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Hydrolyzable polyphenols contained in extracts from *Mentha piperita L.* and antioxidant capacity

Abstract

Mentha piperita L., is one of the most popular and consumed species worldwide because of its secondary metabolites with antioxidant properties, among these metabolites are polyphenolic molecules. These molecules can be extracted using different methodologies. However, the range of bioactive compounds in an extract can vary because of different factors, including the extraction methodology. Recently, use of hybrid technologies for extraction of plant compounds has been increased since a empowerment of various properties in the obtained extracts has been demonstrated. In this study was performed an analysis of the polyphenolic compounds and antioxidants from Mentha piperita L. Polyphenolic compounds were extracted using different extraction techniques which are reported as efficient and friendly to the environment, like ultrasound and microwave, which were compared with the hybrid technology (ultrasound-microwave). Results showed a significant increase on the obtaining of hydrolyzable polyphenolic compounds and antioxidant capacity, highlighting that using extraction by a hybrid technique (ultrasound-microwave) showed a greater % yield of extraction compared to ultrasound or microwave used separately.

Keywords: *Mentha piperita L.*, antioxidant, hydrolyzable polyphenolic, extraction

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Introduction

Nowadays, polyphenols are very important due to their high demand in the formulations of healthy foods, cosmetics, and pharmaceutical products.[1] Polyphenols constitute one of the most common and abundant families of plant compounds that have been found in flowers, stems, leaves, and fruits.[2] They are considered secondary metabolites involved in the chemical defense of plants and are a wide variety of molecules that contain at least one aromatic ring with one or more hydroxyl groups in addition to other substituents.[3] These compounds have been associated to different biological properties such as antimicrobial, anti-inflammatory, anticancer, and antioxidant activities.[4] Polyphenols are classified as condensed polyphenols, phlorotannin, hydroxy stilbenes, hydrolyzables, and flavonoids, and hydrolyzable polyphenols which are divided into two large groups: gallotannins, molecules formed by a glucose nucleus linked and ellagitannins which are non-nitrogen compounds found in the cytoplasm and vacuoles of plant cells as secondary metabolites.[5]

Mentha piperita L., better known as mint, is a highly scented perennial plant that belongs to the Lamiaceae family which does not grow in the wild; however, it tolerates extreme climates, but it has adapted to humid soil.[6] M. piperita is one of the most popular and consumed species worldwide since it has been recognized as one of the most important commercial medicinal plants used in the clinical, pharmaceutical, food, and cosmetic industries.[2] Various scientific studies have revealed numerous biological effects such as antioxidant, antimicrobial, antiviral, anti-inflammatory, biopesticide, larvicide, anticancer, radioprotective, genotoxic, and antidiabetic activity.[2] This is due to its high content of polyphenolic compounds, which promote the formation of flavonoids, flavanones, lignins, and lignans. Some of the phenolic compounds identified in Mentha piperita L. leaves are chlorogenic acid, caffeic acid, and rosmarinic acid. Among the flavones, the presence of luteolin 7-O-glucoside and apigenin is recorded.[2] However, the percentage of extraction of these compounds depends on different variables, one of them being the extraction technique.[2] Multiple conventional methodologies have been registered to obtain biologically active molecules from plants, such as different types of distillation, immersion, pressing, maceration, and sublimation, among others, which, although they have already been widely used, have various disadvantages.[8] Because of the green chemistry concept, development of environmentally friendly extraction technologies has become of vital importance.[9] One of these technologies is the use of sonochemistry, since the entire extraction process can be completed in minutes with high reproducibility, reducing solvent consumption, simplifying handling and work, and eliminating wastewater post-treatment.[10]

The use of microwave has also become very popular in the extraction of biological material since it presents great advantages, being a faster process, smaller equipment, presenting shorter extraction time, less use of solvent, higher rate extraction, and better products with lower costs.[11] Although, these techniques have numerous advantages, recent research has been performed to optimize the results further by creating hybrid methodologies seeking to improve extraction efficiency.[12] Sillero reported that the combination of ultrasound and microwaves is one of the most effective hybridized methods that provide rapid and efficient extraction compared to other hybrid methodologies.[13] In this context, the objective of this research is to perform a comparative study between these three techniques (ultrasound assistance, microwave assistance, and hybrid ultrasound-microwave assistance) in order to determine if there are significant changes on the amount and antioxidant capacity of hydrolyzable polyphenols extracted from *Mentha piperita L.*

Material and methods

Extraction of bioactive compounds from *Mentha piperita L*

Three different treatments were carried out using 100g of dry *Mentha piperita L.* which were dissolved in 800 mL of ethanol 96% using a hybrid Ultrasonic Microwave cooperative Workstation model XO-SM400 for 30 minutes (Table 1).

