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Bioactive Properties of *Pentacalia vaccinioides* (Kunth) Cuatrec. (Asteraceae) Essential Oils: Evaluation of Antimicrobial and Antioxidant Activities

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Abstract: Essential oils (EOs) have unique properties, such as antibacterial, antioxidant, and antiviral activities, which are beneficial in various industries, including cosmetics, food, and pharmaceuticals. In this study, the antioxidant and antimicrobial activities of *Pentacalia vaccinioides* EOs obtained from leaves and flowers (fresh and dried plant material) were evaluated using hydrodistillation (HD), steam distillation (SD), simultaneous distillation–extraction (SDE), and solid-phase microextraction (SPME) techniques. Antimicrobial activity (minimum inhibitory concentration, MIC) and antioxidant capacity (half-maximal inhibitory concentration, IC_{50}) were determined. The identification and quantification of the compounds present in the EOs were conducted by gas chromatography coupled to mass spectrometry (GC-MS). The main secondary metabolites identified in most samples obtained by different extraction techniques included phenol (~18%), 1S-α-pinene (~15%), β-phellandrene (~13%), β-pinene (~12%), 4-terpineol (~10%), γ-terpinene (~10%), trans-nerolidol (~8%), limonene (~8%), and β-thujene (~6%). EOs obtained by HD, SD, and SDE exhibited antioxidant activity, with IC_{50} values between 621.7 and 696.6 µg/mL. Additionally, the EOs demonstrated bactericidal activity against *Bacillus subtilis* and *Staphylococcus aureus*, with MIC values of 5.0 and 45 µg/mL, respectively. *Escherichia coli* and *Pseudomonas aeruginosa* did not show antimicrobial susceptibility to EOs. This study constitutes the first evaluation of *Pentacalia vaccinioides* EOs, demonstrating their bioactive potential and the relevance of the extraction method. The findings highlight this species as a promising source of natural compounds for therapeutic and preservative applications, depending on the type of plant material and extraction technique used. Future research should investigate how microclimatic conditions and plant development affect the chemical composition and elucidate the molecular mechanisms behind the observed bioactivities to better understand their cellular actions. Furthermore, the evaluation of the applications of EOs and hydrolates in the pharmaceutical and food industries, along with the exploration of the bioactive potential of extraction-derived hydrolates, offers a promising avenue to maximize plant utility.

Keywords: *Pentacalia vaccinioides*; antioxidant; antimicrobial; essential oil; volatile compounds; hydrodistillation; steam distillation; simultaneous distillation–extraction; solid-phase microextraction; Colombia

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1. Introduction

Essential oils (EOs) are volatile compounds present in various plant organs including flowers, leaves, bark, roots, and fruits. Their composition is complex, including terpenes, terpenoids, alcohols, phenols, aldehydes, ketones, and esters, among others, and varies according to the plant species, the part used, the extraction method, and the environmental conditions where the plant grows [1,2]. EOs exhibit a range of biological activities, including insecticidal, antioxidant, and antibacterial properties, in addition to carminative, anti-inflammatory, antispasmodic, and analgesic effects [3–5]. EOs have diverse applications in the pharmaceutical, food, cosmetic, and fragrance industries, among others [6,7]. Furthermore, EOs used by these industries are recognized for their safety and health benefits by regulatory bodies such as the Food and Drug Administration (FDA) and the International Fragrance Association (IFRA) [8,9].

In recent years, there has been growing interest in the use of EOs as antioxidants and antimicrobials. Antioxidants are substances that help protect cells or molecules from free radical damage, and antimicrobials are substances that kill or inhibit the growth of microorganisms such as bacteria, fungi, and viruses. EOs, either in combination or alone, show antibacterial, antifungal, antiviral, and antioxidant activities. The effectiveness of these oils increases when combined, taking advantage of synergistic and additive effects [10,11].

In Colombia, EOs extracted from endemic, native, and foreign plants have been used for medicinal, insecticidal, condimentary, culinary, and cosmetic purposes, among others [12–16]. This diversity of applications reflects the valuable potential of the country's plant wealth, where biodiversity not only sustains traditional practices but also drives the development of innovative products with added value [17]. For this reason, the search for new sources of essential oils is not only of national interest, but also a strategic priority for sectors such as health, sustainable agriculture, food industry, and cosmetics [18].

Among the plant species with potential biological activity and developing applications is the genus Pentacalia, which has been the subject of research by our group for the last 30 years. This genus has outstanding ethnobotanical uses, especially in the disinfection and healing of wounds that are difficult to heal. In the departments of Boyacá and Cundinamarca, farmers have traditionally used these plants to treat wounds, combat syphilis, cure persistent pimples and boils, relieve sore throats, and treat ulcers [19].

The species *Pentacalia vaccinioides* is known by several common names, such as "Chiquilla menuda" and "Hierba de páramo" in Cauca, "Tangue" and "Chitón" in Santander, and "Romerillo rusio" and "Panque" in the Nevado del Cocuy, in the departments of Boyacá, Cundinamarca, and Santander [20]. This plant, endemic to the moorlands of Colombia and Venezuela, has not been previously studied in either pharmacognosy or phytochemistry.

To the best of our knowledge, EOs of Pentacalia species have not been studied. Therefore, *Pentacalia vaccinioides* represents a plant species with significant unexplored potential in terms of its chemical composition and bioactive properties.

The objective of this study was to characterize the chemical profile of the EOs of *Pentacalia vaccinioides* obtained by hydrodistillation (HD), simultaneous distillation–extraction (SDE), steam distillation (SD), and solid-phase microextraction (SPME) methods, as well as to evaluate their antioxidant and antimicrobial activity. It is expected that the results obtained will contribute to the scientific knowledge about this plant species and may be useful in the development of pharmaceutical, cosmetic, and agroindustrial applications based on its bioactive properties.

2. Materials and Methods

During the performance of the experiments, the laboratory's health and safety procedures were complied with, following the national and institutional regulations in force. In addition, the necessary precautions were taken to minimize the risks associated with the materials and substances used.

2.1. Reagents and Equipment

The following Sigma-Aldrich reagents and standards were used: C_7–C_{30} Saturated Alkanes Standard (1000 µg/mL each component in hexane, product number 49451-U), n-Tetradecane GC (≥99.5%, product number 87140) olefin-free, 6-hydroxy-2,5,78-tetramethylchroman-2-carboxylic acid (Trolox, product number 238813), 2,2-Azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) (ABTS, product number A9941), 2,6-Di-tert-butyl-4-methylphenol (BHT, >99.0%, product number 34750), L-Ascorbic acid (Vit.C, >99.0%, product number A5960), (±)-α-Tocopherol (Vit.E, 95.5%, product number 258024), sodium sulfate (Na_2SO_4, ≥99.0%, product number 238597), sodium chloride (NaCl, ≥99.0%, product number S9888), ethanol (EtOH, ≥99.5%, product number 459844), dimethyl sulfoxide (DMSO, ≥99.7%, product number 34869), and dicloromethane (CH_2Cl_2, ≥99.8%, product number 270997). The following extraction equipment was used: Clevenger-type hydrodistillation (HD, Labbox, reference 1531), steam distillation (SD, Kasalab, reference TE-2761), simultaneous distillation–extraction (SDE, Likens–Nickerson apparatus, Labxsci, reference Sku:La), solid-phase microextraction (SPME, Supelco Inc., Fiber Holder 57330-U, Fiber Assembly 57300-U, Vial Headspace SU860098, Magnetic Screw Cap SU860103, Septa PTFE/white silicone 27514) using a fused silica fiber of poly(dimethylsiloxane) (PDMS, 100 µm, 57300-U), rotary evaporator (Büchi, R-100), refractometer (Atago RX-i), and UV–Vis spectrophotometer (FluoStar Omega microplate reader, BMG LABTECH GmbH, Ortenberg, Baden-Württemberg, Germany).

