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Original Research

Antimicrobial and Antioxidant Activities of Two Medicinal Plants *Cuphea aequipetala* var. *hispida* (Cav.) Koehne and *Eryngium comosum* Delaroche F Against Bacteria Related to Equine Infections

Tonantzin Díaz Alvarado [a], María Dolores Mariezcurrena Berasain [b], Abdelfattah Z.M. Salem [c], Dora Luz Pinzón Martínez [b, *]

[a] *Maestria en Agroindustri Rural, Desarrollo Territorial y Turismo Agroalimentario, Instituto de Ciencias Agropecuarias y Rurales, Universidad Autonoma del Estado de Mexico, Instituto Literario Avenue, Toluca, Estado de Mexico, Mexico*

[b] *Facultad de Ciencias Agricolas, Universidad Autonoma del Estado de Mexico, Instituto Literario Avenue, Toluca, Estado de Mexico, Mexico*

[c] *Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Instituto Literario Avenue, Toluca, Estado de Mexico, Mexico*

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A B S T R A C T

Functional biocompounds beneficial for animals and humans are in Mexican folk herbs. Cuphea and Eryngium species presented antimicrobial potential. Natural antibiotic uses by ethnoveterinary research with medicinal plants in equine infection or digestive diseases need more scientific evidence. *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* serotype *Enteritidis* are etiological agents in horses responsible for stable infections, abortions, fetal or perinatal deaths, and resistant intrahospital infections. The main objective of the present research was to evaluate the potential of antimicrobial and antioxidant activities of two Mexican medicinal plants *Cuphea aequipetala* var. *hispida* (Cav.) Koehne and *Eryngium comosum* Delaroche F over *Listeria monocytogenes* ATCC 19115, *Staphylococcus* sp., *E. coli* ATCC 25922, and *S. enterica* serotype *Enteritidis* ATCC 13076 bacterium reference strains related to equine infections. Determination of total phenol, saponins, antioxidant activity (ABTS), and antimicrobial activity with diffusion-sensitive discs was performed in triplicate. All the strains were sensitive for both extracts except for *E. coli* strain that was inhibited only by *C. aequipetala*. *Staphylococcus* sp. and *S. enterica* strains were inhibited equally by both extracts. *E. comosum* extracts tested have shown the highest effect over *L. monocytogenes*. In summary, antimicrobial activity was similar to the reported activity of *Eryngium* species extracts with other different solvents. Present extracts are suggested as a potential alternative antibiotic; definitely, more specific equine pathogen inhibition tests are needed in feed additives for horse nutrition research. In conclusion, antimicrobial activities of *Cuphea aequipetala* var. *hispida* (Cav.) Koehne and *Eryngium comosum* Delaroche F over reference strains related to equine infections suggested these medicinal plants as potential antibiotic sources for horse diseases.

1. Introduction

Mexico is a country with great biological wealth, ecosystem diversity, and genetic diversity because of its topography and weather variability which originated many medicinal plants. Medicinal herbs present biological compounds with beneficial effects for animals and humans [1,2]. *Cuphea aequipetala* var. *hispida* (Cav.) Koehne is named as cancer herb in Mexico, and it grows in temperate, tropical, and dried zones [3,4]. *Eryngium* gender especially, *Eryngium comosum* named as toad herb, is found at pine-oak forest from highlands of Mexico, and it grows in open sites, around houses, degraded surfaces, and in cultivated areas [5,6]. *Eryngium* gender plants' biological compounds have shown antimicrobial, antioxidant, anti-inflammatory, and antitumoral activities, along with diuretic, hypotensive, and anticarcinogenic effects [7–10]. Phenols, phenolic acids, flavonoids, and glycosides have been related to that antioxidant activity reported [11–13]. *Streptococcus pneumoniae*, *Listeria monocytogenes,* and *Staphylococcus aureus* or *Salmonella* species have been reported as sensitive pathogens to

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* Corresponding author at: Dora Luz Pinzón Martínez, Facultad de Ciencias Agricolas, Universidad Autonoma del Estado de Mexico, Instituto Literario Avenue, 100, Zip Code 50 000, Toluca, Estado de Mexico, Mexico.

