Quantification of Phenolic Compounds and Determination of Antioxidant Potential in Different Medicinal Plants

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Article

Quantification of Phenolic Compounds and Determination of Antioxidant Potential in Different Medicinal Plants

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Abstract

The study was developed in the context of the search for bioactive compounds of interest present in medicinal plants, among them phenolic compounds, recognized for their functional relevance. To this end, the extraction process was optimized using soursop leaves as a model, combining different particle sizes (<2 mm, 2–6.3 mm, and 6.3–9.5 mm), maceration times (12, 24, and 48 h), and ethanol:water ratios (25:75, 50:50, and 75:25), which generated 27 extracts. The optimal process corresponded to an ethanol:water ratio of 50:50, fine particle size (<2 mm), and 24 h of maceration, reaching 584.64 mg·L^{-1} of gallic acid equivalents (GAE). Additionally, other plant species were evaluated: horsetail, kiswara, matico, muña, and thyme. Antioxidant capacity was determined using the DPPH method through IC_{50} values, where soursop, kiswara, and muña recorded the lowest values (0.52, 0.52, and 0.61 mg·L^{-1}), even lower than ascorbic acid (19.10 mg·L^{-1}). Thyme and horsetail showed intermediate activity, while matico presented the lowest response. The results indicate that these species have high potential as sources of functional bioactive compounds, highlighting the importance of medicinal plants for the development of natural products with antioxidant properties.

Keywords: phenolic compounds; antioxidant activity; folin–ciocalteu; DPPH; free radicals; medicinal plants

1. Introduction

Polyphenols are secondary metabolites widely present in the plant kingdom and can be obtained from plants, antioxidant-rich foods, or supplements, standing out for their potential as nutraceuticals in the prevention of various diseases [1]. Free radicals, in turn, are highly reactive chemical species due to the presence of an unpaired electron in their outer orbitals, making them unstable and prone to react with nearby molecules, causing cellular damage by oxidizing lipids, proteins, carbohydrates, nucleic acids, and DNA [2]. This process generates both radical and non-radical species that are generally harmful, and such damage is associated with the development of chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular disorders, as well as the acceleration of cellular aging processes [3].

It is known that some plants play a key role as antioxidants by acting as hydrogen donors that are captured by reactive radicals, generating less reactive radical and non-radical species [4]. This mechanism contributes to the protection of tissues against oxidative damage, preventing cellular damage, reducing oxidative stress, and preserving the integrity of cells and tissues [5]. However, the use of certain synthetic external antioxidants may pose health risks, making the search for safe natural compounds a priority [6].

In this context, various plant species traditionally used in Bolivia represent a promising alternative as sources of bioactive compounds with positive effects on human well-being. Among them are horsetail (Equisetum arvense), soursop (Annona muricata L.), kiswara (Buddleja coriacea),

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matico (Piper aduncum), muña (Minthostachys mollis), and thyme (Thymus vulgaris), which have been traditionally used for the prevention and treatment of various diseases [7][8].

The aim of this study was to evaluate the concentration of phenolic compounds and the antioxidant capacity of the leaves of these medicinal plants, collected in different regions of the country and during two seasons of the year, contributing knowledge about their bioactive potential. This provides a basis for the development of functional products and strategies for the sustainable use of local biodiversity. To achieve this, an optimized extraction protocol was implemented using specific parameters such as the ethanol:water solvent ratio, particle size, and extraction time. Likewise, the preparation of the Folin–Ciocalteu reagent was standardized to ensure the reproducibility of the analyses. The data obtained were processed and analyzed using SAS software (version 9.0) in order to identify optimal extraction conditions and determine the species with the greatest antioxidant potential.

