

Comparative acaricidal activity of aqueous and ethanolic extracts of medicinal plants against the mite *Psoroptes cuniculi*

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Abstract

Background and Aim. *Psoroptes cuniculi* is one of the most widespread causative agents of psoroptic mange in rabbits, causing significant economic losses in rabbit breeding. Given the increasing need for environmentally friendly alternatives to synthetic acaricides, the investigation of acaricidal properties of medicinal plants represents a promising research direction. The aim of the present study was to conduct a comparative evaluation of the acaricidal activity of aqueous and ethanolic extracts of medicinal plants against *Psoroptes cuniculi* mites under laboratory conditions.

Material and Methods. The study objects included extracts of *Mentha piperita*, *Origanum vulgare* L., *Thymus vulgaris*, *Ferula foetida*, *Cinnamomum cassia* (L.) D. Don, *Curcuma phaeocaulis* Valetton, and *Eugenia caryophyllata* Thunb. at concentrations of 200, 500, and 1000 µl/ml. A 1% dimethyl sulfoxide (DMSO) solution was used as a negative control, while permethrin at concentrations of 1, 2.5, 5, 10, and 20 µl/ml served as a positive control. Acaricidal activity was assessed by mite mortality rates. Statistical analysis was performed using the Kruskal-Wallis test.

Results. It was established that the extraction method significantly influenced the efficacy of the preparations. Overall, ethanolic extracts induced significantly higher mite mortality compared to aqueous extracts (30.33% and 19.70%, respectively; $P < 0.01$). The most pronounced acaricidal activity was demonstrated by the ethanolic extract of *Eugenia caryophyllata* Thunb., which achieved 100% mite mortality at a concentration of 1000 µL/mL, whereas the aqueous extract caused only 28% mortality. High efficacy was also shown by ethanolic extracts of *Cinnamomum cassia* (69%) and *Curcuma phaeocaulis* (48%) at the maximum concentration. Extracts of *Mentha piperita*, *Origanum vulgare*, *Thymus vulgaris*, and *Ferula foetida* were characterized by considerably lower acaricidal activity.

Conclusion. The obtained results indicate the high potential of ethanolic extracts of *Eugenia caryophyllata*, *Cinnamomum cassia*, and *Curcuma phaeocaulis* as sources of natural acaricidal compounds for the development of alternative control agents against *Psoroptes cuniculi*. Further in vivo studies are required to confirm their practical efficacy, focusing on therapeutic effectiveness and safety of application.

Keywords: acaricidal activity; aqueous extracts; ethanolic extracts; medicinal plants; natural acaricides; *Psoroptes cuniculi*.

Introduction

Tick and mite infestations represent one of the most pressing challenges in veterinary parasitology and livestock production worldwide. Among the causative agents of mange in rabbits, particular significance is attributed to mites of the genus *Psoroptes* - the etiological agents of psoroptic mange

(ear mange), which cause severe lesions of the external auditory canal and adjacent skin areas. *Psoroptes cuniculi* (Delafond, 1859) is a non-burrowing mite that does not penetrate the deep layers of the skin but instead localizes on the epidermal surface within the auricular region, where it feeds on lymph, exudate, and desquamated epidermal cells. The life cycle of the parasite lasts approximately 21 days, and mites are capable of surviving off the host for up to 21 days, which accounts for the high contagiousness of the disease [1]. Rabbit psoroptic mange is characterized by the formation of dark-brown crusts in the external ear canal, pruritus, head shaking, and, in severe infestations, the spread of the pathological process to the face, neck, and limbs. The disease leads to a significant reduction in rabbit productivity, deterioration of pelt quality, development of secondary bacterial complications, and, in some cases, animal mortality, all of which result in substantial economic losses for rabbit breeding enterprises.

Currently, the control of *Psoroptes* mites in veterinary practice relies predominantly on the use of synthetic acaricides, such as ivermectin, fipronil, pyrethroids (deltamethrin, cypermethrin), amitraz, and their combinations [2]. However, prolonged and uncontrolled use of these agents has led to the development of resistance in mite populations, significantly reducing the therapeutic efficacy of synthetic compounds [2, 3]. Moreover, synthetic acaricides possess a number of substantial drawbacks, including high toxicity to target and non-target organisms, accumulation of residues in livestock products (meat, pelts), adverse environmental impact, and detrimental effects on beneficial entomofauna [2, 3, 4].

