The Journal of Veterinary Medical Science

FULL PAPER

Parasitology

# Acaricidal activity of *Erigeron acer* L. root against *Haemaphysalis longicornis* and phytochemical profiling by liquid chromatography-tandem mass spectrometry

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*J Vet Med Sci*
86(8): 897–905, 2024
doi: 10.1292/jvms.24-0090

Received: 4 March 2024
Accepted: 19 June 2024
Advanced Epub:
3 July 2024

**ABSTRACT.** The present study is focused on evaluating acaricidal activity and chemical compositions of *Erigeron acer* root, which was identified as a promising candidate among fifteen Mongolian plant extracts tested for acaricidal activity. The acaricidal effect was evaluated against *Haemaphysalis longicornis*, assessed for toxicity to normal human skin fibroblast, and analyzed for its chemical constituents. The acetone extract of *E. acer* root showed significant activity against *H. longicornis*, with a lethal concentration ($LC_{50}$) of 5.31 mg/mL and low toxicity, evidenced by a cytotoxic concentration ($CC_{50}$) of 267.00 µg/mL. Using liquid chromatography-tandem mass spectrometry and molecular networking, thirteen natural compounds were identified, including pyrrolidines, alkaloids, fatty acids, and flavonoids, highlighting the efficacy of *E. acer* root extract as an effective acaricide against *H. longicornis* and offering insights for developing new tick control solutions.

**KEYWORDS:** acaricide, *Erigeron acer* root, *Haemaphysalis longicornis*, liquid chromatography-tandem mass spectrometry, molecular networking

Ticks transmit a variety of important disease, contributing to an escalating public health concern due to their potential to cause severe illness and fatalities in both humans and animals. Additionally, the geographic distribution of tick-borne diseases is expanding, and reported human cases are increasing [32]. Consequently, tick-borne diseases can lead to significant economic losses, impact human and animal health, and directly affect the livestock industry by reducing milk, meat, and leather production, causing economic setbacks [12]. One significant tick species contributing this threat is *Haemaphysalis longicornis*, the Asian long-horned tick. Primarily distributed in East Asia including China, Japan, Korea, and Australia [8, 12], has also been reported in New Zealand, New Caledonia, Fiji, and Tonga, [8]. This tick species is known to transmit several significant disease, including piroplasmosis [8, 12], Q fever, rickettsia infections, Russian spring-summer encephalitis [8], severe fever with thrombocytopenia syndrome virus (SFTSV) [20], and anaplasmosis [15].

Effective management of tick species is crucial for preventing the spread of diseases and reducing the economic burden on public health systems, as well as on the livestock industry. Commercial synthetic acaricides including pyrethroids, deltamethrin, and ivermectin are the most widely employed methods for controlling and preventing tick and tick-borne diseases. However, the extensive and ongoing use of these commercial acaricides has led to the development of acaricide resistance [13]. Furthermore, synthetic acaricides, such as deltamethrin, contaminate the environment due to their extended degradation periods and can pose a threat to mammals, including humans, due to their toxicity [22].

Medicinal plants offer a vast source of highly effective ingredients for tick control. Extracts and essential oils derived from various plant species have shown promising results against various tick species, as documented in publications [2]. For example, Giglioti *et al.* conducted a comprehensive study to explore the acaricidal activity of neem seed extract (*Azadirachta indica*) against the cattle tick *Rhipicephalus microplus* [14]. Based on the results, the engorgement rates were significantly reduced, underscoring the potential of neem extract as a natural tick control method [14]. Compared to synthetic acaricides, plant-based acaricides may cause a lower

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(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

risk to human and animal health and tend to degrade more rapidly in the environment due to their biodegradability and lower toxicity to non-target organisms. Furthermore, their complex mode of action may contribute to a reduced risk of tick resistance [31]. Hence, discovering novel, selective, beneficial, and eco-friendly methods for controlling ticks is of utmost importance.

The genus *Erigeron*, which belongs to the Asteraceae family, is widely distributed in various regions, including Asia, North America, and Europe. Nine species of *Erigeron* are found in Mongolia [11]. *Erigeron acer* L. (*E. acris*), commonly referred to as blue fleabane, holds a prominent place in traditional medicine. This plant has a long history of traditional use in diverse cultures. In traditional Italian medicine, *E. acris* roots are utilized to treat toothaches, arthritis, bruises, digestive issues, and enteritis. In traditional Chinese medicine, the plant is employed to treat conditions such as indigestion, enteritis, epidemic hepatitis, and hematuria [28, 35].

The primary objective of the current study was to examine the acaricidal activity of Mongolian plants. Among these plants, *E. acer* root exhibited significantly higher acaricidal activity. Therefore, we conducted a detailed analysis of its chemical constituents with aim of identifying efficient natural products capable of controlling tick vectors. Furthermore, by concentrating on the acaricidal potential of *E. acer* root, our goal was to contribute to the development of sustainable solutions in the field of tick vector control.

## MATERIALS AND METHODS

### *Plant material*

The plant samples were collected in Mongolia between 2006 and 2013. Plant data, such as the collected date and place for each sample, are provided in Supplementary Table 1. The plant species were identified by Prof. Ch. Sanchir and Prof. Ts. Jamsran at the Institute of Botany, Mongolian Academy of Sciences, Mongolia. Voucher specimens (102.10.9.08A) were deposited in the herbarium of the National University of Mongolia for future reference and verification.

### *Crude extract*

The plant samples were air-dried in a shaded area to protect them from direct sunlight. The dried samples were finely cut into small pieces after the air-drying process. The cut material was subjected to extraction by maceration in 80% acetone (acetone/water ratio of 4:1, v/v) at room temperature for 5 days. Subsequently, the liquid extracts were filtered and concentrated under reduced pressure at 40°C utilizing a rotary vacuum evaporator. The crude extracts were stored at 4°C until further use.

### *Ticks*

Parthenogenetic *H. longicornis* (Okayama strain) ticks were maintained at the National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan. These ticks were sustained by feeding on Japanese white rabbits (SLC Japan, Hamamatsu, Japan). As previously described, the ticks were reared at 25°C in a dark environment [19]. The rabbits were housed in a temperature- and humidity-controlled room according to guidelines approved by the Obihiro University of Agriculture and Veterinary Medicine Animal Care and Use Committee (approval number: 19-74).

### *Acaricidal activity of plant extracts against female ticks*

The acaricidal activity of the crude extract was examined according to a previously described method [5]. First, each crude plant extract was suspended in 80% ethanol containing 2% DMSO at a 50 mg/mL concentration. Next, 150 µL of the extracts were sprayed onto Petri dishes (diameter of 30 mm) padded with filter paper (Whatman No. 1, Whatman Inc., Maidstone, UK). Then, the Petri dishes were air-dried at room temperature for 72 hr to evaporate the solvent completely. Next, the ticks ($n$=10 × 2 replication) were placed into the Petri dishes and kept in desiccators under an optimum temperature of 27 ± 1°C and 80–90% humidity for 72 hr. The mortality of ticks was recorded after 24 and 72 hr, and immovable ticks were recorded as dead. This method was verified using cypermethrin (LKT laboratories, St. Paul, MN, USA) and the test solution (80% ethanol containing 2% DMSO) as positive and negative controls, respectively [29]. Cypermethrin exhibited 100% mortality at a concentration of 1 mg/mL. Mortality concentrations were calculated as follows [9]:

$$\text{Corrected mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

Furthermore, to assess the acaricidal activity of the crude extract of *E. acer*, serial dilutions were prepared at concentrations of 2.5, 5.0, 10, 15, and 25 mg/mL. Concentration-dependent mortality assays were conducted to determine the lethal concentrations for 50% mortality ($LC_{50}$) and 90% mortality ($LC_{90}$) of *E. acer* root. Nonlinear regression analysis was performed using GraphPad Prism 8 software to calculate the $LC_{50}$ and $LC_{90}$ values.

