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Research paper

Chemical characterization and acaricidal activity of *Drimia maritima* (L) bulbs and *Dittrichia viscosa* leaves against *Dermanyssus gallinae*

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ABSTRACT

The emergence of resistance to chemical acaricides in *Dermanyssus gallinae*, together with their toxicity and high costs, has prompted investigations into the use of plant extracts as alternatives to chemical acaricidal treatments. *Drimia maritima* bulbs and *Dittrichia viscosa (D. viscosa)* leaf extracts were here characterized by HPLC-PDA-ESI-MS/MS, and their toxicity against *D. gallinae* was evaluated using contact methods. Twenty-nine compounds were identified in *D. maritima* extracts, with glucoscilliphaeoside derivatives (i.e., quercetin, kaempferol and bufadienolides) as the major components. Twenty-four phenolic compounds, mainly caffeic acid derivatives, were detected in *D. viscosa* extracts. *D. maritima* extracts displayed a significantly higher ($p < 0.05$) acaricidal activity than *D. viscosa* extracts, with 100% of *D. gallinae* mortality at a concentration of 100 mg/mL following 24 h exposure. The mortality rate of *D. gallinae* induced by *D. viscosa* extracts ranged from 25 to 45% following 48 h exposure at a concentration of 200 mg/mL. The acetonic extract of *D. viscosa* and *D. maritima* displayed the highest efficacy against *D. gallinae*. This study provides evidence of the diversity of bioactive compounds present in *D. maritima* bulbs and *D. viscosa* leaf extracts, which are both efficacious against *D. gallinae*. The higher efficacy of *D. maritima* bulb extracts might be linked to the presence of bufadienolides in its extracts.

1. Introduction

The blood-sucking poultry red mite *Dermanyssus gallinae* is one of the most economically important ectoparasites of laying hens worldwide (Sparagano et al., 2014); in heavy infestations, clinical signs include anemia, dermatitis, irritation and skin lesions (George et al., 2015; Koziatek and Sokół, 2015). In addition, this mite is a potential vector of pathogenic viruses (e.g., Flavivirus, Avulavirus) and bacteria (e.g., *Salmonella* spp., avian spirochetes), as well as the aetiological agents of tick-borne encephalitis, Newcastle disease and salmonellosis in livestock (Dehghani-saman et al., 2015; George et al., 2015). Heavy infestations by *D. gallinae* may cause severe damage to the poultry industry, which vary from decreased growth rates, egg production and feed conversion to high animal mortality (Sparagano et al., 2014). Control of *D. gallinae* typically relies on the use of chemical acaricides (i.e., organophosphates, carbamate, amidine and pyrethroid) (Marangi et al., 2012) with an annual cost estimated at around $100 billion (Mohan et al., 2011). Nonetheless, parasiticide resistances of this mite to chemical compounds have been reported (Marangi et al., 2012; Sparagano et al., 2014). In addition, concerns regarding the presence of pesticide residues in poultry meat and/or eggs at the end of the production cycle (Carriger et al., 2006; Marangi et al., 2012) have limited the use of these synthetic products as advised by EU legislation (Sparagano et al., 2014).

Biological control based on the acaricidal effect of plant products is increasingly been investigated as a potential solution against acaricide resistance (Borges et al., 2011; Adenubi et al., 2018). Secondary metabolites in plant preparation (i.e., phenol, flavonoids, terpenes, cardiac glucosides) may interfere with ectoparasites via a range of mechanisms of action (Borges et al., 2011; Adenubi et al., 2018) and several investigations have showed their ability to control acarine mites and insects of veterinary and medical importance (Koziatek and Sokół, 2015). For example, extracts from Mediterranean plants (i.e., *Azadirachta indica*, *Chrysanthemum cinearіaefolium*, *Allium sativum*, *Tanacetum vulgare*,

Abbreviations: m.a.s.l., Meters above sea level; RH, Relative Humidity; RT, Retention Time; sh, shoulder

