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In vitro acaricidal activity of honey bee propolis against *Haemaphysalis* spp.

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Abstract

Ticks are responsible for transmitting of pathogenic microorganisms during their feeding process on the hosts. They also cause significant losses in livestock production and, in many cases, the death of infected animals. In recent decades, many efforts have been carried out to combat ticks by using natural compounds. The *present* study aimed to evaluate the acaricidal effect of the hydroalcoholic extract of honey bee propolis against *Haemaphysalis* spp. *in vitro*. The acaricidal activities of the propolis were considered at concentrations of 25, 50, and 100 mg/ml and negative and positive controls (distilled water and Cypermethrin) following 10, 30, and 60 minutes of exposure. In this experiment the spraying and contact methods were used, and all tests were repeated twice. The chemical composition of propolis was identification by Gas Chromatography-Mass Spectrometry (GC–MS). Data were analyzed using GraphPad Prism software version 5.0. According to the results, propolis had an acaricidal effect; however, this effect was more potent in the spraying. The propolis showed a 100% mortality rate at 100 mg/ml concentrations after 60 min exposure. GC-MS investigation showed that Heptanone (48.65%) was the main ingredient of propolis. The results indicated that the hydroalcoholic propolis extract carry potent acaricidal ingredients and might afford new natural acaricidal compounds for the control of *Haemaphysalis* spp.

Introduction

Ticks are hematophagous ectoparasites of vertebrates and are of medical and veterinary importance worldwide. Ticks cause damage by transmitting diseases to humans and animals, economic harm to domestic animals, reduced livestock production, anemia, poisoning, paralysis, etc (1, 2). *Haemaphysalis* spp. is one of the Ixodidae ticks found on domestic and wild animals worldwide. This tick species needs three hosts to complete its life cycle. The heavy burden of ticks can lead to anemia and even animal death (3).

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Haemaphysalis spp. is present in many parts of the world, mainly due to the extensive use of different habitats and diverse hosts. This tick can transmit many zoonotic pathogens and is, therefore vital for human and animal health (4). The use of pesticides, due to their residual properties in the environment, causes toxicity and adverse effects on human health and their environmental hazards on the other hand, there is a rapid resistance to pesticides (5). It is now claimed that "green pesticides" are helpful for controlling ectoparasites (6). Recently, the use of natural products has been suggesting as an alternative to old chemical pesticides. Due to features such as low cost, low environmental pollution, side effects, and low toxicity, the tendency to use these compounds is increasing daily.

Propolis is a product make by bees, which is a familiar resinous substance that is collected by bees from flowers and substances secreted from plants and combined with bee enzymes, pollen, and wax. Bees use propolis to soften the inner walls of the hive, seal their cavities, and so on. Propolis can also protect the colony from disease due to its antiseptic and antimicrobial properties (7). Recently, immune-stimulating, anti-tumor, antiparasitic, healing, anti-viral, anti-inflammatory, antioxidant, and analgesic activities of various types of propolis have been evaluated worldwide (8, 9). In this study, we examine the acaricidal activity of hydroalcoholic extract of honey bee propolis against *Haemaphysalis* spp. *in vitro*.

Materials and methods

Preparation of propolis

Experimental collection of propolis was conducted, when bees initiated substantial resin collection. Assembly was done from ten hives with the help of beekeepers from villages around Tabriz, Iran. Propolis collected was grounded separately using an electric coffee mill (type MKM6003, Bosch, Germany). 100 g of propolis was assorted with 400 ml of 70% ethanol, and tubes were sonicated for 2 hrs. The solutions were filtered using Whatman cellulose filters. The filtrates were dried on a shaker at room temperature. The powder extracts were weighted and redissolved in 70% ethanol. The working concentrations (25, 50, and 100 mg/ml) of propolis were prepared by diffuse the need quantity of propolis in distilled water to test their acaricidal potential against *Haemaphysalis* spp.

Collection of ticks

Female ticks were collected from the bodies of sheep and cattle. At first, the ticks were placed in wide-mouth rubber containers and then transferred to the parasitology laboratory of the Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran, to determine the species of ticks.

