

Tick repellent activities of supercritical fluid extract from *Perilla frutescens* against *Haemaphysalis longicornis*

Young Su Jang

ys.jang@jbf.kr

Jeonnam Bio Foundation

Research Article

Keywords: *Haemaphysalis longicornis*, *Perilla frutescens*, Supercritical fluid extraction, Repellent, Microcapsules

Posted Date: September 26th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4989189/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Supercritical fluid extraction (SFE) is a rapid, selective, and convenient method for sample preparation prior to the analysis of compounds in a volatile product of plant matrices. In this study, insecticidal activity and repellency were examined in a laboratory study to determine the active constituent of *Perilla frutescens* and a supercritical fluid extract from *Perilla frutescens* against hard tick *Haemaphysalis longicornis*.

The repellency of *P. frutescens* oil extract was evaluated in comparison with two commercial repellents (Icaridin and DEET) against *H. longicornis*. Filter-paper diffusion assay revealed that *P. frutescens* oil (3.2 $\mu\text{g}/\text{cm}^2$) had a 100% repellency and icaridine (3.2 $\mu\text{g}/\text{cm}^2$) had a 96.1% repellency at 120 min. Repellencies of 4 compounds and microcapsule *P. frutescens* against *H. longicornis* were compared with the repellency of Icaridine and DEET at 3.2 $\mu\text{g}/\text{cm}^2$. Responses varied according to the test compound and exposure time. Repellencies of Perillaldehyde and microcapsule *P. frutescens* were 100% at 30 min post-treatment. *P. frutescens* oil merit further study as potential fumigants or lead compounds for the control of *H. longicornis*.

Introduction

The supercritical fluid extraction (SFE) technology has advanced tremendously since its inception. It is a method of choice in many processing industries. Over the last two decades, SFE has been well received as a clean and environmentally friendly “green” processing technique. In some cases, it is an alternative of organic solvent-based extraction of natural products (Mohammed et al., 2012, Jang, 2024). Since the end of the 1970s, SFE has been used to isolate natural products. However, its applications have been concentrated on only a few products for a long time. Now the development of processes and equipment is beginning to pay off and industries are getting more and more interested in SFE techniques (Reverchon and Taddeo, 1995, Jang, 2024).

Plant essential oils have been suggested as alternative materials for insect control because some of them are selective to certain pests. In addition, they are often biodegradable to nontoxic products. Moreover, they have little or no harmful effects on nontarget organisms (Isman, 2001; Mumcuoglu et al., 2002, Jang, 2024). They can be applied to resting and hiding places in the same way as other conventional insecticides. They also provide useful information on resistance management because certain plant extracts or phytochemicals are highly effective against insecticide-resistant insect pests (Lindquist et al., 1990; Ahn et al., 1997, Jang, 2024). In addition, some plant essential oils and their constituents have been proposed as alternatives to commonly used synthetic insecticides because they are exempted as minimum risk pesticides under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requirements (U.S. EPA, 1996). Furthermore, plant essential oils are widely available, with some of them being relatively inexpensive compared with plant extracts (Isman, 2001, Jang, 2024). Because of this, much effort has been focused on plant essential oils or their constituents as potential sources of commercial control agents.

The hard tick, *Haemaphysalis longicornis*, is widely distributed in East Asia and Oceania (Fujisaki et al., 1994; Silva *et al.*, 2005; Tanaka et al., 2012, Jang, 2024). It can act as a vector for viruses, chlamydia, rickettsia, bacteria, and protozoa (Fujisaki et al., 1994; Guan et al., 2002; Jongejan and Uilenberg, 2004; Li et al., 2009, Jang, 2024). In South Korea, this species is the most abundant tick that infests humans and domestic animals, with infestation rates peaking during the summer. At present, common anti-tick measures include spraying synthetic drugs, performing regular medicated bathing of livestock, using smoke agents as acaricidal drugs in forests, and applying synthetic acaricides to the environment and animals (Regassa, 2000; Iori et al., 2005; Patarroyo et al., 2009, Jang, 2024). Anti-tick drugs used usually include chemical agents containing synthetic pyrethroids, organophosphates, and amitraz (Gazim et al., 2011, Jang, 2024). Although these acaricides are beneficial when properly used, misuse has led to poisoning in humans and animals (FAO, 1998; Zorloni et al., 2010, Jang, 2024). Their misuse can also lead to problems such as only partial success of treatment with these agents, development of serious resistance in parasites, drug residue, and environmental hazards (Fernandes and Freitas, 2007; Ribeiro *et al.*, 2007; Nong et al., 2012, 2013, Jang, 2024).

