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Antioxidant properties by differential extraction of L*avandula dentata*

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Abstract

Lavandula dentata has a high content of secondary metabolites that act as antioxidant compounds. Lavender extracts were obtained by differential Soxhlet extraction with different solvents (hexane, ethyl acetate, dichloromethane, ethanol, methanol, and water), ordered from highest to lowest polarity. The ethanolic extract showed the best results with all solvents, indicating its antioxidant capacity for medicinal use. This was mainly due to a high presence of phenols and flavonoids, compared to literature, which suggests the need for further studies to explore its antioxidant potential.

Keywords: *Lavandula Dentata*; Antioxidants; Differential Extraction; Secondary Metabolites

1. Introduction

In Mexico, the use of medicinal plants dates to pre-Hispanic times. This has been passed down to current generations as an ancestral tradition, since society has used certain plants directly or in infusions, using the flowers or leaves, and very rarely the roots and stems. [1] According to the Ministry of Health, 90% of the Mexican population has used one of Mexico's 4,500 medicinal plants at least once in their lifetime. [2] One prominent medicinal plant is lavender (*Lavandula dentata*), a perennial that can reach 60 cm in height. It is characterized by its purple flowers and grayish-green, toothed leaves. [3] Native to the Mediterranean, southern Europe, and the Canary Islands, it is widely used as a medicinal plant, infusions, as a decorative plant, and for its essential oil, which is used to treat illnesses such as diabetes, respiratory infections, and digestive problems. It is also used as a relaxant. [3] It reports metabolites (figure 1) such as polyphenols (anthocyanins), flavonoids (apigenin, luteolin) and terpenes (camphor, linalool and eucalyptol) [4].

These metabolites have a particular characteristic, as they are reported as antioxidants [4-9]; however, the levels of this property depend on the cultivation conditions [9-12]. Therefore, the objective of this study is to analyze the antioxidant levels of *L. dentata* cultivated in the university botanical garden, to compare them with those reported in wild conditions.

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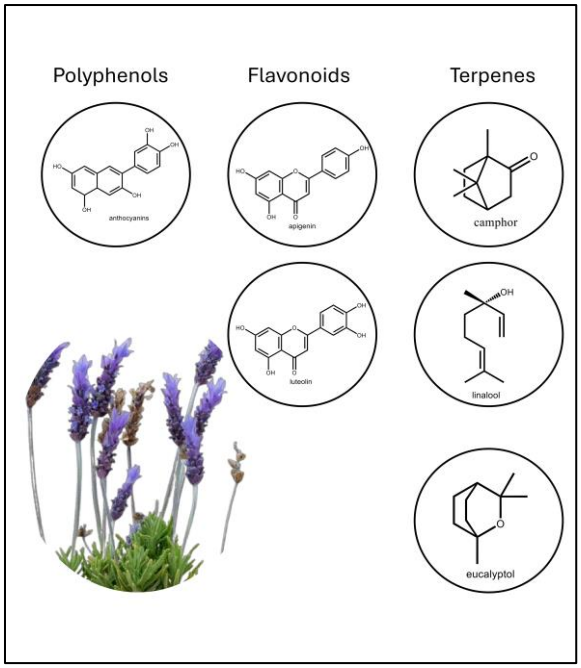

Figure 1 *Lavandula dentata* and secondary metabolites reported.

2. Materials and Methods

2.1. Plant Material Collection

Fresh plant material of *L. dentata* was collected within the BUAP University Botanical Garden, identified as Lavanda (access number) at [altitude missing] m above sea level. Collection took place in the morning between 9 and 11 am. For extraction, cuts were made carefully to preserve new shoots and thus allow for plant regeneration.

