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Bioactive properties of guaco (*Mikania glomerata* Sprengel) leaf extracts produced with different hydroalcoholic solvents

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Abstract

Even though guaco (*Mikania glomerata* Sprengel) is a promising source of active compounds, its antioxidant and antimicrobial activities remain underexplored. Thus, this study aimed to produce guaco leaf hydroalcoholic extracts with significant antioxidant and antimicrobial activities while ensuring their safety (GRAS). Guaco leaf hydroalcoholic extracts were prepared at 60 °C using hydroalcoholic solvents with varying concentrations (0, 20, 40, 60, 80, and 100% v/v) of ethanol or methanol for comparison. Characterization included soluble solids, chlorophylls, carotenoids, phenolic compounds, and flavonoid concentrations, alongside antioxidant activity ($ABTS^{\bullet+}$ and FRAP) and antimicrobial activities. The guaco leaf hydroalcoholic extracts that showed the highest antioxidant activities, as assessed by the FRAP method, were those containing 40% and 60% of ethanol with 28.0 and 26.4 mg Trolox/g dry matter, respectively, as well as the extracts with 60% and 80% of methanol with 27.7 and 23.5 mg Trolox/g dry matter, respectively. Data obtained from ABTS antioxidant assays also showed this same trend. The extracts with the highest antioxidant activities – GLHeE.40, GLHeE.60, GLHmE.60 and GLHmE.80 – generally exhibited simultaneously high soluble solids (1.3; 1.6; 2.3; and 2.6 °Brix, respectively), intermediate carotenoids (0.15; 0.22; 0.12; and 0.16 mg/g dry matter, respectively), the highest phenolic compounds (13.5; 12.4; 11.5, 11.3 mg gallic acid/g dry matter, respectively), and flavonoids (9.11, 8.95, 7.01 and 11.3 mg catechin/g dry matter, respectively). The hydroethanolic extracts containing 40% and 80% ethanol showed antimicrobial activity against *Listeria monocytogenes*, with inhibition zones ranging from 1.0 to 1.5 cm in diameter. These findings indicate that ethanol is an effective solvent for producing guaco leaf hydroalcoholic extracts, thereby enhancing their antimicrobial properties while preserving their antioxidant activity.

Keywords: Antimicrobial activity; Antioxidant activity; Hydroalcoholic extracts; Solvent composition.

Highlights

- Alcohol type and concentration influenced the composition of guaco leaf extracts
- GLHeE.80 and GLHeE.40 showed activity against *L. monocytogenes*
- Only GLHeE.80 exhibited activity against *E. coli*

1 Introduction

The use of antioxidant compounds emerges as a primary strategy to delay or prevent the oxidation of oxidizable substrates in food, thereby preserving nutritional quality and extending the shelf life of products (Luciano et al., 2022; Wang et al., 2024a, 2024b). Additionally, these compounds can function as indirect antimicrobials, inhibiting the growth of pathogenic bacteria responsible for foodborne infections or intoxications, such as *Bacillus cereus* (Li et al., 2024), *Listeria monocytogenes* (Liu et al., 2020), *Staphylococcus aureus* (Soyuçok et al., 2024), *Escherichia coli* (Miera et al., 2022), and Salmonella (Mahlangu et al., 2017). Although antioxidants may be naturally present in food compositions, they are typically added during processing for enhanced effectiveness (Bertan et al., 2021; Luciano et al., 2021a).

The use of synthetic antioxidants has been extensively questioned regarding their safety, prompting research into the use of natural antioxidants, particularly in food processing (Fan & Eskin, 2015; Lourenço et al., 2019). Phenolic compounds are the most common natural antioxidants, which include flavonoids and phenolic acids, which are secondary metabolites of plants that are commonly found in spices and herbs (Lourenço et al., 2019). Their effects against cardiovascular diseases and some neurological disorders also contribute to the growing, considerable interest in phenolic compounds (Rahaman et al., 2023).

Phenolic compounds can be found either in purified form or extracted from their respective raw materials using solvents. Selecting the solvent is a crucial step in the extraction process, since the composition of the extract and its bioactivities (e.g., antioxidant and antimicrobial activities) heavily depend on the type of solvent used (Quezada et al., 2004; Troncoso et al., 2005). No solvent extraction system is efficient in isolating all classes or a specific class of natural bioactive compounds; however, plant extracts always contain a mixture of substances from different classes. A variety of solvents can be employed to extract bioactive compounds from natural sources, including methanol, ethanol, acetone, water, ethyl acetate, acetonitrile, *n*-heptane, propanol, and their combinations (Al Jitan et al., 2018; Alara et al., 2021). Ethanol and methanol are universal solvents broadly used in the extraction of phytochemical compounds (Zhang et al., 2018). Methanol is a popular choice that is often considered the most effective in extracting phenolic compounds from natural sources (Bilal et al., 2024; Cendrowski et al., 2024; Tekin & Küçükbay, 2025). However, ethanol is classified as GRAS (Generally Recognized As Safe) and can produce extracts suitable for use in the food, pharmaceutical, and cosmetic industries (Al Jitan et al., 2018; Albertos et al., 2017). Furthermore, plant extracts are more commonly produced using aqueous solutions of these organic solvents, as they tend to be more effective in extracting phenolic compounds than pure organic solvents (Cendrowski et al., 2024).

Guaco is a sub-shrub plant with a twining habit, belonging to the Asteraceae family. It originates from the southern region of Brazil, as well as Argentina, Uruguay, and Paraguay. Two species are commonly referred to as guaco: *Mikania glomerata* Sprengel (*M. glomerata*) and *Mikania laevigata* Schultz Bip. ex Baker (*M. laevigata*). Both species exhibit medicinal properties working as an expectorant and bronchodilator, confirmed by scientific studies, and having been used for centuries in folk medicine (Gasparetto et al., 2010; Czelusniak et al., 2012; Santana et al, 2014; Della Pasqua et al., 2019; Borghi et al., 2023). Several phenolic compounds (such as phenolic acids, tannins, and flavonoids) are isolated from guaco species (Della Pasqua et al., 2019). However, the Unified Health System (SUS) (Brasil, 2022) highlights *M. glomerata* as particularly notable among the only 12 native plants used in the production of herbal medicines, being considered safe and effective by the National Health Surveillance Agency (ANVISA). Furthermore, the limited studies on the antioxidant activity of guaco leaf extracts suggest that *M. glomerata* leaves exhibit greater antioxidant activity than *M. laevigata* leaves (Borghi et al., 2023). Therefore, *M. glomerata* leaf extracts are considered to have significant potential as a source of active compounds, although their antioxidant activity has not been extensively studied.

Therefore, this study aimed to assess the impact of alcohol type (ethanol or methanol) and its concentration in hydroalcoholic solvents on the properties (color and pH), concentrations of bioactive compounds (carotenoids, phenolic compounds, and flavonoids), and pro-oxidants (chlorophylls), as well as the functional

properties (antioxidant and antimicrobial) of hydroalcoholic extracts from guaco leaves (*M. glomerata*). For being widely recognized as the optimal solvent for plant extract production, methanol was included as a control.

2 Materials and methods

2.1 Material

Dried guaco leaves (*Mikania glomerata* Sprengel), including their stems, were obtained from HerbaFlora (Pirassununga, Brazil). The chemicals ethanol, methanol, acetone, aluminum chloride, sodium hydroxide, hydrochloric acid, sodium acetate, and acetic acid were sourced from Labsynth (São Paulo-SP, Brazil). For the analyses of total phenolic compound content (TPCC) and antioxidant activity, the following chemicals were employed: Folin-Ciocalteu reagent; gallic acid; 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); Trolox, sodium nitrite; 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ); catechin; and ferric chloride. All chemicals were provided by Sigma-Aldrich (St. Louis, USA). Additionally, potassium persulfate and sodium carbonate were obtained from Merck (Darmstadt, DE). Reagents used for the analysis of antimicrobial activity included Brain Heart Infusion (BHI) and Muller Hinton agar from Becton, Dickinson and Company (Rutherford, USA), in addition to Tryptone soy broth (TSB) from Neogen® Culture Media (Lansing, USA). Filter paper (Whatman No. 1) was supplied by GE Healthcare (Maidstone, UK). All other reagents were of analytical grade.

