goger, G., Demirci, B. & Baser, K. H. C. (2015). Characterization and antimicrobial evaluation of the essential oil of *Pinus pinea* L. from Turkey. *Natural Volatiles & Essential Oils*, *2*(2), 39-44.

ESO 2000 (1999). *The Complete Database of Essential Oils. Boelens Aroma Chemical Information Service*: The Netherlands.

Fauci, A. S. & Morens, D.M. (2012). The Perpetual challenge of infectious diseases. *The New England Journal of Medicine*, *366*, 454-461.

Ghosh, S., Ozek, T., Tabanca, N., Ali, A., Rehman, J. R., Khan, I. A. & Rangan, L. (2014). Chemical composition and bioactivity studies of *Alpina nigra* essential oils. *Industrial Crops and Products*, *53*, 111-119.

Haines, A., Kovats, R. S. Campbell-Lendrum, D. & Corvalan, C. (2006). Climate change and human health: Impacts, vulnerability and public health. *Public Health*, *120*, 585-596.

Hemingway, J., Beaty, B. J., Rowland, M., Scott. T. W. & Sharp, B. L. (2006). The innovative vector control consortium: improved control of mosquito-borne diseases. *Trends in Parasitology*, *22*, 308-312.

Iscan, G., Demirci, B., Demirci, F., Goger, F., Kirimer, N., Kose, Y. B. & Baser, K. H. C. (2012). Antimicrobial and antioxidant activities of *Stachys lavandulifolia* subsp. *lavandulifolia* essential oil and its infusion. *Natural Product Communications*, *7*(9), 1241-1244.

Jennings, W. G. & Shibamoto, T. (1980). *Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC*; Academic Pres: New York.

Joulain, D. & König, W. A. (1998). *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*. E.B.-Verlag: Hamburg.

Kaczmarczyk, D., Strub, D., Polowy, A., Wilk, K. A. & Lochynski, S. (2015). Selected essential oils in cosmetic emulsions: process oriented stability studies and antimicrobial activity. *Natural Volatiles & Essential Oils*, *2*(4), 27-39.

Krist, S., Banovac, D., Tabanca, N., Wedge, D. E., Gochev, V. K., Wanner, J., Schmidt, E. & Jirovetz, L. (2015). Antimicrobial activity of nerolidol and its derivatives against airborne microbes and further biological activities. *Natural Product Communications*, *10*, 143-148.

König, W. A., Joulain, D. & Hochmuth, D. H. (2004). Terpenoids and related constituents of essential oils. MassFinder 3. Hamburg, Germany: Hochmuth Scientific Consulting.

Kurkcuoglu, M., Iscan, G., Demirci, F., Baser, K. H. C., Malyer, H. & Erdogan, E. (2010). Composition and antibacterial activity of the essential oil of *Ferulago confusa* Velen. *Journal of Essential Oil Research*, *22*(6), 490-492.

Lepelletier, D., Andremont, A. & Grandbastien, B. (2011). Risk of Highly resistant bacteria importation from repatriates and travelers hospitalized in foreign countries: about the French recommendations to limit their spread. *Journal of Travel Medicine*, *18*, 344-351.

Martinez, L. (2000). Global infectious disease surveillance. *International Journal of Infectious Diseases*, *4*, 222-228.

McLafferty, F. W. & Stauffer, D. B. (1989). *The Wiley/NBS Registry of Mass Spectral Data*; J. Wiley and Sons: New York.

McManus, C. J. & Kelley, S. T. (2005). Molecular survey of aeroplane bacterial contamination. *Journal of Applied Microbiology*, *99*, 502-508.

Ozek, G., Demirci, F., Ozek, T., Tabanca, N., Wedge, D. E., Khan, S. I., Baser, K. H. C., Duran A. & Hamzaoglu, E. (2010). Gas chromatographic-mass spectrometric analysis of volatiles obtained by four different techniques from *Salvia rosifolia* Sm., and evaluation for biological activity. *Journal of Chromatography A*, *1217*, 741-748.

Sadgrove, N., Greatrex, B. & Jones, G. L. (2015). α-Cyclodextrin encapsulation enhances antimicrobial activity of cineole-rich essential oils from Australian species of Prostanthera (Lamiaceae). *Natural Volatiles & Essential Oils*, *2*(2), 30-38.

Samarasekera, U. & Triunfol, M. (2016). Concern over Zika virus grips the world. *The Lancet*, *387*, 521-524.

Staphen, I., Wanner, J., Tabanca, N., Wedge, D. E., Ali, A., Khan, I. A., Kaul, V.K., Lal, B., Jaitek, V., Gochev, V., Girova, T., Stoyanova, A., Schmidt, E. & Jirovetz, L. (2014). Chemical composition and biological effects of *Artemisia maritima* and *Artemisia nilagirica* essential oils from western Himalaya. *Planta Medica*, *80*, 1079-1087.

Staphen, I., Ali, A., Tabanca, N., Khan, I. A., Wanner, J., Gochev, V. K., Singh, V., Lal, B., Jaitek, V., Kaul, V. K., Schmidt, E. & Jirovetz, L. (2015a). Antimicrobial and repellent activity of the essential oils of two Lamiaceae cultivated in western Himalaya. *Current Bioactive Compounds*, *11*, 23-30.

Staphen, I., Wanner, J., Tabanca, N., Wedge, D. E., Ali, A., Kaul, V. K., Lal, B., Jaitek, V., Gochev, V. K., Schmidt, E. & Jirovetz, L. (2015b). Chemical composition and biological activity of essential oils from *Dracocephalum heterophyllum* and *Hyssopus officinalis* from Western Himalaya. *Natural Product Communications*, *10*, 133-138.

Staphen, I., Tabanca, N., Ali, A., Wedge, D. E., Wanner, J., Kaul, V. K., Lal, B., Jaitek, V., Gochev, V. K., Schmidt, E. & Jirovetz, L. (2015c). Chemical composition and biological activity of essential oils from wild growing aromatic plant species of *Skimmia laureola* and *Juniperus macropoda* from western Himalaya. *Natural Product Communications*, *10*, 1071-1074.

Tabanca, N., Bedir, E., Kirimer, N., Baser, K. H. C., Khan, S. I., Jacob, M. R. & Khan, I. A. (2003). Antimicrobial compounds from *Pimpinella* species growing in Turkey. *Planta Medica*, *69*, 933-938.

Tabanca, N., Bedir, E., Ferraira, D., Slade, D., Wedge, D. E., Jacob, M. J., Khan, S. I., Kirimer, N., Baser, K. H. C. & Khan, I. A. (2005). Bioactive constituents from Turkish *Pimpinella* species. *Chemistry and Biodiversity*, *2*, 221-232.

Tabanca, N., Demirci, B., Ali, A., Khan, S. I., Jacob, M. R., Aytac, Z. & Khan, I. K. (2015). Chemical composition, biting deterrent, antimalarial and antimicrobial activity of essential oil from *Hypericum scabrum* L. *Current Bioactive Compounds*, *11*, 62-72.

Tängdén, T., Cars, O., Melhus, Å. & Löwdin, E. (2010). Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum β-lactamases: a prospective study with Swedish volunteers. *Antimicrobial Agents and Chemotherapy, 54*, 3564-3568.

Ud-Daula, A. F. M. S., Demirci, F.**,** Salim, K. A, Demirci, B., Lim, L. B. L., Baser, K. H. C. & Ahmad, N. (2016). Chemical composition, antioxidant and antimicrobial activities of essential oils from leaves, aerial stems, basal stems, and rhizomes of *Etlingera fimbriobracteata* (K.Schum.) R.M.Sm. *Industrial Crops and Products*, *89*, 383-389.

Viljoen, A., van Vuuren S., Ernst, E., Klepser, M., Demirci, B., Baser, K. H. C. & van Wyk, B. E. (2003). *Osmitopsis asteriscoides* (Asteraceae) the antimicrobial activity and essential oil composition of a Cape-Dutch remedy. *Journal of Ethnopharmacology, 88*, 137-143.

Wang, T. & Sun, Y. (1987). Definitions and methods of calculation of the temperature-programmed retention index, ITP. I. The relationship between ITP and the net retention volume for *n*-alkanes. *Journal of Chromatography*, *390*, 261-267.

White, N. J. (2004). Antimalarial drug resistance. *The Journal of Clinical Investigation*, *113*, 1084-1092.

Nat. Volatiles & Essent. Oils, 2016; 3(2): 9-14

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Essential oils of Persian Musk rose (*Rosa moschata* Herrm.) as influenced by drying and harvest times

Akbar Karami* and Samira Jandoust

Department of Horticultural Science, Faculty of Agriculture, Shiraz University, Shiraz, IRAN

*Corresponding author. Email: akbarkarami@shirazu.ac.ir, akarami2004@gmail.com

Abstract

Persian musk rose (*Rosa moschata* Hermm.) is widely used in perfumes and cosmetics industries because of its medicinal properties and pleasant odour. Since synthesis and accumulation of volatile compounds affected by flower harvest time, the current study was conducted to evaluate and monitor the changes of volatiles in the essential oil (EO) of Persian Musk Rose petals harvested at different dates (May 11, May 21 and June 01). GC and GC-MS determined the compositions of EO. In addition, the EOs obtained from fresh and dried flowers harvested at different dates were compared to maximize yield and quality of EO. The highest EO yield was observed in the fresh and dried petals harvested at May11, which was significantly higher than the June samples; however, there was no significant difference between May 11 and May 21 samples. The EO composition at different harvest dates was significantly different in the fresh petals, and the highest phenyl ethyl alcohol (14.3%) was observed at the second harvest date. Monoterpenes increased from 2.4% in the first harvest to 8.5% in the third harvest. Aliphatic hydrocarbons showed an increasing pattern in the petals harvested at May 11 (78.6%) to June (86.4%). Concentration of oxygenated monoterpenes significantly reduced in the EO of the dried petals. After drying, phenylpropanoids reduced at the first and the second harvest dates and increased at the third harvest. However, the concentration of aliphatic hydrocarbons increased at the first and the second harvests and decreased at the third harvest date.

