EXPERIMENTAL RESEARCH ON THE EXTRACTION OF POLYPHENOLS FROM NETTLE, LAVENDER AND SAGE USING THE PERCOLATION METHOD
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CERCETĂRI EXPERIMENTALE PENTRU EXTRACȚIA POLIFENOLILOR DIN URZICĂ, LAVANDĂ ȘI SALVIE PRIN METODA PERCOLĂRII

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ABSTRACT

This article presents the results of experimental research on the extraction of polyphenols from nettle, lavender, and sage using the percolation method. This technique is recognized for its efficiency in extracting bioactive compounds from plants. Polyphenols are a group of natural chemical compounds characterized by the presence of multiple phenolic groups in their molecular structure. They are predominantly found in plants and are recognized in various industries, including agriculture, for their antioxidant, antimicrobial, and antifungal properties. The antioxidant capacity of the extracts from nettle, lavender, and sage refers to the ability of these extracts to neutralize free radicals. The concentrations of polyphenols in the obtained extracts were measured using the Folin-Ciocalteu spectrophotometric method. The impact of pressure on total polyphenol content varied by plant species. Sage showed increased polyphenol content at higher pressures, indicating more efficient extraction with the proposed technology.

REZUMAT

Acest articol prezintă rezultatele cercetărilor experimentale privind extracția polifenolilor din urzică, lavandă și salvie, utilizând metoda percolării. Această tehnică este recunoscută pentru eficiența sa în extragerea compușilor bioactivi din plante. Polifenolii reprezintă un grup de compuși chimici naturali, caracterizați prin prezența multiplelor grupări fenolice în structura lor moleculară. Aceștia se găsesc predominant în plante și sunt recunoscuți în diverse industrii, inclusiv în agricultură pentru proprietățile lor antioxidante, antimicrobiene și antifungice. Capacitatea antioxidantă a extractelor din urzică, lavandă și salvie se referă la abilitatea acestor extracte de a neutraliza radicalii liberi. Concentrațiile de polifenoli din extractele obținute au fost măsurate folosind metoda spectrofotometrică Folin-Ciocalteu. Impactul presiunii asupra conținutului total de polifenoli a variat în funcție de specie. Salvia a arătat un conținut crescut de polifenoli la presiuni înalte, indicând o extracție mai eficientă cu tehnologia propusă.

INTRODUCTION

Medicinal and aromatic plants were the primary source of medicine for humans before the advent of civilization (*Parvin et al., 2023*). Biologically, these plants are remarkable for their rich composition of active substances with recognized medicinal properties according to Western standards (*Mendes et al., 2023*). These active substances are either produced and stored by the plants during their growing season or accumulate in response to stress conditions such as sudden climate changes, excess or insufficient soil water, nutrient deficiency, air pollution, etc. (*Greff et al., 2022*). Among the bioactive compounds present in plants are polyphenols, which are especially recognized for their antioxidant properties (*Pinto et al., 2021*). In agriculture, polyphenols protect crops from diseases and pests, enhance the quality and nutritional value of produce, and reduce the need for chemical pesticides, thereby promoting more sustainable agricultural practices (*Stiller et al., 2021*).

Additionally, these types of plants are crucial for maintaining ecological balance and soil health, protecting other crops, and contributing to reducing the use of harmful chemicals through their efficient use as natural pesticides and organic fertilizers. Promoting ecological processes for extracting active principles from these plants is an important step towards sustainable agriculture, minimizing the environmental impact (*Greff et al., 2022*).

Percolation is a widely used process for extracting compounds from plants. In this process, a solvent passes through a porous material, such as plant material, to extract the desired substances. This method is considered gentle as it occurs at room temperature, making it suitable for extracting thermosensitive compounds from plants (*Zhang et al., 2023*). The percolation process can be conducted either by subjecting the plant material to high pressures or without using pressure variations. Studies have demonstrated that percolation under high pressures enhances the efficiency of extracting valuable compounds from plant materials by increasing solvent penetration and solute dissolution (*Nenciu et al., 2023*). This method often leads to higher yields of target compounds, such as polyphenols, due to the improved extraction dynamics facilitated by elevated pressures. These compounds can then be used as biofertilizers in ecological agriculture, offering promising results for enhancing soil health and plant growth (*Popescu et al., 2023*). Solid-liquid extraction under high pressure regimes enhances the extraction of specific compounds from the plant materials improving solvent penetration and compound solubility, leading to higher yields and faster extraction times (*Butler et al., 2004*).

