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Characterization of bioactive compounds, antioxidants and antimicrobial properties of *Allagoptera leucocalyx*

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ABSTRACT

Allagoptera leucocalyx is a non-studied tropical fruit distributed in the Bolivian Chiquitania region. This study aimed to investigate its bioactive compounds as well as its antioxidant and antimicrobial properties to encourage its consumption and explore potential applications in various industries. The bioactive compounds: polyphenols, flavonoids, and anthocyanins were determined by the Folin-Ciocalteu, AlCl₃, and differential pH methods. Antioxidant activity was assessed by the DPPH $^{\bullet}$ and ABTS $^{\bullet}$ assays, while antimicrobial activity by turbidimetry and antibiogram. The highest results obtained revealed significant levels of polyphenols (209 \pm 12 mg GAE/100 g), and flavonoids (7.9 \pm 0.1 mg QE/100 g). Moreover, the fruit exhibit antioxidant activity (9.3 \pm 0.2 μ mol Trolox/g by ABTS $^{\bullet}$ and 5.9 \pm 0.2 μ mol Trolox/g by DPPH $^{\bullet}$) and antimicrobial activity against Shigella sp. In conclusion, these promising findings suggest that this fruit could be utilized in the development of new products within the cosmetic, food, and pharmaceutical industries.

1. Introduction

In recent years, the consumption of plant-based foods, particularly fruits, has increased in various countries, since they have been shown to have beneficial effects on health, contributing to the prevention of degenerative processes that cause cancer, diabetes, neurodegenerative diseases, cardiovascular dysfunctions, obesity, hypertension, among others. These beneficial effects of fruit consumption have been associated with the biological properties of bioactive compounds (Faustino et al., 2019; Maqsood et al., 2020).

Bioactive compounds refer to molecules that confer health benefits beyond their nutritional value. These compounds could exhibit a range of advantageous properties: antimicrobial, anticancer, antiviral, antihypertensive, antioxidant, anti-inflammatory, vasodilator, and healing attributes (Pérez et al., 2021). The most common bioactive compounds present in fruits are vitamins C (ascorbic acid), vitamin E (tocopherols and tocotrienols), carotenoids, polyphenolic compounds, and especially flavonoids. Several studies have indicated that phenolic and flavonoid compounds play a more significant role in the antioxidant capacity of fruits compared to vitamin C, carotenoids, and other constituents. Consequently, polyphenolic compounds have emerged as focal points in the investigation of natural antioxidant compounds (Baquero et al.,

2016).

The presence of bioactive compounds in fruits, along with their associated biological activities, holds substantial significance across diverse sectors. In the food industry, these compounds find applications as natural additives, colorants, preservatives, and nutraceuticals (Baquero et al., 2016). In the cosmetic industry, they serve as pivotal active ingredients in cosmetic formulation, and in the medicinal and pharmaceutical sectors, these compounds play a role in the development of antibiotics and various other therapeutic agents (Guevara et al., 2019; Sarkar et al., 2022).

The growing consumer preference for products with minimal chemical synthesis inputs, which ensure better health and nutrition, has led to an increasing demand for these bioactive-rich fruits. This trend has particularly gained attention in tropical countries like Brazil, Thailand, and Indonesia where researchers have focused their efforts on characterizing different tropical fruits and assessing their biological properties for industrial uses (Kumoro et al., 2020; Sviech et al., 2022).

Bolivia, located in South America, possesses a rich diversity of tropical fruit species, particularly in the Chiquitania region (Coimbra Molina, 2016). However, these fruits have not been extensively studied or characterized due to limited research in Bolivia, which has primarily focused on Andean products (McNeish, 2002). Consequently, these

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tropical fruits are underutilized and little-known by both the population and the scientific community. Therefore, it is important to study and characterize these fruits, as they may serve as abundant sources of bioactive compounds and contribute to the development of new products, thereby enhancing commercial opportunities and improving the quality of life for communities in the tropical region.