2.2 Proximate composition of Guaco leaves

Initially, the dried guaco leaves were crushed and sieved (48 mesh) to standardize the particle size. The dried and crushed guaco leaves were then placed in sealed polyethylene bags and stored, protected from light, until the analyses. The guaco leaf powder underwent a drying process in an air-circulating oven at 105 °C for 12 hours to determine moisture content. Subsequently, the remaining material was subjected to a 24-hour exposure in a muffle furnace at 550 °C to determine the ash content (Association of Official Analytical Chemists, 2005). Acid followed by basic digestion method was employed (Association of Official Analytical Chemists, 2005) to determine crude fiber content. Lipids were extracted using a Soxhlet apparatus for 6 hours, while crude protein content was assessed by the Kjeldahl method, based on a conversion factor of 6.25 to convert total nitrogen into crude protein (Association of Official Analytical Chemists, 2005). The nitrogen-free extract, primarily composed of carbohydrates, was obtained by subtracting the contents of crude fiber, crude protein, lipids, and ashes from 100 (Association of Official Analytical Chemists, 2005).

2.3 Hydroalcoholic extraction procedure of guaco leaves

To produce guaco leaf hydroalcoholic extracts (GLHEs), hydroalcoholic solutions with ethanol or methanol were used in the following volumetric alcohol proportions: 0, 20, 40, 60, 80, and 100% (v/v). The GLHEs were named according to the volumetric concentration of alcohol used for their production, producing 11 different extracts: 1 aqueous extract, GLHE.0; 5 guaco leaf hydroethanolic extracts (GLHeEs), GLHeE.20, GLHeE.40, GLHeE.60, GLHeE.80, and GLHeE.100; and 5 guaco leaf hydromethanolic extracts (GLHmEs), GLHmE.20, GLHmE.40, GLHmE.60, GLH-mE.80, and GLHmE.100.

The guaco leaf hydroalcoholic extracts (GLHEs) were prepared following Celeghini et al. (2001) and Della Pasqua et al. (2019), with adaptations. Firstly, the guaco leaf powder was dispersed in a hydroalcoholic solution (1 g of guaco leaf powder/10 g of hydroalcoholic solution) and subjected to an ultrasound processing using an ultrasound bath (Ultra Sonic, Odontobrás, Brodowski-SP, Brazil) for 30 minutes at room temperature. Then, the dispersions were heated at 60 °C for 30 minutes on a magnetic stirrer with heating (AA-2050, Gehaka, São Paulo-SP, BR), in the absence of light. Finally, the dispersions were filtered through filter paper and stored in amber bottles under refrigeration (4 °C). The GLHEs were used within a maximum period of 48 hours.

2.4 Characterization of Guaco Leaf Hydroalcoholic Extracts (GLHEs)

2.4.1 Color

Five random measurements were taken for each replicate using a Miniscan colorimeter (MSEZ 1049, HunterLab, Reston-VA, USA), operating with illuminant D65 and daylight, to determine color parameters in the CIELab standard on the EasyMatchQC Software (HunterLab), according to Sobral et al. (2001).

2.4.2 Soluble solids content and pH

The concentration of soluble solids was determined using a benchtop manual refractometer (ABBE). The results of the soluble solids concentration (°Brix) of the GLHEs were expressed as the difference between the °Brix of the GLHEs and the °Brix of the hydroalcoholic solvents used in the extractions. The pH was determined using a digital pH meter (PG2000, Gehaka, São Paulo-SP, BR).

2.4.3 Chlorophyll and carotenoid content

Chlorophyll a and b content and total carotenoids in the GLHE were determined following Luengo et al. (2014), with modifications. To extract the pigments, a 300 µL aliquot of GLHEs was mixed with 3 mL of an 80% aqueous acetone solution (v/v). The mixture was then incubated in the dark at room temperature for 20 minutes and subsequently centrifuged (Thermo IEC, Centra GP8R, Needham Heights-MA, USA) at 1500*x*g for 5 minutes. The supernatant was analyzed using a UV/vis spectrophotometer (Lambda 35, Perkin Elmer, Waltham-MA, USA) at wavelengths of 663.6, 646.6, and 440.5 nm relative to a blank containing only aqueous acetone solution (80% v/v). The contents of chlorophyll a, chlorophyll b, and total carotenoids in the GLHEs were then calculated using Equations 1, 2, and 3 (Yang et al., 1998).

$$Cl_a = 12.25 \times A_{663.6} - 2.55 \times A_{646.6} \quad (1)$$

$$Cl_b = 20.31 \times A_{646.6} - 4.91 \times A_{663.6} \quad (2)$$

$$Ct = 4.69 \times A_{440.5} - 0.267 \times Cl_{ab} \quad (3)$$

Where Cl_a is the content of chlorophyll a (µg/mL); Cl_b is the content of chlorophyll b (µg/mL); Cl_{ab} is the contents of chlorophylls a and b (µg/mL); Ct is the content of carotenoids (µg/mL); A663,6, A646,6, and A440,5 are the absorbances of the samples at 663.6 nm, 646.6 nm, and 440.5 nm, respectively.

2.4.4 Total phenolic compound content (TPCC)

The total phenolic compound content (TPCC) of the GLHEs was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton & Rossi Junior, 1965). The GLHEs were diluted in distilled water at the following concentrations: 100 µL of extract/mL of solution for GLHeE.100; 70 µL of extract/mL of solution for GLHmE.100; and 45.5 µL of extract/mL of solution for the other GLHeEs, GLHmEs, and GLHE.0. Then, an aliquot (0.5 mL) of each diluted extract was added to 2.5 mL of an aqueous solution of the Folin-Ciocalteu reagent (10% v/v) and shaken. After a five-minute rest, this solution was mixed vigorously with 2 mL of an aqueous sodium carbonate solution (7.5% m/v) and kept at room temperature, in the absence of light, for two hours. Subsequently, the absorbance of this solution was determined at 760 nm using a UV/visible spectrophotometer (Lambda 35, Perkin Elmer, Waltham, MA, USA), based on a blank composed of 0.5 mL of distilled water, 2.5 mL of Folin-Ciocalteu reagent, and 2 mL of sodium carbonate solution. The result was expressed in mg of gallic acid equivalent/g of dry matter based on the standard curve

of gallic acid (R^2 = 0.997). The composition of phenolics of the GLHeE.20, GLHeE.40, GLHeE.60 was accessed by UHPLC-HRMS/MS analysis, whose data are available in Bertan et al. (2025).

2.4.5 Total flavonoid content (TFC)

The total flavonoid content (TFC) of the GLHEs was determined following the spectrophotometric method described by Zhishen et al. (1999). The GLHEs were diluted in distilled water at the following concentrations: 62.5 µL of extract/mL of solution for GLHE. 100 and GLHE.80; 100 µL of extract/mL of solution for GLHE.60 and GLHE.40; 125 µL of extract/mL of solution for GLHE.20; and 200 µL of extract/mL of solution for GLHE.0. Then, an aliquot (250 µL) of each diluted extract was added to 1 mL of distilled water and 75 µL of an aqueous sodium nitrite ($NaNO_2$, 5% w/v) solution and shaken. After a five-minute rest, this solution was mixed vigorously with 75 µL of aqueous aluminum chloride ($AlCl_3$, 10% w/v) solution. After another 6 minutes of rest, the solution was added to 500 µL of sodium hydroxide (NaOH, 1M) solution and 600 µL of distilled water. After vigorous shaking, the absorbance of the solutions was determined at 510 nm using a UV/vis spectrophotometer (Lambda 35, Perkin Elmer, Waltham-MA, USA), based on a blank composed of 1 mL of distilled water, 75 µL of 5% $NaNO_2$ solution (w/v), 75 µL of 10% $AlCl_3$ solution (w/v), and 500 µL of 1M NaOH solution. The result was expressed in mg of catechin equivalent/g of dry matter based on the standard curve of catechin (R^2 = 0.999).

