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# High-Pressure Green Technologies for the Recovery and Functionalization of Bioactive Compounds from *Petiveria alliacea*

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**Abstract**

The growing demand for sustainable technologies in the extraction and functionalization of bioactive compounds has driven the development of innovative, eco-efficient methodologies. This study assesses the feasibility of high-pressure green technologies—Enhanced Solvent Extraction (ESE) and Pressurized Liquid Extraction (PLE)—for extracting bioactive compounds from the leaves of *Petiveria alliacea*, a medicinal plant with significant pharmacological potential. The extracts obtained under optimal PLE conditions (100 bar, 75 °C, ethanol/water: 50:50 *v*/*v*) exhibited the highest total phenolic content (76.27 mg GAE/g) and notable antioxidant capacity. The same extract was tested for its antimicrobial activity against *Escherichia coli*, showing a minimum inhibitory concentration (MIC) of 9.48 µg/mL. Furthermore, the extract was successfully impregnated into polylactic acid (PLA) filaments via supercritical $CO_2$ processing, achieving a maximum antioxidant inhibition of 6.81% under mild conditions (100 bar, 35 °C). The combination of pressurized extraction and supercritical impregnation provides a scalable and environmentally friendly pathway for producing functional biomaterials. These findings highlight the potential of integrating green extraction and material functionalization within the context of the circular bioeconomy and industrial biotechnology.

**Keywords:** plants species; enhanced solvent extraction; pressurized liquid extraction; supercritical impregnation; polylactic acid

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## 1. Introduction

Supercritical fluids (SCFs) have emerged as a fundamental tool in sustainable industrial applications, with special relevance in natural product processing and applied biotechnology. A fluid reaches the supercritical state when both pressure and temperature surpass its critical point, acquiring physicochemical properties that are intermediate between those of liquids and gases. Under these conditions, SCFs exhibit high diffusivity, low viscosity, and tunable densities that can be controlled by adjusting pressure and temperature [1]. These distinctive features make them particularly suitable for enhancing selectivity

and efficiency in the extraction, fractionation, and impregnation of bioactive compounds across diverse matrices. Carbon dioxide is the most widely used supercritical fluid thanks to its non-toxicity, availability, low cost, and the fact that it can be easily removed by depressurization. This aligns with the principles of green chemistry and industrial sustainability regulations [2].

In the field of extraction, supercritical fluids (SCFs) have established themselves as a technologically advanced alternative to conventional organic solvent-based methods. They overcome limitations associated with selectivity, the thermal degradation of compounds, and waste generation [3–5]. Supercritical fluid extraction (SFE) has been successfully applied in the flavor and fragrance industry, where it is recognized for its ability to preserve the integrity of volatile compounds' aromatic profiles. The use of this technology has expanded among leading companies worldwide. For instance, FLAVEX Naturextrakte GmbH, Rehlingen-Siersburg, Germany, applies supercritical fluid processes to maintain the sensory quality and bioactivity of extracts obtained from botanical sources and agri-food residues [6,7]. Likewise, DD Williamson & Co., Ginebra, Suiza (currently part of the Givaudan group) employs supercritical fluid extraction (SFE) to produce natural colorants from fruits and vegetables, addressing the increasing demand for clean-label ingredients [8,9]. In the nutraceutical field, firms such as Phasex Coporation utilize SFE to recover functional compounds, including carotenoids, omega-3 fatty acids, and astaxanthin from algae; fish by-products; and plant materials. This yields highly pure extracts that are free of synthetic residues and suitable for use in functional foods and supplements [10,11].

However, the efficient extraction of polar compounds remains a technical limitation of supercritical $CO_2$ processes due to the low affinity of $CO_2$ for hydrophilic molecules. This is particularly evident in the recovery of antioxidants, such as polyphenols and flavonoids, from agro-industrial by-products. Companies such as Eden Labs have developed supercritical processes specifically targeting these bioactive compounds [12–14]. To overcome this limitation, hybrid techniques have been developed. These methods comprise Enhanced Solvent Extraction (ESE), which employs $CO_2$ mixed with significant amounts of polar solvents such as water or ethanol under subcritical conditions, and Pressurized Liquid Extraction (PLE), which applies liquid solvents at elevated temperatures (40–200 °C) and moderate pressures (30–200 bar) [15,16]. Both techniques have demonstrated great potential for recovering polar bioactive compounds from complex plant matrices [17].

In addition, SCF impregnation has emerged as a valuable technique for sustainable industrial applications. In the textile sector, for instance, the use of supercritical $CO_2$ as a transport medium for dyes has eliminated water consumption, reduced effluent generation, and improved dyeing efficiency, as demonstrated by the technology developed by DyeCoo Textile Systems B.V., Weesp, Netherlands. [18,19]. In wood processing, $scCO_2$ impregnation has been used to introduce fungicides or stabilizers into the wood matrix without altering its surface appearance [20–22].

Conversely, SFI in polymeric matrices has become a popular method for developing controlled-release systems, particularly in the field of biomedicine [23]. This process allows active ingredients to be incorporated into a matrix without altering its structure, while also imparting various functions to the resulting material. Key applications of SFI include functionalization of prostheses, antimicrobial dressings, transdermal systems, and bioabsorbable films with anti-inflammatory or anti-tumor properties [24].

The combined use of extraction and impregnation with supercritical fluids represents a particularly useful technological synergy for closing the natural product valorization cycle. The active compounds extracted can be used directly in biomedical applications or integrated into functional biomedical systems via impregnation. This approach ensures full traceability of the compounds from their natural sources to their clinical or therapeutic

applications. *Petiveria alliacea*, commonly known as 'Anamu', is a promising candidate for the integrated application of these technologies in this context.

Native to tropical America and widely used in traditional medicine, *P. alliacea* contains a variety of bioactive compounds, including sulfur compounds, flavonoids, triterpenes, polyphenols, sterols, and alkaloids [25,26]. Studies confirm that *P. alliacea* exhibits a wide range of pharmacological activities, including neuroprotective effects and activity in the central nervous system. Extracts from the plant have been shown to protect neurons and enhance cognitive functions. In particular, studies have shown that a hydroalcoholic extract can counteract scopolamine-induced deficits in learning and memory in animal models by inhibiting acetylcholinesterase activity and reducing oxidative stress in the brain [25,26]. Furthermore, several reports have described the anticancer potential of aqueous extracts obtained through hot water infusion, as these preparations have been linked to decreased tumor growth and metastasis in myeloid leukemia models [27,28].

On the other hand, methanolic extracts have exhibited notable efficacy in type 2 diabetes models, not only facilitating glycemic control but also attenuating diabetes-induced nephropathy through modulation of the Camp/PKA/CREB/Cfos signaling cascade and suppression of renal apoptosis [29]. Ethanolic extracts have shown activity against various microorganisms, including *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. They have also demonstrated antifungal activity against *Rhizopus* sp. and *Aspergillus niger* [30]. Its anti-inflammatory properties are among the reasons for its traditional use in pain relief [31]. Antioxidant activity is one of its most important properties and underlies many other biomedical effects. It is capable of neutralizing free radicals, which helps protect cells from oxidative damage, a critical process involved in the development of cancer, diabetes, and neurodegenerative diseases [32]. Nevertheless, despite the valuable bioactive compounds present in *Petiveria alliacea*, its industrial utilization remains limited. In this context, it is particularly relevant to note that the study of these compounds using high-pressure techniques has yet to be undertaken. Given the high selectivity of these technologies, their application is a promising and necessary area of research. It represents a crucial preliminary step towards their future implementation in industrial processes for developing functional biomedical systems.

Polylactic acid (PLA) has become one of the most promising polymers in biomedical applications due to its biocompatibility, biodegradability, and flexibility in designing functional materials. Incorporating bioactive compounds into the PLA matrix using advanced methods, such as supercritical fluid-assisted impregnation, allows the biological activity of the extracts to be maintained while enabling controlled and targeted release. This approach supports the development of materials with high potential for regenerative medicine, drug delivery, and antimicrobial coatings [33]. Moreover, PLA demonstrates favorable $CO_2$ sorption, plasticization, and swelling behavior under high-pressure conditions, which facilitates the incorporation of active compounds. Consequently, exploring the impregnation of *P. alliacea* bioactive extracts could reveal whether the resulting polymers exhibit antioxidant properties.