Allagoptera leucocalyx, commonly known as motacuchí in the Chiquitania region, is an evergreen palm species widely distributed in the northeast and center-east of Bolivia, the east of Paraguay, the centersouth of Brazil, and the north of Argentina (Moraes, 2009). This palm typically reaches heights of 1.5 to 2 m and features a relatively short underground stem crowned with upright leaves measuring up to 1.5 m in length. The plant produces ovoid fruits that are orange-yellow and can reach dimensions of up to 3 cm in length and 2 cm in diameter (Fig. 1). Both the fruit and the seed are edible. Additionally, the leaves of A. leucocalyx are utilized in the creation of various handicrafts, such as bags, hats, utensils, brooms, and mats, which contribute to the local economy (Coimbra Molina, 2016). Furthermore, the juice extracted from the young shoots of this plant is used in traditional medicine to treat ear and digestive system diseases. Therefore, A. leucocalyx holds the potential to emerge as a valuable fruit species since it is available throughout the entire year and can grow in warm temperate, tropical, subtropical climates, and even in moderate freezes, enduring droughts (Puccio, 2004). These attributes underscore its considerable potential. This study aimed to evaluate the biological properties of A. leucocalyx fruits, such as bioactive compounds, antioxidant, and antimicrobial activity.

2. Materials and methods

2.1. Reagents

Free radical DPPH (1,1-diphenyl-2-picrylhydrazyl), free radical ABTS 2,2-Azinobis- (3-ethylbenzothiazolin-6-sulfonic), Folin-Ciocalteu reagent, gallic acid, were obtained from Sigma Aldrich (Germany); all other chemicals and reagents used in this study were of analytical grade.

2.2. Plant material and sample preparation

Fruits were collected in the Arubai Nature Reserve in the department of Santa Cruz, Bolivia during the summer season in March 2021 and in October 2021 (coordinates: $17^{\circ}41'11.0''S\,63^{\circ}25'15.3''W$). The fruits were selected based on their state of maturity, presenting homogeneous characteristics in color and of various sizes. They were transported in clean and sterile plastic containers to the laboratory.

The bioactive compounds were extracted according to the method of



Fig. 1. Photograph of the fruit of Allagoptera leucocalyx in its ripe state.

Rodríguez-Pérez et al. (2015) with some modifications: The extraction process involved maceration with 70 % ethanol at room temperature, employing two distinct mass-to-solvent (w/w) ratios 1:2 and 1:4. The maceration was carried out for 18 h, with continuous agitation in darkness. Subsequently, the mixture was filtered using Whatman N5 paper and subjected to centrifugation (15 min, 1.4×10^6 g force), resulting in the acquisition of the ethanolic extracts of *A. leucocalyx* (EEA). Both extracts were then stored at 4 °C until the commencement of testing.

2.3. Bioactive compounds

Total polyphenols content was determined according to the methodology proposed by Autor et al. (2022) and results were expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight.

Total flavonoid content was determined by spectrophotometry (Ahn et al., 2004) and results were expressed as mg quercetin equivalent (QE) per 100 g fresh weight. The Shinoda qualitative method was determined following the methodology outlined by Pant et al. (2017).

The quantification of anthocyanins was carried out by the methodology described by Giusti and Wrolstad (2001) and results were expressed as mg cyanidin-3-glucoside equivalent (C-3-G) per 100 g fresh weight.

2.4. Antioxidant activity

The antioxidant capacity was determined by the electron transfer methods: ABTS and DPPH. For the radical method ABTS the methodology of da Silva Santos et al. (2022) was adopted. The DPPH radical scavenging activity was evaluated according to the method of Aoshima et al. (2004) with a slight modification. Briefly, 30 μL of sample extract or standard was added to 3 mL of DPPH reagent (0.1 mmol.L 11 in ethanol 96 %, with an initial absorbance of about 0.70 \pm 0.02 UA). After incubation at room temperature for 30 min, the absorbance was measured at 515 nm using a UV–Vis spectrophotometer (UV–Vis, Lamda-25, Perkin Elmer, Cambridge, UK). The result was calculated as Trolox equivalent per gram fresh weight.

