Mosquito and tick repellency of two *Anthemis* essential oils from Saudi Arabia

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**A B S T R A C T**

The essential oils (EOs) of *Anthemis melampodina* (Am) and *Anthemis scrobicularis* (As) (Asteraceae) were extracted from the aerial parts of the plants by hydrodistillation, and their chemical compositions were analyzed using GC-FID and GC-MS. Fifty-six components representing 85.5% of the oil composition of *Anthemis melampodina* were identified, and the major components were α-pinene (17.1%) and β-eudesmol (13.8%). Forty-one components representing 86% of the oil composition of *Anthemis scrobicularis* were identified, and the major component was β-eudesmol (12.8%). Laboratory bioassays were conducted to determine repellency of Am and As EOs against the yellow fever mosquito *Aedes aegypti* and the lone star tick *Amblyomma americanum*. The minimum effective doses (MEDs) of the Am and As EOs against mosquitoes were 0.187 ± 0.000 and 0.312 ± 0.063 mg/cm² respectively, which were significantly higher than that of DEET (0.023 ± 0.000 mg/cm²) in human-based repellent bioassays. The As EO was more repellent than Am EO against nymphal ticks but was less effective than DEET in vertical paper bioassays.

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**1. Introduction**

Mosquitoes are a major vector for the transmission of several life-threatening diseases (Rajkumar and Jebanesan, 2010). The Kingdom of Saudi Arabia (KSA) is one of the Eastern Mediterranean countries that is part of the World Health Organization. KSA bears 11% of the world burden of vector-borne diseases, such as malaria and several arboviral diseases (WHO, 2004). Malaria has been endemic in KSA since 1900 (Mattingly and Knight, 1956). Since 1995, the most reported arboviral diseases in KSA have been dengue fever (DF) and Rift Valley Fever (RVF); however, other arboviral diseases have also been reported (Khat et al., 2013). There are 46 mosquito species recorded in the Arabian Peninsula, including 15 in the Riyadh Region (Alahmed et al., 2007). Species from the major three genera *Anopheles*, *Culex*, and *Aedes* are present in KSA (Mattingly and Knight, 1956). Ticks are vectors of bacteria, viruses, and protozoa that cause diseases affecting human and animal health (Meng et al., 2016). Given its vast geographical area and being a center of the Islamic world, with millions of pilgrimages visiting the holy sites during the annual sessions of pilgrimage and Umrah, the kingdom of Saudi Arabia faces a high risk of vector-borne disease epidemics (Ahmed, 2015). After the appearance of Rift Valley Fever (RVF) in Saudi Arabia for the first time in September 2000, the Ministry of Health in Saudi Arabia developed and implemented plans to prevent this disease in the Hajj period (Madani, 2005).

Effective vector control and management are vital in the prevention of disease transmission. *Anthemis* L. (Asteraceae) is one...
of the largest genera of Anthemideae tribe (Mohammed et al., 2017). It is represented by 19 species in Saudi Arabia (Konstantinopoulou et al., 2003) and nearly 210 species distributed in different areas around the world (Mohammed et al., 2017). *Anthemis scrobicularis* Yavin and *A. melampodina* Dei are annual herbs growing in sandy areas of the Arabian Peninsula, Jordan, Palestine and Egyptian desert (Mohammed et al., 2017; Ghafoor, 2010; Chaudhary 2000; Takholm, 1974). Studies of the *Anthemis* species have led to the reports on the chemical composition and diverse biological activities, such as their antioxidant, antifungal (Papaioannoua et al., 2007), antiplasmodial, antitumor, chistosomidal, cytotoxic, antihelmintic, phytotoxic, analgesic (Amjad et al., 2012) effects and for the treatment of afflictions and cystitis (Burim et al., 1999). Tinctures, extracts, tisanes, salves, decoction, infusion and other traditional formulations of *Anthemis* species are widely used for the treatment of dysmenorrhea, inflammation, hemorrhoid, hepatotoxicity, abdominal pain and different types of skin inflammation in the European folk medicine (Mann and Staba, 1986; Ugurlu and Secmen, 2008; ManganelliiUncini and Tomei, 1999; Baltaci et al., 2011; Petkeviciute et al., 2010). Several types of active compounds like flavonoids, sesquiterpene lactones, fatty acids, sterols, essential oils and polyacetylenes have been reported in previous reports on *Anthemis* species (Mohammed et al., 2017; Hajdu et al., 2010; Masterova et al., 2005; Pavlovic et al., 2007; Vuckovic et al., 2005). Anti-inflammatory and hepatoprotective activities have been reported previously for *A. scrobicularis* methanol extract (Yusufoğlu et al., 2014), and there are four new sesquiterpene lactones isolated from the same species (Zaghloul et al., 2014). In this present study, *A. melampodina* (Am) and *A. scrobicularis* (As) essential oils (EOs) were tested, for the first time, for repellency against the mosquito *Aedes aegypti* L. (*Ae. aegypti*) and lone start tick *Amblyomma americanum* L. (*Am. americanum*).

