**ORIGINAL ARTICLE**

*Rhanterium epapposum* Oliv. essential oil: Chemical composition and antimicrobial, insect-repellent and anticholinesterase activities

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Received 28 August 2016; accepted 14 October 2016
Available online 1 November 2016

**KEYWORDS**
*Rhanterium epapposum*; Essential oil; Botanical repellents; Antimicrobial activity; AChE and BuChE inhibition

**Abstract** The essential oil (EO) of the aerial parts of *Rhanterium epapposum* Oliv. (Asteraceae), was obtained by hydrodistillation. The oil was subsequently analyzed by both GC-FID and GC-MS, simultaneously. Forty-five components representing 99.2% of the oil composition were identified. The most abundant compounds were camphene (38.5%), myrcene (17.5%), limonene (10.1%) and α-pinene (8.7%). Referring to the ethnobotanical utilization, an insecticidal assay was performed, where the oil repelled the yellow fever mosquito *Aedes aegypti* L. at a minimum effective dose (MED of 0.035 ± 0.010 mg/cm²) compared to the positive control DEET (MED of 0.015 ± 0.004 mg/cm²). Additionally, the *in vitro* antimicrobial activity against a panel of pathogens was determined using a microdilution method. The acetyl- and butyrylcholine esterase inhibitory activities were measured using the colorimetric Ellman method. The bioassay results showed...
1. Introduction

*Rhanterium epapposum* Oliv. (Asteraceae), a perennial bushy shrub (common name Arfaj) is broadly distributed across Saudi Arabia, and is the only species of the genus found in the Middle East (Collenette, 1999). Arfaj is a forage plant, grazed on by sheep and camel in the desert. It is used as a fuel source by bedouins and in local traditional medicine for skin infections and gastrointestinal disturbances. It is also used as an insecticide in various regions of Sudan and other Afro-Asian countries (Shama et al., 2012). *R. epapposum* was reported to have antioxidant activity, which is attributed to the polyphenolic content of its extract (Shahat et al., 2014). The phytochemical studies on the aerial parts of *R. epapposum* showed the presence of flavonoids, tannins, sterols, triterpenes and essential oils (EOs) (Al-Yahya et al., 1990). The composition of *R. epapposum* EO from Iran has been investigated previously and contained 107 volatile components of which 92% were terpenoids. Among the 69 terpenoid compounds identified, the main constituents were α-phellandrene, linalool, camphene, myrcene, geraniol, bulnesol, and β-phellandrene accounting for 55.6% of the oil. Nonterpenoid aliphatic and aromatic compounds were also detected (Yaghmai and Kolbadipour, 1987; El-Nasr and Youssef, 1984).

The yellow fever mosquito *Aedes aegypti* L. (Diptera: Culicidae) transmits several pathogens, which cause severe morbidity and mortality in humans (Hoel et al., 2010; Demirci et al., 2013; Ali et al., 2013). Personal protection from mosquito bites currently relies heavily upon the use of synthetic compounds. The compound N,N-diethyl-3-methylbenzamide, also known as DEET, is the most common insect repellent product (Tabanca et al., 2013a,b). However, due to neurotoxicity and environmental claims, there is a growing concern by the public for safer repellents to substitute the synthetic compounds for controlling a wide variety of insect-vectored diseases.

In the present study, *R. epapposum* EO was tested, for the first time, for mosquito repellency and for a combination of other biological activities. In addition, the chemical composition of the EO of the dried aerial parts of *R. epapposum* growing in Saudi Arabia was revealed by using GC-FID and GC-MS techniques. There are only a few previous antimicrobial studies on *R. epapposum* (Adam et al., 2011; Akbar and Al-Yahya, 2011). Various *R. epapposum* extracts were tested against a panel of pathogenic bacteria such as *B. cereus*, *S. aureus*, and *P. vulgaris*; the methanol extracts were relatively active up to 10 μg/mL (Adam et al., 2011). Akbar and Al-Yahya (2011) also reported antimicrobial activity of *R. epapposum* extracts from screening surveys.