2.2. Collection and Preparation of Plant Material

The plant material (leaves and flowers) was collected in the village San Francisco, municipality of Choachí (department of Cundinamarca, Colombia), between 3000 and 3500 m above sea level. The material was selected taking into account that it should be free of fungi, physiologically intact, free of soil and dust, then dried at room temperature under shade (12 °C) for 15 days. The fresh and dried leaves were crushed to obtain a greater contact surface with the solvent in the different extractive systems (Sharapin et al. 2000). The taxonomic identification was carried out by Botanist Carlos Parra-O. from the Colombian National Herbarium (COL), voucher COL 570482—*Pentacalia vaccinioides* (Kunth) Cuatrec. This research has the authorization for access to genetic resources and their derived products, as well as for the collection of specimens, granted within the framework of the research project entitled "Phytochemical analysis of some plant species with potential antimicrobial, antioxidant, cytotoxic and remineralization activity, among others". The authorization was granted through the Contract for Access to Genetic Resources and their Derived Products No. 347 of 2022, issued by the Ministry of Environment and Sustainable Development of the Republic of Colombia (MinAmbiente, Colombia).

2.3. Obtaining the Essential Oil and Its Physicochemical Properties

Table 1 shows the notation of the extraction techniques, and the different experimental parameters used in each process. Fresh and dried leaves and flowers of *Pentacalia vaccinioides* were used to obtain the EOs. The extraction time for each process was 3 h except for the SPME process, which was 30 min. The obtained EOs were dried over anhydrous

sodium sulfate and stored in amber-colored bottles at 2 °C. Each extractive process and physicochemical test was performed in triplicate.

Table 1. Experimental parameters used in different extraction processes *.

No	Method	Acronym	Mass (g)	Extraction Time (h)	Solvent
1	Hydrodistillation dry leaf	HD-DL	150	3	1000 mL H_2O
2	Hydrodistillation wet leaf	HD-WL	250	3	1000 mL H_2O
3	Steam distillation dry leaf	SD-DL	150	3	1000 mL H_2O
4	Steam distillation wet leaf	SD-WL	250	3	1000 mL H_2O
5	Simultaneous Distillation and Extraction flowers	SDE-WF	250	3	100 mL CH_2Cl_2, 1000 mL H_2O
6	Simultaneous Distillation and Extraction dry leaf	SDE-DL	150	3	100 mL CH_2Cl_2, 1000 mL H_2O
7	Simultaneous Distillation and Extraction wet leaf	SDE-WL	250	3	100 mL CH_2Cl_2, 1000 mL H_2O
8	Solid-Phase Microextraction flowers	SPME-WF	10	0.5	25 mL H_2O
9	Solid-Phase Microextraction dry leaf	SPME-DL	10	0.5	25 mL H_2O
10	Solid-Phase Microextraction wet leaf	SPME-WL	10	0.5	25 mL H_2O

* Time selection was based on preliminary experiments and previous studies. Each extraction process was performed 3 times.

2.3.1. Hydrodistillation

The extraction of EOs was performed using a Clevenger-type hydrodistiller. The sample was deposited in a round-bottomed flask, and then 1000 mL of deionized water containing 50 g of NaCl was added. The extraction was carried out for 3 h [21,22].

2.3.2. Steam Distillation

EOs were obtained by steam distillation using a Clevenger-type apparatus. Fresh and dried leaves, separately, were placed in the extraction system and steam was generated from a volume of 1000 mL of deionized water. The extraction was carried out for 3 h [21–23].

2.3.3. Simultaneous Distillation–Extraction

The homogenized sample with 1000 mL of distilled water was placed in a 2000 mL round bottom flask. Then, 100 mL of CH_2Cl_2 was placed in a 250 mL round-bottom flask. These two flasks were coupled to a Likens–Nickerson apparatus. The solvent and sample mixtures were heated to 60 °C and boiling temperature, respectively. The temperature conditions were maintained for 3 h. After cooling to room temperature, the dichloromethane extract was collected and dried with anhydrous sodium sulfate. The extract was then concentrated to 1.0 mL using a nitrogen flow evaporator/concentrator [21,22,24].

2.3.4. Headspace Solid-Phase Microextraction

The headspace mode solid-phase microextraction (HS-SPME) technique was carried out using a poly(dimethylsiloxane) (PDMS) fiber with a thickness of 100 µm, purchased from Supelco Inc. (Bellefonte, PA, USA). A total of 10.0 ± 0.1 g of sample was deposited into 50 mL vials along with 25 mL of deionized water (containing 10% NaCl). Each vial was heated at 60 °C for 10 min to reach thermal pre-equilibrium. Subsequently, the fiber was exposed to the headspace for 30 min, after which it was transferred to the GC injection port for 5 min [25–27]. These experimental conditions are based on preliminary and previous studies performed by our research group.

2.4. Determination of Physical Properties

The yield of the extractions was calculated as the ratio between the mass of EOs obtained and the dry or wet sample mass. The refractive index was measured using an ABBE Atago refractometer; 2 drops of the EOs were placed on the prism of the refractometer, and the reading was taken at 20 °C. For the solubility of EOs in ethanol, 10 µL of EOs was added to 100 µL of ethanol (70%, *v*/*v*), and the mixture was homogenized in a vortex for 5 min at 20 rpm. The density determination was performed taking into account the mass contained in a volume of 10 µL of the EOs. The methodology with a pycnometer was not used since the yield obtained from the oils was less than 1.0 mL.

