https://doi.org/10.17221/20/2018-VETMED

Composition and acaricidal activity of essential oil from *Elsholtzia densa* Benth against *Sarcoptes scabiei* mites *in vitro*

*Yancheng Zhou[1†], Fei Liao[2†], Jiahua Weng[1], Quan Mo[1], Ruiguang Xu[1], Yong Zhang[1], Zhihua Ren[1], Zhijun Zhong[1], Zhicai Zuo[1], Guangneng Peng[1], Junliang Deng[1], Cheng Tang[3], Yanchun Hu[1]**

[1]*Key Laboratory of Animal Disease and Human Health of Sichuan Province, College of Veterinary Medicine, Sichuan Agricultural University, Wenjiang, China*
[2]*Animal Epidemic Disease Control Center of Qiandongnan Prefectural, Kaili, China*
[3]*College of Life Science and Technology, Southwest University for Nationalities, Chengdu, China*

**Corresponding author: hychun114@163.com*
[†]These authors contributed equally to this work

Citation: Zhou Y, Liao F, Weng J, Mo Q, Xu R, Zhang Y, Ren Z, Zhong Z, Zuo Z, Peng G, Deng J, Tang C, Hu Y (2019): Composition and acaricidal activity of essential oil from *Elsholtzia densa* Benth against *Sarcoptes scabiei* mites *in vitro*. Veterinarni Medicina 64, 178–183.

Abstract: Plant-based natural products represent an alternative to chemical compounds for the control of mites in veterinary medicine. Here, the essential oil of *Elsholtzia densa* (*E. densa*) Benth was extracted using hydrodistillation at a rate of 1.2%. The chemical composition of the essential oil was determined by gas chromatography-mass spectrometry (GC-MS) analysis. The GC-MS analysis indicated that the principal compounds in the volatile oil of the sample were 4-Pyridinol (28.16%) and thymol (26.58%). The acaricidal activity of *E. densa* oil against *Sarcoptes scabiei* (*S. scabiei*) was tested *in vitro*. Toxicity test data were analysed using a complementary log-log (CLL) model. The *E. densa* oil was prepared in five concentrations by dilution with liquid paraffin (1, 2, 4, 8 and 16 mg/ml) and exhibited strong toxicity against *S. scabiei* with LT_{50} values of 16.637, 5.075, 2.884, 1.184 and 0.760 h, respectively. The LC_{50} values were 7.678, 4.623, 2.543, 1.502, 1.298 and 0.981 mg/ml for *S. scabiei* at 1, 2, 4, 8, 16 and 24 h, respectively. Compared to the control, the essential oil showed significant effects against *S. scabiei in vitro.* At 16 mg/ml, *E. densa* oil was found to kill all mites within a 16-h period. The results indicate that *E. densa* oil possesses potential acaricidal activity *in vitro* and may be exploited as a novel drug for the effective control of *S. scabiei*.

Keywords: 4-Pyridinol; thymol; rabbit; botanical acaricides; degradation

Mange caused by *S. scabiei* is a critical veterinary skin disease in rabbits that reduces the quality and the productivity of animal products, even leading to secondary infection or death in severe cases. *S. scabiei* causes infestation in rabbits, affects their feet, results in intense pruritus and loss of hair (Seddiek et al. 2013). It can lead to large economic losses in livestock. In Sichuan Province, south-west China,

Supported by the Science and Technology Support Program of Sichuan Province (Grant No. 2015SZ0201), the Sichuan Provincial Foundation for Leaders of Disciplines in Science (Grant No. 2016JQ0023) and Chang-Jiang Scholars and the Innovative Research Team at the University (Grant No. IRT0848).

https://doi.org/10.17221/20/2018-VETMED

S. scabiei can survive for a long time and remain capable of infestation after they leave their hosts due to unique environmental factors and excessive humidity. As a consequence, it is difficult to eliminate the disease. Taking into account animal welfare, animal ethics and the safety of animal food products, it is therefore recommended that infected rabbits should receive efficient treatment (Alasaad et al. 2012).