The poly- ϵ -caprolactone (PCL) is suitable for controlled drug delivery due to a high permeability to many drugs excellent biocompatibility and its ability to be fully excreted from the body once bio resorbed (De Ávila SC et al., 2017). Biodegradation of PCL is slow in comparison to other polymers, so it is most suitable for long-term delivery extending over a period of more than 1 year. PCL also has the ability to form compatible blends with other polymers, which can affect the degradation kinetics, facilitating tailoring to fulfill desired release profiles (De Ávila SC et al., 2017).

PCL is a biodegradable polymer capable of incorporating various substances in their chemical structure. This polymer is insoluble in water, but it can permeate their polymer chains, and their degradation is slow in an aqueous medium without causing risks to the environment (De Ávila SC et al., 2017). This work presents the preparation and characterization of Microcapsules of poly- ϵ -caprolactone (PCL) containing *P. frutescens* oil of supercritical fluid extraction.

Materials and methods

Ticks

H. longicornis ticks were collected from naturally infested goats in Suncheon, South Korea. After collection, they were maintained in the laboratory without exposure to any known acaricide. They were reared in plastic containers (15cm $\times$ 12cm $\times$ 6 cm) at $25 \pm 1^\circ\text{C}$ with a relative humidity of 75% in the dark. Experimental tick nymphs were derived from post-blood meal larvae that fed on rabbits (New Zealand white rabbits). They were 2–3 weeks old after molting from engorged larvae.

Chemicals

Fore compounds used in this study were as follows: Perillaldehyde, acetic acid, Dimethyl anthranilate and Bergapten supplied by Sigma Aldrich (St. Louis, MO, USA). Icaridine was obtained from the National

Institute of Health, Korea Center for Disease Control and Prevention, Seoul, Korea. Icaridine and *N,N*-diethyl-*m*-toluamide (DEET) were purchased from Aldrich (Milwaukee, WI, USA). Dibutyl phthalate was supplied by Sigma (St. Louis, MO, USA). All other chemicals were of reagent grade.

Materials

PCL ($M_n = 80000$), PVA ($M_w = 85000 \sim 124000$), and NF ($\geq 98\%$) are purchased from Sigma-Aldrich. PEG ($M_w = 2000$) is purchased from Yakuri Pure Chemicals (Japan). NF is 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester, $C_{17}H_{18}N_2O_5$. DCM (99.5%, Samchun Pure Chemical, Korea) is purchased as a solvent and used as received without further purification.

Extraction

Experiments were carried out in a dynamic extraction unit, which mainly consisted of three parts: a CO₂ reservoir, an extractor vessel, and three separator vessels in series, accompanied by a thermostatic bath, a metering pump, a cryostat, the necessary instrumentation to control the pressures, temperatures, mass flow rates, and valves for collecting extract. The operating temperature and pressure can reach up to 60°C and 500 bar, respectively, with a maximum gas mass flow rate of 0.70 ml/min. As a pretreatment, *P. frutescens* were dried and finely ground. A mass of 100 g was weighed in a precise Scaltec instrument balance (precision of ± 0.1 mg). The mean particle diameter of the resulting powder was determined at around 1 mm (Jang, 2024).

The sample was then allowed to reach a constant extraction temperature before charging CO₂ into the high-pressure pump from the storage cylinder. The CO₂ gas was further compressed to the desired pressure of the pump. After 1 h, a time corresponding to the static extraction, the extractor valve was opened and intermediate valves between separators were continuously adjusted in order to regulate the pressure so that a constant flow rate could be kept. Samples were taken every 15 min by placing valves at the bottom of separators. Samples were then weighed to obtain the mass of the essential oil. Dynamic extraction was pursued for 3.5 h, after which it was noted that the extracted mass was very low. Finally, the glass containing the extracted essential oil was kept in a freezer to be used for chromatographic analysis. Operating conditions such as pressure, temperature, particle diameter, and supercritical fluid flow rate were fixed (Jang, 2024).

Extractions were performed at a temperature range of 30–60°C with particle size of 0.5–2 mm at a pressure between 100 and 500 bar. These ranges are the most frequently adopted for similar system types where CO₂ is at its supercritical state. Therefore, the temperature was controlled thermostatically and the pressure was regulated using a membrane pump to reach the extraction pressure. The maximum mass flow rate was 0.70 ml/min through the pump, which was also connected to a cryostat in order to liquefy CO₂. The flow rate of the latter was measured with a Coriolis force flow meter. It indicated the amount of CO₂ used during the extraction. Separation of CO₂/essential oil was carried out at 10°C in the first separator and at 30°C in the other two separators. The temperature of the first separator was set at 10°C to enable recovery of certain secondary components such as waxes, resins,

fatty acids, and so on present in the solid matrix. The CO₂ used in this study was 99.5% (w/w) pure. It was supplied by Hanguk-gas-well corporation. The extracted essential oil was recovered from separators with CO₂ vented to the atmosphere (Jang, 2024).

