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ANTIOXIDANT ACTIVITY OF LINDEN TEAS [*TERNSTROEMIA PRINGLEI* (ROSE) STANDLEY] OBTAINED BY DECOCTION AND MICROWAVE EXTRACTION.

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Abstract

Linden flower (*Ternstroemia pringlei*) is used in traditional medicine to treat insomnia and anxiety, although traditionally the extract was obtained by aqueous decoction, nowadays people also use the microwave oven to obtain herbal teas; however, the qualitative and quantitative composition of the extract can vary depending on the extraction method used. Therefore, it is important to characterize the extracts obtained by various extractive techniques. The aim of this research was to determine the polyphenol content and antioxidant activity of *T. pringlei* extracts obtained by decoction and microwave-assisted extraction. The extracts obtained were evaluated by the Folin Ciocalteu technique to quantify polyphenols and the antioxidant activity was determined by three methods (DPPH, FRAP and hydrogen peroxide reduction). The results obtained show that the extract obtained by microwave has a higher amount of polyphenols (40.4 mg EAG) and a better antioxidant activity than the decocte that contained 27.7 mg EAG of polyphenolic compounds.

Keywords: polyphenols, secondary metabolites, bioactivity.

1 Introduction

Linden flower [*Ternstroemia pringlei* (Rose) Standl.] is used as a decoct in traditional Mexican medicine for the treatment of insomnia and anxiety; but it is also used to reduce rheumatic pain and is among the most consumed and traded medicinal plants in Mexico [1-3]; among the scientific studies carried out to evaluate its activity are those focused on evaluating its anthelmintic, sedative and antioxidant action [2-4]. Nowadays, the microwave oven is frequently used as a tool to obtain herbal teas from medicinal plants, so the quality of the extract obtained by traditional methods such as maceration, decoction and soxhlet extraction must be compared with the herbal teas extracted by alternative methodologies of green chemistry such as ultrasound-assisted extractions, supercritical fluids and microwaves [5].

Microwave-assisted extraction has aroused scientific interest because it achieves high yields with a shorter extraction time and lower energy and solvent consumption; in this sense, a benefit of microwave-assisted extraction is that in addition to increasing the amount of extract, the bioavailability of phytochemicals is increased; that is, of the secondary metabolites of interest as functional compounds [5-7]. Microwave-assisted extraction is an ecological and efficient process that has been used to obtain polyphenolic compounds from medicinal plants; In this process, a significant increase in the amount of polyphenols extracted is observed and their antioxidant activity is not affected, but is also increased or preserved [8-10]. Therefore, the objective of this research was to

evaluate the effect of microwave-assisted aqueous extraction of *Ternstroemia pringlei* on its antioxidant activity and to compare it with the antioxidant activity of decocte.

2 Materials and methods

The plant material was acquired in the market as dry material packaged in sachets for domestic extraction, three boxes of 24 sachets each, same batch and reporting the content of *Ternstroemia pringlei* as the only medicinal plant; the integrity of the product was verified and in the laboratory it was verified that the sachets were intact and in good condition. A weight uniformity test (n=30, 10 packs of each box) of *T. pringlei* sachets was performed, recording the weight of the packaging with vegetable powder and then measuring the weight of the powder alone.

For decoction extraction, 250 mL of purified water was heated to a boil in a beaker and a sachet of *T. pringlei* powder was placed in the boiling water for 15 minutes; while for microwave-assisted extraction, 250 mL of purified water was transferred into a beaker and a sachet of *T. pringlei* was placed in a beakerinside the water, then the glass was introduced into the microwave oven (LG® brand of 1000W power) on the dish and an exposure was applied for 30 s (2 cycles). Nine replicates of each extraction were made, ensuring that three envelopes were taken from each of the boxes.

The extracts were concentrated dry in a rotary steamer and the weight of the total soluble solids contained in each of them was recorded and the percentage of extraction was calculated; Subsequently, the extract was resuspended in distilled water to obtain a concentration of 1000 mg of soluble solids per liter of solvent; Aliquots were taken from this solution for the quantification of polyphenolic compounds and determination of their antioxidant activity.