Chemical drugs including pyrethrins, macrolides, organophosphates and organochlorine play a very important role and have been widely used to treat and control sarcoptic mange in veterinary clinics. They are characterised by relatively satisfactory effectiveness (Krawczak et al. 2011). However, the use of some chemical acaricides to control the pest and diseases of food commodities is limited due to overuse which can increase resistance in target species and pose environmental hazards (Ebadollahi et al. 2015). Environmental contamination and long degradation periods have led to an intense search for more efficient alternatives to acaricidal management.

Botanical acaricides are superior to chemical acaricides because they can decay quickly in the environment reducing the risk of acaricide residues in meat and dairy products (Benelli et al. 2016). For that reason, botanical acaricides have become hot research topics, and medicinal plants have been used as a source of remedies. In the recent years, the essential oils of different plants, like *Melaleuca alternifolia* (Volpato et al. 2016), *Azadirachta indica* (Stara et al. 2011), *Cedrus deodara* (Sharma et al. 1997), *Jatropha curcas* (Abdel-Shafy et al. 2011), *Pongamia glabra* (Sarkar et al. 2009) and *Cinnamomum zeylanicum* (Gauthami et al. 2015), contain active ingredients which can be effective against *S. scabiei*. Research on botanical acaricides could potentially yield environmentally friendly and low toxicity products.

E. densa is a traditional medicinal herb used in the pharmaceutical industry in China (Xuanji et al. 2016). It belongs to the *Elsholtzia* family and harbours multipurpose medicinal value, including antiviral (Liu et al. 2012), antibacterial, antimicrobial, analgesic, free radical scavenging (Khan et al. 2012) and sedative activities. In Tibetan medicine, *E. densa* is widely used in the treatment of gastropathy, coldness, fever, dysentery and parasitic infections. The oil of *E. densa* contains a significant number of compounds. A study showed that the main chemical components were α-bisabolol, elemenes, β-selinene, thymol and carvacrol (Koul et al. 2008). Variation in the components of essential oils may be caused by differences in species, habitats, harvest seasons, extraction and the analytical methods used for composition determination. Until now, the acaricidal activity of *E. densa* has not been studied. Based on the above, we hypothesized that *E. densa* may harbour certain compounds that can kill mites.

We extracted oil from *E. densa* and studied its acaricidal activity against *S. scabiei* under laboratory conditions. Moreover, we determined the chemical composition of the oil using gas chromatography-mass spectrometry (GC-MS).

MATERIAL AND METHODS

Plant material. The fresh plant material was collected from the Tibetan Qiang Autonomous Prefecture of Ngawa (33°33‘N; 102°58‘E, 98 m), Sichuan Province, south-west China, in October 2015. *E. densa* were identified at the College of Veterinary Medicine, Sichuan Agricultural University, China.

Extraction. The flowers and leaves of plants were dried in the shade at room temperature and ground in a knife mill (Manxu, China). The powdered mixture (100 g) of the flowers and leaves was placed in a round-bottomed flask and 1000 ml distilled water were added. Essential oil was extracted by steam distillation in a Clevenger-type apparatus for 4 hours. At the end of each extraction the oil was dried with anhydrous sodium sulphate, transferred to glass flasks and stored at 4 °C pending GC-MS analysis.

GC-MS analysis of essential oils. GC-MS analysis was performed on an Agilent 7890 gas chromatograph (Agilent, United States) equipped with an Agilent 5975-MS detector with a HP-5MS column. Helium was used as a gas carrier at a flow rate of 1 ml/min. Splitless 1 µl injection was used. The split ratio was 10 : 1. The injector and transfer line temperature were both 250 °C. The ion source temperature was set at 230 °C. Oven temperature was held at 55 °C for 1 min, increased to 115 °C by 4 °C/min and finally held at 115 °C for 5 min, ramped at 2 °C /min to 150 °C then ramped at 5 °C/min to 250 °C and held for 2 min. Data were gathered with the Chem station database of the gas chromatograph and the major components of the essential oil were identified on the basis of comparison of their retention indices and mass spectra with known samples or published data.