2.5. Gas Chromatography–Mass Spectrometry (GC/MS)

Briefly, 10 mL of the EOs plus 0.2 mL of the internal standard (n-Tetradecane) was taken, and CH_2Cl_2 was added to a final volume of 1.0 mL. One microliter (1.0 µL) of this solution was analyzed by GC/MS. The analyses were performed on an Agilent 6890 gas chromatograph equipped with an Agilent 5975B VL mass selective detector (electron impact ionization, 70 eV), (Wilmington, DE, USA), a split/splitless injector (1:50 split ratio), and Enhanced ChemStation MSD D.03.00.52 data system with Wiley and Nist spectral libraries. Two capillary columns were used: Agilent HP-5MS (5%-phenyl-poly(methylsiloxane), 60 m × 0.25 mm i.d, 0.25 µm film thickness), and Agilent HP-Innowax (100% cross-linked poly(ethylenglycol), 60 m × 0.25 mm i.d, 0.25 µm film thickness) capillary columns. The oven temperature was programmed from 60 °C (2 min) to 250 °C (2 min) at 50 °C/min, then to 310 °C (2 min) at 20 °C/min and a post-run to 320 °C (1 min). The temperatures of the injection port, ionization chamber, and transfer line were set at 300, 185, and 285 °C, respectively. Helium (99.999%) was used as a carrier gas, with 85 kPa column head pressure and linear velocity at a constant flow rate (1.0 mL/min). Mass spectra, total ionic currents (TIC), and extracted ion (EIC) were obtained with a quadrupole analyzer, by means of automatic radiofrequency scanning (full scan) in the mass range of m/z 30–500 (2.2 spectra/s). The tentative identification criteria were based on the analysis of mass spectra obtained by GC-MS and the linear retention indices in apolar and polar columns, calculated based on the homologous series of n-alkanes C_7–C_{30} (Sigma-Aldrich, Milwaukee, WI, USA) and compared with those from different mass spectral databases (Wiley 7n.1 and Nist 05a.L) and scientific literature data. Linear retention indices (LRI) were calculated using the following formula: LRI = 100n + 100·[(tRx − tRn)/(tRN − tRn)], where "n" is the number of carbon atoms in the n-paraffin eluting before the compound of interest (its retention time is tRx); tRn and tRN are retention times of n-paraffins with the numbers of carbon atoms n and N, respectively, eluting immediately before and after the analyte of interest [11,28–32].

2.6. Antioxidant Activity Determination

Volumes of 2, 5, 8, 8, 11, and 14 µL of the EOs were diluted to a final volume of 10 mL with EtOH: CH_2Cl_2 (9:1) from Sigma-Aldrich. Antioxidant activity assays were performed following the ABTS method proposed by Sequeda et al., 2021 [33].

2.7. Antimicrobial Activity Determination

Bacillus subtilis (ATCC 6638, CMPUJ 75), *Staphylococcus aureus* (ATCC 6538, CMPUJ 80), *Escherichia coli* (ATCC 8739, CMPUJ 76), and *Pseudomonas aeruginosa* (ATCC 9721, CMPUJ 55) bacterial strains obtained from Pontificia Universidad Javeriana Microorganism Collection. The antimicrobial activity assays were performed following the well and disc diffusion method proposed by Ortiz-Ardila et al., 2017, and Sequeda-Castañeda et al., 2019, with some modifications: 20 µL of the EOs at concentrations between 1215, 435, 135, 45, 15,

15, 5.0, and 1.67 µg/mL was dosed into each well and disc using CH_2Cl_2:DMSO (9:1) as solvent, where 20 µL of CH_2Cl_2, DSMO, and H_2O was used as negative controls, and 20 µL of Gentamicin (30 µg/µL) as a positive control [34,35].

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism under defined conditions. For this purpose, the agar diffusion method was used [36–39]. The concentrations of the EOs used ranged from 1.67 to 1215 µg/mL. A volume of 20 µL of the EOs solutions was deposited in 6 mm diameter wells on Mueller–Hinton (MH) agar plates, previously inoculated with each microorganism [34,35].

2.8. Statistical Analysis

Results are representative of six independent replicates and are expressed as mean ± SD. To determine which extraction method identified more compounds, the maximum likelihood method given by Hogg et al. (2019) for proportions was used. It corresponds to a success–failure method applied to the extraction of each component: a value of 1 is given if the extraction method was successful, and the compound is present in the EOs. On the other hand, a failure is considered when the compound is not found in the EOs, and in this case, a value of 0 is assigned. With the estimation of the proportions, the method that allows the extraction of the highest number of compounds, i.e., the highest number of successes (presence of compounds in the essential oil), is determined. According to the maximum likelihood method, this ratio is estimated with the following formula: p = (# of identified compounds)/(# of total compounds) [40].

3. Results

3.1. Obtaining EOs by Different Extraction Techniques and Physicochemical Parameters

EOs obtained by various extraction methods, including hydrodistillation (HD), steam distillation (SD), simultaneous distillation–extraction (SDE), and solid-phase microextraction (SPME), were analyzed. The parameters evaluated were yield (% m/m), density (ρ), refractive index (η), color, odor, and ethanol solubility (Table 2).

Table 2. Extraction yields and physicochemical parameters *.

No	Acronym	% (*m*/*m*)	ρ (g/mL)	η	Color	Smell
1	HD-DL	0.0034 ± 0.003	0.8666 ± 0.0030	1.611 ± 0.002	Yellow	It has penetrating, somewhat spicy, turpentine notes.
2	HD-WL	0.0016 ± 0.002				
3	SD-DL	0.0037 ± 0.004	0.8666 ± 0.0030	1.611 ± 0.002		
4	SD-WL	0.0019 ± 0.003				
5	SDE-WF	0.0012 ± 0.002				
6	SDE-DL	0.0345 ± 0.006	0.8667 ± 0.0030	1.612 ± 0.001		
7	SDE-WL	0.0156 ± 0.007				
8	SPME-WF					
9	SPME-WL					
10	SPME-WL					

* Average ± standard deviation, n = 3. NA = It was not possible to calculate this value due to the difference in weight of the fibril before and after the extraction process. HD-DL: Hydrodistillation dry leaf. HD-WL: Hydrodistillation wet leaf. SD-DL: Steam distillation dry leaf. SD-WL: Steam distillation wet leaf. SDE-WF: Simultaneous Distillation and Extraction flowers. SDE-DL: Simultaneous Distillation and Extraction dry leaf. SDE-WL: Simultaneous Distillation and Extraction wet leaf. SPME-WF: Solid-Phase Microextraction flowers. SPME-DL: Solid-Phase Microextraction dry leaf. SPME-WL: Solid-Phase Microextraction wet leaf.

3.2. Chemical Composition of the Essential Oil Obtained by Different Extraction Techniques

EOs of *Pentacalia vaccinioides* were extracted using various techniques: HD, SD, SDE, and SPME. The chemical composition was analyzed by GC-MS using apolar and polar columns to obtain linear retention indices (LRI) and their mass spectra for compound identification (Table 3, and Supplementary material Figure S1). According to the results obtained, between 18 and 33 compounds were identified in the EOs, depending on the extraction method and the plant part analyzed. The most abundant compounds (>1.0%) include phenol, 1S-α-pinene, β-phellandrene, β-pinene, 4-terpineol, γ-terpinene, trans-nerolidol, limonene, and β-thujene.

