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Evaluation of Five Types of Chinese Herb for Acaricidal Effect of Cattle Tick

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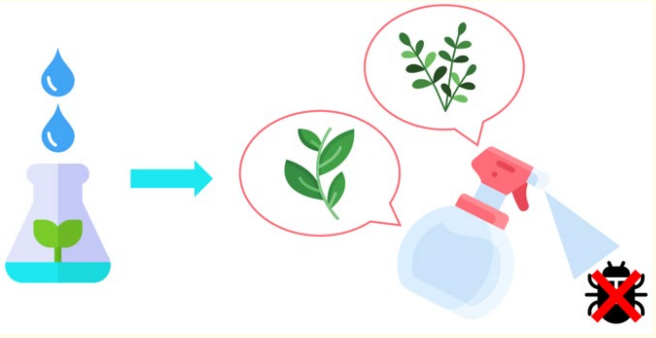
Abstract

Cattle ticks (*Ixodidae*) have caused serious harm to livestock raised in agriculture and animal husbandry and are responsible for severe economic losses. Although chemical products are effective and maintain productivity for a certain period of time, the excessive and uncontrolled use of these synthetic agents poses heavy health losses upon long-term use. However, the need for safer products has stimulated the search for new acaricides such as those obtained from medicinal plants. In the present study, a method based on a transdermal diffusion cell drive system (TDCS) and microwave-assisted extraction (MAE) was evaluated to compare five common Chinese herbs for cattle ticks: rhubarb (*Rheum rhabarbarum*), blume (*Stemona japonica*), clove (*Syzygium aromaticum*), cumin (Foeniculum vulgare), and phellodendronwere (*Phellodendron*). When ethanol was used as the extraction solvent, the concentration of the extract of clove was 0.45% (w/v), indicating good efficacy. These results indicate that the tested extracts exhibited acaricidal activity and could be considered potential agents for the development of alternative natural acaricides.

Keywords: Transdermal diffusion cell drive system; Acaricide; Chinese herb; Cattle tick; clove



Graphical Abstract

Introduction

Ticks are parasitic organisms that may carry viruses, bacteria, and protozoa, leading to serious diseases such as Lyme disease (Bush & Vazquez-Pertejo, 2018). They have a significant impact on human and animal health, particularly in agriculture and animal husbandry. Ticks are known to transmit a wide range of disease-causing arthropods, second only to mosquitoes (*Culicomorpha*) and cockroaches (*Blattodea*) in their ability to spread diseases relevant to human well-being (Sparagano, Földvári, Derdáková, & Kazimírová, 2022; Sykes, 2023). Among them, the cattle tick (*Rhipicephalus microplus*) is particularly aggressive, utilizing humans, domestic animals, and wildlife as hosts throughout its life cycle. These ticks are found worldwide, and some regions have observed an increase in distribution range and population density, resulting in numerous diseases affecting humans and animals (Brites-Neto, Duarte, & Martins, 2015). Chemical acaricides, such as chlorinated hydrocarbons, organophosphorus, carbamates, formamidines, pyrethroids, and avermectins (Nicholson, Sonenshine, Noden, & Brown, 2019; Rajput, Hu, Chen, Arijo, & Xiao, 2006), are used in management of this parasite. Although these chemicals are effective and maintain productivity for a certain period of time, their excessive and uncontrolled long-term use may pose threats to health of animals. In addition, the development of resistance to these chemical agents in ticks is a major challenge. Hence, the need for safer products that are less harmful to both humans and the environment (e.g., innocuous agriculture) has led to an increase in the development of alternative approaches to ectoparasite management (Singh, Mahajan, Kothari, Singh, & Singh, 2023). Moreover, botanical alternatives such as essential oils, herbs, and natural products, are currently receiving growing attention (Benelli & Pavela, 2018; Upadhyay, 2021). The use of natural products can be beneficial in terms not only of reducing the problems associated with commercial acaricides, such as resistance phenomena and environmental contamination (Banumathi et al., 2017; Patoliya et al., 2022). Natural and herbal acaricidal drugs are safe and effective as synthetic agents. Controlling ticks using herbal acaricides is easier, safer, and more cost-effective due to their sustainable availability and biodegradability. Thus, the extraction of natural products is currently the most frequently used technique for the analysis and evaluation of cattle tick activity in biochemistry(Jain, Satapathy, & Pandey, 2021; Khare et al., 2019; Shyma, Gupta, Ghosh, Patel, & Singh, 2014). In this study, a method based on a transdermal diffusion cell drive system (TDCS) and microwave-assisted extraction (MAE) was developed and validated for observing the tick activity of five common Chinese herbs: rhubarb (*Rheum rhabarbarum*), blume (*Stemona japonica*), clove (*Syzygium aromaticum*), cumin (*Foeniculum vulgare*), and phellodendron (*Phellodendron spp*.). To apply it as a controlling agent for cattle ticks in the future, this study aimed to develop contact formulations using Chinese herbs as active ingredients to assess their acaricidal activity.