2.4.6 Antioxidant activity (AoA)

2.4.6.1 ABTS free radical capture method ($ABTS^{\bullet+}$)

For these analyses, the $ABTS^{\bullet+}$ radical was generated by oxidizing ABTS di-ammonium salt with potassium persulfate. Specifically, ABTS (2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) was dissolved in water at 7 mM and reacted with a 2.45 mM potassium persulfate solution in the dark for 16 hours (Re et al., 1999). An aliquot of this solution was then diluted with ethanol to achieve an absorbance of approximately 0.70 at 734 nm to obtain the ABTS working solution. All GLHEs were diluted in absolute ethanol at a concentration of 125 µL of extract/mL of solution. Then, an aliquot (30 µL) of each diluted extract was added to 3 mL of $ABTS^{\bullet+}$ radical solution. After 6 minutes of reaction, protected from light, the absorbance of the solutions was determined at 734 nm using a UV/vis spectrophotometer (Lambda 35, Perkin Elmer, Waltham, MA, USA), based on a blank composed of 30 µL of the diluted sample and 3 mL of ethanol (Re et al., 1999). The AoA was expressed in mg of Trolox equivalent/g of dry matter based on the standard curve of Trolox (R^2=0.988), prepared as a reference to the percentage of $ABTS^{\bullet+}$ inhibition (Bertan et al., 2021).

2.4.6.2 Ferric reducing antioxidant power (FRAP)

The FRAP reagent was prepared in a ratio of 100 mL of 0.3 M acetate buffer, 10 mL of 10 mM TPTZ solution, diluted in 40 mM HCl solution, and 10 mL of 20 mM aqueous ferric chloride solution. The GLHEs were diluted in distilled water at the following concentrations: 100 µL of extract/mL of solution for GLHE.0, GLHmE.20, GLHmE.100, and GLHeE.100; 60 µL of extract/mL of solution for GLHmE.40, GLHmE.60, and GLHmE.80; and 35 µL of extract/mL of solution for the other GLHeEs (GLHeE.20, GLHeE.40, GLHeE.60, and GLHeE.80). Then, an aliquot (90 µL) of each diluted extract was vigorously mixed with 270 µL of distilled water and 2.7 mL of FRAP reagent (Benzie & Strain, 1996; Rufino et al., 2006). The solution was placed in an ultra-thermostatized water bath (MA-184/BX, Marconi, Piracicaba, SP, BR) at 37 °C for 30 minutes. The absorbance of the solutions was then determined at 595 nm using a UV/vis spectrophotometer (Lambda 35, Perkin Elmer, Waltham, MA, USA), based on a blank composed only of the

FRAP reagent (Benzie & Strain, 1996; Rufino et al., 2006). The result was expressed in mg of Trolox equivalents per g of dry matter, based on the standard curve of Trolox (R^2=0.996).

2.4.7 Antimicrobial activity (AmA)

The antimicrobial activity (AmA) of the GLHEs was determined according to Tessaro et al. (2021). The AmA of the GLHEs was tested against the Gram-positive pathogenic bacteria *Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 25923, as well as the Gram-negative bacteria *Salmonella enteritidis* ATCC 13076 and *Escherichia coli* ATCC 25922. The bacterial cultures of *S. aureus*, *S. enteritidis*, and *E. coli* were inoculated in BHI broth, and *L. monocytogenes* was inoculated in TSB broth. All bacterial cultures were incubated at 35 °C for 24 hours. After the incubation period, the absorbance of the bacterial suspensions was standardized between 0.08 and 0.10 at a wavelength of 625 nm to ensure consistent bacterial concentrations in the suspensions. Then, each bacterium was inoculated onto Mueller-Hinton agar plates. Fifteen microliters (µL) of each GLHE were added to sterile filter paper discs (d = 6 mm), which were then placed on the plates inoculated with the bacteria using sterile tweezers. Each plate also contained a positive control disc (d = 6 mm) with the antibiotic chloramphenicol (2.5 mg/mL) and negative control discs (d = 6 mm) containing 15 µL of each hydroalcoholic solvent used in the production of the GLHE. The plates were then incubated upside down at 35 °C for 24 hours. After the incubation period, the inhibition zones (diameter) of bacterial growth were measured for both GLHeE and GLHmE samples and positive and negative controls. We studied the extracts containing solvents, as their presence could enhance the AmA in the extracts. Furthermore, we aimed to use these extracts directly, without any additional processing, in the production of active films (Bertan et al., 2025).

2.5 Statistical analysis

All analyses were carried out in triplicate at a minimum, with three measurements for each replicate. The results were presented as mean ± standard deviation of mean (SD). The experimental data were subjected to statistical analysis using ANOVA and Tukey's test with a significance level of 95%, on the software programs MINITAB® 2019 and ORIGIN® 2022.

3 Results and discussion

3.1 Proximate composition of guaco leaf powder

The determination of the average proximate composition of guaco leaf powder detected no ash-free neutral detergent fiber (NDF), acid detergent fiber (ADF), nitrogen in ADF, or nitrogen in NDF. The guaco leaf powder exhibited 6.7 ± 0.2% (g/100 g of wet sample) moisture; 10.8 ± 0.6% protein; 23.9 ± 0.3% crude fiber; 2.5 ± 0.2% lipids; 10.3 ± 0.4% total ash; and 45.9 ± 0.5% nitrogen-free extract. Various factors in the cultivation system, such as soil type, fertilization, and irrigation, can influence the chemical composition of plant leaves (Braga et al., 2018; Santos et al., 2020). Therefore, significant variability in the proximate composition of plant leaves is expected. Nonetheless, Alvarenga et al. (2009) observed moisture (11%) and total ash content (14%) values for guaco leaves of the species *M. glomerata* that were quite close to those observed herein. Martins et al. (2022) also found a comparable lipid content (2.08%) in *M. glomerata* leaves; however, their reported values for protein (3.28%), fiber (0.033%), and mineral content (1.46%) were lower than our findings. As a result, the nitrogen-free extract value in their study was substantially higher (93.15%).

3.2 Characterization of guaco leaf hydroalcoholic extracts (GLHE)

3.2.1 Color

The solvent composition (alcohol type and alcohol: water ratio) affected both the visual color (Figure 1) and the instrumental color parameters (Table 1) of GLHEs. The aqueous extract (GLHE.0) exhibited a dark brown color (lower L*, a*, and b* values), while the extracts produced with high alcohol concentrations (GLHeE.80, GLHeE.100, and GLHmE.100) were the darkest and most greenish (lower L* and b* values and intermediate a* values). GLHmE.20 and GLHmE.40 showed intermediate L*, a*, and b* values and a brown color. The extracts with higher L*, a*, and b* values displayed colors ranging from orange (GLHeE.20, GLHeE.40, and GLHmE.60) to greenish (GLHeE.60 and GLHmE.80). Significant color differences ($p < 0.05$) were also observed between GLHeEs and GLHmEs produced at the same alcohol concentrations. GLHeE.20 had higher L*, a*, and b* values compared to GLHmE.20, GLHeE.40, and GLHmE.40, which showed very similar L*, a*, and b* values and coloration. For the extracts produced with high alcohol concentrations in the solvent (60 - 100%), GLHeEs exhibited lower L*, a*, and b* values compared to GLHmEs.