3.3. Antimicrobial Activity and Minimum Inhibitory Concentration

Antimicrobial activity was evaluated using the agar diffusion method using a well and disc, and the minimum inhibitory concentration was determined. The agar diffusion method with disc did not show positive results, while the well method was effective. Antimicrobial activity was determined in terms of the relative percentage of inhibition, and variability in biological activity was observed depending on the microorganism and the concentration of the oil, but not as a function of the plant organs from which the oils were obtained (Table 4a).

The EOs obtained by SD produced the highest antibacterial activity, with 90.7% inhibition of *Bacillus subtilis* at 1215 µg/mL, decreasing to 10.1% at 5.0 µg/mL. In contrast, the EOs obtained by HD and SDE showed lower effectiveness, especially at low concentrations. For example, in HD, the inhibition of *Bacillus subtilis* reached 28.5% at 1215 µg/mL and was non-existent at lower concentrations. These results suggest that the extraction technique directly influences the antimicrobial efficacy, probably due to the ability of each method to extract different bioactive compounds, such as monoterpenes and phenols, known for their antimicrobial properties. On the other hand, a concentration dependence was observed, with higher activity at higher concentrations, regardless of the extraction method. For example, in the case of *Staphylococcus aureus*, both in HD and in SD and SDE, inhibitions higher than 30% were observed only at high concentrations (1215 µg/mL and 405 µg/mL), indicating that the antibacterial effectiveness of EOs is dose-dependent.

The Minimum Inhibitory Concentration (Table 4b) of *Pentacalia vaccinioides* EOs shows a variation in antimicrobial efficacy according to the extraction method and the microorganism evaluated. In the case of *Bacillus subtilis* and *Staphylococcus aureus*, low MIC values are observed in the EOs obtained by SD, with a minimum effective concentration of 5.0 µg/mL for *Bacillus subtilis* and 45 µg/mL for *Staphylococcus aureus* in the case of SDE. However, both *Escherichia coli* and *Pseudomonas aeruginosa* showed resistance, with MIC values higher than 1215 µg/mL for all extraction techniques, indicating a low effectiveness of EOs against these Gram-negative bacteria.

3.4. Antioxidant Activity

The antioxidant activity of the EOs obtained from *Pentacalia vaccinioides* was evaluated by different extraction techniques using the ABTS method, which measures the ability of the compounds to neutralize free radicals. The 50% inhibition concentration (IC_{50}) reflects the concentration required to reduce 50% of the ABTS radicals, indicating the antioxidant efficacy of each essential oil. The results obtained are presented in Table 5, where it can be seen that the IC_{50} values vary according to the extraction method and the type of plant organ used.

Table 3. Relative amount (>1.0%) and identification of the main components of *Pentacalia vaccinioides* EOs obtained by different techniques [a].

No.	Compound	Type	LRI		HD		SD		SDE		SPME			
					Leaf		Leaf		Leaf		Flower	Leaf		Flower
			Apola	Polar	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Fresh	Dry	Fresh
1	2-Butanone, 3-hydroxy-	*	775	-	1.6	2.9	3.7	-	3.2	3.7	3.3	-	-	-
2	1-Butanol, 3-methyl-	*	780	-	-	-	4.8	-	1.5	1.8	1.0	-	-	-
3	1-Octene	*	786	-	-	-	-	-	-	-	-	1.4	1.5	1.5
4	Hexanal	*	802	815	1.8	5.5	1.0	1.3	1.4	2.5	2.7	-	-	-
5	Furfural	*	831	1078	-	-	1.1	1.1	-	-	-	-	-	-
6	3-Hexen-1-ol, (Z)-	*	852	-	1.6	1.7	3.2	3.4	1.7	2.0	2.2	-	-	-
7	2-Hexenal, (E)-	*	851	1249	2.3	3.4	2.0	1.3	1.2	1.2	1.4	-	-	-
8	1-Hexanol	*	863	-	1.3	1.5	1.5	1.6	2.3	2.7	3.0	3.0	4.3	4.9
9	Phenol	**	883	1537	11.4	9.4	19.2	18.0	2.9	3.7	1.0	3.1	7.1	3.1
10	Cyclopropane, 1-methyl-2-pentyl-	*	890	1612	1.2	1.4	1.4	1.5	1.8	1.6	2.0	2.3	2.1	2.7
11	Propanal, 3-(methylthio)-	*	907	-	-	-	1.3	1.4	-	-	-	-	-	-
12	2(5H)-Furanone	*	915	1060	3.0	1.7	6.0	5.3	-	-	-	-	-	-
13	Bicyclo [3.1.0]hexane, 4-methyl-1-(1- methylethyl)-, didehydro deriv	*	927	1175	1.8	1.2	-	-	3.1	1.7	3.2	3.7	1.6	4.2
14	1S-.alpha.-Pinene	M	934	1245	7.4	3.8	2.0	2.6	14.6	13.0	14.4	22.8	24.3	22.3
15	Cyclohexanone, 4-methylidene-	*	949	1386	4.4	2.6	3.1	2.3	1.2	1.6	1.8	-	-	-
16	Benzaldehyde	*	961	-	1.8	1.6	1.9	1.1	1.8	1.7	1.8	2.1	2.5	3.1
17	beta.-Phellandrene	M	974	1042	6.6	4.7	1.4	2.6	11.3	9.3	12.5	7.9	8.8	8.3
18	beta.-Pinene	M	978	1683	5.3	4.2	1.3	2.1	10.9	10.1	11.9	7.4	5.8	8.6
19	beta.-Tujene	M	991	1808	3.8	4.2	1.0	1.9	4.3	4.3	5.1	5.7	2.6	5.9
20	alpha.-Phellandrene	M	1006	1069	2.9	2.3	1.8	1.5	3.0	2.4	3.1	3.9	2.1	3.3
21	o-Cymene	M	1025	-	1.2	2.1	1.5	2.1	1.9	2.3	3.0	3.2	3.2	4.0
22	Limonene	M	1029	1323	6.2	6.3	3.2	3.5	7.9	5.4	6.2	8.3	8.1	8.4
23	2,4-Heptadienal, (E,E)-	*	-	-	1.0	1.3	-	-	-	-	-	-	-	-
24	Undecane	*	1045	1484	1.4	1.5	1.9	1.0	-	-	-	-	-	-
25	Gamma Terpinene	M	1059	1646	1.7	2.0	-	1.3	1.7	1.4	1.8	9.9	9.0	8.4
26	1-Octanol	*	1069	1760	1.5	1.6	-	-	-	-	-	-	-	-
27	2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, Cis	*	1073	-	-	-	-	1.1	-	-	-	-	-	-
28	Terpinolene	M	1089	1982	1.5	1.3	2.2	1.1	1.2	1.2	1.0	2.1	1.1	1.2
29	Linalool	MO	1100	2095	2.6	2.0	2.8	2.2	1.2	1.9	2.2	2.0	2.4	3.0
30	Nonanal	*	1104	1048	2.4	2.2	1.2	1.3	1.4	2.0	-	-	-	-
31	Phenylethyl Alcohol	*	1114	-	-	-	1.6	1.6	-	-	-	-	-	-
32	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	MO	1123	-	1.2	1.4	4.7	6.2	-	-	-	-	-	-
33	4-Terpineol	MO	1180	2049	9.4	6.9	7.4	8.0	3.8	2.5	3.0	6.2	4.0	4.9
34	Alpha Terpineol	MO	1192	2200	-	-	1.5	1.7	2.0	2.5	2.7	-	-	-
35	Di-epi-.alpha.-cedrene-(I)	*	1481	2614	-	-	1.7	1.1	-	-	-	-	-	-
36	1-Pentadecene	*	1491	2765	-	-	1.0	1.2	-	-	-	-	-	-
37	2-Fluorobenzyl alcohol	*	1532	-	3.3	1.8	2.3	3.0	2.3	2.7	2.9	-	-	-
38	Trans-Nerolidol	SO	1539	2560	7.2	8.0	6.0	7.1	5.1	5.4	5.5	1.0	1.1	1.1
39	4-(2,3,4,6-Tetramethylphenyl)-3-buten-2- one	*	1653	-	-	-	1.9	2.0	-	-	-	-	-	-

