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# Influence of temperature, solvent and extraction procedure on the content of phenolic compounds and antioxidant activity of *Aloysia citriodora* Palau leaves

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**ABSTRACT**

**Background:** The leaves of *Aloysia citriodora* Palau are a source of phenolic compounds that have antioxidant properties.

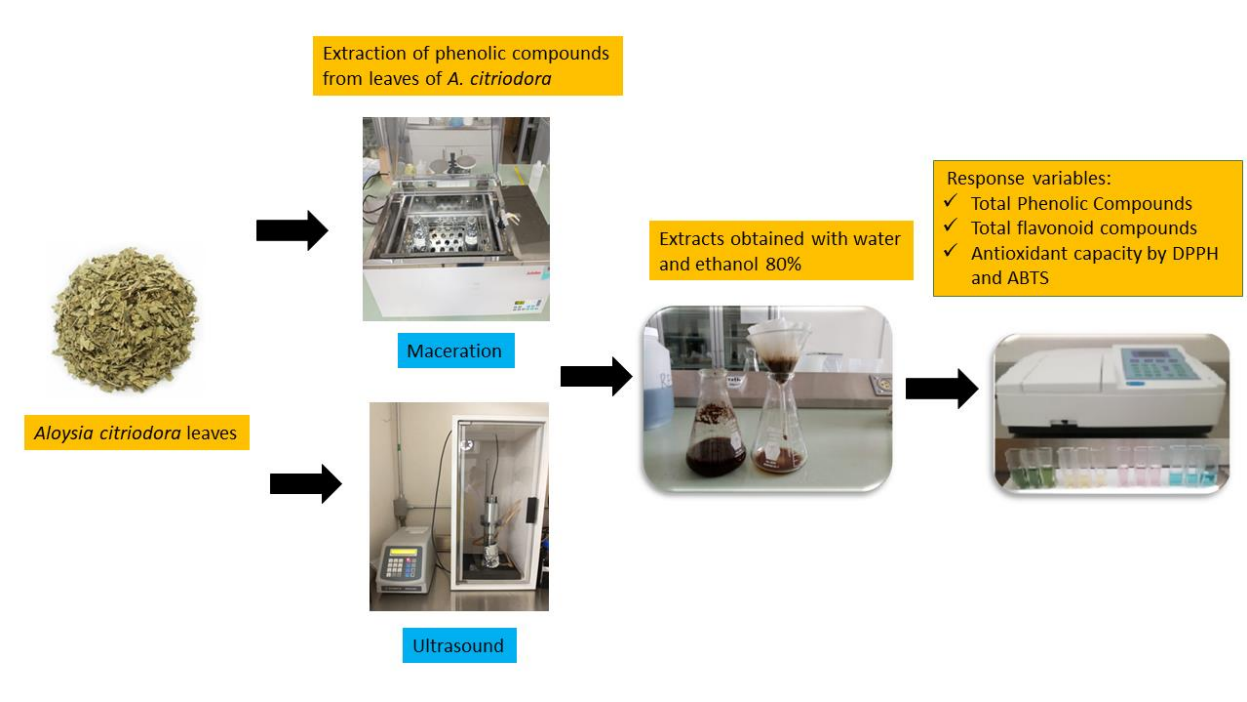
**Objective:** The objective of this study was to compare the effects of temperature, type of solvent, maceration, and ultrasound processes in the extraction of phenolic compounds from *A. citriodora* Palau leaves that allow for maintaining the highest antioxidant capacity.

**Methods:** In this study, phenolic compounds were extracted from *A. citriodora* by two methods, maceration, and ultrasonic treatment, using water and 80% ethanol as solvents and extraction temperatures of 25 °C, 45 °C, and 65 °C. Total phenolic compounds (TPhen), total flavonoid compounds (TFlav), and antioxidant capacity, evaluated by DPPH and ABTS assay, were characterized and reported as inhibition concentration ($IC_{50}$).

