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Antioxidant, antibacterial, and antifungal activities of *Tetraglochin cristata* (Canghy) extracts from the Peruvian Andes

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Abstract

Medicinal plants have gained visibility for their bioactive compounds with antioxidant, antibacterial, and antifungal properties that can be used for various applications such as in the pharmaceutical, food, and biological control industries. Peru is a country rich in flora, and it has a wide variety of plant species, however, the study of bioactive compounds with pharmacological potential is scarce. Therefore, the objective of the present study is to evaluate the antioxidant, antibacterial and antifungal activity of the extract of fresh leaves of *Tetraglochin cristata* known in the Peruvian highlands as Canghy. Canghy samples were collected in the Arequipa and Cusco regions to compare the variability of properties due to environmental and altitude differences. Extracts were tested against two types of bacteria, *Staphylococcus aureus* and *Escherichia coli*, and two fungi, *Candida albicans* and *Penicillium commune*. Antioxidant activity was evaluated using the DPPH method. The results demonstrated high antioxidant activity, with a half-maximal inhibitory concentration (IC_{50}) of (92.3±1.3) µg/mL for the Cusco sample and (98.2±1.7) µg/mL for the Arequipa sample. Furthermore, the extracts effectively inhibited *P. commune*, showing high antifungal activity. This study highlights the pharmacological potential of Canghy bioactive compounds and the importance of geographical diversity in the variability of these properties.

1. Introduction

Medicinal herbs, widely used in alternative medicine, offer a diverse array of bioactive molecules with multiple applications (Adane *et al*., 2020; Tlili and Sarikurkcu, 2020). These practices are particularly common among pregnant women and vary according to cultural beliefs and traditions (Jansen *et al*., 2021). Herbs are used in the cosmetic industry, in the treatment of cardiovascular diseases, atherosclerosis, hypertension and as potential therapies for SARS-CoV-2 infection (Cho *et al*., 2020; Shaito *et al*., 2020; Upadhyay *et al*., 2020). Phenolic compounds, such as phenolic acids and flavonoids, exhibit antioxidant, antibacterial, anti-inflammatory, and anticancer properties, capable of neutralizing free radicals (Benabderrahim *et al*., 2019). Free radicals, which are produced during aerobic metabolic processes, can cause oxidative stress at high concentrations. Plant antioxidants, however, can neutralize these radicals (Kohli *et al*., 2019).

Plants exhibit evolutionary adaptations to their environment, with variations in their characteristics depending on ecological factors, altitude, length, and leaf size (Liu *et al*., 2020). Furthermore, it has been established that the number of active components in plants can vary due to environmental conditions, which directly or indirectly affect the content of secondary metabolites and biological activities (Jugran *et al*., 2016; Adhikari *et al*., 2022).

In addition, infectious microorganisms pose a threat to public health, causing potentially fatal diseases and developing resistance to commercial drugs. *Escherichia coli* bacteria have been reported to cause catheter-associated urinary tract infections (Pavan *et al*., 2017), while *Staphylococcus aureus* can cause serious skin and soft tissue infections (Cong *et al*., 2020). Some fungi, such as *Penicillium commune*, can deteriorate foods such as cheese since it is the main cause of negative effects such as change in flavor and discoloration, as well as degrading hydrocarbon compounds (Hansen *et al*., 2003; Cheong *et al*., 2014; Esmaeili and Sadeghi, 2014).

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Meanwhile, the fungus *Candida albicans* is a common cause of vaginal infections, significantly affecting the quality of life of women (Moreno *et al*., 2018). Consequently, it is crucial to research new antimicrobial molecules (Alam *et al*., 2020).

Tetraglochin cristata (Britton) is an herbaceous plant widespread in South America, in the regions of Chile, Bolivia, Argentina and Peru, growing between 3000 and 4500 m altitude (Cialdella and Pometti, 2017; Bek *et al*., 2023; Fernandez and Alli, 2023). This plant commonly called "Canghy" has various uses, from firewood to food for llamas, alpacas and vicunas in Peru (Dueñas-Huanca *et al*., 2022; Galan de Mera *et al*., 2022). It is considered a shrub that grows 20 to 80 cm high, with thick branches densely arranged mainly at the base, macroblasts and brachy blasts covered by the base covered by petioles; glabrous sheaths, densely hairy on the margin; petioles glabrous. Leaves of macroblasts with persistent petiole-rachis axis about 0.1 to 1cm, and spines 1 to 3 cm long, flowers solitary and axillary on brachy blasts, stamens not seen (Cialdella and Pometti, 2017). However, its pharmacological potential remains largely unexplored. This study aimed to evaluate the antioxidant, antibacterial, and antifungal activities of *Tetraglochin cristata* leaves collected from two different regions in Peru, Arequipa and Cusco, and to assess the impact of geographical variation on these activities.