2. Results

2.1. Optimal Extraction Process

The results indicated that, regardless of the ethanol:water (v/v) solvent ratio used (25:75, 50:50, and 75:25), the use of a fine sieve and an extraction time of 24 hours was sufficient to reach maximum values, thus optimizing both time and yield across all three solvent proportions. The concentration of total phenolic compounds, expressed in mg·L^{-1} gallic acid equivalents (GAE), was: 527.0 mg·L^{-1}, 517.4 mg·L^{-1}, and 505.1 mg·L^{-1}, respectively.

The ANOVA model showed an F value of 1.57 with a p-value = 0.2831 (> 0.05), indicating that there are no statistically significant differences between the means of the three proportions evaluated at the 5% significance level.

2.2. Total Phenolic Content Regarding the Concentration of Sodium Carbonate, 10% and 20% Solutions Were Evaluated

It was observed that with a 10% concentration, the reaction time was longer, whereas with 20% the reaction was completed in a shorter time. After 30 minutes, the reaction had already stabilized and no significant variations were observed over time. Therefore, a 20% sodium carbonate solution was selected for the entire study. The observations obtained from both preparations of the Folin–Ciocalteu reagent are summarized in Table 1.

Table 1. Comparison of the Folin–Ciocalteu reagent with and without lithium sulfate.

Parameter	Result with lithium sulfate	Result without lithium sulfate
Reagent Stability	No variability in the data was observed over time; the reagent remained stable.	Variability in the data over time was observed. (changes in absorbance).
Presencia of precipitates	No precipitates formed in the sample.	Precipitates were observed in the sample.
Reaction Time	30 minutes	60 minutes
Data Consistency	Data were consistent and relatively stable.	Datos similares entre sí, aunque con una leve variación respecto a la absorbancia.

The results for the Folin–Ciocalteu reagent with and without lithium sulfate showed an average percentage error of 19.06%. Despite this difference, the values of total phenolic compounds maintained a consistent pattern depending on particle size and extraction time, reaching similar results. Therefore, the use of the Folin–Ciocalteu reagent with or without lithium sulfate can be considered valid, provided that proper standardization and control of operational variables are maintained, as long as the reagent without lithium sulfate has been freshly prepared and stored for no more than one week. For this reason, it is essential to maintain constant conditions throughout all assays, including reagent concentrations, reaction times, and temperature, in order to obtain reproducible and comparable results.

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The slope (0.0066 ± 0.0015) demonstrated the sensitivity of the Folin–Ciocalteu reagent to phenolic compounds, while the intercept (0.0297 ± 0.0965) corresponded to the residual absorbance of the system. This calibration curve was used as a reference for calculating the total phenolic content in the evaluated plant samples. The results were expressed in milligrams of gallic acid equivalents per liter (mg·L^{-1} GAE), as shown in Table 2.

Table 2. Phenolic compound content expressed in mg·L^{-1} GAE and previous studies on phenolic compounds in different plant species.

Plant	February CFT [mg·L^{-1} EAG]	October CFT [mg·L^{-1} EAG]	Decontions [mg·L^{-1} EAG]	Researchs	Source
Horsetail	138.41±5.50^{bB}	175.36±25.1^{aA}	209.18±16.73^{aA}	396.2 ± 3.2	[9]
Soursop	676.51±2.75^{dB}	482.13±29.29^{bC}	1020.77±4.18^{cA}	372.92 ± 0.15	[10]
Kiswara	881.27±9.91^{eA}	428.99±25.1^{bC}	583.57±29.29^{bB}	No se encontró dato cuantificado	[11]
Matico	73.33±8.25^{aC}	441.06±29.29^{bB}	607.73±4.18^{bA}	12.982	[12]
Muña	865.4±2.75^{eC}	1156.04±16.73^{cB}	1767.15±18.24^{dA}	91.8	[13]
Thyme	638.41±2.75^{cB}	187.44±29.29^{aC}	1044.93±7.25^{cA}	62.196 ± 1.994	[14]

The superscripts a, b, c, and d indicate comparisons of means within the same collection period using p = 0.05. The superscripts A, B, and C indicate comparisons of means between collection periods using p = 0.05. Plants sharing the same letter do not show a statistically significant difference, whereas those with different letters do show a statistically significant difference.