**Results:** The highest CPhen content was obtained with ethanolic extract using the ultrasonic method at 65 °C, with a value of 15.32 mg EAG/mL at 65 °C. For TFlav, the highest value was obtained with the ultrasonic treatment at 45 °C using ethanol as solvent, obtaining a value of 6.52 mg EQ/mL. The best antioxidant capacity was shown by the sonication with ethanol at 65 and 45 °C, with $IC_{50}$ values of 27.10 µg/mL and 20.40 µg/mL, respectively.

**Conclusion:** Ultrasound extraction at higher temperatures (45°C and 65°C) proved more effective than maceration, yielding greater amounts of phenolic and flavonoid compounds and exhibiting better antioxidant capacity.".

**Keywords:** *Aloysia citrodora*, phenolic compounds, antioxidant activity, inhibitory concentration $IC_{50}$.






## INTRODUCTION

*Aloysia citriodora,* a plant from the Verbenaceae family, is native to South America and is known by various names such lemon verbena, Maria Luisa, or aromatic verbena. Its common name is lemon because when it is cut or crushed it gives off an intense smell [1-2]. The infusion of its leaves, stems, flowers, and fruits is used to calm pain, nervous and gastrointestinal disorders, rheumatism, ulcers, menstrual pain. *Aloysia citriodora* also has antioxidants, anticancer and antibacterial properties [3]. The leaves of *A. citriodora,* rich in essential oils, mainly contain citral (38-40%) and geranial [4]. They also have different types of phenolic compounds, such as flavonoids, hydroxycinnamic acid derivatives, derivatives of benzoic acid and lignans [5].

Phenolic compounds are mainly found in fruits, legumes, vegetables, tea, wine, and coffee, contributing to the organoleptic characteristics of plant foods. Some phenolics also contribute to the bitterness of many fruits and vegetables [6]. The phenolic compounds in plants include phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignins [7], which are the most abundant secondary metabolites that have one or more hydroxyl groups attached to an aromatic ring. Phenolic compounds are also antioxidants that neutralize the oxidizing action of free radicals. An antioxidant is a substance that delays or prevents substrate oxidation at low concentrations. In addition, antioxidant compounds play a role in several chemical mechanisms: hydrogen atom transfer, single electron transfer, and the ability to chelate transition metals and others [8]. The most common antioxidant assays used in analyses of food, nutrition, and supplements are free radical diphenylpicrylhydrazyl (DPPH), ferric reducing activity power assay (FRAP), and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) [9].

The phenolic compounds are traditionally extracted from plants, fruits, barks, and trees, using methods such as the Soxhlet method, soaking and stirring, heat reflux, and maceration [10], in which water and organic solvents (ethanol, methanol, acetone, and ethyl acetate) are used. However, extraction by maceration, which can be performed at boiling, room or cold temperatures and with different extraction times, requires a long operation time and higher energy consumption. Thus, other more efficient methods are being studied, such as microwave-assisted extraction, supercritical fluid extraction, and ultrasonic-assisted extraction (UAE) [11]. UAE increases extraction yield and reduces extraction time and solvent usage and is suitable for thermo-sensitive compounds [12]. In UAE, high-frequency sounds between 20 kHz and 40 kHz are used to extract phenolic compounds from biological materials. The effectiveness of ultrasound is based on cavitation, which creates cavities in liquids with high energy behavior due to large pressure variations caused by high temperature and pressure. As a result, the cell wall ruptures and releases the bioactive components toward the solvent [13-14]. Bioactive compounds from plants like A. citriodora can be used as ingredients in functional foods. These are molecules that, though present in small amounts, can promote health through specific physiological effects.[15]. Among the bioactive compounds that already have epidemiological studies that support their physiological effect in humans are phenolic compounds [16].

Therefore, the objective of this study was to extract phenolic compounds from *Aloysia citriodora* by maceration and ultrasonic process, varying extraction time and temperature, and using water and 80% ethanol as solvents. Another objective was to determine the total phenolic compounds and total flavonoid compounds, measuring their antioxidant properties by their ability to scavenge $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals.