The polyphenolic compounds were quantified according to the Folin Ciocalteu method based on the reduction of the reagent (tungstophosphate and molybdophosphate) in alkaline medium, generated by the addition of sodium carbonate to the reaction mixture, producing a blue compound that can be quantified spectrophotometrically; for this, 100 mL of the Folin Ciocalteu 2N reagent is added to 1.0 mL of water, mix well, and add 100 mL of the extract, let this mixture react for 15 minutes and then add 500 mL of 10% sodium carbonate, homogenize and incubate at 40 ºC for 30 minutes and finally read the absorbance at 760 nm; Gallic acid was used to obtain the calibration curve and the results are expressed as mg of gallic acid equivalents per gram of extract (mg EAG/g) [11,12].

The first technique used to determine the antioxidant activity of the extracts was the neutralization capacity of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) according to the method described by Brand Williams, in which 2.9 mL of a 100 mM DPPH radical methanol solution is used and 100 mL of extract is added; a calibration curve of the DPPH is made and a solvent target is run; the results are expressed as the extract concentration (in ppm) that inhibits 50% of DPPH radical, i.e., in IC50 (mg/mL) [11,12].

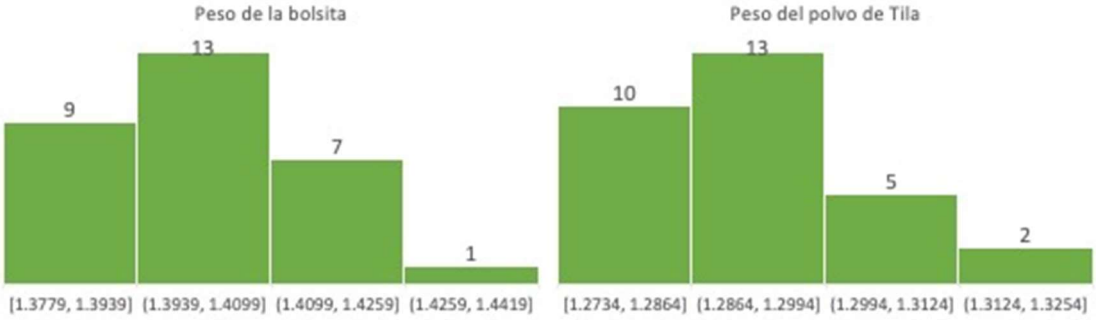
For the determination of the ferric ion reducing power (FRAP), the Benzie and Strain procedure was followed, in which a solution of 200 mL of TPTZ [2,4,6-tri(pyridyl-2)-S-triazine] 10 mM in 40 mM HCl, 200 mL of a 20 mM ferric chloride solution and 2.0 mL of a 300 mM sodium acetate buffer solution (pH=3.6) were used, mixes well and absorbance is measured at 593 nm; then 50 mL of extract is added and stirred in a vortex for 30 s, allowed to react for 15 minutes and absorbance is measured at 593 nm; a calibration curve was performed with $FeSO_4$ $7H_2O$ and the results are expressed as micromoles of Fe^{2+} produced per 100 g of sample [11,13].

Finally, the hydrogen peroxide (H_2O_2) removal capacity was quantified by the Ruch *et al.* method, for which a 2mM H_2O_2 solution was prepared in a phosphate buffer solution (pH 7.4), the peroxide concentration was measured spectrophotometrically at 230 nm considering a molar absorptivity of 81 mol L^{-1} cm^{-1}; 100 mL of extract was added to 2.9 mL of the H_2O_2 solution and allowed to react for 15 minutes at room temperature, then the absorbance of the mixture was measured using the phosphate buffer solution as a target and an extract target was also measured to correct the absorbance of the reaction mixture [14].