Acaricidal activity of propolis in vitro

In an *in vitro* experiment, the acaricidal activity of propolis was studied at 25, 50, and 100 mg/ml concentrations. All weighted propolis were diluted in distilled water to adjust different concentrations. Separately, one ml of each concentration was added to the Petri dishes. Afterward, ten adult female ticks were placed in each plate. Subsequently, separate concentrations of the extract were sprinkled directly on the ticks and they were examined, every 10, 30, and 60 minutes. In this experiment the spraying and contact methods were used and all tests were repeated twice. Distilled water and Cypermethrin (EC 40%, Gyah Corp, Iran) were used as negative and positive controls, respectively. Cypermethrin working solution was used in three concentrations of 25, 50, and 100 mg/ml and three times of 10, 30, and 60 minutes as in the test groups.

Evaluation of the acaricidal effect of propolis by contact method

For the contact method the round filter papers of 4.8 cm in diameter were treated with the provided concentrations of propolis (25, 50, and 100 mg/ml). After drying for 2-3 minutes, ten live ticks were move to the filter paper, water-soaked cotton was placed in petri dishes to provide moisture, and finally, the petri dishes were covered and the parafilms were fasten.

Evaluation of the acaricidal effect of propolis by spraying method

For the spraying method, firstly, ten ticks moved to petri dishes, after which various concentrations of propolis were sprayed directly on the ticks.

Gas-Chromatography/Mass Spectrometry (GC-MS)

Chromatography was performed with (Agilent GC/MS19091S-433, USA). The propolis was mixed with hexane (*Merck KGaA*, Darmstadt, Germany) (1:1), and the solution was placed on the shaker until it was homogeneously mixed. Then, the blend was placed it a separator, and after 15 minutes the separated hexane phase was injected in the GC/MS (10).

Statistical analysis

The data were analyzed using GraphPad Prism software version 5.0, and expressed as a mean ± SEM. Data were analyzed by a two-way ANOVA for the comparison between the test and control.

Results

Based on the results, all concentrations of hydroalcoholic extract of propolis had acaricidal effects against *Haemaphysalis* spp. at all test times, and a concentration of 100 mg/ml of propolis had the highest activity (100%) at 60 min exposure time. The results indicate the spraying method was more potent than the contact method. The mortality rate of ticks at various exposure times of the propolis is presented in Table 1, and Figures 1. Different concentrations of all treatments (propolis and Cypermethrin) had a significant difference ($P < 0.0001$).

Gas chromatography-mass spectrometry (GC-MS) showed that Heptanone (48.65%), Hexane (25.1%), and Hexadecanoic acid (5.03%), respectively, as the main ingredient of propolis. The results of the GC-MS investigation are presented in Table 2 and Figure 2.

Table 1. The acaricidal effect of propolis against *Haemaphysalis* spp. *in vitro*

Concentrations	Times	Positive control	Spraying method	Contact method	Negative control
25 mg/ml	10 min	100 ± 0.0	0 ± 0.0	0 ± 4.76	0.0 ± 0.0
	30 min	100 ± 0.0	20 ± 4.76	10 ± 4.89	0.0 ± 0.0
	60 min	100 ± 0.0	40 ± 4.89	20 ± 0.0	0.0 ± 0.0
50 mg/ml	10 min	100 ± 0.0	40 ± 0.0	20 ± 4.89	0.0 ± 0.0
	30 min	100 ± 0.0	40 ± 4.62	20 ± 4.76	0.0 ± 0.0
	60 min	100 ± 0.0	60 ± 4.89	30 ± 0.0	0.0 ± 0.0
100 mg/ml	10 min	100 ± 0.0	80 ± 0.0	50 ± 4.89	0.0 ± 0.0
	30 min	100 ± 0.0	80 ± 4.89	50 ± 3.57	0.0 ± 0.0
	60 min	100 ± 0.0	100 ± 4.76	60 ± 0.0	0.0 ± 0.0