## METHODS AND MATERIALS

The *A. citriodora* plant was purchased at the Independence Market in the city of Morelia Michoacán, Mexico. Branches and flowers were removed from the plant. The leaves were dried at 20 °C, ground in a blender, and sieved through a 0.8 mm mesh sieve.

**Aqueous and ethanolic extractions by maceration and UAE:** A complete experimental design was carried out in which the independent variables were the extraction method (maceration and UAE), the solvent used (water or 80% ethanol) and the temperature (25 °C, 45 °C, and 65 °C), as shown in Table 1. For the maceration group, the

sample with the solvent (water or 80% ethanol) was placed in a 250 mL Erlenmeyer flask at a ratio of 1:10 (dry mass of leaves/solvent volume). The flask was then placed in a thermobath (Julabo, model SW22, Seelbach, Germany) with stirring at 100 rpm and temperatures of 25 °C, 45 °C, and 65 °C. After 24 hours, the extract was filtered using filter paper # 20 and the supernatants (phenolic extracts) were refrigerated at 4 °C.

For the UAE treatment group, samples were treated at 20 kHz, maximum power of 500 W, and a surface area of 3.8 $cm^2$ using an ultrasonic processor under various temperature conditions of 25 °C, 45 °C and 65 °C, an amplitude of 30% and a time of 20 min. Both the aqueous and ethanolic extracts were obtained by placing the sample and the solvent in a beaker at a ratio of 1:10 (dry mass of the ground leaves/volume of the solvent). An ultrasonic probe was immersed 1.5 cm into the aqueous and ethanolic solutions, while keeping both solutions in the dark. Once the samples were processed, they were centrifuged and filtered for 10 min using filter paper. The supernatants (phenolic extracts) obtained were then stored at 4 °C until analysis.

**Table 1.** Experimental design for extraction of phenolic compounds by maceration and ultrasonication.

| Run | Extraction method | Solvent | Temperature (°C) |
|---|---|---|---|
| 1 | Maceration | Water | 25 |
| 2 | Maceration | Water | 45 |
| 3 | Maceration | Water | 65 |
| 4 | Maceration | Ethanol 80% | 25 |
| 5 | Maceration | Ethanol 80% | 45 |
| 6 | Maceration | Ethanol 80% | 65 |
| 7 | Ultrasound | Water | 25 |
| 8 | Ultrasound | Water | 45 |
| 9 | Ultrasound | Water | 65 |
| 10 | Ultrasound | Ethanol 80% | 25 |
| 11 | Ultrasound | Ethanol 80% | 45 |
| 12 | Ultrasound | Ethanol 80% | 65 |

**Extraction of bioactive compounds:** 10 mL of methanol was added to 1 g of each sample, and then the samples were protected from the light and stirred for 24 h. Subsequently, the samples were centrifuged using a Centrificient IV CRM Globe at 2500 x g at 20 °C for 10 min. The extracts were kept at 4 °C until they were used to determine the total phenolic content, total flavonoid content, and antioxidant capacity.

**Total phenolic and total flavonoid content:** Total phenolic compounds (TPhen) were quantified according to the method described in Tena-Rojas *et al*. [17]. The results were expressed as milligram equivalents of gallic acid per milliliter of extract (mg EAG/mL E). The total flavonoid compounds (TFlav) were determined according to the method described in Tena-Rojas *et al*. [17] TFlav was measured at a wavelength of 415 nm and expressed

in mg quercetin equivalents per ml of extract (mg EQ/mL E).

**Determination of Antioxidant Activity by DPPH and ABTS:** Total antioxidant activity was evaluated using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) scavenging radical assay. This determination was made according to the method described in Tranquilino-Rodríguez and Martínez-Flores [18]. The 2,2-diphenyl-1-picrylhydrazyl radical was prepared at a concentration of 6 x $10^{-5}$ M using methanol as a diluent. After preparing the radical, the absorbance was measured at a wavelength of 517 nm.