2. Materials and methods

Acetylcholinesterase (AChE) from electric eel (C3389-2KU), butyrolycholinesterase (BuChE) from horse serum (E. C.3.1.1.8, Sigma St. Louis, MO, USA), acetylthiocholine iodide and butyrylthiocholine chloride (Sigma St. Louis, MO, USA), 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) (Sigma), galantamine hydrobromide from *Lycoris* sp. (Sigma St. Louis, MO, USA). Instrumentation: (Shimadzu, UV-1700).

2.1. Plant material

The fresh aerial parts of *R. epapposum* Oliv. in the flowering stage were collected from the region of Majmaah (Riyadh-Qassim Road), 90 km, North of Riyadh, Saudi Arabia. The taxonomist Dr. Mohammed Yousuf Yaqoob identified the plant, and a voucher specimen has been deposited at the herbarium (# 15386), College of Pharmacy, King Saud University.

2.2. Isolation of essential oil

The volatile oil was prepared by water distillation of the fresh plant material for about 4–5 h to produce 0.085%, v/w, of EO. The oil was collected, dehydrated over anhydrous sodium sulfate and kept in small well, closed amber colored glass containers at low temperature until analysis.

2.3. GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as the 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.

2.4. GC-FID 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. The analysis results are expressed as mean percentage ± standard deviation (SD) (n = 3) as listed in Table 1.
2.5. Identification of components

Identification of the EO components was carried out by comparison of their relative retention times with those of authentic compounds and components of known oils as well as MS literature data (Joulain and Koenig, 1998; ESO 2000, 1999) was used for the identification.

2.6. Mosquito repellent bioassay

The Ae aegypti mosquitoes used in these studies were obtained from a colony maintained since 1952, originally from Orlando, FL, and now maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL. Repellency was determined as the Minimum Effective Dosage (MED), which is the minimum threshold surface concentration necessary to prevent mosquitoes from biting through the treated surface. The assay was performed based on the previous study (Katritzky et al., 2008). There were three human volunteers in this study and all three provided written informed consent to participate in this study as part of a protocol (636-2005) approved by the University of Florida Human Use Institutional Review Board (IRB-01). DEET (97% N,N-diethyl-3-methylbenzamide) (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control.

2.7. Antimicrobial activity

2.7.1. Microorganisms and growth conditions

All microorganisms were stored at −85 °C in 15% glycerol until use. Strains and strain sources of the test microorganisms are given in Table 2. The bacteria and yeast were re-suspended in Muller Hinton broth at 35–37 °C and then, inoculated on Mueller Hinton Agar (MHA) plates whereas fungi were grown on Sabouraud Dextrose Agar (SDA) at 28 °C for purity check.

2.7.2. Antimicrobial assay

The minimum inhibitory concentrations (MIC) were determined as mg/mL for the oil, on each organism by using a broth microdilution method (Iscan et al., 2002; Winn et al., 2006). Stock solutions of the EO (4 mg/mL) and standard antimicrobials (2 mg/mL) were prepared using Mueller Hinton Broth (MHB, containing 25% DMSO). Serial dilution of the initial concentrations was done on 96-well microtiter plates containing equal amounts of distilled water. Microbial suspension concentrations were standardized to McFarland No:0.5 using a turbidometer (Biolab, Turkey), after incubation for 24 h at 37 °C in MHB. Cultures were mixed with test samples and were further incubated for 24 h. Minimum inhibitory concentrations were detected at the least concentration, where microbial growth was not present. Furthermore, the addition of a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC, Aldrich) was used as an indicator of microbial growth. EO free solutions were used as negative control, whereas standard antimicrobial agents were used as a positive control. All the experiments were performed in triplicate and mean of results is given in Table 2.

2.8. Determination of AChE and BuChE inhibitory activities

AChE and BuChE inhibitor activity was measured by using the method of Ellman et al. and Dohi et al. with minor modifications (Ellman et al., 1961; Dohi et al., 2009).