2.2. Ascending Differential Extraction by Soxhlet

The collected plant material was thoroughly washed to remove any contamination and then dried with sanitary napkins. Differential extraction was performed using the Soxhlet method with six solvents: hexane, ethyl acetate, dichloromethane, ethanol, methanol, and water, based on polarity. 180 ml of each solvent was used with 35 g of plant material for the six extractions, with each extraction lasting 3 hours. Finally, the solvent was separated from the extract of each extraction using a rotary evaporator, proceeding to resuspend the solutions at a concentration of 1g in 10 ml in methanol for antioxidant testing.

2.3. Antioxidant activity

Five tests were performed to analyze the antioxidant potential using the DPPH free radical oxidation inhibitory potential, the determination of total phenols and total flavonoids, and the ability to reduce metal ions by CUPRAC and FRAP [13,14].

2.3.1. Scavenging of DPPH

The DPPH assay was performed in a 96-well plate. 25 µl of blank, control (0.015 mg/ml gallic acid), and extract (hexane, ethyl acetate, dichloromethane, ethanol, and water) were used. Then, 200 µl of DPPH reagent were added, and three replicates were. The plates were incubated in the dark for 40 minutes, and the results were then read using an ELISA at a wavelength of 510 nm, shaking for 10 seconds [15-19]. The antioxidant activity was calculated using the percentage of DPPH inhibition, with the following formula:

$$\%\ DPPH\ scavening\ = \left(\frac{Basal\ absorbance - Sample\ absorbance}{Basal\ absorbance}\right) x\ 100$$

2.3.2. Folin-Ciocalteu phenol determination

For the determination of total phenols, 150.0 µl of blank, positive control (gallic acid 0.1 mg/ml), and extract (hexane, ethyl acetate, dichloromethane, ethanol, methanol, and water) were used 2250.0 µl of distilled water were added to each cell, and the mixture was homogenized. Then, 150.0 µl of Folin-Ciocalteu reagent was added to each cell, and the mixture was allowed to stand for 1–8 minutes. Finally, 450 µl of 20% sodium carbonate was added, the cells were homogenized, and the mixture was incubated for 2 hours. Readings were then taken using a UV-VIS spectrophotometer at a wavelength of 760 nm. The results are expressed in gallic acid equivalents (mg GAE/kg of plant) [20-22].

2.3.3. CUPRAC

For the determination of metal complexes, a 96-well plate was used 25.0 µl of blank, control (0.03 mg/ml gallic acid), and extract (hexane, ethyl acetate, dichloromethane, ethanol, methanol, and water) were added. Then, 25.0 µl of each of the following were added: 10.0 mM $CuBr_2$, 7.5 mM Neucoproin, and 1.0 M ammonium buffer (pH 7). Finally, 100.0 µl of distilled water was added, and the mixture was allowed to stand for 30 minutes. The readings were then taken using an ELISA at a wavelength of 450 nm with slow shaking for 30 seconds. The results are expressed in gallic acid equivalents (mg GAE/kg of plant) [23-25].

2.3.4. FRAP

For the FRAP test, 10 µl of blank, positive control (quercetin 0.04 mg/ml), and extract (hexane, ethyl acetate, dichloromethane, ethanol, methanol, and water) were added to a 96-well plate. 300.0 µl of FRAP reagent was added, the mixture was incubated for 40 minutes, and then the results were read using an ELISA instrument at a wavelength of 595 nm. The results are expressed as quercetin equivalents (mg QE/kg of plant) [26-29].

2.3.5. Total Flavonoids

For the determination of total flavonoid content, 4 ml of distilled water was placed in test tubes. 1 ml of sample (hexane, ethyl acetate, dichloromethane, ethanol, methanol, and water), blank, and control (quercetin 0.1 mg/ml) were added to each tube. The mixture was homogenized using a vortex mixer. Then, 300.0 µl of 1M sodium nitrite was added, and the mixture was homogenized again using a vortex mixer. Next, 300.0 µl of 10% aluminum trichloride was added, and the mixture was homogenized. Finally, 2.0 ml of 1.0 M sodium hydroxide was added, and the mixture was homogenized. Each tube was then brought to the mark with 2.4 ml of distilled water. The tubes were allowed to stand for 40 minutes. Finally, 3 ml of the reaction were placed in each cell, and absorbance readings were taken at 510 nm using a UV-VIS spectrophotometer. The results are expressed in quercetin equivalents (mg QE/kg of plant) [30-33].