2. Materials and methods

2.1. Plant material

The aerial parts of Am and As were collected during the flowering period from the Al-shadida, Province of Alkhari, Saudi Arabia, in February 2014, and March 2015, respectively. The plant species were authenticated by Dr. Osman Almekki using morphological features of the plant samples. The voucher specimen was deposited in the Herbarium, College of Pharmacy (PSAU-CPH-11-2014, PSAU-CPH-5-2015), Prince Sattam bin Abdulaziz University, Al-Kharj, KSA.

2.2. Preparation and analysis of the oil

Air-dried aerial parts of Am (250 g) and As (500 g) were ground and subjected, separately, to hydrodistillation for 4 h using a Clevenger-type apparatus with 5 L rounded-bottomed flask. After decanting and drying over anhydrous sodium sulfate, the corresponding oils were purified, resulting in a yield of 0.095% and 0.25% w/w, respectively.

2.3. GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. An Innowax fused silica capillary column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as the carrier gas (0.8 ml/min). The GC oven temperature was held at 60 °C for 10 min after injection and then ramped to 220 °C at a rate of 4 °C/min, and held at 220 °C for 10 min, followed by a second ramp to 240 °C at a rate of 1 °C/min. The split ratio was set at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded using 70 eV electrons in Electron Ionization (EI) mode. The mass analyzer was scanned from m/z 35–450 at a scan rate of (3.46) s⁻¹.

2.4. GC analysis

The GC analysis was carried out on an Agilent 6890N GC system. The FID detector temperature was set to 300 °C. To obtain the same elution order with GC-MS, simultaneous auto-injection was performed on the GC-MS system using a similar column operated with identical GC parameters. Relative percentages of the separated compounds were calculated from peaks in the GC-FID chromatograms. Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to a series of n–alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (Zaghloul et al., 1989; McAafferty and Stauffer, 1989) and in-house “Baser Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (Koenig et al., 2001; Jouilain and Koenig, 1998), were also used for the identification.

2.5. Bioassays

2.5.1. Insects

Mosquitoes used in all bioassays were female *Ae. aegypti* (Orlando strain, 1952) from the colony maintained at the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE-USDA-ARS) in Gainesville, FL. Pupae were obtained from the onsite colony and maintained in laboratory cages until ready for use in experiments (ESO, 2000). Laboratory-reared nymphs of the lone star tick, *Am. americanum*, maintained at the USDA-ARS, Invasive Insect Biocontrol and Behavior Laboratory in Beltsville, MD, USA. Nymphs were 1–3 months old at the time of the experiments. Technical DEET (97% active ingredient; Sigma-Aldrich, USA) was used as positive control for mosquito and tick bioassays (Tabanca et al., 2016a).