E-mail address: dora_lpm@hotmail.com (D.L. Pinzón Martínez).

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these plants extracts; likewise, *Helicobacter pylori* was reported to be inhibited by *Cuphea aequipetala* Cav or other *Cuphea* species (*Eryngium carlinae* F. Delaroche, *Eryngium foetidum*) [11–13].

Animal infection diseases are one of the most important morbidities and mortality in developing countries. Synthetical antibiotics play a special role in those treatments [14]. Antibiotic diversity and their indiscriminate use have originated bacterial resistant mechanisms [15]. New natural treatment research has become important to treat infectious diseases to improve the pharmaceutical animal industry [14]. Several beneficial effects have been reported from garlic (*Allium sativum*), lavender (*Lavandula angustifolia*), narrow leaf Equinacea (*Echinacea angustifolia*), flaxseed (*Linum usitatissimum*), ginseng (*Panax quinquefolius*), among others. These plants are frequently used in rural communities, and they have been studied in ethnoveterinary research as a more important field [16–18]. Many reports have been published about plant use in feed additives. One of the most studied plants for animals and humans is garlic (*Allium sativum*) because of its antimicrobial and antioxidant properties. In *ab libitum* diets, it should be used carefully because overdosage was related to some blood disorders in horses; nonetheless, in the poultry industry, it is commonly used [19,20]. Another common plant used in horses is ginseng (*Panax ginseng*) because of its stimulation effect over the immune system and the inhibition of proinflammatory cytokines. In rats, the ginseng plant was reported as a plasma adipokine and a lipogenesis reducer; thereby, it was reported as a lipolysis promoter [21,22]. In racing horses, this plant has been reported as an anticancer agent and a reducer of the recovery time [16]. Other ginseng-named plants (*Panax quinquefolius* and *Zingiber officinale*) were reported with antioxidant activity. Antioxidant activity of the milk thistle plant (*Silybum marianum*) was proved even in horses [16]. *Aloe vera* is widely used in humans because of its benefits on gastrointestinal issues, and then, in horses, those effects have been compared with omeprazole effect. It inhibited *Escherichia coli* in some animals as some other plant mixtures with peppermint (*Mentha* x *piperita*), palmarosa (*Cymbopogon martini*), oregano (*Origanum vulgare*), and cloves (*Syzygium aromaticum*) presented antimicrobial activity over *Staphylococcus xylosus* [16,23]. However, there are some outstanding considerations about plant therapy in animals such as interactions with anti-inflammatory drugs, gastrointestinal disorders, and mare's administration to avoid fetal losses, among others [22–24]. In equine diseases treated with natural plants, some research studies expressed that few plants could present some toxic effects [25]. Ethnoveterinary is an interdisciplinary science that studies the local or indigenous knowledge; furthermore, it includes the beliefs, practice, and social structure applied to medicinal plants for animal breeding and health care. Ethnoveterinary has been more accepted, even though it is less formal and represents an animal natural product development opportunity field [16,26]. Some literature information is available to evaluate plant or natural products as feed additives or secure antibiotics for horses' diseases such as digestive problems or other infections [16,27]. However, more scientific evidence is needed to support plant treatments in animals as antioxidants, antimicrobials, or by some other biological function reported. Medicinal plant antioxidant potential could be estimated mainly by using total phenol (TP) determination by the Folin-Ciocalteu test and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) chromogenic methodology, among others. The $ABTS^{\bullet+}$ method could measure the reduction capacity from biological compounds in plant extracts [28]. *Staphylococcus* sp., *E. coli*, *Salmonella enterica* serotype *Enteritidis* are common equine fecal pathogens that cause stable infections, as well as abortions, fetal and perinatal deaths [29]. *E. coli* causes morbidity, and it has been reported in equine hospitals, even though some drug-resistant strains were mentioned in research reports [30–32]]. *Sta. aureus* is another important pathogen in clinic epidemiology, farms, and equine hospitals as an etiological agent from animal and zoonotic infections. Methicillin-resistant *S. aureus* strains are prevalent, although some resistant strains are still routinely sensitive to antibiotics [33–35]. *Salmonella* gender includes many species from the digestive tract or disease etiological agents. *Salmonella* gender transmission modes include animal fecal feces, and its incidence is related to edge, breeding, among other aspects. Salmonellosis is the most common disease transmitted through food that causes economic problems. Ninety percentage of pet's clinic cases and some equine abortions are originated by this bacterium [36,37]. In hospitalized equine, the disease transmission had originated security protocols not only for horses, but also for equine workers. Multidrug-resistant species and *Salmonella* nonresistant strains in hospitalized equines cause high fatality cases and costs, and nonresistant serotypes have been reported in ruminants, beef or dairy cattle, and sheep [36–38]. *L. monocytogenes* is an obliquus bacterium as a human and animal pathogen. It has been related to problems in reproductive systems, as abortion in sheep, goats, pigs, cats, and horses. It has been also related with equine keratoconjunctivitis [39–41]. Then, the main objective of the present research was to evaluate the potential of antimicrobial and antioxidant activities in two Mexican medicinal plants *Cuphea aequipetala* hispida (Cav.) Koehne and *Eryngium comosum* Delaroche F over *L. monocytogenes* ATCC 19115, *Staphylococcus* sp., *E. coli* ATCC 25922, and *S. enterica* serotype *Enteritidis* ATCC 13076 bacterium reference strains related to equine infections.

