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Evaluation acaricidal efficacy of botanical extract from *Eupatorium adenophorum* against the hard tick *Haemaphysalis longicornis* (Acari: Ixodidae)

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HIGHLIGHTS

- *Eupatorium adenophorum* extract acaricidal activity was tested against hard ticks.
- LT_{50} for 1.5 g/ml were 0.790 and 1.018 h for larvae and nymphs.
- LT_{50} for 1.0 g/ml were 1.445 and 1.313 h for larvae and nymphs.
- LT_{50} for 0.5 g/ml were 2.22 and 2.651 h for larvae and nymphs.

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ABSTRACT

The aim of this study was to evaluate the acaricidal activity of a botanical extract from *Eupatorium adenophorum* against the hard tick *Haemaphysalis longicornis*. This could result in developing effective extracts of *E. adenophorum* as a source of natural, low-toxicity plant-based acaricidal drugs. Adult engorged females of *H. longicornis* were collected from naturally infected goats. The engorged females were reared in the laboratory and their offspring (larvae and nymphs) were used as test ectoparasites. The toxic effects of botanical extracts from *E. adenophorum* against larvae and nymphs of *H. longicornis* were evaluated. The results showed that the extracts with 1.5 and 1.0 g/ml (w/v) concentrations were toxic for *H. longicornis*, comparable to a toxic effect of 2% chlorpyrifos (positive control). The median lethal time (LT_{50}) for larval and nymphal ticks with 1.5 g/ml (w/v) concentration of extract were 0.790 (LT_{99} = 1.065) and 1.018 (LT_{99} = 10.608) hours, respectively, whereas the LT_{50} of 1.0 g/ml (w/v) concentration were 1.445 (LT_{99} = 6.047) and 1.313 (LT_{99} = 29.932) hours for larval and nymphal ticks, respectively. At a concentration of 1.5 g/ml (w/v), an acaricidal effect of 100% was achieved for both larval and nymphal ticks, while a concentration of 1.0 g/ml (w/v) resulted in 100% (for larvae) and 93% (for nymphs) within a 6 h period. In additional, we found that the relatively low concentration (0.5 g/ml) also obtained a good acaricidal effect during the short experimental period, with 2.22 and 2.651 h LT_{50} for larval and nymphal ticks, respectively. These results indicate that *E. adenophorum* contains potent acaricidal ingredients against the hard tick *H. longicornis*.

1. Introduction

Ticks are ectoparasites of various vertebrate hosts. Following a tick bite, the skin tissue of a host is damaged (Ribeiro et al., 2011), which can lead to a small wound and cause dermatitis (Roshdy, 1974), erythematous nodules (Kim et al., 2003), erosion, and ulcers due to secondary systemic infections. Ticks parasitizing livestock can reduce the quality of animal products and can even cause livestock death (Tian et al., 2011; Elango and Rahuman, 2011). Ticks are also important vectors of various pathogens, such as bacteria, viruses, and protozoa. Ticks are second only to mosquitoes as vectors of disease-causing agents in humans, domestic, and wild animals (Boldbaatar et al., 2006; Kocan et al., 2011). Furthermore, as blood-feeding arthropods, ticks have caused great harm in humans and animals (Kaufman, 2010; Zheng et al., 2012; Kiss et al., 2012). It has been reported that, globally, approximately 80% of

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1.2 billion cattle are at risk of ticks and tick-borne diseases, thus causing a global annual loss of US$ 7 billion (Bagavan et al., 2009; Zahir et al., 2010).

The hard tick *Haemaphysalis longicornis*, which is widely distributed in East Asia and Oceania (Fujisaki et al., 1994; Silva et al., 2005; Tanaka et al., 2012), 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). In Northern China, this species is the most abundant tick and infests humans and domestic animals, with infestation rates peaking during the summer (Zheng et al., 2012).

At present, common anti-tick measures include the spraying of synthetic drugs, regular medicated bathing of livestock, using smoke agents in forests as acaricidal drugs, and applying synthetic acaricides both in the environment and to animals (Regassa, 2000; Iori et al., 2005; Patarroyo et al., 2009). The use of anti-tick drugs usually includes chemical agents containing synthetic pyrethroids, organophosphates, and amitraz (Gazim et al., 2011). Although these acaricides are beneficial when properly used, misuse has led to poisoning of humans and animals (FAO, 1998; Zorloni et al., 2010). It also leads to the problems of the agents being only partially successful, of the parasites developing serious resistance, of drug residue, and environmental hazards (Fernandes and Freitas, 2007; Ribeiro et al., 2007; Nong et al., 2012, 2013). To overcome these obstacles, the development of effective and environmentally friendly replacement drugs of low toxicity is required. This has led to the assessment of low-cost, safe and environmentally benign plant-based alternatives to commercial pesticides.