3. Results and discussion

Antioxidant properties can be classified into three types: first, the ability to inhibit the formation or stability of free radicals; second, the ability to stabilize metals associated with redox processes, such as iron (Fe^{2+}) and copper (Cu^{+1}); and finally, the content of secondary metabolites associated with antioxidants, such as flavonoids and total phenols. Regarding the first test, we have the DPPH radical scavenging study, associated with ROS stress. As shown in Figure 2, the methanolic and ethanolic extracts have the greatest capacity to affect the DPPH free radical, while hexane shows the least, indicating that the effect is primarily due to polar metabolites. This is consistent with results obtained in previous studies, in which it was possible to identify a wide range of metabolites associated with this type of activity in methanol extracts, such as hydrocaffeic acid, caffeic acid, rosmarinic acid, among others [35]. In this same study, flavonols were also identified, such as quercetin, specifically glycosylated wiht some hexose, or phenolic acids such as coumaric acid in its glycosylated form, which could explain the remarkable result in aqueous extract, Taken together, these data reinforce that despite the expected diversity due to natural variables such as climate, latitude, altitude, and nutrient availability, the generation of metabolites in this plant species maintains a marked tendency toward the generation of polar metabolites.

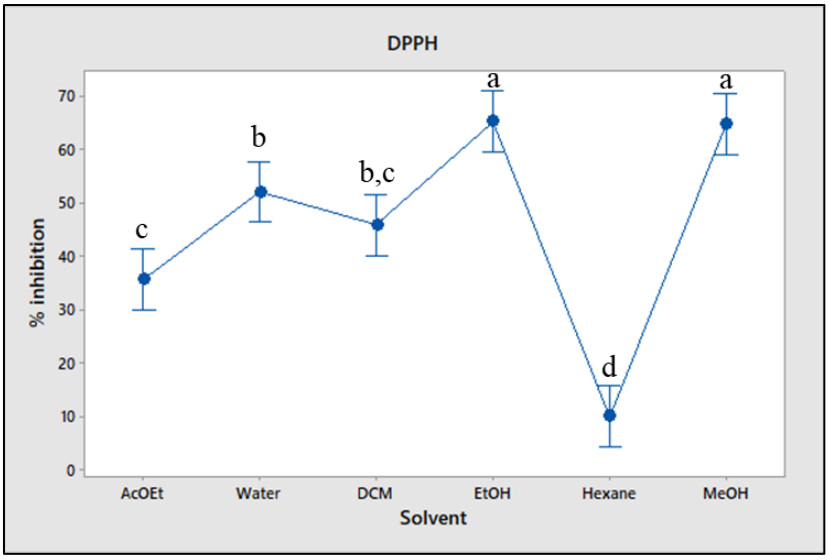

Figure 2 Determination of antioxidant potential with respect to the % elimination of the DPPH free radical of extracts (hexane, ethyl acetate, dichloromethane, ethanol, methanol and water) at 1.0 mg/ml. Kruskal-Wallis test (p<0.05)

The second group of studies is the ability to affect metal ions such as copper and iron, as shown in Table 1. In this case, it is exclusively the ethanolic extract that shows a significant difference over the rest of the extracts. This implies that the exclusive metabolites affinity for methane has the chelating potential on these metals, while the metabolite, although it could inhibit free radicals, does not have the dual effect on metals, limiting its potential.

Table 1 Results obtained for studies of inhibition of these metallic oxidation processes, for Iron (FRAP) and copper (CUPRAP). Kruskal-Wallis test (p<0.05).