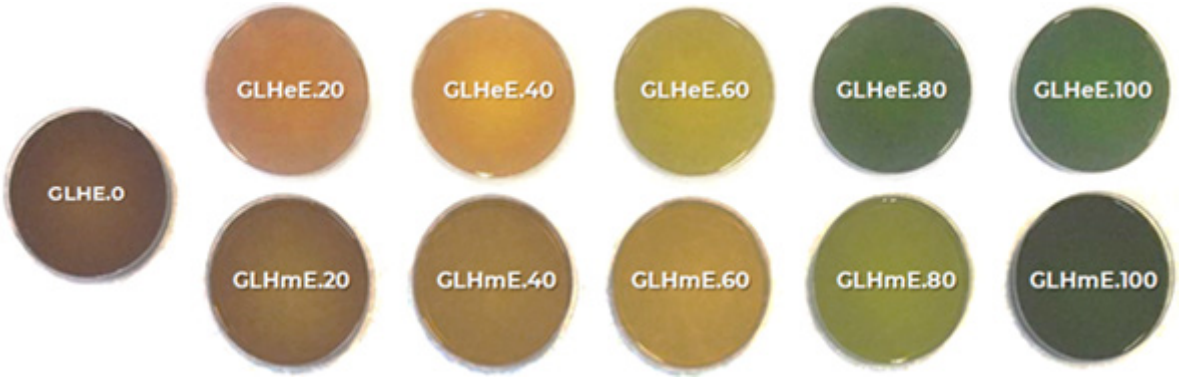

Figure 1. Visual color of aqueous extract (GLHE.0), hydroethanolic extracts (GLHeE.20, GLHeE.40, GLHeE.60, GLHeE.80, and GLHeE.100), and hydromethanolic extracts (GLHmE.20, GLHmE.40, GLHmE.60, GLHmE.80, and GLHmE.100) of guaco leaves.

Table 1. Color parameters (CIELab) and soluble solids content (SSC) of guaco leaf hydroalcoholic extracts (GLHE).

Alcohol concentration in solvent (%)	L*		a*	
	GLHeE	GLHmE	GLHeE	GLHmE
0	0.52 ± 0.05^{D}	0.52 ± 0.05^{C}	0.52 ± 0.05^{E}	0.52 ± 0.05^{D}
20	8.26 ± 0.68^{Aa}	4.21 ± 0.47^{Bb}	4.33 ± 0.25^{Ba}	2.58 ± 0.10^{Cb}
40	4.76 ± 0.13^{Ba}	4.62 ± 0.49^{Ba}	7.48 ± 0.82^{Ab}	10.57 ± 0.59^{Ba}
60	1.90 ± 0.17^{Cb}	9.02 ± 0.39^{Aa}	3.57 ± 0.24^{BCb}	16.87 ± 1.31^{Aa}
80	1.06 ± 0.08^{Db}	8.69 ± 0.65^{Aa}	1.78 ± 0.07^{Db}	10.88 ± 0.67^{Ba}
100	0.49 ± 0.05^{Db}	0.82 ± 0.07^{Ca}	3.14 ± 0.04^{Ca}	2.13 ± 0.09^{CDb}
	b*		SSC (°Brix)	
0	0.72 ± 0.04^{E}	0.72 ± 0.04^{D}	2.7 ± 0.1^{A}	2.7 ± 0.1^{A}
20	9.01 ± 0.61^{Aa}	4.37 ± 0.41^{Cb}	1.8 ± 0.3^{Ba}	1.8 ± 0.0^{Da}
40	6.95 ± 0.27^{Ba}	6.76 ± 0.76^{Ba}	1.3 ± 0.3^{Bb}	2.1 ± 0.1^{Ca}
60	3.05 ± 0.25^{Cb}	14.63 ± 0.92^{Aa}	1.6 ± 0.3^{Bb}	2.3 ± 0.0^{Ca}
80	1.76 ± 0.12^{Db}	14.19 ± 1.70^{Aa}	1.6 ± 0.1^{Bb}	2.6 ± 0.1^{ABa}
100	0.83 ± 0.09^{Eb}	1.36 ± 0.09^{Da}	0.7 ± 0.1^{Cb}	2.3 ± 0.1^{BCa}

Different letters indicate significant differences by one-way ANOVA/Tukey's test ($p < 0.05$). Capital letters: differences within the same column between GLHEs produced with the same alcohol type at different alcohol concentrations in the solvent (0-100% v/v). Lowercase letters: differences between GLHEs produced with different alcohol types (ethanol and methanol) at the same alcohol concentration in the solvent.

Pigments are molecules that absorb radiation at specific wavelengths and reflect only certain wavelengths in the visible region, imparting particular colors to foods. Chlorophylls are lipid-soluble molecules that impart a green color; carotenoids are lipid-soluble molecules that provide yellow, orange, or red colors; and others, like flavonoids (e.g., anthocyanins), are water-soluble and contribute to blue, purple, red, and orange hues (Elias et al., 2018). The mixture of water with ethanol or methanol alters the polarity of the hydroalcoholic solvent produced (Tibkawin et al., 2022). Therefore, the different colors of the GLHEs may indicate that each extraction system proposed in this study enabled the extraction of different classes of compounds (mainly pigments) from guaco leaves, according to the polarities of these compounds. For instance, pure ethanol and methanol are among the most effective solvents for chlorophyll extraction (Viñas-Ospino et al., 2023); therefore, it is highly likely that the greener extracts contain higher concentrations of chlorophylls compared to others. The dark coloration of the GLHE may also be linked to light scattering caused by some phenolic compounds extracted from guaco leaves (Luciano et al., 2021b)

Bertan et al. (2021) observed a progressive decrease in L*, a*, and b* values in extracts of boldo (*Peumus boldus*) leaves with increasing ethanol concentrations in the hydroethanolic solvents used for extraction (0, 25, 50, 75, and 100% v/v), corresponding to a color change from orange to green. Conversely, Tibkawin et al. (2022) reported a progressive increase in L*, a*, and b* values in extracts of teak (*Tectona grandis*) leaves with increasing ethanol concentrations in the hydroethanolic solvents used for extraction (0, 25, 50% v/v), corresponding to a color change from light brown to orange. Despite differences in the variation of L*, a*, and b* values of leaf extracts with alcohol concentration in the solvent, it is confirmed that extracts produced with more water in the solvent tend to have colors closer to brown, while those produced with more alcohol tend to have colors closer to green.

3.2.2 Soluble solids content

The aqueous extraction (GLHE.0) and the extraction using 80% methanol (GLH-mE.80) resulted in the highest increase ($p < 0.05$) in soluble solids in the extract (2.6-2.7 °Brix), indicating that these treatments improved the solubilization of guaco leaf compounds during extraction (Table 1). All GLHeEs showed similar ($p > 0.05$) soluble solids content (1.3-1.8 °Brix), except for GLHeE.100 (0.7 ± 0.1 °Brix). Differently, the soluble solids content in GLHmEs decreased in the following order: GLH-mE.80 (2.6 ± 0.1 °Brix) > GLHmE.40, GLHmE.60, and GLHmE.100 (2.1 - 2.3 °Brix) > GLHmE.20 (1.8 ± 0.0 °Brix). However, in general, GLHeEs had a lower concentration of soluble solids ($p < 0.05$) than GLHmEs produced with the same alcohol concentration. Therefore, the aqueous solvent demonstrated greater efficiency in solubilizing compounds from guaco leaves compared to hydroalcoholic solvents, whereas hydromethanolic solvents exhibited higher efficiency than hydroethanolic ones. These findings suggest that guaco leaves comprise predominantly polar compounds, given that water has greater polarity than ethanol and methanol (Nawaz et al., 2018; Nawaz et al., 2020). Vargas et al. (2016) determined soluble solids content in aqueous extract of rosemary leaves (2.6 °Brix) close to that of GLHE (2.7 °Brix), whereas it was higher for aqueous extract of pitanga (*Eugenia uniflora* L.) leaves (3.6 °Brix). A higher content of soluble solids may indicate larger quantities of antioxidant substances (Vargas et al., 2016).