The three plants studied in this article—nettle, lavender and sage—were selected for their beneficial effects in horticulture, particularly as biofertilizers and biopesticides.

Nettle (*Urtica dioica*) –, is native to Europe and Asia, and was later spread to other parts of the world, including North America and North Africa. Although nettle has adapted to the climate and environmental conditions of many regions, Europe and Asia are still considered its regions of origin (*Subba and Pradhan, 2022; Bhusal et al., 2022*). Nettle is a perennial plant that can grow between 0.4 and 4 meters tall (*Subba and Pradhan, 2022; Bhusal et al., 2022; Mueen Ahmed and Parsuraman, 2014; Devkota et al., 2022*), with oval or elongated, opposite leaves that have strongly serrated edges, a cordate base, and a pointed tip (*Bhusal et al., 2022; De Vico et al., 2018; Subba and Pradhan, 2022*). Nettle leaves are a rich source of bioactive compounds, including flavonoids, phenolic acids, essential amino acids, terpenoids, carotenoids, lutein, as well as vitamins, tannins, polysaccharides, sterols, and minerals (*Kregiel et al., 2018.; Devkota et al., 2022; Koraqi, 2023*). Nettle flowers are small, dioecious, and borne in separate inflorescences. They can be brown to greenish and appear in the axils of the upper leaves (*De Vico et al., 2018; Subba and Pradhan, 2022*).

Nettle (*Urtica dioica)* - Figure 1, is used as a food additive in the food industry, in shampoos and lotions in the cosmetic industry, as supplements in the pharmaceutical industry (*Koraqi, 2023*), and its extracts are also used as biofertilizers/bioinsecticides in agriculture. In agriculture, nettle extracts act as biofertilizers and bioinsecticides, enhancing plant growth and soil fertility by improving nutrient absorption, stress resistance, and microbial activity. They also positively impact soil conductivity, boosting nutrient availability for plants (*Maricic et al., 2022*).

Fig. 1 - Nettle (*Urtica dioica*)

Lavender (*Lavandula angustifolia*) - Figure 2, is a perennial plant in the Lamiaceae family and originates from Mediterranean regions, which is why it shows adaptability to dry and warm climates, preferring sandy soils and sunny locations (*Oroian et al., 2019; Adam, 2018; Kimbrough and Swift, 2006*). Lavender grows as shrubs between 20 and 60 cm tall. It has leaves of various shapes, including sessile, linear, and lanceolate, and its stems are branched and irregular. The roots are woody, and the flowers, predominantly blue-violet, are spirally arranged and have a two-lipped form (*Katarzyna, et al., 2014; Fakhriddinova et al., 2020*). Lavender

flowers are rich in various essential compounds such as phytochemicals (triterpenoids, phenolic acids, flavonoids) (*Héral, et al., 2021*), anthocyanins, phytosterols (*Batiha et al., 2023*), and essential oils (*Kozuharova et al., 2023; Diass et al., 2023; Voicea, et al., 2022*). The benefits of lavender extracts in agriculture are remarkable and can bring significant improvements in plant health and agricultural system efficiency (*Crișan et al., 2023*).