Keywords: *Rosa moschata*, drying, essential oils, harvest date

Introduction

Rosa has sixteen wild species in Iran of which *R. moschata* Herrm. with the common names of Persian Musk rose, Nastrane Shiraz and Rose Anbar is one of the most strongly scented rose species with characteristic floral scent molecules such as terpenoids, phenylpropanoids/benzenoids and fatty acid derivatives (Mozaffarian 2013; Jandoust & Karami, 2015). Persian Musk rose is distributed in many local regions of Iran; its wild origins are uncertain but are suspected to lie in the western Himalayas (Khosh-Khui 2014, Honarvar et al., 2011). As, Persian Musk rose has not been confirmed clearly in history, but the supposition is that it is a parent of Damask rose (Jandoust & Karami, 2015). In traditional medicine, hydrosol of Persian Musk rose has been used to strengthen heart muscles, stomach, liver, spleen, nerves, and gums and to strengthen intelligence (Honarvar et al., 2011; Jandoust & Karami, 2015). The quantity and composition of the rose oil distilled from the rose petals are strongly affected by the genotypes, the climatic conditions, diurnal variability, storage conditions, the time of rose petals harvesting, and the technology used for processing and distillation (Baydar & Baydar 2005; Carvalho-Filho et al., 2006; Baydaret al., 2008; Barbosa et al., 2011; Sharmaet al., 2012; Karami et al., 2013; Kumar et al., 2013; Jandoust & Karami, 2015). Therefore, in this research, the seasonal variations of EOs of fresh and dried flowers were studied by GC and GC/MS techniques.

Materials and Methods

Plant material

The fresh flowers of Persian Musk rose were collected from College of Agriculture gardens (Shiraz – 59° 35´E, 29°43´ N, Altitude 1810 m) during their flowering period (May 11, May 21 and June 01, 2014). A specimen (Collector Number: PC 87-23) has been deposited in the Herbarium of the Faculty of Sciences, Shiraz University.

Analysis of the oil

The aerial parts were air-dried at ambient temperature in the shade and air-dried and fresh flower hydrodistilled by using a Clevenger-type apparatus for 3 h. It was dissolved in*n*-hexane (Merck), dried over anhydrous sodium sulphate and stored at 4°C ± 2°C.GC analysis was performed using an Agilent gas chromatograph series 7890-A with a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 column (30 m × 0.32 mm *i.d.*; film thickness 0.25 mm). The injector and detector temperatures were kept at 250 ◦C and 280 ◦C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min; oven temperature program was 60-210 ◦C at the rate of 4◦C/min and then programmed to 240 ◦C at the rate of 20 ◦C/min and finally held isothermally for 8.5 min; split ratio was 1:50. GC-MS analysis was carried out by use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm *i.d.*; film thickness 0.25 m) coupled with 5975-C mass spectrometer. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 230 ◦C and 280 ◦C, respectively. Mass range was from 45 to 550 *amu*. Oven temperature program was the same given above for the GC.

Identification of Compounds

The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C8-C25) and the essential oil on a HP-5 column under the same chromatographic conditions. Identification of individual compounds made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Results and Discussion

In general, seasonal variation and drying had a significantly effect on the EOs contents and composition of Persian musk rose as discussed more below.

Essential oil content

The EO content of both fresh and dried Persian musk rose flowers extracted during their flowering period (May 11, May 21 and June 01). The highest EO content was observed in the fresh and dried petals harvested at May 11, which was significantly higher than the June samples; however, there was no significant difference between May 11 and May 21 samples.

GC-MS analysis

Seasonal variation

In the current study, at the selected time, EOs were collected for periods of 3hrs and analyzed by GC/MS. In this study, a total number of 79 EOs compounds were detected by GC/MS from FW and DW of *R. moschata* at different season (Table 1). In overall, identified components in the subsequent season was representing 97.4–99.9 % of total EOs. The major compounds at different season were identified as 1-nonadecene (5.90-34.80%), *n*-heneicosane (18.8-53.8 %), *n*-nonadecane (9.5-34.4) and phenyl ethyl alcohol (0.1-14.27 %). The EO composition at different harvest dates was significantly different in the fresh petals, and the highest phenyl ethyl alcohol (14.27 %) was observed at the second harvest date. Monoterpenes increased from 2.39% in the first harvest to 8.54% in the third harvest. Aliphatic hydrocarbons showed an increasing trend in the petals harvested at May 11 (78.6%) to June (86.4%). The yield and chemical composition of essential oils (EO) from medicinal plants are related to a variety of internal and external factors such as harvest time and postharvest processing, due to spontaneous conversions and their unstable nature. The effect of harvest time on yield and quality of EO has been widely investigated. Baydar & Baydar, 2005 reported that yield and EO composition of *R. damascena* flowers was significantly different on May 8 and 24. They obtained more EO on May 24, which was about 0.04%. Kumar et al., 2013 showed that harvesting *R. damascena* at different times might affect its EO composition and yield. The highest EO yield in *Thymus vulgaris* have been reported on December. However, the monoterpenic phenols, thymol and carvacrol were higher after blooming on summer (McGimpsey et al., 2006).

Effects of drying

The EO composition of dried petals was significantly dissimilar than the fresh ones at different harvest dates. Concentration of oxygenated monoterpenes significantly reduced in the EO of the dried petals (Table 1). After drying, phenylpropanoids reduced at the first and the second harvest dates and increased at the third harvest. However, the concentration of aliphatic hydrocarbons increased at the first and the second harvests and decreased at the third harvest date. Number of components and composition of the EO obtained from fresh and dried flowers harvested at different times were different. The GC-MS analyses revealed that Persian Musk rose EO is mainly rich of aliphatic hydrocarbons such as *n*-nonadecane, *n*-heneicosane, 1-nonadecane, *n*-tricosene; however, components such as geraniol, citronellol, nerol, comprise lower proportion of the EO. Aliphatic hydrocarbons in fresh petals of EO were about 78.6%, 75.3%, and 86.4% in May 11, May 21 and June. Therefore, aliphatic hydrocarbon increased from first harvest to third harvest. On the other hand, the highest phenyl ethyl alcohol (14.2%), which causes the odor of the rose flowers, was obtained from the fresh tissues harvested in May 21. Hence, it can be concluded that efficiency of EO extraction and quality of EO obtained from flowers harvested on May 21 is significantly higher. Although phenyl ethyl alcohol, or 2-phenylethanol, is the major scent compound of the fresh flower, its content is around 1% in the hydrodistilled rose oil due to the high solubility in residue water or rose water, by-products of hydrodistillation (Baydar et al., 2008). It appears that such investigations are useful for optimizing EO extraction and obtain products with established composition as a market demand. On the other hand, postharvest processing and preserving methods may also influence amount and composition of EO of the harvested material. Drying is widely used for controlling microbial infections, insect pest management and preserving the medicinal plant tissues for long time (Schweiggert et al., 2007). However, drying may influence the amount and composition of essential oil. Barbosa et al., 2006 reported that the citral level in *Lippia alba* dried leaves was significantly increased, however nerol

content showed a significant decrease and geraniol oxidized into geranial after drying. Carvalho-Filho et al., 2006 showed significant changes in the *Ocimum basilicum* L. EO composition during drying.

Table 1. Seasonal changes in essential oil compounds (%) of *Rosa moschata* Herrm.

Components	**RI**	**May 11**		**May 21**		**June**	
		FW	**DW**	**FW**	**DW**	**FW**	**DW**
α-Pinene	930	t	t	-	-	t	-
Myrcene	988	t	-	-	-	-	-
n-Octanal	1001	-	-	-	-	t	-
p-Cymene	1021	-	t	-	-	t	0.2
trans-Rose oxide	1124	-	-	-	-	t	-
Limonene	1025	t	t	-	-	t	0.2
1,8-Cineole	1028	-	t	-	-	-	-
(*Z*)-β-Ocimene	1033	t	-	-	-	-	0.3
Benzene acetaldehyde	1040	t	0.2	-	-	t	-
(*E*)β-Ocimene	1044	t	-	-	-	-	-
dihydro-Tagetone	1048	-	-	-	-	-	3.1
γ-Terpinene	1055	-	t	-	-	-	-
n-Octanol	1066	t	t	-	-	t	-
Linalool	1066	0.1	0.2	-	-	0.1	-
n-Nonanal	1097	0.2	0.7	-	-	0.5	-
Terpinene-4-ol	1174	-	-	-	-	t	-
Phenylethyl alcohol	1110	1.9	1.3	14.3	1.7	0.1	0.8
Camphor	1141	-	t	-	-	-	-
(2*E*)-Nonen-1-al	1155	-	0.3	-	-	t	-
n-Nonanol	1167	t	0.1	-	-	t	0.7
α-Terpineol	1187	t	t	-	-	t	-
n-Dodecane	1196	-	-	-	-	t	-
n-Decanal	1202	0.1	0.5	-	-	0.1	0.5
Citronellol	1225	0.9	0.2	4.1	-	7.6	5.4
Pulegone	1235	-	0.1	-	-	-	-
Neral	1237	0.1	-	-	-	t	-
Geraniol	1251	1.1	0.2	0.4	-	0.7	-
2-Phenylethyl acetate	1253	0.3	0.1	0.3	-	0.2	-
Geranial	1267	0.1	-	-	-	t	-
Nonanoic acid	1267	-	-	-	-	-	6.4
Undecanal	1303	0.1	0.4	-	-	0.1	1.4
Methyl geranate	1320	-	-	-	-	t	-
Citronellyl acetate	1350	-	-	-	-	0.1	-
Eugenol	1353	2.2	1.5	1.2	-	0.4	3.0
Geranyl acetate	1381	-	-	-	-	0.1	-
β-Elemene	1388	-	-	-	-	t	-
n-Decanoic acid	1364	-	0.9	-	-	-	3.0
n-Tetradecane	1396	-	-	-	-	t	-

Methyl eugenol	1401	-	-	-	-	0.4	-
Dodecanal	1405	0.1	0.4	-	-	t	0.5
(*E*)-Caryophyllene	1415	0.8	0.3	-	-	0.2	-
dihydro-β-Ionone	1434	0.5	-	1.1	-	-	-
α-Guaiene	1434	-	-	-	-	0.1	-
α-Humulene	1449	-	-	-	-	0.2	-
Geranyl acetone	1449	0.4	0.7	-	-	-	0.8
(*E*)-β-Farnesene	1453	0.5	-	-	-	-	-
Germacrene D	1476	-	-	-	-	0.1	-
(*E*)-β-Ionone	1482	-	2.0	-	-	0.1	-
n-Pentadecane	1495	-	0.5	-	-	0.4	-
(*E,E*)-α-Farnesene	1505	-	-	-	-	0.1	-
Tridecanal	1506	-	0.2	-	-	-	1.2
(*E*)-Nerolidol	1560	-	-	-	-	0.1	-
Caryophyllene oxide	1577	-	0.2	-	-	t	2.8
2-Phenylethyl tiglate	1581	-	-	-	-	t	-
n-Hexadecane	1595	0.1	0.2	-	-	0.1	-
Tetradecanal	1608	-	-	-	-	t	-
β-Eudesmol	1645	-	-	-	-	0.1	-
α-Eudesmol	1648	-	-	-	-	0.1	-
1-Heptadecene	1695	2.4	1.1	1.2	-	0.4	2.7
(6Z,9E)-Heptadecadiene	1719	1.2	-	-	-	-	-
n-Heptadecane	1695	2.7	4.1	1.6	1.0	3.2	-
(*Z,Z*)-Farnesol	1717	-	-	-	-	0.4	-
Benzyl benzoate	1758	-	0.9	-	0.3	0.3	-
n-Octadecane	1795	-	0.4	-	0.1	0.6	-
Phenylethyl octanoate	1846	-	2.7	-	2.5	0.4	-
1-Nonadecene	1868	34.8	19.6	21.3	7.6	8.6	5.9
n-Nonadecane	1892	12.7	16.6	13.0	9.5	27.4	30.4
2-Phenylethyl phenyl acetate	1902	-	0.8	-	0.5	-	-
1-Eicosene	1970	0.7	0.3	0.7	-	0.5	-
Ethyl palmitate	1995	-	-	-	-	0.3	-
n-Eicosane	1999	0.8	1.4	-	0.9	4.5	2.1
n-Octadecanol	2073	6.1	2.8	3.0	1.7	1.4	-
n-Heneicosane	2103	21.1	32.7	30.9	53.9	26.1	18.8
n-Docosane	2197	0.3	0.6	0.4	1.8	1.5	-
1-Tricosene	2287	0.2	0.2	-	0.5	0.8	-
n-Tricosane	2296	1.6	2.7	5.1	10.5	7.8	-
n-Tetracosane	2400	-	-	-	0.5	0.5	-
n-Pentacosane	2500	0.2	0.4	0.7	1.9	2.9	-
Total		97.4	98.5	99.3	98.9	99.9	99.8