https://doi.org/10.17221/20/2018-VETMED

Collection of mites. In this study, the mites were collected (the morphological characteristics of mites were identified under the microscope) from scabs which were collected from the infested legs of naturally infected rabbits in Wenjiang, Sichuan Province, China. None of the rabbits had been treated with any anti-acariasis drug before their mites were collected. The collected scabs were placed in Petri dishes, then incubated immediately at 35 °C for 30 min in an incubator (at a temperature of 35 °C the mites will escape from the scabs, making it easy to collect without causing mites to die) (Liao et al. 2014). The motile adult mites were immediately collected for testing under a stereomicroscope. Adults have eight legs, which make them easily distinguishable from larvae which have six legs. After collection of the samples, all the infected rabbits were treated with ivermectin immediately. The sampling protocol adhered to ethical and animal care guidelines and all processes were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Acaricidal activity of essential oil *in vitro*. For *in vitro* applications, the essential oil was mixed with paraffin liquid to obtain five concentrations: 1, 2, 4, 8 and 16 mg/ml. Then, 0.1 ml of sample were directly added to the Petri dishes (5 cm in diameter, 2 cm deep), and the mites were placed in the sample with 10 mites per dish. Ivermectin (1%) was used as a positive control and liquid paraffin as a negative control. All experiments were performed in six replicates. All dishes were incubated at 25 °C under 75% relative humidity and were observed under a stereomicroscope at 1, 2, 4, 8, 16 and 24 hours. Mites were considered to have died when they exhibited no reaction in response to stimulation with a needle.

Data analysis. The obtained data were analysed with statistical software (SPSS, version 20.0) and are expressed as means ± SD. The median lethal concentration value (LC_{50}) (95% fiducial limits) and the median lethal time value (LT_{50}) (95% fiducial limits) were calculated using the complementary log-log (CLL) model.

RESULTS

Chemical composition of essential oil

The essential oil from the flowers and leaves of *E. densa* is yellow with a rich herbaceous odour and density (0.95 g/ml). The extraction rate of *E. densa* oil was 1.2%. The chemical composition of the essential oil is given in Table 1. Based on GC-MS investigations, 18 components were identified in *E. densa* oil. 4-Pyridinol (28.16%) and thymol (26.58%) were recorded as the most abundant components in *E. densa* oil.

Acaricidal activity of the essential oil

We studied the acaricidal activity of *E. densa* oil against *S. scabiei in vitro*. Treatment with a high concentration (16 mg/ml essential oil) caused 100% mortality in test mites within 16 h (Figure 1). In response to negative control treatment (paraffin liquid only), mortality was only 1.67% and most mites remained alive after 24 h treatment. However, in the positive control group of ivermectin some *S. scabiei* mites remained alive in the sample after 24 h, probably because they were resistant to the product. Compared to the control, the essential oil showed significant effects ($P < 0.05$) against

Table 1. Chemical composition of essential oil from *Elsholtzia densa*

Compound	Formula	Relative content (%)
1-Methyl-4-(1-methylethyl)benzene	$C_{10}H_{14}$	0.76
D-Limonene	$C_{10}H_{16}$	0.84
3-Carene	$C_{10}H_{16}$	0.61
Acetophenone	C_8H_8O	1.16
3-Methyl-4-isopropylphenol	$C_{10}H_{14}O$	9.86
1,6-Octadien-3-ol,3,7-dimethyl-,2-aminobenzoate	$C_{17}H_{22}O_2$	1.09
Cyclopentylacetylene	C_7H_{10}	2.43
4-Pyridinol	C_5H_5NO	28.16
2-Allyl-4-methylphenol	$C_{10}H_{12}O$	0.80
1,4-Pentadiene	C_5H_8	1.07
Thymol	$C_{10}H_{14}O$	26.58
trans-Chrysanthemol	$C_{10}H_{18}O$	0.61
Caryophyllene	$C_{15}H_{24}$	10.72
α-Caryophyllene	$C_{15}H_{24}$	5.68
2-(2-Methylprop-2-enyl)phenol	$C_{10}H_{12}O$	0.62
Isoledene	$C_{15}H_{24}$	5.86
Bicyclogermacrene	$C_{15}H_{24}$	0.74
Caryophyllene oxide	$C_{15}H_{24}O$	1.08
2-Tridecanone	$C_{13}H_{26}O$	0.62
Total		99.29