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and others) were efficacious in controlling *D. gallinae* (Maurer et al., 2009; Sparagano et al., 2014; Camarda et al., 2018). Amongst plants of the Asparagaceae and Asteraceae families growing in the Mediterranean area, *Dittrichia viscosa* and *Drimia maritima* are active against several pests (i.e, *Tribolium castaneum*, Varroa mite (*Varroa destructor*), Carmine spider mite (*Tetranychus cinnabarinus*), yellow mealworm beetle (*Tenebrio molitor*), mainly due to the high levels of cardiac glycosides, sesquiterpene and phenolic compounds that they contain (El-Seedi et al., 2013; Hamouda et al., 2015; Knittel et al., 2014; Sofou et al., 2017). However, the efficacy of these plant extracts against *D. gallinae* has yet to be tested. This study aimed i) to investigate the chemical composition of different alcoholic extracts of *D. maritima* bulbs and *D. viscosa* leaves and ii) to evaluate their acaricidal activity against *D. gallinae*.

2. Materials and methods

2.1. Plant extracts preparation and chemical characterization

D. maritima bulbs and *D. viscosa* leaves were collected from the Sidi Thabet region of Tunisia (36° 55′ 45″ N, 10° 06′ 02.10″ E, altitude 30 m a.s.l.) and identified at the Department of botany, National Institut of Agronomic Research, Tunis. Leaves and bulbs were dried at 40 °C and manually ground to powder. Each powdered plant (20 g) was macerated sequentially in 100 mL of different solvents (i.e., methanol, ethanol, acetone, and butanol) for 48 h. Each extract solution was vacuum filtered through a Buchner funnel (diameter ~4.25 cm) with Whatman filter paper n°1 (Fisher Scientific UK Ltd, Loughborough, UK). The filtrates were left to evaporate until dry using a rotary evaporator under reduced pressure at 40 °C and the extract was stored at 4 °C until further use. The chromatographic separation method was performed as previously reported with slight modifications (Mahmoudi et al., 2015). Briefly, 5 mg of each extract from *D. maritima* bulbs and *D. viscosa* leaves were dissolved in HPLC grade methanol (1 mL), then ultrasonicated for 10 min and filtered using 0.2 µm syringe filters (Whatman International Ltd., Maidstone, Kent, UK). An aliquot of 20 µL of sample was subjected to liquid chromatography analysis (HPLC-PDA-ESI-MS/MS) on a Waters Alliance e2695 HPLC system (Bedford, MA) equipped with photodiode array detector (PDA) and interfaced with a triple quadruple mass spectrometer (MSD 3,100, Waters) coupled with an electrospray ion source (ESI) in the negative mode. Compounds were separated on a RP-xTerra MS column (150 × 4.6 mm i.d., 3.5 µm particle size). The mobile phase consisted of an aqueous solution acidified with 0.1% formic acid (solution A) and acetonitrile acidified with formic acid 0.1% (solution B) with a flow rate of 0.5 mL/min. The multistep linear solvent gradient was programmed as follows: 0–40 min: 14–26% B; 40–60 min: 15% B; 60–75: 0% B; 75–80 min: 14% B. The desolvation temperature was 380 °C, and the ion source temperature was set at 120 °C. The capillary and cone voltage was set at 3500 V and 2500 V respectively. MS scans were operated in full scan mode from 100 to 1000 amu. Photodiode array (PDA) detection measured wavelength range from 200 to 800 nm. The identification of compounds in the tested extracts was confirmed via comparison with reference compounds or using structural information available in the literature (Bravo et al., 2007; Liu et al., 2007; Wang et al., 2012; Abdel-Hameed et al., 2013; Spiridon et al., 2013; Knittel et al., 2014; El Sayed et al., 2016; Li et al., 2016; Marczak et al., 2016; Maazoun et al., 2017; Chernonosov et al., 2017; Rhimi et al., 2017).

