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Research article

Antibacterial activity of plant extracts against *Streptococcus equi* subsp. *zooepidemicus* isolates from guinea pigs with lymphadenitis in Ecuador

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ABSTRACT

Lymphadenitis is a commonly occurring and contagious disease in guinea pigs caused by different pathogens, including *Streptococcus* sp., *Staphylococcus* sp., and *Corynebacterium* sp. This study aimed to characterize the bacteria isolated from pus extracted from abscessed mandibular lymph nodes of diseased guinea pigs in Ecuador in 2019 and evaluate the in vitro antibacterial activity of the total extracts of three plant species. Isolates were recovered from three diseased guinea pigs with Lymphadenitis on a farm in Imbabura, Ecuador province. The bacteria were characterized through microbiological, biochemical, and molecular tests as *Streptococcus equi* subsp. *zooepidemicus*. Furthermore, the susceptibility of *S. equi* subsp. *zooepidemicus* to three plant extracts belonging to the Asteraceae family, *Acmella ciliata, Bidens andicola, and Gazania splendens* collected in Ecuador, were assessed in vitro by the microdilution method. Our data indicate that all the evaluated extracts showed activity, with a Minimum Inhibitory Concentration (MIC) of 22.50 mg/mL for *Acmella ciliata*, 11.25 mg/mL for *Bidens andicola*, and 5.60 mg/mL for *Gazania splendens*. *Bidens andicola* extract showed the highest efficacy with a % inhibition of 63.90 at the highest tested concentration (45 mg/mL). This is the first report on the bioactivity of these plant species against *S. equi* subsp. *zooepidemicus*.

1. Introduction

The Caviidae family comprises approximately 23 species of South American rodents distributed in five genera. The guinea pig (*Cavia porcellus*) belongs to this family, is a mammal rodent native to the Andean region of Ecuador, Peru, Colombia, and Bolivia, domesticated 2500 to 3500 years ago [1]. It is crucial to produce meat with high nutritional value [2].

The guinea pig is a species of zootechnical interest that is attacked by infectious and parasitic diseases, which are associated with

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sudden changes in temperature, humidity, air currents, dirty pools, and changes in their diet [3]. These diseases are caused by bacteria, viruses, and fungi that cause the death of the animals [2]. Among the most frequent diseases are respiratory diseases (frequently caused by *Bordetella*, *Streptococcus*, and adenovirus), salmonellosis, chlamydiosis, pneumonia, and lymphadenitis [1,4,5]. The latter is a severe and persistent chronic disease observed in guinea pigs. It is distinguished by abscesses primarily in the lymph nodes, with a higher likelihood of affecting the cervical region [6,7]. A notable feature of this disease is the manifestation of abscesses in the lymph nodes, particularly in the cervical region. Given its gravity, appropriate attention and diligent management are necessary to ensure effective treatment and minimize its impact on the affected guinea pigs' overall health and well-being. Affected guinea pigs show weight loss, hunched posture, abnormal gait, sunken abdomen, dull coat, or respiratory distress and may become unresponsive to stimuli; it is well-established that bacterial transmission can transpire through various routes, including the oral route, skin contact, mucous membranes with minor abrasions, aerosol droplets, or even through genital contact [6,8]. These diverse modes of transmission highlight the versatility and adaptability of bacteria in their ability to infect and spread through different entry points. The most common conditions are respiratory and gastrointestinal [1,4]. The pathogens associated with lymphadenitis frequently include pyogenic bacteria *Streptococcus* sp., *Staphylococcus* sp., and *Corynebacterium* sp [2,5,9].

The most commonly used antibiotics by guinea pig producers to treat lymphadenitis are enrofloxacin, penicillin, and oxytetracycline [2]. Including antibiotics as a mechanism to cure infectious diseases led to a significant medical breakthrough, making them a fundamental tool in science that has significantly improved animal health. However, bacteria resistant to the antibacterial effect of these drugs have been reported [10], and many researchers have felt compelled to seek new strategies to develop effective drugs against bacteria and to identify new sources of antibiotics.