### *Liquid chromatography-tandem mass spectrometry analysis*

The crude extract was dissolved in water:methanol (25:75) at a concentration of 1.0 mg/mL for LC–MS analysis. Samples were diluted with water to obtain a final concentration of 0.1 mg/L. Analyses were performed on a Q Exactive high-resolution mass spectrometer connected to an Ultimate 3000 RSLC high-performance liquid chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an InertSustain AQ-C18 (2.1 × 150 mm; 3 µm-particle, GL Science, Tokyo, Japan). Elution was conducted with a mobile phase consisting of $H_2O$ + 0.1% FA (A) and ACN (B), which were pumped at a rate of 0.2 mL/min. The gradient program was set as follows: 2% B (0–3 min), 2–98% B (3–30 min), 98% B (30–35 min), 98–2% B (35–35.1 min), and 2% B (35.1–40 min). The column oven was set at 40°C, and the injection volume was 2 µL. LC–MS/MS analyses were achieved by coupling the LC system to an Orbitrap MS (Q Exactive™, Thermo Fisher Scientific). The relative content ratio of each main peak was determined by identifying the peaks corresponding to the metabolites of interest, extracting the peak intensities for each metabolite, and normalizing

the peak intensities by dividing each value by the total sum of all peak intensities (316883033.26). The values were expressed as percentages. The detected compounds were annotated and identified using several databases: PubChem (https://pubchem.ncbi.nlm.nih.gov/compound), the UC2 database (which includes KNApSAcK at http://kanaya.naist.jp/KNApSAcK/), the Human Metabolome Database (http://www.hmdb.ca), and Dictionary Natural Products (https://dnp.chemnetbase.com/).

### *Molecular networking*

The raw data obtained from the LC–MS/MS system were converted to mzXML format using the ProteoWizard tool (Vanderbilt University, Nashville, TN, USA) [17]. The MZmine workflow for feature-based molecular networking on Global Natural Product Social (GNPS) was utilized. A molecular network was created using the online workflow [34] on the GNPS website (http://gnps.ucsd.edu). First, the data were filtered by removing all MS/MS fragment ions within ± 17 Da of the precursor m/z. Next, MS/MS spectra were window-filtered by choosing the top 6 fragment ions in the ± 50 Da window throughout the spectrum. The precursor ion mass tolerance was set to 0.02 Da, and the MS/MS fragment ion tolerance was 0.02 Da. The MS/MS fragment ion tolerance was set to 0.5 Da, and the precursor ion mass tolerance was set to 2.0 Da. A network was created in which the edges were filtered to have a cosine score above 0.7 and more than 6 matched peaks. Furthermore, edges between two nodes were only left in the network if each node appeared in each other's respective top 10 most similar nodes. Finally, the maximum size of a molecular family was set to 100, and the lowest-scoring edges were removed from molecular families until the molecular family size was below this threshold. The spectra in the network were then searched against GNPS spectral libraries. The library spectra were filtered in the same manner as the input data. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 6 matched peaks. The resulting molecular network is available at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=e3431d1161a84396a15499c0e86c6ff8.

Data output was imported into Cytoscape version 3.9.1 for visualization and further analysis (https://cytoscape.org/) [33].

### *Cytotoxic activity tests*

The cytotoxic activity test was conducted to evaluate the potential cytotoxic effect of *E. acer* root. Normal human skin fibroblast (NB1RGB, RCB0222, RIKEN BRC, Ibaraki, Japan) were cultured in Dulbecco's modified Eagle's medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and 1% penicillin. The cells were maintained at 37°C under 5% $CO_2$.

The experiments were carried out according to previously described methods [6, 7]. NB1RGB were seeded in 96-well plates at a density of $1 \times 10^4$ cells/well and grown for 48 hr at 37°C under 5% $CO_2$. After incubation, various concentrations (25–500 µg/mL) of the test extracts were added to the wells. Then, the treated cells were incubated with the extracts for 72 hr. After 72 hr, cell counting kit-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added and the plate was further incubated for 3 hr, the resulting water-soluble formazan dye intensity was then measured at 450 nm using an MTP-120 microplate reader (Corona Electric, Hitachinaka, Japan). DMSO-treated cells as a diluent of extracts served as negative controls and the wells containing medium alone were used to correct for background signals. The test was performed in triplicate and repeated two times.

To determine the 50% and 90% cytotoxic concentration ($CC_{50}$, $CC_{90}$) values, extracts were diluted by a serial dilution ranging from 50 to 500 µg/mL. The $CC_{50}$ and $CC_{90}$ were calculated using nonlinear regression with GraphPad Prism 8 software.

## RESULTS

### *Acaricidal activity*

During the prescreening phase, in which we evaluated the acaricidal activity of fifteen different plants, *E. acer* root was the most promising candidate, demonstrating the highest mortality percentage of 100% at a concentration of 50 mg/mL. The next best candidates were *Euphrasia hirtella* and *Schultzia crinita*, which achieved mortality rates of 80% each (Table 1).

To further assess the acaricidal potency of *E. acer* root against *H. longicornis* female ticks, we conducted experiments using various concentrations of the plant extract, as outlined in Supplementary Table 2. The findings revealed that *E. acer* root exhibited notable acaricidal activity, with an $LC_{50}$ value of 5.31 mg/mL and an $LC_{90}$ value of 5.64 mg/mL against female ticks. In contrast, the negative control, represented by the test solution, did not induce any mortality among the ticks. These results indicate that *H. longicornis* was susceptible to the acetone extract of *E. acer* root and support the potential of *E. acer* root as an effective acaricide against this tick species.

**Table 1.** Acaricidal activity of the screened plants at 50 mg/mL

| No. | Plant name | Part | Mortality, % |
|---|---|---|---|
| 1 | *Amaranthus retroflexus* | Root | 20 |
| 2 | *Arctogeron gramineum* | Aerial part | 20 |
| 3 | *Axyris amaranthoides* | Leaf | 60 |
| 4 | *Erigeron acer* | Root | 100 |
| 5 | *Euphrasia hirtella* | Leaf | 80 |
| 6 | *Hedysarum alpinum* | Stem | 20 |
| 7 | *Lagochilus ilicifolius* | Whole part | 0 |
| 8 | *Leontopodium leontopodioides* | Fur + seed | 20 |
| 9 | *Oxytropis trichophyla* | Aerial part | 20 |
| 10 | *Peucedonum baicalence* | Leaf | 40 |
| 11 | *Potentilla astragalifolia* | Root | 40 |
| 12 | *Salsola passerine* | Whole part | 0 |
| 13 | *Saxifraga hirculus* | Aerial part | 0 |
| 14 | *Schultzia crinita* | Whole part | 80 |
| 15 | *Spirea salicifolia* | Whole part | 40 |

### *Cytotoxic effect*

To examine the cytotoxicity of the crude extract, a cell viability assay against human foreskin fibroblasts was performed with the crude extract at different concentrations (final concentrations 25–500 µg/mL), and the $CC_{50}$ and $CC_{90}$ were determined. As a result, the crude extract of *E. acer* root showed relatively low toxicity, with a $CC_{50}$ of 267.00 µg/mL and a $CC_{90}$ of 595.80 µg/mL.