3.2.3 pH

The pH of the GLHeEs (5.68-6.13) was either equal to ($p > 0.05$) or slightly higher than ($p < 0.05$) the pH of their respective hydroethanolic solvents (5.68-6.08). In contrast, the pH of the GLHmEs (5.68-6.39) was either equal to ($p > 0.05$) or slightly lower than ($p < 0.05$) the pH of their respective hydromethanolic solvents (Table 2). The pH of the hydroethanolic solvents with 40% to 100% ethanol (~6.0) was slightly higher ($p < 0.05$) than the pH of the solvents with 0-20% ethanol (5.68). Among the GLHeEs, GLHeE.80 had the highest pH (6.13 ± 0.04), while the pH of the other extracts ranged

from 5.68 (GLHE.0) to 6.00 (GLHeE.60). The pH of the hydromethanolic solvents followed the order: 100% methanol (7.52 ± 0.02) > 60% - 80% methanol (6.55 - 6.87) > 0 - 40% methanol (~6.0). As for the GLHmEs, GLHmE.60, GLHmE.80, and GLHmE.100 exhibited higher pH values (6.27 - 6.39) compared to the other extracts (~6.0).

Table 2. pH of the hydroalcoholic solvents and respective guaco leaf hydroalcoholic extracts.

Alcohol concentration in solvent (%)	pH			
	Hydroethanolic solvent	GLHeE	Hydromethanolic solvent	GLHmE
0	5.68 ± 0.01^{Ba}	5.68 ± 0.02^{Da}	5.68 ± 0.01^{Fa}	5.68 ± 0.02^{Fa}
20	5.68 ± 0.04^{Bb}	5.91 ± 0.02^{Ca}	5.95 ± 0.05^{Ea}	5.95 ± 0.01^{Ea}
40	6.08 ± 0.09^{Aa}	5.93 ± 0.03^{BCb}	6.05 ± 0.03^{Db}	6.13 ± 0.01^{Da}
60	5.98 ± 0.07^{Aa}	6.00 ± 0.02^{Ba}	6.55 ± 0.04^{Ca}	6.27 ± 0.02^{Cb}
80	6.02 ± 0.04^{Ab}	6.13 ± 0.04^{Aa}	6.87 ± 0.03^{Ba}	6.39 ± 0.01^{Ab}
100	5.96 ± 0.12^{Aa}	5.86 ± 0.05^{Ca}	7.52 ± 0.02^{Aa}	6.36 ± 0.01^{Bb}

Different letters indicate significant differences by one-way ANOVA/Tukey's test ($p < 0.05$). Capital letters: differences within the same column between solvents or GLHEs produced with the same alcohol type at different concentrations of alcohol in the solvent (0-100% v/v). Lowercase letters: differences between solvents and GLHEs produced with the same type of alcohol and at the same alcohol concentration in the solvent.

It is not straightforward to assign a pH value to distilled water, pure ethanol or methanol, hydroalcoholic solvents, or compounds dissolved in these solvents. However, an increase in pH with a higher alcohol concentration in hydroalcoholic solvents is an expected behavior. Additionally, comparing the acidity of compounds dissolved in different hydroalcoholic solvents is a very complex task (Deleebeeck et al., 2021; Kotrba & Schilling, 2017). Despite this complexity, it is evident that different solvents facilitate the extraction of different classes of compounds from guaco leaves. A GLHE with a higher pH than its solvent (e.g., GLHeE.20, GLHeE.80, and GLHmE.40) may indicate the extraction of slightly more basic compounds from guaco leaves. Conversely, a GLHE with a lower pH than its solvent (e.g., GLHeE.40, GLHmE.60, GLHmE.80, and GLHmE.100) suggests the extraction of more acidic compounds, such as phenolic acids (Vargas et al., 2016).

Bertan et al. (2021) also observed an increase in the pH of extracts from boldo leaves with an increase in the ethanol concentration in solvents (0, 25, 50, 75, and 100% v/v), ranging from 5.1 to 5.7, which aligns closely with the pH values observed in this study. Vargas et al. (2016) found a similar pH for aqueous extracts of rosemary leaves (5.75) but a lower pH for aqueous extracts of pitanga leaves (4.53). Therefore, the pH of plant extracts is quite variable and can depend on the concentration of acidic compounds dissolved by each extraction system, influenced by factors such as temperature and the extraction solvent, among others.

3.2.4 Chlorophyll and carotenoid content

The chlorophyll content of both a and b in GLHEs was minimal ($p < 0.05$) in extractions carried out with low alcohol concentrations in the solvent (0 - 40% ethanol and 0 - 60% methanol). However, it increased sharply ($p < 0.05$) until reaching its peak in extractions performed with 100% alcohol (Figure 2). The concentrations of chlorophylls in GLHeEs were consistently higher ($p < 0.05$) than those in GLHmEs when produced with the same concentrations of alcohol in the solvent. Moreover, they increased significantly with higher alcohol concentrations used in the solvent, except for extractions performed with pure alcohol (GLHeE.100 and GLHmE.100), which exhibited the opposite trend. These results confirm the previous suggestion that the greener extracts (GLHeE.80, GLHeE.100, and GLHmE.100) contained higher concentrations of chlorophylls.