Dried *P. frutescens* leaves (5 kg) were purchased as a commercially available product. They were finely powdered, extracted twice with methanol (10 L) at room temperature, and filtered with Tokyo filter paper No. 2 (Tokyo Roshi, Japan).

GC/MS analysis

The *n*-hexane fraction of *P. frutescens* supercritical fluid extract was analyzed on a gas chromatograph-mass spectrometer (GCMS-QP2010, Shimadzu). The GC column was a 30 m × 0.25 mm i.d. VF-5MS (0.25 μ m) fused silica capillary column (J&W Scientific, Folsom, CA, USA). GC conditions were as follows: injector temperature, 50°C; column temperature, isothermal at 230°C for 5 min, then programmed to 280°C at 3°C/min, and held at this temperature for 19 min; and ion source temperature, 200°C. Helium was used as the carrier gas at a rate of 1.0 mL/min. The effluent of the GC column was introduced directly into the source of the MS. Spectra were obtained in the EI mode with ionization energy of 70 eV. The sector mass analyzer was set to scan from 50 to 800 amu for 2 s. Compounds were identified by comparison with retention times. Mass spectra were obtained with authentic standards on the GC-MS system used for analysis. When an authentic sample was unavailable, identification was carried out by comparing mass spectra with those in the mass spectra library (The Wiley Registry of Mass Spectral Data, 8th ed.).

Microcapsule Synthesis

The 2 ~ 8 wt% PCL solutions dissolved in DCM are prepared by stirring for 24 h at a speed of 500 rpm and then mixed with *P. frutescens* oil (10 mg) to obtain a homogeneous solution. PCL and PCL/PEG solutions containing weight ratios in the range of 10/0 to 6/4 are prepared. PVA dissolved in distilled water is refluxed at 500 rpm and 80 °C to obtain a viscous homogeneous solution (De Ávila SC et al., 2017). The as-prepared PCL and PCL/PEG solutions are placed in a 20 mL B-D luer-lok syringe attached to the syringe pump (KDS-200, Stoelting Co., USA) and are fed into the 22-gage blunt-end metal needle at a flow rate of 0.25 mL/min. They are injected into PVA aqueous solution (1.0 wt%) and stirred with a high-speed agitator (HD-312, HsiangTai, China) for 4 h at speed in the range of 3000 to 5000 rpm to obtain the desired microcapsules (De Ávila SC et al., 2017). The microcapsules are screened through a 25 μ m sieve. The capsules are then cleaned in ethanol solution for 0.5 h and subsequently in distilled water for 0.5 h to remove the remnant solvent. The microcapsules are dried overnight in a vacuum oven (De Ávila SC et al., 2017). Prior to use, their storage and handling are conducted cautiously without light due to photosensitivity of *P. frutescens* oil.

Vibrational spectroscopy, infrared (IR)

The infrared analyses were performed in a Perkin Elmer Instruments FT-IR Spectrometer, in the range 4000-650cm⁻¹. The samples were analysed by UATR accessory.

Scanning electron microscopy (SEM)

The analysis of morphology and surface of the samples was performed using the Scanning Electron Microscope (SEM), MIRA 3 LMU model (TESCAN) with a resolution of 3.5nm (secondary electron) and increases the range of 1000-4000times, 20kV acceleration voltage using gold plating the samples (De Ávila SC et al., 2017).

Bioassay

A filter-paper repellent bioassay was used to evaluate toxicities of *P. frutescens* oil, 4 test compounds, *P. frutescens* derived material, and two repellents, DEET and Icaridine, to *H. longicornis*. A volume of 1 ml of the concentration was added to Petri dishes (10 cm diameter, 2 cm height) containing filter paper disks to absorb the liquid. Ten larval ticks were placed on the filter paper, sprayed twice with the extract to be tested, and incubated at 25–27 °C with a relative humidity of 70–80%, with each treatment consisting of three replicates (Jang, 2024).

Repellency was calculated according to the following formula: % repellency = $[(Ta - Tb) / Ta] \times 100$, where *Ta* was the number of hard ticks in the control group and *Tb* was the number of hard ticks in the treated group. Repellency percentages were transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by the Scheffé test or Bonferroni multiple-comparison method at $P = 0.05$. Means $\pm$ SEs of untransformed data are reported (SAS Institute, 2004) (Jang, 2024).

Results

Chemical constituents

Compositions of *P. frutescens* oil extracted with the supercritical CO₂ extraction method were analyzed. A total of 4 compounds were identified in the *P. frutescens* oil. Perillaldehyde, Acetic acid, Dimethyl anthranilate and Bergapten were major components in the *P. frutescens* oil extracted at a pressure of 150 bar, a temperature of 50°C, and a particle size of 0.5 mm (Table 1).