For instance, studies conducted in East and West African countries have revealed moderate to high levels of resistance to chlorfenvinphos and amitraz in populations of *Rhipicephalus microplus*, *Amblyomma variegatum*, and *Rhipicephalus appendiculatus*; notably, *R. microplus* demonstrated high resistance to alpha-cypermethrin and cypermethrin, while populations of *A. variegatum* (West Africa) and *R. appendiculatus* (Uganda) exhibited moderate resistance to ivermectin [3]. These findings underscore the urgent need for alternative, environmentally safe methods for tick and mite control. Given the limited resources of chemical acaricides and the growing problem of resistance, plant-derived products -- including phytopreparations, essential oils, and plant extracts - are attracting increasing research interest [4, 5]. Plants synthesize a wide array of secondary metabolites (monoterpenes, sesquiterpenes, phenylpropanoids, flavonoids, alkaloids, tannins) that possess insecticidal, repellent, and acaricidal activities. Botanical acaricides are characterized by multi-component mechanisms of action, which substantially reduces the risk of resistance development in parasites; they are less toxic to mammals and non-target organisms, degrade rapidly in the environment, and leave no residues in livestock products [4, 5, 6]. Review studies confirm that natural products, including plant extracts and essential oils, represent promising agents for the control of ticks, mange mites, and other ectoparasites of animals [7].

Despite the fact that aqueous and ethanolic extracts of medicinal plants are simpler to produce and have lower production costs, their acaricidal activity has been significantly less investigated [8, 9]. Aqueous extracts predominantly contain polar compounds - phenolic acids, flavonoids, glycosides, tannins, and saponins -- whereas ethanolic extracts enable the recovery of a broader spectrum of compounds, including less polar alkaloids, terpenoids, coumarins, and phenylpropanoids.

In this context, the aim of our study was to conduct a comparative evaluation of the acaricidal activity of aqueous and ethanolic extracts of medicinal plants against the mite *P. cuniculi*.

Materials and Methods

Collection and identification of plant materials

Plant materials from seven species were used. The inner bark of *Cinnamomum cassia* (L.) D. Don was purchased from Jiangsu Yabang Chinese Medicine Co., Ltd. (Jiangsu Province, China) and ground before extraction. Roots of *Ferula foetida* L. were collected in the vicinity of Shymkent in late September 2025. The aerial parts of *Thymus vulgaris* L. were collected in the Almaty Region in July 2025. The aerial parts of *Origanum vulgare* L. were collected in the Trans-Ili Alatau, Almaty Region, in 2025. Leaves of *Mentha piperita* L. were collected in the vicinity of Shymkent in early July 2025. Rhizomes of *Curcuma phaeocaulis* Valetton and dried unopened flower buds of *Eugenia caryophyllata* Thunb. were purchased from Jiangsu Yabang Chinese Medicine Co., Ltd. (Jiangsu Province, China).

The botanical identity of the field-collected specimens was confirmed at the Research Center for Medicinal Plants of Al-Farabi Kazakh National University. Taxonomic identification was based on morphological characteristics using appropriate botanical identification keys. Following collection, plant materials were sorted, washed when necessary, air-dried to remove surface moisture, and dried in a drying oven at 40–50 °C until completely dry. The materials were cooled to room temperature, ground to a homogeneous powder using a laboratory mill, and stored in sealed moisture-resistant containers at 20–25 °C, protected from direct sunlight, until extraction.

Preparation of plant extracts by ultrasonic extraction. Thirty grams of dried, ground plant material was mixed with 240 mL of extractant (1:8, w/v) in a 500-mL conical flask. Extraction was performed using a KQ5200B ultrasonic bath at 40 kHz and room temperature (20–25 °C) for 30 min and was repeated three times. Between extraction cycles, the mixtures were maintained at room temperature and mixed to ensure uniform contact between the plant material and extractant.