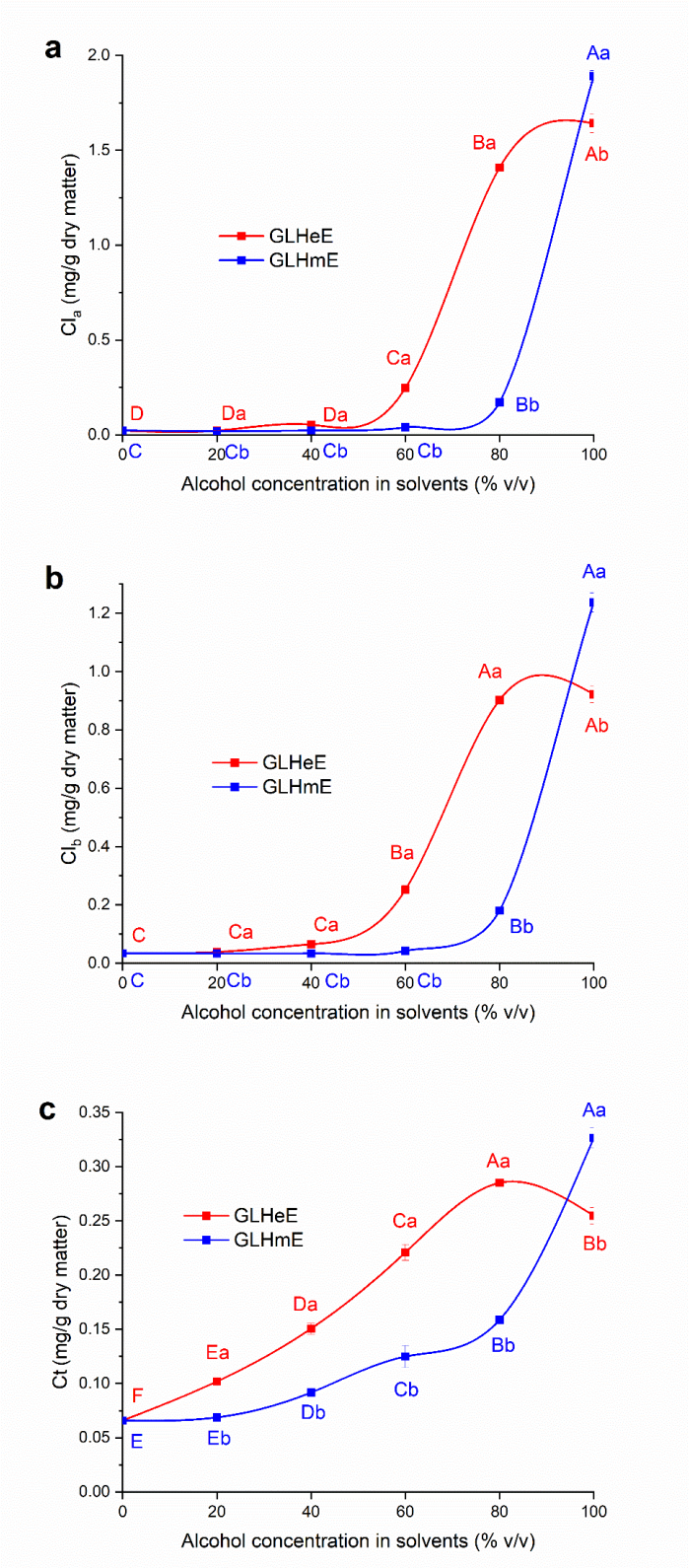

Figure 2. Contents of (a) chlorophyll a (Cl_a) and (b) b (Cl_b), and (c) total carotenoids (Ct) in guaco leaf hydroalcoholic extracts (GLHEs). The results are presented as the mean ± standard deviation of mean (n = 3). Different letters indicate significant differences by one-way ANOVA/Tukey's test ($p < 0.05$). Capital letters: differences between GLHEs produced with the same alcohol type at different alcohol concentrations in the solvent (0 - 100% v/v). Lowercase letters: differences between GLHEs produced with different alcohol types (ethanol and methanol) at the same alcohol concentration in the solvent.

The concentration of chlorophyll in leaves is directly linked to the nitrogen content in plant cultivation, whereas the concentration of carotenoids depends on the need to shield chlorophyll from photo-oxidation due to excessive light exposure. Consequently, the levels of chlorophylls and total carotenoids observed in plant leaves heavily rely on soil fertilization (Wang et al., 2019), light conditions during plant growth (Souza et al., 2011), water stress, or temperature fluctuations (Elango et al., 2023), as well as leaf drying conditions (Branisa et al., 2017). Souza et al. (2011) examined guaco (*M. laevigata*) leaves for their chlorophyll and total carotenoid contents, yielding values of 1.53 - 2.76 mg chlorophyll a/g dry weight, 0.75 - 2.24 mg chlorophyll b/g dry weight, and 15.74 - 17.65 mg carotenoids/g dry weight. Hence, while the concentration of these compounds in plant leaves is influenced by various factors, the higher chlorophyll contents observed for GLHeE.100 and GLHmE.100 align with expected values for guaco leaves, whereas the total carotenoid contents presented by GLHES can only account for approximately 2% of the total carotenoids found in guaco leaves.

3.2.5 Total phenolic compound content (TPCC) and total flavonoid content (TFC)

The GLHEs displayed TPCC levels close to 12 mg of gallic acid/gram of dry matter, except the GLHE.100 and GLHmE.100 samples, which showed lower values (Figure 3). Among the GLHeEs, the highest ($p < 0.05$) concentrations of phenolic compounds were observed in GLHeE.40, GLHeE.20, and GLHeE.60. Regarding GLHmEs, only GLHmE.100 did not exhibit the highest ($p < 0.05$) TPCC. GLHeEs and GLHmEs almost always demonstrated comparable ($p > 0.05$) TPCC when produced with the same alcohol concentration. GLHeE.40 is a noteworthy exception for presenting higher phenolic content ($p > 0.05$) than GLHmE.40. Therefore, hydroethanolic solvents are as effective as hydromethanolic solvents in extracting phenolic compounds from guaco leaves and may even outperform them when 40% ethanol is used.

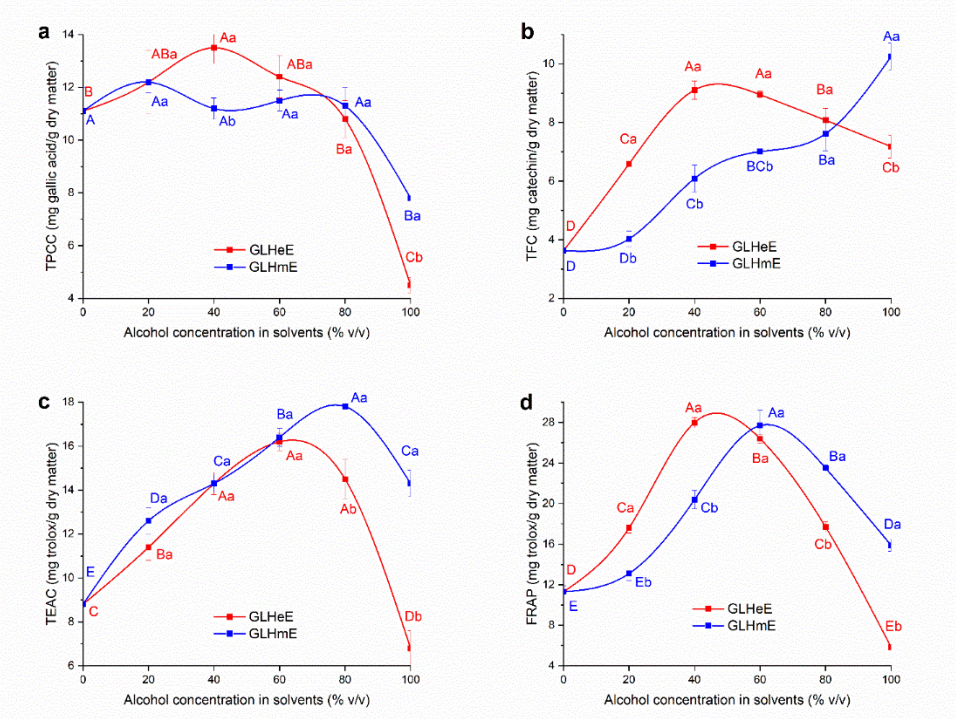

Figure 3. (a) Total phenolic compound content (TPCC), (b) total flavonoid content (TFC), and antioxidant activity, measured as (c) Trolox equivalent antioxidant capacity (ABTS) and (d) ferric reducing antioxidant power (FRAP) of the hydroalcoholic extracts of guaco leaves (GLHEs). The results are presented as the mean ± standard deviation of mean (n = 3). Different letters indicate significant differences by one-way ANOVA/Tukey's test ($p < 0.05$). Capital letters: differences between GLHEs produced with the same alcohol type at different alcohol concentrations in the solvent (0 - 100% v/v). Lowercase letters: differences between GLHEs produced with different alcohol types (ethanol and methanol) at the same alcohol concentration in the solvent.

Regarding the total flavonoid content (TFC) of GLHEs (Figure 3), GLHeE showed a peak ($p > 0.05$) in TFC for GLHeE.40 and GLHeE.60, while the TFC of GLHmE was directly proportional to the methanol content in the extraction solvent, reaching its maximum in GLHmE.100. GLHeEs consistently exhibited higher ($p < 0.05$) TFC than GLHmEs, except for those extracts produced with pure alcohols. Comparing the results of TPCC and TFC to the concentration of soluble solids in GLHEs, it is evident that the extraction condition enabling the highest solubilization of compounds from guaco leaves (GLHE.0) did not correspond to the optimal extraction systems for the active compounds of interest in GLHE (mainly phenolic compounds and flavonoids). However, all proposed extraction systems succeeded in extracting phenolic compounds and flavonoids from guaco leaves, indicating that hydroalcoholic mixtures, which alter the polarity of the solvents, facilitated the dissolution of antioxidant compound classes with different polarities from guaco leaves.