The geographic location and variety of microclimates allow Ecuador to be one of the most biodiverse countries [11,12]. Ecuador holds significant potential as a reservoir of bioactive molecules, which can serve either independently or as raw materials for the development of products targeting the pharmaceutical, nutraceutical, or agricultural industries. There has been a notable increase in scientific research on Ecuador's biodiversity in recent years. However, given the abundant biodiversity found in this country, the current phytochemical and pharmacological studies are still relatively limited [13].

Over centuries, ancestral wisdom has guided the diverse applications of plant species, particularly in treating both human and animal ailments [14,15]. Exploring plant derivatives in veterinary medicine represents an exciting frontier with significant potential for further exploration and innovation [16]. The antimicrobial effects of certain plant extracts have been studied, unveiling their potential to combat bacterial, fungal, and viral infections in animals. These natural compounds offer an alternative or complementary approach to traditional antibiotic therapies, potentially reducing the risk of resistance development and promoting sustainable treatment practices [17]. In addition, plant extracts have demonstrated insecticidal and acaricidal activities. These botanical agents can effectively repel or eliminate pests that afflict animals, such as fleas, ticks, mites, and other ectoparasites [16,18].

Among the different families of plants, we found the Asteraceae (Compositae) family, one of the largest and most diverse plant families, with 1620 genera and 23,699 species [19]. Plant species from this family has been used in traditional medicine due to their biological and pharmacological properties [20,21]. *Acmella ciliata* (Kunth) Cass., *Bidens andicola* Kunth, and *Gazania splendens* Lem. are members of this family and are used in the present investigation.

A. ciliata is a species native to northern South America, considered a weed. It has been used in traditional medicine to relieve dental pain, sore throats, gum infections, stomach acidity, and gastrointestinal disorders and to control gastritis, headaches, and diarrhea [22]. Previous research has reported its antibacterial [23], immunomodulatory, and antihistaminic activities [23] and its uses as a biocide [24,25].

B. andicola is a widely distributed species in the Ecuadorian Andes, it is native to Ecuador and is used by indigenous communities to treat urinary infections and kidney stones as a hepatic protector, cystitis, prostatitis, laryngitis anti-flu, and anti-rheumatic [26]. Its analgesic, antioxidant [27], anti-inflammatory [28], and antibacterial activities [29] have been reported.

G. splendens is an ornamental species. It is an introduce species in Ecuador. The Gazania genus has a role in popular medicine to prevent dental pain and spontaneous abortion and is incorporated into purgative preparations [30]. They also treat ear pain in babies and infertility [31].

This study aims to isolate and characterize the bacteria extracted from the lymph nodes of guinea pigs infected with lymphadenitis. Furthermore, the in vitro antibacterial activity of the total extracts of three species, *A. ciliata*, *B. andicola*, and *G. splendens*, was evaluated against, *Streptococcus equi* subsp. *zooepidemicus*. To date, we have not found reports about the effect of extracts of the three plants species selected in this study against *Streptococcus equi* subsp. *zooepidemicus*.

2. Materials and methods

2.1. Collection and taxonomic identification of plant material

The aerial parts of the plant species under study, *A. ciliata* (0°21′03.63″N 78°06′21.45″W), *B. andicola* (0°20′57.74″N 78°06′26.08″W), and *G. splendens* (0°20′56.33″N 78°06′26.08″W), were collected in the Imbabura province of Ecuador and taxonomically identified. Herbarium specimens of *A. ciliata* (No. 6736), *B. andicola* (No. 6734), and *G. splendens* (No. 6735) were deposited at the Herbarium of the Pontificia Universidad Católica del Ecuador Ibarra, Ecuador, under the codes No. 6736 for *A. ciliata*, No. 6734 for *B. andicola*, and No. 6735 for *G. splendens*. The specimens were collected with official authorization 006-2019-IC-FAU-FLO-DPAI/MAE.

2.2. Plant extraction

Samples of the collected plants were dehydrated using a dryer with air flow at 50 °C for 24 h. The dried and crushed plants were then extracted using dynamic maceration. The plant material was mixed with ethyl acetate at a ratio of 1:10 (m/v) and was constantly stirred at 250 revolutions per minute at room temperature for 24 h (3 times). The resulting extracts were filtered, combined, and then evaporated under reduced pressure until all the solvent was removed, and a dry extract was obtained [32].