https://doi.org/10.17221/20/2018-VETMED

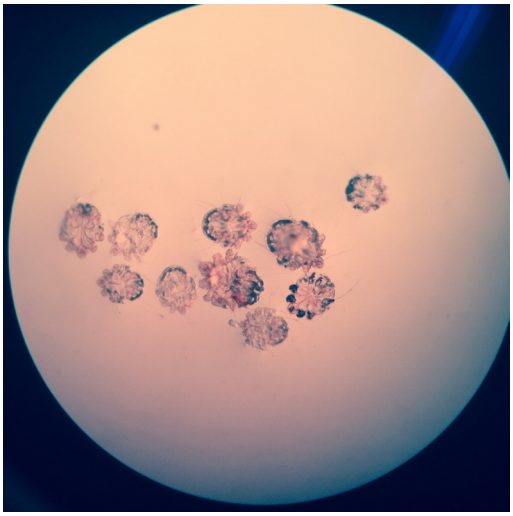

Figure 1. Dead *Sarcoptes scabiei*, observed by microscopy (× 400) following treatment with essential oil after 24 h

S. scabiei in vitro, and doses of 16, 8, 4 and 2 mg/ml could kill 100.0, 98.3, 81.7, and 73.3% of mites, respectively, by 24 h (Table 2). The toxicity of the oil was evaluated using a CLL model, Pearson's Chi-square test and the Hosmer-Lemeshow goodness-of-fit statistic indicated that the data fitted the CLL model. The LC_{50} and LT_{50} values of the essential oil against *S. scabiei* are shown in Table 3 and Table 4, respectively.

DISCUSSION

As a traditional Chinese medicine, *E. densa* has been used to treat skin diseases of animals for thousands of years. Especially in ancient Tibetan areas, the herb has long been used by local people in order to expel the parasite. In addition, *E. densa* is an aromatic plant commonly used as a herbal tea, in food, as a spice, in perfumeries, aromatherapies, cosmetics, as a nectar source and in folk medicine. In the latter, the herb has also been used for the treatment of headaches, colds, fever, diarrhoea, pharyngitis, digestion disorders, nephritises, rheumatic arthritis and nyctalopia (Chen et al. 2011). However, not all the bioactivities of this herb have been studied using modern technologies. To our knowledge, this study is the first to demonstrate that the essential oil of *E. densa* has acaricidal activities against *S. scabiei*.

Natural acaricides have the potential to be satisfactory substitutes to synthetic acaricides, because they have low toxicity in animals and exert little environmental effects (Esteves Filho et al. 2013). Isolating and finding new acaricidal medicines among traditional Chinese herbs has be-

Table 3. Probit regression analysis of the toxicity (median lethal time, LT_{50}) of essential oil from *Elsholtzia densa* against *Sarcoptes scabiei in vitro*

Concentration	Regression line	LT_{50} (h) (95% FL)	Pearson chi-square
1 mg/ml	$y = 0.709x - 0.865$	16.637 (10.209–40.278)	8.746
2 mg/ml	$y = 0.818x - 0.577$	5.075 (3.375–0.510)	5.256
4 mg/ml	$y = 0.866x - 0.399$	2.884 (1.753–4.151)	11.529
8 mg/ml	$y = 1.043x + 0.077$	1.184 (0.612–1.782)	24.404
16 mg/ml	$y = 1.531x + 0.183$	0.760 (0.413–1.103)	19.549

FL = fiducial limits

Table 2. The acaricidal activity of essential oil from *Elsholtzia densa* against *Sarcoptes scabiei in vitro*