*RI: Retention indices analysed on HP-5; " – ": not detected; t: trace amount; DW: Dry weight; FW: Fresh weight

Conclusion

In general, it is clear that during seasonal variation and emission timing, Persian musk rose EOs varied significantly over time. GC/MS analysis was performed to define both similarities and differences across different seasons and fresh and dried flower in Persian Musk rose. Therefore, drying of plant material and seasonal variations of EOs has essential function and application in agriculture. Consequently, it can be concluded that efficiency of EO extraction and quality of EO obtained from flowers of this plants harvested on May 21 is significantly higher than other harvest times.

REFERENCES

Barbosa, F.F., Barbosa, L.C.A., Melo, E.C., Botelho, F.M. & Santos, R.H.S. (2006). Influence of drying air temperature on the content and chemical composition of essential oil of *Lippia alba* (Mill) N. E. Brown. *Quim Nova*, *29*, 1221-1225.

Baydar, H. & Baydar, N.G. (2005). The effects of harvest date, fermentation duration and Tween 20 treatment on essential oil content and composition of industrial oil of Damask rose (*Rosa damascena* Mill.). *Industrial Crops and Products*, *21*, 251-255.

Baydar, H., Schulz, H., Kruger, H., Erbas, S. & Kineci, S. (2008). Influences of fermentation time, hydro-distillation time and fractions on essential oil composition of Damask rose (*Rosa damascena* Mill.). *Journal of Essential Oil Bearing Plants*, *11*, 224-232.

Carvalho-Filho, J.L.S., Blank, A.F., Alves, P.B., Ehlert, P.A., Melo, A.S., Cavalcanti, S.C. & Silva-Mann, R. (2006). Influence of the harvesting time, temperature and drying period on basil (*Ocimum basilicum* L.) essential oil. *Brazilian Journal of Pharmacognosy*, *16*, 24-30.

Honarvar, M., Javidnia, K. & Khosh-Khui, M. (2011). Essential oil composition of fresh and dried flowers of *Rosa moschata* from Iran. *Chemistry of Natural Compounds*, *47*, 826-828.

Jandoust, S. & Karami, A. (2015). Seasonal variation in floral scent of Persian Musk rose (*Rosa moschata* Hermm.). *Journal of Medicinal Plants and By-products*, 4, 243-247.

Karami, A., Khosh-Khui, M., Salehi, H. & Saharkhiz, M.J. (2013). Headspace analysis of floral scent from two distinct genotypes of Iranian Damask rose (*Rosa damascena* Mill.). *Journal of Essential Oil Bearing Plants*, *16*, 489-498.

Khosh-Khui, M. (2014). Biotechnology of scented roses: a review. *International Journal of Horticultural Science and Technology*, *1*, 1-20.

Kumar, R., Sharma, S., Sood, S., Agnihotri, V.K. & Singh, B. (2013). Effect of diurnal variability and storage conditions on essential oil content and quality of damask rose (*Rosa damascena* Mill.) flowers in north western Himalayas. *Scientia Horticulturae*, *154*, 102-108.

McGimpsey, J.A., Douglas, M.H., VanKlink, J.W., Beauregard, D.A. & Perry, N.B. (2006). Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L. in New Zealand. *Flavour and Fragrance Journal*, *9*, 347-352.

Mozaffarian, V. (2013). *Identification of Medicinal and Aromatic Plants of Iran*. Farhang Moaser Publishing, IR.

Sharma, B., Singh, B., Dhyani, D., Verma, P.K. & Karthigeyan, S. (2012). Fatty acid composition of wild growing rose species. *Journal of Medicinal Plants Research*, *6*, 1046–1049.

Nat. Volatiles & Essent. Oils, 2016; 3(2): 15-19

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Composition of the essential oil of the *Hyssopus officinalis* L. subsp. *angustifolius* (Bieb.) Arcangeli

Mine Kürkçüoğlu[1,*], Şenay Arıkan Eser[1,2] and K. Hüsnü Can Başer[1,3]

[1] Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskisehir, TURKEY
[2] AUBIBAM (Plant, Drug and Scientific Research Center), Anadolu University, Eskisehir, TURKEY
[3] Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Near East Boulevard, ZIP: 99138, Nicosia, N. CYPRUS

*Corresponding author. Email: mkurkcuo@anadolu.edu.tr

Abstract

Hyssopus officinalis subsp. *angustifolius* (Bieb) Arcangeli growing in North East and South Anatolia is the only member of this genus growing in Turkey. Aerial parts of the plant material were hydrodistilled and the resulting oil was analyzed by GC and GC/MS simultaneously. Main components were identified as pinocarvone (27.1%), β-pinene (19.0%), and isopinocamphone (13.6%), respectively.

Key words: *Hyssopus officinalis*, Labiatae, Essential oil, pinocarvone, β-pinene, isopinocamphone

Introduction

Members of the genus *Hyssopus* L. (Lamiaceae) are aromatic semi-woody perennials (Mill, 1982). The most widespread species *H. officinalis* is naturalized in Western and Central Europe. It is known and recognised as "hyssop". In some regions, it is used as a spice to flavour soups (Small, 1997) or steeped in water to make a purgative infusion (Mill, 1982). Hyssop is considered a reasonable effective remedy for mild irritations of the respiratory tract that accompany the common cold (Baytop, 1994). *H. officinalis* subsp. *angustifolius* (Bieb) Arcangeli growing in North East and South Anatolia is the only member of this genus growing in Turkey. This species is locally known as 'çördük' (Mill, 1982) and 'zulfa otu'. Contrary to its use Europe, it is not a common medicinal plant in Turkey (Baytop, 1994).

According to the literature survey, the chemical composition of the essential oil of *H. officinalis* of various origins have been investigated. Letessier, et al., 2001; Kızıl et al., 2008; Kızıl et al. 2010; Moro et al., 2011; Fathiazad & Hamedeyazdan, 2011; Mohan et al., 2012; Figueredo et al., 2012; Dzami´c et al., 2013; Pandey et al., 2014; Hristova et al., 2015; Figueredo et al., 2015; Stappen et al., 2015, Schultz & Stahl-Biskup (1991), Gorunovic et al. (1995), Vallejo et al. (1995), Garg et al. (1999), Piccalgia et al. (1999), Salvatore et al. (1998) have determined the composition of various oils of hyssop collected from Turkey, Germany, Montenegro, Spain, India, Italy and France.

Materials and Methods

Plant Material

Aerial parts of *Hyssopus officinalis* subsp. *angustifolius* were collected in August 2003 from Kastamonu: Ihsangazi in Turkey. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University in Eskisehir, Turkey (ESSE 14254).

Isolation of the Essential Oil

Air dried aerial parts were hydrodistilled for 3 h using a Clevenger-type apparatus to yield 2.2 % oil.

GC and GC/MS Analyses

The GC analysis was carried out using an Agilent 6890N GC system. Flame ionization detector (FID) temperature was 300°C. To obtain the same elution order with GC/MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m*/*z* 35 to 450.

Identification of the Volatile Compounds

The components of the sample were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Adams Library (Adams, 2007), MassFinder Library (Hochmuth, 2008), Wiley GC/MS Library (McLafferty & Stauffer, 1989) and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Alkanes (C8-C22) were used as reference points in the calculation of relative retention indices (RRI) (Curvers, Rijks, Cramers, Knauss, & Larson, 1985). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Results and Discussion

As shown in Table 1, overall 51 components were identified, constituting 98.6 % of the oil of *Hyssopus officinalis* ssp. *angustifolius*. The relatively high yielding characteristic oil contained pinocarvone (27.1%), β-pinene (19.0%), isopinocamphone (13.6%), and pinocamphone (13.6%) as main constituents. According to the literature, so far three types of *Hyssopus* oils have been encountered, namely; monoterpene ketone-type, 1,8-cineole type, and methyl eugenol-type (Gorunovic et al., 1995; Vallejo et al. 1995; Lawrence, 1993), respectively.

The oil examined in this present study is rich of monoterpene ketones and among these, the content of pinocarvone is remarkably high. This value is higher than the oils of *Hyssopus* previously studied, except for the oil from Gümüşhane, with 36.3% pinocarvone (Özer et al. 2005). According to the ISO 9847/1991 standard, commercial oil should contain 40-67.5 % monoterpene ketones and 13.5-23.0 % β-pinene (Mazzanti et al. 1998). The values obtained in this study (55 % monoterpene ketones and 19 % β-pinene) are compatible with the standard values.