3 Results and discussion

The analysis of the uniformity of the weight of the sachets and the dust of the linden flower (*T. pringlei)* shows that its distribution, although not normal, tends towards normality (Figure 1); the total weight of the sachets studied ranged from 1.3779 to 1.4419 g (X=1.40029 g, SD= 0.01378 g) and that of linden powder was 1.2734 to 1.3254 g (X=1.2917 g, SD= 0.0117 g); These results allow us to estimate that the variation in the content of linden flower powder does not exceed 12 mg, which represents less than 1% of the total weight, and consequently would not significantly influence the amount of solids extracted by the solvent.

Figure 1 Distribution of the weight of sachets and linden dust contained (n=30).



Source: Authors' own elaboration with the results of the research.

The yield obtained in each extraction process is shown in Figure 2, where it can be seen that microwave-assisted extraction significantly improves the solids obtained from the plant material and, consequently, the extraction yield. However, sometimes the yield of extraction does not significantly improve the quality of the extract, or sometimes the technique used may affect the biological activity of the extract when breaking down, especially if a greater amount of heat is used that affects the thermolabile metabolites; In the case of microwaves, it has been reported that it generally improves the extraction performance of secondary metabolites and tends to retain or enhance their biological activity [15,16].

Figure 2 Total soluble solids (mg solute/ 100 mL tea) and yield (%) of the extractive process (n=9) for obtaining linden tea by decoction and microwave.

Source: Authors' own elaboration with the results of the research.

The amount of polyphenols determined in the decocte of *T. pringlei* (Table 1.1) coincides with that reported for species of the same genus [17,18], a significantly higher amount of polyphenolic compounds was obtained in the extract obtained by microwaves, with about 45% more of this type of secondary metabolites, this is in agreement with other studies where it was evaluated that the extraction performance of polyphenols is better with microwaves than with others conventional methods, which is why it is considered an effective technique for the recovery of bioactive compounds for the food and pharmaceutical industries [19-23].

Board 1.1 Polyphenols and Antioxidant Activity of Extracts *T. pringlei.*

Test	Decoction	Microwave
Polifenoles (mEAG/g)	27.7±0.9	40.4 ± 1.5
DPPH (IC50 mg/mL)	45.9±2.0	37.7 ± 1.7
FRAP (mmol Fe^{2+}/L)	29.0 ± 1.1	35.6± 1.9
H_2O_2 (IC50 mg/mL)	85 ± 3	72 ± 2

Source: Authors' own elaboration with the results of the research.

Likewise, it was found that the antioxidant activity corresponds to the amount of polyphenols determined, in the sense that, as reported in the literature, a higher amount of polyphenols implies a greater antioxidant activity [17,23]; in the DPPH radical neutralization assay, the IC50 of the microwave extract was lower than that of the decocte, meaning that the first extract contains a higher amount of antioxidant compounds that require a lower concentration than the decocte to produce the same effect [24].

Another antioxidant test that was evaluated was the reducing power of the ferric ion, this was carried out with the FRAP technique, in this test it is determined how much ferric ion can be reduced by the extract, in such a way that a greater production of ferrous ion means a greater reducing power of the extract [25]; In this sense, the microwave extract reduced a greater amount of ferric ion and exhibited better antioxidant action. Finally, the microwave extract also showed increased antioxidant activity in the hydrogen peroxide reduction assay; This test is complementary to the previous ones because, although hydrogen peroxide is not properly considered a reactive

oxygen species, it is biochemically and physiologically a precursor of highly reactive species that are harmful to biological systems; Therefore, it is important to measure the peroxide-reducing capacity [26].

Although some authors assume that microwave extraction not only improves the extraction of secondary metabolites but also tends to preserve or improve their biological activity [15,16], in this case there is only an increase in antioxidant activity due to a greater amount of phenolic compounds obtained; But this does not represent an increase in its antioxidant activity due to the quality of the metabolites, but proportional to the amount of polyphenols contained in the extract.

4 Conclusions

Microwave-assisted aqueous extraction of linden flower (*T. pringlei*) allowed for a higher amount of polyphenolic compounds and better antioxidant activity of the extract compared to decocte from the same part of the plant.

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