Despite the extraction method being optimized, the concentration of phenolic compounds decreased or increased depending on the season of the year. This variation may be due to the flowering stage and other physiological processes that modified metabolic dynamics, which directly affected the concentration of phenols in plant tissues [15]. Compared with other studies in which extractions were performed using alcoholic solutions, the optimized process achieved higher concentrations.

In decoctions, muña showed the highest concentration, with 996.16 mg·L^{-1} gallic acid equivalents (GAE), followed by the other plant species. This behavior indicates that each species has a different capacity to synthesize and accumulate phenolic compounds, which play a fundamental role in protecting against oxidative damage and in neutralizing free radicals.

2.3. Antioxidant Capacity

It was observed that when preparing the reagent with 80% ethanol, DPPH did not dissolve properly, and there was an insufficient development of the characteristic intense purple color of DPPH. In contrast, when 96% ethanol was used, dissolution was effective and can be considered an alternative for spectrophotometric analysis.

When comparing absorbance values measured in the spectrophotometer at 517 nm, the recorded values were: DPPH prepared with 80% ethanol, 1.136 Abs; with 96% ethanol, 1.106 Abs; and with 80% methanol, 0.963 Abs. However, it is important to note that slight variations in absorbance may influence the sensitivity of the method and, consequently, the accuracy of antioxidant capacity quantification.

The antioxidant capacity of the evaluated plant species showed significant differences in free radical scavenging activity. These variations reflect differences in the phytochemical composition of each plant, highlighting those with greater potential for food, pharmaceutical, and cosmetic applications, such as soursop (66.56 ± 0.01 % inhibition), kiswara (59.2 ± 0.01 % inhibition), and muña (55.84 ± 0.01 % inhibition), which showed the highest values at a 1:100 dilution. They also maintained notable antioxidant activity at 1:1000, positioning them as the most promising within the analyzed set.

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Table 3 presents the percentage inhibition of the extracts at different volumes.

Table 3. Percentage inhibition of the 1:100 v/v and 1:1000 v/v dilutions of the different plant species.

Plants	%I a 1:100 v:v	%I a 1:1000 v:v
Horsetail	34.6 ± 0.01^{bA}	4.1 ± 0.01^{bB}
Soursop	66.56 ± 0.01^{fA}	38.52 ± 0.12^{eB}
Kiswara	59.2 ± 0.01^{eA}	37.12 ± 1.45^{eB}
Matico	19.45 ± 0.01^{aA}	0.95 ± 0.01^{aB}
Muña	55.84 ± 0.01^{dA}	33.82 ± 0.12^{dB}
Thorntail	49.81 ± 0.24^{cA}	16.16 ± 0.97^{cB}

The superscripts a, b, c, and d represent comparisons of means within the same collection period using p = 0.05. The superscripts A, B, and C represent comparisons of means between collection periods using p = 0.05. Plants sharing the same letter do not show a statistically significant difference, whereas those with different letters do show a statistically significant difference.

At the 1:1000 dilution, a generalized decrease in relative antioxidant activity (RAA) was observed, which was expected due to the reduced concentration of active compounds. Using these preliminary data, calibration curves were prepared to determine the median inhibitory concentration (IC_{50}) for the extracts of six plant species, using ascorbic acid as a reference. The reported IC_{50} for ascorbic acid was 19.10 mg·L^{-1}. The results are summarized in Table 4.

Table 4. IC_{50} values of the different plant species.