Phenolic compounds are often associated with other biomolecules in nature, such as polysaccharides, proteins, terpenes, chlorophyll, or inorganic compounds, which may interfere with their extraction using solvents (Ghasemzadeh et al., 2011). Nevertheless, higher contents of phenolic compounds from leaves and spices are generally obtained with more polar solvents (Ghasemzadeh et al., 2011; Nawaz et al., 2018). Hydroalcoholic solvents demonstrate greater efficiency in extracting these compounds compared to water and pure alcohol separately (Azman et al., 2016), and the extraction of flavonoids is usually higher in methanol than in other solvents due to its higher polarity compared to ethanol (Ghasemzadeh et al., 2011; Przygodzka et al., 2014), which is consistent with our findings.

Bertan et al. (2021) observed higher TPCC in hydroethanolic extracts of boldo produced with 0, 50, and 75% ethanol (6.6 - 7.3 mg gallic acid/g dry matter) and lower TPCC for extraction with 100% ethanol (3.6 mg gallic acid/g dry matter). Tibkawin et al. (2022) found higher TFC in teak leaf extracts produced with 50% ethanol (40.9 mg rutin/g dry matter) compared to those produced with 0, 25, and 75% ethanol (10.8 - 22.9 mg rutin/g dry matter). Siddhuraju & Becker (2003) concluded that hydroalcoholic solvents with 80% methanol showed higher TPCC (8.87-12.33 mg gallic acid/g dry matter) and TFC (5.76 - 14.07 µg rutin/g dry matter) in moringa tree (*Moringa oleifera* Lam.) leaves compared to solvents with 70% ethanol and 100% water. Nawaz et al. (2018) analyzed extracts of red cabbage leaves (*Brassica oleracea* Convar Capitata Var L.) and found that aqueous extraction (5.2 mg gallic acid/g dry matter) showed higher TPCC than methanolic extraction (2.2 - 2.3 mg gallic acid/g dry matter). These findings closely resemble the results obtained for the GLHE.

3.2.6 Antioxidant activity (AoA)

The highest ($p < 0.05$) ABTS values were exhibited by GLHeE.40, GLHeE.60, and GLHeE.80 among GLHeEs, and by GLHmE.60 and GLHmE.80 among GLHmEs (Figure 3). The ABTS of GLHeEs was close ($p > 0.05$) to GLHmEs when produced with 0 - 60% alcohol in the solvent; however, the ABTS of GLHmEs was higher ($p < 0.05$) than GLHeEs for the higher alcohol concentrations (80 and 100%) (Figure 3). Therefore, we can suggest that hydroethanolic solvents can replace hydromethanolic solvents in obtaining GLHEs without any difference in AoA, as long as the alcohol concentration in the solvent does not exceed 60%. Similar results were obtained for AoA by the FRAP method (Figure 3). GLHeE.40 and GLHeE.60 showed the highest FRAP values ($p < 0.05$) among GLHeEs, while GLHmE.60 and GLHmE.80 exhibited the highest FRAP values among GLHmEs. In low and intermediate alcohol concentrations (0 - 40%), GLHeEs exhibited higher ($p < 0.05$) FRAP than GLHmEs, and the opposite behavior was observed in high alcohol concentrations in the solvent (80 and 100%). It is worth highlighting that the extracts produced with 60% ethanol or methanol showed similar ABTS and FRAP values. The agreement between both methods allows us to infer that the highest AoA occurred in GLHeE.40 and GLHeE.60 among the GLHeEs, as well as for GLHmE.60 and GLHmE.80 among the GLHmEs. Additionally, GLHmEs show higher AoA when produced with high alcohol concentrations.

Comparing the AoA results (ABTS and FRAP) with the total phenolic compound content (TPCC) and total flavonoid content (TFC), it is evident that GLHEs exhibiting high TPCC and TFC simultaneously demonstrated

the highest AoA, both for GLHeEs (GLHeE.40 and GLHeE.60) and GLHmEs (GLHmE.60 and GLHmE.80). This relationship stems from the direct proportional correlation between total phenolic and flavonoid contents and the AoA of plant leaf extracts (Ghasemzadeh et al., 2011; Ho et al., 2010; Przygodzka et al., 2014; Siddhuraju & Becker, 2003). Despite GLHEs showing nearly identical TPCC for extractions of 0-60% alcohol, there is a progressive increase in AoA with the rise in alcohol concentration under these conditions. This elevation is likely attributed to the gradual increase in the content of carotenoids and flavonoids observed with the increase in alcohol concentration in the solvent between 0 and 60%. For their potent bioactivity, carotenoids undoubtedly contributed to the AoA of these GLHEs (Vargas et al., 2016).

Among the extracts produced with 80% alcohol, GLHmE.80 exhibited higher AoA than GLHeE.80, despite both containing the same ($p > 0.05$) TPCC and TFC, with GLHeE.80 having higher ($p < 0.05$) carotenoid content. This outcome is likely attributed to the significantly higher ($p < 0.05$) concentration of chlorophylls a and b in GLHeE.80 compared to GLHmE.80, as chlorophylls can act as prooxidant molecules under light exposure, leading to the formation of reactive oxygen species that can oxidize other molecules, explaining its lower antioxidant activity (Vargas et al., 2016).

Extracts produced solely with ethanol or methanol showed a similar trend in antioxidant activity (AoA) to that of hydroalcoholic extracts prepared with 80% alcohol, likely due to the contribution of the polarity of water in enhancing the extraction of polar antioxidant compounds. GLHeE.100 showed the lowest ($p < 0.05$) AoA due to its low TPCC and TFC, and the highest ($p < 0.05$) concentration of chlorophylls a and b simultaneously. Despite GLHmE.100 having higher ($p < 0.05$) contents of flavonoids and carotenoids, its low AoA is likely linked to the minimal TPCC and maximum chlorophyll content in this extract. When comparing the extracts with different alcohol types, GLHmE.100 showed higher AoA than GLHeE.100 due to its higher contents of phenolic compounds, flavonoids, and carotenoids.

The varied concentrations of phenolics, flavonoids, and carotenoids in GLHEs with the varying alcohol concentration in the solvents indicate that guaco leaves contain active compounds of different polarities, which likely differ in their AoA. In other words, regardless of the analysis of compound concentrations in GLHEs, a specific solvent may have extracted a group of compounds with higher AoA than those extracted by other solvents, thus imparting greater AoA to a GLHE compared to others (Ghasemzadeh et al., 2011; Nawaz et al., 2018). Antioxidant activity studies on guaco leaves are rare in the literature but the AoA of guaco leaf extracts has been attributed to the presence of cinnamic acid and coumarin derivatives, which are antioxidants and the main compounds responsible for the pharmacological properties of guaco (Santana et al., 2014). Therefore, it is likely that GLHeE.40, GLHeE.60, GLHmE.60, and GLHmE.80 contain the highest contents of cinnamic acid and coumarin derivatives from guaco leaves, thus exhibiting high AoA, as previously discussed.

3.2.7 Antimicrobial activity (AmA)

Figure 4 shows the AmA of GLHEs against Gram-positive (*L. monocytogenes* and *S. aureus*) and Gram-negative (*Salmonella enteritidis* and *E. coli*) bacteria. The GLHEs showed no significant inhibition zones (diameters of 0.6 - 1.5 cm) compared to chloramphenicol (3.2 - 4.0 cm); hence, they were considered weakly active against the bacteria. None of the GLHmEs showed AmA against any of the bacteria studied. Neither the GLHEs nor the hydroalcoholic solvents exhibited AmA against *S. aureus*; only GLHeE.100 and GLHeE.80 showed AmA against *E. coli* (0.7 - 0.8 cm); GLHeE.80, GLHeE.40, and pure ethanol showed AmA against *L. monocytogenes* (1.0 - 1.5 cm); GLHeE.100 and the solvents composed of 100% methanol, 100%, 80%, and 60% ethanol showed AmA against *Salmonella enteritidis*. The AmA of GLHeE.100 against *Salmonella enteritidis* may have been caused by the action of the solvent itself (100% ethanol). Therefore, only GLHeE.100, GLHeE.80, and GLHeE.40 exhibited effective AmA (*E. coli* and/or *L. monocytogenes*), i.e., not attributable to the solvent itself, and likely contained antimicrobial compounds.