*Metabolite profiling of E. acer determined by liquid chromatography-tandem mass spectrometry*

To gain comprehensive knowledge of the chemical composition of the crude extract of *E. acer*, we performed LC–MS/MS analysis using the data-dependent acquisition technique in positive ionization mode. The obtained total ion chromatogram (TIC) of the crude extract is depicted in Fig. 1, providing an overview of the detected compounds. Subsequently, an autoMS$^2$ process was conducted, selectively targeting the most prevalent MS$^1$ ions for fragmentation during MS$^2$ analysis. Finally, by manually examining the resulting MS/MS spectra, we putatively identified the chemical components present in the crude extract of *E. acer*. A detailed list of these identifications is provided in Table 2, suggesting insights into the potential constituents of the extract.

Furthermore, to facilitate the annotation and identification of the detected compounds, we utilized several databases, including PubChem, UC2 database (which includes KNApSAcK), the Human Metabolome Database, and Dictionary Natural Products. Through these comprehensive resources, we assigned potential identities to the compounds detected in the crude extract of *E. acer*.

Through this process, 17 substances were detected, and upon further analysis, we identified 13 compounds, as presented in Table 2. These identified metabolites belong to nine distinct families of natural compounds, namely, pyrrolidines, benzofurans, cyclic ketones, fatty acids, phenylpropanoids, and quinazolines. Through utilizing these databases and performing manual examination and analysis, we could gain insights into the chemical diversity and composition of the crude extract of *E. acer*.

*Tandem mass spectrometry-molecular networking-based dereplication*

In addition to the manual examination, we employed the molecular networking (MN) approach that utilizes the GNPS website (http://gnps.ucsd.edu) to analyze the crude extract of *E. acer*. Through this advanced technique, we could explore the intricate relationships between the precursor ions present in the extract. We identified 303 nodes representing these precursor ions through molecular network analysis, as depicted in Fig. 2. These nodes formed 18 clusters (nodes ≥2) in the network, with six main clusters emerging as the most prominent groups.

The compounds within the molecular network were classified based on their structural similarity, providing valuable insights into the chemical diversity of the crude extract. The six main clusters, designated Clusters A, B, C, D, E, and F, were determined by examining the relative contributions of peak regions at the network nodes. Cluster A predominantly consisted of terpenoids, while Cluster B contained phenylpropanoids. Cluster C was characterized by the presence of DEHP (bis (2-ethylhexyl) phthalate), while Cluster D featured a combination of phenylpropanoids and terpenoids. Cluster E contained small peptides, and Cluster F was enriched with fatty acids.

For a more detailed analysis of these clusters and their constituent compounds, please refer to Supplementary Fig. 2 (Cluster A) and Supplementary Fig. 3 (Clusters C, D, E, and F). These Supplementary Figures provide comprehensive information regarding the annotation of compounds within each cluster. In total, we identified an additional 60 compounds through the GNPS database, expanding our knowledge of the chemical composition and diversity of the crude extract of *E. acer* (Table 3).

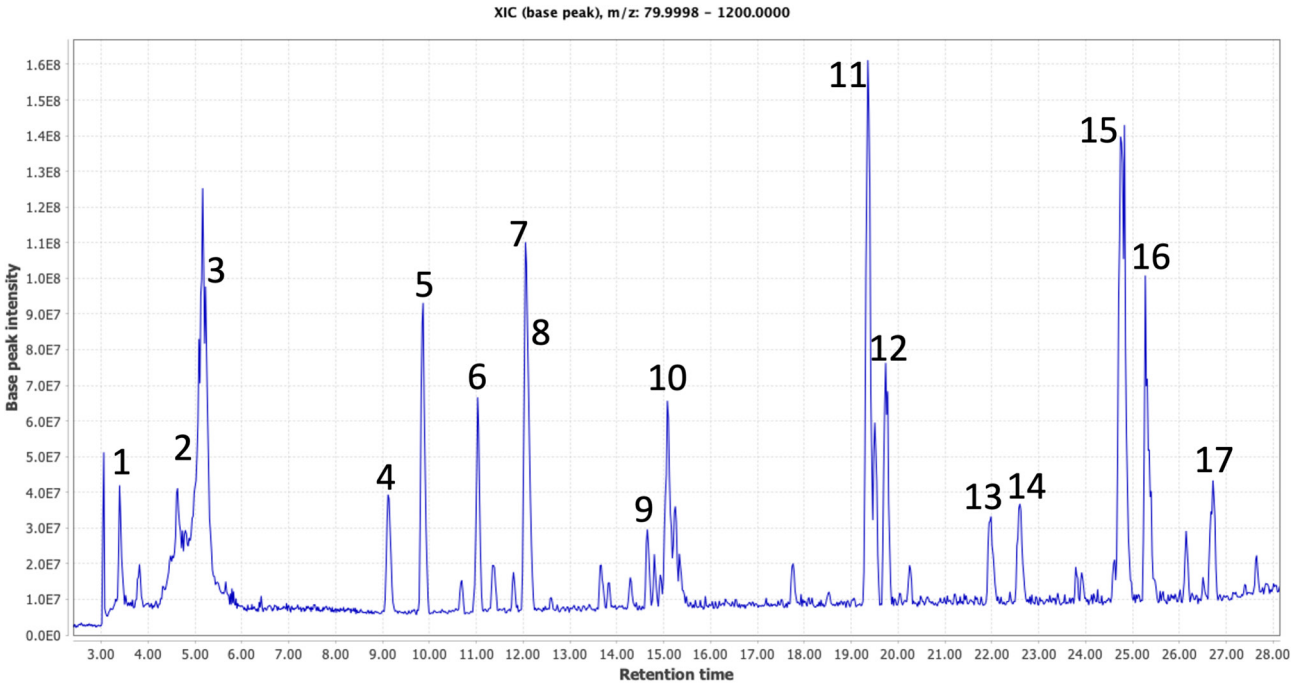


**Fig. 1.** Total ion chromatogram in positive ion mode for acetone extract of *Erigeron acer* obtained from an Orbitrap mass spectroscopy (Q Exactive, scan range m/z 80-1,200). The indicated number corresponds to the peak numbers in Table 2.

## DISCUSSION

The utilization of plant-derived natural compounds and essential oils has gained significant attention as a promising approach for the development of acaricides, as these compounds exhibit inherent advantages in tick control due to their relative safety and efficacy. Natural products derived from plants offer a sustainable and environmentally friendly alternative to synthetic acaricides, addressing concerns related to environmental contamination and the potential risks posed to human and animal health. By harnessing this advantage, these plant-derived acaricides provide a compelling solution that aligns with the principles of sustainable agriculture and integrated pest management. Based on the growing interest in exploring and harnessing the potential of natural products, further research is needed to determine their mechanisms of action, optimize their effectiveness, and safely integrate them into tick management strategies. By prioritizing the development and utilization of natural products, we can pave the way for safer and more sustainable approaches to tick control, minimize the environmental impact, and promote the well-being of both ecosystems and human populations.

In our study, we comprehensively evaluated the acaricidal properties of *E. acer* root among fifteen Mongolian plants, focusing on its activity against *H. longicornis*. The results were highly encouraging, as *E. acer* root demonstrated excellent acaricidal properties against *H. longicornis* (Table 1). The mortality of *H. longicornis* female ticks reached 90% after 24 hr of treatment at 10 mg/mL, while after 72 hr of treatment, mortality reached 100%. Moreover, tick mortality was not observed in the repellent assay with the negative control, highlighting that *E. acer* root shows potential as a natural acaricide against *H. longicornis*. Similarly, in a previous report, *E. acer* root showed moderate acaricidal activity against *Dermacentor nuttalli* [5]. Based on these results, *E. acer* root may exhibit acaricidal properties against various tick species. However, additional evidence is required.