Plant	February IC_{50} [mg·L^{-1} EAA]	October IC_{50} [mg·L^{-1} EAA]	Decoctions IC_{50} [mg·L^{-1} EAA]	Researchs IC_{50}	Sources
Horsetail	8.19 ± 0.07^{dA}	1.19 ± 0.02^{bB}	0.52 ± 0.01^{aC}	12.3 ± 0.7 mg·L^{-1}	[9]
Soursop	2.66 ± 0.13^{aA}	0.48 ± 0.01^{aB}	0.52 ± 0.01^{aB}	907.7 mg·L^{-1}	[16]
Kiswara	4.94 ± 0.01^{cA}	1.35 ± 0.03^{cB}	0.52 ± 0.01^{aC}	11.99 mg·L^{-1}	[11]
Matico	14.12 ± 0.38^{eA}	0.57 ± 0.01^{aB}	0.50 ± 0.01^{aB}	0.790 ± 0.5 µmol TEAC/g	[17]
Muña	3.56 ± 0.20^{bA}	2.17 ± 0.09^{dB}	0.61 ± 0.03^{bC}	25 ± 2 mg/g	[18]
Thortail	4.85 ± 0.38^{eA}	0.44 ± 0.03^{aB}	0.50 ± 0.01^{aB}	8.49 ± 0.02 mg·L^{-1}	[14]

The superscripts a, b, c, and d indicate comparisons of means within the same collection period using p = 0.05. The superscripts A, B, and C indicate comparisons of means between collection periods using p = 0.05. Plants sharing the same letter do not show a statistically significant difference, whereas those with different letters do show a statistically significant difference.

The results in Table 4 showed that all species presented IC_{50} values lower than that of ascorbic acid (19.10 mg·L^{-1} AAE), demonstrating a high antioxidant capacity.

3. Discussion

Although the optimal combination in terms of phenolic compound concentration corresponded to the ethanol:water ratio of 25:75, particle size <2 mm, and 24 hours of extraction, additional variables were identified that influenced the yield and behavior of the extracts during the process. These include the concentration time in the rotary evaporator, the amount of recovered sample, and the color of the extract, as detailed in Table 5.

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Table 5. Diferencias de extractos entre las proporciones etanol:agua.

..Proporción etanol:agua	Tamaño de partícula	CFT [mg·L⁻¹ EAG]	Tiempo en rotavapor (h)	Volumen recuperado (mL)	Coloración del extracto	Additional observations
25:75	G	384.4±12^{c}	3	80	Dark Yellow	Longer concentration time during the process.
	M	441.6±9.9^{b}				
	F	478.1±16.5^{a}				
50:50	G	298.7±7.3^{c}	2	50	Café claro	Acceptable yield and good stability.
	M	368.6±16.5^{b}				
	F	609.8±18^{a}				
75:25	G	367.0±7.3^{c}	1	20	Dark Green	Higher concentration, but lower recovered volume; associated with a higher percentage of error.
	M	475±11^{b}				
	F	527.3±13.7^{a}				

The superscripts a, b, c, and d represent comparisons of means applied using the same solvent ratio at p = 0.05. Plants sharing the same letter do not show a statistically significant difference, whereas those with different letters do show a statistically significant difference.

The 25:75 ratio, particle size <2 mm, and 24-hour extraction time allowed for greater sample recovery and produced an extract with a yellow coloration, which is favorable for industrial applications requiring higher volumes. To minimize errors, the concentration time in the rotary evaporator and the amount of recovered extract should be considered, suggesting that any of the evaluated combinations could be used for practical purposes. This flexibility would allow industry to select the proportion that best suits its needs, considering volume, concentration, or sensory characteristics of the extract.

On the other hand, extracts with the 75:25 ratio, although they present lower recovered volumes, exhibit a high concentration of phenolic compounds, making them suitable for industries that require concentrated formulations and reduced volumes, such as cosmetics, pharmaceuticals, or nutraceuticals. In contrast, the 25:75 proportion is more efficient in terms of extract recovery, with applications in large-scale food or phytotherapeutic uses. Although no statistically significant differences were found, the 50:50 ratio maintains an optimal balance between concentration, yield, stability, processing time, and versatility. Additionally, its light brown coloration indicates good stability and lower oxidation. It is recommended to develop specific calibration curves for each preparation of the Folin–Ciocalteu reagent, especially if the reagent was freshly prepared or modifications were introduced.