Table 1. Chemical composition of the essential oil of *Hyssopus officinalis* ssp. *angustifolius*

RRI[a]	**Compounds**	**%**
1018	Methyl 2-methyl butyrate	<0.1
1032	α-Pinene	1.0
1035	α-Thujene	0.3
1076	Camphene	0.1
1078	5-Methyl 3-hexanone	<0.1
1118	β-Pinene	19.0
1132	Sabinene	2.1
1159	δ-3-Carene	<0.1
1174	Myrcene	4.2
1188	α-Terpinene	0.1
1195	Dehydro 1,8-cineole	<0.1
1203	Limonene	1.0
1213	1,8-Cineole	6.3
1232	(*Z*)-3-Hexenal	<0.1
1246	(*Z*)-β-Ocimene	1.2
1255	γ-Terpinene	0.2
1266	(*E*)-β-Ocimene	0.2
1266	5-Metil 3-heptanone	0.1
1280	*p*-Cymene	0.1
1290	Terpinolene	0.1
1394	Myrtenyl methyl ether	4.1
1452	1-Octen-3-ol	0.2
1474	*trans*-Sabinene hydrate	0.3
1500	Bicycloelemene	<0.1
1563	Pinocamphone	13.6
1553	Linalool	0.1
1562	Isopinocamphone	13.6
1586	Pinocarvone	27.1
1603	Nopinone	0.2
1612	β-Caryophyllene	0.5
1638	*cis*-*p*-Menth-2-en-1-ol	0.1
1648	Myrtenal	0.4
1670	*trans*-Pinocarveol	0.4
1682	δ-Terpineol	0.1
1687	α-Humulene	0.1
1706	α-Terpineol	0.1
1719	Borneol	0.2
1726	Germacrene D	0.3
1755	Bicyclogermacrene	0.1
1773	δ-Cadinene	<0.1
1804	Myrtenol	0.5
1830	2,6-Dimethyl-3[*E*],5[*E*],7-octatriene-2-ol	0.1

1838	[*E*]-β-Damascenone	<0.1
1845	*trans*-Carveol	<0.1
1864	*p*-Cymen-8-ol	<0.1
2008	Caryophyllene oxide	0.2
2029	Perillyl alcohol	0.1
2073	*p*-Mentha-1,4-dien-7-ol	0.1
2113	Cumin alcohol	<0.1
2114	Spathulenol	0.1
2186	Eugenol	<0.1

[a] Relative Retention Index

REFERENCES

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Carol Stream, IL: Allured Publ. Corp.

Baytop, T. (1994). Türkçe Bitki Adları Sözlüğü, p. 294, Ankara.

Curvers, J., Rijks, J., Cramers, C., Knauss, K. & Larson, P. (1985). Temperature pro-grammed retention indexes: calculation from isothermal data. Part 1: Theory. *Journal of High Resolution Chromatography, 8*, 607–610.

Dzami´c, A.M., Sokovi´c, M.D., Novakovi´c, M., Jadranin, M., Risti´c, M.S., Tesevi´c,V. & Marin, P.D. (2013) . Composition, antifungal and antioxidant properties of *Hyssopus officinalis* L. subsp. *pilifer* (Pant.) Murb. Essential oil and deodorized extracts, *Industrial Crops and Products, 51*, 401– 407.

Fathiazad, F. & Hamedeyazdan, S. (2011). A rewiev on *Hyssopus officinalis* L.: Composition and biological activities, *African Journal of Pharmacy and Pharmacology, 5*(17), 1959-1966.

Figueredo, G., Özcan, M.M., Chalchat, J.C., Bagci, Y. & Chalard, P. (2012). Chemical Composition of Essential Oil of *Hyssopus officinalis* L. and *Origanum acutidens*, *Journal of Essential Oil Bearing Plants, 15*(2), 300 – 306.

Figueredo, G., Özcan, M. M., Chalchat, J. C., Bagci, Y., Chalard, P., Al Juhaimis, F. & Dogu, S. (2015). Chemical composiition of essential oil from aerial parts and fruits of *Hyssopus officinalis* L., *Origanum acutidens*, *Prangos uechritzii*, *H-hircinum* L. and *Hypericum polyphyllum* subsp. *polyphyllum*, *Zeitschrift fur Arznei-& Gewurzpflanzen, 20*(4), 185-191.

Garg, S. N., Naquvi, A., A., Singh, A Ram, G. & Kumar, S. (1999). Composition of Essential Oil from an Annual Crop of *Hyssopus officinalis* Grown in Indian Plains, *Flavour and Fragrance Journal, 14*, 170-172.

Gorunovic, M. S., Bogavic M. P., Chalcat, J. C. & Chabart, J. L. (1995). Essential Oil of *Hyssopus officinalis* L., Lamiaceae of Montenegro Origin, *Journal of Essential Oil Research, 7*, 39-43.

Hochmuth, D. H. (2008). *MassFinder-4*, Hamburg, Germany: Hochmuth Scientific Consulting.

Hristova, Y., Wanner, J., Jirovetz, L., Stappen, I., Iliev, I. & Gochev, V. (2015). Chemical composition and antifungal activity of essential oil of *Hyssopus officinalis* L. from Bulgaria against clinical isolates of Candida Species, *Biotechnology & Biotechnological Equipment, 29* (3), 592-601.

Kızıl, S., Toncer, O , Ipek, A. , Arslan, N. , Saglam, S. & Khawar, K. M. (2008). Blooming stages of Turkish hyssop (*Hyssopus officinalis* L.) affect essential oil composition, *Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 58*: 273-279.

Kızıl, S., Haşimi, N., Tolan, V., Kılınç, E. & Karataş, H. (2010). Chemical Composition, Antimicrobial and Antioxidant Activities of Hyssop (*Hyssopus officinalis* L.) Essential Oil, *Not. Bot. Hort. Agrobot. Cluj 38* (3) 2010, 99-103.

Lawrence, M. B. (1993). Labiatae Oils-Mother Nature's Chemical Factory, in: Essential Oils, M.B. Lawrence (ed.), p. 188-197. Allured Publishing Co.

Letessier, M.P., Svoboda, K.P. & Walters, D.R. (2001). Antifungal Activity of the Essential Oil of Hyssop (*Hyssopus offcinalis*), *Journal of Phytopathology, 149*, 673-678.

Mazzanti, G., Lu, M. & Salvatore, G. (1998). Spasmolytic Action of the Essential Oil from *Hyssopus officinalis* L. var. *decumbens* and it's Major Components, *Phytotherapy Research Suppl., 12*, 92-94.

McLafferty, F.W. & Stauffer, D.B. (1989). *The Wiley/NBS Registry of Mass Spectral Data*, New York: J. Wiley and Sons.

Mill R.R. (1982). *Hyssopus* L. in Flora of Turkey and the East Eagean Islands, P.H. Davis (ed.) Univ. Pres Edinburgh.

Mohan, M., Seth, R., Singh, P., Lohani, H. & Gupta, S. (2012). Composition of the Volatiles of *Hyssopus officinalis* (L.) and *Thymus serpyllum* (L.) from Uttarakhand Himalaya, *Natl. Acad. Sci. Lett*.; doi: 10.1007/s40009-012-0075-1.

Moro, A., Zalacain, A., Hurtado de Mendoza, J. & Carmona, M. (2011). Effects of Agronomic Practices on Volatile Composition of *Hyssopus officinalis* L. Essential Oils, *Molecules 16*, 4131-4139; doi:10.3390/molecules16054131.

Ozer H., Şahin F., Kılıç H. & Güllüce M. (2005). Essential Oil Composition of *Hyssopus officinalis* L. subsp. *angustifolius* (Biab.) Arcangeli from Turkey. *Flavour and Fragrance Journal*, 20, 42-44.

Pandey, V., Verma, R.S., Chauhan, A. & Tiwari, R. (2014). Compositional variation in the leaf, flower and stem essential oils of Hyssop (*Hyssopus officinalis* L.) fromWestern-Himalaya, *Journal of Herbal Medicine, 4*, 89–95.

Piccalgia, R., Pace, L. & Tammaro F. (1999). Characterization of Essential Oils From Tree Italian Ecotypes of [*Hyssopus officinalis* L. subsp. *aristatus* (Gordon) Briq.], *Journal of Essential Oil Research, 11*, 693-699.

Schultz, G. & Stahl-Biskup, E. (1991). Essential Oils and Glycosidic Bound Volatiles from Leaves, Stems, Flowers and Roots of *Hyssopus officinalis* L. (Lamiaceae), *Flavour and Fragrance Journal*, *6*, (1), 69-73.

Vallejo, M. C. C., Herraiz, J. G., Alonso, M. J. P. & Negueruela, A.V. (1995). Volatile Oil of *Hyssopus officinalis* L. from Spain, *Journal of Essential Oil Research,* 7, 567-568.

Salvatore, G., D'andrea, A. & Nicoletti, M. (1998). A Pinocamphone Poor Oil of *Hyssopus officinalis* L. var. *decumbens* from France (Banon), *Journal of Essential Oil Research*, *10*, 563-567.

Small, E. (1997). Culinary Herbs, p. 291-294. NRC Research Press, Ottava.

Stappen, I., Wanner, J., Tabanca, N., Wedge, D. E., Ali, A., Kaul, V., Lal, B., Jaitak, V., Gochev, V. K., Schmidt, E. & Jirovetz, L. (2015). Chemical Composition and Biological Activity of Essential Oils of *Dracocephalum heterophyllum* and *Hyssopus officinalis* from Western Himalaya, *Natural Product Communication*, *10*(1), 133-138.

Nat. Volatiles & Essent. Oils, 2016; 3(2): 20-25

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Antique lavender essential oil from 1945, its chemical composition and enantiomeric distribution

Prabodh Satyal* and Robert S. Pappas

Essential Oil University, New Albanay, IN 47150, USA

*Corresponding author. Email: prabodh@essentialoils.org

Abstract

Properly stored lavender from 1945 was collected in French market and Gas Chromatography and Mass Spectrometry (GCMS) analysis was carried out to see possible chemical transformation and chemical composition. Total of 102 peaks (100% components) were identified, out of them 10 were oxidized minor components (total 0.41%). The lavender essential oil was dominated by the linalool (40.8%), terpinen-4-ol (9.9%), α-terpineol (3.5%), linalyl acetate (16.4%), lavandulyl acetate (3.8%), and *trans*-caryophyllene (2.2%). Enantiomeric distribution of major monoterpenoids were also measured.

Keywords: Lavender oil-1945, linalool, linalyl acetate, terpinen-4-ol, oxidation, enantiomeric distribution, storage of essential oils.

Introduction

Lavandula angustifolia Mill. (also known as lavender) comprised of more than 39 known species is one of the most popular essential oils in aromatherapy from Lamiaceae family. The flowering plant of lavender (*Lavandula angustifolia* or *Lavandula officinalis*) is used to produce lavender essential oils. It is sweet aromatic and colourless essential oil. It is widely used in aromatherapy and fragrance industry because of its biological activities and aromatic potentials.

Proper storage of essential oils is always mandatory since lavender does not contain any antioxidant molecules in its essential oil (Hagvall et al., 2008). Presence of water molecules and aerial exposure causes severe oxidation of essential oils (Guenther, 1948).

The major components of lavender are linalool, linalyl acetate and caryophyllene (Sköld et al., 2007). Out of them *trans*-caryophyllene is most susceptible to oxidation to produce oxidized product (caryophyllene oxide) which is the least sensitizing molecule (Sköld et al., 2006). Highly oxidized lavender produces hydroxide, epoxides and hydroperoxides of linalool, linalyl acetate, and caryophyllene (Nilsson et al., 2008). Autooxidation happens in the allylic positions of double bonds in linalool and linalyl acetate (C6-C7) (Sköld et al 2007). They are found to possess sensitizing effect on skin (Nilsson et al., 2008), however there are few contact allergies have been reported (Letizia et al., 2003). So, proper handling and storage of fragrance compounds are always advised (Turek & Stintzing, 2013).