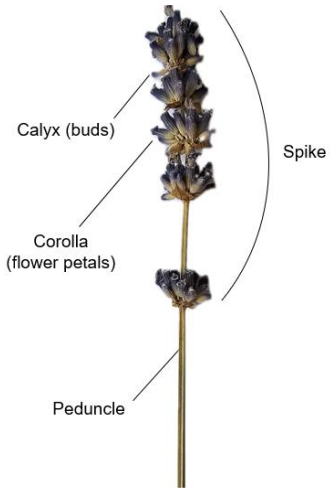

Fig. 2 - Lavender (*Lavandula angustifolia*)

Sage (Salvia officinalis) - Figure 3, is an aromatic and medicinal plant in the Lamiaceae family, originating from Mediterranean regions (*Ghorbani and Esmaeilizadeh, 2017; Hamidpour et al., 2014; Ben Khader et al., 2017*). Sage plants grow up to 60 cm tall, with grey-green leaves that are hairy on the underside, and they range from 3 to 6.5 cm long and 1.5 to 2.5 cm wide (*Jakovljevic et al., 2019*). The flower colour varies from red-violet-blue and they are grouped in panicles or racemes (*Ben Akacha et al., 2023*). The flowers, leaves, and stems of *Salvia officinalis* contain numerous chemical substances, the most important being polyphenols, alkaloids, fatty acids, terpenes, steroids, glycoside derivatives, and essential oils (*Ghorbani and Esmaeilizadeh, 2017*).

Extracts from *Salvia officinalis* can bring multiple benefits to agriculture. Through their antifungal and antibacterial action, these extracts help prevent and combat plant diseases, ensuring their health. Additionally, the antioxidant properties of sage protect plants against oxidative stress and promote their growth and development. By repelling insects and other harmful organisms, sage extracts protect crops, reducing the need for chemical pesticides (*Speranza et al., 2023; Busato et al., 2022*).

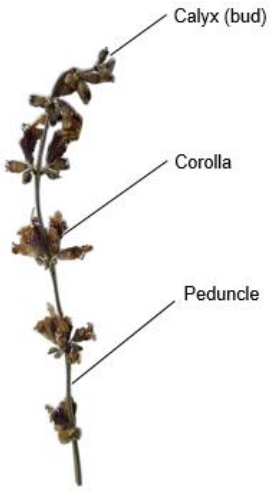

Fig. 3 - Sage (*Salvia officinalis*)

This paper presents experimental results obtained through the pressure percolation extraction method, obtaining extracts from nettle, lavender and sage and assessing their polyphenol content and antioxidant activity with the purpose of assessing the extracts' suitability to be used as amendments in agriculture.

MATERIALS AND METHODS

The plants used for experimentation in this study were: nettle (*Urtica dioica*), lavender (*Lavanda angustifolia*) and sage (*Salvia officinalis*).

To ensure the vegetal material for experiments, plants were harvested using a mechanized equipment. The plants were dried for 12 hours in a laboratory oven at 105°C and were cut at dimensions no larger than 3 cm using a plant cutting equipment.

The three plants were chosen because of their ecological and agricultural benefits. Nettle improves soil fertility, having the capacity to act as an organic fertilizer that stimulates plant growth. Lavender and sage are rich in essential compounds with properties to repel harmful insects, acting as natural pesticides that protect crops, thus eliminating the need for synthetic chemical substances.

The active principles from the medicinal and aromatic plants (nettle, lavender, sage) were extracted using the TIMATIC percolator (Figure 4).