Table 1 Extraction conditions

Sample	Ultrasound Power (W)	Microwave Power (W)
A1	800	800
A2	800	0
A3	0	800

Determination of total phenolic content using the Folin-Ciocalteu method

The *Mentha piperita L.,* extracts obtained by different methodologies were prepared at a concentration of 1000 ppm. Subsequently, 800 µL of the sample was placed in a test tube. Next, 800 µL of Folin-Ciocalteu reagent were added and allowed to react for 5 min. After, 800 µL of sodium carbonate (0.01M) were added and allowed to react for 5 minutes. Finally, the solution was diluted with 5mL of distilled water, and its absorbance was read at 790 nm. To determine the content of total phenols, a calibration curve was carried out with gallic acid at concentrations of 0, 50, 100, 150, 200, 250 and 500 ppm.[14]

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

A calibration curve was performed with TROLOX (Sigma-Aldrich) with different concentrations ranging from zero to 0.5, in the three antioxidant capacity techniques, and 1:10 dilutions of the extracts were prepared. The DPPH reagent was prepared by weighing 1.18 mg of DPPH which were placed in 50 mL of ethanol. Subsequently, 190 µL of the DPPH reagent and 10 µL of the extract dilution were added to a microplate and allowed to stand for 30 min in the dark and finally read in a spectrometer at an absorbance of 517 nm. Equation 1.

$$\%\ \text{inhibition} = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} x100 \qquad \text{eq. 1}$$

ABTS free radical scavenging assay (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)

ABTS free radical scavenging assay (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) The test was carried out by preparing 5 mL of persulfate with ABTS radical, which was allowed to stand in the dark at room temperature for 16h before use. It was subsequently diluted with ethanol until an absorbance value of 0.7 ± 0.02 was obtained at a wavelength of 734 nm. Next, 950 µL of the adjusted ABTS solution was placed in the test tube, and 50 µL of the sample was added. Finally, the absorbance was read immediately at a wavelength of 734 nm using distilled water as a control.

LOI Oxygen limiting index

Previously, a linoleic acid solution was prepared with ethanol 96% and Tween 20. Then, 50 µL of the extract was taken and mixed with 100 µL of linoleic acid solution and 1.5 mL of 0.02 M acetate buffer, pH 4.0. Controls contained 50 µL of distilled water. All samples were homogenized with shaking for 3 min. The solutions obtained were incubated at 37°C for 1 min, and then 750 µL of 50M FeCl2 solution was added. Subsequently, sample was incubated again at 37°C and allowed to rest for different times, these being one hour and 24 hours. After that, 250 µL of the mixture was taken, and 1 mL of 0.1M NaOH was added to stop the oxidation process. Finally, 2.5 mL of 10% ethanol was added, and the absorbance was measured at 232 nm against a black of 10% ethanol.[15]

Results

Table 2 shows yield of extraction (%) of polyphenols using different methods, highlighting that the highest extraction percentage was obtained using the hybrid (A1) method, ultrasound-microwave. Solid powder extracts of different shades were obtained, where green/brown colors stand out, showing a more intense coloration when the hybrid method was employed.

Table 2 Yield of extraction using three different technologies

Sample	Ultrasound Power (W)	Microwave Power (W)	% Extraction
A1	800	800	24
A2	800	0	14
A3	0	800	14

The total hydrolyzable polyphenols content revealed that the extract obtained by hybrid assistance (A1) has a greater amount of hydrolyzable polyphenols compared to the extracts obtained by the other techniques (A2 and A3) (Table 3), while the amounts obtained using the ultrasound and microwave methodologies present similarities, being slightly greater when using ultrasound.

Table 3 Quantificantion of soluble hydrolyzable phenols in the extracts of *Mentha piperita L*

Sample	Total phenols (mg EAG/g)
A1	329.68
A2	153.08
A3	126.58

The antioxidant capacity of the extracts of dried leaves from *Mentha piperita L.* showed varied results because these tests involve different reactions. The DPPH and ABTS techniques use the same principles: a synthetic reagent that generates a colored radical or a redox-active compound which is reacted with the ability of a biological sample to eliminate the radical or to reduce the redox-active compound, which is then visualized using a sperctrophotomer.[16] However, the lipoperoxidation technique is responsible for measuring the inhibition of lipid oxidation using linoleic acid as the lipid source and a FeCl2 solution to induce oxidation, so, in the presence of antioxidants, the accumulation of peroxide lipid should be minimal until all antioxidant compounds present in the samples are exhausted.[15] A higher percentage of inhibition is observed in all techniques when using extract A1 (Table 4), while for A2 and A3 similar results are observed between them, but lower that A1. The above is attributed to the presence of a greater number of polyphenols. In this context, the increase in the capacity of the extracts when using the combination of microwave ultrasound is due to the mechanical effect caused by the waves transmitted during sonication since it facilitates the extraction of soluble compounds by breaking the plant cell walls through cavitation phenomena. In turn, the entire sample is heated with microwave waves very quickly, which triggers the movement of dissolved molecules, promoting a mass movement that allows access to the cellular material by the solvents.[17]