Numerous studies on lavender essential oils have been carried out, however this is the first report on chemical composition and enantiomeric distribution of 71 year old lavender so far from our understanding.

Materials and Methods

Plant Material

Essential oil sample was purchased in 1945 in Paris, France. The essential oil was stored on amber glass container in refrigerator until analysis was carried out.

Gas Chromatographic – Mass Spectral Analysis

The essential oil of lavender was analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ultra operated in the EI mode ((electron energy = 70eV), scan range = 3.0 scans/sec), and GCMS Solution software. The GC column was Zebron ZB-5MS fused silica capillary column with a (5% phenyl)-polymethyl siloxane stationary phase a film thickness of 0.25 mm. The career gas was helium with a column head pressure 80 psi and flow rate of 1.37 ml/min. Injector temperature was 250 ^{o}C and the ion source temperature was 200 ^{o}C, increase in temperature rate 2 ^{o}C/min to 260 ^{o}C. The GC oven temperature program was programmed for 50 ^{o}C initial temperature, increase in rate 2 ^{o}C/min to 260 ^{o}C. A 5% w/v solution of the sample in CH_2Cl_2 was prepared and 0.1 µL was injected in splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of n-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007), and stored in the MS library.

Chiral Gas Chromatographic-Mass Spectral Analysis

Chiral analysis of the lavender essential oils was performed on a Shimadzu GCMS-QP2010S operated in the EI mode ((electron energy=70eV), scan range = 3.0 scans/sec). GC equipped with a Restek B-Dex 325 capillary column (30 m×0.25 mm ID×0.25 _m film). Oven temperature was started at 50 ◦C, and then gradually raised to 120 ◦C at 1.5 ◦C/min. The oven was then raised to 200 ◦C at 2 ◦C/min and held for 5 min. Helium was the carrier gas and was flow rate was maintained at 1.8ml/min. Samples were diluted 3% w/v with CH_2Cl_2 and then a 0.1 µL sample was injected in a split mode with a split ratio of 1:45.

Results and Discussion

Essential Oil Composition

The lavender oil was purchased commercially in 1945 and stored in dark bottle for 71 years. A total of 102 peaks (100% components) were identified, out of them 10 oxidized minor components (total 0.41%) were detected (see Table 1). The studied lavender is the least oxidized as compared to studies carried out in stability studies of tea tree oil (Trachida et al., 2010). The lavender essential oil was dominated by the linalool (40.8%), terpinen-4-ol (9.9%), α-terpineol (3.5%), linalyl acetate (16.4%), lavandulyl acetate (3.8%), and *trans*-caryophyllene (2.2%). These results are quantitatively different than any result previously published in a significant review paper (Lawrence, 2012) and from ISO standard of French lavender (ISO lavender, 2016). Out of the published research high terpinen-4-ol, high linalool, and low linalyl acetate has not previously been reported (Lawrence, 2012), the maximum amount of terpinen-4-ol was reported as 5.0-6.2% (Chemat et al., 2006; Pavela, 2006).

Increase in oxidation products like linalool oxide, caryophyllene oxide indicates autoxidation of linalool and caryophyllene (Misharina et al., 2003; Sköld et al., 2008).

Linalool readily undergoes autooxidation to form cyclized molecule: furanoids and pyranoids (Sköld et al., 2008), so the concentration of furanoids and pyranoids in this report is relatively high as compared to freshly distilled lavender (Lawrence, 2012). Whereas due to large bulky acetate group, linalyl acetate is unable to form cyclized furanoid, and pyranoids. Sesquiterpene oxide is formed from oxidation of sesquiterpene (Hădărugă et al., 2014). Ascaridole glycol *cis* and *trans* along with epoxide are observed as oxidized product of terpinen-4-ol (Tranchida et al 2010).

Enantiomeric distribution of lavender essential oil was also studied (Table-2), which is similar to previously studied lavender essential oil (Baser et al., 2005). Total 70.64% of monoterpene was enantiomerically analyzed to see the distribution. L-linalool, L-linalyl acetate, D-Terpinen-4-ol, and L-α-Terpineol were the major enantiomer in the studied lavender.

In addition to genetic variation, other factors such as age, vegetative cycle stage, climate, season, soil composition, etc. are among several things responsible for the considerable variation in essential oil compositions (Satyal & Pappas, 2016). Based on the observed chemical composition, this variety of French lavender may be treated as a distinct chemical composition. The large variation of chemical composition might be related to different distillation technique or could have plant material was genetically modified on time.

Table 1. Chemical composition of lavender EO purchased in 1945 in France

RI	**Compound**	**%**
795	*n*-Octane	0.02
863	*n*-Hexanol	0.03
922	Tricyclene	0.05
924	α-Thujene	0.1
932	α-Pinene	0.3
949	Camphene	0.3
971	Sabinene	0.02
977	Octen-3-ol	0.6
983	3-Octanone	0.9
988	Myrcene	0.4
989	*trans*-dehydroxy-Linalool oxide	0.04
995	Butyl butanoate	0.05
996	3-Octanol	0.2
1005	*cis*-dehydroxy-Linalool oxide	0.03
1008	δ-3-Carene	0.2
1011	Hexyl acetate	0.2
1019	*o*-Cymene	0.08
1024	*p*-Cymene	0.5
1028	Limonene	0.3
1030	β-Phellandrene	0.05
1031	1,8-Cineole	1.6
1034	*cis*-β-Ocimene	0.8
1045	*trans*-β-Ocimene	0.7
1057	γ-Terpinene	0.04
1069	*cis*-furanoid-Linalool oxide	0.4
1084	Terpinolene	0.04
1086	*trans*-furanoid-Linalool oxide	0.2
1091	Rosefuran	0.02
1100	**Linalool**	**40.8**
1103	Hotrienol	0.2
1104	Hexyl-propionate	0.03
1106	Octen-3-yl acetate	2.3
1118	3-Octanol acetate	0.2

1124	*cis*-p-Menth-2-en-1-ol	0.04
1127	*allo*-Ocimene	0.06
1138	Nopinone	0.03
1140	*trans*-Pinocarveol	0.02
1147	Camphor	0.8
1150	Nerol oxide*	0.02
1162	Lavandulol	0.9
1169	*cis*-pyranoid-Linalool oxide	0.04
1172	Borneol	2.3
1174	*trans*-pyranoid-Linalool oxide	0.04
1180	**Terpinen-4-ol**	**9.9**
1183	*p*-methyl-Acetophenone	0.04
1186	Cryptone+ p-Cymenol [2:1]	0.4
1187	2,6-dimethyl-octa-3,7-diene-2,6-diol*	0.09
1191	Hexyl butanoate	0.2
1194	**α-Terpineol**	**3.5**
1200	3-*cis*-Octenyl acetate	0.04
1207	3-*trans*-Octenyl acetate	0.09
1209	Verbenone	0.03
1211	Linalool formate	0.3
1216	γ-Geraniol	0.07
1218	*trans*-Carveol	0.02
1223	Nerol	0.5
1228	Bornyl formate	0.1
1241	Cumin aldehyde	0.1
1243	Carvone	0.03
1249	**Linalyl acetate**	**16.4**
1253	Piperitone	0.01
1266	Geranial	0.04
1271	Linalool hydroperoxide*	0.03
1273	*trans*-Ascaridol glycol*	0.03
1275	Neryl formate	0.06
1277	Phellandral	0.05
1282	**Lavandulyl acetate**	**3.8**
1283	Bornyl acetate	0.4
1290	*p*-Cymen-7-ol	0.08
1296	Geranyl formate	0.2
1312	Epoxylinalyl acetate*	tr
1331	Hydroperoxy linalyl acetate*	0.08
1338	Hydroxylinalyl acetate*	0.06
1344	Benzyl butanoate	0.03
1345	α-Terpinyl acetate	0.04
1357	Neryl acetate	1.00
1359	*epoxy*-Ascaridole*	0.03
1377	Geranyl acetate	2.0
1379	3,4-dihydro-Coumarin	0.03
1383	α-Bourbonene	0.04

1384	Hexyl hexanoate	0.03
1386	7-*epi*-Sesquithujene	0.03
1412	α-*cis*-Bergamotene	0.05
1419	*tran*-Caryophyllene	2.2
1432	α-*trans*-Bergamotene + Coumarin (tr)	0.2
1434	Isoamyl octanoate + Elemene <γ->	0.03
1443	*cis*-β-Farnesene	0.04
1446	*epi*-β-Santalene	0.03
1451	*trans*-β-Farnesene	0.5
1455	α-Humulene	0.05
1456	Sesquisabinene	0.04
1480	Germacrene D	0.1
1483	Neryl isobutanoate	0.06
1485	*p*-Menthane-1,2,4-triol*	0.03
1506	β-Bisabolene	0.06
1512	δ-Amorphene	0.1
1517	Isobicyclogermacrene	0.2
1581	Caryophyllene oxide	0.4
1598	Oxidized sesquiterpene*	0.04
1640	*Epi*- α-Cadinol	0.09
1746	α -Bisabolol oxide A	0.05
1897	Thiogeraniol	0.04
	Total compound identified (102)	100%
	Compounds produced by oxidation (10)	0.41%

Where RI Retention Index determined to a series of n-Alkanes on ZB-5 column; compounds are listed in order of elution (Increasing RI), % refers to Percent of total oil, "tr" indicates trace components present less than 0.01% of total oil composition. Those components with asterisk "*" are oxidized products.

Table 2. Enantiomeric distribution of major monotepenes in Lavender essential oil from 1945.

Compounds	**Relative %**	**ee (%)**	**ed [d to l] (%)**
Linalool	40.8	78	11 to 89
Linalyl acetate	16.4	52	2 to 98
Terpinen-4-ol	9.9	100	0 to 100
α- Terpineol	3.5	48	26 to 74
Total %	**70.6**		

Where "ee" stands for enantiomeric excess and "ed" stands for enantiomeric distribution

ACKNOWLEDGMENT

PS and RSP are grateful to Aaron Sorensen for his valuable suggestion during manuscript preparation.

REFERENCES

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography*. Illinois: Allured.

Baser, K. H., Özek, T. & Konakchiev, A. (2005). Enantiomeric Distribution of Linalool, Linalyl Acetate and Camphor in Bulgarian Lavender Oil. *Journal of Essential Oil Research, 17*(2), 135-136.

Chemat, F., Lucchesi, M., Smadja, J., Favretto, L., Colnaghi, G. & Visinoni, F. (2006). Microwave accelerated steam distillation of essential oil from lavender: A rapid, clean and environmentally friendly approach. *Analytica Chimica Acta, 555*(1), 157-160.

Guenther, E. (1948). *The essential oils Vol. I: History origin in plants production analysis.* Newyork: Van Nostrand.