Table 3. *Cont.*

No.	Compound	Type	LRI		HD		SD		SDE		SPME			
					Leaf		Leaf		Leaf		Flower	Leaf		Flower
			Apola	Polar	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Fresh	Dry	Fresh
Total compounds					**29**	**29**	**33**	**33**	**26**	**26**	**25**	**18**	**18**	**18**
Total identified (%)					98.8	90.5	98.4	94.5	94.6	90.5	98.6	96.1	91.5	98.7
Compound family					**Relative quantity (%)**									
Monoterpene hydrocarbons (M)					36.6	31.0	14.4	18.7	56.7	49.3	59.0	71.1	64.9	70.1
Oxygenated monoterpenes (MO)					13.2	10.3	16.3	18.1	7.1	6.9	7.9	8.2	6.4	7.9
Sesquiterpene hydrocarbons (S)					-	-	-	-	-	-	-	-	-	-
Oxygenated sesquiterpenes (SO)					7.2	8.0	6.0	7.1	5.1	5.4	5.5	1.0	1.1	1.1
Phenol **					11.4	9.4	19.2	18.0	2.9	3.7	1.0	3.1	7.1	3.1
Other compounds *					30.4	31.8	42.5	32.6	22.8	25.2	25.3	12.6	12.0	16.4

[a] Identification made by (1) Linear retention indices determined experimentally and compared with databases. (2) Experimental mass spectra (EI, 70 eV), fragmentation pattern analysis, and comparison with database mass spectra (Nist 05a.L and Wiley 7n.1). Apolar: Agilent HP-5MS column (5%-phenyl-poly(methylsiloxane). Polar: Agilent HP-Innowax column (100% cross-linked poly(ethylenglycol). EOs were obtained using hydrodistillation (HD), steam distillation (SD), simultaneous distillation–extraction (SDE), and solid-phase microextraction (SPME). Other compounds *. Phenol **.

Table 4. (a) Antimicrobial from *Pentacalia vacciniodes* essential oils (μg/mL) obtained by different extraction techniques. Agar diffusion method. (b) The Minimum Inhibitory Concentration (μg/mL) (*).

(a) Relative Percentage of Inhibition (RI, %)							
Microorganism	**HD-DL, HD-WL-μg/mL**						
	1215	**405**	**135**	**45**	**15**	**5.0**	**1.67**
B. subtilis	28.5 ± 4.4	21.4 ± 2.2	-	-	-	-	-
S. aureus	34.0 ± 2.2	26.2 ± 2.5	17.5 ± 2.7	-	-	-	-
E. coli	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-
Microorganism	**SD-DL, SD-WL-μg/mL**						
	1215	**405**	**135**	**45**	**15**	**5.0**	**1.67**
B. subtilis	90.7 ± 3.8	75.2 ± 5.4	59.0 ± 7.7	37.3 ± 5.4	20.9 ± 2.1	10.1 ± 1.6	-
S. aureus	35.7 ± 4.1	24.5 ± 3.5	15.3 ± 2.8	-	-	-	-
E. coli	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-
Microorganism	**SDE-WF, SDE-DL, SDE-WL-μg/mL**						
	1215	**405**	**135**	**45**	**15**	**5.0**	**1.67**
B. subtilis	73.7 ± 5.2	69.9 ± 7.6	53.5 ± 6.6	37.1 ± 5.4	-	-	-
S. aureus	49.0 ± 2.4	39.8 ± 3.7	33.6 ± 2.6	32.7 ± 2.1	-	-	-
E. coli	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-
(b). The Minimum Inhibitory Concentration (μg/mL)							
Microorganism	**HD-DL**	**HD-WL**	**SD-DL**	**SD-WL**	**SDE-WF**	**SDE-DL**	**SDE-WL**
B. subtilis	405	405	5.0	5.0	45	45	45
S. aureus	135	135	135	135	45	45	45
E. coli	>1215	>1215	>1215	>1215	>1215	>1215	>1215
P. aeruginosa	>1215	>1215	>1215	>1215	>1215	>1215	>1215

(*) HD-DL: Hydrodistillation dry leaf. HD-WL: Hydrodistillation wet leaf. SD-DL: Steam distillation dry leaf. SD-WL: Steam distillation wet leaf. SDE-WF: Simultaneous Distillation and Extraction flowers. SDE-DL: Simultaneous Distillation and Extraction dry leaf. SDE-WL: Simultaneous Distillation and Extraction wet leaf. (a) When evaluating the antibacterial activity of EOs obtained from different organs of *Pentacalia vaccinioides* (leaves and flowers), no significant variation was observed depending on the part of the plant used. Antimicrobial activity was only observed when a well diffusion method was used. Relative Percentage of Inhibition: Average ± standard deviation, n = 6 determinations. Positive control: Gentamicin (30 μg/mL). Negative control: dichloromethane, dimethylsufoxide and water, 20 μL of each in well. (b) The Minimum Inhibitory Concentration Bactericidal: Average ± standard deviation, n = 6 determinations.

Table 5. Antioxidant activity from *Pentacalia vaccinioides* essential oils (μg/mL) obtained by different extraction techniques—ABTS method *.

Inhibition Concentration—IC_{50} (μg/mL)						
HD-DL	**HD-WL**	**SD-DL**	**SD-WL**	**SDE-WF**	**SDE-DL**	**SDE-WL**
633.82 ± 20.98	621.62 ± 23.55	668.83 ± 21.28	658.24 ± 20.42	673.39 ± 26.21	696.59 ± 25.50	682.54 ± 30.03

* Average ± standard deviation, *n* = 6. Positive control: Trolox (154.08 ± 3.91 μg/mL), BHT (248.47 ± 6.8 μg/mL), Vitamin E (160.45 ± 5.7 μg/mL), Vitamin C (128.74 ± 4.21 μg/mL). HD-DL: Hydrodistillation dry leaf. HD-WL: Hydrodistillation wet leaf. SD-DL: Steam distillation dry leaf. SD-WL: Steam distillation wet leaf. SDE-WF: Simultaneous Distillation and Extraction flowers. SDE-DL: Simultaneous Distillation–Extraction dry leaf. SDE-WL: Simultaneous Distillation and Extraction wet leaf.