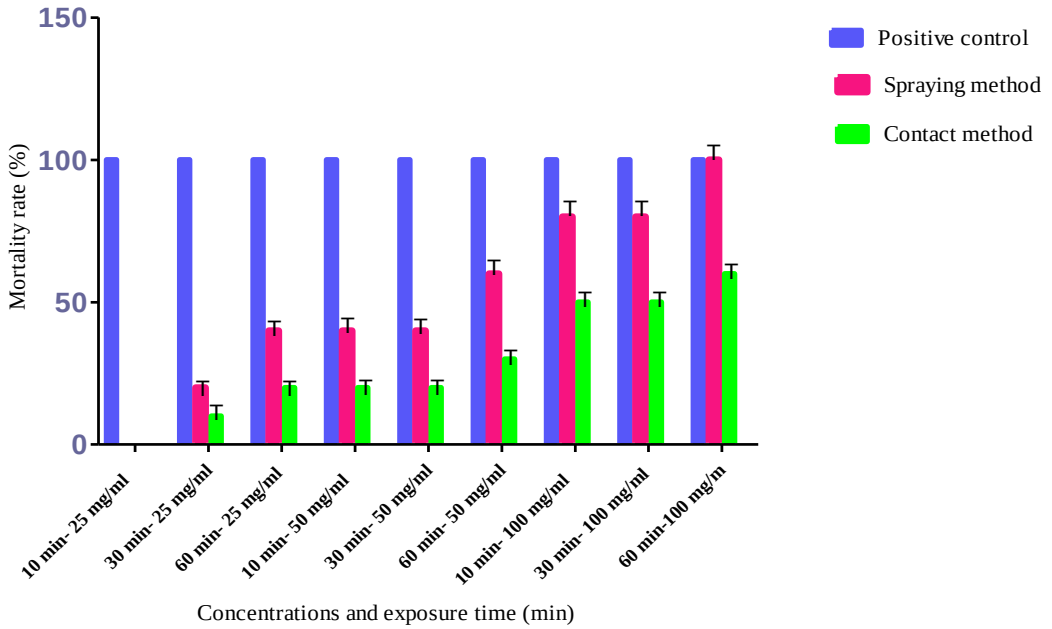

Fig. 1. Acaricidal effects of the propolis against *Haemaphysalis* spp. by spraying and contact method

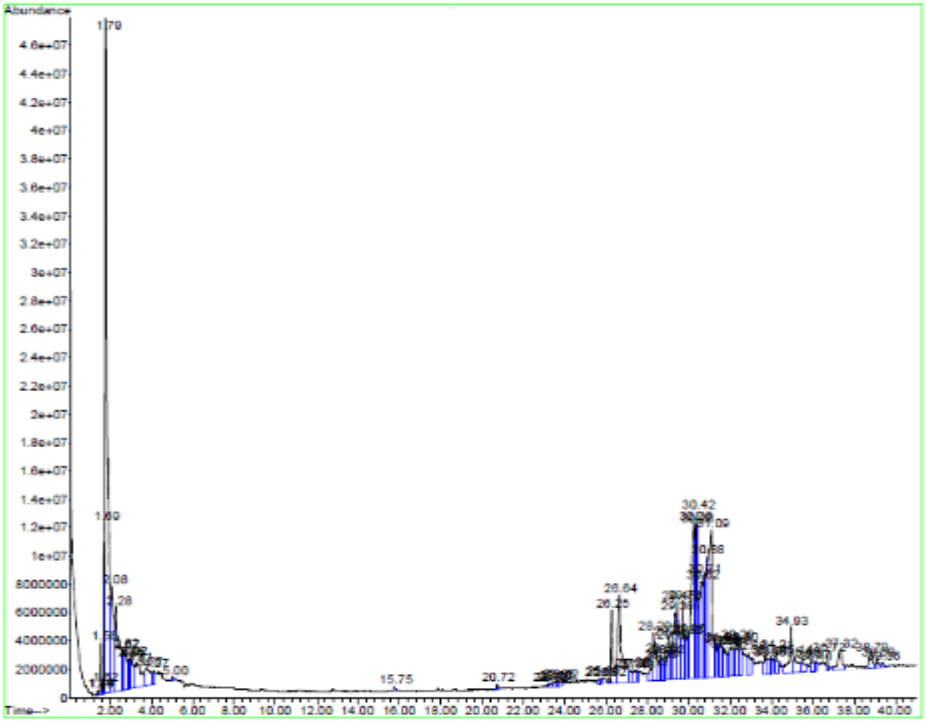

Fig. 2. Gas chromatography/mass spectrometry (GC-MS) analysis of propolis

Table 2. GC-MS results of ingredients and percent (%) of propolis.