This raises the following scientific question: Can high-pressure techniques such as ESE and PLE be used to obtain bioactive compounds from *P. alliacea*. Additionally, it is relevant to evaluate the possibility of incorporating these extracts into polymers commonly employed in the biomedical filed, such as PLA, using supercritical fluid impregnation.

This research aimed to evaluate non-conventional extraction methods, such as ESE and PLE, to determine the optimal solvent, pressure, and temperature conditions for improving the total extraction yield, phenolic composition, and antioxidant capacity of all the extracts obtained. This work also includes an assessment of the antimicrobial activity against *E. coli* of the extract with the highest bioactivity. Finally, SFI was tested as a methodology to

develop functionalized polymers based on PLA with the most bioactive *Petiveria alliacea* extract obtained to propose them as candidates for biomedical applications.

## 2. Materials and Methods

### 2.1. Raw Material and Chemicals

The *Petiveria alliacea* material used in this study was collected from the Pimpiguasí Sector, Abdón Calderón Parish, Portoviejo Canton, Manabí Province, Ecuador. The samples were gathered in 2023 from a comprehensive farm located in the lower Chico River basin (coordinates: −1.012624, −80.365781). The plants grew in sandy loam soil under irregular rainfall conditions, with a maximum annual precipitation of 1700 mm. Temperature records from the area have indicated an increasing trend since 2013 (maximum 27 °C) [34]. The leaves were sun-dried under controlled environmental conditions until they reached a moisture content of approximately 11%, determined using a BOECO (Hamburgo, Germany) thermobalance (model: BMA I50). The samples were then crushed using a domestic blender (OSTER, Monterrey, México) and homogenized to obtain small, uniform particles without pulverizing them in order to avoid obstructions in the extraction system. The collected leaves were air-dried at room temperature and ground prior to use.

Figure 1 illustrates the key steps of the experimental procedure performed with *Petiveria alliacea*, covering sample collection and conditioning, extract preparation and characterization, as well as the impregnation of PLA filaments. This visual representation provides a comprehensive overview of the methodological workflow applied in this study.

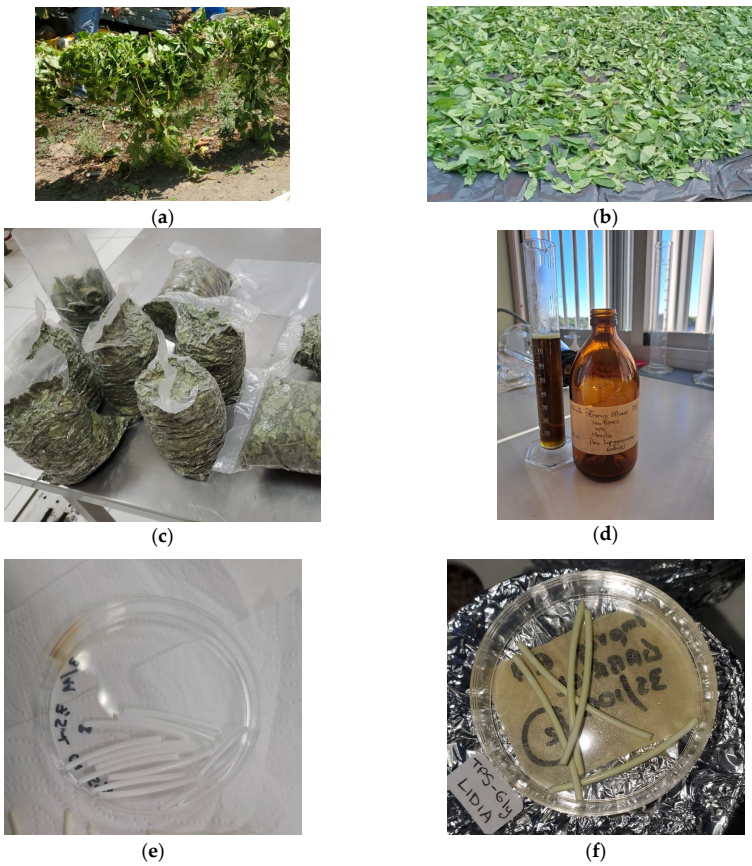



**Figure 1.** Experimental procedure applied to *Petiveria alliacea*: raw material processing, extract preparation, and impregnation into a polymeric matrix: (**a**) sample collection of *Petiveria alliacea*; (**b**) drying of samples; (**c**) sample packaging; (**d**) extracts obtained; (**e**) unimpregnated filaments; (**f**) impregnation with the extract.

For the extraction and impregnation procedures, high-purity carbon dioxide (99.99%), obtained from Abello-Linde S.A. (Barcelona, Spain), and ethanol (>99%), supplied by Panreac (Barcelona, Spain), were employed. The reagents, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate ($K_2S_2O_8$, 99.0%), and Folin–Ciocalteu's reagent, were obtained from Sigma-Aldrich (Steinheim, Germany). Additionally, sodium carbonate ($Na_2CO_3$) and gallic acid ($C_7H_6O_5$), both obtained from Sigma-Aldrich, were used for the quantification of total phenolic content by the Folin–Ciocalteu method, with gallic acid as a calibration standard.

For microbial assays, peptone, sodium chloride, and yeast extract for culture media preparation were purchased from Panreac (Barcelona, Spain). The 2,3,5 Triphenyltetrazolium chloride solution used in the microbiology test was obtained from Sigma-Aldrich. The attenuated strain of *Escherichia coli* (CECT 101) employed to evaluate antimicrobial activity was sourced from the Spanish Type Culture Collection (CECT, Valencia, Spain). Bacterial growth was promoted using an orbital shaking incubator (Shaking Incubator, LBX Instruments, Mataró, Spain), and pH measurements were carried out with a GLP 2 potentiometer (Crison Instruments, Barcelona, Spain).

For the impregnation experiments, PLA filaments with a diameter of 1.6 mm were acquired from Mundo Reader S.L. (Madrid, Spain). The polymer consisted entirely of PLA, with a density of 1.24 g/cm$^3$, a melting point of 145–160 °C, and a glass transition temperature of 56–64 °C, according to the manufacturer's specifications.

### 2.2. High-Pressure Extraction

The extraction protocol used in this study was adapted from a previously validated methodology to ensure methodological comparability but focused on a different plant species characterized by a distinct phytochemical profile.

Extraction was performed using a high-pressure system (model: SF100 (Thar Technologies, Pittsburgh, PA, USA)). This equipment comprises a 100 mL stainless steel extraction vessel. Based on the internal volume and design specifications, the vessel presents an internal diameter (cm)/height of the vessel (cm) = 0.2. Temperature was maintained using a thermostatic jacket, while pressure was precisely regulated with a back pressure regulator (BPR). Two independent flow pumps, each delivering 50 g/min, supplied carbon dioxide ($CO_2$) and the liquid solvents.

This study employed two extraction methods: Enhanced Solvent Extraction (ESE), combining $CO_2$ with a solvent, and Pressurized Liquid Extraction (PLE), with only liquid solvents. Pressure and temperature were adjusted within the ranges of 100 to 250 bar and 55 to 75 °C, respectively, and the extraction was carried out in batch mode for 3 h.

Extracts were collected in a cyclone separator, then transferred to amber bottles and stored at 4 °C in the dark to minimize degradation of bioactive compounds. All experiments were conducted in duplicate.

The operating conditions described above were established based on previous experimental studies conducted by the authors on different plant species, which yielded favorable outcomes for the evaluated parameters, thereby supporting their applicability in the present work [35]. Table 1 summarizes the factors, variables, and levels used in multi-factorial experimental designs for each extraction method (ESE and PLE).