2. Materials and Methodology

2.1. Vegetative Material

Plant material was collected from Ejido Los Padres location at Villa Victoria, Mexico state using purposive sampling [42]. Vegetative material was dried at 40°C and milled (300 MACSA Hammer mill) at the laboratory of analysis of agricultural products, Agricultural Faculty Science, Universidad Autonoma del Estado de Mexico (UAEMex). The collected *Cuphea aequipetala* var. *hispida* and *Eryngium comosum* specimens had a botanical identification confirmed by "Eizi Matuda" Herbarium, Agricultural Faculty Science (CODAGEM), where a specimen per plant was deposited and registered as *Cuphea aequipetala* var. *hispida* (Cav.) Koehne (19668) and *Eryngium comosum* Delaroche F (19669).

2.2. Microbial Strains Tested

Four reference strains were used to study some equine etiological pathogens. Gram-positive strains were *L. monocytogenes* ATCC 19115 and *Staphylococcus* sp. Gram-negative bacteria were *E. coli* ATCC 25922 and *S. enterica* serotype *Enteritidis* ATCC 13076. Strains were donated by the Food Microbiology Laboratory, Food Research and Development Unit (UNIDA), Veracruz Technological Institute, Veracruz, Mexico. The strains were stored at −40°C using Luria-Bertani (LB) broth at 40% glycerol solutions. Luria-Bertani broth incubations were carried out at 37°C for 24 hours to reactivate the microorganisms used. Then, a second growth took place at 37°C for 18 hours to continue with microbiological tests.

The preparation of 50% ethanolic extracts was carried out with a 125 mg/mL dried matter plant concentration. Extracts reposed for 72 hours at room temperature in amber flasks. Then, incubation was performed for 1 hour at 39°C and filtered. Extracts were stored in amber flasks at 4°C [43].

2.3. Physicochemical Analysis of 50% of Ethanol Extracts of the Plant

2.3.1. Total Phenol Folin-Ciocalteu Determination

Folin-Ciocalteu determination to estimate TP equivalents based on a gallic acid standard curve was performed by the method given by Arizmendi et al. [44] and Archundia et al. [45] with slight modifications. Total phenolic equivalents were expressed as gallic acid equivalents per g dry matter (DM) through 760 nm absorbance measures.

2.3.2. Total Phenol and Saponin Quantification by Salem et al.'s [43] Methodology

Total phenol and saponin (SP) quantification was performed by Salem et al.'s [43] methodology, where TP and SP phases obtained were estimated as mg/g DM. All physicochemical plant extract analyses were carried out in triplicate.