Scanning electron microscopy (SEM)

For microcapsules prepared by using PVA emulsifier, uniform and highly stable spherical PCL, PCL/ PVP, and PCL/PEG microcapsules (~ 150 µm) with a narrow size distribution are successfully synthesized.5 This may be due to high affinity of the PVA for the shell materials and the core material. The *P. frutescens* oil release rate of PCL/PVP and PCL/PEG capsules increases with increasing PVP and PEG concentration due to the increase in capsule size. The 1 wt% PVA emulsifier is determined to be the best composition for the formation of uniform microcapsule. In the present study, PCL and PCL/ PEG microcapsules using 1 wt% PVA emulsifier are studied by the ESE technique using the high-speed agitator. The PCL capsule size decreases gradually from $10.1 \pm 0.85 \mu\text{m}$ to $3.7 \pm 0.25 \mu\text{m}$ with increasing

the stirring speed from 500 to 3000 rpm (Fig. 1). The microcapsule size is controlled by the balance between the turbulence to break the microcapsules, which are emulsion particles stabilized by PVA, and the internal viscosity and interfacial tension to hold the microcapsules. Fast stirring speed increases the turbulent energy acting on each microcapsule, increasing the rate at which the capsules becomes smaller, thereby reducing the size of the microcapsules (De Ávila SC et al., 2017). The capsules prepared at 3000 rpm are selected in the present study.

Vibrational spectroscopy, infrared (IR)

The infrared spectroscopy (IR) was used to check the encapsulation of the citronella oil and no formation of secondary phase. The essential oil of citronella infrared spectrum Fig. 3 showed characteristics absorptions, like the axial deformation of the OH (600cm^{-1}) also related with water absorption (Fig. 2) (De Ávila SC et al., 2017).

Repellency

The repellency of *P. frutescens* oil was evaluated in comparison with two commercial repellents (Icaridin and DEET) against *H. longicornis* (Table 3). In filter-paper diffusion method, *P. frutescens* oil ($3.2\text{ }\mu\text{g}/\text{cm}^2$) gave 100% repellency at 120 min after exposure, whereas Icaridine ($3.2\mu\text{g}/\text{cm}^2$) gave 96.1% repellency at 120 min.

Repellency effects of 4 compounds against *H. longicornis* were compared with the repellency of Icaridine and DEET at $3.2\text{ mg}/\text{cm}^2$ (Table 3). Responses varied according to the test compound and exposure time. At 10 min post-treatment, Perillaldehyde gave 100% repellency. At 120 min, they gave 67.4.% repellency, respectively. At 10 min post-treatment, Acetic acid, Dimethyl antranilate and Bergapten gave 44.2%, 23.5% and 91.5% repellency, respectively. However, these compounds showed significantly lower repellency rates at 30 min. Whereas Icaridine ($3.2\mu\text{g}/\text{cm}^2$) gave 96.1% repellency at 120 min.

Repellency effects of microcapsule against *H. longicornis* were compared with the repellency of Icaridine and DEET at $3.2\text{ mg}/\text{cm}^2$ (Table 4). Responses varied according to the test microcapsule and exposure time. At 10 min post-treatment, microcapsule gave 100% repellency. At 120 min, microcapsule gave 85.4.% repellency, respectively. Also, Icaridine and DEET gave 96.1%, 100% repellency at 120 min.

Discussion

Supercritical fluid extraction (SFE) has attracted enormous interest nowadays, with thousands of references dealing with SFE. It is now a real option for product development, mainly products that will be used for human consumption, such as new foods, food ingredients/additives, insecticides or pharmaceutical products. Moreover, SFE has also demonstrated some advantages in the environmental field. For example, it can be used to reduce solvent waste, to get new useful compounds from industrial by-products, and to allow quantification and/or removal of toxic compounds from the environment. SFE can be used to extract volatile components from plant materials. Emphasis is placed on optimization of

extraction parameters (temperature, pressure, extraction time, modifier, etc.) for complete recovery of analytes from their matrices (Jang, 2024). In this study, supercritical carbon dioxide (SC-CO₂) extraction was used to determine essential oil compositions of leaves of *P. frutescens*. Optimal extraction conditions for improving essential oil yield within the experimental range of variables examined were: particle size (0.5–2.0 mm), temperature (40–60°C), and pressure (100–500 bar). Chemical compositions of its essential oils were analyzed by GC-MS. Major components were found to be Perillaldehyde, Acetic acid, Dimethyl anthranilate and Bergapten.