Table 2. GC-MS results of ingredients and percent (%) of propolis.

Ingredients	Percent (%)
Heptanone	48.65
Hexane	25.1
Hexadecanoic acid	5.03
n-Hexane	3.85
Cyclohexane	3.27
Pyrrolidine	2.16
Pentane, 2-methyl- (CAS)	2.16
2-phenyl-3-ethyl-6-methoxyindeno	1.74
Di-(2-ethylhexyl)phthalate	1.40
18-methyl-19-oxoicosanoic acid	0.67
Benzene Ethan amine	0.57
Benzene	0.02
Acetic acid	0.03
Pentane	0.39
Acebutolol	0.38
1,10-diethylpyrido1	0.23
2-phenyl-3-ethyl	0.13
Butane	0.12
1,3-diethyl-2-phenyl-6	0.11
7,12a-Dimethyl-1	0.11
Dillapiole	0.1
2,6-Octadien-1-ol	0.08
Hydrogen bromide	0.07
1-Di(tert-butyl)silyloxy-3	0.05

Discussion

Infestation with ticks causes adverse effects in animals, decline in livestock production and transmission of important diseases in humans, and animals. In recent decades, the number of studies on natural products, plant extracts, and plant essential oils that can be used to control ticks has increased (11, 12). Because the use of natural products is safe, environmentally friendly and inexpensive, resistance and side effects are less (13). Present study aimed to assess the acaricidal activity of honey bee propolis against *Haemaphysalis* spp. *in vitro*. Our hypothesis for the acaricidal activity of propolis is confirmed by the obtained results.

Bees are insect species that can exploit almost any habitat on earth. This success is due to the particular products they produce: honey, wax, poison, propolis, pollen, and royal jelly. Propolis is one of the chemical weapons of bees against pathogenic microorganisms. Humans have also used propolis for centuries to treat wounds and burns, sore throats, stomach ulcers, and more (14). For this reason, propolis has been an exciting topic for biological, pharmacological, and chemical studies for the past 30 years. The chemical composition of propolis is different in each region because the plant origin that bees use in the production of propolis is different. The use of different parts of plants to produce propolis also makes a difference. In fact, the plant origin of propolis regulate its chemical variety (14). Many publications have discussed propolis antimicrobial compounds by gas chromatography-mass spectrometry (GC–MS) (15-21). All of them contained mainly flavonoids and esters of caffeic and ferulic acids. The present study showed that Heptanone (48.65%), Hexane (25.1%), and

Hexadecanoic acid (5.03%), respectively, as the main ingredient of propolis. Because of the difference in the propolis of each region and the difference in the device used to analyze the propolis, the results of this study are different from other studies.

Some studies have been performed on the antiparasitic avtivity of propolis, such as *Leishmania tropica* (22), *Giardia intestinalis* (23), *Trypanosoma cruzi* (24, 25), *Naegleria* and *Balamuthia* (26), *Plasmodium falciparum* (27), *T. brucei brucei* (28), *Leishmania donovani* (29), *Trichomonas vaginalis* (30) and *Nosema ceranae* (31).

Limited studies have been performed on the antiparasitic effect of propolis on ectoparasites. Drescher et al. (2017) used natural propolis on the mite *Varroa destructor*. Their study did not show any significant effect of propolis on mite survival and infection levels (32). Madja dos Santos Silva et al. (2021) investigated the effect of propolis alcoholic extract on *Rhipicephalus (Boophilus) microplus*is. They concluded that the viability of propolis as an alternative for the control of cattle ticks, with the 70% extract concentration being most efficient and the most effective for controlling *R. microplus* under laboratory conditions (33). The difference between the results of this study, and our study can be explained by the difference in propolis, the difference in the type of tick, the concentration and the time of exposure. For example, in the present study, the concentration of mg/ml was used, but in other studies, percentage, µ/ml, etc., were used, or the tests were performed at different times.

Conclusion

In this work, the preliminary tests demonstrated that propolis has significant acaricidal activity against *Haemaphysalis* spp. *in vitro*, and was found to be the active fraction of propolis. Anyway, further studies need to be conducted in an *in vivo* condition.

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Conflict of interest statements

The authors declare that there is no conflict of interest.

Ethical approval

Not applicable.

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