In addition, antioxidant capacity was compared with other studies, and the obtained IC_{50} values were slightly lower, suggesting greater effectiveness of the extracts in this study. Likewise, it was observed that a higher concentration of phenolic compounds does not directly correspond to higher antioxidant capacity, as factors such as the chemical structure of the compounds, extraction conditions, and harvest season influenced the observed antioxidant response. In decoction samples evaluated using the DPPH method and expressed as IC_{50}, thyme showed the most notable result, presenting the lowest value (0.44 ± 0.03 mg·L^{-1}), reflecting a high efficiency in free radical inhibition, while the other plants showed lower antioxidant capacity. These results revealed that a higher concentration of phenolic compounds does not always translate into greater antioxidant activity, which may be attributed to the structural diversity and specific reactivity of phenolic compounds present in each extract.

Finally, seasonal variation in phenolic compounds and antioxidant activity may be influenced by harvest time, climate, and the physiological state of the plant, highlighting the importance of considering seasonality when evaluating the bioactive potential of medicinal plants [19]. Therefore, the observed differences may be partly attributed to the environmental conditions of the collection period, emphasizing the importance of considering seasonality and botanical origin when assessing the bioactive potential of medicinal plants.

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4. Materials and Methods

4.1. Reagents and Chemicals

The following reagents were used: gallic acid ($C_7H_6O_5$), ethanol (C_2H_5OH), sodium tungstate (Na_2WO_4), sodium molybdate (Na_2MoO_4), concentrated hydrochloric acid (HCl), 85% phosphoric acid (H_3PO_4), 2,2-diphenyl-1-picrylhydrazyl (DPPH), from Merck ($C_{18}H_{12}N_5O_6$) and Sigma-Aldrich, 80% methanol, and lithium sulfate (Li_2SO_4).

4.2. Collection of Plant Material

The plants were selected based on their availability, their health-related properties, and the limited scientific information available about them. The literature search was conducted mainly using the Google Scholar database and books from the Bolivian Ministry of Health.

The collection of soursop leaves (Annona muricata) was carried out in the department of Santa Cruz. The species horsetail (Equisetum arvense), kiswara (Buddleja coriacea), matico (Piper aduncum), muña (Minthostachys mollis), and thyme (Thymus vulgaris) were collected in different regions of Bolivia (Figure 1).