For ethanolic extraction, 96% ethanol was used as the extractant; distilled water was used for aqueous extraction. After extraction, the mixtures were filtered through paper filters. Ethanolic filtrates were concentrated using an EYELA N-1300 rotary evaporator at 45 °C until the solvent was removed and a concentrated extract was obtained. Aqueous filtrates were concentrated under the same temperature conditions and, when necessary, subjected to gentle additional drying. The extracts were stored in sealed containers at 4–8 °C until testing.

Preparation of working extract solutions. Concentrated extracts were weighed and used to prepare 10 mg/mL stock solutions. Water-soluble extracts were dissolved in distilled water. When an extract was insufficiently soluble in water, DMSO was used as a co-solvent. For example, 40.65 mg of extract was dissolved in a total volume of 4.065 mL, including 0.4065 mL DMSO, to obtain a 10 mg/mL stock solution containing 10% (v/v) DMSO.

The 1000 µg/mL working solution was prepared by mixing 0.1 mL of the 10 mg/mL stock solution with 0.9 mL distilled water. The 500 µg/mL solution was prepared by mixing 0.1 mL of the 1000 µg/mL solution with 0.1 mL water, and the 200 µg/mL solution was prepared by mixing 0.1 mL of the 1000 µg/mL solution with 0.4 mL water. The final DMSO concentrations in these solutions did not exceed 1% (v/v).

Mite collection

P. cuniculi mites were collected from auricular lesions of six naturally infested New Zealand White rabbits. Three rabbits had lesions predominantly involving the external surface of the ear, whereas three had lesions involving the internal surface of the pinna. The collected material was transferred to sterile glass Petri dishes and incubated at 35 °C for 30 min. Active adult mites were selected under a stereomicroscope using a fine entomological needle. After sample collection, the rabbits were treated with ivermectin. The procedures were conducted in accordance with the regulations of the Gansu Provincial Experimental Animal Centre and the applicable national requirements in China.

In vitro acaricidal assay

Filter paper was placed in 60-mm Petri dishes and treated with 30 µL of the corresponding working solution. Twenty active adult mites were transferred to each dish. Each treatment was tested in five independent replicates, corresponding to 100 mites per treatment condition. Permethrin, a synthetic pyrethroid acaricide manufactured in China, was used as the positive control at 1, 2.5, 5, 10, and 20 µL/mL. A 1% (v/v) aqueous DMSO solution without plant extract, representing the maximum vehicle concentration used in the assay, served as the negative control.

The dishes were sealed and incubated at 25 °C and 75% relative humidity. Mortality was assessed 24 hours after exposure. Mites were considered dead when no movement was observed, including after mechanical stimulation. The experiment included 42 plant-extract treatment conditions (seven plants × two extraction methods × three concentrations), five permethrin conditions, and one negative-control condition. Overall, 4800 mites were examined: 4200 in the plant-extract groups, 500 in the permethrin groups, and 100 in the DMSO control group.

Statistical analysis

Analyses were performed using R version 4.4.2. Mortality was calculated separately for each Petri dish and expressed as mean percentage ± standard deviation (SD) across five independent replicates. Overall mortality associated with aqueous and ethanolic extraction was compared using the paired

Wilcoxon signed-rank test, with plant species and concentration treated as matched conditions. The effect of concentration was evaluated using the Friedman test, with plant species–extraction method combinations treated as blocks. Differences among plant species were evaluated using the Kruskal–Wallis test. Where pairwise comparisons were performed, p-values were adjusted using the Holm method. Statistical significance was defined as $p < 0.05$. Because mortality in the negative control was 7%, the table reports observed, uncorrected mortality values [10, 11, 12].

Results

Mortality varied substantially among plant species, extraction methods, and concentrations (Figure 1). Across all plant–concentration combinations, ethanolic extracts produced a mean mortality of 27.43%, compared with 13.67% for aqueous extracts. The overall difference between extraction methods was statistically significant (paired Wilcoxon signed-rank test, $p = 0.0033$).