2.3. Sample collection and isolation of bacteria

We examined *S. equi* subsp. *zooepidemicus* isolates from three guinea pigs with lymphadenitis on a farm in the Imbabura province in 2019. Veterinarians aspirate pus from abscessing mandibular lymph nodes of diseased animals. Subsequently, the samples were systematically examined using standard bacterial culture methods. Briefly, using sterile swabs, the pus was inoculated onto a 5 % (v/v) blood agar base and incubated at 37 °C ± 1 °C for 24 h. During this time, colonies with beta-hemolysis characteristic of pathogenic *Streptococcus* were observed. After 24 h, a single colony of the bacteria was taken with a bacteriological loop and inoculated onto a Petri dish containing 20 mL of Mueller-Hinton agar and incubated at 37 °C ± 1 °C for 24 h [33].

2.4. Characterization of bacteria

Bacterial isolates were characterized at both the species and subspecies levels through the evaluation of growth characteristics on Mueller-Hinton, examination of cell morphology via Gram staining, analysis of biochemical reactions, and identification of marker DNA sequences [34].

2.4.1. Microbiological characterization

We analyzed the bacteria after 24 h of growth in Mueller-Hinton agar. A sterile drop of distilled water was placed on a microscope slide, and an isolated colony was picked and placed on the drop of water, making circular movements. Once the slide dried, drops of crystal violet were added to cover the entire sample for 45 s. After this time, the slide was washed with running water. Drops of Lugol were added to cover the entire sample for 45 s, and the slide was rewashed with running water. Acetone was added for approximately 10 s and immediately washed with running water. Safranin was added drop by drop to cover the sample for 30 s, and the slide was rewashed with running water. Finally, the microscope slides were left to dry, and immersion oil was placed on the sample to observe it under the microscope. This process was carried out with isolated colonies from all Petri dishes. Gram-positive bacteria appear as dark blue to purple, while Gram-negative bacteria are stained red to pink [35].

2.4.2. Biochemical characterization: three tests described by McFarland (2003) [36] were used for the biochemical identification of *Streptococcus* strains

Motility test: It is used to determine whether a microorganism is mobile or immobile. Mobile bacteria have flagella, while immobile bacteria lack them. 1.5 g of SIM medium was dissolved in 50 mL of distilled water in a Boeco flask. After autoclaving the medium, 10 mL was dispensed into screw-capped test tubes, which were autoclaved again without tightening the caps. Once the tubes were autoclaved, they were left to cool vertically [37].

Hemolysis test: The culture media that favor the growth of the cocci are sheep blood agar, chocolate agar (solids) as well as thioglycolate broth (liquid), growth generally occurring after 18–24 h of incubation at a temperature of 37 °C, in blood agar the presence of hemolysis is characteristic. Some bacteria show beta hemolysis as well as the characteristic yellow pigment. For the test, a seed of the pure culture was carried out using a bacteriological loop on a blood agar plate, incubated for 24 h at 37 °C ± 1 °C [36].

Catalase test: This test is used to differentiate *Streptococcus* (−) from *Micrococcus* (+) and/or *Staphylococcus* (+). The catalase enzyme is present in most aerobic and facultative anaerobic bacteria that contain cytochrome, except mainly in the genus Streptococcus. A 4 % hydrogen peroxide drop was dropped onto a microscope slide, and the bacteria were placed on the drop. The production of bubbles indicates the presence of catalase [38,39].

Oxidase test: This test determines the presence of oxidase enzymes, and most Gram-positive bacteria are oxidase-negative. A colony was placed on the oxidase disk, and the results were observed after approximately 3 min [39].

Mannitol fermentation test: The bacteria were sown in mannitol salt medium, the medium was streaked on the surface, incubated for 24 h at 35 °C. The result would be considered positive, if there is the presence of growth and change in color. It is considered negative when there is no color change in the medium and it remains pink or reddish [36].

2.4.3. Molecular characterization

The analysis was conducted by aligning the 16S rRNA gene sequence with GenBank records from NCBI. The sequencing of the 16S rRNA gene took place at Zurita & Zurita Laboratories in Ecuador.