**Table 1.** Experimental design of the extraction process for the two techniques studied.

| Extraction Method | Factor | Variable | Levels |
|---|---|---|---|
| ESE | A | Solvent | $CO_2$/ethanol (1:1 $v/v$, $CO_2$/water (1:1 $v/v$), $CO_2$/ethanol/water (1:0.5:0.5 $v/v/v$) |

**Table 1.** *Cont.*

| Extraction Method | Factor | Variable | Levels |
|---|---|---|---|
| ESE | B | Pressure (bar) | 100, 250 |
| | C | Temperature (°C) | 55, 75 |
| PLE | A | Solvent | Ethanol, water, ethanol/water (50:50 *v*/*v*) |
| | B | Pressure (bar) | 100, 250 |
| | C | Temperature (°C) | 55, 75 |

### *2.3. Characterization of the Extracts*

#### 2.3.1. Extraction Yields

The extraction yields for both ESE and PLE were expressed as the percentage of dry extract relative to the initial dry weight of the plant material. This calculation was performed using Equation (1), where *md* corresponds to the mass of the recovered dry extract and *mi* denotes the initial dry mass of the leaves. All determinations were performed in duplicate to ensure data reproducibility.

$$\%Y = \frac{md}{mi} \times 100 \quad (1)$$

#### 2.3.2. Total Phenolic Content

The obtained extracts were analyzed using spectrophotometric methods. Total phenolic content (TPC) was measured following the Folin-Ciocalteu procedure [36], with gallic acid serving as the calibration standard. Standard solutions of gallic acid ranging from 25 to 300 µg/mL were prepared to construct a calibration curve according to Equation (2), where A represents the absorbance at 725 nm and C is the gallic acid equivalent concentration in µg/mL. For the assay, 12.5 µL of each extract was combined with 12.5 µL of Folin-Ciocalteu reagent, followed by the addition of 200 µL of double-distilled water and mixing for 5 min. Subsequently, 25 µL of 20% (*w*/*v*) sodium carbonate solution was added, and the mixture was stirred for an additional 5 min. After incubation for 60 min at room temperature in the dark, absorbance was recorded at 725 nm using a Synergy HTX multimode microplate reader (BioTek Instruments, Winooski, VT, USA). To eliminate background absorbance from reagents and solvents, specific blanks were prepared for each extract type by replacing the sample with the corresponding solvent (ethanol, water, or hydroalcoholic mixture), while maintaining the exact proportions of Folin-Ciocalteu reagent and sodium carbonate. The average absorbance of each blank was subtracted from the respective sample readings prior to concentration determination. The results were expressed as mg of gallic acid equivalents per gram of dry extract (mg GAE/g).

$$A = 0.0777 \times C - 0.0101; \quad R^2 = 0.9997 \quad (2)$$

#### 2.3.3. Antioxidant Activity

The antioxidant activity of the extracts was assessed using the ABTS radical cation assay (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) [37]. The ABTS solution was prepared by combining 7.4 µM ABTS diammonium salt with 2.6 µM potassium persulfate in a 1:1 ratio and incubating the mixture in the dark for at least 16 h.

Prior to the assay, the solution was diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 750 nm. For the test, 3 µL of extracts was mixed with 300 µL of ABTS solution at concentrations ranging from 150 to 9000 µL/mL. The samples were incubated in the dark for 60 min, and absorbance was subsequently measured at 750 nm using a Synergy HTX multimode microplate reader (BioTek Instruments, Winooski, VT, USA). The

percentage of inhibition (% $I$) was calculated by comparing the control absorbance ($A_0$) with the absorbance after 1 h ($A_i$) according to Equation (3).

$$\%I = \frac{(A_0 - A_i)}{A_0} \times 100 \quad (3)$$

2.3.4. Determination of the Antimicrobial Activity of the Extract

The antimicrobial capacity (AC) against *Escherichia coli* was evaluated using the extract selected from the previous analysis, specifically, the hydroalcoholic extract obtained by PLE at 100 bar and 75 °C. The AC was quantitatively determined by microdilution in liquid medium using the reagent 2,3,5-Triphenyl-tetrazolium chloride solution for microbiology (TTC) as a colorimetric marker, following the procedure described in [38].

The experiments were carried out in Luria–Bertani (LB) liquid medium (5 g of sodium chloride, 5 g of yeast extract, and 10 g of peptone bacteriological, adjusted to a pH of 7.2). The extract was prepared at various concentrations of 0.38–18.95 µg/mL and added to the culture media before inoculating *E. coli* at a concentration of $10^6$ UFC/mL. The experiment was run in a 96-well multiplate, and each sample was prepared in triplicate. The wells contained 200 µL of inoculum + 20 µL of each solution of the extract in the different concentrations. Additionally, blanks were prepared by placing 200 µL of the culture medium without bacteria + 20 µL of the extract. All samples were left in the incubator for 24 h at 32 °C. Once that time had passed, 20 µL of the TTC reagent prepared at 5 mg/mL was added to the wells for determining the cell viability. The plate was read using a spectrophotometer, the Synergy™ HTX Multi-Modal Microplate Reader (BioTek Instruments, Winooski, VT, EE. UU.), at 500 nm, and the inhibition of growth was calculated considering that 100% corresponds to the control without adding any extract.

In the antimicrobial activity test, growth inhibition was calculated considering that 100% corresponds to the positive control 1, which consisted of 200 µL of bacterial inoculum without the addition of any extract or solvent. Additionally, a positive control 2 was included, consisting of 200 µL of inoculum plus 20 µL of the solvent used to dissolve the extracts, in order to verify that the solvent did not exert antimicrobial effects on its own. A blank was also used, prepared with 200 µL of culture medium and 20 µL of the extract without inoculum, to rule out possible optical interference from the extract during reading.

*2.4. Impregnation at High Pressure*

The impregnation process was carried out using the same high-pressure equipment used for the extractions. A quantity of 3 mL of the extract selected (i.e., hydroalcoholic extract ethanol/water (1:1 $v/v$) was poured into the 100 mL vessel. Five PLA filaments, each 30 mm in length, were placed in a steel support basket to avoid direct contact with the extract. Impregnation was conducted in batch mode for 2 h, after which the system was depressurized at a rate of 100 bar/min. The filaments were then gently wiped with a sterile cloth to remove any residual extract. All experiments were performed in duplicate, and the impregnated samples were stored in the dark to maintain their stability and prevent degradation.

A $2^2$ factorial design was evaluated to study the effect of temperature (35–55 °C) and pressure (100–400 bar) on the impregnation loading (Table 2). The operations conditions for the supercritical impregnation of *Petiveria alliacea* extract into PLA filaments were likewise determined based on the authors' previous research, which demonstrated their effectiveness in similar experiments settings [35].

The amount of *Petiveria alliacea* extract loaded into the PLA filaments was evaluated by measuring antioxidant activity spectrophotometrically. For this purpose, 15 mg of each impregnated filament was immersed in 4 mL of ABTS solution. After 60 min of incubation

at room temperature in the dark, the decrease in absorbance at 725 nm was recorded. The percentage of inhibition was calculated using Equation (3).

**Table 2.** Designs of experiments for the supercritical impregnation of PLA filaments.

| Factor | Variable | Levels |
|---|---|---|
| A | Pressure (bar) | 100, 400 |
| B | Temperature (°C) | 35, 55 |

### 2.5. Statistical Analysis

A multivariate factorial design was applied to evaluate the effects of three independent variables: solvent type (ethanol, water, and a hydroalcoholic mixture), temperature (55 and 75 °C), and pressure (100 and 250 bar) on extraction yield, phenolic composition, and antioxidant activity of the extracts.

A $2^2$ factorial design was employed for the impregnation process to evaluate the statistical significance of pressure and temperature on the antioxidant activity of the impregnated filaments.

The data were analyzed statistically using Statgraphics Centurion XVII software (StatPoint Technologies, Inc., Princeton, NJ, USA). The standardized effect is defined as the estimated effect of each factor divided by its standard error, enabling a direct comparison of their relative influences. Pareto diagrams were created to visualize the magnitude and importance of each variable regarding the evaluated responses. The F- and *p*-values were reported for each factor, and experimental errors were used to validate the adequacy of the model.

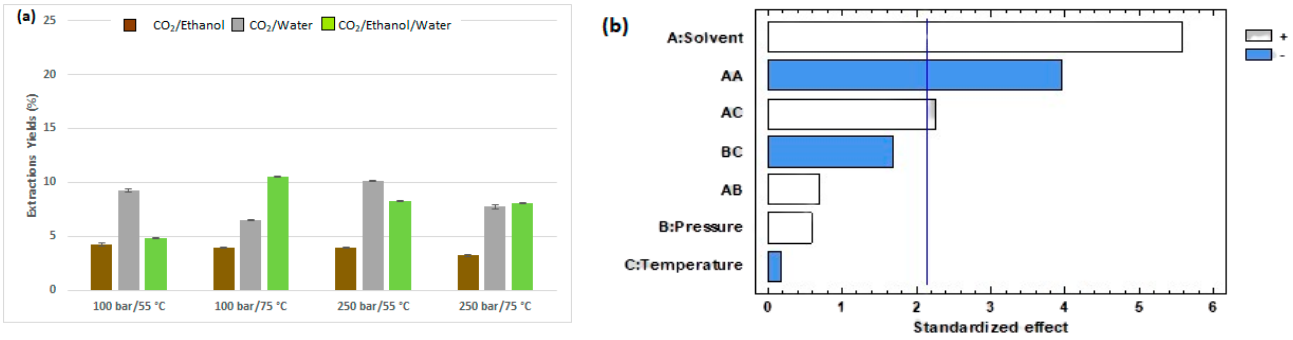
In addition, an ANOVA was performed, followed by a Tukey test, to identify homogeneous subgroups while taking into account the polyphenol composition and antioxidant activity of the extracts.