Plants are potential products for dust mite control because many of them are selective to pests, showing no or little harmful effects on nontarget organisms and the environment (Pollart et al., 1987; Lee, 2004; Jang, 2024). They might be applied to control dust mite nests such as beds, carpeted floors, furniture, and sofas in the same way as other conventional acaricides (Tovey, 1981). Many plant extracts and phytochemicals are known to possess acaricidal activities against house dust mites (Lee, 2004; Jang, 2024). Reported naturally occurring acaricidal compounds against dust mites include fenchone, estragol, and thymol derived from *Foeniculum Vulgare* (Lee, 2004), anisaldehyde and 3-carene from leaves of *Pimpinella anisum* (Lee, 2004), and cinamaldehyde, cinnamyl alcohol, and salicylaldehyde from *Cinnamomum cassia* (Kim, 2001).

Elucidation of the mode of action of essential oils and their constituents is of practical importance for insect control because it may give useful information on the most appropriate formulation, delivery means, and resistance management. Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids (Coat *et al.*, 1991; Visser, 1983; Jang, 2024). They exhibit fumigant activities (Ahn et al., 1998; Yang et al. 2003; Kim et al. 2001; Jang, 2024). Structure-activity relationships of plant compounds against insect pests have been well studied. Rice *et al.* (1994) and Tsao et al. (1995) have attempted to enhance the potency of selected monoterpenes and phenols through derivatization of the hydroxyl group. They found that enhanced bioactivities of derivatives appeared to result from increased vapor pressure, leading to greater fumigant action and/or increased lipophilicity, which result in better penetration and bioavailability in an insect's body.

In this study, repellency of *P. frutescens* oil and microcapsule were evaluated in comparison with two commercial repellents (Icaridin and DEET) against *H. longicornis*. In filter-paper diffusion method, *P. frutescens* oil (3.2 µg/cm²) gave 100% repellency at 120 min after exposure, whereas Icaridine (3.2 µg/cm²) gave 96.1% repellency at 120 min. Repellencies of 4 compounds against *H. longicornis* were compared with the repellency of Icaridine and DEET at 3.2 mg/cm². Responses varied according to test compound and exposure time. At 30 min post-treatment, α-terpinene, terpinene-4-ol, and α-terpineol gave 100% repellency.

P. frutescens oil described merit further study as potential repellents or lead compounds for the control of *H. longicornis*. For practical use of *P. frutescens* oil as novel repellent, further research is required on its safety for human health. Other areas requiring attention are insecticide's mode of action and

formulations, including a carrier that can slowly release the active material, to improve insecticidal potency and stability and to reduce cost.

Declarations

Author Contribution

I wrote the main manuscript text and table. I prepared all figures.

References

1. Ahn Y.J., Kwon M., Park H.M., and Han C.G. 1997. Potent insecticidal activity of *Ginkgo biloba*-derived trilactone terpenes against *Nilaparvata lugens*. In: Phytochemical Pest Control Agents, Hedin P. A., Hollingworth R., Miyamoto J., Masler E. and Thompson D. G. Eds., ACS Symposium Series 658, American Chemical Society, Washington, DC., pp. 90–105.
2. Ahn, Y. J.; Lee, S. B.; Lee, H. S.; Kim, G. H. 1998. Insecticidal and acaricidal activity of carvacrol and a-thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. *J. Chem. Ecol.*, 24, 81-90.
3. Coats, J. R.; Karr, L. L.; Drewes, C. D. 1991. Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In *Naturally Occurring Pest Bioregulators*; Hedin, P. A., Ed.; ACS Symposium Series 449; American Chemical Society: Washington, DC, pp 305-316.
4. De Ávila SC,; Holz JP,; Carone CLP,; Morisso FD,; Ligabue RA,; Pighinelli L. 2017. Microcapsules PCL with essential oil citronella. *Adv Tissue Eng Regen Med Open Access.*; pp 159–162.
5. Guan G.Q., Yin H., Luo J.X. 2002. Transmission of *Babesia* sp. to sheep with fieldcollected *Haemaphysalis qinghaiensis*. *Parasitology Research* 88: S22–S24.
6. FAO-Food and Agriculture Organization. 1998. Regional coordination for the integrated tick and tick-borne disease control programme in Eastern, Central and Southern Africa. Report for the OAU/IBAR organization of Africa unit/interbureau for animal resources.
7. Fernandes F.F., Paula Souza Freitas E. 2007. Acaricidal activity of an oleoresinous extract from *Copaifera reticulata* (Leguminosae: Caesalpinioideae) against larvae of the southern cattle tick, *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae). *Veterinary Parasitology* 147: 150–154.
8. Fujisaki K., Kawazu S., Kamio T. 1994. The taxonomy of the bovine *Theileria* spp. *Parasitology Today*. 10: 31–33.
9. Gazim Z.C., Demarchi I.G., Lonardon M.V., Amorim A.C., Hovell A.M., Rezende C.M., Ferreira G.A., Lima E.L., Cosmo F.A., Cortez D.A., 2011. Acaricidal activity of the essential oil from *Tetradenia riparia* (Lamiaceae) on the cattle tick *Rhipicephalus* (Boophilus) *microplus* (Acari; Ixodidae). *Experimental Parasitology* 129: 175–178.
10. Iori A., Grazioli D., Gentile E., Marano G. 2005. Acaricidal properties of the essential oil of *Melaleuca alternifolia* Cheel (tea tree oil) against nymphs of *Ixodes ricinus*. *Veterinary Parasitology* 129: 173–176.