Hagvall, L., Sköld, M., Bråred-Christensson, J., Börje, A. & Karlberg, A. (2008). Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis, 59*(3), 143-150.

Hădărugă, D. I., Hădărugă, N. G., Costescu, C. I., David, I. & Gruia, A. T. (2014). Thermal and oxidative stability of the *Ocimum basilicum* L. essential oil/β-cyclodextrin supramolecular system. *Beilstein Journal of Organic Chemistry Beilstein J. Org. Chem., 10*, 2809-2820.

International Standard ISO 3515 (2002), Oil of lavender (*Lavendula angustifolia* Mill.)

Lawrence, B. M. (2012). *Essential Oils 2008-2011*. Carol Stream, IL: Allured.

Letizia, C., Cocchiara, J., Lalko, J. & Api, A. (2003). Fragrance material review on linalyl acetate. *Food and Chemical Toxicology, 41*(7), 965-976.

Letizia, C., Cocchiara, J., Lalko, J. & Api, A. (2003). Fragrance material review on linalool. *Food and Chemical Toxicology, 41*(7), 943-964.

Misharina, T., Polshkov, A. N., Ruchkina, E. L. & Medvedeva, I. B. (2003). Changes in the composition of the essential oil of Marjoram during storage. *Applied Biochemistry and Microbiology, 39*(3), 311-316.

Nilsson, J., Carlberg, J., Abrahamsson, P., Hulthe, G., Persson, B. & Karlberg, A. (2008). Evaluation of ionization techniques for mass spectrometric detection of contact allergenic hydroperoxides formed by autoxidation of fragrance terpenes. *Rapid Communications in Mass Spectrometry Rapid Commun. Mass Spectrom., 22*(22), 3593-3598.

Pavela, R. (2006). Insecticidal Activity of Essential Oils Against Cabbage Aphid Brevicoryne brassicae. *Journal of Essential Oil Bearing Plants, 9*(2), 99-106.

Satyal, P. & Pappas, R. S. (2016). First Reporting on the Chemistry and Biological Activity of a Novel Boswellia Chemotype: The Methoxy Alkane Frankincense. *Global Journal of Frontier Science Research B, 16*, 1-9.

Sköld, M., Borje, A., Matura, M. & Karlberg, A. (2002). Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis, 46*(5), 267-272.

Sköld, M., Börje, A., Harambasic, E. & Karlberg, A. (2004). Contact Allergens Formed on Air Exposure of Linalool. Identification and Quantification of Primary and Secondary Oxidation Products and the Effect on Skin Sensitization. *Chemical Research in Toxicology, 17*(12), 1697-1705.

Sköld, M., Karlberg, A., Matura, M. & Börje, A. (2006). The fragrance chemical β-caryophyllene—air oxidation and skin sensitization. *Food and Chemical Toxicology, 44*(4), 538-545.

Sköld, M., Hagvall, L. & Karlberg, A. (2007). Autoxidation of linalyl acetate, the main component of lavender oil, creates potent contact allergens. *Contact Dermatitis, 58*(1), 9-4.

Tranchida, P. Q., Shellie, R. A., Purcaro, G., Conte, L. S., Dugo, P., Dugo, G. & Mondello, L. (2010). Analysis of Fresh and Aged Tea Tree Essential Oils by Using GCxGC-qMS. *Journal of Chromatographic Science, 48*(4), 262-266.

Turek, C. & Stintzing, F. C. (2013). Stability of essential oils: a review. *Comprehensive Reviews in Food Science and Food Safety*, *12*(1), 40-53.

Nat. Volatiles & Essent. Oils, 2016; 3(2): 26-34

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Composition & biological activity of *Cyperus rotundus* L. tuber volatiles from Saudi Arabia

Shaza Al-Massarani[1*], Fedha Al-Enzi[1], Maram Al-Tamimi[1], Nourah Al-Jomaiah[1], Roaa Al-amri[1], K. Hüsnü Can Başer[2], Nurhayat Tabanca[3,4], Alden S. Estep[4,5], James J. Becnel[4], Jeffrey R. Bloomquist[3] and Betül Demirci[6]

[1]Department of Pharmacognosy, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, SAUDI ARABIA
[2]Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Nicosia, N. CYPRUS
[3]Department of Entomology&Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL32610,USA
[4]USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608 USA
[5]Navy Entomology Center of Excellence, CMAVE Detachment, 1700 SW 23rd Drive, Gainesville, FL 32608-1069 USA
[6]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, 26470, TURKEY

*Corresponding author. Email: shazamas@yahoo.com

Abstract

The present study was conducted to investigate the composition of the oil of *Cyperus rotundus* L. rhizomes collected in Saudi Arabia, and to evaluate *in vitro* cytotoxic, antimicrobial and mosquito mortality activities of both the oil and the alcoholic extract of the aforementioned plant. Nineteen compounds were identified by GC-FID and GC-MS, of which α-cyperone (21.1%) and 4-oxo-α-ylangene (12.8%) were the major components. The essential oil recovered with diethyl ether demonstrated potent cytotoxic activities against colon (HCT-116), hepatocellular (Hep G-2) and breast (MCF-7) human cancer cell lines with IC_{50}s of 1.06, 1.17 and 2.22 µg/mL, respectively. This oil also showed a comparable zone of inhibition against the gram positive bacteria *Staphylococcus epidermidis* and the gram negative bacteria, *Klebsiella pneumoniae*, *Shigella flexneri* and *Salmonella enteritidis* when compared to the standard drugs ampicillin and gentamycin. The ethanolic extract showed moderate activity against human cancer cells and pathogens. The essential oil was significantly more toxic to 1st instar *Aedes aegypti* larvae than the ethanol extract; however, the ethanol extract was more toxic to adult *Ae. aegypti* than the essential oil. Based on the results obtained, *C. rotundus* collected from Riyadh city could provide a potential and cheap source of biologically active compounds.

Keywords: *Cyperus rotundus*, Essential oil, GC-MS, Cytotoxicity, Antimicrobial, Larvicidal, Adulticidal, *Aedes aegypti*

Introduction

Cyperus rotundus L., (Cyperaceae), is a perennial glabrous herb commonly known as Nut Sedge, Purple Nut Sedge or as Nabtat Alsa'ad in Arabic. It is a highly invasive weed, widely distributed in tropical, subtropical, and temperate regions around the world (Parsons & Cuthbertson, 1992). The bulbous roots (tubers) store starch as a food reserve and give rise to new rhizomes. The tubers are around 1- 3.5 cm in length, reddish white inside and brownish black externally (Aghassi et al., 2013).The presence of *C. rotundus* in a field considerably decreases crop yield due to its strong competition for ground resources, and because the roots of *C. rotundus* release substances that are harmful to other plants ''allelopathic'' (El-Rokiek et al., 2010). Despite the allelopathic effects, the tubers have been used as an occasional nutrient source and have a long history in traditional medicine. Historically, Arabs used roasted *C. rotundus* tubers or hot ashes from burned tubers, to treat wounds, bruises, carbuncles, and other related complaints (Imam et al., 2014). Ayurvedic and Islamic herbal medicine practitioners have described *C. rotundus* tubers to treat uterine disorders, fever, delayed menstruation and dysmenorrhea, removal of obstructions and as stomachic and emollient plasters

(Mohsin et al., 1989; Pirzada et al., 2015). The tubers of *C. rotundus* are used in traditional Chinese medicine as an antidiarrheal, antidepressant, analgesic, antiinflammatory and antiemetic remedy for dysentery and women's diseases (Chen et al., 2014; Oh et al., 2015).

The results of a clinical study involved ninety one female volunteers suffering from androgenic hair and supported the traditional use of topical Egyptian *C. rotundus* essential oil for treatment of moderate degrees of hirsutism and axillary hair. The hair growth was effectively and safely decreased with no effect on serum testosterone (Mohammed, 2014). Furthermore, compounds isolated from *C. rotundus* tubers showed significant antidepressant (Zhou et al., 2016), anti-hepatitis B virus (Xu et al., 2015), antimalarial (Weenen et al., 1990), antidiarrheal (Uddin et al., 2006) and antioxidant activities (Yazdanparast & Ardestani, 2007).

Plant extracts as well as their essential oils have been the major source of natural products with potential biological importance to be used as alternative remedies for the treatment of infectious diseases (Hemaiswarya et al., 2008). Concerns about the toxicity of many synthetic insecticides and the development of resistance in insects are great impetus for the development of alternative insecticides and repellents from isolated natural products. Novel natural products can help manage disease vectors such as *Aedes aegypti* (L.), which transmits the pathogens of many diseases such as yellow fever, dengue, and zika virus (Campos, et al., 2015; Benelli & Mehlhorn, 2016). We conducted this study with the aim of finding naturally derived, safe, and easily obtainable substituents to be used medically for treating contemporary diseases. To the best of our knowledge, this is the first report evaluating the chemical composition and biological activities of the essential oil (EO) *C. rotundus* tubers growing in Saudi Arabia.

Materials and Methods

Plant Material

The fresh tubers of *Cyperus rotundus* Linn. were collected in March 2016 from a farm near Riyadh city. A voucher specimen (No. 19010) has been deposited in the Herbarium of the Department of Pharmacognosy, King Saud University.

Essential Oil Isolation and Extract Preparation

The fresh tubers (300 g) were crushed, directly immersed in water and hydrodistilled for 6-7 hrs using Clevenger apparatus. The oil was obtained by extraction with diethyl ether from the aqueous distillate, dried over anhydrous Na_2SO_4, and the ethereal layer was finally evaporated at room temperature. In parallel, the air dried tubers (100 g) were powdered and extracted with 85% ethanol by cold maceration until exhaustion. The ethanol extract was evaporated using a rotary evaporator to give a dark residue (2 g).

GC–MS Analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

GC Analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (McLafferty & Stauffer, 1989; Koenig et al., 2004) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & Koenig, 1998; ESO 1999), was used for the identification.

Cytotoxic Activity

The mammalian cell lines HepG-2 (human liver cancer), HCT-116 (human colon cancer), and MCF-7 cells (human breast cancer) were obtained from the ATCC. The cells were propagated in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Co., St Louis, MO, USA), 1% L-glutamine, HEPES buffer, and 50 µg/mL gentamicin (Sigma Chemical Co., St Louis, MO, USA).

Cytotoxic activity was evaluated by the crystal violet staining (CVS) method in a three cell line-single concentration (50 mg/mL) anticancer assay by adapting the method described by Itagaki et al.-(Itagaki et al., 1991). Treated samples were compared with the negative cell control. All the experiments were carried out in triplicate. The standard antitumor drug used was doxorubicin.

