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Ixodicidal compounds from pre-domesticated *Lavandula luisieri*

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ABSTRACT

Several extracts from pre-domesticated *Lavandula luisieri* (Rozeira) Riv-Mart. (Lamiaceae) (essential oil, EO; hexane, Hx and the organic fraction of the residual hydrolate, WRO) showed larvicidal effects against the hard tick *Hyalomma lusitanicum*, being WRO the most active. Their content in the necrodane-type monoterpenes (2,2,3,4-tetramethyl-5-oxocyclopent-3-en-1-yl)-methyl acetate (**2**), 5-hydroxymethyl-2,3,4,4-tetramethylcyclopent-2-en-1-one (**1**) and 2-(hydroxymethyl)-3,4,4-trimethyl-5-methylenecyclopent-2-en-1-one (**5**) correlated with these effects. Among the isolated compounds, 3,3,4,5-tetramethyl-2H-pyran-2,6(3H)-dione (**7**) and (2,2,3,4-tetramethyl-5-oxocyclopent-3-en-1-yl)-methyl acetate (**2**) were the most potent ones, with activity levels within the range of the positive control thymol. These compounds represent a new class of ixodicidal agents.

1. Introduction

Lavandula luisieri (Rozeira) Riv-Mart. (Lamiaceae) (Rivas-Martínez, 1979), is a small aromatic shrub endemic to the Iberian Peninsula. Previous studies showed that *L. luisieri* essential oil contained 1,8-cineole, lavandulol, linalool and their acetates, also present in other *Lavandula* species, in addition to a series of compounds with a 1,2,2,3,4-pentamethylcyclopentane (necrodane) structure (García-Vallejo et al., 1994; Lavoine-Hanneguelle and Casabianca, 2004; Baldovini et al., 2005; Julio et al., 2016) and showed antifungal and antibacterial effects (Lavoine-Hanneguelle and Casabianca, 2004; Zuzarte et al., 2012).

L. luisieri essential oils from central and southern populations of the Iberian Peninsula had camphor, 1,8-cineole and 2,3,4,4-tetramethyl-5-methylen-2-cyclopenten-1-one as their major components (Sanz et al., 2004). The essential oils of western *L. luisieri* populations had *trans*-α-necrodyl acetate as the major component and showed less variability in their composition (González-Coloma et al., 2011a). These oils showed moderate insect antifeedant effects (González-Coloma et al., 2006; González-Coloma et al., 2011a). The supercritical extraction (SCE) of *L. luisieri* improved the concentration of necrodane-type ketones and exhibited stronger insect antifeedant effects than the essential oil and ethanolic extract (Julio et al., 2014).

Given the potential value of this plant species to be developed as a biopesticide, a domestication programme has been established to obtain a chemically stable *L. luisieri* variety. Furthermore, necrodane-type compounds from a pre-domesticated population of *L. luisieri* have been recently identified as being phytotoxic and nematicidal (Julio et al., 2016). However, further bio-valorization of extracts from this plant is undergoing.

Hard ticks (Ixodidae) are a broad group of hematophagous ectoparasites with 720 species that colonize a wide range of cold- and hot-blooded vertebrate hosts. Ticks can transmit a great variety of pathogens to vertebrates, being the first vectors affecting wild and domestic animals and livestock and the second most important vectors affecting humans after mosquitoes (Guglielmone et al., 2014).

The genus *Hyalomma* is a relatively young phylogenetic group of ixodid ticks, well adapted to arid biotopes of the Old World. The domestication and development of cattle-breeding stimulated the evolution and biological progress of this group (Kolonin, 2009). *Hyalomma lusitanicum*, Koch 1844 (Ixodida: Ixodidae) is abundant in the Mediterranean region (Apanaskevich et al., 2008), has a widespread distribution some regions of Southern Spain (Encinas-Grandes, 1986), and it is becoming much more common in central Spain (Basco-Basco et al., 2008; Barandika et al., 2011). *H. lusitanicum* is one of the vectors of