In addition to the promising results observed with *E. acer* root, other *Erigeron* species have demonstrated insecticidal activities against various pests and organisms. For instance, *E. canadensis* has shown activities against *Aedes aegypti* [1], *Culex quinquefasciatus* [30], *Tribolium castaneum*, and *Aspergillus flavus* [4], while *E. annuus* has shown larvicidal activity against *Aedes albopictus* [18]. In addition, *E. speciosus* has shown molluscicidal activity against *Planobdella trivolvis* [23]. These findings suggest that the genus *Erigeron* exhibits potential as a valuable source of bioactive compounds for pest control.

The acaricidal activity of *E. acer* root can be partially attributed to its diverse chemical constituents that exhibit different modes of action. While previous studies have extensively described the constituents found in the aerial parts of *E. acer* [24–26, 35, 36], limited information is available regarding the constituents of the root. In a study by Nazaruk *et al.* [28], the essential oils of the root was extracted by hydrodistilation method and revealed the presence of 54 compounds with polyacetylene esters as the dominant components [27]. In the present study, we utilized maceration method to obtain the acetone extract of the root, and identified 13 and 60 compounds using two different analytical approaches. The observed differences in constituents between our study and Nazaruk *et al.* [28] may attributed by several factors. Most importantly, different extraction methods were used, along with potential influences from genetic diversity, geographical location, and the developmental stage of the plant. Additionally, our findings indicated that certain compounds, apigenin, kaempferol, luteolin, quercetin, and chlorogenic, were present in both the aerial part and root of *E. acer.* These compounds have been previously reported in aerial parts of the plant [24, 26]. To enhance potential therapeutic application of *E. acer* roots, it is important to identify the secondary metabolites present within them. This identification can facilitate their utilization in various contexts.

**Table 2.** Identification of compounds from crude acetone extract of *Erigeron acer* by liquid chromatography-tandem mass spectrometry in positive ion mode

| Peak Number | RT (min) | Relative contents ratio (%) | *m/z* detected | Exact mass | Molecular formula | Adduct | Compound identification | Compound class |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.42 | 0.64 | 152.12 | 151.11 | Unknown | $[M+H]^+$ | Unknown | Unknown |
| 2 | 5.18 | 1.18 | 275.08 | 274.07 | $C_{11}H_{14}O_8$ | $[M+H]^+$ | Unknown | Unknown |
| 3 | 5.18 | 3.72 | 113.02 | 274.07 | $C_6H_{11}O_3N_8P$ | $[M-(Hexose-H_2O)+H]^+$ | Unknown | Unknown |
| 4 | 9.14 | 1.08 | 120.08 | 119.07 | Unknown | $[M+H]^+$ | Unknown | Unknown |
| 5 | 9.86 | 0.31 | 185.129 | 184.121 | $C_9H_{16}O_2N_2$ | $[M+H]^+$ | *N* (3-acetamidopropyl) pyrrolidin-2-one | Pyrrolidine |
| 6 | 11.04 | 1.32 | 188.07 | 204.09 | $C_{11}H_{12}O_2N_2$ | $[M-NH_3+H]^+$ | Vasicinol | Quinazoline |
| 7 | 12.06 | 2.53 | 163.04 | 162.03 | $C_9H_6O_3$ | $[M+H]^+$ | Umbelliferone | Phenylpropanoid |
| 8 | 12.06 | 1.02 | 193.05 | 354.10 | $C_{16}H_{18}O_9$ | $[M-(Hexose-H_2O)+H]^+$ | 4-caffeoylquinic acid | Phenylpropanoid |
| 9 | 14.71 | 0.69 | 189.09 | 188.08 | $C_{12}H_{12}O_2$ | $[M+H]^+$ | Trigoforin | Phenylpropanoid |
| 10 | 15.09 | 2.02 | 499.12 | 516.13 | $C_{25}H_{24}O_{12}$ | $[M-H_2O+H]^+$ | 3,4-di-*O*-caffeoylquinic acid | Phenylpropanoid |
| 11 | 19.36 | 4.58 | 203.11 | 202.10 | $C_{13}H_{14}O_2$ | $[M+H]^+$ | Tremetone | Benzofuran |
| 12 | 19.53 | 1.56 | 205.12 | 204.12 | $C_{13}H_{16}O_2$ | $[M+H]^+$ | Norpinguisone | Benzofuran |
| 13 | 22.02 | 1.30 | 161.06 | 160.05 | $C_{10}H_8O_2$ | $[M+H]^+$ | 6-methylcoumarin | Phenylpropanoid |
| 14 | 22.64 | 1.40 | 163.08 | 162.07 | $C_{10}H_{10}O_2$ | $[M+H]^+$ | β-dolabrin | Cyclic ketone |
| 15 | 24.72 | 5.66 | 175.08 | 174.07 | $C_{11}H_{10}O_2$ | $[M+H]^+$ | Wutaifuranol | Benzofuran |
| 16 | 25.39 | 2.84 | 177.09 | 176.08 | $C_{11}H_{12}O_2$ | $[M+H]^+$ | 2,2-dimethyl-2H-chromen-5-ol | Phenylpropanoid |
| 17 | 26.75 | 1.34 | 279.23 | 278.23 | $C_{18}H_{30}O_2$ | $[M+H]^+$ | Lamenallenic acid | Fatty acid |

RT: retention time.