2.5. Determination of the biological activity of plant extracts by the microdilution method

Susceptibility of *S. equi* subsp. *zooepidemicus* to three plant extracts was assessed by the microdilution method. Bacteria were cultured in Mueller-Hinton broth for 24 h at 37 °C. The inoculum was adjusted using the McFarland scale, which requires an absorbance of 0.08–0.10 at a wavelength of 625 nm, and the observed value corresponds to a homogeneous suspension of 1.5×10^8

cfs/mL [40].

The Minimum Inhibitory Concentration (MIC) was evaluated using the microtiter broth dilution method in sterile flat-bottom 96-well polystyrene plates. To determine the MIC of the extracts, six serial dilution were employed, at concentrations of 45.00, 22.50, 11.25, 5.62, 2.81, and 1.41 mg/mL after 18 h growth. Negative controls (cells + Mueller-Hinton), positive controls (cells + Mueller-Hinton + antibiotics−enrofloxacin), vehicle controls (cells + Mueller-Hinton + ethyl acetate), and media controls (Mueller-Hinton) were included. Positive controls for antibiotics were prepared by mixing 15 μL of enrofloxacin and 95 μL Mueller-Hinton + cells. All tests were performed in triplicate. Optical density readings were obtained with a microplate reader at 650 nm at both 0 and 18 h after-inoculation. The reported results represented the MIC for growth at 18 h post-inoculation. The MIC was recorded as the lowest extract concentration that prevented bacterial growth on the appropriate agar plate after incubation. As previously described, Equation (1) was used to calculate the percent of inhibition [41].

$$\%\ \text{Inhibition} = \left[1 - \left(\frac{OD_{t18} - OD_{t0}}{OD_{vc18} - OD_{vc0}}\right)\right] \times 100 \quad (1)$$

where, OD_{t18} represents the optical density measured in the test well 18 h after inoculation. OD_{t0} is the optical density observed in the test well at the initial time of inoculation (0 h). OD_{vc18} indicates the optical density recorded in the vehicle control well 18 h after inoculation. OD_{vc0} denotes the optical density noted in the vehicle control well at the starting point of inoculation (0 h).

2.6. Statistical analysis

Results are expressed in the form of mean values, and each procedure was replicated three times. The results obtained in the experimental phase of the research were analyzed using the statistical program R project.

3. Results and discussion

3.1. Plant extracts

Total extracts in ethyl acetate were obtained from three plant species collected in Ecuador's Imbabura province. The extraction process yielded (mass of extract/mass of dry matter) 3.4 % of *A. ciliata*, 2.8 % for *B. andicola*, and 4 % for *G. splendens*.

3.2. Identification of the bacteria isolated from guinea pigs with lymphadenitis

All guinea pigs included in this study showed typical clinical signs of lymphadenitis, including hunched posture, abnormal gait, sunken abdomen, and enlargement or abscessation in lymph nodes of the neck [1,4].

The isolated bacterium from the lymph nodes of sick guinea pigs with lymphadenitis were subjected to microbiological, biochemical, and molecular analysis. After 24 h of incubation on Mueller-Hinton agar, the bacterial colonies exhibited specific characteristics. The colonies were tiny, measuring approximately 1–2 mm in diameter, and had a circular shape. They appeared translucent and displayed a mucoid texture. Furthermore, each colony exhibited a broad halo of beta-hemolysis. Gram staining indicated that the bacterial morphology was characterized as *Gram-positive cocci* in chains. Biochemical tests confirmed these results, including motility, where bacterial growth was only observed in the inoculation line; in the Catalase test, the isolated bacteria showed no bubbles on the slide, while in the Oxidase test, no color change was observed after the reaction. In the mannitol fermentation test, the medium retained its reddish color with no discernible alteration. According to the molecular identification, the isolated bacteria were classified as *Streptococcus equi* subsp. *zooepidemicus*, it was corroborated through the comparison of the 16s rRNA gene sequence with the GenBank of NCBI (See Supplementary Material).

Streptococcus equi subsp. *zooepidemicus* is recognized as a zoonotic pathogen [33], a facultative pathogen capable of affecting both animals and humans [1]. It is the most important bacterium among the main infectious agents affecting guinea pigs raised commercially. Research reveals its involvement in cases of lymphadenitis in Cusco, Peru with frequencies of 70 and 100 % in guinea

Table 1
Antibacterial activity of *A. ciliata*, *B. andicola* and *G. splendens* extracts against *S. equi* subsp. *zooepidemicus*.