2.2. Mite specimens

Adults of *D. gallinae* were collected from laying hen farms in two cities in southern Italy: Valenzano (41° 02′ 46.14″ N 16° 53′ 1.79″ E, 83 m a.s.l.) and Bitritto (41°03′00″N 16∘50′00″E, 102 m a.s.l.) at three different times. Laying hens were naturally infested and had received no standard acaricidal treatments over two months prior to sampling. Mites were transported in sealed plastic bags to the Unit of Parasitology and Mycology (Department of Veterinary Medicine, University of Bari, Italy), identified using morphological keys (Di Palma et al., 2012) and kept at 22 ± 1 °C until the beginning of the experiment, within 24 h from collection (Immediato et al., 2015).

2.3. Laboratory bioassays and data analysis

D. maritima and *D. viscosa* extracts (i.e, methanolic, ethanolic, acetonic, butanolic) were dissolved in ethanol at concentrations (w/v) from 10 to 200 mg/mL for *D. viscosa* (i.e., 10, 50,100 and 200 mg/mL) and from 0.1 to 100 mg/mL for *D. maritima* (i.e., 0.1, 10, 50 and 100 mg/mL) and used to evaluate acaricidal activity as previously described (Immediato et al., 2015). Adult *D. gallinae* (n = 3960) were divided into three groups: TG1: group treated with *D. viscosa* extracts (n = 1920); TG2: group treated with *D. maritima* extracts (n = 1920) and CG: group treated with ethanol (extract solvent) (n = 120).

Each group was composed of four subgroups of 20 mites each. Mites were subjected to the same treatment and placed into bioassay rooms (BR) composed of Petri dishes (60 mm diameter) containing filter paper (Whatman n°1, 10 × 10 mm, Tecnochimica Moderna, Italy). The filter paper was soaked in 0.2 mL of each *D. maritima* and *D. viscosa* solution extracts or ethanol. The Petri dishes were covered with a lid, sealed with parafilm and stored at 25 ± 1 °C (RH 80 ± 5%). Mortality was evaluated after 24 h and 48 h of incubation. Mites were considered dead if they showed no movement after repeated mechanical stimulation with an entomological pin by three different examiners. All experiments were conducted in duplicate and repeated three times on different days. The mortality observed was expressed as corrected mortality rates using the Schneider-Orelli's Formula:

Corrected mortality % = (Mortality % in treated plot- Mortality % in control plot)/ (100- Mortality % in control plot) × 100

Where the mortality (%) = (number of mites die after treatment/ 20) × 100.

2.4. Statistical analysis

Mite mortality rates at different times in three independent experiments were compared using Chi-square tests ($p < 0.05$). Subsequently, means were calculated and the rates of mortality of *D. gallinae* following contact with different extracts were expressed as corrected mortality rate as indicated above. Analysis of variance (ANOVA) was performed to test any differences between the plants, solvent, concentrations and mite origin. The difference was considered as significant when $p < 0.05$. Statistical analyses were carried out using IBM SPSS Statistics 21.

3. Results

Twenty nine phenolic and bufadienolides compounds were identified in *D. maritima* extracts with flavonols (kaempferol and quercetin derivative) and bufadienolides (glucoscilliphaeoside (gamabufotalin derivatives) as the most abundant (Table 1; Figs. 1 and 2).

The presence of coumaric acid, chlorgenic acid, kaempferide, viscidulin I 2-O-Glucoside, isorhamnetin-rhamnoside were identified in methanolic, acetonic and butanolic extracts of *D. maritima* (Table 1). On the other hand, 24 phenolic compounds were detected in *D. viscosa* extracts, with caffeic acids derivatives (i.e., chlorgenic acid, di-caffeoylquinic acids, 3,4,5-tri-O-caffeoylquinic acid) as the dominant compounds (Table 2). Kaempferol derivatives and salvianolic acid A were detected only in acetonic extracts of *D. viscosa* (Table 2). The total caffeoylquinic acid content of *D. viscosa* and *D. maritima* extracts ranged from 57.11 ± 0.98 to 87.6 ± 1.06 µg /mg extract and 1.38 ± 0.13 to 8.09 ± 0.11 µg /mg extract, respectively (data not shown). The total

Table 1
Retention time, UV and mass spectral data and tentative identification of the phenolic and bufadienolides components in *Drimia maritima* bulb extracts.