Experimental

Chemicals, reagents, and collection of cattle tick

The extraction solvents including HPLC grade methanol (MeOH), ethanol (EtOH) from Sigma-Aldrich (St. Louis, MO, USA), and ultrapure water from a ELGA Option-R 15 Water purification system (18.2MΩ-cm at 25°C) were obtained from ELGA-LabWater (Wycombe, United Kingdom). The evaluated herbs, rhubarb, blume, clove, cumin, and phellodendron, were obtained from a local Chinese medicine pharmacy (Tainan, Taiwan). The herbal samples were ground using an RT-04 high-speed pulverizing machine (Rong-Tsong Precision Technology, Taichung City, Taiwan), and the solvent was added before the extraction process. All experimental protocols involving animals were reviewed and approved by the Institutional Animal Experimentation Committee of Chia Nan University of Pharmacy and Science. Cattle tick samples were captured from adult large-bred dogs (*Canines*) from two different places, one in the Kaohsiung Animal Protection Office (Kaohsiung City, Taiwan) and one in the Tainan Animal Health Inspection and Protection Office (Tainan City, Taiwan). The same sample collection and preparation methods were used at each study site, and the collected ticks were placed in clean plastic containers with perforated lids to allow ventilation. Tick larvae were transported to a laboratory rearing box and reared until adults, and all ticks were fed blood beef (twice a day). The adult ticks were transferred into a vial until analysis (feeding was maintained during this period) and discarded after one test.

Extraction process

One gram of the ground herbal sample was poured into a microwave tank and extracted with 15 mL ethanol (homogenized by ultrasonic mixing). The extraction was conducted at 80°C temperature and 800 W power by CEM Mars 6 i-Wave Microwave Digestion System (CEM Corporation, Matthews, NC, USA), respectively. The warm-up time was 15 min, the working time was 30 min, and cool down time was 20 min. The supernatant was poured into a centrifuge tube and centrifuged at 8500 rpm for 10 min at 27 °C (Hettich Universal 320R centrifuge, Westfalen, Germany). Subsequently, the supernatant was filtered through a polytetrafluoroethylene (PTFE) syringe filter (Sigma-Aldrich, St. Louis, MO, USA) before subjecting it to dialysis assay. The absorbances of the samples were measured at 240, 250, 263, 277, and 339 nm using a UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All experiments were performed in triplicates.

Transdermal diffusion cell drive system (TDCS)

The transdermal diffusion cell drive system for a Valia-Chien Cell (PermeGear, Hellertown, PA, USA) consists of a single solution compartment, which represents the central compartment for systemic circulation, and two openings (orifice diameters of 10 mm each), which were used for mounting the MW 3500 series OrDial D35 standard-grade regenerated cellulose (Orange Scientific, Braine-l'Alleud, Belgium). The central compartment has a solution volume of 3.5 mL, used magnet rotating at a constant speed (500 rpm) (Variomag POLY 15 Multi position stirrer, Thermo scientific, Waltham, MA, USA) and the temperature can be kept at 37 °C by circulating thermostated water through the water jacket surrounding the central compartment. The extraction solution was collected each time (0.5, 1, 2,3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h) and the absorbance was measured using a UV-visible spectrophotometer.