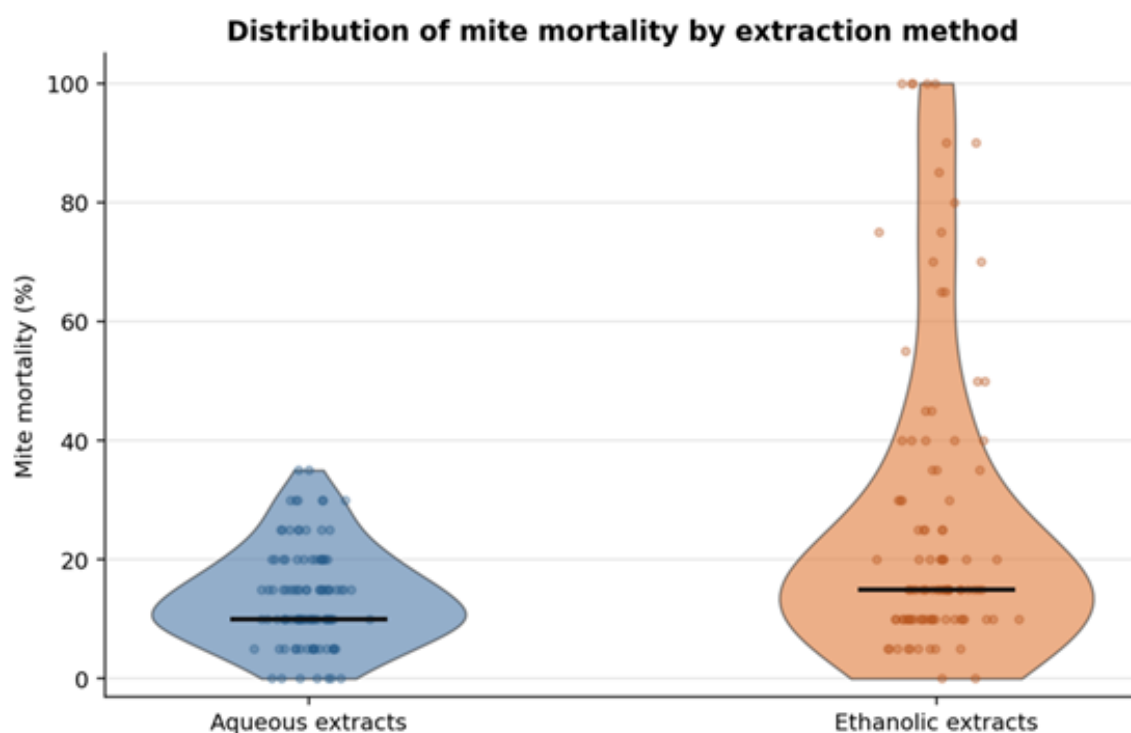


Figure 1. Distribution of *P. cuniculi* mortality following exposure to aqueous and ethanolic plant extracts. Points represent replicate-level mortality values across the seven plants and three concentrations; horizontal lines indicate medians

Concentration had a significant overall effect on mortality (Friedman $\chi^2 = 19.44$, $p < 0.001$), and acaricidal activity differed significantly among plant species (Kruskal–Wallis $H = 22.76$, $p < 0.001$). Concentration-dependent responses for each plant and extraction method are shown in Figure 2.