The following formula was used to obtain the percentage of inhibition:

$$\%inhibition = \frac{A_c - A_m}{A_c} * 100$$

$A_c = Absorbance\ of\ DPPH A_m = Absorbance\ of\ extract + DPPH$

The antioxidant activity was also evaluated using the ABTS•+ (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) scavenging radical assay. This determination was carried out according to the methodology described in Tranquilino-Rodríguez and Martínez-Flores [18]. The ABTS•+ (2,2'-azino-bis(3-ethylbenzothiazoline 6-sulfonic acid) radical was prepared at a concentration of 7 mM using ethanol as diluent. After the preparation of the radical, the absorbance was measured and adjusted to 754 nm. The following formula was used to obtain the inhibition percentage:

$$\%inhibition = \frac{A_c - A_m}{A_c} * 100$$

$A_c = Absorbance of ABTS A_m = Absorbance of extract + ABTS$

**Inhibitory concentration (IC50):** The $IC_{50}$ (µg/mL) is the concentration of the antioxidant required to scavenge 50% DPPH• or ABTS•+ radical. The $IC_{50}$ was first calculated using the equation of the straight line obtained from the reference curve of the standard analyzed (gallic acid) and substituted (y) by 50. Then, a regression analysis of the percentage of radical inhibition versus the concentration of extracts necessary to inhibit 50% of the DPPH• or ABTS•+ radical [18]. and linear regression analysis were used to determine the $IC_{50}$values, which are also used to indicate antioxidant capacity.

**Statistical analysis:** The experimental data were analyzed by analysis of variance (ANOVA). Tukey's test was considered for all variables with a significance level ($p < 0.05$). Correlation coefficients between response variables were calculated using Pearson's test. Triplicate analyses were evaluated using JMP version 11 software.

## RESULTS AND DISCUSSION

Phenolic compounds are present in vegetables, fruits, trees, and in agro-industrial wastes. These compounds are important for human health because they have antioxidant properties that neutralize free radicals and help prevent diseases, such as cancer, and cardiovascular and dyslipidemia diseases. For this reason, measuring the content of phenolic compounds in A. citriodora extracts, and correlating them with antioxidant activity in both aqueous and ethanolic extractions obtained via maceration and ultrasound processes, was an important focus of this study.

**Extraction by maceration:** Overall, for the macerated extracts of *A. citriodora*, the highest amounts of TPhen were present in the samples obtained with ethanol and the samples processed by ultrasound. In addition, the levels of TPhen increased with higher temperatures. The TPhen value of the sample with the ultrasonic treatment was15.32 ± 0.18 mg EAG/g of sample and the TPhen value of the ethanolic extraction was 12.46 ± 0.90 mg EAG/g of

sample when the extraction was aqueous. In the case of maceration, the highest TPhen values were obtained with ethanolic extraction by ultrasound at 65 °C (3.21 mg EAG/g sample), followed by extraction by ultrasound at 45 °C (2.76 EAG/g sample) and aqueous extraction at 65 °C (2.07 EAG/g sample). On the other hand, the highest TFlav values were obtained by ultrasonic treatment with ethanol at 45 °C (6.52 mg EQ/g sample), then ultrasonic treatment with ethanol at 65°C (6.23 mg EQ/g sample) and aqueous by ultrasound at 65 °C (6.31 mg EQ/g sample). It is important to note that the ethanolic extract at 45 °C and the aqueous extract at 65 °C are cost-effective because less energy is used and process costs are reduced, respectively. Some authors reported that temperature has a positive effect on the extraction of phenolic compounds in different vegetables by solvent extraction due to the increase in solubility and diffusion rates of the compounds [19]. Aguado [20] reported that the maximum phenolic compound extraction in ethanol for the *Aloysia polystachya* by maceration was 2.32 mg EAG/mL extract, while the total flavonoid compounds was 0.37 mg EQ/mL extract, which are slightly lower values compared to our results for extraction with ethanol and, specifically much lower than our results for extraction with ethanol performed by ultrasound.