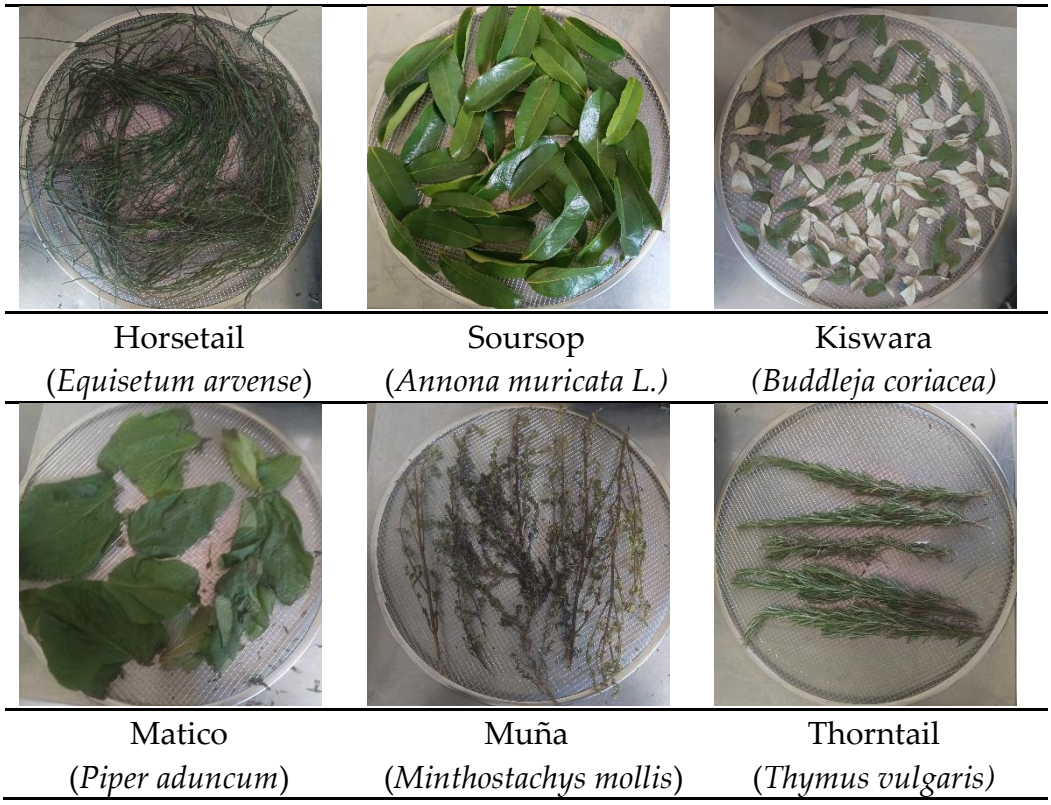

Figure 1. Leaves of selected and collected plants.

4.3. Leaf Dehydration

The collected leaves were subjected to a drying process in a dehydrator (DEHYDRAROE, ST-22, China) at a controlled temperature of 45 °C, since high temperatures may cause the loss of bioactive compounds [20]. Once dehydrated, the samples were crushed using a processor (Skymsen, LAR-06MB, Brazil) and sieved to obtain different particle sizes, classified using sieves of 9.5–6.3 mm (coarse sample), 6.2–2 mm (medium sample), and less than 2 mm (fine sample).

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4.4. Extraction of Phenolic Compounds

Soursop leaves were used as the basis for optimizing the extraction process. The dehydrated and ground leaves were suspended in the extraction solvent at a 1:20 m/v ratio. The solvent consisted of ethanol:water mixtures in volumetric proportions of 25:75, 50:50, and 75:25 (v/v). Maceration was carried out at 45 °C and 300 rpm using a thermo-shaker (DLAB, MS7-H550-Pro, China) for 12, 24, and 48 hours, applied to the different particle sizes.

The macerated suspension was vacuum-filtered, and the filtrate was concentrated using a rotary evaporator (BUCHI, with I-100 interface, Switzerland) at 140 mbar. The concentrated extract was filtered again to remove possible suspended particles, and the volume was adjusted to 100 mL of concentrated aqueous extract. Samples were stored in amber glass bottles at 4 °C prior to analysis.

The other plant species—horsetail (Equisetum arvense), kiswara (Buddleja coriacea), matico (Piper aduncum), muña (Minthostachys mollis), and thyme (Thymus vulgaris)—were extracted using the optimal parameters determined for soursop leaves.