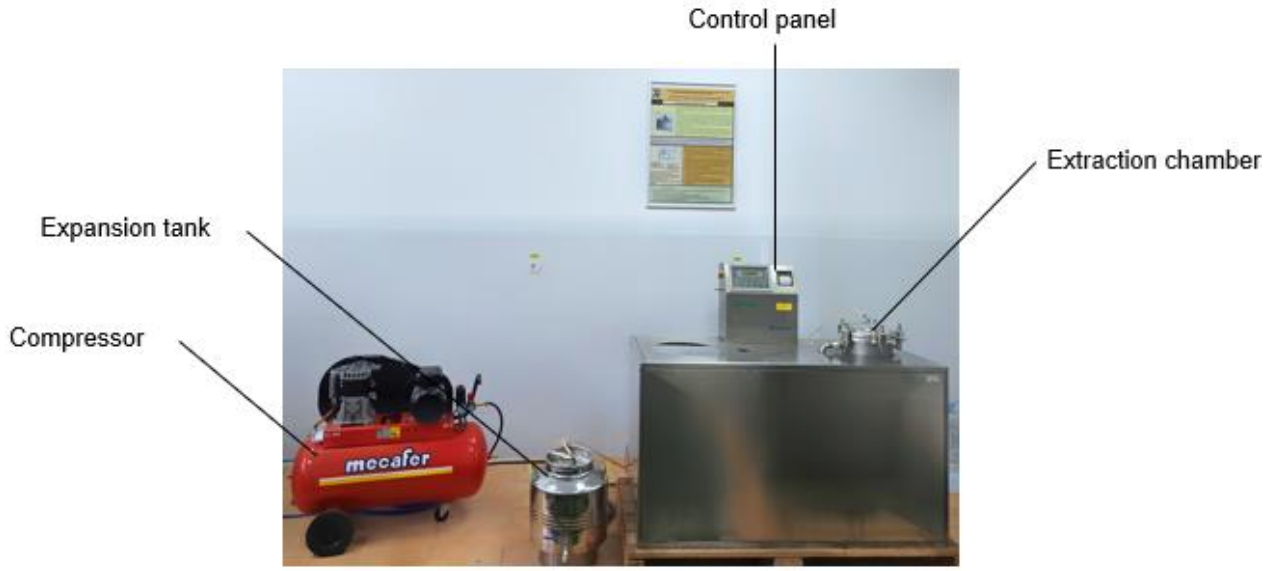

Fig. 4 - TIMATIC percolator

The TIMATIC Percolator model is a high-performance equipment designed for the extraction of active compounds from plants, combining automation with the ability for manual adjustments. It supports a maximum working pressure of 8 bar and operates efficiently within a temperature range of 5-45°C. Its maximum power consumption is 500 W, optimized to deliver high performance and energy efficiency under various operating conditions. The total extraction time, cycles, compression time (TP1) and decompression time (TP0) can be adjusted according to the user's requirements. It operates through a pressure percolation process in two distinct phases.

The equipment consists of the following main components: the extraction chamber - allocated for the introduction of plant batches, ensuring precise handling of the plant material; control panel - displays the extraction process parameters; expansion tank - used to release air from the system during priming operation and also serves as the reservoir from which the necessary amount of solvent for the entire process is drawn; compressor - used to regulate the pressure at which the extraction process takes place.

Each sample involved using 400 grams of dried plant material, with a solvent (water) volume of 12 litres, plus an additional 7 litres to ensure the proper functioning of the process and to eliminate air from the system. The moisture content of the plants before extraction was determined using the Shimadzu MOC63u moisture analyser, and the values obtained were as follows (Figures 5-7): 10.19% for nettle, 9.84% for lavender, and 10.29% for sage.

Fig. 5 - Moisture content of dried nettle

Fig. 6 - Moisture content of dried lavender

Fig. 7 - Moisture content of dried sage

The extraction process begins with the preparation of the plant material, which is weighed to ensure a correct ratio between the plant material and the solvent used. The weighed and dosed plants are then placed into a special cloth bag (Figure 8) and placed in the extraction chamber of the TIMATIC equipment (Figure 9). The solvent is added over the plant material up to the base of the compartment, after which the lid is closed and secured with clamps and nuts to ensure a tight seal.

Fig. 8 – Preparation of plant material: packing in special cloth bag and weighing

Fig. 9 –Sample prepared for introduction into the extraction chamber

The initiation of the percolator's operation involves using an expansion tank to eliminate air from the system and provide the necessary amount of solvent. After completing the preparatory operations, the percolator is started by setting the time and low/high pressure for the percolation operation.

During the extraction process, the percolator alternates between dynamic and static phases, controlling the pressure to facilitate the transfer of the extract into the solvent. The extraction process is optimized by alternately varying the high and low pressures, favouring the release of bioactive principles from the plants.

