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Capacidad antioxidante y contenido fenólico total del extracto acuoso del tallo de matico (*buddleja globosa*)

Antioxidant capacity and total phenolic content of aqueous extract from matico (*buddleja globosa*) stem

Capacidade antioxidante e teor fenólico total do extrato aquoso do caule de matico (buddleja globosa)

ARTÍCULO ORIGINAL

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RESUMEN

Buddleja globosa (matico) es una planta medicinal nativa de Chile, ampliamente reconocida por las propiedades terapéuticas de sus flores y hojas. Sin embargo, su tallo ha sido poco explorado y generalmente descartado, a pesar de su potencial como fuente de compuestos bioactivos. El objetivo de este estudio fue investigar la concentración de polifenoles y la capacidad antioxidante del tallo de esta planta medicinal. Se cuantificaron compuestos bioactivos, como fenoles totales, flavonoides, vitamina C y catequina. La capacidad antioxidante se evaluó mediante las metodologías ABTS, DPPH y ORAC-H. Los resultados mostraron una elevada concentración de polifenoles totales (2749,78 mg de ácido gálico/100 g PS), flavonoides (2096,53 mg de catequina equivalente/100 g PS) y una significativa capacidad antioxidante en los ensayos ORAC-H (35661,73 µmol Trolox equivalente/100 g PS), ABTS (13404,39 µmol Trolox equivalente/100 g PS) y DPPH (8268,45 µmol Trolox equivalente/100 g PS). Estos hallazgos destacan el valor del tallo de Matico como una rica fuente de compuestos bioactivos. Este es el primer reporte sobre la composición del tallo de Buddleja globosa, sugiriendo que partes de la planta comúnmente descartadas poseen un alto potencial para el desarrollo de productos antioxidantes y terapéuticos.

Palabras clave: Antioxidantes; Buddleja globosa; Capacidad antioxidante; Compuestos bioactivos; Hierbas medicinales

ABSTRACT

Buddleja globosa (matico) is a medicinal plant native to Chile, widely recognized for the therapeutic properties of its flowers and leaves. However, its stem has been little explored and generally discarded, despite its potential as a source of bioactive compounds. The aim of this study was to investigate the polyphenol concentration and antioxidant capacity of the stem of this medicinal plant. Bioactive compounds, such as total phenols, flavonoids, vitamin C and catechin, were quantified. The antioxidant capacity was assessed using ABTS, DPPH and ORAC-H methodologies. The results showed a high concentration of total polyphenols (2749.78 mg gallic acid/100 g DW), flavonoids (2096.53 mg catechin equivalent/100 g DW) and a significant antioxidant capacity in the ORAC-H (35661.73 µmol Trolox equivalent/100 g DW), ABTS (13404.39 µmol Trolox equivalent/100 g DW) and DPPH (8268.45 µmol Trolox equivalent/100 g DW) assays. These findings highlight the value of Matico stem as a rich source of bioactive compounds. This is the first report on the stem composition of Buddleja globosa, suggesting that commonly discarded parts of the plant possess a high potential for the development of antioxidant and therapeutic products.

Key words: Antioxidants; Antioxidant capacity; Bioactive compounds; Buddleja globose; Medicinal herbs

RESUMO

Buddleja globosa (matico) é uma planta medicinal nativa do Chile, amplamente reconhecida pelas propriedades terapêuticas de suas flores e folhas. Porém, seu caule tem sido pouco explorado e geralmente descartado, apesar de seu potencial como fonte de compostos bioativos. O objetivo deste estudo foi investigar a concentração de polifenóis e a capacidade antioxidante do caule desta planta medicinal. Compostos bioativos, como fenóis totais, flavonóides, vitamina C e catequina, foram quantificados. A capacidade antioxidante foi avaliada pelas metodologias ABTS, DPPH e ORAC-H. Os resultados mostraram uma alta concentração de polifenóis totais (2.749,78 mg de ácido gálico/100 g de peso corporal), flavonóides (2.096,53 mg de equivalente de catequina/100 g de peso corporal) e uma capacidade antioxidante significativa no ORAC-H (35.661,73 µmol equivalente de Trolox/100 g de peso corporal).), ABTS (13404,39 µmol equivalente Trolox/100 g DW) e ensaios DPPH (8268,45 µmol equivalente a Trolox/100 g DW). Estas descobertas destacam o valor do caule do Matico como uma rica fonte de compostos bioativos. Este é o primeiro relato sobre a composição do caule de Buddleja globosa, sugerindo que partes da planta comumente descartadas possuem alto potencial para o desenvolvimento de produtos antioxidantes e terapêuticos.