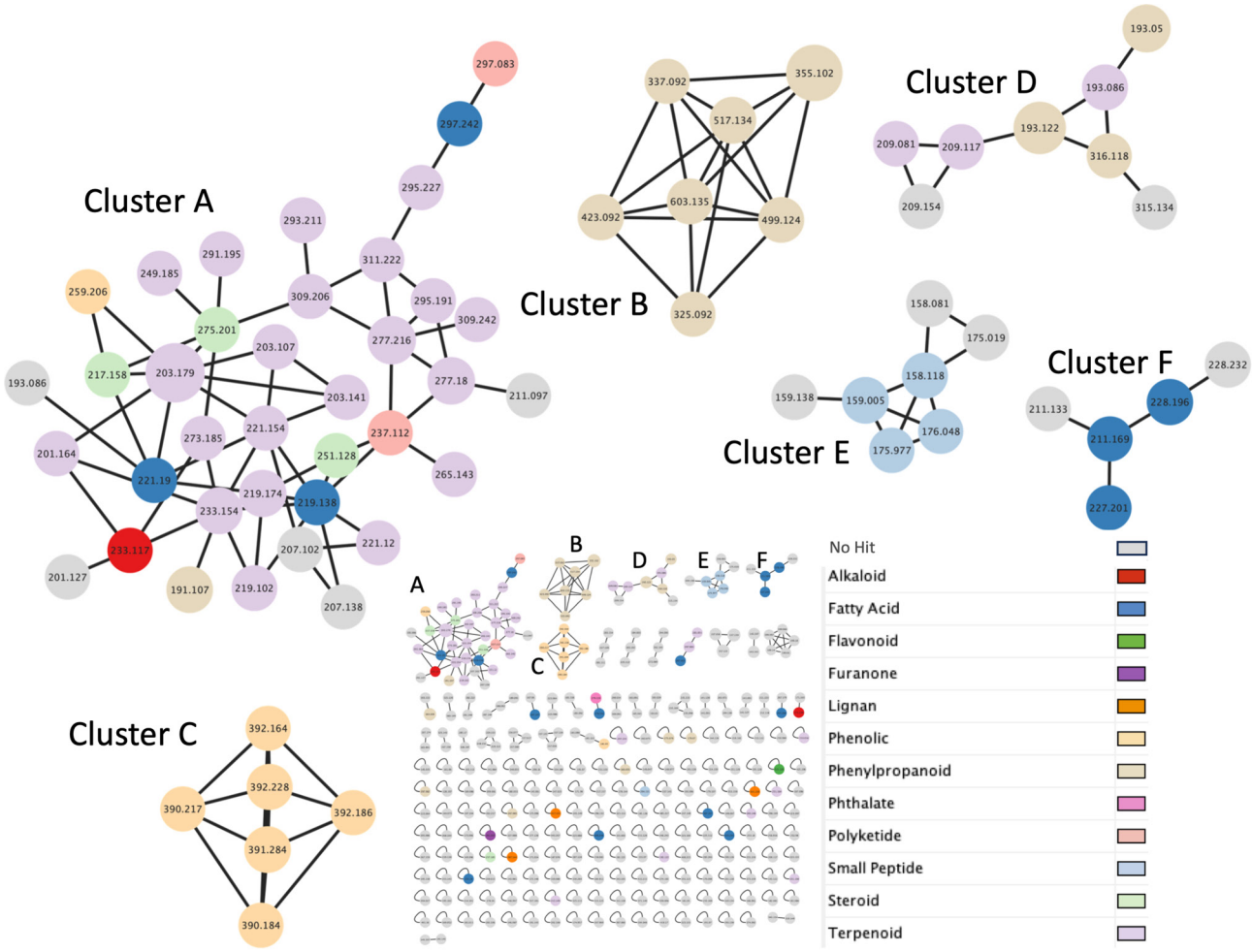


**Fig. 2.** Global Natural Product Social classic molecular networking of *Erigeron acer*. Nodes represent parent ions, and the color represents compound classes as indicated in the legend.

Incorporating molecular networking into this study will result in several key advantages over traditional methods of compound identification. Firstly, it allows for the high-throughput identification of compounds. This means compounds within an extract can rapidly detected and cataloged, including new ones, without requiring pure reference standards for each substance. Secondly, this approach aids in identifying families of compounds, making it easier to spot new potent compounds by their structural resemblance to compounds already known to be active. This method enhances the data analysis to uncover and understand the intricate mixture of bioactive components in natural products. Among the phenylpropanoids identified in Tables 2 and 3, Cluster B (Fig. 3) from MN contained 4-caffeoylquinic acid and 3,4-di-*O*-caffeoylquinic acid and was putatively identified from both approaches, corresponding to 1.02% and 2.02% relative content ratios, respectively. Figure 3 provides a visual representation of the molecular relationships within *E. acer* acetone extract, illustrating how compounds are interconnected. The lines, or edges, connecting the nodes in this molecular network indicate structural similarities or shared molecular fragments between the compounds. These connections are crucial as they can be used for identification of the compounds that are likely to be structurally related or belong to the same biochemical family. Interest in studying caffeoylquinic acids and their derivatives has increased, as these compounds exhibit pharmacological properties and are potential candidates for drug development. The presence of caffeoylquinic acids as the main ingredients has been detected in various *Erigeron* species, including *E. multiradiatus, E. annuus*, and *E. breviscapus* [10, 16, 37]. These secondary metabolites are widely found in edible and medicinal plants and are reported to exhibit a wide range of bioactivities, such as antioxidant, antibacterial, antiparasitic, neuroprotective, anti-inflammatory, anticancer, antiviral, anti-Alzheimer, and antidiabetic effects [3, 21]. In contrast, these compounds have not been reported to exhibit insecticidal or acaricidal activity. The synergistic or additive effects of different compounds within the plant extract should also be considered. The acaricidal activity observed likely results from a complex interaction between multiple compounds, each exerting unique effects on ticks. Therefore, the presence of these phenylpropanoids, along with other unidentified compounds, may collectively enhance the overall acaricidal activity of *E. acer* root.

The development of a compatible acaricide involves multiple factors, with toxicity playing a crucial role. Thus, a toxicity test was performed against human foreskin fibroblasts. In the results, *E. acer* root demonstrated a low toxic effect against human foreskin fibroblasts, with a $CC_{50}$ value of 267.00 µg/mL. This finding aligns with a previous report that the essential oil of *E. acer* root exhibited relatively low toxicity on normal skin fibroblasts (CRL-1474), with an $IC_{50}$ value of >50 µg/mL [28]. Additionally, this study

**Table 3.** Annotated compounds on the Global Natural Product Social classical molecular network of acetone extract of *Erigeron acer*