Development of the formulation

After adding the Chinese herbal extract, the pharmaceutical formulation was an oil/water (O/W) emulsion. The emulsion flasks were stored under ambient conditions and refrigerated. After 15 days of refrigeration, no phase separation was observed. For the same period, the product containing clove remained stable from an organoleptic point of view in room temperature. However, by analyzing the formulations with added clove extract, concentrations of 0.25–0.45% (w/v) was observed with no changes under ambient conditions (no phase separation).

Figure 1: Schematic diagram of the cattle tick used for present study.

Results and Discussion

Optimization of the extraction solvent

The epicuticle characteristic of ticks is differentiated into three distinct layers: the cuticulin layer, inner protein layer, and the outer waxy layer, which is composed of lipoproteins, and the outer waxy layer comprised of esters and derivatives (Hackman & Filshie, 1982; Koch & Ensikat, 2008), which are endowed with multiple functions (e.g., prevent maximum transpiratory water loss and provide protection from external hazards and risk). A number of plant solvents, including hexane, acetone, ethanol, ethyl acetate, methylenedichloride, chloroform, and distilled water, have been used as extractants in a previous study (Chuo et al., 2022; Jha & Sit, 2022). Among them, methanol and acetone was the most commonly used solvent (Fouche et al., 2017). In this study, several commonly used extraction reagents, including ultrapure water, methanol, and ethanol, were evaluated. Our results showed that ethanol was the most toxic solvent, whereas methanol was moderately toxic (Fig. 2). While aqueous solvents are widely used in biochemistry, organic solvents may work better in acaricidal bioassays, as the cuticle of ticks is formed externally, mainly by waxes, and internally by proteins; hence, the more non-polar a chemical compound is, the greater its ability to penetrate the cuticle (O. T. Adenubi, Fasina, McGaw, Eloff, & Naidoo, 2016). Ravindran et al. (Reghu Ravindran et al., 2011) tested various solvents in vitro for their acaricidal effects based on adult mortality, inhibition of fecundity, and blocking of egg hatching. Their results showed that ethanol is less polar and highly penetrable into the epicuticle, while methanol is highly polar and less penetrable than all the other solvents, showing the lowest rate of adult mortality in treated ticks. Previous report (R Ravindran et al., 2011), indicated that the high concentrations of glycerol absorb water and cause dehydration. Owing to its viscosity, it can remain on the tick cuticle for a prolonged period, causing slow dehydration, and resulting in moderately higher mortality than methanol and ethanol. In general, the more polar the solvent, the lower the acaricidal effect.

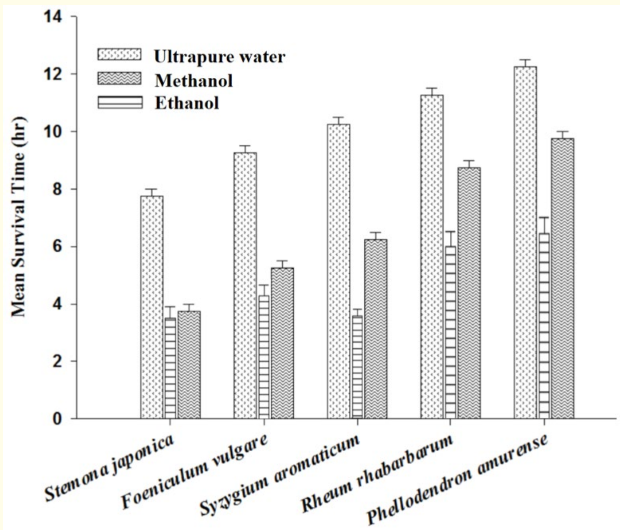


Figure 2: Effect of different solvents versus ticks mean survival time.