11. Isman M.B. 2001. Pesticides based on plant essential oils for management of plant pests and diseases. In International Symposium on Development of Natural Pesticides from Forest Resources; Korea Forest Research Institute: Seoul, Korea, pp 1-9.
12. Jang Y.S. 2024. Tick repellent activities of supercritical fluid extract from *Chamaecyparis obtusa* against *Haemaphysalis longicornis*. Journal of Asia-Pacific Entomology 27
13. Jongejan F., Uilenberg, G. 2004. The global importance of ticks. Parasitology 129: S3–S14.
14. Kim H.K. 2001. Acaricidal activities of phenylpropenes identified in *Cinnamomum cassia* bark against *Dermatophagoides* spp. (Acari: Pyroglyphidae). M.S. Thesis, Seoul National University, Suwon, Republic of Korea.
15. Kim, D. H.; Ahn, Y. J. 2001. Contact and fumigant activities of constituents of *Foeniculum Vulgare* fruit against three coleopteran stores-product insects. Pest Manag. Sci. 57, 301-306.
16. Lee H.S. 2004. Acaricidal activity of constituents identified in *Foeniculum Vulgare* fruit oil against *Dermatophagoides* spp. (Acari: Pyroglyphidae). J. Agric. Food Chem. 52: 2887-2889.
17. Lee H.S. 2004. *p*-Anisaldehyde: Acaricidal Component of *Pimpinella anisum* L. Seed Oil against the House Dust Mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Planta Med. 70: 279-281.
18. Li Y., Luo J., Guan G. 2009. Experimental transmission of *Theileria uilenbergi* infective for small ruminants by *Haemaphysalis longicornis* and *Haemaphysalis qinghaiensis*. Parasitology Research 104: 1227–1231.
19. Lili X., Xiaori Z., Zhaowu Z., Rong C., Haifeng L., Tian X. and Shuling W. 2011. Recent advances on supercritical fluid extraction of essential oils. African Journal of Pharmacy and Pharmacology. Vol. 5(9), pp. 1196-1211
20. Lindquist R.K., Adams A.J., Hall F.R., Adams I.H.H. 1990. Laboratory and greenhouse valuations of Margosan-O against bifenthrin-resistant and -susceptible greenhouse whiteflies, *Trialeurodes Vaporarium* (Homoptera: Aleyrodidae). Proceedings of the USDA Neem Workshop; USDA-ARS 86; USDA: Beltsville, MD. pp 91-99.
21. Mohammed J.H., Mohammed Z.S., Sahena F., Mohd Y.A.M., Nik N.N.A.R, Mohd O. A.K. 2012. Applications of supercritical fluid extraction (SFE) of palm oil and oil from natural sources. Molecules. 17: 1764-1794.
22. Mumcuoglu K. Y., Miller J., Zamir C., Zentner G., Helbin V., Ingber A. 2002. The in vivo pediculicidal efficacy of a natural remedy. Isr. Med. Assoc. J. 4: 790-793.
23. Nong, X., Ren, Y.J., Wang, J.H., Fang, C.L., Xie, Y., Yang, D.Y., Liu, T.F., Chen, L., Zhou, X., Gu X.B., Zheng W.P., Peng X.R., Wang S.X., Lai S.J., Yang G.Y. 2013. Clinical efficacy of extract from *Eupatorium adenophorum* against the scab mite, *Psoroptes cuniculi*. Veterinary Parasitology 192, 247–252.
24. Patarroyo J.H., Vargas M.I., Gonz  les C.Z., Gusm  n F., Martins-Filho O.A., Afonso L.C.C., Valente F.L., Peconick A.P., Marciano A.P., Patarroyo V.A.M., Sossai S. 2009. Immune response of bovines stimulated by synthetic vaccine SBm7462 against *Rhipicephalus* (*Boophilus*) *microplus*. Veterinary Parasitology 166: 333–339.