| No | Adduct type | Compound | Cosine score | *m/z* detected | Compound class |
|---|---|---|---|---|---|
| 1 | $[M+H]^+$ | *D*-camphor | 0.98 | 153.02 | Terpenoid |
| 2 | Unknown | Trans-nerolidol | 0.96 | 203.18 | Terpenoid |
| 3 | $[M+H]^+$ | DEHP (bis (2-ethylhexyl) phthalate) | 0.95 | 391.28 | Phenolic |
| 4 | $[M+Na]^+$ | Gibberellin A4&A7 | 0.95 | 295.23 | Terpenoid |
| 5 | $[M+H]^+$ | 3,4-di-*O*-caffeoylquinic acid | 0.95 | 603.14 | Phenylpropanoid |
| 6 | $[M+H]^+$ | Clovamide | 0.95 | 355.10 | Phenylpropanoid |
| 7 | $[M+H]^+$ | Phellopterin | 0.94 | 517.13 | Phenylpropanoid |
| 8 | $[M-H_2O+H]^+$ | Isolongifolol | 0.94 | 201.16 | Terpenoid |
| 9 | $[M+H]^+$ | Nootkatone | 0.93 | 277.22 | Terpenoid |
| 10 | $[M+H]^+$ | Esculin | 0.93 | 275.08 | Phenylpropanoid |
| 11 | $[M+Na]^+$ | Progesterone | 0.91 | 275.20 | Steroid |
| 12 | $[M+H]^+$ | 4-[(*E*)-3-(3,4-dihydroxyphenyl) prop-2-enoyl]oxy-2,3-dihydroxy-2-methylbutanoic acid | 0.90 | 325.10 | Phenylpropanoid |
| 13 | $[M+Na]^+$ | Mollugin | 0.90 | 237.11 | Polyketide |
| 14 | $[M+H]^+$ | Walleminone | 0.90 | 213.15 | Terpenoid |
| 15 | $[M-H_2O+H]^+$ | Coronaric acid | 0.90 | 211.17 | Fatty acid |
| 16 | $[M+NH_4]^+$ | Glechomafuran | 0.90 | 265.14 | Terpenoid |
| 17 | $[M+H]^+$ | Costunolide | 0.89 | 219.17 | Terpenoid |
| 18 | $[M-H_2O+H]^+$ | 17-epioxandrolone | 0.89 | 237.19 | Steroid |
| 19 | $[M-H_2O+H]^+$ | Jaeschkeanadiol | 0.88 | 203.11 | Terpenoid |
| 20 | $[M-H_2O+H]^+$ | Methyl jasmonate | 0.87 | 219.14 | Fatty acid |
| 21 | $[M+H]^+$ | Methyl-1-testosterone | 0.85 | 251.13 | Steroid |
| 22 | $[M+H]^+$ | 9-hydroxy-1,4a-dimethyl-7-propan-2-yl-2,3,4,9,10,10a-hexahydrophenanthrene-1-carboxylic acid | 0.85 | 291.20 | Terpenoid |
| 23 | $[M-H_2O+H]^+$ | Sinapyl alcohol | 0.85 | 193.12 | Phenylpropanoid |
| 24 | $[M+NH_4]^+$ | Mucic acid | 0.85 | 228.20 | Fatty acid |
| 25 | $[M-H_2O+H]^+$ | Calusterone | 0.84 | 221.12 | Terpenoid |
| 26 | $[M-H_2O+H]^+$ | 2,6-di-tert-butyl-4-hydroxymethylphenol | 0.84 | 259.21 | Phenolic |
| 27 | $[M+H]^+$ | 6-α (*H*)-santonin | 0.84 | 247.13 | Terpenoid |
| 28 | $[M-H_2O+H]^+$ | Vanillylmandelic acid | 0.84 | 160.06 | Phenylpropanoid |
| 29 | $M+2H]^+$ | Yohimbinic acid monohydrate | 0.83 | 171.14 | Alkaloid |
| 30 | $[M+H]^+$ | Dibutyl phthalate | 0.83 | 279.16 | Phthalate |
| 31 | $[M+Na]^+$ | 1,2-dihydroxyheptadec-16-en-4-yl acetate | 0.82 | 433.24 | Fatty acid |
| 32 | $[M-H_2O+H]^+$ | Genipin | 0.82 | 193.09 | Terpenoid |
| 33 | $[M+H]^+$ | Annosquamosin C | 0.81 | 293.21 | Terpenoid |
| 34 | $[M+H]^+$ | Citrulline | 0.81 | 159.01 | Small peptide |
| 35 | $[M+H]^+$ | 6-methylcoumarin | 0.80 | 191.11 | Phenylpropanoid |
| 36 | $[M+NH_4]^+$ | 3a-hydroxy-3,5a,9-trimethyl-3,4,5,6,7,9b-hexahydrobenzo[*g*] [1]benzofuran-2,8-dione | 0.80 | 297.08 | Terpenoid |
| 37 | $[M+Na]^+$ | Ellipticine | 0.79 | 233.12 | Alkaloid |
| 38 | $[M+H]^+$ | Ralfuranone L | 0.79 | 265.11 | Furanone |
| 39 | $[M-H_2O+H]^+$ | Cis-9-hexadecenoic acid | 0.78 | 221.19 | Fatty acid |
| 40 | $[M+H]^+$ | 4-methylesculetin | 0.78 | 207.07 | Phenylpropanoid |
| 41 | $[M+H]^+$ | Stearidonic acid | 0.78 | 307.23 | Fatty acid |
| 42 | $[M+H]^+$ | Syringaresinol | 0.78 | 417.16 | Lignan |
| 43 | $[M+H]^+$ | 3-phenyllactic acid | 0.77 | 163.04 | Phenylpropanoid |
| 44 | $[M+H]^+$ | Undecylenoyl glycine | 0.77 | 167.11 | Fatty acid |
| 45 | $[M+H]^+$ | α-humulene | 0.77 | 193.12 | Terpenoid |
| 46 | $M+H]^+$ | Androsterone | 0.77 | 217.16 | Steroid |
| 47 | $[M+K]^+$ | 1-[2-hydroxy-4-(3-hydroxy-5-methylphenoxy)-6-methylphenyl]-3-methylbutane-2,3-diol | 0.77 | 297.08 | Polyketide |
| 48 | $[M+H]^+$ | 5,7-dihydroxy-6-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one | 0.77 | 323.13 | Flavonoid |
| 49 | $[M+H]^+$ | *N*-fructosyl isoleucine | 0.76 | 294.16 | Small peptide |
| 50 | $[M-H_2O+H]^+$ | Monolaurin | 0.76 | 253.18 | Fatty acid |
| 51 | $[M+K]^+$ | Thymol-β-*D*-glucoside | 0.76 | 265.15 | Terpenoid |
| 52 | $[M+H]^+$ | Caffeic acid phenethyl ester | 0.75 | 193.05 | Phenylpropanoid |
| 53 | $[M+H]^+$ | Santonin | 0.75 | 231.14 | Terpenoid |
| 54 | $[M+H]^+$ | Matairesinol | 0.74 | 387.14 | Lignan |
| 55 | $[M+K]^+$ | Luvangetin | 0.74 | 297.08 | Phenylpropanoid |
| 56 | $[M+NH_4]^+$ | Nortrachelogenin | 0.74 | 341.14 | Lignan |
| 57 | $[M+H]^+$ | Linoleic acid | 0.73 | 297.06 | Fatty acid |
| 58 | $[M+H]^+$ | Gallic acid | 0.73 | 181.05 | Phenolic |
| 59 | $[M+H]^+$ | Monolinolein | 0.72 | 297.24 | Fatty acid |
| 60 | $[M+H]^+$ | Coniferyl aldehyde | 0.72 | 179.07 | Phenylpropanoid |

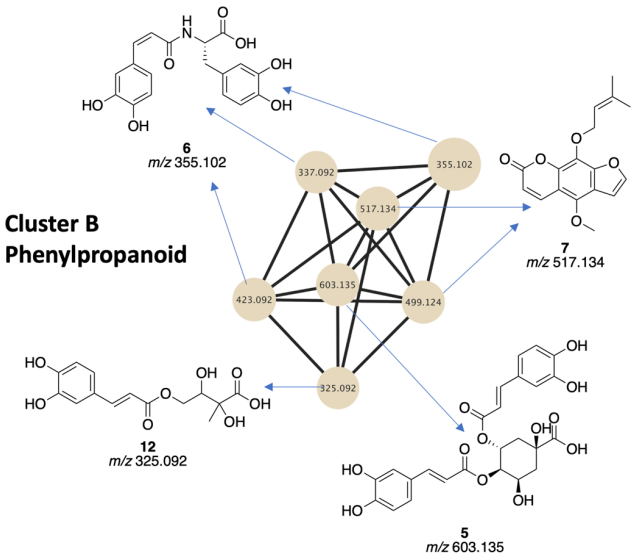


**Fig. 3.** Putative annotation of Cluster B, which contained phenylpropanoid compounds. The indicated number corresponds to the compound numbers in Table 3.

[28] highlights the essential oil's high anti-proliferative activity in MCF-7 cells. Our findings revealed that the crude extract of *E. acer* root exhibited notably low toxicity toward human foreskin fibroblasts. Furthermore, cytotoxic effects were only observed in the tested cells when the concentration of crude extract was relatively high. These results signify that the safety profile of the crude extract is favorable, suggesting its potential as a viable option for further exploration as a natural acaricide. Notably, determining the cytotoxicity of the crude extract against nontarget cells, such as human foreskin fibroblasts, is of utmost importance, as the results will indicate whether the extract exhibits selective toxicity against the target organism without causing significant harm to beneficial organisms or human health. The observed low toxicity toward human foreskin fibroblasts further supports the potential of the crude extract of *E. acer* root as a biocontrol candidate against *H. longicornis* ticks, as the risk of adverse effects on nontarget organisms is low.