Concentration (mg/ml)	Time (h)					
	1 h mean mortality (%) ± SD	2 h mean mortality (%) ± SD	4 h mean mortality (%) ± SD	8 h mean mortality (%) ± SD	16 h mean mortality (%) ± SD	24 h mean mortality (%) ± SD
1 mg/ml	18.3 ± 7.53^{Ce}	23.3 ± 8.17^{Cc}	36.7 ± 8.17^{Dc}	45.0 ± 5.48^{De}	46.7 ± 7.53^{Ed}	53.3 ± 8.17^{Dd}
2 mg/ml	28.3 ± 4.08^{BCd}	38.3 ± 7.53^{Bb}	45.0 ± 8.37^{CDc}	56.7 ± 5.16^{Cd}	63.3 ± 5.16^{Dc}	73.3 ± 5.16^{Cc}
4 mg/ml	33.3 ± 8.17^{Bcd}	45.0 ± 8.37^{Bb}	58.3 ± 7.53^{BCb}	63.3 ± 8.17^{BCcd}	70.0 ± 11.0^{CDc}	81.7 ± 7.53^{BCb}
8 mg/ml	53.3 ± 8.17^{Ab}	61.7 ± 7.53^{Aa}	61.7 ± 9.83^{Bb}	73.3 ± 10.3^{Bb}	88.3 ± 7.53^{Bb}	98.3 ± 4.08^{Aa}
16 mg/ml	63.3 ± 10.3^{Aa}	70.0 ± 11.0^{Aa}	83.3 ± 10.3^{Aa}	91.7 ± 7.53^{Aa}	100 ± 0.00^{Aa}	100 ± 0.00^{Aa}
Positive control	38.3 ± 13.3^{Bc}	41.7 ± 9.83^{Bb}	65.0 ± 12.2^{Bb}	71.7 ± 7.53^{Bbc}	80.0 ± 8.94^{Bcb}	85.0 ± 8.37^{Bb}
Negative control	0.00 ± 0.00^{Df}	0.00 ± 0.00^{Dd}	0.00 ± 0.00^{Ed}	1.67 ± 4.08^{Ef}	1.67 ± 4.08^{Ff}	1.67 ± 4.08^{Ee}

The differences between values with different capital letters within a column are highly significant ($P < 0.01$), and the differences between data with different lowercase letters within a column are significant ($P < 0.05$)

https://doi.org/10.17221/20/2018-VETMED

Table 4. Probit regression analysis of the toxicity (median lethal concentration, LC_{50}) of essential oil from *Elsholtzia densa* against *Sarcoptes scabiei in vitro*

Time (h)	Regression line	LC_{50} (mg/ml) (95% FL)	Pearson chi-square
1 h	$y = 1.050x - 0.930$	7.678 (5.487–12.481)	8.277
2 h	$y = 1.026x - 0.682$	4.623 (3.298–6.699)	9.079
4 h	$y = 0.982x - 0.398$	2.543 (1.600–3.592)	11.870
8 h	$y = 1.077x - 0.190$	1.502 (0.834–2.159)	11.180
16 h	$y = 1.610x - 0.182$	1.298 (0.868–1.709)	13.322
24 h	$y = 1.950x + 0.016$	0.981 (0.643–1.288)	11.069

FL = fiducial limits

come a major strategy in this regard. Many plants have been described to have acaricidal properties. *Azadirachta indica* extracts and oil fractions exhibit a significant acaricidal effect against *S. scabiei* (Deng et al. 2012). A new active compound extracted from *Eupatorium adenophorum*, 9-oxo-10,11-dehydroageraphorone, also showed acaricidal effect against *S. scabiei* and *P. cuniculi* (Liao et al. 2014). The essential oil of Mexican oregano has high thymol and carvacrol content, which are toxic for *Rhipicephalus* (*Boophilus*) *microplus* (Martinez-Velazquez et al. 2011). In summary, carvacrol and thymol are the main constituents of the essential oils from the majority of herbs that exhibit acaricidal activity.