The inhibition percentage was calculated using the Eq. (1):

$$\% Inhibition = 100(A - B)/A$$
 (1)

where, A is the absorbance of the control and B is the absorbance of the sample.

2.5. Antimicrobial activity

Antimicrobial activity was tested against four Gram-negative pathogenic bacteria: Shigella sp., Escherichia coli, Pseudomonas sp. and Salmonella sp. A broad-spectrum antibiotic meropenem (QUIMFA BOLIVIA S.A., Santa Cruz, Bolivia) was used as a positive control and 70 % ethanol as a negative control to verify that the effect antimicrobial was not due to the solvent used.

2.5.1. Antibiogram

The antibiogram was assessed following the methodology outlined by Enan et al. (2021), with slight modifications: Bacteria in the exponential phase of growth were cultivated on sterile Mueller-Hinton agar in Petri dishes. Four filter paper discs, each impregnated with different substances, were introduced into the Petri dishes containing the bacterial cultures. These substances included: (1) 1:2 ethanolic extract, (2) 1:4 ethanolic extract, (3) the positive control (meropenem antibiotic), and 4) the negative control (70 % ethanol). Subsequently, the Petri dishes were incubated for 24 h at 37 °C. Following incubation, visual examination of the Petri dishes took place, and the diameter (mm) of the inhibition zone was meticulously measured.

2.5.2. Turbidimetry

The methodology employed was adapted from Modak et al. (2002) with certain modifications: Initially, 20 samples were prepared, each one containing 10 mL of peptone water. Subsequently, for each bacterial strain, four tubes were allocated; and in each of these tubes, 50 μL of bacteria in the exponential growth phase, with an optical density of 0.23 \pm 0.02 AU, were inoculated. Additionally, 20 μL of the following substances were added to their respective tubes: 1:2 ethanolic extract to the first tube, 1:4 ethanolic extract to the second tube, the positive control to the third tube, and the negative control to the fourth tube. This procedural sequence was replicated for each bacterium.

A blank was used with all the components except the bacteria inoculation. Subsequently, the liquid cultures were incubated at 35 $^{\circ}$ C for 5 days. The absorbance at 650 nm was quantified for turbidity. Finally, the analysis of colony-forming units (CFU) was conducted using serial dilutions to validate the obtained results.

2.6. Statistical analysis

The experimental results obtained were expressed as mean with its standard deviation. Sigmaplot 12 (Trial version, Systat Software Inc, Germany) was used.

3. Results and discussion

3.1. Bioactive compounds

The content of polyphenols, flavonoids, anthocyanins and antioxidant activity of the *A. leucocalyx* are presented in Table 1.

3.1.1. Polyphenols

The polyphenol content determined for 1:2 ethanolic extract was 171 \pm 5 mg GAE/100 g and for 1:4 ethanolic extract was 209 \pm 12 mg GAE/100 g. A. leucocalyx presented higher contents than those reported for Manilkara zapota, Caryocar Brasiliense, Hymenaea courbaril, Diospyros kaki, among others (Otero et al., 2020). According to Vasco et al. (2008), the polyphenol content in fruits can be classified into three different categories: low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g) and high (> 500 mg GAE/100 g), following these parameters, we can suggest that A. leucocalyx fruit is a medium source of polyphenols.

Polyphenol compounds exhibit recognized antimicrobial, anti-inflammatory, antitumor, anti-obesity, antihypertensive, antidiabetic, and antioxidant properties, playing crucial roles in preserving overall health. Additionally, they contribute to various sensorial characteristics: color (anthocyanins and flavonols for reddish-bluish and yellowish hues), flavor (neohesperidin and naringin for bitter taste) and aroma (eugenol), thereby directly influencing the overall quality of the products (Issaoui et al., 2020).