2.3.3. Antioxidant Activity

Antioxidant capacity (AC) ABTS methodology [46] was carried out with modifications in the method given by Archundia et al. [45]. The absorbance at 734 nm was measured, and a Trolox curve pattern was prepared using TAEC mmol/g DM.

2.3.4. Antimicrobial Activity

Antimicrobial activity was performed using the agar diffusion susceptibility test disc methodology by the Clinical and Laboratory Standards Institute [36,47], for *L. monocytogenes* ATCC 19115, *Staphylococcus* sp., *E. coli* ATCC 25922, and *S. enterica* serotype *Enteritidis* ATCC 13076. The control test was performed using the disc only with the solvent without any plant extract in accordance with the method given by Archundia et al. [45] with no effect over bacterial growth.

Luria-Bertani broth and 1 mL of activated bacterium strain were incubated for 18 hours at 37°C. One milliliter was taken to inoculate a second LB broth for 2 hours at 37°C until 1×10^5 CFU/mL count was achieved (Petri count verification). Luria-Bertani agar was poured into Petri dishes after microorganisms were placed. About 10 μL plant extract sample was poured onto a 6-mm diameter paper disc. Discs were incubated for 13–14 hours at 4°C followed by another incubation period (37°C, 24 hours). Inhibition clear zone diameter (mm) from each disc was considered as the antimicrobial activity. All tests were carried out in triplicate.

2.4. Experimental Design

A completely random design with three repetitions of analysis of variance with 5% significance level was carried out. Extracts of plants *Cuphea aequipetala* var. *hispida* (Cav.) Koehne and *Eryngium comosum Delaroche* F in 50% ethanol solutions were considered as treatments. Determination of SP (mg/g), TP content by Folin-Ciocalteu (GAEmg/mL), and AC (μM TAEC/g), together with antimicrobial tests was carried out in triplicate. Statistical differences ($P \geq .05$) were analyzed by a Tukey test at 5%.

Table 2
Antimicrobial activities from Cuphea aequipetala hispida (Cav.) Koehne and Eryngium comosum Delaroche F of 50% ethanolic extracts.

Plant	Inhibition Halo sizes (mm)			
	EC◆	SE◆	SA◆	LM◆
CEH	8 ± 0.03ay	8.0 ± 1.0ax	10 ± 1.0bx	7.0 ± 0.0ax
ECD	0± 0ax	8.0 ± 0.0bx	10.66 ± 0.57cx	9.66 ± 0.57by

Abbreviations: CEH, Cuphea aequipetala var. hispida (Cav.) Koehne; EC, Escherichia coli ATCC 25922; ECD, Eryngium comosum Delaroche F.; LM, Listeria monocytogenes ATCC 19115; SA, Staphylococcus sp.; SE, Salmonella enterica serotype Enteritidis ATCC 13076.
a, b, c letters within columns and x, y, z letters within lines are significantly different ($P \leq .05$), cells that do not share letters are not significantly different ($P \leq .05$).
◆ Control tests were discs only with the solvent and no plant extract added according to Archundia et al. [45].

3. Results and Discussion

3.1. Total Phenol by Folin-Ciocalteu, Saponins, Total Phenol Content, and Antioxidant Capacity

Saponins, TP content, and AC presented significant statistical differences ($P \geq .05$) (Table 1). Results of total phenol and SP determination were higher for *Cuphea aequipetala* var. *hispida* (Cav.) Koehne than for *Eryngium comosum*. Nevertheless, *Eryngium comosum* AC had the highest of the results observed (Table 1). *Cuphea* species has been reported as an ornamental plant, soil stabilizer, and as a Mexican folk medicinal herb outstanding for the Herbal Pharmacopoeia of the United Mexican States [5,12]. Some *Cuphea* species have shown anti-inflammatory, gastroprotective, and antioxidant activities; then, they were used for gastrointestinal disorders [48,49,50,51]. Cardenas et al. [52] reported *Cuphea aequipetala* hexane, methanol, or ethanol extracts that presented 38.04–61.86 mg GAE/mL of TP content. However, present Folin-Ciocalteu equivalent results for *Cuphea aequipetala* var. *hispida* (Cav.) Koehne (Table 2) resulted slightly lower than other *Cuphea* species. Present results were in 50% ethanol solutions and could present functional compounds, which made them candidates for further research for feed additives or human consumption due to low toxicity of 50% ethanol solutions as Archundia et al. [45] reported. *Eryngium comosum* scientific reports are very few; in the same way, there are many research articles or cyber graphic publications or articles from other *Eryngium* species extracts. These have shown genotoxic, cytotoxic, hypoglycemic, anti-inflammatory, and antibacterial potential, and they are used for diarrhea, poisons, tapeworms, venereal or kidney diseases and even as an aphrodisiac [53–56]. Present results from TP quantifications (22.1 mg GAE/mL) are higher than those for *E. foetidum*, *E. maritumum*, and *E. planum* (1.82–8.9 mg GAE/mL). Nonetheless, more sophisticated extraction methodologies reported higher TP results (58.8–105.5 GAE/mL) as it was cited for *E. caucasicum* [56,57].