2. Materials and methods

2.1 Botanical material and extract preparation

In 2020 and 2021, with necessary permits, the leaves of *Tetraglochin cristata* (Britton) (Figure 1) were collected from the Reserva Nacional de Salinas y Aguada Blanca, Tambo Cañahuas- Arequipa (3956 masl, -16° 0.868'N, -71° 22.464'E), and the rural community of Acco Acco Phalla-Sicuani-Cusco (3570 masl, 14° 13.68S', 71°11.63'W). The plant was taxonomically identified by the Michael Owen Dillon Institute of Science (MODI).

Figure 1. *Tetraglochin cristata* plant collected from a) Arequipa and b) Cusco regions.

The collected leaves were washed with distilled water to remove soil residues and other contaminants. A subsample of eight leaves was dried in the shade for microscopic analysis. The remaining leaves were ground and macerated in distilled water (1:10 w/v) for 24 hours at room temperature. The resulting suspension was filtered to separate the aqueous extract from the plant material. The extract was stored at -10°C and subsequently lyophilized in a CHRIST Alfa 1-2 LDplus freeze-dryer for 48 hrs. The lyophilized extract was labeled and stored at -7°C for further analysis.

2.2 Leaf characterization by scanning electron microscope

Eight fully developed *Tetraglochin cristata* leaves, characteristic in size and shape, were randomly selected from the midsection of the plant. Specifically, 8 leaves were collected from the Arequipa region and 8 from the Cusco region. The selected leaves showed no signs of damage or deterioration. The fresh leaves were processed for scanning electron microscopy (SEM). For this purpose, samples were mounted on aluminum supports using double-sided carbon tape and coated with a conductive gold layer of approximately 20 nm thickness using a 2-minute sputtering process in a Quorum Q150R ES Plus metallizer. The metallized samples were observed in a Thermo Scientific Scios 2 Dual Beam SEM operating at an acceleration voltage of 10 to 15 kV. Electron micrographs were acquired at different magnifications using a secondary electron detector (SE) and the ETD detector in compound mode (BF+SE) to evaluate the general morphology. The working distance was adjusted between 10 and 35 mm to optimize resolution and depth of field. Morphometric measurements were performed to quantify the length (L) and width (W) of the leaves.

2.3 Determination of antioxidant activity by UV-visible spectroscopy

The antioxidant activity of *Tetraglochin cristata* was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical neutralization technique described by (Alara *et al*., 2018).

DPPH is a stable radical with an intense purple color and the sample is read in a maximum UV-VIS spectrum at 515 nm (Khalid *et al*., 2022). This free radical behaves like reactive oxygen species (ROS); the intensity of the purple color decreases as it is neutralized by antioxidants, changing to a yellow color as it gains electrons from the antioxidants.

A solution of 100 µM of DPPH in methanol was prepared, the mixture was shaken vigorously, and the absorbance changes of the samples were measured after 30 minutes in a UV-Vis spectrophotometer (Thermo Scientific Evolution 220) at 515 nm (Wong and Tan, 2020; Khalid *et al*., 2022). Methanol was applied as a

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blank. The experiments were repeated at least three times. The percentage of DPPH inhibitory activity is estimated with the following equation:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The inhibition coefficient (IC_{50}) value was calculated by determining the concentration of the sample at which the DPPH radical absorbance was reduced by 50%, as determined by the linear regression of the DPPH absorbance curve.

2.4 Determination of antimicrobial activity

The evaluation of the antibacterial and antifungal activity was performed at the Laboratory of Applied Physics in Medicine and Biology at the Universidad Nacional de San Agustín de Arequipa-Peru and the evaluation of the bacterial strains was repeated with cultures that were collected as pure culture from the Universidad Federal Grande Dourados-Brazil.

The evaluation of the antimicrobial effects of Canghy leaves was carried out against two bacterial strains and two fungi. The data were obtained from three individual experiments (n = 3).

2.4.1 Antibacterial activity

The antibacterial activity was obtained from a Gram-positive bacterial strain *Staphylococcus aureus* (ATCC BAA-1708) and a Gram-negative *Escherichia coli* (ATCC 33876) used for the evaluation of the antibacterial effects of each extract. The well diffusion method was used (Subramaniam *et al*., 2020).