25. Pollart S.M., Chapman, M.D. and Platts-Mills T.A.W. 1987. House dust sensitivity and environmental control. *Prim. Care.* 14: 591-603.
26. Regassa A., 2000. The use of herbal preparations for tick control in western Ethiopia. *Journal of the South African Veterinary Association* 71: 240–243.
27. Reverchon E., Taddeo R. 1995. Extraction of sage oil by supercritical CO₂: Influence of some process parameters. *J. Supercrit. Fluids.* 8: 302–309.
28. Ribeiro V.L.S., Santos J.C.D., Bordignon S.A.L., Apel M.A., Henriques A.T., von Poser G.L. 2010. Acaricidal properties of the essential oil from *Hesperozygis ringens* (Lamiaceae) on the cattle tick *Rhipicephalus* (Boophilus) microplus. *Bioresource Technology* 101, 2506–2509.
29. Rice, P. J.; Coats, J. R. 1994. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae) and the southern corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 87, 1172-1179.
30. Silva Vaz Jr I.D., Imamura S., Nakajima C., Cardoso F.C., Ferreira C.A.S., Renard G., Masuda A., Ohashi K., Onuma M. 2005. Molecular cloning and sequence analysis of cDNAs encoding for Boophilus microplus, *Haemaphysalis longicornis* and Rhipicephalus appendiculatus actins. *Veterinary Parasitology.* 127: 147–155.
31. Tanaka T., Maeda H., Matsuo T., Boldbattar D., Umemiya-Shirafuji R., Kume A., Suzuki H., Xuan X., Tsuji N., Fujisaki K. 2012. Parasitocidal activity of *Haemaphysalis longicornis* longicin P4 peptide against *Toxoplasma gondii*. *Invertebrate Neuropeptides XII* 34: 242–250.
32. Tsao, R.; Lee, S.; Rice, P. J.; Jensen, C.; Coats, J. R. 1995. Monoterpenoids and their synthetic derivatives as leads for new insectcontrol agents. In *Synthesis and Chemistry of Agrochemicals IV*; Baker, D. R., Basarab, G. S., Fenyves, J. H., Eds.; ACS Symposium Series 584; American Chemical Society: Washington, DC, pp 312-324.
33. U.S. EPA. 1996. Exemption of certain pesticide substances from federal insecticide, fungicide, and rodenticide act requirements. U.S. Environmental Protection Agency, Final Rule, 40 CFR 152.25(g), May 6.
34. Visser, J. H. 1983. Differential sensory perceptions of plant compounds by insects. In *Plant Resistance to Insects*; Hedin, P. A., Ed.; ACS Symposium Series 208; American Chemical Society: Washington, DC, pp 215-230.
35. Yang, Y. C.; Lee, S. H.; Lee, W. J.; Choi, D. H.; Ahn, Y. J. 2003. Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *J. Agric. Food Chem.*, 51, 4884-4888.
36. Zorloni A., Penzhorn B.L., Eloff J.N., 2010. Extracts of *Calpurnia aurea* leaves from southern Ethiopia attract and immobilise or kill ticks. *Veterinary Parasitology.* 168: 160–164.

Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

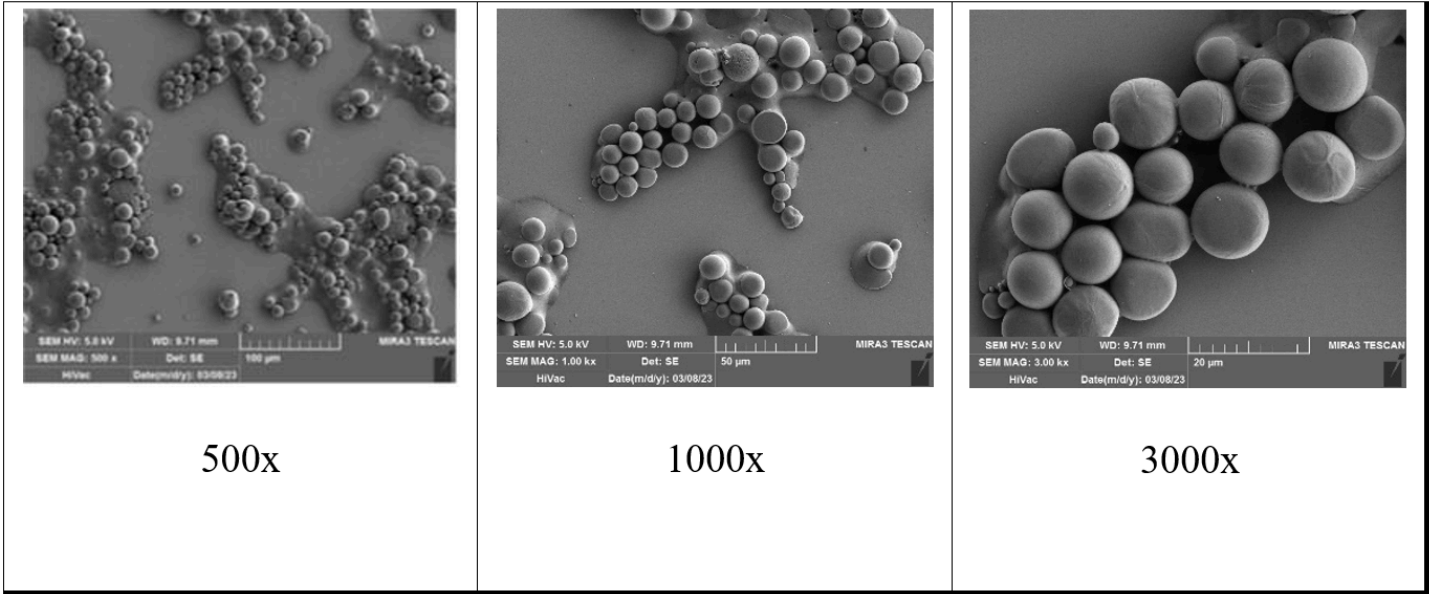
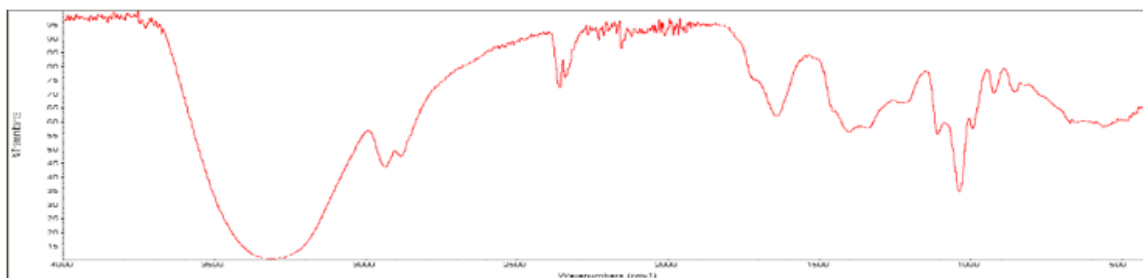
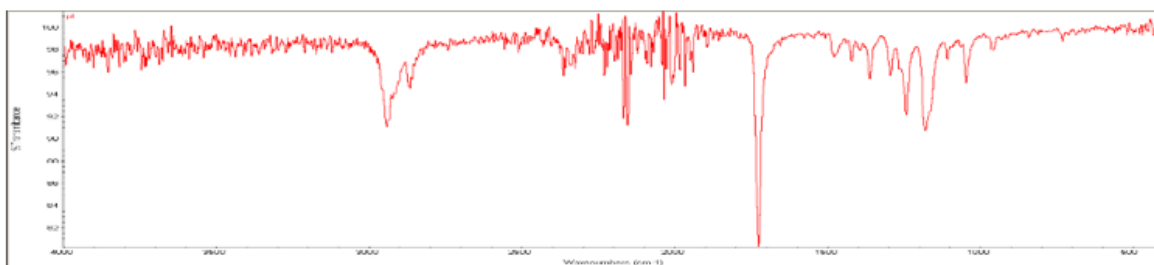


Figure 1

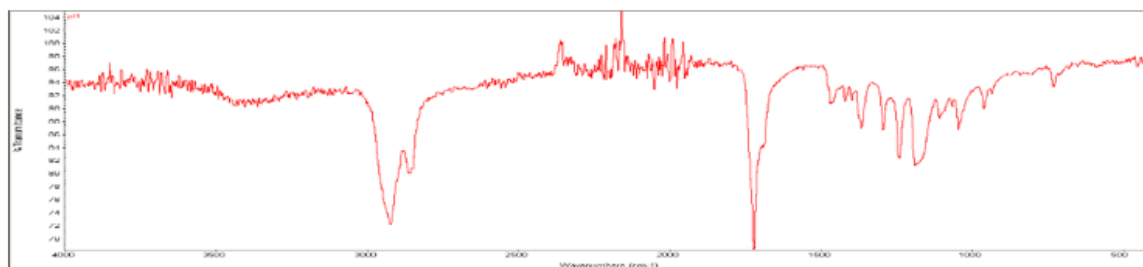
PCL Microcapsules with *Perilla frutescens* oil, at 500x, 1000x and 3000x magnification.



FT-IR Spectra of *Perilla frutescens*



FT-IR Spectra of PCL microcapsule(PCL)



FT-IR Spectra of PCL microcapsule containing *Perilla frutescens* (0.5 wt%)

Figure 2

FT-IR Spectra of PCL microcapsule containing *Perilla frutescens* (0.5 wt%)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstractJANG.docx](#)
- [TableJang.docx](#)