Palavras-chave: Antioxidantes; Buddleja globosa; Capacidade antioxidante; Compostos bioativos; Ervas medicinais

INTRODUCTION

The exponential growth of the food industry has generated significant challenges, including food waste management (1). According to the Food and Agricultural Organization (FAO) (2), more than 1.5 billion tons of food produced are lost or wasted annually, generating a carbon footprint of more than 3.5 billion tons of carbon dioxide. A large part of this waste comes from the processing and transformation of raw materials (3), including waste of animal and plant origin (4). The plant-based food industry generates substantial horticultural waste, such as leaves, peels, pulps, seeds, roots and stems, which often contain valuable bioactive compounds (5).

In the case of medicinal plants, leaves and flowers are commonly used, while stems, bark and other inedible parts are discarded despite being rich in phytochemicals such as polyphenols, alkaloids, carotenoids, and vitamins (6,7). Phenolic compounds, in particular, have demonstrated antioxidant properties and protective effects against neurodegenerative, cardiovascular, and inflammatory diseases, as well as cancer, diabetes and autoimmune conditions (8,9,10). Globally, more than 30,000 medicinal plants with therapeutic properties have been documented, significantly boosting their commercialization (11).

According to the World Health Organization (WHO), up to 80% of the populations in developing countries use medicinal plants to treat various conditions (12). In Europe, at least 25% of the population uses phytotherapy treatments (13), and a study in Germany revealed that 81% of respondents prefer home remedies, such as hot drinks, with lemon, honey and chamomile, instead of pharmaceutical drugs (14). Similarly, in Chile, medicinal herbs are integral to folk medicine, with 70% of the population using phytotherapy or complementary medicine (15). This importance is reflected in the recognition of 103 medicinal plant species by the Chilean Ministry of Health, documented in the "Traditional Herbal Medicines (MHT)" report (16), which includes boldo (*Peumus boldus*), cedrón (*Aloysia citrodora*), maitén (*Maytenus boaria*), and matico (*Buddleja globosa*).

Matico is a medicinal plant widely distributed in Chile and other South American countries, such as Argentina, Bolivia, and Perú (17). Previous studies have identified phenolic compounds in its flowers, such as catechins and epicatechins, associated with high antioxidant capacity (18). Additionally, extracts from its leaves have been traditionally used as a poultice for wound healing (19), as well as in the treatment of ulcers and gastric disorders (20). Nevertheless, the stem, which may represent a rich source of antioxidants and bioactive compounds, is often discarded. To our knowledge, no literature reports are available on the bioactive compound and antioxidant capacity of matico stems.

This study aims to address the underutilization of agricultural by-products by evaluating the polyphenol concentration and antioxidant capacity of matico stems using the ABTS, DPPH, FRAP, and ORAC methods. The results of this research will not only contribute to the revalorization of an agricultural by-product but also open new perspectives for its application in the nutraceutical and food industries.

MATERIALS AND METHODS

Plant Material and sample preparation

The matico stem (*Budleja globosa*) was collected manually in the commune of Fresia, Los Lagos Region (Chile). After collection, the plant material was stored in perforated polypropylene bags. It was then freeze-dried and transported to the laboratory for the corresponding analyses. For the preparation of the extract, 1.0 g of freeze-dried matico stem was dissolved and homogenized in 20 mL of water acidified with 0.1% HCl, using an Ultra-Turrax (IKA-WERK©) for 45 seconds. The sample obtained was subjected to centrifugation at 5000 rpm for 12 minutes at room temperature. Finally, the supernatant was collected for use in subsequent analyses.