In a Petri dish was placed the Müller-Hinton Agar medium (15 ml approx.) in this the bacteria were seeded; four perforations were made to the agar to place 200 μL of the aqueous extract of *Tetraglochin cristata* using a micropipette.

The plates were inverted and incubated at 37°C for 24 hrs for optimal bacterial growth. Antibacterial activity was observed with inhibited growth of the microorganisms giving a clear and distinct zone of inhibition around the discs. Finally, the diameter of the inhibition zone was measured.

2.4.2 Antifungal activity

The antifungal potential of *Tetraglochin cristata* against a single-cell fungus *Candida albicans* (ATCC 10231) and the multicellular fungus *Penicillium commune* was investigated using the well diffusion method. The fungi were seeded by filling in Petri dishes containing sterile Potato Dextrose Agar (PDA), four punctures were made to the agar and then 200 μl of *Tetraglochin cristata* extract was placed, those containing *Candida albicans* were incubated at 37°C for 48 hours and those containing *Penicillium commune* were incubated at 25°C for 5 days.

2.5 Statistical analysis

Data were presented as mean ± standard deviation (SD) from at least three independent experiments. Statistical comparisons between the two groups (*Tetraglochin cristata* from Arequipa and Cusco) were performed using an unpaired t-test to compare mean values, with significance set at $p < 0.05$. Effect sizes were calculated to provide additional insight into the magnitude of differences between groups.

3. Results and discussion

The microscopic image obtained from SEM analysis indicates that the average size of the eight leaves of *Tetraglochin cristata* grown in Arequipa is 3.637 mm long and 0.500 mm thick, with a rough surface and a fold in the central part (Figure 2). In contrast, the leaves of *Tetraglochin cristata* grown in Cusco are slightly larger, measuring 3.693 mm in length and 0.544 mm in thickness, with a smooth surface and a fold in the central part.

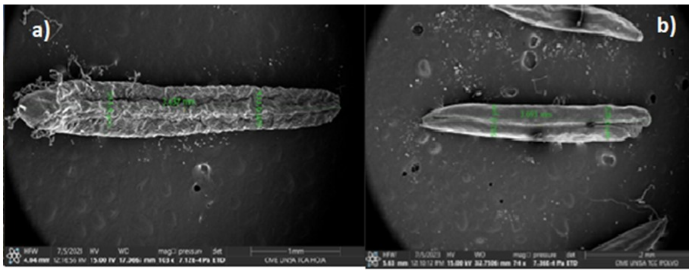

Figure 2. Morphological characterization of a *Tetraglochin cristata* leaf using SEM, plant collected from a) Arequipa and b) Cusco regions.

The size of the leaves of *Tetraglochin cristata* appears to reflect the plant's adaptation to its growing environment. The average leaf size varies with altitude, with plants grown at higher altitudes, such as in Arequipa, exhibiting smaller leaves compared to those at lower altitudes, like in Cusco (Liu *et al*., 2018; Liu *et al*., 2020). This observation is consistent with the higher altitude and solar irradiation in Arequipa. Climate plays a significant role in determining leaf size, as over 50% of the variation in stomata length is attributed to climate factors (Tian *et al*., 2016). Thus, leaf size in the same species grown at different altitudes, climates, and solar irradiance varies and decreases to adapt to the environmental conditions prevalent in high-altitude environments.

The variation in leaf size between the Arequipa and Cusco samples may not only reflect adaptation to different environmental conditions but could also be associated with differences in the accumulation of secondary metabolites responsible for antioxidant and antimicrobial activities. Although speculative, the larger leaves in the Cusco sample might suggest a higher capacity for photosynthesis and potentially greater production of these bioactive compounds. However, further studies are needed to establish a direct correlation between leaf size and the observed variations in biological activities.

The results of the present study demonstrate the effect of altitude on the morphology of *Tetraglochin cristata* leaves. Additionally, there was a marked variation in active metabolites, antioxidants, and antimicrobial activity among plant samples collected at different altitudes (3570-3956 m), habitats, and environmental conditions.