There are few reports about *Cuphea* SP production; furthermore, there are other reports about its antioxidant, antimicrobial, and antiproliferative or apoptotic tumor capacities. Nevertheless, its SP quantification has not been reported in extension [52,50,58,59].

Table 1
Saponins, total phenol content, and antioxidant capacity determinations from Cuphea aequipetala var. hispida (Cav.) Koehne and Eryngium comosum Delaroche F of 50% ethanolic extracts.

Plant	Total Phenol (mg/mg)	Total Phenol (mg GAE/g Dry Plant)	Saponins (mg/g)	Antioxidant Capacity (μM TAEC/g)
ECD	22.1 ± 3.1a	4.33 ± 0.2a	62.2 ± 2.9a	1972.42 ± 7.5a
CEH	41.4 ± 15.9 b	33.2 ± 3.2b	154.2 ± 6.1 b	1756.59 ± 1.9a

Abbreviations: CEH, Cuphea aequipetala var. hispida (Cav.) Koehne; ECD, Eryngium comosum Delaroche F.
a, b, c letters within columns are significantly different ($P \leq .05$), and cells that do not share letters are not significantly different ($P \leq .05$).

Saponins and TPs, used as feed additives, are considered as anti-methanogens for ruminants; besides, SP production in horses and ruminants was reported mainly by archaea metabolism. Methane reduction by feed additives has been related to the availability of dietary energy in equines [27,60]. High SP concentration was related to toxic effect in horses, too. However, some *in vitro* and *in vivo* studies are needed to achieve more specific information. This was cited by Salem et al. [27] in their aguamiel (*Agave atrovirens*) addition effect over some forage species cecal fermentation kinetics report, where the authors mentioned methane and CO_2 production modifications. The plant studied and aguamiel presented even higher SP doses than the results of this study. AA and TP in *Cuphea aequipetala* hexane, methanol, or methanolic extracts have shown the presence of triterpenoids, tannins, flavonoids, and polyphenols, among others as antibacterial compounds. Present TP and SP quantifications were close to some other determinations from forages or feed additives for horses' diet such as *Moringa oleifera* or *Salix babylonica* [16]. *Cuphea aequipetala* var. *hispida* (Cav.) Koehne SP content was higher than *Psidium guava* leaf extracts reported by Archundia et al. [61]. Besides, the AC of the present plant (Table 1) was similar to *Cuphea aequipetala* methanol extract results (1, 743.21 μM TAEC/g) [52] and higher than that in *Psidium guava* reported by Archundia et al. [45] that was cited as a potential natural antibiotic's alternative for animal production.

In sum, physicochemical determinations performed suggested important antioxidant chemical compounds for a qualitative further research. In accordance with the present results, *Eryngium comosum* F. Deraloche and *Cuphea aequipetala* var. *hispida* (Cav.) Koehne resulted as folk medicinal plants with potential antioxidant and antimicrobial biochemical compounds, whose more specific research has not been achieved [62,63,53]. After that, the phenolic and antioxidant activities were found in both medicinal herbs and suggested for more *in vitro* or *in vivo* research.