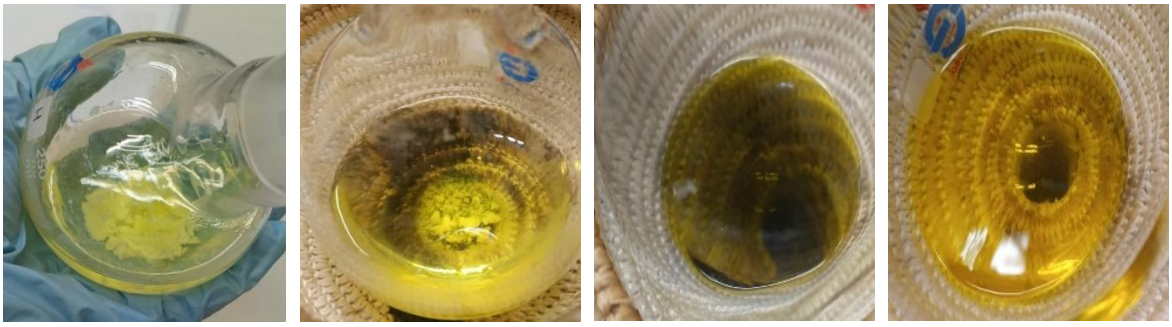
4.5. Traditional Decoction Analysis

Infusions constitute a traditional and widely used method for obtaining aqueous extracts from plant material. To evaluate the effect of this procedure on the bioactive properties transferred to the liquid medium, 5 g of leaves were used in 100 mL of distilled water, boiled for 5 minutes under controlled conditions. After cooling, the solution was adjusted to the initial volume and filtered. The obtained extracts were directly analyzed for the quantification of phenolic compounds and antioxidant activity.

4.6. Determination of Total Phenolic Content (TPC)

Both the original Folin & Ciocalteu (1927) protocol [21] and its adaptations proposed by Singleton & Rossi (1965) [22] and Singleton et al. (1999) [23] have been widely used for the quantification of total phenolic content (TPC). The most frequent modifications relate to assay volume or the amount of Folin–Ciocalteu (FC) reagent used, the order of reagent addition, sodium carbonate concentration (10% to 20%), the wavelength used for spectrophotometric reading, and reaction time (30 to 120 minutes). In addition, two versions of the Folin–Ciocalteu reagent were prepared, one with and one without lithium sulfate, in order to evaluate possible variations in analytical performance during phenolic compound quantification.

The reagent preparation was adapted for 50 mL of solution: 1.25 g sodium molybdate (Na_2MoO_4), 5.00 g sodium tungstate (Na_2WO_4), 35 mL distilled water (dH_2O), 2.50 mL phosphoric acid (95%), 5 mL hydrochloric acid (36%), 7.50 g lithium sulfate, and, in case of contamination (appearance of a greenish or bluish coloration after reflux), drops of 30% hydrogen peroxide (followed by boiling to remove excess). The solution was refluxed for eight hours (Figure 2), during which a progressive intensification of yellow color was observed. It was then cooled to approximately 50 °C, lithium sulfate (Li_2SO_4) was added, and finally the volume was completed with distilled water to reach the initial volume, ensuring the required concentration for analytical application.



a) b) c) d)

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Figure 2. a. FC reagent before reflux.b. FC reagent during reflux.c. FC reagent after completion of reflux (semi-green coloration).d. FC reagent with drops of 30% hydrogen peroxide (intense yellow coloration).

The concentration of a substance was determined using the equation of the straight line obtained from a calibration curve [23].

It is expressed as the equation of the straight line:

$$\text{Concentration} = \frac{\text{Abs - A}}{\text{B}} \cdot \text{f} \quad (1)$$

Where:

A is the intercept (y-intercept) of the calibration curve line, B is the slope of the calibration curve line, Abs is the absorbance obtained from the sample using a spectrophotometer at 765 nm, Concentration is the amount of phenolic compounds present in the sample expressed as the standard used (mg·L^{-1} GAE), and f is the dilution factor.

0.5 mL of Folin–Ciocalteu reagent (with lithium sulfate, used throughout the study), 0.5 mL of previously diluted sample at a 1:100 ratio, and 1 mL of 20% sodium carbonate were used. The mixture was made up to a final volume of 10 mL, and gallic acid standard solutions at concentrations of 20, 50, 100, 150, and 200 mg·L^{-1} GAE were prepared. The reactions were incubated for 30 minutes at room temperature and protected from light. Absorbance was measured using a UV/VIS spectrophotometer at 765 nm [24].