Reagents and equipment used

Reagent solvents were purchased from Sigma-Aldrich® (St. Louis, USA) and Merck (Germany). Ultraviolet-visible (UV-Vis) spectrum measurements were performed on a Multiskan Spectrum spectrophotometer (Thermo Scientific®). For the ORAC assay, a Perkin-Elmer LS-55 spectrofluorometer (UK) was used. Chromatographic analyses were carried out on a Shimadzu® Prominence UFLC high-performance liquid chromatograph (HPLC) (Japan).

Quantification of bioactive compounds

Total phenols were quantified using the Folin-Ciocalteu colorimetric method, following the protocol described by (21). Results were expressed in gallic acid equivalents per 100 g of dry sample (mg GAE/100 g DW). The quantification of (+)-catechin and (-)-epicatechin was performed by HPLC-DAD (liquid chromatography with diode array detection). A Shimadzu LC-20AD/T HPLC was used, equipped with an SPD-6A UV detector and a C18 Pinnacle II column (5 µm, 250 × 4.6 mm, Restek®, USA). The mobile phase consisted of methanol (A) and water acidified with formic acid (0.1%) (B), using a gradient elution: 60% A at the beginning; 80% A between 5-12 minutes; and 60% A between 13-14 minutes. The mobile phase flow was 1.0 mL/min. Total flavonoids were determined following the colorimetric method proposed by (22). The results were expressed in quercetin equivalents per 100 g of dry sample (mg QE/100 g DW). Ascorbic acid determination was carried out by high-performance liquid chromatography (HPLC), following the protocol of (23). A mobile

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phase composed of purified water acidified with 0.1% formic acid was used, with an isocratic elution system. The mobile phase flow was 0.8 mL/min, and the column was kept at a constant temperature of 35°C. Detection was performed at a wavelength of 245 nm. Identification and quantification of ascorbic acid was carried out using calibration curves prepared with standard solutions of known concentrations of the compound.

Determination of antioxidant activity

The antioxidant activity against the DPPH radical was evaluated according to the method of (24), with modifications. 10 µL of sample was mixed with 990 µL of DPPH solution, and left to react for 30 minutes at room temperature. The decrease in absorbance, associated with the reduction of DPPH, was measured at 517 nm. The results were expressed in trolox equivalents per 100 g of dry sample (µmol TE/100 g DW). The antioxidant activity by the ABTS assay was determined following the protocol of (25), with adaptations. 10 µL of sample was added to 990 µL of ABTS•+ solution. The reduction in absorbance was recorded after 30 minutes of reaction at 732 nm. The results were compared with a reference curve constructed with trolox, and reported as TEAC (trolox equivalents in µmol per 100 g DW). The ORAC assay was performed according to the methods described by (26, 27). Solutions were prepared with 30 µL of sample, 21 µL of fluorescein (1×10^{-2} M) in PBS (75 mM) and 50 µL of AAPH (0.6 M) in PBS. The reaction was maintained at 37°C and pH 7.4. Readings were made at 493 nm (excitation) and 515 nm (emission). Antioxidant capacity was calculated as the difference in area under the curve (AUC) between the blank (no sample) and the sample, comparing with a trolox curve. Results were expressed in TEAC (µmol trolox per 100 g DW) according to equation 1.

$$ORAC = \frac{AUC - AUC^{\circ}}{AUC_{Trolox} - AUC^{\circ}} f[Trolox] \quad (1)$$

Where AUC is the area under the curve for the sample, AUC° area under the curve for the control, AUCTrolox area under the curve for Trolox, f is the dilution factor for the extracts.

RESULTS AND DISSCUSION

The metabolite content is presented in Table 1, where the concentration of total phenols was determined as total gallic acid equivalents. Flavonoids, vitamin C and catechin were also quantified. The presence of epicatechin, p-coumaric acid or caffeic acid was not reported. The antioxidant capacity of matico stem was evaluated by different methodologies (ABTS, DPPH, ORAC-H), the results of which can be consulted in Table 2. It is worth mentioning that the results are presented as the mean ± standard deviation and are expressed in terms of dry basis (DW).

Tabla 1. Bioactive compounds quantified in matico steam.

Compound	Matico steam
Total phenols (mg gallic ac. Eq.)	2749.78
Flavonoids (mg catechin Eq.)	2096.53
Ascorbic acid (mg)	3,8
Catechin (mg)	266,61

*The results of each analysis are presented as the mean ± standard deviation (N = 3). **Results expressed in 100 g DW.