## 3. Results and Discussion

### 3.1. Enhanced Solvent Extraction

In ESE, $CO_2$ is combined with polar liquid solvents, including water, ethanol, and hydroalcoholic mixtures. The extraction is performed under subcritical conditions, meaning that the pressure and temperature remain below the critical point of the solvent mixtures. This creates a two-phase system with enhanced solvation properties that is highly effective at extracting polar metabolites. The $CO_2$ acts as an expanding phase, reducing the system's viscosity and surface tension. This facilitates solvent penetration into the plant matrix and enhances mass transfer [39], which can lead to improved recovery of phenolic compounds.

Figure 2a shows the total extraction yields at various pressures and temperatures when using the ESE method with $CO_2$/ethanol, $CO_2$/water, and $CO_2$/ethanol/water mixtures. The results show that the extraction yield depends greatly on the solvent system used. The $CO_2$/ethanol (1:1 $v/v$) mixture produced the lowest yields (3.22–4.23% $\pm$ 0.44%), with no significant differences observed across various operating conditions. The highest overall yield (10.14% $\pm$ 0.62%) was achieved using $CO_2$/water (1:1 $v/v$) at 250 bar and 55 °C. Hydroalcoholic mixtures are commonly employed in ESE to minimize the use of organic solvents. Using the $CO_2$/ethanol/water system (1:0.5:0.5 $v/v/v$) strikes an effective balance between efficiency and sustainability, yielding results comparable to those of the $CO_2$/water (1:1 $v/v$) system. According to the literature, mixing $CO_2$ with water and/or alcohol generates an expanded gas phase that favors the extraction of polar compounds by promoting the formation of carbonic acid, which decreases the pH. This positively influences the stability and yield of the compounds.




| | Sum of Squares | Df | Mean Square | F-Ratio | *p*-Value |
|---|---|---|---|---|---|
| A: Solvent | 66.4225 | 1 | 66.4225 | 31.21 | 0.0001 |
| B: Pressure | 0.742017 | 1 | 0.742017 | 0.35 | 0.5636 |
| C: Temperature | 0.0704167 | 1 | 0.0704167 | 0.03 | 0.8581 |
| AA | 33.5002 | 1 | 33.5002 | 15.74 | 0.0012 |
| AB | 1.01002 | 1 | 1.01002 | 0.47 | 0.5014 |
| AC | 10.8241 | 1 | 10.8241 | 5.09 | 0.0395 |
| BC | 6.02002 | 1 | 6.02002 | 2.83 | 0.1133 |
| Blocks | 0.04335 | 1 | 0.04335 | 0.02 | 0.8884 |
| Total error | 31.9199 | 15 | 2.128 | | |
| Total (corr.) | 150.553 | 23 | | | |

(**c**)

**Figure 2.** (**a**) Total yield extraction using $CO_2$/ethanol (1:1 *v*/*v*), $CO_2$/water (1:1 *v*/*v*), and $CO_2$/ethanol/water (1:0.5:0.5 *v*/*v*/*v*) under varying operating conditions. (**b**) Pareto chart results for the total extraction yield. (**c**) ANOVA of the results for the total extraction yield.

The influence of temperature depends on the solvent used. For a $CO_2$/ethanol/water mixture (1:0.5:0.5 *v*/*v*/*v*) at 100 bar, increasing the temperature from 55 °C to 75 °C led to a significant increase in the extraction yield. However, at 250 bar, this effect was less pronounced. With $CO_2$/water, a different trend was observed: increasing the temperature decreased the yield. These findings suggest that solute–matrix interactions (such as hydrogen bonds and dipolar forces) are more effective at moderate temperatures (55 °C) in matrices like *Petiveria alliacea*, promoting the release of bioactive compounds without damaging their thermal stability.

Temperature plays a critical role in determining extraction efficiency. Elevated temperatures decrease the surface tension and viscosity of the solvent, improving its ability to penetrate the plant matrix and typically increasing the extraction rate [40]. However, increased temperature can also lead to more co-extracted analytes, which may decrease extraction selectivity [41,42]. Furthermore, high temperatures may damage thermolabile compounds that are susceptible to thermal degradation, thereby compromising their bioactivity [43].

The application of pressure during extraction reduces the solvent's surface tension, which improves the interaction between the solvent and analytes and facilitates solvent penetration into the porous structure of the plant matrix. Higher pressure also helps to prevent air bubbles forming inside the matrix, which could obstruct the solvent's access to the target sites [44]. Nevertheless, multiple studies have indicated that pressure generally has a limited effect on the recovery of bioactive compounds compared to other operational parameters [35,45]. In agreement with these findings, the data shown in Figure 2b demonstrate that pressure does not significantly influence the extraction yield of *Petiveria alliacea*, whereas the choice of solvent is the primary factor affecting process efficiency.

Previous studies have used the ESE technique and similar ranges of pressure, temperature, and solvent combinations to assess the extractive behavior of *Prestonia mollis*. The results show similar patterns for both plant matrices, indicating that the solvent type is the most influential factor, followed by its quadratic interaction. Conversely, the individual effects of pressure and temperature were not statistically significant ($p > 0.05$ at a 95% confidence level). As illustrated in Figure 2c, ANOVA results confirm that solvent type had a statistically significant effect on extraction yield from *P. alliacea* ($p < 0.05$), whereas pressure and its interaction had no significant influence. The low residual error and high F-values support the adequacy and reliability of the fitted model.

Temperature did not play a significant role in the SFE of polar compounds from *Camellia sinensis* leaves. The study found that temperature changes did not significantly impact extraction performance. Instead, the composition of the ethanol/water modifier played a more influential role in recovering target compounds such as caffeine and catechins [46]. In the study conducted on Salvia officinalis, a decrease in extraction yield was observed with increasing temperature, indicating that higher temperatures did not enhance the recovery of key compounds [47].

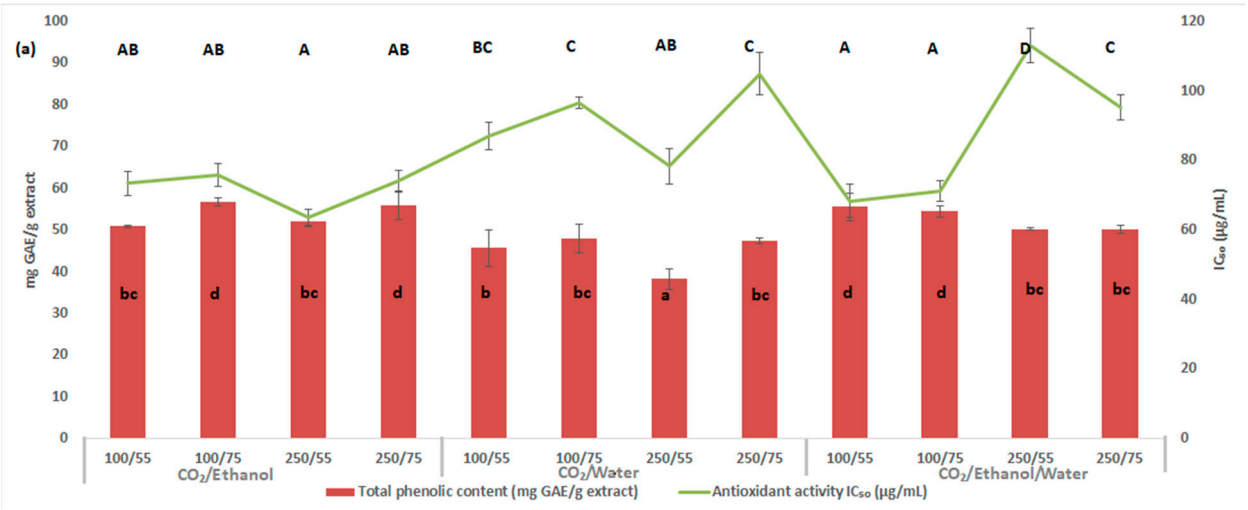
As shown in Figure 3a, the total phenolic compound content varied between 38.25 and 56.73 $\pm$ 5.22 mg GAE/g of dry extract. The values were higher in extracts obtained using hydroalcoholic mixtures. Although the extraction yields were low for extracts obtained using the $CO_2$/ethanol (1:1 $v/v$) mixture (Figure 2a), the concentration of phenolic compounds was high, suggesting the system's high selectivity for this family of compounds. The ABTS assay showed lower $IC_{50}$ values for extracts produced using $CO_2$/ethanol/water (1:0.5:0.5 $v/v/v$) at 100 bar and $CO_2$/ethanol at 250 bar and 55 °C, indicating higher antioxidant capacity. The absence of a linear correlation between total phenolic content and $IC_{50}$ values suggests the presence of synergistic effects between phenolic compounds and other metabolites with antioxidant capacity [48].