Our results from the GC-MS analysis show that thymol (26.58%) and 4-Pyridinol (28.16%) are the main constituents of *E. densa* essential oil. Previous studies have reported that the essential oils of many *Elsholtzia* contain thymol (Liu et al. 2006). Interestingly, previous studies have shown thymol's potential as an acaricide against different target species such as *Rhipicephalus sanguineus* (Daemon et al. 2012), *Rhipicephalus microplus* (Scoralik et al. 2012) and *Amblyomma cajennense* (da Silva Mendes et al. 2011), but the acaricidal activity of 4-Pyridinol has not been reported. The acaricidal activity found in this study and those reported in the literature is likely to be linked to the presence of thymol in the essential oil.

In conclusion, the present study shows that the essential oil of *E. densa* has the potential to control *S. scabiei in vitro*. *E. densa* oil is easily available, low-cost and eco-friendly. Further studies with this oil could result in its establishment as a possible alternative to chemical acaricides and lead to the development of plant-sourced acaricides. However, further assays will be needed to isolate and identify the important and active components from the *E. densa* oil and to appraise its efficacy and safety in clinical trials. In further in-depth studies, we will study the acaricidal mechanism of the active components, and we believe that *E. densa* may be exploited as a novel drug for the effective control of *S. scabiei*.

REFERENCES

Abdel-Shafy S, Nasr SM, Abdel-Rahman HH, Habeeb SM (2011): Effect of various levels of dietary Jatropha curcas seed meal on rabbits infested by the adult ticks of Hyalomma marginatum marginatum I. Animal performance, anti-tick feeding and haemogram. Tropical Animal Health and Production 43, 347–357.

Alasaad S, Schuster RK, Gakuya F, Theneyan M, Jowers MJ, Maione S, Min AM, Soriguer RC, Rossi L (2012): Applicability of molecular markers to determine parasitic infection origins in the animal trade: a case study from Sarcoptes mites in wildebeest. Forensic Science, Medicine, and Pathology 8, 280–284.

Benelli G, Pavela R, Canale A, Mehlhorn H (2016): Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? Parasitology Research 115, 2545–2560.

Chen ZW, Wen-Man WU, Liu H, Wan Y, Luo YM (2011): Review of chemical constituents of medicinal herbs Herba Moslae. Chinese Journal of Experimental Traditional Medical Formulae 17, 160–164.

da Silva Mendes A, Daemon E, de Oliveira Monteiro CM, Maturano R, Brito FC, Massoni T (2011): Acaricidal activity of thymol on larvae and nymphs of Amblyomma cajennense (Acari: Ixodidae). Veterinary Parasitology 183, 136–139.

Daemon E, Maturano R, de Oliveira Monteiro CM, Goldner MS, Massoni T (2012): Acaricidal activity of hydroethanolic formulations of thymol against Rhipicephalus sanguineus (Acari: Ixodidae) and Dermacentor nitens (Acari: Ixodidae) larvae. Veterinary Parasitology 186, 542–545.

Deng Y, Shi D, Yin Z, Guo J, Jia R, Xu J, Song X, Lv C, Fan Q, Liang X (2012): Acaricidal activity of petroleum ether

https://doi.org/10.17221/20/2018-VETMED

extract of neem (Azadirachta indica) oil and its four fractions separated by column chromatography against Sarcoptes scabiei var. cuniculi larvae in vitro. Experimental Parasitology 130, 475–477.

Ebadollahi A, Sendi J, Aliakbar A, Razmjou J (2015): Acaricidal activities of essential oils from Satureja hortensis (L.) and Teucrium polium (L.) against the two spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae). Egyptian Journal of Biological Pest Control 25, 171–176.

Esteves Filho AB, Oliveira JVD, Matos CHC (2013): Residual efficacy of synthetic acaricides and natural products for Tetranychus urticae Koch on cotton. Revista Brasileira De Ciencias Agrarias 8, 583–586.

Gauthami M, Srinivasan N, Goud NM, Boopalan K, Thirumurugan K (2015): Synthesis of silver nanoparticles using Cinnamomum zeylanicum bark extract and its antioxidant activity. Nanoscience & Nanotechnology-Asia 5, 2–7.