4.7. Determination of Antioxidant Capacity

The preparation of the DPPH reagent was carried out using ethanol at 80% and 96%, and methanol at 80% as solvents, at a concentration of 0.1 mM. The ability to reduce the DPPH radical was quantified by the decrease in absorbance at 517 nm. This reaction is evidenced by a color change from purple to yellow. One of the most commonly used reference compounds is ascorbic acid [25].

Relative antioxidant activity (% inhibition or RAA) was calculated using the following formula [26].

$$\%I = \frac{A\text{-}A_1}{A} \times 100 \quad (2)$$

Where:

I is the antioxidant capacity expressed as a percentage (%), A is the absorbance of the blank (control), and A_1 is the absorbance of the sample.

The calibration curve was prepared using ascorbic acid (AA) and solvent, with a final volume prepared in a 1:1 ratio for each sample, using four points. The blank consisted of 2 mL of solvent. Each concentration was analyzed in triplicate.

Plant samples were previously diluted in distilled water, considering this as their original solvent. For each assay, equal volumes of reagent and sample were mixed (1:1 ratio), allowing the reaction to proceed for 30 minutes in the dark at room temperature (approximately 20 °C), in order to prevent photodegradation or alterations in radical activity.

The IC_{50} was calculated, representing the concentration required to inhibit 50% of the DPPH free radical activity. This value indicates antioxidant effectiveness: the lower the IC_{50}, the higher the inhibitory power, and it is represented by the following expression [26]:

$$50 = Slope\ (X) + intercept \quad (3)$$

$$IC_{50} = X$$

Where:

50: Represents the value of 50% inhibition.

Slope: Slope of the calibration curve of the standard or sample.

Intercept: Intercept of the calibration curve of the standard or sample.

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X: The unknown value representing the concentration at which 50% of radical scavenging activity is achieved.

The model used for the extracts was based on Sebaugh (2011) [27].

$$Y=d+\frac{a-d}{1+\left(\frac{x}{c}\right)^{b}} \quad (4)$$

The lower asymptote is a, known as the minimum, and the upper asymptote is d, known as the maximum. The steepness of the linear portion of the curve is described by the slope factor b. The parameter c is the concentration corresponding to the response halfway between a and d. When this model failed to provide accurate predictions, polynomial regression was applied.

4.8. Statistical Analysis

All observations were performed in duplicate, and the data were expressed as mean ± standard error of the mean. The results were analyzed using analysis of variance (ANOVA), considering a significance level of $p < 0.05$ using Fisher's test, and means were compared using multiple comparison tests with SAS software (version 9).

5. Conclusions

The analysis of phenolic compounds in the different medicinal plants collected in February and October showed differences of at least 13%. Some species, such as matico and kiswara, even presented higher variations. This may be due to changes in the way plants produce phenolic compounds; therefore, the optimized extraction parameters should be recalculated throughout the year, which is an important consideration for the industrial use of these resources.

Compared with decoctions, similar differences were observed, with decoctions exceeding the ethanol:water extraction method in several cases. This suggests that water-based extraction can achieve the desired yield and that many of these compounds may be more polar.

Regarding antioxidant capacity, decoctions showed significantly higher values than ethanol:water solvent extraction, except for thyme, which in October showed higher antioxidant capacity with the solvent-based method, followed by soursop leaves, which were very similar to their water-extracted counterparts. Additionally, it was observed that there is not always a direct correlation between phenolic compound concentration and antioxidant capacity. Therefore, further research using instrumental methods is necessary, since the molecules involved may differ depending on the extraction method and season of collection.

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Abbreviations

CFT	Compuestos fenólicos totales
EAG	Equivalentes de ácido gálico
EAA	Equivalentes de ácido ascórbico
AAR	Actividad antioxidante relativa
DPPH	2,2-difenil-1-picrilhidrazil
FC	Folin–Ciocalteu
UV–Vis	Espectroscopía ultravioleta–visible

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