Table 1
Bioactive compounds (polyphenols (mg GAE/100 g), flavonoids (mg QE/100 g) and anthocyanins (mg C-3-G /100 g)) and antioxidant activity (ABTS● (μmol Trolox/g) and DPPH● (μmol Trolox/g)) determined for the ethanolic extract of Allagoptera leucocalyx in the 1:2 and 1:4 ratios.

Properties	Extracts	
	1:2	1:4
Polyphenols (mg GAE/100 g)	171 ± 5	209 ± 10
Flavonoids (mg QE/100 g)	5.9 ± 0.1	7.9 ± 0.1
Anthocyanins (mg C-3-G /100 g)	0.43 ± 0.03	0.72 ± 0.03
Antioxidant activity		
ABTS [●] (μmol Trolox/g)	4.5 ± 0.1	9.3 ± 0.2
% Inhibition	92.4 ± 0.9	95±2
DPPH [●] (μmol Trolox/g)	3.9 ± 0.1	5.7 ± 0.2
% Inhibition	51 ± 1	$\textbf{72} \pm \textbf{2}$

3.1.2. Flavonoids

Flavonoids content presented was for 1:2 ethanolic extract (5.9 \pm 0.1 mg QE/100 g) and for 1:4 ethanolic extract (7.9 \pm 0.1 mg QE/100 g), these values were higher than Euterpe oleracea, Psidium guajava, Averrhoa carambola, Spondias mombin, Byrsonima crassifolia (Aniceto et al., 2021; Otero et al., 2020), which makes the fruit a major source of flavonoids. In the Shinoda qualitative test, a cherry red coloration was observed, which indicates the presence of flavonoids of the flavonol type. Flavonols are widely distributed in all yellow pigments of plants (Liu et al., 2021). Therefore, we can assume that, since the A. leucocalyx present has a yellow color, this can be attributed in part by flavonols.

Flavonoids are crucial for health due to their essential antiallergenic, antiviral, anti-inflammatory, antimutagenic, anticancer, and vasodilating properties. Moreover, their ability to modulate vital cellular enzymatic functions is indispensable. Consequently, numerous flavonoids find extensive applications in nutraceutical, pharmaceutical, agricultural, medicinal, and cosmetic areas (Rahaman & Mondal, 2020).

3.1.3. Anthocyanins

In the anthocyanin measurement, A. leucocalyx showed lower contents in the 1:2 ethanolic extract $(0.43 \pm 0.03 \text{ mg/100 g})$ and the 1:4 ethanolic extract $(0.72 \pm 0.03 \text{ mg/100 g})$. This is in line with the literature, which reports that anthocyanins are typically found in higher concentrations in red and bluish fruits (Hidalgo & Almajano, 2017). However, the anthocyanin content of A. leucocalyx ethanolic extract is significant compared with other yellow fruits such as Eugenia stipitate, Rheedia brasiliensis, Buchenavia tomentosa, Byrsonima crassifolia, among others, where the anthocyanin content was not detected (Siriano et al., 2022).

The obtained results indicate a higher concentration of polyphenols, flavonoids, and anthocyanins in 1:2 ethanolic extract compared to 1:4 ethanolic extract. These findings corroborate previous research affirming that an increased solvent ratio enhances mass transfer, facilitating the extraction of polar compounds like polyphenols (Brighenti et al., 2014).