HD of both dried leaves (HD-DL) and wet leaves (HD-WL) showed outstanding results in terms of antioxidant activity, with HD-WL being the most efficient, with an IC_{50} of 621.62 μg/mL. This value suggests that fresh leaves provide a higher concentration of antioxidant compounds, possibly because the technique better preserves bioactive compounds such as oxygenated terpenes and phenols. HD-DL, with an IC_{50} of 633.82 μg/mL, also showed good activity, although slightly lower. This could be due to the loss of some volatile compounds during leaf drying. HD seems to be a suitable method to maximize the extraction of antioxidant compounds. SD showed a similar trend to HD, although with slightly higher IC_{50} values, indicating lower antioxidant activity. SD-DL presented an IC_{50} of 668.83 μg/mL, while SD-WL was more efficient, with an IC_{50} of 658.24 μg/mL. The difference between fresh and dried leaves in this method suggests that the presence of moisture could help to preserve or extract antioxidant compounds more efficiently. However, compared to HD, SD is less efficient, which could be related to differences in heat transfer and volatilization of certain compounds. The technique, which combines

SDE, presented the highest IC_{50} values, indicating a lower efficiency in the extraction of antioxidant compounds. Both leaves and flowers showed similar results, with IC_{50} of 673.39 µg/mL for fresh flowers (SDE-WF) and 696.59 µg/mL for dried leaves (SDE-DL). Wet leaves (SDE-WL) also presented a relatively high IC_{50} of 682.54 µg/mL. These values indicate that, although this technique is useful for extracting EOs, it is not the most efficient for obtaining antioxidant compounds compared to the other methods. This could be due to the fact that the combination of SDE does not favor the preservation of antioxidant compounds as much. Grouping the results by technique, HD proved to be the most effective technique for extracting antioxidant compounds, especially when using fresh leaves.

SD is also effective, but less than hydrodistillation, while SDE showed the highest IC_{50} values, indicating a lower antioxidant capacity of the oils obtained by this method. These results highlight the importance of properly selecting the extraction technique according to the specific objective of the product, as different methods could affect the composition and efficacy of the EOs obtained.

4. Discussion

4.1. Performance and Physical Parameters

The yield of EOs, expressed as the mass percentage with respect to the original sample, presented low values in all extractions (Table 2). Yields ranged from 0.0012 to 0.0345%, with the highest value recorded for the SDE-DL method (0.0345 ± 0.006%). This behavior reflects the nature of EOs, which are obtained in small quantities due to their volatile character and high concentration of aromatic compounds. The SDE method produced the highest yields compared to HD and SD, being notably more efficient in the extraction of EOs from dry and wet leaves. On the other hand, the SPME method does not provide a sufficient amount of oil to calculate yield values. The density (ρ) of essential oils was relatively uniform in the samples. These data are consistent with the literature, as essential oils usually have lower densities than water due to their lipophilic nature and high proportion of terpenes, key components in these oils. The homogeneity in the density values suggests that, despite the different extraction methods used, the volumetric properties of the essential oils are similar.

The refractive index (η) is a key property that can be related to the composition and purity of the EOs, providing an indication of its content of terpenes and other volatile aromatic compounds. The similarity in η values indicates that the oils extracted by these methods have comparable optical characteristics [41]. The color in all samples presented a yellow hue. This color may be related to the presence of certain compounds such as carotenoids or oxygenated sesquiterpenes, which contribute shades to the essential oils [42]. The odor of the essential oil in the samples was described as penetrating, with pungent notes and reminiscent of turpentine. These olfactory characteristics are typical of essential oils with high contents of monoterpenes and sesquiterpenes, compounds responsible for the strong and spicy aromas usually found in oils obtained from leaves and resins [43].

All EOs samples evaluated were soluble in 70% (v/v) ethanol. Their solubility in ethanol, a common solvent in perfumes, cosmetics, and food, allows homogeneous mixtures without turbidity. Moreover, this property facilitates its use in analytical studies and in the preparation of therapeutic or industrial extracts, as ethanol is an efficient extraction medium for volatile compounds. It also suggests the presence of low molecular weight apolar compounds, such as monoterpenes and sesquiterpenes, which is useful in the characterization of the main components of samples [44–46].

In summary, the results obtained show that the SDE technique provides the highest yields of EOs, especially in dry and wet leaves. Physicochemical parameters, such as density and refractive index, were consistent in the samples, suggesting a similar chemical

composition among the extracted oils. Sensory analysis of odor and color reinforces the presence of characteristic volatile compounds, responsible for the penetrating aromas.

4.2. Analysis of the Main Bioactive Compounds and Their Industrial and Pharmaceutical Applications

The following is an analysis of the main compounds identified, emphasizing their bioactive properties and their industrial and pharmaceutical relevance. These compounds, commonly used in fragrances, cosmetics, and cleaning products, also exhibit antimicrobial, antioxidant, and anti-inflammatory activities, positioning them as promising candidates for natural therapies and preservation solutions.

Among these compounds, phenols stand out for their antioxidant and antimicrobial properties. Found in essential oils from plants such as oregano, thyme, and cloves, they are widely used in food preservation, cosmetics, and medicine. Despite their benefits, phenols require careful handling due to potential health risks [47–49]. Similarly, 1S-α-pinene, a monoterpene present in conifers and rosemary, is valued for its fresh aroma and antimicrobial, anti-inflammatory, and antioxidant properties. It also serves as a precursor in industrial synthesis and shows potential in respiratory treatments [50–52].

Another notable compound is β-phellandrene, a cyclic monoterpene found in eucalyptus and lavender, which contributes to fragrances and exhibits anticancer and anti-inflammatory properties, although its toxicity necessitates caution [53–55]. β-pinene, primarily in pines, plays a key role as a precursor for derivatives used in cosmetics and pharmaceuticals, particularly against antibiotic-resistant bacteria [51,52,56].

Furthermore, 4-terpineol, a major component of tea tree oil, is recognized for its antimicrobial efficacy, especially against resistant bacteria, making it invaluable in pharmaceuticals and cosmetics [57–59]. γ-terpinene, present in oregano and citrus, provides antioxidant and antimicrobial benefits, with applications in food, cosmetics and cleaning products [60–62].

Lastly, trans-Nerolidol, a sesquiterpene alcohol, is used in fragrances and skin care due to its anticancer and neuroprotective potential [63–65]. Limonene, abundant in citrus peels, is a versatile and eco-friendly solvent with antioxidant properties [66–69], while β-thujene, found in conifers, is useful in perfumes and disinfectants but requires further research to confirm its therapeutic effects [70–72].