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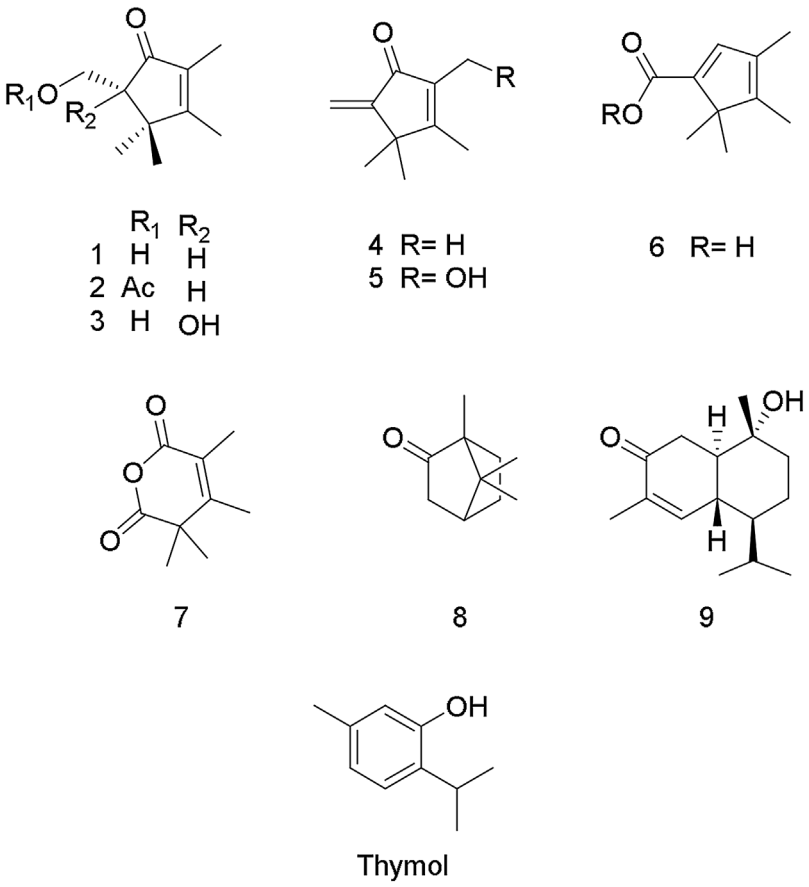

Fig. 1. Chemical structures of compounds **1**-**9** and thymol.

Theileria annulata that causes Mediterranean theileriosis in cattle and it is a potential vector of several zoonotic bacterial agents such as *Anaplasma* spp., *Ehrlichia* spp., *Bartonella* spp., *Borrelia* spp., *Coxiella burnetii*, *Francisella* spp. and *Rickettsia* spp. (Toledo et al., 2008; Toledo et al., 2009) including Crimean-Congo hemorrhagic fever virus (C-CHFV) (Estrada-Peña et al., 2012).

Tick control relies mostly on rapid-acting synthetic pesticides (Ostfeld et al., 2006), increasing the selection of acaricide-resistant ticks and causing environmental contamination (Kiss et al., 2012). Plant extracts, including essential oils and their terpene components have been reported as being toxic and/or repellent to ticks (Kiss et al., 2012; Cruz et al., 2013; Kröber et al., 2013) and therefore are a promising new source of natural ixodicidal agents.

In this work we have studied the ixodicidal effects on *H. lusitanicumm* larvae of different extracts from pre-domesticated *L. luisieri* plants (essential oil, EO; hexane and ethanolic extract, Hx, EtOH and the organic fraction from the aqueous residue, WROE) and their chemical composition. The compounds isolated from the active extracts have been also tested (Fig. 1).

2. Materials and methods

2.1. Plant material and cultivation

L. luisieri plants have been cultivated in an experimental field located in Comarca del Campo de Cariñena, Aguarón (Zaragoza, Spain) (16 m, 41°19′13.33″N; 1°19′53.9″W) as described (Julio et al., 2016).

The experimental design consisted of three random blocks (2 m between blocks), containing four 10 m rows with 104 plants per row (49.92 m^2) at a distance of 1.20 × 0.40 m (0.48 m^2/plant). The experimental field was established in March 2008 with plants produced from seeds collected in June 2007 from a wild population located in Pueblo Nuevo del Bullaque (Ciudad Real, Spain; latitude: 39° 27′41″N, longitude: 4°24′34″W, altitude: 733 m) and germinated in a commercial nursery. The aerial parts of the cultivated plants collected during 2009–2012 were dried in the absence of light at room temperature.

2.2. Extraction

The hexanic (Hx) and ethanolic extractions (EtOH) were performed in a Soxhlet apparatus with *n*-hexane or EtOH and concentrated in vacuo (1.2 and 12.5% yield respectively).

Laboratory scale hydrodistillation (essential oil, EO) was performed in a Clevenger-type apparatus (0.8% yield) according to the method recommended by European Pharmacopoeia (http://www.edqm.eu/en/Homepage-628.html). Pilot plant vapor pressure extraction (PEO, 0.2% yield) was carried out in a stainless steel distillation plant equipped with a 100 kg distillation chamber, a 500 L vessel and a pressure range of 0.5-1.0 bar. The water collected after the essential oil was decanted and filtered to give an oil-free water residue (WR, 4.5 mg/mL of organic components, pH 3.2). This aqueous residue (WR, 1000 mL) was extracted with dichloromethane (800 mL × 3) to give the organic extract WRO (3.42 g, 0.34% yield).

2.3. Extract fractionation

The organic extract WRO was chromatographed by flash chromatography on a 2.5 cm diameter silica cartridge (40–70 μm) eluted with a DCM:MeOH gradient (100:0-95:5, 20 mL/min) (Jones Flash Chromatography) to give four fractions (WROE 1–4: 1.1%, 12.0%, 4.3% and 6.6% yield respectively).The fractions were monitored by TLC (silica gel 60 F254, 0.25 mm, Merck) and analyzed by GC–MS.