Peak n	RT (min)	[M-H]$^-$	Fragments (m/z)	UV max	Tentative of identification	Presence in the extracts
1	5.447	199	163(100)	260-339	Coumaric acid	MM
2	10.784	284	284(100)-179 (40) -165(80)-151(60)	282 -320	Kaempferol	MM, ME, MA, MB
3	11.901	615	299(30)-284(100)-227(28)-137(55)	286 -320	kaempferol-hexosyl- pentoside	MM, ME, MA
4	12.934	593	413(50)-284(100)-256(40)	272 sh-332	Kaempferol-3-O-hexose-deoxyhexose	MA
5	15.144	501	465(100) -437-303(20) -285(65)-275(40)-259-152(38) 125.1(90)	288.5 -332	Dihydroquercetin O-hexoside	MM, ME, MA, MB
6	15.983	300	284 (100)- 137(60)	272-334	Quercetin derivative	MM, ME, MA, MB
7	16.541	723	609(10)-465(100)- 303(30)-285(80)-125(90)	298 -320	Quercetin-3-O-rutinoside-7-O-rhamnoside	ME, MA, MB
8	17.370	773	561(46)- 465(30)-284(100)-249(50)	286-320	Kaempferol 3-O-glucosyl-rutinoside	MA
9	18.835	301	301(100)-284 (35)-249 (28)-151(36)	290-330	Quercetin	MM, ME, MA
10	20.274	627	564(30) -301(100)-284(55)-249(60)-151(35)	290-328	Quercetin-rutinoside (Rutin)	MM, MA, MB
11	21.866	643	643 (100)	290	Viscidulin I 2-O-Glucoside	MA
12	24.772	353	191(100)	271-334	Chlorgenic acid	MM
13	25.075	577	431(83)-284(100)	286 sh	kaempferol-3-O-rutinoside	MA, MM
14	27.090	869	707(100)-545 (20)	289-320	Glucoscilliphaeoside-hexoside	MM, ME, MA, MB
15	28.757	869	707(100)-545 (20)	295	Glucoscilliphaeoside-hexoside isomer	MM, ME, MA, MB
16	30.747	871	709(100) -547(29)	295	Gamabufotalin-rhamnosido-glucoside	MM, ME, MA, MB
17	32.322	828	791(100)-709(30)-	291	Gamabufotalin derivative	MM,M E, MA, MB
18	34.896	639	639(100)	295	hydroxyscilliglaucosidin-glucoside	MA
19	35.362	709	707(90)-643 (100)-545(20)	293	Scillarenin-glucose-glucoside	MM,M E, MA, MB
20	37.579	869	707(100)-639(80)-525(20)	292	Scillarenin-glucose-glucoside–hexoside	MM,M E, MA, MB
21	39.756	790	707(60)-559 (40)-397(100)	292	Scilliglaucosidin dervatives	MM, ME, MA, MB
22	40.992	735	707-577-397-299-284-271	298	Glucoscilliphaeoside	MM, ME, MA, MB
23	42.084	739	605(100)-559(70) -533-397-329-249	292	Scilliglaucoside/altoside	MM, ME, MA,M B
24	43. 690	749	707 (100)-545(50)-413(40)	294	Glucoscilliphaeoside	MM, ME, MA, MB
25	45.658	707	627(100)-577(20)-559(20)-397(40)	291	Scilliglaucoside/altoside	MM, ME, MA, MB
26	46.490	317	299(100)-284(80)	267-320	Kaempferide	MA
27	47.352	479	315(40)-300(100)-283(40)-151(20)	268-330	Isorhamnetin-rhamnoside	MB
28	48.849	625	625 (100)-609(20)-329(50) -315(40)-299(80)	290sh	Quercetin O-hexoside-hexoside	ME, MA, MB
29	49.856	625	625 (100)-609(20)-343(20) -315(40)-299(80)	290	Quercetin O-hexoside-hexoside	MA,M B