Antimicrobial Activity

Antimicrobial tests were carried out by agar well diffusion according to the National Committee for Clinical Laboratory Standards (NCCLS) criteria. Bacterial and fungal suspensions were prepared and cultivated on Mueller-Hinton agar and Sabouraud dextrose media punched with 6-mm diameter wells. Then, 100 µL of 10% of tested samples were added to the wells, with 10% DMSO used as the negative control. Amphotericin B, ampicillin and gentamicin (30 µg/mL) were used as standard agents against fungi, Gram-positive bacteria, and Gram-negative bacteria, respectively. After incubation of the plates at 37 °C for 18 to 24 h, the antimicrobial activity was evaluated by measuring the diameter of the inhibition zones. Each test was performed in triplicate and the average of the results calculated. The extraction solvents were used as negative controls (NCCLS, 2004).

Mosquito Activity

Mosquito colony: Aedes aegypti L. used for testing were pesticide susceptible and provided from the CMAVE insectary. The "Orlando" strain was collected near Orlando, Florida, USA in 1952 and has been in continuous laboratory colony for 64 years. Rearing procedures are standardized and have been described previously (Meepagala et al., 2015).

Larvicidal Activity Assay

Larvicidal activity testing was performed essentially as described previously, except with the noted exceptions (Meepagala et al., 2015). Embryonated eggs are hatched overnight from oviposition papers in

approximately 200 mL of deionized water with approximately 40 mg of a finely ground mixture (1:1) of alfalfa powder and pig chow. The next day, five first instar larva were gently aspirated by pipette in 180 µL of liquid and transferred to one well of a 96-well flat bottom tissue culture plate. The larva are provided with 10 µL of the supernatant from a 2% solution of 1:1 alfalfa powder: pig chow to stimulate natural feeding. Two microliters of a specific compound diluted to 100 µg/mL in ethanol or DMSO was added to the well and mixed gently with the larva. Further dilutions of each compound were made by addition of 1 µL, 0.5 µL, or 0.2 µL of stock into other wells. For each assay, a positive control of 41.36 ng/ml permethrin stock and a negative control of ethanol or DMSO was included. Assays were repeated at least three times on separate days using different hatches of eggs.

Adult Topical Activity

The toxicity of compounds was tested in assays against adult *Aedes aegypti* using cohorts of 3-6 day post-emergence females as described previously (Meepagala et al., 2015). Mosquitoes were cold anesthetized on ice and groups of 10 females sorted into individual plastic cups. Application of 0.5 µL of the appropriate dilution of the test chemical was made by repeater pipettor (Hamilton PB600) with a 25 µL blunt tip glass syringe (Hamilton 7100 series) to at least twenty females at each dose. Controls again included a solvent only negative and doses of 0.19 ng/organism and 0.86 ng/organism of permethrin as positive controls. After treatment, cohorts had access to a cotton ball saturated with 10% sucrose. Mortality was scored at 24 hours after application and a mosquito unable to right itself was scored as dead. The LD_{50} of the ORL strain was approximately 0.15 ng/organism, so a valid assay required complete mortality at 0.86 ng/org and approximately 50% mortality at 0.19 ng/organism in the Orlando strain positive controls and <10% mortality in the negative control. There was complete mortality in the higher permethrin dose and 60±10% mortality at the dose slightly above the LD_{50}. Assays were repeated at least three times on different days.

Results and Discussion

Oil Composition

Steam distillation of *C. rotundus* tubers yielded 0.2% of yellowish oil with a strong aromatic odor. The analysis of the essential oil by GC-MS allowed the identification of 17 compounds accounting for 55.0% of the total composition of oil. The oil composition is displayed in Table 1 and constituents are listed in order of their elution. Sesquiterpenes (41.2 %) and oxygenated monoterpenes (13.8 %) were the chief classes of identified compounds. The major compounds identified were the sesquiterpenes α-cyperone (21.1%), 4-oxo-α-ylangene (12.8%) and caryophyllene oxide (3.5%); as well as the monoterpene *trans*-pinocarveol (3.6%). The α-cyperone (11.0%) was also the major compound detected in the South African *C. rotundus* tubers essential oil (Lawal & Oyedeji, 2009). Cyperene and cyperotundone were the major compounds reported from *C. rotundus* tubers oil in the South of Tunisia as well as in Iran. In our current study, cyperene was found in minor amounts (0.3%), while cyperotundone was not detected (Lawal & Oyedeji, 2009; Aghassi et al., 2013). On the other hand, 4-oxo-α-ylangene was reported as one of the constituents in *C. rotundus* oil samples analyzed in Egypt and South Africa. However, in both studies, they were reported in smaller amounts (9.35 and 1.9 %) than those found in our study (12.8%) (El-Gohary, 2004; Lawal & Oyedeji, 2009).

Table 1. The composition of the essential oil of *Cyperus rotundus* L. rhizomes

RRI	Compound	%
1544	Cyperene	0.3
1586	Pinocarvone	0.2
1648	Myrtenal	1.7
1670	*trans*-Pinocarveol	3.6
1683	*trans*-Verbenol	1.5
1706	α-Terpineol	0.8
1725	Verbenone	1.7
1751	Carvone	0.1
1804	Myrtenol	2.8
1845	*trans*-Carveol	0.5
1864	*p*-Cymen-8-ol	0.9
1900	*epi*-Cubebol	0.4
1957	Cubebol	0.5
2008	Caryophyllene oxide	3.5
2071	Humulene epoxide-II	2.6
2289	4-oxo-α-Ylangene	12.8
2304	α-Cyperone	21.1
	Total	55.0

RRI: Relative retention indices calculated against n-alkanes, with % calculated from FID data

Cytotoxic Activity

Cytotoxic activity was determined for the total extract of *C. rotundus* tubers, as well as the essential oil against HepG-2, HCT-116 and MCF-7 carcinoma cell lines, using the CVS method employing doxorubicin as reference drug. The response parameter (IC_{50}) was calculated for each cell line. From the results shown (Table 2), both samples possessed a dose-dependent cytotoxic effect against the three cell lines. However, the volatile oil sample showed remarkable cytotoxic activity against all the cell lines, with IC_{50} values ranging from 1.06 to 2.22 µg/mL while the total alcoholic extract showed less selective cytotoxic activity (IC_{50} = 38.3–48.5 µg/mL) in comparison with doxorubicin (IC_{50} = 0.55–0.67 µg/mL). Several previous studies have reported equally remarkable cytotoxic and apoptotic activities for the oil of *C. rotundus* (Kilani et al., 2008a; Kilani et al., 2008b; Kumar & Khanum, 2013; Kilani et al., 2014).

Table 2. Cytotoxic activity of *C. rotundus* EO and total extract against three cancer cell lines

Sample concentration (µg/mL)		100	50	25	12.5	6.25	3.125	1.56	IC_{50}[2] µg/mL
Tumor cell line		% Inhibition[1]							
Hep G-2	EO	96.06±0.19	92.40±0.44	88.21±0.56	82.94±2.28	75.26±3.44	67.76±3.86	56.66±3.86	1.17
	Total extract	63.41±2.85	55.16±2.74	36.71±2.47	16.96±2.67	5.15±1.80	0.42±0.73	NT	43
	Doxorubicin	95.79±0.53	90.98±0.97	86.36±1.13	81.24±1.60	75.10±1.35	67.56±1.85	60.81±0.91	0.67
HCT-116	EO	95.96±0.46	92.72±0.90	90.06±1.60	85.14±3.57	80.00±1.60	75.16±1.30	61.01±1.95	1.06
	Total extract	68.09±2.85	57.17±2.65	41.90±3.45	28.94±3.10	16.34±3.10	7.21±1.47	NT	38.3
	Doxorubicin	96.21±0.28	93.67±0.44	89.38±1.29	82.64±0.77	76.89±1.27	71.19±1.81	65.13±1.75	0.55
MCF-7	EO	94.42±1.09	87.12±1.13	80.69±1.46	73.15±1.08	65.13±1.29	57.88±2.18	44.29±3.99	2.22
	Total extract	63.81±2.73	50.70±3.84	39.40±3.71	21.89±2.43	11.30±2.65	4.14±2.55	NT	48.5
	Doxorubicin	95.74±0.26	94.51±0.13	90.38±0.58	83.86±0.16	75.23±6.73	71.39±0.45	64.20±1.10	0.62

[1] The percent of cell survival inhibition at 50 µg/mL, compared to control; [2] IC_{50s} are expressed in (µg/mL); NT, not tested; $p < 0.01$, compared to reference drug.

Antimicrobial Activity

The antimicrobial activities of *C. rotundus* essential oil and the total ethanolic extract were evaluated by determining their zone of inhibition against four fungi, four Gram-positive bacteria and four Gram-negative bacteria (Table 3). The volatile oil exhibited noticeable *in vivo* antifungal efficacy against the pathogenic fungi *Absidia corymbifera*, *Geotricum candidum* and *Candida albicans* with 21.0, 24.0 and 23.0 mm zones of inhibition diameter, respectively. These results compared favorably to the standard drug amphotericin with 23, 27 and 26 mm zones of inhibition diameter, respectively. On the other hand, the maximum antimicrobial activity for the essential oil against the Gram positive bacteria *Staphylococcus epidermidis* (25±0.11 mm zone of inhibition diameter) and *Streptococcus pyogenes* (26.7±0.17 mm zone of inhibition diameter); and the Gram negative bacteria *Klebsiella pneumoniae* (26±0.33 mm zone of inhibition diameter) and *Salmonella enteritidis* (25.4±0.25 mm zone of inhibition diameter) showed results comparable to the standard drugs ampicillin and gentamycin.

These findings are in agreement with previous antimicrobial activity studies on volatile oil of *C. rotundus* tubers, which demonstrated inhibitory activity against a large number of microorganisms such as *S. aureus, S. pyogenes, E. coli, P. vulgaris, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Uddin et al., 2006; Pirzada et al., 2015). On the other hand, the total extract was moderately active against most of the tested organisms, but it was inactive against *Listeria innocua* and *Proteous vulgaris*.

Table 3. Antimicrobial activities (zone of Inhibition, mm) of *C. rotundus* EO and total extract against selected clinical pathogens

Tested microorganisms Fungi	**Test sample**	**Zone of Inhibition (mm) ±SD**	**Amphotericin B**
Absidia corymbifera (RCMB 02564)	EO	21±0.14	23±0.33
	Total extract	15±0.32	
Trichophyton mentagrophytes (RCMB 0925)	EO	18.67±0.11	24±0.24
	Total extract	17.34±0.12	
Geotricum candidum (RCMB 05097)	EO	24±0.34	27±0.22
	Total extract	21±0.14	
Candida albicans (RCMB 05036)	EO	23±0.27	26±0.18
	Total extract	20±0.23	
Gram Positive Bacteria			**Ampicillin**
Staphylococcus aureus (RCMB 010027)	EO	22.7±0.14	27.4±0.15
	Total extract	20.33±0.31	
Staphylococcus epidermidis (RCMB 010024)	EO	25±0.11	25±0.22
	Total extract	21.7±0.32	
Streptococcus pyogenes (RCMB 010015)	EO	26.7±0.17	26.4±0.25
	Total extract	20±0.10	
Listeria innocua (RCMB 010052)	EO	18±0.15	22.3±0.10
	Total extract	NA	
Gram Negative Bacteria			Gentamycin
Proteous vulgaris (RCMB 010085)	EO	16.4±0.18	23.3±0.26
	Total extract	NA	
Klebsiella pneumoniae (RCMB 0010093)	EO	26±0.33	26.67±0.31
	Total extract	16±0.16	
Shigella flexneri (RCMB 0100542)	EO	25±0.35	27±0.26
	Total extract	13.7± 0.19	
Salmonella enteritidis (RCMB 010084)	EO	25.4±0.25	25.3±0.17
	Total extract	20±0.31	

Data are expressed in the form of mean ± SD.