3.2. Antioxidant activity

The antioxidant activity determined by the ABTS® method was for 1:2 ethanolic extract ($4.5\pm0.1~\mu mol~Trolox/g$) and 1:4 ethanolic extract ($9.3\pm0.2~\mu mol~Trolox/g$), these values are higher than those found in Caryocar brasiliense, Manilkara zapota, Bactris gasipaes, Diospyros kaki, Theobroma grandiflorum, Passiflora edulis, Selenicereus undatus, Euterpe oleracea, Manilkara zapota. While by the DPPH® method it was for 1:2 ethanolic extract ($3.9\pm0.1~\mu mol~Trolox/g$) and 1:4 ethanolic extract ($5.9\pm0.2~\mu mol~Trolox/g$), greater than the contents for Physalis peruviana, Mangifera indica, Passiflora edulis, Solanum quitoense, Passiflora ligularis, Euterpe oleracea, Manilkara zapota (Enriquez et al., 2020; Otero et al., 2020; Vasco et al., 2008). In terms of percentage, the inhibition of free radicals by the ABTS® method is 92 % for 1:2 extract and 95 % for 1:4 extract, while for DPPH® it is 51 % for 1:2 extract and 72 % for 1:4 extract. These results indicate that the A. leucocalyx ethanolic extract has good antioxidant capacity.

The ABTS[®] assay showed higher antioxidant activity than the DPPH[®] assay, possibly due to several factors. Firstly, the assays were conducted at different wavelengths (734 nm for ABTS[®] and 515 nm for DPPH[®]), where compounds like carotenoids and anthocyanins in the sample could interfere with the DPPH[®] measurement. Secondly, reversible reactions of DPPH[®] with specific phenols like eugenol and its derivatives may result in lower values. Lastly, the antioxidant compounds in the A. leucocalyx extract could be highly hydrophilic (sugars, organic acids, vitamin C and B) and lipophilic (vitamins A and E, phytosterols, and carotenoids), making them more sensitive to the ABTS[®] technique (Casagrande do Nascimento et al., 2020; Maina et al., 2021).

The content of bioactive compounds shows a positive correlation with antioxidant capacity, potentially explaining the higher antioxidant

activity observed in the 1:4 ethanolic extract.

Research on natural antioxidants derived from plants has been crucial in recent decades to replace synthetic with natural alternatives. Consequently, considerable emphasis has been placed on fruits and vegetables. Antioxidants from fruits play a key role in inhibiting lipid and protein oxidation, in preventing microbial activity in various food products like meats and beer, and in the formulation of cosmetics and pharmaceuticals (Manessis et al., 2020; Sathya et al., 2023).

3.3. Antimicrobial activity

For the antibiogram analysis, Table 2 presents findings on the antimicrobial activity. The 1:4 ethanolic extract demonstrated efficacy against *Shigella* sp., *E. coli*, and *Pseudomonas* sp. Notably, its pronounced activity against *Shigella* sp. is evident, as indicated by a larger inhibition halo in comparison to the other tested strains.

In Turbidimetry, Fig. 2 demonstrates the efficacy of 1:2 ethanolic extract against *E. coli* and *Shigella sp,* while 1:4 ethanolic extract is effective against *Shigella sp, E. coli,* and *Pseudomonas sp,* as shown by lower absorbances compared to the positive control and higher absorbances than the negative control. Fig. 3 provides an alternative perspective on antimicrobial effects, showing bacterial growth curves on the 5th day with absorbances exceeding 1, indicating the extracts' control over bacterial growth. Additionally, Fig. 4 illustrates the calculation of colony-forming units for positive results.

Various flavonoid structures, such as quercetin, apigenin, catechin, myricetin, naringenin, epigallocatechin gallate, luteolin, among others, exhibit antimicrobial activity primarily by inhibiting bacterial growth through mechanisms including membrane disruption, nucleic acid synthesis inhibition, ATP synthesis inhibition, and bacterial toxin inhibition, among others (Dias et al., 2021).

Consequently, our findings propose that the ethanolic extract of *A. leucocalyx* demonstrates antimicrobial activity attributed to identified polyphenols and flavonoids in the preliminary phytochemical analysis. Furthermore, a positive correlation between the bioactive compounds and the antimicrobial capacity is observed. In both methods, 1:4 ethanolic extract outperformed 1:2 ethanolic extract, attributed to its higher polyphenol and flavonoid concentrations.