Table 1 The composition of the R. epapposum EO.

| RRI  | Compounds                  | %    |
|------|----------------------------|------|
| 1032 | α-Pinene                   | 8.73 ± 0.06 |
| 1035 | α-Thujene                  | 0.30 ± 0   |
| 1076 | Camphene                   | 38.47 ± 0.23 |
| 1118 | β-Pinene                   | 3.70 ± 0   |
| 1132 | Sabinene                   | 5.30 ± 0   |
| 1174 | Myrcene                    | 17.53 ± 0.06 |
| 1176 | α-Phellandrene             | 0.37 ± 0.06 |
| 1188 | α-Terpine                  | 0.20 ± 0   |
| 1203 | Limonene                   | 10.12 ± 0.06 |
| 1218 | β-Phellandrene             | 0.17 ± 0.06 |
| 1255 | γ-Terpine                  | 0.40 ± 0   |
| 1266 | (E)-β-Ocimene              | 0.10 ± 0   |
| 1280 | p-Cymene                   | 0.50 ± 0   |
| 1290 | Terpinolene                | 0.40 ± 0   |
| 1457 | Hexyl-3-methyl butyrate    | 0.10 ± 0   |
| 1474 | trans-Sabinene hydrate     | 0.10 ± 0   |
| 1480 | Nerol oxide                | 0.10 ± 0   |
| 1495 | Bicycloelemene             | 0.10 ± 0   |
| 1533 | Linalool                   | 0.90 ± 0   |
| 1556 | cis-Sabinene hydrate       | 0.10 ± 0   |
| 1571 | trans-p-Menth-2-en-1-ol     | 0.10 ± 0   |
| 1588 | Bornyl formate             | 0.90 ± 0   |
| 1591 | Bornyl acetate             | 0.10 ± 0   |
| 1598 | Camphene hydrate           | 0.20 ± 0   |
| 1631 | Terpinene 4-ol             | 1.00 ± 0   |
| 1626 | 2-Methyl-6-methylene-3,7-octadien-2-ol | 1.00 ± 0 |
| 1628 | Citronellyl formate         | 1.30 ± 0   |
| 1638 | cis-p-Menth-2-en-1-ol       | 0.15 ± 0.07 |
| 1668 | Citronellyl acetate         | 0.60 ± 0   |
| 1700 | p-Mentha-1,8-dien-4-ol      | 0.10 ± 0   |
| 1706 | α-Terpineol                | 0.90 ± 0   |
| 1715 | Geranyl formate             | 1.07 ± 0.06 |
| 1733 | Neryl acetate              | 1.10 ± 0   |
| 1758 | cis-Piperitol              | 0.10 ± 0   |
| 1772 | Citronellol                | 0.30 ± 0   |
| 1773 | δ-Cadinene                 | 0.10 ± 0   |
| 1776 | γ-Cadinene                 | 0.10 ± 0   |
| 1808 | Nerol                      | 0.30 ± 0   |
| 1857 | Geranol                    | 0.10 ± 0   |
| 1864 | p-Cymen-8-ol               | 0.13 ± 0.06 |
| 2144 | Spathulenol                | tr      |
| 2174 | Fokienol                   | tr      |
| 2187 | τ-Cadinol                  | 1.30 ± 0   |
| 2257 | β-Eudesmol                 | 0.60 ± 0   |
| 2931 | Hexadecanoic acid          | 0.10 ± 0   |
| Total|                          | 99.23 ± 0.12 |

RRI: relative retention indices calculated against n-alkanes; % calculated from FID data; tr: trace (<0.1%).
2.8.1. AChE inhibitory activity

Different concentrations of samples were prepared in methanol. 20 μL of enzyme (1 U/mL) and 10 μL of sample were added to 2.4 mL of buffer and the mixture was incubated at 37 °C for 15 min. After incubation, 50 μL of 0.01 M DTNB and 25 μL of 75 mM ATCI were added, and the final mixture was incubated at room temperature for 30 min. A control mixture and blank were prepared by using 10 μL of methanol instead of the oil sample, with all other procedures. Absorbances were measured at 412 nm and 37 °C using polystyrene cuvettes with a spectrophotometer (Shimadzu, UV-1700).

The inhibition (%) was calculated for both of the activities using the following equation:

\[
I (%) = 100 - \frac{(\text{OD}_{\text{sample}}/\text{OD}_{\text{control}})}{100}
\]

\(I\) = inhibition in percentage, \(\text{OD}\) = optical density.