*MM: methanolic extract of *D. maritima*; ME: ethanolic extract of *D. maritima*; MA: acetonic extract of *D. maritima*; MB: butanolic extract of *D. maritima*.

flavonoids content of *D. viscosa* and *D. maritima* extracts ranged from 30.86 ± 1.28 to 58.03 ± 1.85 µg /mg extract and 5.33 ± 0.62 to 11.01 µg /mg extract respectively (data not shown).

The acaricidal activity of extracts from *D. maritima* bulbs and *D. viscosa* leaves at 24 h and 48 h against adult *D. gallinae is* reported in Table 3. The mortality rate of *D. gallinae* was positively correlated with the concentration of extracts and with exposure time. *D. maritima* extracts had a significantly higher ($p < 0.05$) acaricidal activity than *D. viscosa* extracts, with 100% of mite mortality achieved at the concentration of 100 mg/mL after 24 h of exposure (Table 3). The corrected mortality rate of *D. viscosa* extracts ranged from 0 to 45% after 48 h of exposure (Table 3). Mite mortality varied according to the solvent extracts, and the acetonic extracts from both *D. viscosa* and *D. maritima* were the most effective (Table 3).

4. Discussion

This study characterized the phenolic and bufadienolides compounds from *D. maritima* bulbs and *D. viscosa* leaves and demonstrated, for the first time, their efficacy against *D. gallinae*. In particular, kaempferol derivatives (i.e, kaempferol-glycoside, kaempferol-O-pentose, kaempferol-O-hexoside) and salvianolic acid in *D. viscosa* acetonic

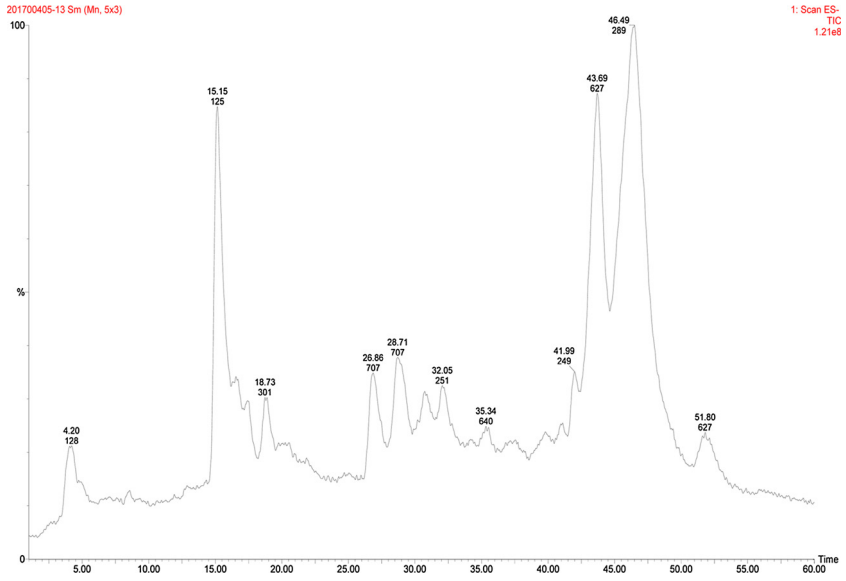

Fig. 1. Representative HPLC-PDA-ESI-MS Analysis of acetonic extract of *Drimia maritima* bulbs (assignment of peaks are given in Table 1).