In our study, extractions with ethanol and higher temperatures led to more TPhen detected, with15.32 mg EAG/mL of ethanolic extract at 65 °C, being statistically different ($p<0.05$) from the extracts at 45 °C and 25 °C. For the extractions with water, there were no significant differences between the extract at 65 °C (12.46 mg EAG/mL) and extract at 45 °C (11 mg EAG/mL). For TFlav, the highest levels were obtained at 65 °C for the aqueous extract, which were significantly different ($p<0.05$) from those obtained at 45 °C. In the case of the ethanolic extract, there were no significant differences ($p<0.05$) between the extract obtained at 45 °C (6.52 mg EQ/mL) and that at 65 °C (6.23 mg EQ/mL).

**Table 2.** Total phenolic and total flavonoids compounds in extracts of *Aloysia citriodora* leaves.

| | Temperature | | |
|---|---|---|---|
| | 25 °C | 45 °C | 65 °C |
| **Maceration** | | | |
| TPhen (mg EAG /mL sample) | | | |
| Aqueous extract | 1.03 ± 0.06$^{c}$ | 1.23 ± 0.03$^{b}$ | 2.07 ± 0.02$^{a}$ |
| Ethanolic extract | 1.49 ± 0.13$^{c}$ | 2.76 ± 0.16$^{b}$ | 3.21 ± 0.03$^{a}$ |
| TFlav (mg EQ /mL sample) | | | |
| Aqueous extract | 0.06 ± 0.021$^{b}$ | 0.13 ± 0.018$^{b}$ | 0.18 ± 0.009$^{a}$ |
| Ethanolic extract | 0.22 ± 0.002$^{c}$ | 0.51 ± 0.026$^{b}$ | 0.61 ± 0.024$^{a}$ |
| **Ultrasonic** | | | |
| TPhen (mg EAG /mL sample) | | | |
| Aqueous extract | 7.59 ± 0.20$^{b}$ | 11.11 ± 0.07$^{a}$ | 12.46 ± 0.90$^{a}$ |
| Ethanolic extract | 8.04 ± 0.34$^{c}$ | 11.21 ± 0.45$^{b}$ | 15.32 ± 0.18$^{a}$ |
| TFlav (mg EQ /mL sample) | | | |
| Aqueous extract | 2.51 ± 0.12$^{c}$ | 3.89 ± 0.12$^{b}$ | 6.31 ± 0.41$^{a}$ |
| Ethanolic extract | 3.84 ± 0.15$^{b}$ | 6.52 ± 0.34$^{a}$ | 6.23 ± 0.17$^{a}$ |

Different letters in a column indicate a significant difference analyzed by Tukey's statistical test ($p < 0.05$). N = 3.

**Inhibitory concentration (IC50) of DPPH and ABTS tests:** The $IC_{50}$ of the extracts was evaluated using the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging assays (Table 3). The $IC_{50}$ value obtained refers to the amount of µg of the extract required to inhibit the DPPH$^{\bullet}$ or ABTS$^{\bullet+}$ radical by 50%. A lower value of $IC_{50}$ indicates greater antioxidant activity.

The lowest $IC_{50}$ value for the DPPH assay was 11.20 µg/mL for the aqueous extract obtained by maceration at 65 °C, which was significantly different ($p<0.05$) from the values obtained at 45 °C and 25 °C. For the ethanolic extract, the lowest $IC_{50}$ value was 26.83 µg/mL at 45 °C, followed by an$IC_{50}$ value of 32.71 µg/mL at 65 °C. Both values were significantly different ($p<0.05$). Overall, all ultrasound treatments, both aqueous and ethanol extractions, showed higher $IC_{50}$ values than the maceration treatments.