Concentration-dependent acaricidal activity of plant extracts

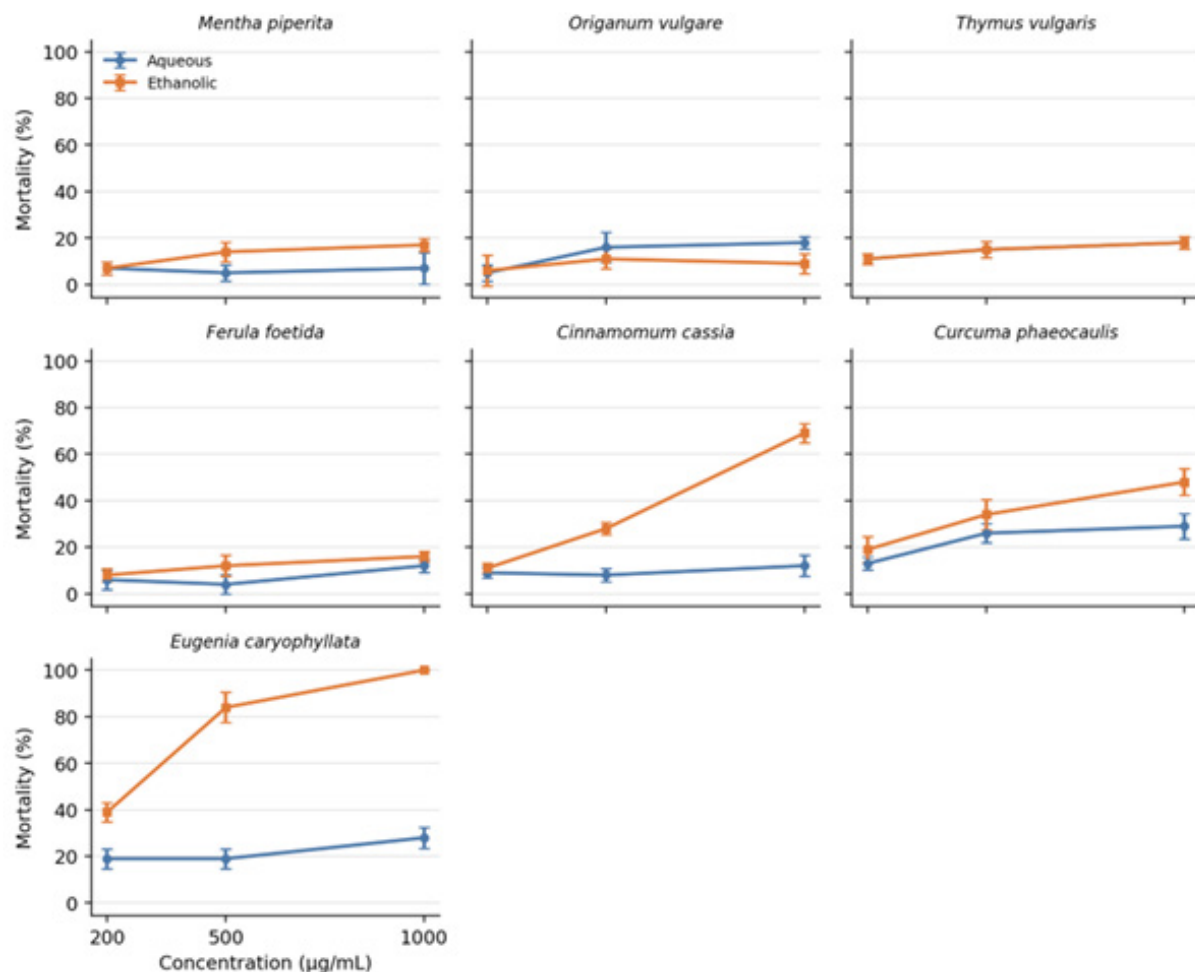


Figure 2. Concentration-dependent acaricidal activity of aqueous and ethanolic plant extracts against *P. cuniculi*. Values are means $\pm$ SD from five independent replicates, with 20 mites per replicate

The highest mortality was produced by the ethanolic extract of *Eugenia caryophyllata* at 1000 µg/mL ($100.00 \pm 0.00\%$), whereas the corresponding aqueous extract produced $28.00 \pm 4.47\%$ mortality. At the same concentration, the ethanolic extract of *Cinnamomum cassia* produced $69.00 \pm 4.18\%$ mortality, compared with $12.00 \pm 4.47\%$ for its aqueous extract. *Curcuma phaeocaulis* showed a consistent concentration-related increase, reaching $48.00 \pm 5.70\%$ mortality with the ethanolic extract and $29.00 \pm 5.48\%$ with the aqueous extract at 1000 µg/mL.