3.2. Antimicrobial Activity

Cuphea aequipetala var. *hispida* (Cav.) Koehne and *Eryngium comosum* Delaroche F extracts revealed antimicrobial activity against all the reference microorganisms tested except for *E. coli* ATCC 25922. The *E. coli* ATCC 25922 strain was sensitive only to *Cuphea aequipetala* extracts (Table 2). Even though some phenols, phenolic acids (ferulic and caffeic acids), quercitin, isoquercitin, tannins, and alkaloids reported as *E. coli* growth inhibitors have been reported in other *Eryngium* species, there was no antimicrobial activity over *E. coli* ATCC 25922 from present *Eryngium comosum* Delaroche F extracts. Patra et al. [54] cited that phenolic acids did not inhibit *E. coli* and *Salmonella* sp., and then authors reported that *E. coli* antimicrobial activity effectiveness was related to the bacterium strain used. Takahashi et al. [55] cited no antimicrobial effect over *E. coli* O 157:H7 and *S. enterica* strains from ferulic acid. Mohamadipour et al. [56] reported *Eryngium caucasicum* Trautv. essential oil with inhibition growth effect over *E. coli* and other gram-negative and gram-positive strains. Then, further antimicrobial tests and qualitative determination are suggested to these *Eryngium comosum* Delaroche F plant extracts. *Staphylococcus* sp. have shown higher inhibition halo sizes than all the microorganism's reference strains tested with both plant extracts. *Staphylococcus* sp. and *S. enterica* serotype *Enteritidis* ATCC 13076 have shown no statistical differences between antimicrobial sensitivities to the effect of both plants ($P \leq .05$). Nonetheless, *L. monocytogenes* ATCC 19115 presented higher inhibition halo sizes from the effect of *Eryngium comosum* Delaroche F extracts (Table 1).

Cuphea aequipetala hispida (Cav.) *Koehne* extracts have shown close to inhibition halos from other medicinal plants (*Terminalia arjuna* and *Camellia sinensis*) against *E. coli* and *S. aureus* (11–18 mm); likewise, *Moringa oleifera* leaf extracts reported by Semeneh & Kang [64] had very similar results in ethanol and aqueous extracts (8.0–8.5 mm) against the same strains and *L. monocytogenes*. However, there are more intense antibacterial effects from other medicinal plants; besides, many pieces of scientific evidence for *Cuphea aequipetala* var. *hispida* (Cav.) Koehne have not been reported. Extract of *Cuphea aequipetala* could be suggested as a potential natural antibacterial agent for *E. coli* ATCC 25922 and *Staphylococcus* sp. because some of these two species strains were reported as multidrug-resistant microorganisms in equine hospitals. Even though *E. coli*–resistant species infections are less common, in particular, cross-transmissions in horses and risk of infection in equine coworkers were observed [65,57]. The present result of *Eryngium comosum* Delaroche F extracts has shown less antimicrobial effect than *E. foetidum* methanol and chloroform extracts over *E. coli* and *S. aureus* (18 and 15 mm, respectively)[66]. Antimicrobial activities found were in accordance with other reports of *Eryngium* species extracts over gram-negative or gram-positive strains, such as *Salmonella* species, *Str. pneumoniae*, and *L. monocytogenes* [13,66–69]. However, extracts made of 50% ethanol, which made them available for *in vivo* or *in vitro*, are more specific for animal probes and provide more scientific evidence from herbs as an alternative antibiotic or feed additive useful for horse nutrition [45]. These extracts could be proved more specific in further antimicrobial tests over keratoconjunctivitis etiological agents (*L. monocytogenes*) or other horse disease agents (*S. aureus* or *Salmonella* species) [16,40,41].

4. Conclusions

The extracts of *Cuphea aequipetala hispida* (Cav.) *Koehne* and *Eryngium comosum* Delaroche F are rich in TP and SP and considered as potential antimicrobial agents and additives for further animal health and nutrition. The effect of antimicrobial activities over reference strains related to equine infections tested is suggested as scientific evidence for further *in vitro* and *in vivo* animal tests, especially equines. Plant extracts are recommended for *in vitro* tests to explore the gas regulation or other tests related to animal nutrition.

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