Compared with efficient chemical agents, botanical extracts have the advantage of biodegradation. Phytotherapy is considered a viable alternative to the use of synthetic compounds for the control of tick infestations in livestock (Moyo and Masika, 2009; Madzimure et al., 2011). Therefore, the development of plant-based acaricidal drugs represents a focus of research. Based on promising results obtained by other researchers with other plant species against ticks (Fernandes et al., 2007), plant extracts provide a potential alternative to existing acaricides. Essential oils from *Tetradenia riparia* (Lamiaceae), extracts of *Calpurnia aurea* leaves, from *Copaifera reticulata* (Leguminosae: Caesalpinioideae), from the aerial parts of *Hypericum polyanthemum*, and *Tephrosia vogelii* leaf extracts have been tested against *Rhipicephalus* (*Boophilus*) *microplus*, *Rhipicephalus pulchellus*, and *Rhipicephalus appendiculatus* ticks (Fernandes and Freitas, 2007; Ribeiro et al., 2007; Zorloni et al., 2010; Nana et al., 2010; Gazim et al., 2011; Kalume et al., 2012).

Eupatorium adenophorum (Asteraceae: Compositae) (Auld and Martins, 1975) is a perennial herbaceous plant, mainly distributed in Central America, Mexico and other tropical and mild zones (Sun et al., 2004; Chen et al., 2012). Because of its rapid dispersal, it has become a harmful weed of crops, natural environments, and forests around the world. *E. adenophorum* has caused environmental and ecological damage in at least 30 countries (Lei et al., 2012) and has become the most important invasive alien species in China (Li et al., 2009; Sang et al., 2010; Shi et al., 2012). Therefore, how to explore/use such huge resource of harmful invasive plants and change waste into valuables need more methodologies. In recent years, researchers have begun to realize the potential of *E. adenophorum* as a source of plant-derived drugs. Methanolic extracts of *E. adenophorum* leaves contain an active compound preventing infection with the parasite *Trypanosoma evansi* (Shaba et al., 2012). The extracts of *E. adenophorum* were found to be highly effective against coccidian oocysts in chicken (Yang et al., 2012), and have also been used against *Psoroptes cuniculi* and *Sarcoptes scabiei* mites in rabbits (Nong et al., 2012, 2013). Additionally, the bioactive compound of cadinene sesquiterpenes extracted from leaves of *E. adenophorum* was used *in vitro* against four phytopathogenic fungi (Kundu et al., 2013). However, no information is available on the use of *E. adenophorum* extracts against ticks thus far. Based on this, the aim of this study was to evaluate the acaricidal efficacy of botanical extracts from *E. adenophorum* against the hard tick *H. longicornis*.

2. Materials and methods

2.1. Plant material and extraction

2.1.1. Plant material

E. adenophorum plants (Fig. 1) were collected from Xichang, Sichuan Province of China, in September 2011. The morphological identification of the plant was performed based on the taxonomic key (Li, 1998).

2.1.2. Extraction

The aerial parts of *E. adenophorum* were dried and crushed in a knife mill (Type: GX-10A, YongKang Gao Xiang Food machinery CO., LTD, Zhejiang, China). *E. adenophorum* extracts were created by ethanol thermal circumfluence. The plant material was soaked in 95% ethanol (600–800 ml 95% ethanol per 100 g plant material) for 30 min at room temperature. It was then twice extracted with boiling ethanol under reflux (1 h per cycle). After that, the extracts were merged and concentrated by evaporation using a rotary evaporation apparatus. Finally, the concentration of the extract was diluted in distilled water and glycerol (1:1) to give the concentration of 2 g/ml for storage. During the procedure of the experiments, this concentration was diluted into 1.5 g/ml, 1.0 g/ml, and 0.5 g/ml for use.

2.2. Ticks

Engorged females of *H. longicornis* were collected from naturally infested goats in Cangxi Country, Guangyuan City, Sichuan Province, China. The use of acaricidal treatment on the infested goats had been suspended for at least 45 days prior to the start of the study (Gazim et al., 2011). After collection, ticks were put into glass vials filled with fine sand and a piece of filter paper, and the glass vials were sealed with rubber rings to prevent the ticks from escaping. The engorged females were incubated at 25–27 °C and 70–80% relative humidity for about 15 days until oviposition had occurred (Gazim et al., 2011). After larval ticks had hatched, they were fed on rabbits (Matsuo et al., 2003) and molted into nymphal ticks.

Fig. 1. *Eupatorium adenophorum*. Note: Collected from Xichang city of Sichuan province, China in September 2011.