2.5.2. Mosquito repellent bioassays (Cloth patch assay)

Repellency was determined as the minimum effective dosage (MED) of Am and As EOs against *Ae. aegypti* mosquitoes. The methods are as described in the literatures (Posey and Schreck, 1981; Schreck et al., 1977; Katritzky et al., 2010). Tests were conducted on each control or treated patch for 1 min. A control patch (acetone solvent only) was tested before the start of experiments and after every 10 tests. If fewer than five landings occurred on the control patch in 30 s, then tests were discontinued for 60 min. At the conclusion of testing, the control patch was tested again. If five landings were not received within 30 s, the data for the replicate was discarded. When testing a patch treated with a candidate repellent, if ≈1% or five mosquito bites were received during this one min test, this compound was considered to have failed, that is, was not repellent at that concentration. Observed Minimum Effective Dosage (MED) values for each candidate compound were averaged across participants and reported as a mean MED ± SE. Additional explanation of this type of bioassay can be found in (Tabanca et al., 2016b). Written informed consent was obtained for all human subjects used in this study in accordance with protocol #636-2005, as approved by the University of Florida Institutional Review Board (IRB-01).

2.5.3. Tick repellency bioassay

Tick repellency against *Am. americanum* was determined by using a bioassay technique (vertical paper assay) described by
Carroll et al. (Carroll et al., 2011). Briefly, a 4 × 7 cm rectangle of Whatman No. 4 filter paper was prepared by treating the central 4 × 5 cm zone with a volume of 165 μL of test solution. After drying, the paper strip was suspended from a bulldog clip hung from a holder. Ten *Am. americanum* nymphs were released from a glass vial on the lower untreated end of the paper strip. Locations of the nymphs were recorded at 1, 3, 5, 10 and 15 min. Ticks were considered repelled if they stayed on the lower untreated zone or fell off the filter paper without having crossed into the upper untreated zone (Meng et al., 2016). Each treatment/concentration included three replicates. The mean percent repellency was generated for DEET and each essential oil concentrations for comparison.

### 3. Results and discussion

The hydrodistillation of the herbal parts of As and Am yielded 0.095% w/w (calculated to dry weight) and 0.25% w/w (calculated to fresh weight) with dark brownish colored oils, respectively. In the As EO, the GC-MS revealed at least 56 components representing 85.5% of the As EO (Table 1). The major component was oxygenated sesquiterpene \( \beta \)-eudesmol (12.8%). The oil was characterized by a high percentage of oxygenated sesquiterpenes (55.4%), followed by oxygenated monoterpenes (20.1%). Monoterpene hydrocarbons and sesquiterpene hydrocarbons represented only 1.7% and 4.0% of the oil, respectively (Table 1).

The chemical composition of Am EO from Saudi Arabia and Egypt has been previously investigated (Al-Humaidi, 2015; Grace, 2002), however, we found significant quantitative and qualitative differences in Am EO from region to regions even in the same country of origin. The GC-MS analyses of Am EO revealed at least 41 components representing 86% of the volatile Am EO (Table 1). The oil was characterized by a high percentage of monoterpene hydrocarbons (31.6%), with \( \alpha \)-pinene (17.1%) as the main component, followed by oxygenated monoterpenes (23.7%) and oxygenated sesquiterpenes (22.2%). The same plant which collected from Tabuk (Saudi Arabia), the northwest part of the kingdom and has been reported by Al-Humaidi (2015) has shown 42 components representing 86% of the volatile Am EO (Table 1).