In another study by Girondi et al. (2017), the antimicrobial effect of *A. leucocalyx* leaves was tested against *E. coli*, and *Pseudomonas aeruginosa*, obtaining negative results. The ineffectiveness would indicate that the fruit has bioactive compounds that the leaves do not have.

The significance of natural sources, particularly plants, in possessing antimicrobial properties is notable. This is crucial in the ongoing quest for novel antimicrobial therapies as substitutes for established antibiotic treatments due to the rising challenge of bacterial resistance. Accordingly, plants exhibit a considerable potential for the creation of innovative antimicrobial drugs, treatment of infectious diseases, and in product preservation (Khare et al., 2021). Some fruits serve as successful preservatives without altering the authentic flavor of food items, presenting an alternative within the food industry (Amiri et al., 2021). Therefore, our fruit can have a great potential especially against *Shigella* sp.

Table 2Antimicrobial activity of Allagoptera leucocalyx ethanolic extracts (1:2 and 1:4 ratios) by antibiogram.

Bacteria	1:2 extract	1:4 extract
Shigella sp.	_	++
E. Coli	_	+
Pseudomonas sp.	_	+
Salmonella sp.	_	_

^{&#}x27;++' Positive bioactivity (inhibition diameter >12 mm).

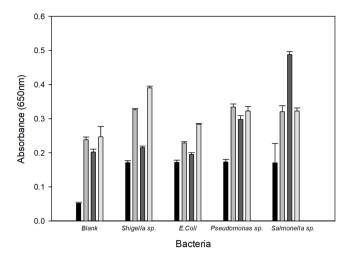


Fig. 2. Antimicrobial activity of *Allagoptera leucocalyx* ethanolic extracts by turbidimetry against Gram negative bacteria, () positive control, () 1:2 extraction, () 1:4 extraction, () negative control.

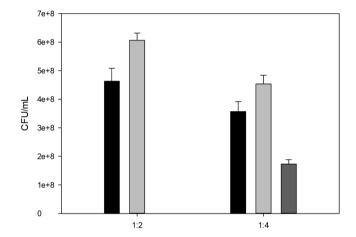


Fig. 3. Colony-forming units (CFU/ml) of () Shigella sp., () E. coli, () Pseudomonas sp. for the Allagoptera leucocalyx ethanolic extracts, 1:2 and 1:4 ratio.

4. Conclusions

The results of this study clearly demonstrate that *A. leucocalyx* is a rich source of bioactive compounds, including polyphenols and flavonoids, and exhibits significant antioxidant activity. Additionally, the *A. leucocalyx* extract shows antimicrobial activity, particularly against Shigella sp. These findings suggest potential applications in various fields, opening prospects for agro-industrialization and expanding its production and marketing. However, further studies are necessary to evaluate the fruit comprehensively and to explore its potential applications. Future research could focus on investigating its vitamin and mineral content, dietary fiber content, anti-inflammatory properties, and its ability to modulate the immune system. These additional studies would provide a more comprehensive understanding of the fruit's health benefits and its potential uses in the food or pharmaceutical industries.

CRediT authorship contribution statement

Ayelen J. Camacho Crespo: Writing – original draft, Visualization, Investigation. **Natalia Montellano Duran:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

^{&#}x27;+' Positive bioactivity (inhibition diameter >8 mm).

^{&#}x27;-' No bioactivity.

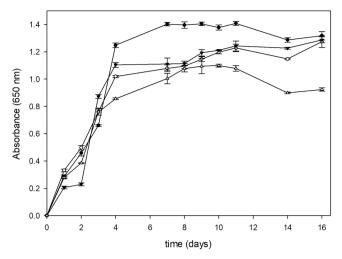


Fig. 4. Growth curves of bacteria, () Shigella sp., () E. coli, (∇) Pseudomonas sp., (Λ) Salmonella sp.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Natalia Montellano Duran reports financial support was provided by International Development Research center. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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