Assessment of herbal species and concentration

Natural botanical compounds are relatively safe, flora- and fauna-friendly, biodegradable, and contain a range of chemically active ingredients that can intervene in all biological processes of insects, thus interrupting their life cycle and dispersal. Hence it can be used as alternatives for synthetic compounds. The selected herb has been proven to perform acaricidal activity behaviors in the past (Varadharajan & Gnanasekar, 2019; Voronova, Horban, & Bohatkina, 2022; Zaman et al., 2012); however, the difference changed with time, and the survival rate of the sample ticks gradually produced apparent differences with various herbs. In the present study, five herbal species included in the assessed approach were compared with the survival rates of ticks: rhubarb, blume, clove, cumin, and phellodendron. The experimental results showed that the combination of clove and ethanol resulted in the best response, which led to the selection of the herb for use in subsequent analyses. The acaricidal activity of clove is attributed to known major components and the resulting synergistic or antagonistic actions (Batiha et al., 2019; Thorsell, Mikiver, & Tunon, 2006). Several biological activities have been reported for the clove herb: the most noteworthy activities that have been previously demonstrated are the antibacterial effect against pathogenic bacteria causing infections in animals (Karuppiah & Rajaram, 2012), the fungicidal activity against plant pathogen fungi in for agricultural activity (Chee & Lee, 2007), and the insecticidal activity against acarine-arthropod pests of economic importance. In this study, we demonstrated the potent acaricidal activity of cloves, which produced toxic effects in cattle ticks at any concentration. Eugenol accounts for a high proportion of clove components and is the source of the fragrance; it also has the function of acaricidal ticks, true ticks (de Monteiro et al., 2012), and *Lymantriidae* (Mango), and can resist various fungi, such as *Salmonella typhi* (Devi, Nisha, Sakthivel, & Pandian, 2010), as well as the characteristics of resisting fungi (Cheng, Liu, Chang, & Chang, 2008). Acetylcholine (ACh) and acetylcholinesterase (AChE) exist in ticks as antidotes to excrete toxic substances from the body, and are carried out simultaneously in the nervous system (Colovic, Krstic, Lazarevic-Pasti, Bondzic, & Vasic, 2013). When eugenol enters ticks, it interrupts the action of AChE. In the state where no antidote is used, eugenol continues to react with AChE, causing the hydrolysis step of AChE to stop, allowing ACh to continue to be produced, and causing the tick to appear poisoned (Floris, Galloni, Conte, & Sabuzi, 2021; Perry, Yamamoto, Ishaaya, & Perry, 2013). The acaricidal activity of eugenol has an inseparable relationship with ACh and AChE; this also interferes with the action potential of the tick and has a strong inhibitory effect on AChE activity (Olubukola Tolulope Adenubi et al., 2018), causing ACh to continue to act in the body to form a stacking effect and interfere with the operation of the central nervous system and peripheral nervous system (Cepeda et al., 2022; Vatanparast, Khalili, & Naseh, 2017). A large amount of choline damages the nerve function, causes severe antagonism of the nervous system, and gradually leads to death (Na, 2017; Selles et al., 2021).

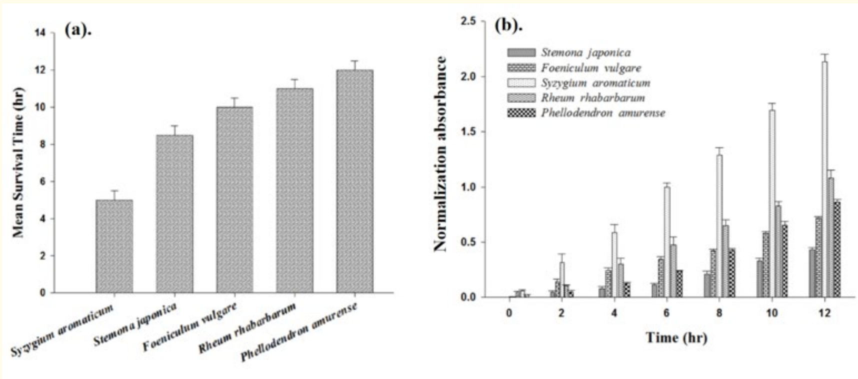


Figure 3: The absorbance signals of different herbal type were obtained after TDCS (a) and ticks mean survival time (b).