**Table 3.** $IC_{50}$ values of DPPH and ABTS from extracts of *Aloysia citriodora* leaves.

| | Temperature | | |
|---|---|---|---|
| | 25 °C | 45 °C | 65 °C |
| **Maceration** | | | |
| DPPH | | | |
| Aqueous extract | 42.50 ± 0.07$^{b}$ | 63.66 ± 0.76$^{a}$ | 61.20 ± 0.06$^{a}$ |
| Ethanolic extract | 68.66 ± 0.40$^{a}$ | 26.83 ± 0.09$^{c}$ | 32.71 ± 0.12$^{b}$ |
| ABTS | | | |
| Aqueous extract | 100.25 ± 0.51$^{b}$ | 112.72 ± 0.93$^{a}$ | 37.87 ± 0.87$^{b}$ |
| Ethanolic extract | 153.03 ± 2.65$^{a}$ | 56.06 ± 0.05$^{b}$ | 60.60 ± 0.15$^{b}$ |
| **Ultrasound** | | | |
| DPPH | | | |
| Aqueous extract | 192.55 ± 0.27$^{b}$ | 150.55 ± 0.04$^{a}$ | 117.30 ± 0.16$^{c}$ |
| Ethanolic extract | 105.23 ± 0.40$^{a}$ | 85.55 ± 0.29$^{b}$ | 62.41 ± 0.20$^{c}$ |
| ABTS | | | |
| Aqueous extract | 42.50 ± 0.34$^{a}$ | 40.50 ± 0.19$^{a}$ | 30.75 ± 0.12$^{b}$ |
| Ethanolic extract | 35.20 ± 0.09$^{a}$ | 28.40 ± 0.02$^{b}$ | 27.10 ± 0.10$^{b}$ |

Different letters in a column indicate a significant difference analyzed by Tukey's statistical test ($p < 0.05$). N = 3.

The best results were the ABTS$^{\bullet+}$ from the extracts obtained using ultrasound treatment, with the ethanolic extracts at 45 °C and 65 °C being the most efficient as they presented the lowest $IC_{50}$ values of 28.40 µg/mL and 27.20 µg/mL, respectively, in addition to the aqueous extract at 65 °C, which presented an $IC_{50}$ value of 30.75 µg/mL. For maceration to obtain the ABTS$^{\bullet+}$ from the extracts, the lowest values of $IC_{50}$ were obtained with the aqueous extract at 65 °C, followed by the ethanolic extracts at 45 °C and 65 °C with values of 37.87 µg/mL, 56.06 µg/mL and 60.60 µg/mL, respectively.

Our results demonstrate that all the extracts had higher values in the ABTS$^{\bullet+}$ radical capture technique compared to the DPPH$^{\bullet}$ technique due to the low selectivity of the ABTS$^{\bullet+}$ radical, which reacts with any hydroxylated aromatic compound regardless of its real antioxidant potential. On the other hand, if the antioxidant capacity of the extracts is due to the presence

of phenolic acids, flavonoids, and other polyphenols, as reported in the literature, then DPPH$^{\bullet}$ is more selective than ABTS$^{\bullet+}$ because DPPH$^{\bullet}$ does not react with flavonoids lacking hydroxyl groups in the B-ring, nor with aromatic acids containing a single hydroxyl group.

**Correlation Analysis of Spearman of Phenolic Compounds and Antioxidant Activity:** Higher antioxidant activity demonstrated a stronger correlation with the ABTS radical scavenging. The ABTS radical scavenging had very strong negative correlations with total phenolic compounds (-0.8741) and total flavonoid compounds (-0.8111), indicating that lower concentrations of phenolic compounds and total flavonoids are required to reduce and stabilize ABTS and DPPH radicals to prevent their chain action from producing more harmful free radicals.