2.8.2. BuChE inhibitory activity

Different concentrations of samples were prepared in methanol. 20 μL of enzyme (1 U/mL), and 10 μL of sample were added to 1.9 mL of buffer, after which the mixture was incubated at 37 °C for 15 min. After the 15 min incubation, 50 μL of 0.01 M DTNB and 25 μL of 10 mM BTCI were added, and the final mixture was incubated at room temperature for 30 min. A control mixture and blank were prepared by using 10 μL of methanol instead of the oil sample, with all other procedures similar to those used in the case of the sample mixture.

2.9. Statistical analysis

Experiments were performed in triplicate. Data are expressed as mean ± standard deviation (SD) calculated by SPSS 11.5.

3. Results and discussion

Essential oil was obtained by hydrodistillation from the aerial parts of R. epapposum. The EO contained 45 compounds in which the major components were identified as camphene, myrcene, limonene, α-pinene, sabine and β-pinene, and these accounted for 78.6% of the oil. Aromatic and fatty acid (non-terpenoid) compounds were also detected in the oil content. All identified compounds are listed in Table 1, along with their peak numbers and their relative percentage in the composition. From examination of the mass spectra, compounds present in the hydrocarbon and the oxygenated fractions account for 40% and 55.6% of the content of the oil, respectively. Overall, terpenoids constituted the majority of the oil (93.8%), of which monoterpenoids (91.58%) were the most predominant.

Among the hydrocarbons in the EO, 12 monoterpenes were detected: camphene, myrcene, limonene, α-pinene, sabine and β-pinene, which were present in considerable quantities (38.5%, 17.3%, 10.1%, 8.7%, 5.3% and 3.7%, respectively). Three sesquiterpene hydrocarbons were detected (0.3%); these were bicycloelemene, γ-cadinene and δ-cadinene (0.1%), respectively. The oxygenated part of the oil consisted of 23 monoterpenes (10.92%), where citronellyl formate was the most abundant compound, and T-cadinol was detected as the major sesquiterpene component and 1.9% of the total. Notably only a few non-terpenoid fatty acids were detected in small traces, one of these was hexadecanoic acid.

Yaghmai and Kolbadipour reported that α-phellandrene (15.7%), linalool (14.1%), geraniol (11.1%), bulnesol (9.0%), β-phellandrene (5.4%) were found as the main constituents in aerial parts of the EO oil of R. epapposum from Iran (Yaghmai and Kolbadipour, 1987). Awad and Abdelwahab also reported a total of 51 compounds representing 76.35–94.86% of flowers, leaves and stems oil composition were identified and the chemical profile of the fractions revealed the dominance of monoterpenes but there are a qualitative and quantitative differences between flower oil, leaves oil and stems oil (Awad and Abdelwahab, 2016). When the previous studies are compared with our present results, the differences from the previously reported studies could be attributed to differences in geographic location, climate, soil, altitude, harvesting time, etc.

According to the insecticidal ethnobotanical uses (Shama et al., 2012) we have aimed to evaluate the biological activities initially in this direction, where the repellency assays of R. epapposum EO against Aedes aegypti were conducted by measuring the Minimum Effective Dosage (MED) at which the oil was repellent. The EO repelled at a MED of 0.035 ± 0.010 mg/cm² compared to standard compound DEET (0.015 ± 0.004 mg/cm²).

Additionally, we aimed to complement the insecticidal activity on enzymatic level. Our study showed that the R. epapposum EO exhibited a weak inhibitory effect on AChE and
BuChE (14.55 ± 0.32%, 4.45 ± 0.64% at 80 μg/mL, respectively) compared to galantamine (96.56 ± 0.66, 68.51 ± 1.63 at 8 μg/mL, respectively).

DEET is known as the gold standard of insect repellents and it has been reported as an inhibitor of acetylcholinesterase enzyme (Debboun et al., 2015). Acetylcholinesterase (AChE) catalyzes the hydrolysis of neurotransmitter acetylcholine (ACh) that when released from synaptic vesicles briefly depolarizes the postsynaptic cell membrane. Thus, AChE terminates the signal transmission (Deletre et al., 2013). The mechanism of action for many synthetic chemical pesticides, including organophosphates (OPs) and carbamates, is inhibition of the acetylcholinesterase (AChE) enzyme. Both OPs and carbamates are known to bind to and inhibit the AChE enzyme, although OPs bind irreversibly and carbamates bind reversibly. ACh accumulation in the synapse causes overstimulation of the neurons, which leads to rapid twitching of the muscles, convulsions, and insect death (Corbel et al., 2009).