### Table 1

| RRI | Compound                | Am (%) | As (%) | IM   |
|-----|-------------------------|--------|--------|------|
| 1032| \( \alpha \)-Pinene      | 17.1   | 1.0    | RRI, MS |
| 1076| Camphene                | 2.5    | 0.2    | RRI, MS |
| 1118| \( \beta \)-Pinene       | 0.6    | 0.2    | RRI, MS |
| 1132| Sabine                 | 0.1    | –      | RRI, MS |
| 1174| Myrcene                | 5.6    | –      | RRI, MS |
| 1203| Limonene               | 4.5    | 0.3    | RRI, MS |
| 1213| 1,8-Cineole            | 4.7    | 1.5    | RRI, MS |
| 1244| 2-Pentyl furan        | –      | 0.1    | MS   |
| 1280| \( \beta \)-Caryophyllene | 0.2 | – | RRI, MS |
| 1285| Isoamyl isovalerate | tr    | tr     | MS   |
| 1299| 2-Methylbutyl isovalerate | 0.3 | – | MS |
| 1482| (\( \alpha \))-3-Hexenyl-2-methyl butyrate | 2.9 | 0.3 | MS |
| 1494| (\( \alpha \))-3-Hexenyl-3-methyl butyrate | 0.5 | – | MS |
| 1499| \( \alpha \)-Campholene aldehyde | –   | 0.5 | MS |
| 1534| Campher                | –      | 1.0    | RRI, MS |
| 1541| Benzaldehyde           | 1.8    | 2.7    | RRI, MS |
| 1562| Isopinocamphone        | 0.3    | 0.5    | MS   |
| 1586| Pinocarvone            | –      | 0.4    | RRI, MS |
| 1591| Bornyl acetate         | 0.1    | –      | RRI, MS |
| 1611| Terpinen-4-ol          | 0.5    | –      | RRI, MS |
| 1612| 1,8-Caryophyllene      | 0.2    | –      | RRI, MS |
| 1648| Myrtenol               | 0.1    | 0.5    | MS   |
| 1668| (\( \alpha \))-Farnesene | 1.2 | – | MS |
| 1670| trans-Pinocarveol      | 0.3    | 1.2    | RRI, MS |
| 1683| trans-Verbolen        | 0.8    | 1.2    | RRI, MS |
| 1704| \( \gamma \)-Murolene  | –      | 0.2    | MS   |
| 1719| Borneol                | 4.8    | 4.0    | RRI, MS |
| 1725| Verbeneone             | 0.7    | 0.7    | MS   |
| 1740| \( \alpha \)-Murolene  | –      | 1.0    | MS   |
| 1742| \( \beta \)-Selinene   | 2.1    | –      | MS   |
| 1773| \( \gamma \)-Cadinene  | –      | 1.8    | MS   |
| 1776| \( \gamma \)-Cadinene  | tr     | 0.8    | MS   |
| 1786| Kessane                | –      | 0.3    | MS   |
| 1797| \( \beta \)-Methyl acetonaphenone | – | 0.3 | MS |
| 1804| Myrtenol               | 0.4    | 0.7    | MS   |
| 1838| (\( \alpha \))-\( \beta \)-Damascenone | – | 0.3 | MS |
| 1845| trans-Carveol          | 0.3    | 0.8    | RRI, MS |
| 1849| Calameneene            | –      | 0.2    | MS   |
| 1864| \( \beta \)-Cadinene-8-ol | – | 1.1 | RRI, MS |
| 1871| Neryl isovalerate      | –      | 0.3    | MS   |
| 1872| \( \alpha \)-Methone    | –      | 0.2    | MS   |
| 1896| cis-Jasmine            | 1.1    | 0.4    | MS   |
| 2008| Caryophyllene oxide    | 1.7    | 3.1    | RRI, MS |
| 2050| (\( \alpha \))-Nerolidol | –    | 0.4    | MS   |
| 2080| Cubenol                | –      | 0.5    | MS   |
| 2144| Spalthulenol           | 0.6    | 2.9    | MS   |
| 2148| (\( \alpha \))-3-Hexenyl-1-yl benzoate | 1.6 | 0.7 | MS |
| 2187| T-Cadinol              | 3.2    | 6.7    | MS   |
| 2200| \( \alpha \)-Guaiol     | –      | 0.3    | MS   |
| 2204| Eremoligenol           | 0.6    | 1.9    | MS   |
| 2209| T-Murolol              | –      | 2.3    | MS   |
| 2214| (\( \alpha \))-Turmerol | –    | 0.7    | MS   |
| 2219| \( \delta \)-Cadinol   | –      | 0.4    | MS   |
| 2250| \( \alpha \)-Eudesmol  | 0.9    | 3.2    | MS   |
| 2255| \( \alpha \)-Cadinol    | –      | 7.2    | MS   |
| 2257| \( \beta \)-Eudesmol   | 13.8   | 12.8   | MS   |
| 2264| Intermedeol            | –      | 8.7    | MS   |
| 2278| \( \alpha \)-Kessy alcohol | 1.4 | – | MS |
| 2298| Decanoic acid          | 1.4    | 1.3    | RRI, MS |
| 2312| 9-Geranyl-\( \beta \)-cymene | 1.1 | 0.4 | MS |
| 2365| (\( \alpha \))-Methyl jasmonate | 1.7 | 0.8 | MS |
| 2388| (\( \alpha \))-Nuciferal | 0.5    | 1.9    | MS   |
| 2503| Dodecanoic acid        | 0.5    | 0.5    | RRI, MS |
| 2586| (\( \alpha \))-Nuciferal | –      | 0.5    | MS   |
| 2604| \( \alpha \)-Costol     | 0.9    | 0.8    | MS   |
| 2931| Hexadecanoic acid      | 2.1    | 1.4    | RRI, MS |