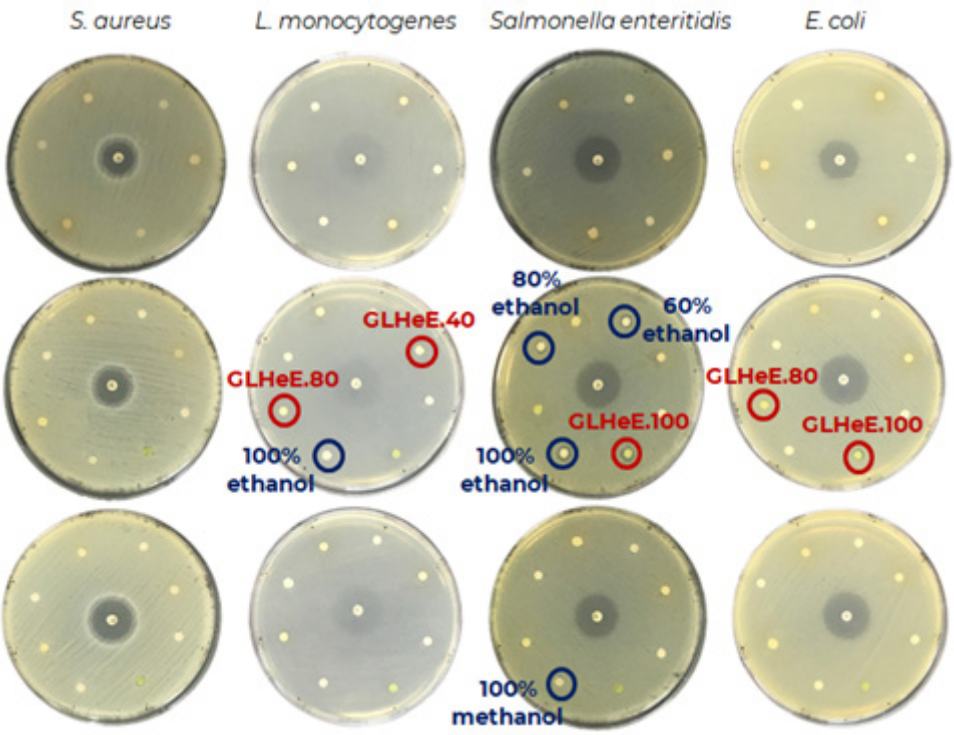

Figure 4. Antimicrobial activity of hydroalcoholic solvents (high) and guaco leaf hydroethanolic (center) and hydromethanolic (bottom) extracts (GLHEs) against the bacteria *S. aureus*, *L. monocytogenes*, *Salmonella enteritidis*, and *E. coli*.

The AmA of guaco leaf extracts varies considerably and is influenced by the species of guaco, whether *M. glomerata* or *M. laevigata*, as well as the composition of the solvent used for extraction. Baratto et al. (2008) assessed ethanol extracts of *M. laevigata* guaco leaves using the disk diffusion technique and found no AmA against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25992), among other bacteria. Otherwise, Gasparetto et al. (2010) reviewed literature studies regarding the AmA of guaco extracts and reported that i) methanolic extracts of *M. glomerata* were inactive against *S. aureus*, while hydroethanolic extracts (70% v/v) of *M. laevigata* were strong inhibitors of this bacterium; ii) hydroalcoholic extracts of *M. glomerata* are weak inhibitors of *E. coli*, while ethanol extracts of *M. laevigata* were inactive.

L. monocytogenes and *E. coli* are among the main bacteria causing foodborne infections or intoxications. *L. monocytogenes* is a facultative aerobic Gram-positive bacillus that grows in fruits, vegetables, meat products, and dairy, even at refrigeration temperatures. This bacterium causes listeriosis, which can result in abortion, conjunctivitis, and meningitis, among others. Meanwhile, *E. coli* is a Gram-negative coliform used as an indicator of fecal contamination in food. In addition to being a foodborne pathogen, *E. coli* is responsible for causing undesirable reactions in food (Osés et al., 2015). Therefore, the AmA for GLHeE.100, GLHeE.80, and GLHeE.40 can be of great interest to the food industry and showed no relationship with the Gram classification of bacteria proposed in some literature studies (Albertos et al., 2017; Bonilla & Sobral, 2017; Ebrahimi et al., 2022; Weerakkody et al., 2010) since these GLHEs showed AmA only against one of the Gram-positive (*L. monocytogenes*) and one of the Gram-negative (*E. coli*) studied bacteria.

Some studies link the AmA of plant extracts to their concentration in anthocyanins and phenolic compounds (TPCC) (Puškárová et al., 2017; Weerakkody et al., 2010), while others report a low correlation between AmA and TPCC and associate the AmA of herb and spice extracts with non-phenolic compounds, such as organic acids and aldehydes (Weerakkody et al., 2010). We also observed this weak correlation between AmA and TPCC, as the AmA against the studied bacteria was found in extracts with varied TPCC: GLHeE.40 (13.5 ± 0.6 mg of gallic acid/g of dry matter), GLHeE.80 (10.8 ± 0.7 mg of gallic acid/g of dry

matter), and GLHeE.100 (4.5 ± 0.3 mg of gallic acid/g of dry matter). Several compounds present in guaco leaves exhibit AmA and can contribute to functional activity in the extracts. For example, coumarin is considered one of the substances responsible for the antimicrobial activity of guaco, along with cupressic acid, diterpenic acid, and kaurenoic acid, as well as some components of guaco essential oil, such as 1,8-cineole, limonene, linalool, geranial, germacrene-D, and menthol (Baratto et al., 2008). Since the type of solvent used in extraction can impact the AmA of plant extracts significantly (Weerakkody et al., 2010), it is suggested that some of these components may have been extracted in higher concentration in GLHeE.100, GLHeE.80, and GLHeE.40.

Our findings allow us to conclude that guaco leaves are indeed a valuable source of bioactive compounds, and the extraction of these compounds from guaco leaves is highly dependent on the hydroalcoholic solvent used. Among the guaco leaf extracts, GLHeE.40 and GLHeE.60, as well as GLHmE.60 and GLHmE.80, exhibited the highest antioxidant activity (ABTS and FRAP, concurrently). These extracts appeared the clearest (with higher L*, a*, and b* values), while those with more orange hues contained minimal chlorophyll (such as GLHeE.40 and GLHmE.60), and the greener extracts (GLHeE.60 and GLHmE.80) had intermediate chlorophyll content. These extracts boasted a high concentration of soluble solids, intermediate carotenoid content, and a lower pH compared to their solvents (GLHeE.40, GLHmE.60, and GLHmE.80), suggesting the extraction of phenolic acids from guaco leaves.

4 Conclusions

All extracts simultaneously exhibited the highest levels of total phenolic compounds and total flavonoids, confirming a close correlation between the concentration of these compounds and the antioxidant activity of vegetable leaf extracts. Notably, GLHeE.80 and GLHeE.40 also demonstrated antimicrobial activity against *L. monocytogenes*. Concerning the type of alcohol used in the solvent, ethanol can effectively replace methanol in the production of hydroalcoholic extracts of guaco leaves, while maintaining or even enhancing the bioactivities of the extracts.

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Data Availability Statement

The data supporting this study are available from the corresponding author upon reasonable request.

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