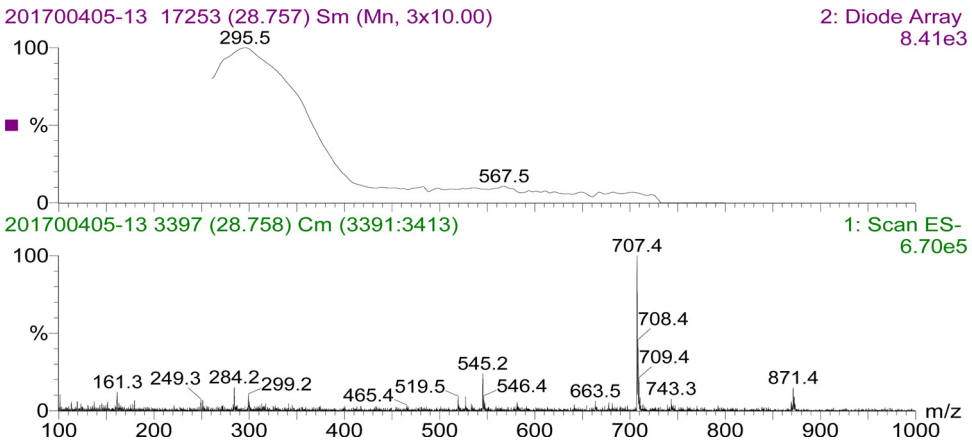

Fig. 2. MS fragmentation pattern and corresponding UV spectra of Glucoscilliphaeoside-hexoside isomer present in *Drimia maritima* (peak no. 29).

extracts are here reported for the first time in addition to other active compounds already known (Knittel et al., 2014; Trimech et al., 2014)

D. maritima bulb extracts were characterized by abundant quercetin derivatives, kaempferol derivatives and bufadienolides. The presence of bufadienolide compounds, exclusively found in *D. maritima* plants, confirms previous results obtained with *D. maritima* from Egypt, Italy and Japan (Iizuka et al., 2001; Kopp et al., 1996; Knittel et al., 2014), suggesting that these compounds might be considered hallmarks of *D. maritima*. Similarly, the dominance of the caffeic acids derivatives (i.e., chlorogenic acid, caffeic acid, dicaffeoylquinic acids) in *D. viscosa* extracts confirms the results obtained from other Mediterranean countries (Danino et al., 2009; Trimech et al., 2014), and suggests that *D. viscosa* leaf extracts might be an important sources of phenolic compounds independently from geographical origin (Danino et al., 2009; Knittel et al., 2014; Trimech et al., 2014). However, this is the first study reporting the presence of kaempferol derivatives and salvianolic acid A only in *D. viscosa* leaf acetonic extract. This is most likely due to the high solubility of these compounds in the solvent used (Lu and Foo, 2001; Munhoz et al., 2014), the geographical location (Djouahri et al., 2015) and/or the harvesting time (Yesil-Celiktas et al., 2007). The presence of these compounds might suggest that their acaricidal activity is due to their enzymatic antioxidants properties (i.e., superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and polyphenol oxidase), as reported for *Adonis coerulea* and *Syzygium aromaticum* (Shang et al., 2017; Matluobi et al., 2018).

In this study, both *D. maritima* and *D. viscosa* extracts, although with different efficacy, caused mortality of *D. gallinae*. The potential use of these plants in the control of several ectoparasites has previously been reported (El-seedi et al., 2013; Hamouda et al., 2015; Topakci, 2016) and phenolic compounds (i.e, caffeic acid derivatives, kaempferol derivatives and quercetin derivatives) and/or cardiac glycoside compounds (bufadienolides) are the likely active compounds (El-Seedi et al., 2013; Knittel et al., 2014; Hamouda et al., 2015).