**Table 4.** Spearman correlation analysis of the total phenolic and total flavonoid compounds with the antioxidant capacity to scavenge DPPH and ABTS radicals.

| | DPPH | ABTS |
|---|---|---|
| CPhenT | 0.468531469 | -0.874125874 |
| CFlavT | 0.552447552 | -0.81118881 |

Through this research, it was found that it is possible to obtain a high extraction yield of phenolic compounds with antioxidant capacity from Aloysia citriodora leaves using the ultrasound-assisted extraction technique. The ultrasound technique is feasible to be used for practical purposes as it uses short extraction times, mild temperature and is environmentally friendly as it is a green technology.

## CONCLUSION

The ethanolic extract of *A. citriodora* leaves obtained by the ultrasonic method showed a higher content of total phenolic compounds. The highest yields of these compounds were obtained at a °C. *A. citriodora* leaves also exhibited the best $IC_{50}$ for ABTS in the ethanolic extraction at 65 °C by the ultrasonic method. A strong correlation was found between $IC_{50}$ from DPPH and ABTS scavenging radicals and the total phenolic compounds of the extracts. The ultrasonic extraction process allowed us to obtain higher yields in a shorter amount of time compared to the maceration method. Therefore, our study will contribute to the expansion of scientific knowledge on the extracts of the species *A. citriodora*. However, further research is needed to investigate the detailed chemical composition of *A. citriodora* extracts and their antioxidant properties through vivo evaluations.

**List of Abbreviations:** ABTS•+, (2,2'azinobis-3-ethylbenzothiazoline 6-sulfonic acid); ANOVA–Analysis of variance; DPPH•, (1,1-diphenyl-2-picryl-hydrazyl); $IC_{50}$, inhibition concentration to scavenge 50% DPPH$^{\bullet}$ or ABTS$^{\bullet+}$ radical; TPhen, Total phenolic compounds; TFlav, Total flavonoid compounds; UAE, Ultrasonic-assisted extraction; GAE, gallic acid equivalents; QE, Quercetin equivalents.

**Author Contributions:** M.G.G.R and H.E.M.F. designed the experiments, discussed, and analyzed the data obtained during the experiments. C.I.S.P. performed the experiments. All authors approved the paper.