Tabla 2. Antioxidant activity in Matico steam

Compound	Matico steam
ABTS (µmol Trolox Eq.)	13404.39
DPPH (µmol Trolox Eq)	8268.45
ORAC-H (µmol Trolox Eq.)	35661,73

*The results of each analysis are presented as the mean ± standard deviation (N = 3). **Results expressed in 100 g DW.

Secondary metabolites

Several studies have documented high concentrations of metabolites in different parts of the matico plant (18) pointed out that the flower is a promising source of bioactive compounds with potent antioxidant capacity. Regarding the leaves, significant concentrations of polyphenols have been reported, which highlights the medicinal value of this plant species (28).

In our study, it was determined that the concentration of total polyphenols in the matico stem (2749.78 mg gallic acid/100 g DW) is comparable and even higher than that reported for several widely recognized medicinal plants. This finding is particularly relevant, since bioactive compounds, such as polyphenols, have been associated with chemoprotective, anti-inflammatory, antioxidant, and anticancer effects, among others (29-31). For example, in East Asia, korean mint (*Agastache rugosa*) is known for both its medicinal and ornamental properties (32), standing out for its anticancer and antimicrobial action, which has been attributed to the presence of phenolic compounds (33,34). Reported that a stem extract of korean mint showed remarkable bactericidal and/or bacteriostatic capacity against *Escherichia coli*, *Staphylococcus hemolyticus*, and *Cronobacter sakazakii* (35). These effects were mainly attributed to the presence of anthocyanins and flavonoids, with a total polyphenol concentration of 765 mg GAE/100 g DW. It should be noted that this amount is approximately 3 times lower than that determined in our study, suggesting a higher concentration of these compounds in the matico stem, with potential antioxidant and antimicrobial benefits. Furthermore, the

concentration of catechins quantified in our study (266.61 mg/100 g) was significantly higher than the 0.4 mg/100 g reported by (35) for korean mint. This finding is important, since catechins possess antioxidant properties, in addition to antimicrobial, anti-inflammatory, and UV protection effects (36-40).

Compared to *Jatropha curcas*, an Egyptian plant known for its therapeutic effects derived from its stem extracts, which have shown benefits in the treatment of pyorrhea, dental and gum problems (41,42), as well as in cases of malaria (43), dysentery (41), and in wound healing and relief of muscle pain (44), our values are slightly higher, since (45) reported a total polyphenol concentration of 2235 mg GAE/100 g. Similarly, the jarilla plant (*Barkleyanthus salicifolius* (Kumth) H. Rob & Brettell), is known for its anti-inflammatory, nephroprotective, and hepatoprotective properties (46). In aqueous and methanolic extracts of Jarilla, total polyphenol concentrations of 199 mg GAE/100 g DW and 1831 mg GAE/100 g DW, respectively, have been reported (47). These concentrations are considerably lower than those reported for matico stem. It is worth noting that, in the study carried out by (47), both extracts inhibited the growth of *Phytophthora capsici* and *Colleototrichum gloeosporioides*, which could indirectly suggest the potential of matico stem polyphenols in pathogen control. Regarding vitamin C, the concentration in the matico stem (3.8 mg ascorbic acid/100 g DW) is similar to that reported by (48) for cherry stem, which presented values of 3.11 and 3.02 mg of ascorbic acid/100 g. However, in that same study, a concentration of total polyphenols of 11800 mg GAE/100 g was reported in the Cherry stem, a figure considerably higher than that of the matico stem. On the other hand, the determined amount of flavonoids determined in the matico stem (2096.53 mg catechin Eq/100 g DW) suggests that these compounds constitute one of the main groups of polyphenols present, contributing significantly to the antioxidant capacity detected in our study.

Antioxidant Capacity

In this research, the antioxidant capacity of matico stem was evaluated using 3 different methodologies (ABTS, DPPH, and ORAC). The combined application of these techniques is of great relevance, since it offers information and a comprehensive view on the nature and characteristics of the bioactive compounds present in the samples analyzed. The results are detailed in Table 2.