**RRI**: Relative retention indices calculated against n-alkanes; % calculated from FID data; tr Trace (<0.1%); IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

**Table 1** Composition of the Essential Oils of *Anthemis melampodina* (Am) and *A. scrobicularis* (As).
to our study, \(\beta\)-eudesmol was the second major constituent (13.8%) in Am EO and the most abundant compound (12.8%) of As EO. It is difficult to attribute the repellency of Am EO to \(\beta\)-eudesmol because As EO has that component at a similar percentage and the EO exhibited weak repellency.

In tick bioassays, *A. melampodina* and *A. scrobicularis* EOs showed 80.0% and 96.7% repellency against *Am. americanum* at a concentration of 2.50% As EO (80.0%) showed higher repellency than Am EO (26.7%) and the two plants showed significantly lower repellency when compared with the positive control DEET, which was 95% repellent at a concentration of 0.625% (Table 3). The moderate repellency of As EO is likely due to its high sesquiterpene content (59.4%).

According to previous studies on essential oils derived from plants, the repellency was attributed to the high percentage of sesquiterpenes, especially oxygenated sesquiterpenes, like farnesol. Farnesol is the major component of oxygenated sesquiterpenes of the essential oil isolated from *Pluchea dioscoridis* (Grace, 2002). Germacrene D, pregeijerene and geijerene namely norsequitperenes, the major constituents isolated from the oil of *Chloroxylon swietenia* leaves, were repellent against *Ae. aegypti* and *Anopheles gambiae* larvae (Krivpa and Pushpalatha). The sesquiterpene alcohol, (−)-10-epi-\(\beta\)-eudesmol, components of essential oils isolated from some plant species were found to be repellent against *Am. americanum* (Tabanca et al., 2013). Previous studies indicated that terpenoids (sesquiterpenes) that contain two functional groups, are biologically active against mosquitoes. One of these functional groups on the negatively charged end contains an ester/ether bond or an ethanol hydroxyl group and the other group on the positively charged end contains an alkyl group (Kalita et al., 2013).

In conclusion, this is the first report of a comprehensive study where GC–FID and GC–MS were employed to characterize the volatile constituents of *A. melampodina* and *A. scrobicularis* essential oils. Differences in the chemical compositions of these two species play an important role in the repellent activities. The essential oil of *A. melampodina* as a mosquito repellent was more effective than that of *A. scrobicularis*, but both were less efficacious than DEET. On the contrary, the essential oil of *A. scrobicularis* performed moderately as a repellent against ticks, while the essential oil of *A. melampodina* was less repellent. In tests with mosquitoes, both EOs were less repellent than DEET. As we mentioned above the mosquito repellency cannot be attributed to the high percentage of \(\alpha\)-pinene (17.1%) in the Am EO, but the high amount of monoterpane hydrocarbons (31.6%), oxygenated monoterpenes (23.7%) and oxygenated sesquiterpenes (22.2%) may be acting synergistically to produce the repellency. The tick repellency of As EO is likely due to the high relative amount of \(\beta\)-eudesmol or possibly to the effect additional compounds in the mixture acting together. This EO contains a high amount of oxygenated sesquiterpenes (55.4%) and oxygenated monoterpenes (20.1%). These Am and As EOs will be further evaluated in bioassay-guided studies to determine the active compound(s) that are responsible for their repellency against mosquitoes and ticks.

### Declaration

No financial/commercial conflicts of interest are reported by the authors.

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