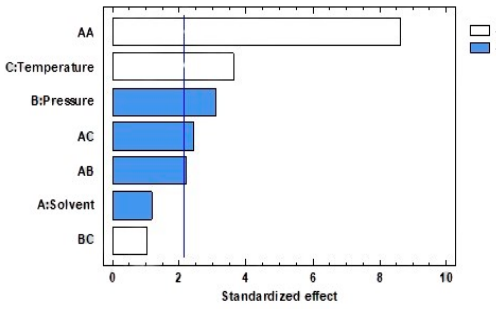
Various plant species used in traditional Ecuadorian medicine have been studied for their potential bioactivity. For example, *Mimusops coriacea*, traditionally used as an analgesic and anti-inflammatory, has demonstrated antioxidant capacity with an $IC_{50}$ value of 197.00 µg/mL in the ABTS assay. Similarly, *Corynaea crassa* Hook. f. (Balanophoraceae) had an $IC_{50}$ value of 138.52 µg/mL in the same assay. In contrast, *Petiveria alliacea* extracts obtained in the present study using high-pressure technologies showed an $IC_{50}$ of approximately 65 µg/mL, indicating stronger antioxidant activity. This higher activity could be due to the phytochemical richness of the species and the efficiency of the extraction process under high-pressure conditions.

Various studies have identified bioactive metabolites in *Petiveria alliacea*, including capreoside, narcissine, indane, (-)-isocaryophyllene, (-)-β-pinene, (E)-tagetone, and peonidin derivatives, which are linked to its antioxidant activity. Additional reports confirm the presence of flavonoids, terpenoids, tannins, phenols, alkaloids, carotenoids, and sulfur-containing compounds like dibenzyl-disulfide and dibenzyl-trisulfide. The consistent detection of phenolic compounds, such as isoarborinol, myricetin, and quercetin, supports the extract's antioxidant potential [30,49–51].

Other phytochemical studies have identified a broad range of bioactive compounds in *Petiveria alliacea*. These include flavonoids such as astilbin, myricitrin, and engeletin; triterpenes like barbinergic acid and α-friedelinol; and steroids such as daucosterol. Lipids such as lignoceric, nonadecanoic, and oleic acids have also been isolated, along with compounds such as allantoin and trithiolaniacin. In the roots, coumarins have been reported, along with benzaldehyde, benzoic acid, β-sitosterol, potassium nitrate, glucose, and glycine. Additionally, the species is rich in phenolic compounds, especially salicylic,

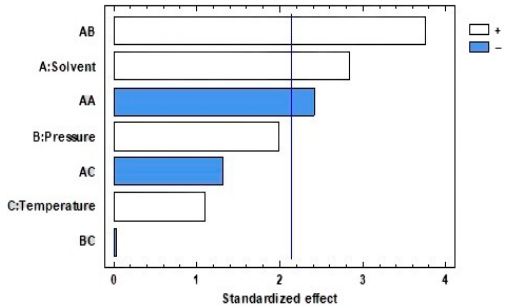
ferulic, *p*-coumaric, 4-hydroxybenzoic, gallic, protocatechuic, caffeic, syringic, and vanillic acids, which reflect its antioxidant potential [52–54].




| | Sum of Squares | Df | Mean Square | F-Ratio | *p*-Value |
|---|---|---|---|---|---|
| A: Solvent | 6.85131 | 1 | 6.85131 | 1.36 | 0.2622 |
| B: Pressure | 48.5357 | 1 | 48.5357 | 9.62 | 0.0073 |
| C: Temperature | 66.7 | 1 | 66.7 | 13.22 | 0.0024 |
| AA | 374.586 | 1 | 374.586 | 74.22 | 0.0000 |
| AB | 24.7258 | 1 | 24.7258 | 4.90 | 0.0428 |
| AC | 29.8389 | 1 | 29.8389 | 5.91 | 0.0280 |
| BC | 5.4245 | 1 | 5.4245 | 1.07 | 0.3163 |
| Blocks | 31.3959 | 1 | 31.3959 | 6.22 | 0.0248 |
| Total error | 75.707 | 15 | 5.04713 | | |
| Total (corr.) | 663.765 | 23 | | | |

(**b**)




| | Sum of Squares | Df | Mean Square | F-Ratio | *p*-Value |
|---|---|---|---|---|---|
| A: Solvent | 926.746 | 1 | 926.746 | 8.11 | 0.0122 |
| B: Pressure | 453.184 | 1 | 453.184 | 3.96 | 0.0650 |
| C: Temperature | 139.25 | 1 | 139.25 | 1.22 | 0.2872 |
| AA | 669.387 | 1 | 669.387 | 5.85 | 0.0287 |
| AB | 1614.23 | 1 | 1614.23 | 14.12 | 0.0019 |
| AC | 196.35 | 1 | 196.35 | 1.72 | 0.2098 |
| BC | 0.0805042 | 1 | 0.0805042 | 0.00 | 0.9792 |
| Blocks | 0.127604 | 1 | 0.127604 | 0.00 | 0.9738 |
| Total error | 1715.03 | 15 | 114.335 | | |
| Total (corr.) | 5714.38 | 23 | | | |

(**c**)

**Figure 3.** (**a**) Total phenolic compounds and antioxidant activity of *Petiveria alliacea* leaf extracts obtained by ESE with $CO_2$/water (1:1 *v*/*v*), $CO_2$/ethanol/water (1:0.5:0.5 *v*/*v*/*v*), and $CO_2$/ethanol (1:1 *v*/*v*) at different pressures and temperatures (different letters show significant differences among samples (multiple range test, $\alpha = 0.05$)). (**b**) Pareto chart results for the total phenolic compounds. (**c**) Pareto chart results for the antioxidant activity.

A thorough assessment of the effects of the variables on total polyphenol content and antioxidant activity for each extract underscores the complexity of the systems analyzed (Figure 3b,c). The Pareto chart of total phenolic compounds (Figure 3b) shows that pressure and temperature, as well as the interactions between solvent pressure and temperature, have a significant influence on the process. According to the ANOVA results shown in Figure 3b, the type of solvent had the most significant effect on total phenolic content ($p < 0.05$), followed by its quadratic contribution. Pressure and interaction terms had a negligible influence. These results emphasize the pivotal role of solvent polarity in the recovery of phenolic compounds.

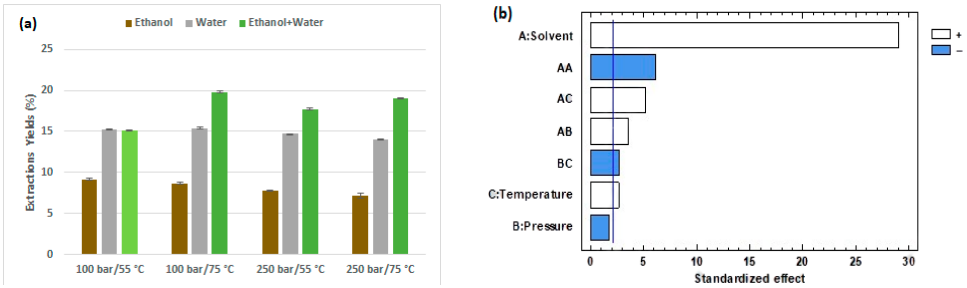
Figure 3c shows the analysis of variance (ANOVA) for antioxidant activity in the form of a Pareto chart. The solvent type ($p = 0.0122$), its quadratic effect ($p = 0.0287$), and the interaction between the solvent and pressure ($p = 0.0019$) were identified as statistically significant factors ($p < 0.05$). These findings highlight the significant influence of solvent polarity and its interaction with process parameters on the antioxidant capacity of the extracts.