Using the ORAC assay, the antioxidant capacity of matico stem (35661.73 µmol Trolox Eq/100 g DW) was of the order of magnitude or even higher than those Andean plants reported by (49), such as hierba santa (*Cestrum auriculatum*), alcachofa (*Cynara scolimus*), and cuturrumaza (*Rumex crispus L.*), whose antioxidant capacities

were reported at 12300, 33860, and 29800 µmol Trolox/100 g DW, respectively. The ORAC method is particularly valuable because it measures the ability of phenolic compounds to capture the peroxyl radicals generated in situ through a hydrogen atom transfer (HAT) proton transfer mechanism (50). Therefore, these results could indicate that the polyphenols present in the matico stem have a considerable potential to act specifically as ROO• peroxide radical scavengers.

On the other hand, the results obtained by the ABTS assay showed an antioxidant capacity of 13404.39 µmol Trolox/100 g, a value significantly higher than those reported for other plant species of recognized medicinal importance. For example, (51), reported antioxidant capacities of 3347 and 4528 µmol Trolox/100 g for the stems of *Lonicera japonica* Thunb and *Morus alba* L., respectively. Comparatively, the values obtained for the matico stem are notably higher. For this reason, it is relevant to mention that *Lonicera japonica* Thunb is part of the Pharmacopoeia of the People's Republic of China, and that it is recognized for its antibacterial, anti-inflammatory, and antiviral properties, which have been directly related to its bioactive compounds (52,53). Similarly, stem extracts of *Morus alba* L. have shown anti-inflammatory, antibacterial properties, and positive effects in the treatment of antiosteoarthritis (54-56). Likewise, our results far exceed those reported for the stem of other important medicinal plants, such as *Perilla frutescens* (L.) Britt (1680 µmol Trolox/100 g DW) and *Stemona sessilifolia* (Miq.) Franch. (1543 µmol Trolox/100 g DW) (51).

The high antioxidant capacity determined by the ABTS methodology suggests that the antioxidant compounds present in the matico stem are highly hydrophilic. Furthermore, it is important to take into account that the ABTS assay reacts with a broad spectrum of antioxidants, due to its high reactivity and low selectivity in reactions towards hydrogen donor atoms (57). This could, in certain cases, lead to an overestimation of the real antioxidant capacity of the samples, since ABTS can react with any hydroxylated aromatic compound, regardless of its true antioxidant potential (58).

In line with other investigations, in our study it was observed that the antioxidant capacity values measured by DPPH were considerably lower than those obtained with ABTS (Table 2). For example, (49), reported an antioxidant capacity of 2760 µmol Trolox/100 g DW for artichoke leaves using DPPH, while the value obtained with ABTS was significantly higher, reaching 33860 µmol Trolox/100 g DW). This trend is commonly observed and is attributed to the greater selectivity and stability of the DPPH radical, since its molecular conformation and high steric hindrance limit its ability to interact with certain antioxidant compounds (59,26).

Finally, unlike what was pointed out by (18), who indicated that the antioxidant compounds present in the matico flower are predominantly lipophilic,

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in this study we observed that the compounds present in the stem show greater solubility in aqueous media. This behavior is reflected in the greater sensitivity of the compounds to the ABTS methodology, which reinforces the idea that different parts of the plant present polyphenols with specific properties, structural characteristics, mechanisms and functions (60).

CONCLUSION

The present study successfully achieved its objective of evaluating the bioactive composition and antioxidant capacity of the often-discarded stem of matico (*Buddleja globosa*), demonstrating significant concentration of polyphenols, flavonoids, and vitamin C. The antioxidant assays (ABTS, DPPH, ORAC-H) confirmed its potential to neutralize free radicals, supporting its possible inclusion in food and medicinal applications. These findings provide a basis for re-evaluating the use of plant by-products, promoting sustainability, and reducing agricultural waste. The results contribute to the growing body of evidence supporting the valorization of underutilized plant parts in the nutraceutical and food industries. Future research should focus on optimizing extraction methods and conducting in-depth in vitro and in vivo studies to further elucidate their therapeutic potential and mechanisms of action.

CONFLICT OF INTEREST. The authors declare that there is no conflict of interest for the publication of this scientific article.

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