Caffeic acid and quercetin are known as the most bioactive molecules for the control of adult acarids and insects, due to their acetylcholinesterase nerve action inhibition activities (Maazoun et al., 2017). However, the dose of caffeic acid and quercetin which caused arthropod mortality, was relatively higher (i.e., 100 mg/mL) (Ravindran et al., 2017), when compared to findings from our study (lower than 17.4 mg/mL for caffeic acid and lower than 11.6 mg/mL for quercetin). These data confirm the higher efficacy of crude plant preparations when compared to individual compounds, which is most

Table 2
Retention time, UV and mass spectral data and tentative identification of the phenolic components in *Dittrichia viscosa* leaves extracts.

Peak n	RT (min)	[M-H]$^-$	Fragments (m/z)	UV max	Tentative of identification	Presence in the extracts
1	3.920	387	341(100)-179 (60)	260-330	Caffeic acid-O-hexoside	DM
2	4.868	377	317(70)-163(100)	294sh-320	P-coumaric acid derivative	DA
3	7.992	353	191(100)-135(10)	325	Chlorogenic acid	DM, DE, DA, DB
4	11.725	469	467(60)-429(10)-299(20)-284(100)-267(40)	274sh-330	Kaempferol -glycoside derivative	DA
5	14.711	389	284(100)-267(40)-137(40)	273sh-333	Kaempferol-O-pentose	DA
6	16.431	446	429(20)-284(100)-137(40)	273sh-333	Kaempferol-O-pentose	DA
7	17.413	489	299(20)-284(100)-137(30)	273sh-333	Kaempferol-O-hexoside	DA
8	18.986	609	301(45)-284(100)-191(40)	273sh-333	Rutin (quercetin-3-O-rutinoside)	DM, DE, DA, DB
9	21.117	477	301(100)-179 (20)-151(20)	260-335	Quercetin-3-glucuronide	DE, DA, DB
10	22.9	477	315(40)-301(100)-179(75)	328	Quercetin-3-glucuronide	DM, DE, DA,D B
11	23.368	463	357(30)-315(50)-301(100)- 179(80)-161(50)	327	Quercetin 3-O-diglucoside	DA
12	24.592	515	353(35)-191(100)-179(30)	328sh	3,4-dicaffeoylquinic acid	DM, DE, DA, DB
13	26.565	677	353(20) -191(100)-179(40)-135(25)	328.5	1,3, 5-tricaffeoyl quinic acid	DM,D E, DA, DB
14	27.893	677	509(20) 284(50)- 191(100)-179(50)	328.5	3,4,5-tricaffeoyl quinic acid	DM, DE, DA, DB
15	28.891	537	515(30)-353(40) -191(65)-173(100)-135(40)	330sh	3,5-dicaffeoylquinic acid	DM, DE, DA, DB
16	29.373	515	353(25)-284(40)-191(60)-179(100)-135(50)	329sh	4,5-dicaffeoylquinic acid	DM, DE, DA, DB
17	30.794	677	591(20)-374 (20) -284(40)-191(80)-179 (100)	328.5	3,4,5-tricaffeoyl quinic acid	DE, DA
18	31.843	826	790(100) -299(40)-284 (100)-209(40)-179(55)	260-328	Dehydrodicaffeic acid	DM, DE, DA
19	32.105	826	790 (100)-405(30)-209(50)-191(50)	260-328	Dehydrodicaffeic acid	DM, DE, DA, DB
20	33.612	826	790(100)- 549(30) -371(50)- 241(80)- 161(80)	260-328	Dehydrodicaffeic acid	DM, DA, DB
21	39.850	369	299(100)-271(73)	260-329	Rhamnocitrin derivatives	DA
22	41.585	493	493(100)	289.5 sh	Salvianolic acid A	DA
23	43.380	493	493(100)	289.5 sh	Salvianolic acid A	DA
24	43.395	493	315(40)-300(100)- 165 (20)	288-333	Methylquercetin aglycon	DM, DB

*DM: methanolic extract of *D. viscosa*; DE: ethanolic extract of *D. viscosa*; DA: acetonic extract of *D. viscosa*; DB: butanolic extract of *D. viscosa*.