Table 1. Mortality of *P. cuniculi* following exposure to plant extracts and control treatments

Treatment	Concentration	Aqueous extract, mortality (%) $\pm$ SD	Ethanolic extract, mortality (%) $\pm$ SD
Control treatments			
1% DMSO	1% (v/v)	7.00 ± 4.47	
Permethrin	1 µL/mL	25.00 ± 3.54	
Permethrin	2.5 µL/mL	42.00 ± 5.70	
Permethrin	5 µL/mL	52.00 ± 8.37	
Permethrin	10 µL/mL	53.00 ± 25.88	
Permethrin	20 µL/mL	64.00 ± 10.84	

Continuation of Table 1

Plant extracts			
<i>Mentha piperita</i>	200 µg/mL	7.00 ± 2.74	7.00 ± 2.74
<i>Mentha piperita</i>	500 µg/mL	5.00 ± 3.54	14.00 ± 4.18
<i>Mentha piperita</i>	1000 µg/mL	7.00 ± 6.71	17.00 ± 2.74
<i>Origanum vulgare</i>	200 µg/mL	5.00 ± 3.54	6.00 ± 6.52
<i>Origanum vulgare</i>	500 µg/mL	16.00 ± 6.52	11.00 ± 4.18
<i>Origanum vulgare</i>	1000 µg/mL	18.00 ± 2.74	9.00 ± 4.18
<i>Thymus vulgaris</i>	200 µg/mL	11.00 ± 2.24	11.00 ± 2.24
<i>Thymus vulgaris</i>	500 µg/mL	15.00 ± 3.54	15.00 ± 3.54
<i>Thymus vulgaris</i>	1000 µg/mL	18.00 ± 2.74	18.00 ± 2.74
<i>Ferula foetida</i>	200 µg/mL	6.00 ± 4.18	8.00 ± 2.74
<i>Ferula foetida</i>	500 µg/mL	4.00 ± 4.18	12.00 ± 4.47
<i>Ferula foetida</i>	1000 µg/mL	12.00 ± 2.74	16.00 ± 2.24
<i>Cinnamomum cassia</i>	200 µg/mL	9.00 ± 2.24	11.00 ± 2.24
<i>Cinnamomum cassia</i>	500 µg/mL	8.00 ± 2.74	28.00 ± 2.74
<i>Cinnamomum cassia</i>	1000 µg/mL	12.00 ± 4.47	69.00 ± 4.18
<i>Curcuma phaeocaulis</i>	200 µg/mL	13.00 ± 2.74	19.00 ± 5.48
<i>Curcuma phaeocaulis</i>	500 µg/mL	26.00 ± 4.18	34.00 ± 6.52
<i>Curcuma phaeocaulis</i>	1000 µg/mL	29.00 ± 5.48	48.00 ± 5.70
<i>Eugenia caryophyllata</i>	200 µg/mL	19.00 ± 4.18	39.00 ± 4.18
<i>Eugenia caryophyllata</i>	500 µg/mL	19.00 ± 4.18	84.00 ± 6.52
<i>Eugenia caryophyllata</i>	1000 µg/mL	28.00 ± 4.47	100.00 ± 0.00

Values are presented as mean mortality (%) ± standard deviation (SD) from five independent replicates, with 20 mites per replicate. Observed, uncorrected mortality values are shown. Plant-extract concentrations are expressed in µg/mL; permethrin concentrations are expressed in µL/mL

Mortality remained comparatively low for *Mentha piperita*, *Thymus vulgaris*, *Ferula foetida*, and *Origanum vulgare*. At 1000 µg/mL, ethanolic-extract mortality was 17.00 ± 2.74% for *M. piperita*, 18.00 ± 2.74% for *T. vulgaris*, 16.00 ± 2.24% for *F. foetida*, and 9.00 ± 4.18% for *O. vulgare*. For *T. vulgaris*, aqueous and ethanolic extracts produced identical mean mortality at all three concentrations. *O. vulgare* showed a different pattern, with higher mortality for the aqueous extract at 500 and 1000 µg/mL.