Concentration/Control	% Inhibition		
	Acmella ciliata	*Bidens andicola*	*Gazania splendens*
45.00 mg/mL	13.70 ± 0.2	63.90 ± 0.07	20.30 ± 0.19
22.50 mg/mL	4.44 ± 0.4	56.31 ± 0.41	15.56 ± 0.18
11.25 mg/mL		34.73 ± 0.30	12.26 ± 0.13
5.62 mg/mL			2.31 ± 0.20
Control (+) enrofloxacin	–	–	–
Control (−)	+	+	+
Vehicle control	+	+	+
Media control	+	+	+

Data are expressed as mean ± SD (n = 3). Significant differences were found.

pigs sampled with lesions [42]. However, other bacterial agents have also been identified, including *Salmonella enterica serovar Typhimurium*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Micrococcus* spp, *Streptococcus* spp and *Corynebacterium* spp [43]. In addition to lymphadenitis *S. equi* subsp. *zooepidemicus* has been implicated in various other pathologies, including otitis, hemorrhagic pneumonia, peritonitis, chronic nephritis, metritis, abortions, and dermatitis [6,7,44]. *S. equi* subsp. *zooepidemicus* is classified as a Lancefield's group C streptococcus, as described by Timoney et al., in 1997 [45]. This gram-positive, exhibiting β-hemolysis, features an antiphagocytic capsule (M-like antigen) and generates various exotoxins, such as hyaluronidase, a protease, and streptokinase [45].

3.3. Determination of biological activity of plant extracts by microdilution method

The three plant extracts used in the present investigation were tested at six different concentrations, 45.00, 22.50, 11.25, 5.62, 2.81, and 1.41 mg/mL. *A. ciliata, B. andicola, and G. splendens*, showed inhibition of the growth of *S. equi* subsp. *zooepidemicus* (see Table 1). The total extract of *A. ciliata* inhibited the growth of the bacteria by 13.70 % at a concentration of 45.00 mg/mL and 4.44 % at a concentration of 22.50 mg/mL. No inhibition of bacterial growth was reported with the four lowest concentrations tested. The total extract of the plant *B. andicola*, native to Ecuador, reported the highest inhibition percentages, inhibiting the growth of *S. equi* subsp. *zooepidemicus* by 63.90 %, 56.31 %, and 34.73 % at 45.00, 22.50, and 11.25 mg/mL concentrations, respectively. The total extract of *G. splendens* inhibited the growth of the *S. equi* subsp. *zooepidemicus* bacteria at the four highest concentrations tested, with inhibition of bacterial growth of 20.30 % at 45.00 mg/mL, 15.56 % at 22.50 mg/mL, 12.26 % at 11.25 mg/mL, and 2.31 % at 5.60 mg/mL. No inhibition of bacterial growth was reported at concentrations of 2.83 and 1.41 mg/mL.

The positive control enrofloxacin inhibited the growth of the bacteria, whereas the vehicle control (ethyl acetate), negative control, and medium control did not exhibit inhibitory effect on the growth of *S. equi* subsp. *zooepidemicus.*

The Minimum Inhibitory Concentration (MIC) displayed for the total extract of *A. ciliata* was 22.50 mg/mL, for the extract of *B. andicola* was 11.25 mg/mL, and for the total extract of *G. splendens* was 5.60 mg/mL against *S. equi* subsp. *zooepidemicus.*

The pharmacological potential of *A. ciliata* against other microorganisms has been reported. Studies with *A. ciliata* extracts showed inhibition of the growth of *E. coli, S. aureus, S. epidermidis*, and *C. albicans*; essential oils obtained from this species have demonstrated inhibition of the growth of gram-positive bacteria *S. aureus* and *S. epidermidis* at concentrations of 25 and 15 mg/mL. However, lower activity was reported against gram-negative bacteria [23]. Endophytic fungi and bacteria isolated from *A. ciliata* have been reported to be potentially used as biocontrol agents against *F. decemcellae* and *C. gloeosporioides* [24]. Preliminary phytochemical studies report the presence of alkaloids, flavonoids, coumarins [46], phenolic compounds, glucosides, and terpenoids [25], which could be responsible for the biological activity of *A. ciliata*. The species *B. andicola* has shown analgesic, antioxidant [27], and anti-inflammatory activity [28]; furthermore, the antibacterial activity of the ethanolic extract against *S. aureus* has been also reported [29]. *B. andicola* has been shown to contain active components such steroids, flavonoids, tannins, saponins, alkaloids, cardiotonic glucosides, anthocyanins [29]. Within the genus Gazania, the presence of hepatoprotective and antioxidant properties has been documented [30,31]. However, no available reports have been found regarding the biological activity or phytochemical studies conducted on total extracts of the *G. splendens* specie.

