Saudi Pharmaceutical Journal xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

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

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ARTICLE INFO

Article history:
Received 19 January 2018
Accepted 25 March 2018
Available online xxxx

Keywords:
Anthemis melampodina
Anthemis scrobicularis
Natural repellents
Aedes aegypti
Amblyomma americanum

ABSTRACT

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 melampdina* 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* L. and the lone star tick *Amblyomma americanum* L. The minimum effective doses (MEDs) of the Am and As EOs against mosquitoes were 0.187 ± 0.000 and 0.312 ± 0.063 mg/cm^2 respectively, which were significantly higher than that of DEET (0.023 ± 0.000 mg/cm^2) 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.

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 (Khater 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

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2018.03.012
1319-0164/

Please cite this article in press as: Yusufoglu, H.S., et al. Mosquito and tick repellency of two *Anthemis* essential oils from Saudi Arabia☆. Saudi Pharmaceutical Journal (2018), https://doi.org/10.1016/j.jsps.2018.03.012

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, schistosomicidal, cytotoxic, anthelmintic, 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; ManganenelliUncini 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* methanolic extract (Yusufoglu 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 Alkharj, 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 Election 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; McLafferty and Stauffer, 1989) 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 (Koenig et al., 2004; Joulain 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

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