4.3. Comparison of Extraction Techniques

The extraction techniques used had a significant influence on the composition and concentration of the compounds (Table 3). Significant differences were observed in the number of compounds identified and in the relative abundance of chemical groups. By HD, between 29 and 33 compounds were identified, with a predominant profile of monoterpene hydrocarbons (36.6% in fresh leaves, and 31.0% in dry leaves). This method showed high concentrations of compounds such as α-pinene (7.4% in fresh leaves) and phenol (11.4% in fresh leaves), highlighting the ability of HD to extract monoterpenes and phenols. Using SD (similar to HD), up to 33 compounds were identified, but with a higher concentration of phenols (19.2% in dry leaves). A decrease in the amount of monoterpene hydrocarbons (14.4%) was observed compared to HD, suggesting that steam may be less efficient in the extraction of these compounds in certain cases. SDE was the most efficient technique in the extraction of monoterpene hydrocarbons (56.7% in fresh leaves and 59.0% in flowers). It stands out for the high concentration of α-pinene (14.6% in fresh leaves), β-pinene (10.9% in fresh leaves), and β-Phellandrene (12.5% in fresh flowers) indicating that SDE is particularly effective in extracting volatile terpenes. However, it presented a lower amount of phenols (2.9% in fresh leaves). SPME showed a more specific profile, with a lower number of compounds identified (up to 18), and a high concentration of monoterpene

hydrocarbons (71.1% in fresh flowers). This method proved to be the most selective for volatile compounds such as α-pinene (22.8% in fresh leaves) and γ-Terpinene (9.9% in fresh leaves), but less efficient for extracting oxygenated compounds and sesquiterpenes.

The extraction techniques used to obtain essential oils have advantages and limitations that condition their use and efficiency. HD, a traditional and accessible method, allows the economical extraction of essential oils on a small scale. Additionally, the water used partially protects volatile compounds from overheating. However, it has low yields and can cause degradation of heat-sensitive compounds during prolonged distillations.

SD improves efficiency by avoiding direct contact of the material with water, thus better preserving the volatile compounds. This makes it suitable for industrial applications, although it requires more precise temperature control and more expensive equipment. SDE stands out for its high yields because it combines the use of solvents and distillation to minimize the loss of volatile compounds. However, this method can increase operating costs because of the use of solvents and specialized equipment.

Finally, SPME is ideal for trace analysis of volatile compounds, as it does not require solvents and allows for working with small samples. However, its main limitation is the small amount of oil obtained, which restricts its usefulness to analytical rather than productive purposes.

The distribution of chemical groups within the essential oil of *Pentacalia vaccinioides* provides key insights into its bioactive and therapeutic profile. The high proportion of monoterpene hydrocarbons, particularly in SPME and SDE techniques, highlights the importance of these compounds in the antimicrobial and antioxidant properties of the oil. Oxygenated monoterpenes, which play critical roles in anti-inflammatory and relaxant actions, present significant variations among extraction methods, suggesting that some techniques, such as HD and SD, are more efficient in extracting these compounds. On the other hand, the presence of oxygenated sesquiterpenes and phenols, although in smaller proportion, adds important therapeutic value, particularly due to their antiseptic and antioxidant properties. These results suggest that the choice of extraction method has a direct impact not only on the chemical composition, but also on the functionality and potential application of the essential oil in different medicinal and cosmetic contexts.

In addition to the influence of the extraction method on the composition and properties of EOs, biotic and abiotic factors also play a crucial role in determining their chemical profile. The chemical profile of EOs is determined by biotic and abiotic factors that affect plant metabolism. Variables such as harvest time, soil type, climatic conditions, and cropping system directly influence the synthesis and accumulation of specialized metabolites. These dynamics highlight the importance of considering ecological and agricultural management conditions to understand and optimize the composition of essential oils [73–80].

In summary, each extraction method has specific advantages and disadvantages that make it suitable for different objectives. If the objective is to obtain large quantities of EOs, SDE is the most efficient technique, although it is more complex and requires the use of solvents. On the other hand, if simplicity and low cost are sought, HD remains a viable option, although with lower yield. For analytical purposes, SPME is highly effective, although it does not produce enough material for large-scale studies. Finally, SD balances efficiency and protection of volatile compounds, being a preferred option in industrial applications. The extraction method and the plant part used are determining factors in the chemical composition of *Pentacalia vaccinioides* EOs. HD and SD provide a more balanced extraction of monoterpene and oxygenated compounds, while SDE is more efficient for the extraction of monoterpene hydrocarbons. SPME, on the other hand, specializes in the extraction of volatile compounds. These results provide valuable information for the

selection of the most suitable extraction method, depending on the desired chemical profile and the application of the essential oil in the cosmetic, pharmaceutical, or food industry.

4.4. Antimicrobial Activity

Evaluation of antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* provides a comprehensive understanding of the effectiveness of antimicrobial compounds due to the various biological characteristics of these microorganisms. Their clinical and industrial relevance further underscores their importance in such evaluations [81].

Bacillus subtilis, a Gram-positive model bacterium, is particularly useful for assessing the efficacy of antimicrobial agents against sporulating bacteria, which are important in environmental and food contamination [82]. Similarly, *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), represents a critical challenge in human infections, highlighting the need for effective solutions against resistant and systemic pathogens [83]. In contrast, Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* present unique obstacles due to their outer membrane structures. These barriers limit the penetration of antimicrobial compounds, making *Escherichia coli* a significant target in intestinal and urinary tract infections [84], while *Pseudomonas aeruginosa* is renowned for its resistance mechanisms and biofilm formation, which complicate treatment, particularly in hospital settings and immunosuppressed patients [85].

The insights gained from such evaluations extend beyond efficacy measurement; they are critical for the development of safe and effective antimicrobial agents with applications in the medical and industrial sectors. In this study, the well diffusion method demonstrated clear effectiveness in inhibiting bacterial growth, whereas the disk diffusion method did not exhibit detectable antimicrobial activity. This disparity may be attributed to the deeper penetration of active compounds in agar in the well diffusion method, compared to the limited diffusion and increased evaporation of compounds in the disk method [35,86].

Antimicrobial activity, quantified as the relative percentage of inhibition (Table 4), varied depending on the microorganism and the extraction technique. *Bacillus subtilis* showed sensitivity to EOs obtained by all methods, with the highest inhibitory activity observed using SD, followed by SDE and HD. Similarly, *Staphylococcus aureus* was inhibited, especially by oils extracted through SDE. However, *Escherichia coli* and *Pseudomonas aeruginosa* showed no susceptibility at the concentrations tested. This resistance in Gram-negative bacteria can be attributed to their outer membrane, which acts as a protective barrier against lipophilic compounds found in EOs [87]. Efflux pumps, such as the ACRAB-TOLC system in *Escherichia coli*, further reduce susceptibility by actively expelling antimicrobial agents [88]. Furthermore, the formation of *Pseudomonas aeruginosa* biofilms creates an additional layer of protection, significantly limiting the efficacy of EOs [89]. These findings reaffirm the structural differences between Gram-positive and Gram-negative bacteria as key determinants of the antimicrobial activity of EOs.