Finally, after the percolation process is complete, the DISCHARGE operation follows, in which the extract and active principles are collected in the expansion tank for subsequent use in various applications.

A total of 18 tests were performed using the percolator (6 for each type of plant used) at both low pressure (total extraction time – 60 min, number of percolations – 8, and 6 cycles) and high pressure (total extraction time – 60 min, number of percolations – 8, and 6 cycles).

For the low-pressure tests, each cycle included a compression time (TP1) of 4 minutes (at 5 bar pressure) and a decompression time (TP0) of 6 minutes. The decompression time was divided as follows: 1 minute at 0.06 bar, followed by 7 repetitions of 10 seconds at 0.06 bar and another 2 minutes and 30 seconds at the same pressure. These cyclic phases alternated with 8 repetitions of 10 seconds at 0.69 bar. This sequence was repeated for the following 5 cycles.

For the high-pressure tests, each cycle included a compression time (TP1) of 4 minutes (at 7 bar pressure) and a decompression time (TP0) of 6 minutes. The decompression time was divided as follows: 1 minute at 0.12 bar, followed by 7 repetitions of 10 seconds at 0.12 bar and another 2 minutes and 30 seconds at the same pressure. These cyclic phases alternated with 8 repetitions of 10 seconds at 0.87 bar. This sequence was repeated for the following 5 cycles.

Determining the total polyphenol content in the plant extracts (nettle, lavender, sage) was performed using the Folin-Ciocalteu spectrophotometric method. The method involves extracting the total polyphenols in a methanol: water mixture (1:1), treating them with the Folin-Ciocalteu reagent, and measuring the absorbance of the complex formed at a wavelength of λ = 755 nm. The quantification of phenolic compounds was based on the calibration curve of gallic acid, in the concentration range 0-0.2 mg/mL, with results expressed in mg GAE/100g.

The antioxidant capacity of the nettle, lavender, and sage extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, at a wavelength of λ = 517 nm. The Trolox calibration curve was created in the concentration range of 0-0.4375 mmol/L.

a) b) c)

Fig. 10 – Extracts from: a) nettle; b) lavender; c) sage

RESULTS

Table 1 summarizes relevant information about the type of plant, the pressure used in percolation, the degree of plant chopping, extraction time, number of cycles, and number of percolations. This data is essential for evaluating the effectiveness of the extraction process in obtaining polyphenols from each type of plant.

Table 1

Input parameters for the extraction process of active principles from medicinal and aromatic plants

Plant	Extraction method*	Chopping degree (cm)	Pressure (bar)	Time (min)	TP1 (min:sec)	TPO (min:sec)	Cycles	No. of percolations
NETTLE	P1	3	5	60	4	6	6	8
	P2	3	5	60	4	6	6	8
	P3	3	5	60	4	6	6	8
	P4	3	7	60	4	6	6	8
	P5	3	7	60	4	6	6	8
	P6	3	7	60	4	6	6	8
LAVENDER	P1	3	5	60	4	6	6	8
	P2	3	5	60	4	6	6	8
	P3	3	5	60	4	6	6	8
	P4	3	7	60	4	6	6	8
	P5	3	7	60	4	6	6	8
	P6	3	7	60	4	6	6	8
SAGE	P1	3	5	60	4	6	6	8
	P2	3	5	60	4	6	6	8
	P3	3	5	60	4	6	6	8
	P4	3	7	60	4	6	6	8
	P5	3	7	60	4	6	6	8
	P6	3	7	60	4	6	6	8

**P1, P2, P3– Low-pressure percolation; P4, P5, P6 – High-pressure percolation;*

The average results of the experimental analysis of polyphenol content and antioxidant capacity of extracts from the three types of plants are summarized and presented in Tables 2 and 3.