Solvent	CUPRAC	FRAP
Hexane	61.66 ± 18.81 ^{B}	8.29 ± 3.37 ^{B}
Ethyl acetate	158.0 ± 141.9 ^{B}	155.4 ± 152.1 ^{B}
Dichloromethane	63.7 ± 55.7 ^{B}	61.3 ± 68.7 ^{B}
Ethanol	970.0 ± 674 ^{A}	404.7 ± 260.2 ^{A}
Methanol	99.6 ± 78.3 ^{B}	98.7 ± 101.6 ^{B}
Water	351.0 ± 0.1161 ^{B}	259.8 ± 0.2024 ^{B}

[1]Results expressed as mg gallic acid equivalent/kg of plant; [2]Results expressed as mg quercetin equivalent/kg of plant; A-B. Mean values in the same column with different letters are significantly different.

Given the results observed in both test groups, the overall content of secondary metabolites is important, as the greatest effects are seen in polar solvents. Total phenols and flavonoids are the most important, as shown in Table 2, where again the highest values are found in the ethanolic extract, which is statistically different from the rest of the extracts. Compared to what is reported in the literature, for *L. multifida* in a methanolic extract of flowers is reported 7.9 ± 0.2 mg EAG/g DW and leaves 23 ± 1.1 mg EAG/g DW, while for L. dentata, 15.8 ± 0.9 mg EAG/g DW is reported in leaves and 16.4 ± 0.5 mg EAG/g DW in flowers [36]. The difference can be explained both by the water contained in the sample in our present study and by the fact that it is a differential extraction, in which many polar metabolites were already extracted by ethanol prior to methanol. Thefore, by analyzing and normalizing the units, we would have a result comparable to that of *L. multifida* leaves, but still distant from the result reported in the same species. Based on these data, it can be inferred that different species exhibit a distribution of secondary metabolites that is preferentially localized in leaves, flowers, or is balanced between both organs, which is a starting point for the analysis of the variables that influence the ideal extraction of phenols, beyond the origin of the sample (wild or captive).

This confirms that total phenols, in conjunction with flavonoids, are responsible for the antioxidant effect on both metals and free radicals, while the methanolic extract shows a reduced effect. This suggests that other metabolites have the exclusive potential to combat free radicals, rather than iron and copper.

Table 2 Determination of total phenolics and flavonoids. Kruskal-Wallis test ($p<0.05$).

Solvent	Total phenolics[1]	Total flavonoids[2]
Hexane	137.9 ± 117.7 B	308.8 ± 45.3 B
Ethyl acetate	1491.7 ± 137.0 B	450.3 ± 37.5 B
Dichloromethane	1012.6 ± 110.4 B	302.6 ± 18.4 B
Ethanol	6463.3 ± 563.4 A	2056.5 ± 144.3 A
Methanol	1213.2 ± 109.1 B	511.7 ± 45.3 B
Water	257.5 ± 1.677 B	201.8 ± 9.65 B

[1]Results expressed as mg gallic acid equivalent/kg of plant; [2]Results expressed as mg quercetin equivalent/kg of plant; A-B. Mean values in the same column with different letters are significantly different

The ethanolic extract exhibits the greatest capacity for both scavenging free radicals and stabilizing Cu+ and Fe2+ metal cations, due to the high level of phenols and flavonoids extracted by this solvent. This supports previous studies reported in the literature [4-9] and paves the way for future research on the potential of this lavender species.

4. Conclusion

Extracts of *Lavandula dentata* showed differences in antioxidant capacity determined by each method. It was observed that most extracts—hexane, ethyl acetate, dichloromethane, methanol, and water—had significantly lower antioxidant capacity compared to the ethanolic extract. These comparisons were evident in all antioxidant capacity tests, with the ethanolic extract showing a greater hydrogen atom transfer capacity. This data indicates that lavender has a high content of secondary metabolites, which are important antioxidant compounds that were successfully extracted by the ethanolic solvent due to its polarity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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