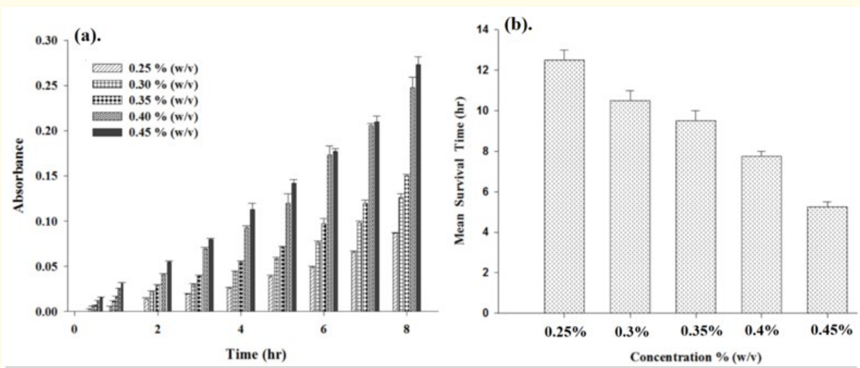


Figure 4: The absorbance signals of different concentration clove were obtained after TDCS (a) and ticks mean survival time (b) in O/W emulsion.

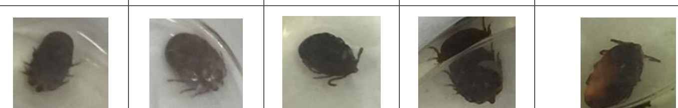
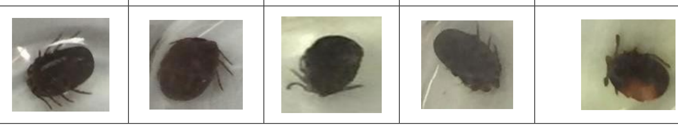
Species / *Time*	*Clove*	*Blume*	*Cumin*	*Rhubarb*	*Phellodendron*
0.5 hr					
1 hr					
2 hr					
3 hr					
4 hr					
5 hr					
6 hr					
7 hr					
8 hr					
9 hr					
10 hr					
11 hr					
12 hr					

Table 1: Observation images of evaluated 5 types of herb using TDCS.

Extract add in O/W emulsion

The survival times of ticks in cloves treated with five different concentrations of the extract were compared (Fig. 4a-b), and the extracts of these agents were optimized. Finally, 0.45% (w/v) was selected as the final concentration to be added to the oil-in-water (O/W) emulsion. In published studies, it was observed that clove extract was incorporated in organic solvents without adding adjuvants, which provided stability to the product and adherence to the tegument of the ticks (de Mello et al., 2014). With this information, this study aimed to assess the acaricidal activity of these herbs added to contact formulations that resulted in stability, low levels of environmental toxicity, reduced cost, and easy handling. The obtained product was an emulsion formed by adding an extract to an aqueous base containing a polymer. The addition of this polymer to the formulation aimed to increase the viscosity and pellicle formation on the tick tegument, and increase the retention and action time of the extract (Ansel, Popovich, & Alen Jr, 2000; Rodríguez-Rojo, Varona, Núñez, & Cocero, 2012). The addition of oil to water requires the presence of surfactants to favor the miscibility of physically incompatible components, increase the stability, and avoid the separation of the oil and aqueous phases (Ansel et al., 2000; de Mello et al., 2014; Rodríguez-Rojo et al., 2012).

Conclusion

The present study proposes an alternative, cheap, environmentally friendly, and safe approach using a natural herb and pesticide composition that also achieves acaricidal results. The study concluded that cloves show high efficacy against cattle ticks. Moreover, the ethanol extract exhibited a strong acaricidal effect on adult cattle ticks after 5 h of application and continued for up to 12 h after treatment, resulting in 100% mortality. The present data provide a platform for the development of environmentally friendly and non-toxic acaricidal herbs that can be used on a large scale in agriculture and animal husbandry.

Conflict of interest

The authors declare no conflict of interest with this work.

Acknowledgments

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