### 3.2. Pressurized Liquid Extraction

PLE is a technique that employs liquid solvents, such as water or ethanol, at elevated temperatures (40–200 °C) and moderate pressures (30–200 bar) to maintain the solvent in the liquid phase above its normal boiling point. Compared to conventional methods such as maceration or Soxhlet extraction, this technology improves the extraction of bioactive compounds, enhances process kinetics, and significantly reduces extraction time and solvent consumption [55].

Figure 4a shows the total extraction yield for PLE investigations. Comparing Figures 1a and 3a, PLE achieves higher extraction yields than ESE for each solvent used: 7.18–19.78% for PLE versus 3.22–10.55% for ESE. Similarly, Olomieja (2021) [30] reported that cold extraction of *Petiveria alliacea* leaves using hexane and ethanol yielded 11.02% and 8.75%, respectively. These findings further highlight the superiority of high-pressure techniques like PLE, which outperform conventional extraction methods regarding both extraction efficiency and the utilization of plant material. A pattern similar to that observed for ESE was noted when ethanol was employed as the solvent, resulting in the lowest extraction efficiency among the three solvents evaluated. This behavior has been consistently reported in previous studies [56–58]. In contrast, hydroalcoholic mixtures (ethanol/water at a 1:1 $v/v$ ratio) significantly improved extraction yields, particularly at 75 °C. This improvement is likely due to the synergistic action of the two solvents: ethanol increases the solubility of moderately polar metabolites, while water promotes solute desorption from the plant matrix. This behavior has been widely documented in the literature for polyphenol-rich matrices [59–61].

A comparison of the Pareto diagrams in Figures 2b and 4b shows that PLE was more sensitive to the operational variables than ESE. Figure 4b in particular shows that the type of solvent was the most influential factor affecting extractive yield, reaching statistical significance at the 95% confidence level. Although temperature had a less pronounced effect, it also contributed notably, particularly in ethanol/water mixtures, where recovery was higher. In contrast, as was also observed with ESE, pressure had a limited impact on the process. These findings are consistent with previous reports indicating that pressure typically has a smaller effect on the extraction of bioactive metabolites compared to temperature. Figure 4c (ANOVA) shows that both the solvent type and its quadratic effect significantly influence total phenolic content ($p < 0.05$), while pressure showed no statistical significance. The model's adequacy is supported by a high F-value and low residual error. These results align with the Pareto analysis, confirming solvent dominance in the system.




| Source | Sum of Squares | Df | Mean Square | F-Ratio | *p*-Value |
|---|---|---|---|---|---|
| A: Solvent | 377.914 | 1 | 377.914 | 841.57 | 0.0000 |
| B: Pressure | 1.4357 | 1 | 1.4357 | 3.20 | 0.0940 |
| C: Temperature | 3.2047 | 1 | 3.2047 | 7.14 | 0.0174 |
| AA | 17.0408 | 1 | 17.0408 | 37.95 | 0.0000 |
| AB | 5.49902 | 1 | 5.49902 | 12.25 | 0.0032 |
| AC | 12.25 | 1 | 12.25 | 27.28 | 0.0001 |
| BC | 3.21934 | 1 | 3.21934 | 7.17 | 0.0172 |
| Blocks | 0.00120417 | 1 | 0.00120417 | 0.00 | 0.9594 |
| Total error | 6.73589 | 15 | 0.449059 | | |
| Total (corr.) | 427.3 | 23 | | | |

(**c**)

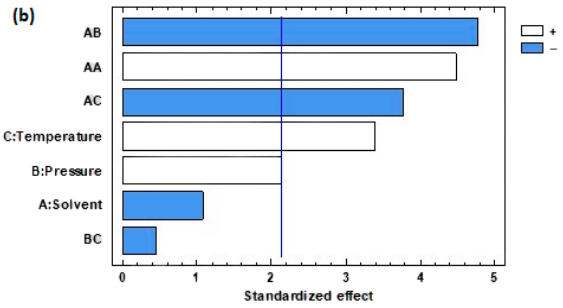
**Figure 4.** (**a**) Total yield extraction using water, ethanol/water, and ethanol at different pressures and temperatures. (**b**) Pareto chart results for the total yield extraction. (**c**) ANOVA of the results for the total extraction yield.

Previous studies by Luthria [62] and Mukhopadhyay et al. [59] concluded that, when evaluated as an independent variable, pressure does not significantly affect the efficiency of natural compound extraction.

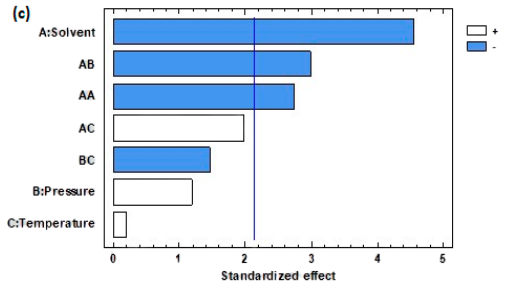
Regarding the chemical composition of the extracts (see Figure 5a), the total phenolic content measured in this study varied between 45.99 and 76 ± 2.27 mg GAE/g of dry extract, depending on the solvent and extraction method used. These values are significantly higher than those reported by Navarro et al. [54], who obtained 13.45 mg GAE/g of dry extract using ultrasound-assisted extraction with a methyl tert-butyl ether and methanol mixture (90:10 *v*/*v*). This discrepancy underscores the effectiveness of the high-pressure technologies applied in this study for enhancing the recovery of bioactive compounds. These values exceed those obtained with ESE (see Figure 3a), suggesting that $CO_2$ limits the recovery of highly polar metabolites. The highest phenolic content was achieved using ethanol at 250 bar and 75 °C, highlighting the solvent's high selectivity under subcritical conditions. However, extracts obtained with hydroalcoholic mixtures exhibited higher antioxidant capacity (lower $IC_{50}$ values), suggesting that, in addition to phenolic compounds, other redox-active metabolites were efficiently co-extracted under these conditions. Several studies have demonstrated that the ethanol-to-water ratio in binary solvent systems significantly influences both the phenolic profile and antioxidant capacity of plant extracts [63]. For instance, Scarano et al. [64] observed a higher total phenolic content when a hydroalcoholic mixture (ethanol/water: 70:30 *v*/*v*) was used to extract *Arbutus unedo* (strawberry tree), compared to water alone. Similarly, Jacotet-Navarro et al. [65] investigated how different ethanol concentrations influenced the extraction yield of antioxidant compounds from *Rosmarinus officinalis* (rosemary). They reported that raising the ethanol concentration from 0% to 60% significantly enhanced the extraction yield; however, beyond this range, particularly between 60% and 80%, no further improvement was observed and the yield decreased from 26% to 15%. These results suggest the existence of an optimal

ethanol concentration at which the analyte is both highly soluble and effectively desorbed from the plant matrix.