**Competing Interests:** There are no conflicts of interest to declare.

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## REFERENCES

1. Al-Maharik N, Salama Y, Al-Hajj N, Jaradat N, Jobran NT, Warad I, Hamdan L, Alrob MA, Sawafta A, Hidmi, A: Chemical composition, anticancer, antimicrobial activity of Aloysia citriodora Palau essential oils from four different locations in Palestine. BMC Complement Med Ther 2024, 24(1):94. DOI: https://doi.org/10.1186/s12906-024-04390-9
2. Mohammadhosseini M, Frezza C, Venditti A, Mahdavi B: An overview of the genus Aloysia Paláu (Verbenaceae): Essential oil composition, ethnobotany and biological activities. Nat Prod Res 2022, 36(19):5091-5107. DOI: https://doi.org/10.1080/14786419.2021.1907576
3. Alrub MA: Unveiling the chemical profiling, antioxidant, anticancer and antibacterial activities of essential oils derived from fennel *(Foeniculum vulgare), M. fruticose and Alyosia citriodora*. Ph.D. thesis. Faculty of Graduate Studies, An-Najah National University; 2023.
4. Sprea RM, Fernandes LH, Pires TC, Calhelha RC, Rodrigues PJ, Amaral JS: Volatile compounds and biological activity of the essential oil of Aloysia citrodora Paláu: Comparison of hydrodistillation and microwave-assisted hydrodistillation. Molecules 2023, 28(11):4528. DOI: https://doi.org/10.3390/molecules28114528
5. Bahramsoltani R, Rostamiasrabadi P, Shahpiri Z, Marques AM, Rahimi R, Farzaei MH.: Aloysia citrodora Paláu (Lemon verbena): A review of phytochemistry and pharmacology. J Ethnopharmacol 2018, 222:34-51. DOI: https://doi.org/10.1016/j.jep.2018.04.021
6. Liu S, Grierson D, Xi W: Biosynthesis, distribution, nutritional and organoleptic properties of bitter compounds in fruit and vegetables. Crit Rev Food Sci Nutr 2024, 64(7):1934-195 3. DOI: https://doi.iorg/10.1080/10408398.2022.2119930
7. Ahlawat YK, Singh M, Manorama K, Lakra N, Zaid A, Zulfiqar F: Plant phenolics: neglected secondary metabolites in plant stress tolerance. Braz J Bot 2024, 47(3):703-721. DOI: https://doi.org/10.1007/s40415-023-00949-x
8. Mittal A, Vashistha VK, Das DK: Recent advances in the antioxidant activity and mechanisms of chalcone derivatives: A computational review. Free Radic Res 2022, 56(5-6):378-397. DOI: https://doi.org/10.1080/10715762.2022.2120396
9. Parcheta M, Świsłocka R, Orzechowska S, Akimowicz M, Choińska R, Lewandowski W: Recent developments in effective antioxidants: The structure and antioxidant properties. Materials 2021, 14(8):1984. DOI: https://doi.org/10.3390/ma14081984
10. Panja P: Green extraction methods of food polyphenols from vegetable materials. Curr Opin Food Sci 2017, 23:173-182. DOI: https://doi.org/10.1016/j.cofs.2017.11.012
11. Shen L, Pang S, Zhong M, Sun Y, Qayum A, Liu Y, Rashid A, Xu B, Liang Q, Ma H, Ren X: A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. Ultrason Sonochem 2023:106646. DOI: https://doi.org/10.1016/j.ultsonch.2023.106646
12. Yusoff IM, Taher ZM, Rahmat Z, Chua LS: A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. Food Res Int 2022:157, 111268. DOI: https://doi.org/10.1016/j.foodres.2022.111268
13. Lama-Muñoz A, Contreras MDM: Extraction systems and analytical techniques for food phenolic compounds: a review. Foods 2022, 11(22):3671. DOI: https://doi.org/10.3390/foods11223671
14. Bucur MP, Radulescu MC, Radu GL, Bucur B: Cavitation-effect-based treatments and extractions for superior fruit and milk valorisation. Molecules 2023, 28(12):4677. DOI: https://doi.org/10.3390/molecules28124677
15. Martirosyan D, Stratton, S.: Quantum and tempus theories of function food science in practice. Funct Food Sci - Online ISSN: 2767-3146, 2023 3(5):55-62. DOI: https://doi.org/10.31989/ffs.v3i5.1122
16. Martirosyan D, Lampert T, Lee MA: comprehensive review on the role of food bioactive compounds in functional food science. Funct Food Sci – Online ISSN: 2767-3146, 2022:2(3), 64-78. DOI: https://doi.org/10.31989/ffs.v2i3.906

17. Tena-Rojas KFF, Martínez-Flores HE, Garnica-Romo MG, de Dios Figueroa-Cárdenas J, Meléndez-Herrera E, Salgado-Garciglia R: Influence of factors and interactions in ultrasound-assisted extraction and conventional maceration on aqueous extract of Psidium guajava leaves. Bioact Compd Health Dis 2022 5(10):186-201.
DOI: https://doi.org/10.31989/bchd.v5i10.969
18. Tranquilino-Rodríguez E, Martínez-Flores HE: Ultrasound-assisted extraction of phenolic compounds from Moringa oleifera leaves by response surface methodology. Bioact Compd Health Dis 2023 6(11):325-337.
DOI: https://doi.org/10.31989/bchd.v6i11.1229
19. Antony A, Farid M: Effect of temperatures on polyphenols during extraction. Appl Sci 2022, 12(4):2107.
DOI: https://dor.org/10.3390/app12042107
20. Aguado MI, Nuñez MB, Bela AJ, Okulik NB, Bregni C: Caracterización fisicoquímica y actividad antioxidante de un extracto etanólico de *Aloysia polystachya* (Griseb.) Mold. (Verbenaceae). Rev Mex Cienc Farm 2013, 44(3): 46-51.