Table 4 Inhibition percentages obtained using different techniques for measuring antioxidant capacity

	% Inhibition		
Sample	**DPPH**	**ABTS**	**LOI**
A1	70.60	99.25	53.15
A2	50.79	97.59	50.15
A3	60.68	99.14	11.39

Discussion

Extraction of bioactive compounds from *Mentha piperita L*

The extraction of bioactive compounds with the hybrid technology is higher in comparison to the other extraction techniques, because in the hybrid method is created an interaction between ultrasound and microwave technologies, where the cavitation bubble and the breakdown of the cell wall caused by the ultrasound waves, and the increase in temperature by microwaves, promoted the solvation and release of secondary metabolites that are present in plant leaves. The high % is highlighted using the hybrid methodology, and this is due to the sum of the influence of ultrasound and microwaves.

Determination of total phenolic content using the Folin-Ciocalteu method

In the determination of Folin-Ciocalteu method, it has been reported that the extracts of dry leaves from *Mentha piperita L.,* obtained by ultrasound or microwave assistance showed mainly flavonoids. Currently, there are a total of 49 flavonoids identified in the extracts from *Mentha piperita L.,* obtained by these techniques such as Flavones (luteolin, luteolin- O-diglucuronide, luteolin O-glucuronide, luteolin 7-O-□-glucuronide, luteolin 7-O-rutinoside and isorhoifoline), flavanones (eriodictyol, naringin, eriodictyol-glycopyranosyl rhamnopyranoside, naringenin-7-O-□-glucodide, hesperidin and eriocitrin), methoxyflavones (gardenin B, 5,6-dihydroxy-7,8,3,4-tetramethoxyflavone and salvigenin) and flavonols (catechin, rutin, [-]-epicatechin, quercetin, quercetin-4-glucoside, kaempferol 7-O-rutinoside, and myricetin-O-glucoside)[18] However, this type of polyphenols belongs to condensed polyphenols, which are not captured by the Folin-Ciocalteu. On the other hand, hydroxycinnamic acids, which are hydrolyzable polyphenols, such as caffeic, rosmarinic hydroxycinnamic, and chlorogenic acids, are acids that are found in abundance in *Mentha piperita L.,* and therefore in this context, it is attributed that the amount of hydrolyzable polyphenols content of the extract obtained from the dry leaves of *Mentha piperita L*. is greater when using hybrid technologies due to a greater carryover of this type of molecules, however, it was not possible to rule out the presence of polyphenols condensed in the extracts.

Antioxidant activity

The antioxidant activity values obtained in this study reflected better inhibition percentages than those reported in the literature. Al-Mijalli[19] consignee antioxidant activity percentages around 49.83% using DPPH and 61.19% employing ABTs for extracts from *Mentha piperita L.* while Bui-Phuc et al mentioned percentages of 52% for DPPH and ABTS,[20] all of which are lower than those achieved in the extracts obtained by the hybrid methodology. However, it should be noted that the percentage of inhibition of lipid oxidation is lower compared to the scavenging of free radicals since all the results are less than 60% inhibition. In this sense, it is assumed that hydrolyable polyphenolic compounds can resist the peroxidation of linoleic acid under the test conditions but have a better capacity to eliminate free radicals.

Conclusion

The comparison among the individual assistance of ultrasound and microwaves and the hybrid technology to obtain natural extracts from dried leaves of *Mentha piperita L.,* showed that hybrid extraction is the most adequate for obtaining % yields greater than 10%, and showing greater antioxidant capacity than using ultrasound and microwave individually. In the literature, both ultrasound and microwave are reported as more efficient and environmentally friendly techniques. However, the hybrid ultrasound-microwave extraction technology showed more favorable results, with significantly greater obtaining of hydrolyzable polyphenols and, in turn, greater antioxidant properties, so, in this context, hybrid assistance is a very viable alternative to enhance the benefits that both techniques have demonstrated countless times separately.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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