| Source | Sum of Squares | Df | Mean Square | F-Ratio | *p*-Value |
|---|---|---|---|---|---|
| A: Solvent | 17.619 | 1 | 17.619 | 1.16 | 0.2978 |
| B: Pressure | 69.0883 | 1 | 69.0883 | 4.56 | 0.0496 |
| C: Temperature | 173.236 | 1 | 173.236 | 11.44 | 0.0041 |
| AA | 304.164 | 1 | 304.164 | 20.08 | 0.0004 |
| AB | 346.611 | 1 | 346.611 | 22.89 | 0.0002 |
| AC | 217.047 | 1 | 217.047 | 14.33 | 0.0018 |
| BC | 3.05307 | 1 | 3.05307 | 0.20 | 0.6599 |
| Blocks | 6.4896 | 1 | 6.4896 | 0.43 | 0.5226 |
| Total error | 227.164 | 15 | 15.1443 | | |
| Total (corr.) | 1364.47 | 23 | | | |




| | Sum of Squares | Df | Mean Square | F-Ratio | *p*-Value |
|---|---|---|---|---|---|
| A: Solvent | 1052.68 | 1 | 1052.68 | 20.73 | 0.0004 |
| B: Pressure | 72.7668 | 1 | 72.7668 | 1.43 | 0.2498 |
| C: Temperature | 1.86484 | 1 | 1.86484 | 0.04 | 0.8506 |
| AA | 381.49 | 1 | 381.49 | 7.51 | 0.0152 |
| AB | 453.69 | 1 | 453.69 | 8.94 | 0.0092 |
| AC | 198.246 | 1 | 198.246 | 3.90 | 0.0668 |
| BC | 107.231 | 1 | 107.231 | 2.11 | 0.1667 |
| Blocks | 109.099 | 1 | 109.099 | 2.15 | 0.1633 |
| Total error | 761.529 | 15 | 50.7686 | | |
| Total (corr.) | 3138.59 | 23 | | | |

**Figure 5.** (**a**) Total phenolic compounds and antioxidant activity of *Petiveria alliacea* leaf extracts obtained by PLE with water, ethanol/water (50:50 *v*/*v*), and ethanol at different pressures and temperatures (different letters show significant differences among samples (multiple range test, $\alpha = 0.05$)). (**b**) Pareto chart results for the total phenolic compounds. (**c**) Pareto chart results for the antioxidant activity.

In the present study, *Petiveria alliacea* extracts obtained through high-pressure technologies exhibited notable antioxidant capacity, with an $IC_{50}$ value of approximately 65 µg/mL

in the ABTS assay. These findings demonstrate that both the extraction technique and the operating conditions play a crucial role in determining the antioxidant performance of the species. In previous studies, an $IC_{50}$ of 120 µg/mL was reported in the DPPH assay using maceration with methanol for 72 h in darkness, followed by filtration and concentration by rotary evaporation [66]. Likewise, a hydroalcoholic extract (methanol/water: 20:80 *v*/*v*) obtained by maceration with constant agitation for 72 h presented $IC_{50}$ values of 38.62 ± 1.15 µg/mL with DPPH and 59.33 ± 1.32 mg ascorbic acid equivalents per g of extract using the FRAP method [67].

Other authors have reported antioxidant activities with $IC_{50}$ > 1000 µg/mL in extracts prepared with a mixture of methyl tert-butyl ether and methanol 90:10 (*v*/*v*) at 25 °C for 30 min in ultrasound [54]. Similarly, Zavala et al. [26] reported an antioxidant activity of 612.96 mEq Trolox/100 g for ABTS assays from extracts obtained by supercritical fluid extraction with ethyl acetate as a cosolvent. Such variations highlight the critical role of both the extraction technique and the solvent type in determining antioxidant capacity [68].

The Pareto diagrams (Figure 5b,c) indicate that the variability in phenolic content and antioxidant activity is significantly affected by both solvent–pressure interaction and the quadratic effect of the solvent. It was also observed that temperature and its interaction with the solvent are critical factors in maximizing the recovery of phenolic compounds.

Figure 5b,c summarize the ANOVA results for phenolic composition and antioxidant activity, respectively. For phenolic composition, temperature ($p$ = 0.0041), the quadratic effect of the solvent ($p$ = 0.0004), and its interactions with pressure ($p$ = 0.0002) and temperature ($p$ = 0.0031) were statistically significant ($p < 0.05$), highlighting the predominant role of thermal and interactive effects. In contrast, antioxidant activity was significantly influenced by the solvent ($p$ = 0.0004), its quadratic term ($p$ = 0.0152), and the solvent–pressure interaction ($p$ = 0.0092), indicating that solvent polarity and its synergistic or non-linear interactions with pressure drive antioxidant performance, whereas temperature had no significant effect.

In comparative terms, PLE exhibits greater sensitivity to operational variables than ESE, offering broader potential for process optimization. The use of an ethanol/water mixture at 100 bar and 75 °C was determined to be the most effective condition for enhancing both the extraction yield and antioxidant activity of the *Petiveria alliacea* extracts. This configuration simultaneously enhances the solubility of polar and semipolar compounds found in *Petiveria alliacea*, due to the mixed nature of the solvent, and improves diffusion as a result of the elevated temperature. Additionally, a moderate pressure of 100 bar is sufficient to maintain the pressurized liquid phase without causing thermal degradation, which may occur at higher pressures. These conditions were therefore selected for subsequent supercritical impregnation steps in polymeric matrices.

### 3.3. Microbial Capacity

Compounds of plant origin offer a valuable alternative for developing new antimicrobial agents, as they act through diverse mechanisms that can overcome bacterial resistance and contribute to reducing the excessive use of antibiotics in treating infections [69]. *E. coli* bacteria are a very versatile species that can inhabit water, soil, and plants, and they are the cause of many diseases in humans, causing disease outbreaks around the world, because they can invade any part of the body and even lead to death [70], so in the frame of biomedicine, *E. coli* can be explored as a target microorganism. Table 3 represents the minimum concentration required to inhibit *Escherichia coli* growth in the presence of the *Petiveria alliacea* extract selected.

**Table 3.** Experimental data of the microbiological analysis of the hydroalcoholic extract of *Petiveria alliacea* obtained by PLE at 75 °C and 100 bar pressure.

| Concentration (μg/mL) | Inhibition of Growth (%) ± SD |
|---|---|
| 0.38 | 6.87 ± 0.061 |
| 0.95 | 31.02 ± 0.076 |
| 1.90 | 46.70 ± 0.001 |
| 4.74 | 56.04 ± 0.069 |
| 9.48 | 100.71 ± 0.002 |
| 14.21 | 100.41 ± 0.000 |
| 18.95 | 99.78 ± 0.001 |

Table 3 shows that the minimum inhibitory concentration (MIC), i.e., the concentration at which the bacteria's growth is inhibited, is 9.48 μg/mL, demonstrating the antimicrobial potential of this species against *E. coli*.

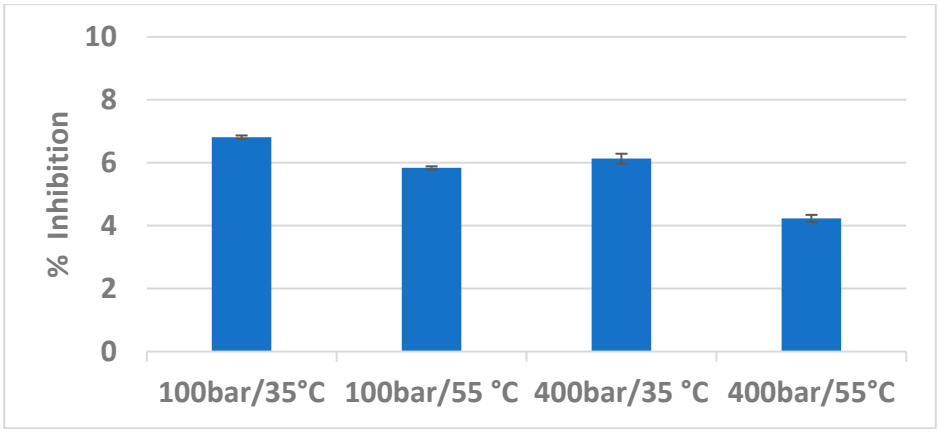
In this study, *Petiveria alliacea* extracts showed notable antimicrobial effects against *E. coli*, achieving 100% inhibition at a concentration of 9.48 μg/mL. The results obtained in this study greatly surpass those reported in the literature, where minimum inhibitory concentrations (MICs) of up to 3690 μg/mL have been observed in crude extracts obtained through dynamic maceration with 70% ethanol. Additionally, some studies report no activity in extracts prepared with methanol and hexane, emphasizing the importance of the solvent type and extraction method. Silva et al. suggested that the antimicrobial activity of *Petiveria alliacea* is likely related to the presence of compounds such as dibenzyltetrasulfide, isoarborinol, and sulfur metabolites, which can disrupt microbial membranes. They also found that the combined effects of these phytochemicals can produce synergistic effects against both Gram-positive and Gram-negative bacteria, supporting their potential as sources of natural antimicrobial agents [71]. Furthermore, the MIC values obtained in this study are considerably lower than those reported by Kim et al. [72] and Mulyani et al. [73], who documented an MIC of 256 μg/mL for *E. coli* using macerated extracts from the plant's fresh roots. This difference indicated that both the type of extract and preparation conditions significantly influence antimicrobial activity. Compared to previous studies, the extract produced here exhibited much more potent antimicrobial effects when using high-pressure techniques, which likely enhance extraction by facilitating cell penetration. These variations in results could also stem from differences in extraction methods, solvent types, bioactive compounds concentrations, *E. coli* strains, determination methods, and environmental and experimental factors influencing the MIC. *Petiveria alliacea* is rich in polysulfides (also known as petiveriines), along with phenolic compounds, which are primary contributors to its antimicrobial activity. While the roots contain higher levels of organosulfur compounds [74], the leaves have greater amounts of phenolic compounds. Gram-negative bacteria have an outer membrane containing lipopolysaccharides, which confer increased resistance to certain antimicrobials; thus, the combination of these compounds may be key to inhibiting *Escherichia coli*. Overall, the results show that *Petiveria alliacea* has great industrial potential for its antimicrobial activity against *E. coli*, meaning that it could be applied in sectors such as pharmaceuticals and the food industry, with high-pressure techniques representing a promising method for producing potent antimicrobial extracts.