Previous research has documented the effectiveness of plant extracts, specifically essential oils, against *S. equi* subsp. *zooepidemicus*. For instance, the essential oil derived from *Thymus vulgaris* exhibited activity against *S. equi* subsp. *zooepidemicus*, with a minimum inhibitory concentration (MIC) of 0.07 mg/mL. Thyme extracts contain antimicrobial compounds such as thymol and carvacrol, which have demonstrated potent antimicrobial effects against a range of bacteria [47]. Furthermore, the essential oil from the leaves of *Lavandula dentata*, commonly known as lavender, displayed a 90 % inhibition against the *S. equi* subsp. *zooepidemicus* at concentrations of 2048 and 4096 μL/mL [48]. Furthermore, an investigation conducted on a population of horses in Iran highlighted a concerning trend of antibiotic resistance among *S. equi* isolates. The study revealed a relatively high frequency of antibiotic-resistant strains, with resistance observed against amoxicillin and penicillin [48].

S. equi subsp. *zooepidemicus* is a facultative pathogen with the potential to impact both animals and humans. It has been identified as the cause of infections in many species, including horses, pigs, ruminants, guinea pigs, monkeys, cats, and dogs [49,50]. Although cases of zoonotic transmission are infrequent, there have been reported instances of human patients becoming infected with *S. equi* subsp. *zooepidemicus* and subsequently developing serious medical conditions. These complications include aortic aneurysm, septic arthritis, pneumonia, and meningitis. In this context, this investigation presents promising results, as it demonstrates the potential of natural products to inhibit the growth of a concerning bacteria [49–52].

Antimicrobials from medicinal plant extracts can be applied alone or in combination with standard antibiotics to achieve bactericidal synergism [53,54] to broaden the antimicrobial spectrum, prevent the emergence of drug-resistant mutants, and minimize toxicity.

Despite all the benefits associated with using antimicrobials from medicinal plant extracts, there are some challenges when using them in a practical setting, including the absence of standardization in treatments and difficulties in reproducing the consistent composition of plant extracts. To overcome these limitations, studies on the pharmacology of medicinal plant-derived compounds must be studied to achieve the standardization of the therapeutic regimens and the characterization of bioactive compounds to establish quality control procedures [55].

4. Conclusions

All the guinea pigs from this study presented symptoms of lymphadenitis. The isolated bacteria were characterized as *S. equi* subsp. *zooepidemicus*. The findings of this study present compelling evidence regarding the antibacterial properties exhibited by three distinct

plant species collected in Ecuador and belonging to the Asteraceae family. These plants have demonstrated their effectiveness in combatting *S. equi* subsp. *zooepidemicus*, a harmful microorganism that leads to lymphadenitis in guinea pigs; they exhibited a Minimum Inhibitory Concentration (MIC) of 22.50 mg/mL for *Acmella ciliata*, 11.25 mg/mL for *Bidens andicola* and 5.60 mg/mL for *Gazania splendens*. We reported for the first time the bioactivity of these species against *S. equi* subsp. *zooepidemicus*. Additional investigations will be focused on bio-guided studies to isolate and characterize the active compounds responsible for the antibacterial effects. Moreover, it is crucial to evaluate the safety and efficacy of these compounds in vivo, ensuring they can be utilized as potential treatments in the future. Finally, our findings add to the scientific evidence regarding the therapeutic potential of medicinal plants from Ecuador.

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CRediT authorship contribution statement

Yadira F. Ordóñez: Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Estefanía Miranda:** Investigation. **María Fernanda López:** Investigation. **Paola E. Ordóñez:** Writing – review & editing, Visualization, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25226.

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