In conclusion, our research demonstrated that *E. acer* root collected from Mongolia exhibits high efficacy in controlling the *H. longicornis* tick. This study is the first report on the acaricidal activity against *H. longicornis* of the specific plant. Additionally, the plant demonstrated lower toxicity against human foreskin fibroblasts, indicating its relative safety. Therefore, *E. acer* root could be a promising biocontrol candidate against *H. longicornis*. The molecular network generated in this study also presents a valuable tool for future research into natural acaricides. It enables the rapid identification of promising compounds in related extracts, aids in streamlining bio-guided fractionation by highlighting potentially synergistic compound groups, and assists in the design of assays focused on specific classes of compounds. This approach complements traditional methods, offering a broader understanding of the complex interactions within natural product mixtures. As a result, it guides the way for more efficient discovery and utilization of acaricidal substances, thereby enhancing the development of natural pest control strategies. However, complementary experiments are necessary to fully evaluate the acaricidal activity and identify the key active compounds of the plant, including *in vivo* tests using animal models and a bioactivity-guided fractionation approach. These investigations would provide a more comprehensive knowledge of the potential of the plant as a source of acaricidal agents.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. We would like to express our gratitude to multiple generations of students at the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia who contributed to the collection of plant samples: Selenge Erdenechimeg, Oyunjargal Tumurbaatar, Tsevelmaa Nanjidsuren, Delgerbat Boldbaatar, Odonbayar Batsukh, Byambasukh Dendev, Baasanmunkh Shuherdorj, Mungunshagai Baatarsuren, Bolortuya Munkhgerel, Orgilkhatan Mukhuu, Ankhlkhagva Ganbaatar, Bolortuya Byambajav, Bertsetseg Dalaijargal, and Batbold Badarch. We are grateful to Inplanta Innovations Inc. (Yokohama, Japan) for assisting with the LC-MS/MS analysis. This study was supported by KAKENHI Grants from the Japan Society for the Promotion of Science (20F20402 [Y.N.]). In addition, N.R.A. is supported by the Japan Society for the Promotion of Science (JSPS) Postdoctoral Fellowship for Overseas Researcher (P20402). This study was partially funded by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan as a project for the Joint Usage/Research Center.

## REFERENCES

1. Abbas MG, Haris A, Binyameen M, Nazir A, Mozūratis R, Azeem M. 2022. Chemical composition, larvicidal and repellent activities of wild plant essential oils against *Aedes aegypti*. *Biology (Basel)* **12**: 1–16. [Medline]
2. Adenubi OT, McGaw LJ, Eloff JN, Naidoo V. 2018. *In vitro* bioassays used in evaluating plant extracts for tick repellent and acaricidal properties: A critical review. *Vet Parasitol* **254**: 160–171. [Medline] [CrossRef]
3. Alcázar Magaña A, Kamimura N, Soumyanath A, Stevens JF, Maier CS. 2021. Caffeoylquinic acids: chemistry, biosynthesis, occurrence, analytical challenges, and bioactivity. *Plant J* **107**: 1299–1319. [Medline] [CrossRef]
4. Azeem M, Zaman T, Abbasi AM, Abid M, Mozūratis R, Alwahibi MS, Elshikh MS. 2022. Pesticidal potential of some wild plant essential oils against grain pests *Tribolium castaneum* (Herbst, 1797) and *Aspergillus flavus* (Link, 1809). *Arab J Chem* **15**: 103482. [CrossRef]
5. Banzragchgarav O, Battur B, Battsetseg B, Myagmarsuren P, Murata T, Batkhuu J. 2019. Acaricidal activity of Mongolian plants against *Dermacentor nuttalli*. *Mong. J. Agric. Sci.* **28**: 26–33. [CrossRef]
6. Banzragchgarav O, Ariefta NR, Murata T, Myagmarsuren P, Battsetseg B, Battur B, Batkhuu J, Nishikawa Y. 2021. Evaluation of Mongolian compound library for potential antimalarial and anti-*Toxoplasma* agents. *Parasitol Int* **85**: 102424. [Medline] [CrossRef]
7. Banzragchgarav O, Batkhuu J, Myagmarsuren P, Battsetseg B, Battur B, Nishikawa Y. 2021. *In vitro* potently active anti-plasmodium and anti-toxoplasma Mongolian plant extracts. *Acta Parasitol* **66**: 1442–1447. [Medline] [CrossRef]
8. Chen Z, Yang X, Bu F, Yang X, Liu J. 2012. Morphological, biological and molecular characteristics of bisexual and parthenogenetic *Haemaphysalis longicornis*. *Vet Parasitol* **189**: 344–352. [Medline] [CrossRef]