This is the first report on the repellent and anticholinesterase evaluation of the R. epapposum oil. The repellent activity is promising and will likely lead to a more in-depth study involving bioassay guided fractionation.

As seen in Table 2, a panel of Gram positive, Gram negative and the yeast Candida parapsilosis pathogens were evaluated for the antimicrobial potential of R. epapposum EO. Minimum inhibitory concentrations (MIC, in mg/mL) were observed using an in vitro microdilution method (Iscan et al., 2002; Winn et al., 2006) where chloramphenicol, ampicillin and tetracycline were standard antimicrobials and ketoconazole was the standard antifungal control.

Except for the clinical MRSA isolate, all microorganisms were standard strains. When compared to the standard antimicrobial compounds, the R. epapposum EO was relatively inactive, in the tested range with maximum MIC values of 0.5 mg/mL against P. vulgaris and S. epidermidis.

Dedication

Prof. Dr. Mohammed Abdulaziz Al-Yahya, Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia (Born in: 3/7/1943 – passed away in: 27/6/2016).

Acknowledgments

We thank Greg Allen (USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL) for performing the mosquito bioassays. This study was supported partially by the Deployed War-Fighter Protection Research Program Grant funded by the U.S. Department of Defense through the Armed Forces Pest Management Board.

References

Adam, S.I.Y., El-Kamali, H.H., Adama, S.E.I., 2011. Phytochemical screening and antibacterial activity of two Sudanese wild plants, Rhanterium epapposum and Trichodesma africanum. J. Fac. Sci. Technol. 2, 83–96.

Akbar, S., Al-Yahya, M.A., 2011. Screening of Saudi plants for phytoconstituents, pharmacological and antimicrobial properties. Aust. J. Med. Herbalism 23, 76–87.

Ali, A., Demirci, B., Kiyan, H.T., Bernier, U.R., Tsikolia, M., Wedge, D.E., Khan, I.A., Baser, K.H.C., Tabanca, N., 2013. Biting deterrence, repellency, and larvicidal activity of Ruta chalepensis (Sapindales: Rutaceae) essential oil and its major individual constituents against Mosquitoes. J. Med. Entomol. 50, 1267–1274.

Al-Yahya, M.A., Al-Meshal, I.A., Mossa, J.S., Al-Badr, A.A., Tariq, M., 1990. Saudi Plants, A Phytochemical and Biological Approach. General Directorate of Research Grants Programs. KACST, Riyadh, Saudi Arabia, pp. 75–80.

Awad, M., Abdelwahab, A., 2016. Chemical diversity of essential oils from flowers, leaves and stems of Rhanterium epapposum Oliv. growing in northern border region in Saudi Arabia. Asian Pac. J. Trop. Biomed. 6, 767–770.

Colletette, S., 1999. Wild Flowers of Saudi Arabia. National Commission for Wildlife Conservation and Development, Riyadh, Saudi Arabia, East Anglian Engraving Co. Ltd., Norwich, UK, p. 213.

Corbel, V., Stankiewicz, M., Pennetier, C., Fournier, D., Stojan, J., Girard, E., Dimitrov, M., Molgo, J., Hougard, J.M., Lapied, B., 2009. Evidence for inhibition of cholinesterases in insect and mammalian nervous systems by the insect repellent DEET. BMC Biol. 7, 47–58.

Debboun, M., Frances, S.P., Strickman, D.A., 2015. Insect Repellent Handbook. CRC Press, Taylor & Francis, NY.

Deletre, E., Martin, T., Campagne, P., Bourguet, D., Cadin, A., Menut, C., Bonafos, R., Chandre, F., 2013. Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector Anopheles gambiae mosquito. PLoS ONE 8, e82103.

Demirci, B., Tsikolia, M., Bernier, U.R., Agramoto, N.M., Alqasoumi, S.I., Al-Yahya, M.A., Al-Rehailly, A.J., Yusufoglu, H.S., Demirci, F., Baser, K.H.C., Khan, I.A., Tabanca, N., 2013. A new natural source: Phoenix dactylifera L. spathae essential oil: chemical composition and repellent activity against the yellow fever mosquito, Aedes aegypti. Acta Trop. 128, 557–560.