Khan M, Ganie SA, Wani IH, Ganai BA, Masood A, Zargar MA, Malik AH, Hamid R (2012): Free radical scavenging activity of Elsholtzia densa. Journal of Acupuncture & Meridian Studies 5, 104–111.

Koul O, Walia S, Dhaliwal G (2008): Essential oils as green pesticides: potential and constraints. Biopesticides International 4, 63–84.

Krawczak FDS, Buzatti A, Pivoto FL, Sangioni LA, Vogel FSF, Botton SDA, Zanetti GD, Manfron MP (2011): Acaricide activity of leaves extracts of Sambucus australis Schltdl. (Caprifoliaceae) at 2% on engorged females of Rhipicephalus (Boophilus) microplus. Ciencia Rural 41, 2159–2163.

Liao F, Hu YC, Tan H, Wu L, Wang Y, Huang Y, Mo Q, Wei YH (2014): Acaricidal activity of 9-oxo-10,11-dehydroageraphorone extracted from Eupatorium adenophorum in vitro. Experimental Parasitology 140, 8–11.

Liu G, Wang H, Zhou BH, Song JC (2006): GC-MS analysis of essential oils from elsholtzia ciliata. Chinese Journal of Experimental Traditional Medical Formulae 12, 18–21.

Liu Y, Jian-Yong SI, Cao L, Jia XG (2012): Chemical composition, antimicrobial and antiviral activities of the essential oil of Elsholtzia densa Benth. Natural Product Research & Development 24, 1070–1074.

Martinez-Velazquez M, Rosario-Cruz R, Castillo-Herrera G, Flores-Fernandez J, Alvarez A, Lugo-Cervantes E (2011): Acaricidal effect of essential oils from Lippia graveolens (Lamiales: Verbenaceae), Rosmarinus officinalis (Lamiales: Lamiaceae), and Allium sativum (Liliales: Liliaceae) against Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). Journal of Medical Entomology 48, 822–827.

Sarkar P, Talukdar B, Somchoudhury A (2009): Relative efficacy of diafenthiuron (50 WP) alone and with two different adjuvants against adult and egg stages of tea red spider mite, Oligonychus coffeae (Nietner) under laboratory condition. Journal of Entomological Research 33, 109–112.

Scoralik MG, Daemon E, De Oliveira Monteiro CM, Maturano R (2012): Enhancing the acaricide effect of thymol on larvae of the cattle tick Rhipicephalus microplus (Acari: Ixodidae) by solubilization in ethanol. Parasitology Research 110, 645–648.

Seddiek SA, Khater HF, El-Shorbagy MM, Ali AM (2013): The acaricidal efficacy of aqueous neem extract and ivermectin against Sarcoptes scabiei var. cuniculi in experimentally infested rabbits. Parasitology Research 112, 2319–2330.

Sharma D, Saxena V, Sanil N, Singh N (1997): Evaluation of oil of Cedrus deodara and benzyl benzoate in sarcoptic mange in sheep. Small Ruminant Research 26, 81–85.

Stara J, Nesvorna M, Hubert J (2011): The toxicity of selected acaricides against five stored product mites under laboratory assay. Journal of Pest Science 84, 387–391.

Volpato A, Lorenzetti W, Zortea T, Giombelli L, Baretta D, Santos R, Vaucher R, Raffin R, Souza M, Stefani L (2016): Melaleuca alternifolia essential oil against the lesser mealworm (Alphitobius diaperinus) and its possible effect on the soil fauna. Revista Brasileira de Ciencia Avicola 18, 41–46.

Xuanji X, Zengjun G, Hui Z, Xia L, Jun L, Dandan L, Jun L (2016): Chemical composition, in vitro antioxidant activity and α-glucosidase inhibitory effects of the essential oil and methanolic extract of Elsholtzia densa Benth. Natural Product Research 30, 2707–2711.

Received: February 5, 2018
Accepted after corrections: November 21, 2018