### 3.4. Impregnation into PLA Filaments

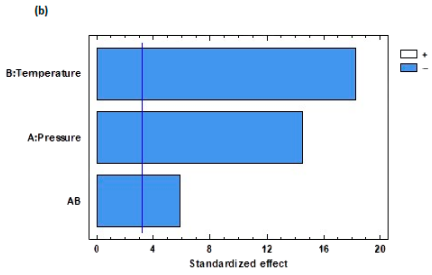
During supercritical impregnation, it is essential to consider the nature of both the polymer and the extract, as well as their interactions with supercritical carbon dioxide ($scCO_2$). Key parameters determining process efficiency include the solubility of active compounds in $scCO_2$, the polymer's capacity to swell and absorb in the presence of this

fluid, and the affinity between the active compounds and the polymer matrix. Given that these factors are strongly affected by processing conditions, it is critical to assess the impact of pressure and temperature on the biological activity of the resulting materials.

The extract used in the impregnation experiments was obtained by PLE at 75 °C and 100 bar using an alcoholic solvent, and this was the extract with the highest yield and antioxidant activity. The maximum percentage of inhibition was observed at 100 bar and 35 °C, reaching 6.81 ± 0.06% in 15 mg of polymer (Figure 6a). As shown in Figure 6b, the Pareto chart indicates that both temperature and pressure significantly affect the impregnation process. The subsequent ANOVA (Figure 6c) results confirm these findings, with temperature ($p$ = 0.0004) and pressure ($p$ = 0.0007) exhibiting strong statistical significance. These results demonstrate that precise control of these parameters is crucial to enhance compound loading and ensure optimal performance of the PLA filament impregnation.




(**a**)




| | Sum of Squares | Df | Mean Square | F-Ratio | *p*-Value |
|---|---|---|---|---|---|
| A: Pressure | 0.0002610 | 1 | 0.0002610 | 210.89 | 0.0007 |
| B: Temperature | 0.0004132 | 1 | 0.0004132 | 333.85 | 0.0004 |
| AB | 0.00004278 | 1 | 0.00004278 | 34.56 | 0.0098 |
| Blocks | $5.5125 \times 10^{-7}$ | 1 | $5.5125 \times 10^{-7}$ | 0.45 | 0.5523 |
| Total error | 0.000003713 | 3 | 0.000001237 | | |
| Total (corr.) | 0.000721389 | 7 | | | |

(**c**)

**Figure 6.** PLA filament impregnation under varying operating conditions. (**a**) Percentage of impregnation at different pressure and temperature levels (mean value ± SD). (**b**) Pareto chart showing the effect of pressure and temperature on impregnation percentage. (**c**) ANOVA of the results for the impregnations.

In general, elevating the pressure of $scCO_2$ increases both its polarity and solvent density, thereby enhancing the solubility of target compounds in the supercritical phase. However, at elevated pressures, the diffusional properties of $scCO_2$ decrease, which hinders interaction between solubilized compounds and the polymeric matrix. The efficiency of the impregnation process is governed by the partitioning behavior of the active compounds between the supercritical and polymeric phases [75]. At 400 bar, the solubility of the extract's active constituents in the supercritical phase exceeds their affinity for the polymeric matrix. This causes lower impregnation percentages (% I), as shown in Figure 6a.

Under isobaric conditions, raising the temperature reduces the density of the $scCO_2$, meaning fewer compounds can contact the polymer matrix. As a result, the quantity of compounds incorporated into the polymer decreases. The impregnation of PLA filaments with piperine and black pepper extract was performed using $scCO_2$ at temperatures of

40 °C and 50 °C and pressures of 100 and 120 bar, with 5% ethanol as a co-solvent. Optimal loading was achieved under these conditions, yielding 5.61% for piperine and 5.28% for the black pepper extract. These results indicate efficient incorporation of bioactive compounds into the polymer matrix facilitated by $scCO_2$-assisted impregnation [76].

The literature review emphasizes the use of PLA in filament form for impregnation studies involving compounds with antioxidant activity. One study impregnated PLA filaments with *Olea europaea* extract using $scCO_2$, resulting in antioxidant inhibition percentages between 6% and 15% [77]. In another study, PLA filaments were impregnated with *Prestonia mollis* extract using high-pressure technologies, showing inhibition values of 10% and 11% [35]. Similarly, a study using mango leaf extract applied PLA filaments measuring 35 mm in length and 1.6 mm in diameter, which displayed high antioxidant activity with an $IC_{50}$ of 8.24 μg/mL and an Antioxidant Activity Index (AAI) of 2.80 μg DPPH/μg extract [78].

The present research delivers novel scientific insights into *Petiveria alliacea*, a plant species sourced from Ecuadorian lands, highlighting its phenolic composition, antioxidant activity, and antimicrobial properties. Furthermore, the most promising extract was, for the first time, impregned into PLA filaments, producing favorable results that offer new opportunities for its use in the development of materials with biomedical applications.

## 4. Conclusions

This work demonstrates the technological feasibility of employing *Petiveria alliacea* as a source for the sustainable production of bioactive extracts and functional biomaterials. The application of pressurized green extraction methods, PLE and ESE, enabled the selective recovery of phenolic compounds with high antioxidant and antimicrobial activities. Of the conditions evaluated, PLE using a hydroalcoholic mixture (ethanol/water: 50:50 *v*/*v*) at 100 bar and 75 °C yielded the highest extract performance, with a total phenolic content of 76.27 mg GAE/g and a minimum inhibitory concentration (MIC) of 9.48 μg/mL against *Escherichia coli*.

Similarities can be observed when comparing ESE and PLE. Although temperature and pressure can influence the total amount of phenolic compounds extracted in both methods, solvent choice modulates the extraction yield, particularly when employing PLE, as well as the recovery of compounds, which is determined by the polarity of the solvent mixture. When $CO_2$ is employed, which adjusts the polarity towards lower values, it decreases both the extraction yield and bioactivity. In conclusion, this parameter is crucial when the goal is to obtain higher bioactive extracts with potential biomedical applications.

The integration of this extract into PLA filaments through $scCO_2$ impregnation achieved promising levels of antioxidant functionality (6.81% inhibition at 100 bar and 35 °C), without compromising the integrity of the polymer matrix.

The use of *P. alliacea*, a species with a broad ethnopharmacological background and which is underused in industry, presents a strategic chance to create high-value functional polymers with biomedical uses, like antimicrobial packaging, wound dressings, or implantable devices with controlled drug release. The combination of green extraction and supercritical impregnation technologies provides a sustainable and adaptable approach for producing bioactive materials that align with circular bioeconomy principles and clean-label innovation. Future efforts should focus on scaling up the process, ensuring regulatory compliance, and verifying the long-term stability of the impregnated systems to support their integration into industrial biotechnological and biomedical markets.

**Author Contributions:** Conceptualization, L.C.-C. and C.M.-S.; methodology, G.A.B.-B. and L.C.-C.; formal analysis, L.C.-C., A.A.D.-R., and C.C.-B.; investigation, G.A.B.-B., C.C.-B., and L.C.-C.; writing—

review and edition, L.C.-C. and C.C.-B.; supervision, C.M.-S. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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