9. Cristiani Z, Galhardo I, Valdrinez M, Lonardoni C, Carolina A, Amorim L, Maria A, Hovell C, Moraes C, Alves G, Luiz E, Lima D, Antunes F, Cosmo D, Aparício D, Cortez G. 2011. Experimental parasitology acaricidal activity of the essential oil from Tetradenia riparia (Lamiaceae) on the cattle tick Rhipicephalus (Boophilus) microplus (Acari; Ixodidae). **129**: 175–178.
10. Dong X, Qu S. 2022. *Erigeron breviscapus* (Vant.) Hand-Mazz.: a promising natural neuroprotective agent for Alzheimer's disease. *Front Pharmacol* **13**: 877872. [Medline] [CrossRef]
11. Elias TS, Grubov VI. 1983. Key to the vascular plants of Mongolia (with an Atlas). *Brittonia* **35**: 315. [CrossRef]
12. Galay RL, Miyata T, Umemiya-Shirafuji R, Maeda H, Kusakisako K, Tsuji N, Mochizuki M, Fujisaki K, Tanaka T. 2014. Evaluation and comparison of the potential of two ferritins as anti-tick vaccines against *Haemaphysalis longicornis*. *Parasit Vectors* **7**: 482. [Medline] [CrossRef]
13. Ghosh S, Sharma AK, Kumar S, Tiwari SS, Rastogi S, Srivastava S, Singh M, Kumar R, Paul S, Ray DD, Rawat AKS. 2011. *In vitro* and *in vivo* efficacy of *Acorus calamus* extract against *Rhipicephalus* (Boophilus) *microplus*. *Parasitol Res* **108**: 361–370. [Medline] [CrossRef]
14. Giglioti R, Forim MR, Oliveira HN, Chagas ACS, Ferrezini J, Brito LG, Falcoski TORS, Albuquerque LG, Oliveira MCS. 2011. *In vitro* acaricidal activity of neem (*Azadirachta indica*) seed extracts with known azadirachtin concentrations against *Rhipicephalus microplus*. *Vet Parasitol* **181**: 309–315. [Medline] [CrossRef]
15. Guo WP, Zhang B, Wang YH, Xu G, Wang X, Ni X, Zhou EM. 2019. Molecular identification and characterization of *Anaplasma capra* and *Anaplasma* platys-like in *Rhipicephalus microplus* in Ankang, Northwest China. *BMC Infect Dis* **19**: 434. [Medline] [CrossRef]
16. Jang DS, Yoo NH, Kim NH, Lee YM, Kim CS, Kim J, Kim JH, Kim JS. 2010. 3,5-Di-*O*-caffeoyl-*epi*-quinic acid from the leaves and stems of *Erigeron annuus* inhibits protein glycation, aldose reductase, and cataractogenesis. *Biol Pharm Bull* **33**: 329–333. [Medline] [CrossRef]
17. Kessner D, Chambers M, Burke R, Agus D, Mallick P. 2008. ProteoWizard: open source software for rapid proteomics tools development. *Bioinformatics* **24**: 2534–2536. [Medline] [CrossRef]
18. Kilickaya Selvi E, Usta A, Akiner MM. 2019. Larvacidal activity of some medicinal plants naturally growing in turkey against *Aedes albopictus* (Diptera: Culicidae). *J. Anatol. Environ. Anim. Sci.* **4**: 53–59.
19. Kuniyori M, Sato N, Yokoyama N, Kawazu SI, Xuan X, Suzuki H, Fujisaki K, Umemiya-Shirafuji R. 2022. Vitellogenin-2 accumulation in the fat body and hemolymph of babesia-infected *Haemaphysalis longicornis* Ticks. *Front Cell Infect Microbiol* **12**: 908142. [Medline] [CrossRef]
20. Li, DX 2011. Fever with thrombocytopenia associated with a novel bunyavirus in China. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*. **25**: 81–84.
21. Liu W, Li J, Zhang X, Zu Y, Yang Y, Liu W, Xu Z, Gao H, Sun X, Jiang X, Zhao Q. 2020. Current advances in naturally occurring caffeoylquinic acids: structure, bioactivity, and synthesis. *J Agric Food Chem* **68**: 10489–10516. [Medline] [CrossRef]
22. Lu Q, Sun Y, Ares I, Anadón A, Martínez M, Martínez-Larrañaga MR, Yuan Z, Wang X, Martínez MA. 2019. Deltamethrin toxicity: A review of oxidative stress and metabolism. *Environ Res* **170**: 260–281. [Medline] [CrossRef]
23. Meepagala KM, Sturtz G, Wise D, Wedge DE. 2002. Molluscicidal and antifungal activity of *Erigeron speciosus* steam distillate. *Pest Manag Sci* **58**: 1043–1047. [Medline] [CrossRef]
24. Nalewajko-Sieliwoniuk E, Nazaruk J, Antypiuk E, Kojło A. 2008. Determination of phenolic compounds and their antioxidant activity in *Erigeron acris* L. extracts and pharmaceutical formulation by flow injection analysis with inhibited chemiluminescent detection. *J Pharm Biomed Anal* **48**: 579–586. [Medline] [CrossRef]
25. Nalewajko-Sieliwoniuk E, Pliszko A, Nazaruk J, Barszczewska E, Pukszta W. 2019. Comparative analysis of phenolic compounds in four taxa of *Erigeron acris* s. l. (Asteraceae). *Biologia (Bratisl)* **74**: 1569–1577. [CrossRef]
26. Nazaruk J. 2006. Flavonoid aglycones and phytosterols from the Erigeron acris L. herb. *Acta Pol Pharm* **63**: 317–319. [Medline]
27. Nazaruk J, Kalemba D. 2009. Chemical composition of the essential oils from the roots of *Erigeron acris* L. and *Erigeron annuus* (L.) Pers. *Molecules* **14**: 2458–2465. [Medline] [CrossRef]
28. Nazaruk J, Karna E, Wieczorek P, Sacha P, Tryniszewska E. 2010. In vitro antiproliferative and antifungal activity of essential oils from Erigeron acris L. and Erigeron annuus (L.) Pers. *Z Naturforsch C J Biosci* **65**: 642–646. [Medline] [CrossRef]
29. Novato TP, Milhomem MN, Marchesini PBC, Coutinho AL, Silva IS, de Souza Perinotto WM, de Azevedo Prata MC, Ferreira LL, Lopes WDZ, Costa-Júnior LM, de Oliveira Monteiro CM. 2022. Acaricidal activity of carvacrol and thymol on acaricide-resistant *Rhipicephalus microplus* (Acari: Ixodidae) populations and combination with cypermethrin: Is there cross-resistance and synergism? *Vet Parasitol* **310**: 109787. [Medline] [CrossRef]
30. Pavela R. 2009. Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Ind Crops Prod* **30**: 311–315. [CrossRef]
31. Rosado-Aguilar JA, Arjona-Cambranes K, Torres-Acosta JFJ, Rodríguez-Vivas RI, Bolio-González ME, Ortega-Pacheco A, Alzina-López A, Gutiérrez-Ruiz EJ, Gutiérrez-Blanco E, Aguilar-Caballero AJ. 2017. Plant products and secondary metabolites with acaricide activity against ticks. *Vet Parasitol* **238**: 66–76. [Medline] [CrossRef]
32. Rosenberg R, Lindsey NP, Fischer M, Gregory CJ, Hinckley AF, Mead PS, Paz-Bailey G, Waterman SH, Drexler NA, Kersh GJ, Hooks H, Partridge SK, Visser SN, Beard CB, Petersen LR. 2018. Vital signs: trends in reported vectorborne disease cases — United States and Territories, 2004–2016. *MMWR Morb Mortal Wkly Rep* **67**: 496–501. [Medline] [CrossRef]
33. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **13**: 2498–2504. [Medline] [CrossRef]
34. Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, Nguyen DD, Watrous J, Kapono CA, Luzzatto-Knaan T, Porto C, Bouslimani A, Melnik AV, Meehan MJ, Liu WT, Crüsemann M, Boudreau PD, Esquenazi E, Sandoval-Calderón M, Kersten RD, Pace LA, Quinn RA, Duncan KR, Hsu CC, Floros DJ, Gavilan RG, Kleigrewe K, Northen T, Dutton RJ, Parrot D, Carlson EE, Aigle B, Michelsen CF, Jelsbak L, Sohlenkamp C, Pevzner P, Edlund A, McLean J, Piel J, Murphy BT, Gerwick L, Liaw CC, Yang YL, Humpf HU, Maansson M, Keyzers RA, Sims AC, Johnson AR, Sidebottom AM, Sedio BE, Klitgaard A, Larson CB, P CAB, Torres-Mendoza D, Gonzalez DJ, Silva DB, Marques LM, Demarque DP, Pociute E, O'Neill EC, Briand E, Helfrich EJN, Granatosky EA, Glukhov E, Ryffel F, Houson H, Mohimani H, Kharbush JJ, Zeng Y, Vorholt JA, Kurita KL, Charusanti P, McPhail KL, Nielsen KF, Vuong L, Elfeki M, Traxler MF, Engene N, Koyama N, Vining OB, Baric R, Silva RR, Mascuch SJ, Tomasi S, Jenkins S, Macherla V, Hoffman T, Agarwal V, Williams PG, Dai J, Neupane R, Gurr J, Rodríguez AMC, Lamsa A, Zhang C, Dorrestein K, Duggan BM, Almaliti J, Allard PM, Phapale P, Nothias LF, Alexandrov T, Litaudon M, Wolfender JL, Kyle JE, Metz TO, Peryea T, Nguyen DT, VanLeer D, Shinn P, Jadhav A, Müller R, Waters KM, Shi W, Liu X, Zhang L, Knight R, Jensen PR, Palsson BO, Pogliano K, Linington RG, Gutiérrez M, Lopes NP, Gerwick WH, Moore BS, Dorrestein PC, Bandeira N. 2016. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nat Biotechnol* **34**: 828–837. [Medline] [CrossRef]
35. Wu G, Fei DQ, Gao K. 2007. Aromadendrane-type sesquiterpene derivatives and other constituents from *Erigeron acer*. *Pharmazie* **62**: 312–315. [Medline]
36. Wu G, Fei D, Gao K. 2006. Two new butenolide derivatives from *Erigeron acer*. *Chem Res Chin Univ* **22**: 33–35. [CrossRef]
37. Zhang Z, Liu Y, Ren X, Zhou H, Wang K, Zhang H, Luo P. 2016. Caffeoylquinic acid derivatives extract of *Erigeron multiradiatus* alleviated acute myocardial ischemia reperfusion injury in rats through inhibiting NF-KappaB and JNK activations. *Mediators Inflamm* **2016**: 7961940. [Medline] [CrossRef]