The antimicrobial activity of a plant depends on the type of microorganisms against which the antimicrobial activity is tested (bacteria or fungi) (Abdelkhalek *et al*., 2020). Regarding the antibacterial activity, the extract of *Tetraglochin cristata* leaves collected in Cusco and Arequipa did not show any inhibition halo against the bacteria under study. The bactericidal efficacy was determined by the zone of inhibition around the well. As for bacteria, the extract is not very sensitive against *E. coli*, showing discontinuity in the striations of the microorganism as observed by the formation of some diffuse colonies around the halos containing the extract under study (Figure 3).

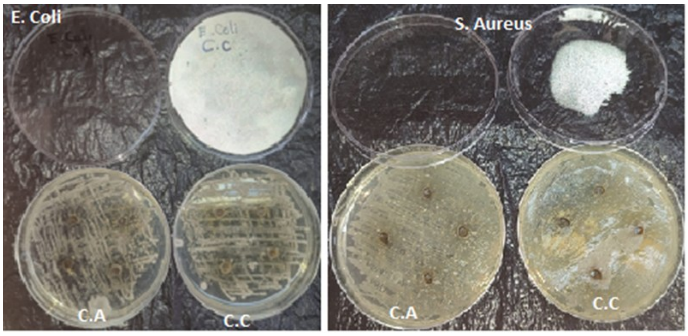

Figure 3. Antibacterial activity of samples grown in Cusco and Arequipa. On the left, the extract against *E. coli* and on the right against *S. aureus*.

In reference to *S. aureus*, it was observed that the extract collected from the Arequipa and Cusco regions did not inhibit the development of gram-positive bacteria, showing the growth of colonies around the halos containing the study extract, with greater continuity noticeable in the samples from the Cusco region.

In addition, the antifungal activity of the extract grown in Arequipa and Cusco against the unicellular fungus *C. albicans* did not show any inhibition (Figure 4). According to the studies reported on *Tetraglochin Andina* which belongs to the same family as *Tetraglochin cristata* did show relevant antifungal activity against *C. albicans* (Moreno *et al*., 2018). This result shows that plants of the same family have different properties.

However, the extracts showed excellent fungicidal activity against the fungus *P. commune* (Figure 4). Table 1 shows the zone of inhibition for the extracts. A significant difference (p = 0.03) indicated greater antifungal activity in the Cusco extract compared to Arequipa. The antioxidant activity, measured by the DPPH assay, showed a significant difference between the regions, with higher potential in the Cusco extract. This suggests that the geographical origin of the plant may influence its antifungal properties. The fungicidal activity of *Tetraglochin cristata* extracts against *P. commune* was notable, with the Cusco sample showing a larger inhibition zone compared to the Arequipa sample. This suggests that the extract has potential as a natural antifungal agent, particularly in food preservation. The lack of significant antibacterial activity against *S. aureus* and *E. coli* could be due to the specific composition of secondary metabolites in the extracts, which may not be effective against these bacteria. Further studies are needed to identify the specific compounds responsible for the observed activities and to explore their mechanisms of action.

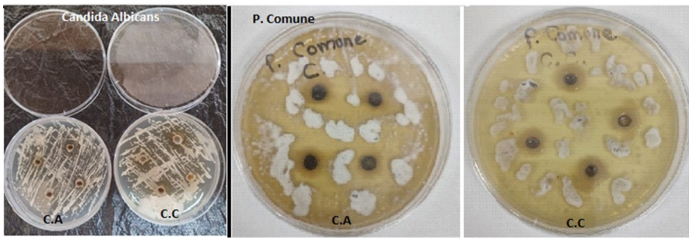

Figure 4. Antifungal activity of the extracts grown in Cusco and Arequipa, on the left the extract against the fungus *Candida albicans* and on the right against *P. commune*.

Table 1. Inhibition zone (mm) against the fungus *P. commune* by *Tetraglochin cristata* extracts. TC stands for *Tetraglochin cristata*.

Fungus	Inhibition zone (mm)	
P. commune	TC Arequipa	40.63±3.25
	TC Cusco	43.13±3.47

Values are presented as mean±SD.

Cheese is known to be very susceptible to fungal growth, which can alter its production and consumption, and some fungi could have serious health consequences for consumers. Fungal growth in cheese is largely due to its ability to grow at refrigeration temperatures, low oxygen concentrations, low pH, and low water activity (Neri *et al*., 2017). To prevent fungal spoilage, antifungal compounds are incorporated. In the last decade, there has been a consumer demand for food products containing

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natural compounds, leading to the direct application of antimicrobial agents through various techniques, such as spraying, coating, or immersion, to prevent the growth of fungi that deteriorate cheese (Torrijos *et al*., 2022). *Penicillium commune* is one of the most common *Penicillium* species known to cause cheese spoilage. For this reason, *Tetraglochin cristata* can serve as a natural preservative to prevent the growth of *P. commune* in cheese and can also be evaluated for use as a shelf-life enhancer in cheese by direct application as an aqueous solution on the surface of the cheese or the manufacture of an edible antifungal film containing the extract.