The negative control produced 7.00 ± 4.47% mortality. Permethrin mortality increased from 25.00 ± 3.54% at 1 µL/mL to 64.00 ± 10.84% at 20 µL/mL. Because plant extracts were expressed as mass concentrations and permethrin as volume concentrations, dose-matched inferential comparisons between these agents were not performed.

Discussion

The results demonstrate that in vitro acaricidal activity against *P. cuniculi* depended on plant species, extraction method, and concentration. Overall, ethanolic extracts produced significantly higher mortality than aqueous extracts (27.43% vs. 13.67%; $p = 0.0033$), although the magnitude and direction of the extraction-method difference varied among plants.

Ethanol can extract a broader range of moderately polar and less polar constituents than water, whereas aqueous extracts are enriched in highly polar compounds. These differences in phytochemical composition may explain the higher overall activity of the ethanolic preparations. However, the present assay did not quantify individual constituents, and the contribution of specific compounds cannot be established from the mortality data alone [13].

The strongest response was observed for the ethanolic extract of *Eugenia caryophyllata*, which produced complete mortality at 1000 µg/mL. Ethanolic extracts of *Cinnamomum cassia* and *Curcuma phaeocaulis* also showed marked concentration-related activity. By contrast, *Mentha piperita*, *Origanum vulgare*, *Thymus vulgaris*, and *Ferula foetida* showed low or modest activity under the present conditions.

Eugenia caryophyllata flower buds are rich in eugenol, and clove essential oil has previously shown dose- and time-dependent activity against *P. cuniculi* [14, 15]. These findings support the biological plausibility of the high activity observed here. Nevertheless, an essential oil, a crude ethanolic extract, and purified eugenol are chemically distinct preparations; direct quantitative comparison among them should therefore be made cautiously.

Cinnamomum cassia bark contains cinnamaldehyde-rich volatile fractions, and both extracts and essential oils of *C. cassia* have shown acaricidal activity against *Haemaphysalis longicornis* [16, 17, 18]. The present results extend this evidence to a crude ethanolic extract tested against *P. cuniculi*, but differences in target species, extraction procedures, chemical composition, and exposure conditions preclude direct comparison of potency.

The *Curcuma* species examined in the present study was *Curcuma phaeocaulis*, whereas much of the published acaricidal literature concerns *Curcuma longa* [19–21]. These are distinct species and should not be considered interchangeable. Evidence for *C. longa* is therefore cited only to indicate the broader acaricidal potential of *Curcuma*-derived constituents. Species-specific phytochemical characterization of *C. phaeocaulis* is required before its active components and mechanisms can be defined.

Conclusions

Under the conditions of this in vitro assay, the ethanolic extracts of *Eugenia caryophyllata*, *Cinnamomum cassia*, and *Curcuma phaeocaulis* showed the greatest acaricidal potential against *P. cuniculi*. The ethanolic extract of *E. caryophyllata* produced complete mortality at 1000 µg/mL. These findings identify promising candidates for further investigation but do not establish therapeutic efficacy or safety in animals. Future studies should characterize the active constituents, assess repeatability and toxicological safety, and evaluate efficacy in controlled in vivo trials.

Authors' Contributions

BO: conceived and designed the study, defined the aims and objectives, provided scientific supervision, coordinated the research process, and approved the final version of the manuscript. GI, AD: performed the literature review and analysis, developed the research methodology, participated in the preparation of plant material and extraction procedures, drafted the initial version of the manuscript, prepared the “Materials and Methods” and “Results” sections, and prepared the manuscript for submission.

ED: performed the statistical analysis of the data, analysed and interpreted the results, and prepared the tables and figures. DKh: critically revised the manuscript, contributed to the discussion of the results, verified the scientific content, performed linguistic and scientific editing, and approved the final version of the article. XSh: conducted the in vitro acaricidal activity experiments, prepared the biological material, and recorded the experimental data. BV: collected the medicinal plant raw material, performed extraction and fractionation of the plant extracts, and supervised the laboratory procedures.

All authors made a substantial contribution to the study, participated in the discussion of the results, read and approved the final version of the manuscript, and agreed to be accountable for the accuracy and integrity of the data presented.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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