Larvicidal and Adulticidal Activity

Bioassay of the essential oil and total alcohol extract from *C. rotundus* was conducted on 1st instar permethrin-susceptible *Ae. aegypti* "Orlando" strain larvae and adult females (Table 4). In the larval assay, the essential oil produced 100% mortality at 1, 0.5, 0.25 and 0.1 μg/μL, whereas the ethanol extract was slightly less active. It produced 100% mortality at 1 and 0.5 μg/μL, 93% mortality at 0.25 μg/μL and significantly less mortality at 0.1 μg/μL. However, the ethanol extract had slightly higher toxicity than the essential oil in adult topical assays.

Table 4. Adulticidal and larvicidal activities of *C. rotundus* EO and total extract

samples	Adult	Larval			
		Dose (μg/μL)% mortality			
	5 μg/mosq.	1	0.5	0.25	0.1
EO	90 ± 10	100	100	100	100
Total extract	96.7 ± 5.8	100	100	93.3 ± 11.5	33.3 ± 23.1

Positive control permethrin at 41.36ppb resulted in 100% mortality and negative solvent control (DMSO) had 0% mortality for larval bioassays. In adult bioassays, permethrin at 0.86 ng/org resulted in 100% mortality. The acetone and untreated controls both had an average mortality of 6.7 ± 5.8 percent

Conclusion

The essential oil from *C. rotundus,* obtained from the Riyadh region, revealed some variations in the composition and percentage of compounds when compared with other *C. rotundus* essential oils from different areas around the world. This demonstrates chemical diversity within the *C. rotundus* species possibly due to chemotypicity, geographic location, harvesting period, and storage time. The essential oil of *C. rotundus* showed remarkable cytotoxic and strong antimicrobial activities against the tested cancer cell lines and microbial strains. This plant might provide a potentially available and cheap source of antibiotics and anticancer compounds and should be considered for further phytochemical investigation and pharmacological evaluation. The 1st instar *Ae. aegypti* larvae activity showed 100% mortality at the lowest dosage in initial screening assays. Further bioassay-guided studies are planned to follow up on compounds of significant interest. To the best of our knowledge, the current study highlights the chemical composition and biological activities of the essential oil and alcoholic extract of *C. rotundus* rhizomes growing in Saudi Arabia for the first time.

ACKNOWLEDGMENT

This study was in part funded by the Deployed War-Fighter Protection Research Program via grants from the U.S. Department of Defense through the Armed Forces Pest Management Board. We thank Miss Jessica Louton, (USDA-ARS, CMAVE, Gainesville FL) for mosquito bioassays.

REFERENCES

Aghassi, A., Naeemy, A. & Feizbakhsh, A. (2013). Chemical composition of the essential Oil of *Cyperus rotundus* L. from Iran. *Journal of Essential Oil Bearing Plants, 16*(3), 382-386.

Benelli, G. & Mehlhorn, H. (2016). Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitology Research*, *115*, 1747-1754.

Campos, G. S., Bandeira, A. C. & Sardi, S. I. (2015). Zika virus outbreak, Bahia, Brazil**.** *Emerging Infectious Diseases*, *21*, 1885-1886.

Chen, H. Y., Lin, Y. H., Su, I. H., Chen, Y.C., Yang, S. H. & Chen, J. L. (2014). Investigation on Chinese herbal medicine for primary dysmenorrhea: implication from a nationwide prescription database in Taiwan. *Complementary Therapies in Medicine*, *22*(1), 116-125.

El-Gohary, H. (2004). Study of essential oils of the tubers of *Cyperus rotundus* L. and *Cyperus alopecuroides* Rottb. *Bulletin of Faculty of Pharmacy*, Cairo University*, 42*, 157-164.

El-Rokiek, K., El-Din, S. & Sharara, F. (2010). Allelopathic behaviour of *Cyperus rotundus* L. on both *Chorchorus olitorius* (broad leaved weed) and *Echinochloa Crus-Galli* (Grassy weed) associated with soybean. *Journal of Plant Protection Research, 50*(3), 274-279.

ESO 2000. The Complete database of essential oils, Boelens Aroma chemical information service, the Netherlands, 1999.

Hemaiswarya, S., Kruthiventi, A. K. & Doble, M. (2008). Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*, *15*(8), 639-652.

Imam, H., Zarnigar, Sofi, G., Seikh, A. & Lone, A. (2014). The incredible benefits of Nagarmotha (*Cyperus rotundus*). *International Journal of Nutrition, Pharmacology, Neurological Diseases, 4*(1), 23-27.

Itagaki, H., Hagino, S., Kato, S., Kobayashi, T. & Umeda, M. (1991). An in vitro alternative to the Draize eye-irritation test: Evaluation of the crystal violet staining method. *Toxicology in Vitro, 5*(2), 139-143.

Joulain, D. & Koenig, W. (1998). *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*, Hamburg: EB-Verlag.

Kilani, S., Ben Sghaier, M., Limem, I., Bouhlel, I., Boubaker, J., Bhouri, W., Skandrani, I., Neffatti, A., Ben Ammar, R., Dijoux-Franca, M.G., Ghedira, K. & Chekir-Ghedira, L. (2008a). *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus*. *Bioresource Technology, 99*(18), 9004-9008.

Kilani, S., Ledauphin, J., Bouhlel, I., Ben Sghaier, M., Boubaker, J., Skandrani, I., Mosrati, R., Ghedira, K., Barillier, D. & Chekir-Ghedira, L. (2008b). Comparative study of *Cyperus rotundus* essential oil by a modified GC/MS analysis method. Evaluation of its antioxidant, cytotoxic, and apoptotic effects. *Chemistry & Biodiversity*, *5*(5), 729-742.

Kilani, S., Zied, G., Nouha, N., Mounira, K., Kamel, G., Genvieve, F.D. & Leila, G.C. (2014). Evaluation of *in vitro* antioxidant and apoptotic activities of *Cyperus rotundus*. *Asian Pacific Journal of Tropical Medicine, 7*(2), 105-112.

Koenig, W., Joulain, D. & Hochmuth, D. (2004). *Terpenoids and Related Constituents of Essential Oils*, Hamburg, Germany: MassFinder 3.

Kumar, K.H. & Khanum, F. (2013). Hydroalcoholic extract of *cyperus rotundus* ameliorates H_2O_2-induced human neuronal cell damage via its anti-oxidative and anti-apoptotic machinery. *Cellular and Molecular Neurobiology, 33*(1), 5-17.

Lawal, O. A. & Oyedeji, A. O. (2009). Chemical composition of the essential oils of *Cyperus rotundus* L. from South Africa. *Molecules, 14*, 2909-2917.

McLafferty, F. & Stauffer, D. (1989). *The Wiley/NBS Registry of Mass Spectral Data,* New York: Sons JWa.

Meepagala, K. M., Becnel, J. J. & Estep, A. S. (2015). Phomalactone as the active constituents against mosquitoes from *Nigrospora spherica*, *Agricultural Science*, *6*, 1195-1201.

Mohammed, G. F. (2014). Topical *Cyperus rotundus* oil: a new therapeutic modality with comparable efficacy to Alexandrite laser photo-epilation. *Aesthetic Surgery Journal, 34*(2), 298-305.

Mohsin, A., Shah, A., Al-Yahya, M., Tariq, M., Tanira, M. & Ageel, A. (1989). Analgesic antipyretic activity and phytochemical screening of some plants used in traditional Arab system of medicine. *Fitoterapia, 60*, 174-177.

National Committee for Clinical Laboratory Standards (NCCLS) (2004). Performance standards for antimicrobial susceptibility testing, 16th informational supplement, M100-S14. NCCLS, Wayne, PA.

Oh, G. S., Yoon, J., Lee, G. G., Kwak, J. H. & Kim, S. W. (2015). The Hexane fraction of *Cyperus rotundus* prevents non-alcoholic fatty liver disease through the inhibition of liver X receptor alpha-mediated activation of sterol regulatory element binding protein-1c. *American Journal of Chinese Medicine*, *43*(3), 477-494.

Parsons, W. & Cuthbertson, E. (1992). *Noxious Weeds of Australia*, Melbourne, Australia: CSIRO Publishing.

Pirzada, A. M., Ali, H. H., Naeem, M., Latif, M., Bukhari, A. H. & Tanveer, A. (2015). *Cyperus rotundus* L.: Traditional uses, phytochemistry, and pharmacological activities. *Journal of Ethnopharmacology, 174*, 540-560.

Uddin, S J., Mondal, K., Shilpi, J. A. & Rahman, M. T. (2006). Antidiarrhoeal activity of *Cyperus rotundus*. *Fitoterapia, 77*(2), 134-136.

Weenen, H., Nkunya, M. H., Bray, D. H., Mwasumbi, L. B., Kinabo, L. S., Kilimali, V. A. & Wijnberg, J. B. (1990). Antimalarial compounds containing an alpha,beta-unsaturated carbonyl moiety from Tanzanian medicinal plants. *Planta Medica, 56*(4), 371-373.

Xu, H. B., Ma, Y. B., Huang, X. Y., Geng, C. A., Wang, H., Zhao, Y., Yang, T. H., Chen, X. L., Yang, C. Y., Zhang, X. M. & Chen, J. J. (2015). Bioactivity-guided isolation of anti-hepatitis B virus active sesquiterpenoids from the traditional Chinese medicine: Rhizomes of *Cyperus rotundus*. *Journal of Ethnopharmacology, 171*, 131-140.

Yazdanparast, R. & Ardestani, A. (2007). *In vitro* antioxidant and free radical scavenging activity of *Cyperus rotundus. Journal of Medicinal Food, 10*(4), 667-674.

Zhou, Z. L., Yin, W. Q., Yang, Y. M., He, C. H., Li, X. N., Zhou, C. P. & Guo, H. (2016). New iridoid glycosides with antidepressant activity isolated from *Cyperus rotundus*. *Chemical and Pharmaceutical Bulletin*, *64*, 73-77.