Table 3
Corrected mortality rate of *Dermanyssus gallinae* exposed to *D. maritima* and *D. viscosa* at different concentration of solvent extracts after exposure of 24 h and 48h.

Plant	C(mg/ml)	Time exposure	Corrected mortality (%)			
			Methanol	Ethanol	Acetone	Butanol
D. maritima	0.1	24 h	0^{a}	0^{a}	0^{a}	0^{a}
		48 h	0^{a}	0^{a}	0^{a}	0^{a}
	10	24 h	0^{a}	2.32^{b}	0^{a}	0^{a}
		48h	2.32^{a}	4.9^{b}	1.03^{c}	7.48^{d}
	50	24 h	5.13^{a}	2.57^{b}	14.1^{c}	11.54^{d}
		48 h	8.76^{a}	13.76^{b}	36.78^{c}	27.63^{d}
	100	24 h	100^{a}	100^{a}	100^{a}	100^{a}
		48 h	100^{a}	100^{a}	100^{a}	100^{a}
D. viscosa	10	24 h	0^{a}	0^{a}	0^{a}	0^{a}
		48 h	0^{a}	0^{a}	0^{a}	0^{a}
	50	24 h	0^{a}	2.32^{b}	2.32^{b}	0^{a}
		48h	0^{a}	2.32^{b}	4.64^{c}	0^{a}
	100	24 h	1.03^{a}	2.32^{b}	2.32^{b}	2.32^{b}
		48 h	21.63^{a}	16.5^{b}	29.36^{c}	26.84^{d}
	200	24 h	8.53^{a}	4.82^{b}	2.32^{c}	2.32^{c}
		48 h	35.7^{a}	25.44^{b}	45.87^{c}	40.77^{d}

*C: concentration; Values followed by the same superscript letter along the row are not significantly different ($p > 0.05$).

likely due to their additive and synergetic activities (Laurin and Murray, 2001). Additionally, in this study, phenolic compounds (i.e., caffeic acid derivatives, kaempferol derivatives and quercetin derivatives) were detected in the extracts of both plants. However, bufadienolide compounds were found exclusively in *D. maritima* extracts. The presence of bufadienolides in *D. maritima* extracts might account for its higher acaricidal activity than *D. viscosa*. Indeed, the insecticidal activity of bufadienolides was previously reported against the third instar larvae of silkworm (*Bombyx mori*) L. (Lepidoptera: Bombycidae) and attributed to its essential structural elements (i.e., orthoacetate and alphapyrone) that act through inhibition of Na^{+}/K^{+} ATPase enzyme and cellular alteration, such as cell volume, free calcium concentration and membrane potential (Hidayat et al., 2014; De Sousa et al., 2017). A concentration of 100 mg/mL of *D. maritima* extracts led to 100% efficacy against *D. gallinae*, as demonstrated for other essential oils (i.e., thyme, sweet basil, common juniper, atlas cedar, coriander, blue gum, silver fir, common lavender, lemon, peppermint, scots pine, summer savory) (Ebrahimi et al., 2015; Magdaş et al., 2010). This concentration is lower than that of organophosphates (1gr/mL) that are currently administered for *D. gallinae* control (Dehghani-saman et al., 2015).

5. Conclusion

This study provides data on the diversity of bioactive compounds in *D. maritima* bulbs and *D. viscosa* leaves and demonstrates that they are both effective in controlling *D. gallinae*. The acaricidal activity of *D. maritima* extracts was higher than that observed for *D. viscosa*, thus suggesting that the former extracts are potentially suitable in controlling mite populations in the environment. Nonetheless, further laboratory and field studies are required to determine the most viable application routes and treatment frequency of poultry establishments.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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