Table 2

Total polyphenol content of nettle, lavender, and sage extracts

No.	SAMPLES	Total polyphenol content (mg GAE/100g)
1.	P1 (nettle)	5.021±0.126
2.	P2 (nettle)	5.227±0.114
3.	P3 (nettle)	5.351±0.129
Average results		**5.199±0.123**
4.	P4 (nettle)	6.445±0.161
5.	P5 (nettle)	6.219±0.170
6.	P6 (nettle)	6.083±0.186
Average results		**6.249±0.172**
7.	P1 (lavender)	14.184±0.355
8.	P2 (lavender)	14.471±0.201
9.	P3 (lavender)	14.711±0.315
Average results		**14.455±0.290**
10.	P4 (lavender)	15.042±0.376
11.	P5 (lavender)	15.094±0.282
12.	P6 (lavender)	15.088±0.311
Average results		**15.074±0.323**
13.	P1 (sage)	17.23±0.43
14.	P2 (sage)	17.05±0.22
15.	P3 (sage)	17.31±0.11
Average results		**17.19±0.25**
16.	P4 (sage)	20.87±0.52
17.	P5 (sage)	20.51±0.43
18.	P6 (sage)	20.88±0.19
Average results		**20.75±0.38**

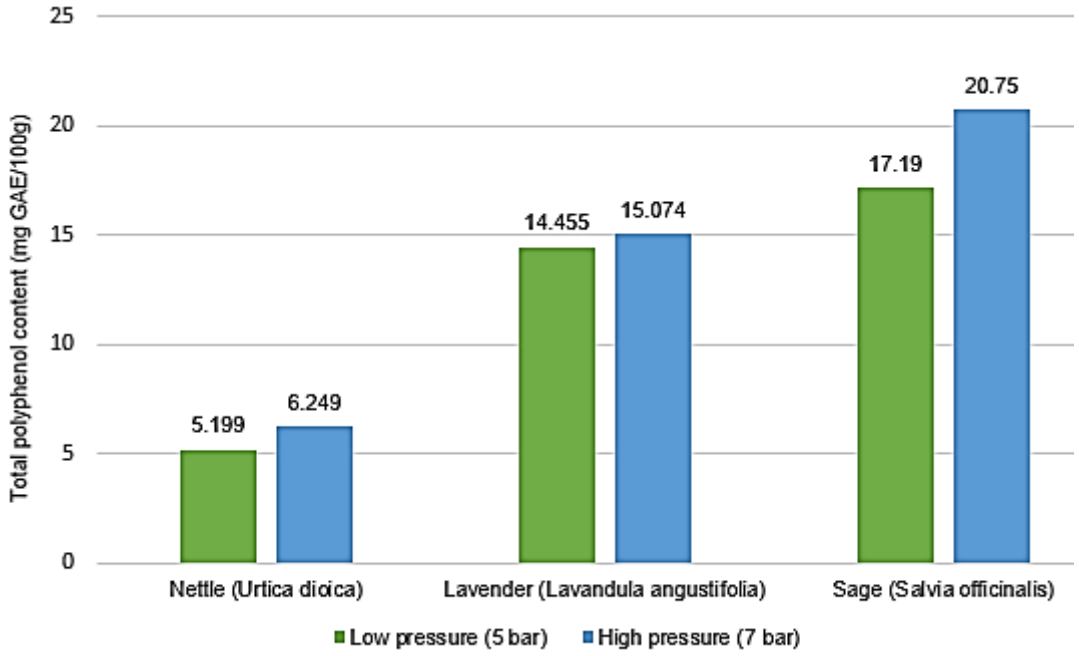

Fig. 11 – Graphical representation of average results obtained for total polyphenol content of nettle, lavender and sage extracts

The results in Figure 11 demonstrate that the effect of pressure on polyphenol extraction varies significantly depending on the type of plant. Data shows that the percolation method (at both 5 and 7 bar pressure) is more suitable for sage, as it more effectively extracts the polyphenols contained in the plant. When analyzed from a perspective of percentage extraction depending on the two pressures, the differences between the two pressure regimes are not significantly different.