Table 2 illustrates the antioxidant potential of *Tetraglochin cristata* based on the DPPH method. The plant collected in Arequipa and Cusco showed a dose-dependent activity in the inhibition of the DPPH radical. However, the plant grown in Cusco exhibited a higher capacity to eliminate the DPPH radical by hydrogen donation, demonstrating through the highest concentration tested (200 µg/mL) a 95.4% inhibition of the DPPH radical, while the extracts of the plant grown in Arequipa showed a 92.5% inhibition of DPPH at the same concentration. In addition, the concentration to inhibit 50% of the DPPH radical (IC50) was also calculated. The antioxidant activity of *Tetraglochin cristata* extracts, as measured by the DPPH assay, was influenced by the geographical origin of the plant. The antioxidant activity of *Tetraglochin cristata* extracts, as measured by the DPPH assay, showed a significant difference between the Arequipa and Cusco samples ($p = 0.03$), with the Cusco extract exhibiting a higher antioxidant potential compared to the Arequipa extract. This variation could be attributed to differences in environmental conditions, such as altitude, temperature, and solar irradiation, which can affect the synthesis and accumulation of secondary metabolites (Liu *et al*., 2020). The higher antioxidant potential of the Cusco sample suggests a higher content of phenolic compounds, which are known to contribute to antioxidant activity (Benabderrahim *et al*., 2019). According to many researchers, diets rich in antioxidants neutralize free radicals, which improves health. Therefore, the antioxidant activity of a biological material has been found to be related to the phenolic content of the material (Vidal-Gutierrez *et al*., 2020; Rumpf *et al*., 2023).

Table 2. Antioxidant activity of *Tetraglochin cristata* extracts from Arequipa and Cusco, measured by DPPH assay (IC_{50} values in µg/mL). TC stands for *Tetraglochin cristata*.

Treatment	
TC Arequipa	98.2± 1.7
TC Cusco	92.3±1.3

Values are presented as mean±SD. Indicates a significant difference compared to Arequipa ($p < 0.05$).

The observed geographical variation in the bioactivity of *Tetraglochin cristata* extracts highlights the importance of considering environmental factors in the pharmacological evaluation of plants. Altitude, in particular, has been shown to influence the biosynthesis of secondary metabolites in plants, potentially leading to differences in their pharmacological properties (Adhikari *et al*., 2022). Future studies should investigate the chemical composition of the extracts from different regions to identify the compounds responsible for the observed activities.

The present study is the first to show the antioxidant activity of *Tetraglochin cristata*, so the findings are relevant. This indicates that the plant could prevent or stop the formation of a chain of reactive oxygen species, which could prevent this reactive species from damaging macromolecules and human genetic material.

The findings suggest that this plant could be an excellent source of extracts or phenolic compounds with high antioxidant potential. This could be an important option for the pharmaceutical sector to develop new natural strategies to combat oxidative stress, such as natural phytopharmaceuticals. Due to its unique cultivation techniques and exceptional resistance to environmental and temperature conditions, this plant is a potential domestic crop for the production of specific industrial metabolites.

4. Conclusion

This study evaluated the antioxidant, antibacterial, and antifungal activities of *Tetraglochin cristata* leaves collected from Arequipa and Cusco, Peru. The results indicate that the leaves possess notable antioxidant and antifungal activities, with the Cusco sample exhibiting higher potency. In contrast, no significant antibacterial activity was observed against *S. aureus* and *E. coli*. Notably, the extracts demonstrated excellent antifungal activity against *P. commune*, making *Tetraglochin cristata* a promising natural agent for controlling fungal growth, particularly in food preservation. The geographical origin of the plant significantly influenced the antioxidant and antifungal activities, suggesting that environmental factors such as altitude and climate play a crucial role in the biosynthesis of secondary metabolites. These findings highlight the potential of *Tetraglochin cristata* as a source of natural antioxidants and antifungal agents, particularly for applications in food preservation and pharmaceuticals. Future research should focus on identifying the specific compounds responsible for these activities and exploring the ecological and genetic factors that influence their production.

Conflict of interest

The authors declare no conflict of interest.

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