Dohi, S., Terasaki, M., Makino, M., 2009. Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils. J. Agric. Food Chem. 57, 4313–4318.

Ellman, G.L., Courtney, K.D., Andres, V.J.R., Fethers-Stone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

El-Nasr, M.M.S., Youssef, M.A.M., 1984. GLC/MS analysis of the volatile oil of Rhanterium epapposum Oliv. Pharmazie 39, 181–182.

ESO 2000, 1999. The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service, The Netherlands.

Hoel, D., Prideon, J.W., Bernier, U.R., Chauhan, K., Meepagala, K., Cantrell, C.L., 2010. Departments of defense and agriculture team up to develop new insecticides for mosquito control. Wingbeats 21, 19–34.

Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K.H.C., Demirci, F., 2002. Antimicrobial screening: Mentha piperita essential oil. J. Agric. Food Chem. 50, 3943–3946.

Joulain, D., Koenig, W.A., 1998. The Atlas of Spectra Data of Sesquiterpene Hydrocarbons. EB-Verlag, Hamburg.

Katritzky, A.R., Wang, Z., Slavov, S., Tsikolia, M., Dobchev, D., Akhmedov, N.G., Hall, C.D., Bernier, U.R., Clark, G.G., Sinoumi, S.I., Al-Yahya, M.A., Al-Rehailly, A.J., Yusufoglu, H.S., Koenig, W.A., Joulain, D., Hochmuth, D.H., 1998. The Atlas of Spectra Data. MassFinder 3, Hamburg, Germany.

Krafft, K., Vona, Z., Korszun, W., 1997. The Wiley/NBS Registry of Mass Spectral Data. J. Wiley and Sons, New York.

Revay, E.E., Kline, D.L., Xue, R.D., Qualls, W.A., Bernier, U.R., Kravchenko, V.D., Ghattas, N., P stygo, L., Miller, G.C., 2013. Reduction of mosquito biting-pressure: spatial repellents or mosquito traps? A field comparison of seven commercially available products in Israel. Acta Trop. 127, 63–68.

Rhanterium epapposum Oliv. essential oil
Shahat, A.A., Ibrahim, A.Y., Elsaid, M.S., 2014. Polyphenolic content and antioxidant activity of some wild Saudi Arabian Asteraceae plants. Asian Pac. J. Trop. Med. 7, 545–551.
Shama, I.Y., Shama, I.Y.A., Adam, S.E.I., 2012. Comparative toxicity of Trichodesma africanaum and Rhanterium epapposum aerial parts aqueous and methanolic extracts on Wistar rats. J. Pharmacol. Toxicol. 7, 128–138.
Tabanca, N., Wang, M., Avonto, C., Chittiboyina, A.M., Parcher, J. F., Carroll, J.A., Kramer, M., Khan, I.A., 2013a. Bioactivity-guided investigation of Geranium essential oils as natural tick repellents. J. Agric. Food Chem. 61, 4101–4107.
Tabanca, N., Bernier, U.R., Ali, A., Wang, M., Demirci, B., Blythe, E. K., Khan, S.I., Baser, K.H.C., Khan, I.A., 2013b. Bioassay-guided investigation of two Monarda essential oils as repellent of yellow fever mosquito against Aedes aegypti. J. Agric. Food Chem. 61, 8573–8580.
Swale, D.R., Sun, B., Tong, F., Bloomquist, J.R., 2014. Neurotoxicity and mode of action of N,N-diethyl-meta-toluamide (DEET). PLoS ONE 9, 1–11.
Winn, C., Allen, S.D., Janda, W.M., Koneman, E.W., Procop, G.W., Schreckenberger, P.C., Woods, G.L., 2006. Koneman’s Color Atlas and Textbook of Diagnostic Microbiology. Lippincott Williams & Wilkins, Philadelphia, USA.
Yaghmai, M.S., Kolbadipour, S., 1987. Volatile components of Rhanterium epapposum Oliv. Flav. Fragr. J. 2, 29–32.