Table 3

Antioxidant capacity of nettle, lavender, and sage extracts

No.	SAMPLE	Antioxidant capacity (mg Trolox/100g)
1.	P1 (nettle)	11.760±0.294
2.	P2 (nettle)	11.799±0.213
3.	P3 (nettle)	11.802±0.344
Average results		**11.787±0.283**
4.	P4 (nettle)	11.088±0.277
5.	P5 (nettle)	11.194±0.269
6.	P6 (nettle)	11.207±0.302
Average results		**11.163±0.282**
7.	P1 (lavender)	38.563±0.964
8.	P2 (lavender)	38.405±0.787
9.	P3 (lavender)	38.221±0.555
Average results		**38.396±0.768**
10.	P4 (lavender)	28.578±0.714
11.	P5 (lavender)	28.502±0.636
12.	P6 (lavender)	29.495±0.622
Average results		**28.525±0.657**
13.	P1 (sage)	61.99±1.55
14.	P2 (sage)	61.83±2.21
15.	P3 (sage)	61.56±1.58
Average results		**61.79±1.78**
16.	P4 (sage)	66.21±1.66
17.	P5 (sage)	66.11±1.59
18.	P6 (sage)	66.02±1.18
Average results		**66.11±1.47**

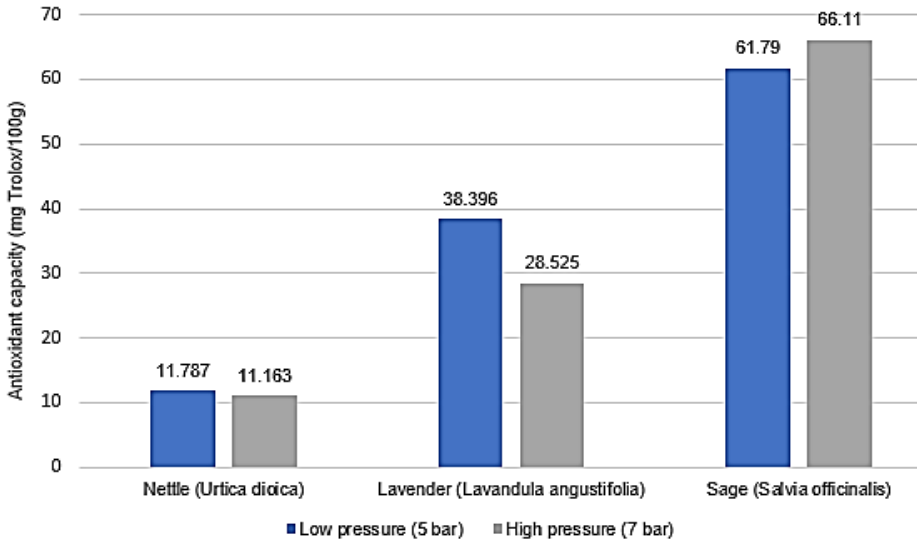

Fig. 12 – Graphical representation of average results obtained for the antioxidant capacity of extracts

According to Figure 12, the results of the antioxidant capacity analysis of nettle, lavender, and sage extracts obtained through percolation at different pressures show that sage has the strongest antioxidant capacity, regardless of pressure. The antioxidant capacity of lavender decreases at high pressure, while for nettle remains relatively constant. A possible explanation could be that high pressure percolation can induce stress on the delicate antioxidant compounds present in lavender, such as certain flavonoids and essential oils. These compounds are more sensitive to high pressure, leading to their degradation or structural alteration, which reduces their effectiveness as antioxidants.

CONCLUSIONS

The impact of pressure on the total polyphenol content varied depending on the plant species. For nettle and lavender, no significant differences were observed between low and high pressure, suggesting stability in

polyphenol content regardless of the applied pressure. In the case of sage, an increase in total polyphenol content was observed at higher pressures, indicative of a more efficient extraction at elevated pressures.

These findings suggest that the properties of the three types of plants can be utilized in various agricultural applications: sage for its robust antioxidant potential, and lavender and nettle for their antimicrobial and antifungal properties.

The results obtained underscore the importance of optimizing extraction conditions based on the plant species to maximize the extraction of various compounds such as polyphenols and the antioxidant activity of the obtained extracts.

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