The growing interest in the antimicrobial properties of EOs reflects their potential as natural alternatives to synthetic additives in food, cosmetics, and pharmaceuticals, particularly in response to the increasing resistance of microorganisms to antibiotics. The antimicrobial activity of EOs is largely due to their bioactive constituents, such as monoterpenes, sesquiterpenes, phenols, and alcohols. These compounds exhibit various antimicrobial potencies, with phenols showing the highest activity, followed by aldehydes, ketones, alcohols, esters, and hydrocarbons [90,91]. Antimicrobial activity is recognized in EOs such as *Origanum vulgare* (oregano), *Cymbopogon citratus* (lemongrass), *Thymus vulgaris* (thyme), *Salvia officinalis* (sage), *Rosmarinus officinalis* (rosemary), *Syzygium aromaticum* (clove), *Coriandrum sativum* (coriander), *Allium sativum* (garlic), and *Allium cepa* (onion), among others, and in

their individual compounds such as carvacrol, thymol, citral, eugenol, 1–8 cineol, limonene, pinene, linalool, and other precursors [92,93]. For example, lemongrass EOs, rich in citral, citronellal, and geraniol, have demonstrated activity against *Escherichia coli*, *Bacilluis subtilis*, and *Staphylococcus aureus* [94,95]. Similarly, *Melaleuca alternifolia* EO (Tea tree), which contains terpinen-4-ol and linalool, is highly effective against various pathogens [96,97].

Interestingly, the antimicrobial efficacy of EOs often exceeds that of their isolated components, highlighting the role of synergistic interactions among their minor constituents [92]. For example, combinations of citral with vanillin, thymol, or carvacrol enhance inhibitory effects against *Zygosaccharomyces bailii* [98], while carvacrol and thymol demonstrate synergistic effects against other microorganisms [90,99–101]. Studies on compounds such as 1,8-cineole and camphor of *Osmitopsis asteriscoides* reveal stronger antimicrobial activity in combination than when tested independently [102].

Although the precise mechanisms of action of EOs are not fully elucidated, their primary target is proposed to be the bacterial cell membrane. Terpenoids and phenolic compounds disrupt membrane integrity and permeability, causing ion leakage, cytoplasmic content release, and eventual cell death [103,104]. Phenolic terpenoids, such as carvacrol and thymol, are particularly effective, leading to oxidative respiration inhibition and membrane swelling [105,106]. Compounds like cinnamaldehyde further disrupt cellular processes by inhibiting key enzymes and altering permeability [107]. These mechanisms, combined with factors such as pH, concentration, and microorganism type, dictate the antimicrobial effectiveness of EOs and their active compounds.

In conclusion, this study highlights the significant potential of EOs as antimicrobial agents, highlighting their varied efficacy in different bacterial strains and extraction techniques. The structural and compositional diversity of EOs underscores the need for continued research into their mechanisms of action, synergistic interactions, and practical applications in the food, cosmetic, and pharmaceutical industries.

4.5. Antioxidant Activity

Evaluation of the antioxidant activity of EOs is of great importance in sectors such as the food, cosmetic, pharmaceutical, and medical industries due to their ability to neutralize free radicals and prevent cell damage. These antioxidant properties are essential to mitigate oxidative stress, which is associated with premature aging and chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders [108,109].

EOs contain compounds such as phenols, terpenes, and flavonoids, which act as natural antioxidants capable of neutralizing free radicals and preventing cell damage. These properties are key in the prevention of cellular aging and degenerative diseases [110]. In the food industry, EOs are used to delay fat oxidation, prolonging the shelf life of products, while in cosmetics they stabilize formulas and protect active ingredients [111]. In the pharmaceutical field, their antioxidant properties position them as promising therapeutic agents for the treatment of diseases related to oxidative stress. Similarly, in natural medicine and cosmetics, antioxidant-rich EOs stand out as protecting the skin against UV radiation and pollution. Evaluation of its antioxidant activity is crucial to identify compounds with potential in various applications [112,113].

To evaluate the antioxidant activity of EOs, methods such as ABTS, DPPH, and FRAP, which measure their ability to neutralize free radicals or reduce metals, are used. Among them, the ABTS method is particularly useful for EOs because of its versatility in hydrophilic and lipophilic media, which better reflects the complexity of their compounds, both polar and apolar. This method is more sensitive, faster, and cheaper than others such as DPPH, making it an effective tool for evaluating the antioxidant activity of complex mixtures of terpenes, phenols, and flavonoids [33].

In our work, when comparing the antioxidant activity with the chemical composition of *Pentacalia vaccinioides*, a relationship was observed between the presence of certain groups of compounds and the antioxidant capacity of the EOs. The essential oils evaluated showed an antioxidant capacity measured by IC_{50}, which varied according to the extraction technique and the plant organs used. However, this behavior can be explained in part by the variation in chemical composition observed in these oils. Monoterpenes, such as α-pinene, β-pinene, and limonene, constituted the largest proportion of EOs in most extraction techniques, especially in SDE extraction. These compounds are known for their moderate antioxidant capacity [114]. EOs with a higher proportion of phenolic compounds and oxygenated terpenes, such as linalool and 4-terpineol, showed greater antioxidant activity. These compounds are potent antioxidants due to their ability to donate electrons and neutralize free radicals [115]. The presence of phenols, which was especially notable in the EOs obtained by HD, correlates with the lowest IC_{50} values. These results indicate that HD techniques are more effective in extracting compounds with high antioxidant capacity. Although in smaller amounts, oxygenated sesquiterpenes, such as trans-nerolidol, also play a role in the antioxidant activity of EOs. These compounds were found in higher concentrations in samples extracted by SD, where the antioxidant activity was moderate. The contribution of oxygenated sesquiterpenes could slightly improve antioxidant capacity, although their effect is less pronounced compared to oxygenated phenolic compounds and terpenes [116].

Research on EOs has also highlighted the contributions of individual compounds to their antioxidant activity. Compounds such as γ-terpinene and sabinene are known to delay lipid peroxidation and scavenge free radicals effectively, whereas α-pinene and limonene have shown lower antioxidant activity. In contrast, terpinene and terpinolene exhibit high hydrogen-donating capacity, and citronellal demonstrates strong protection against lipid peroxidation [90,117]. These findings indicate that minority components often play a crucial role in the total antioxidant activity of EOs. In addition, synergistic interactions between EO components have been reported. For example, terpinen-4-ol in *Melaleuca teretifolia* EOs showed enhanced antioxidant activity compared to oils dominated by methyleugenol or 1,8-cineole [90,118]. These synergies may significantly improve antioxidant efficacy beyond the sum of their individual components.

The growing interest in natural antioxidants, especially those derived from polyphenol-rich plant extracts, reflects their potential as safer and more sustainable alternatives to synthetic preservatives [119–121]. These natural compounds are highly valued in the cosmetic and food industries for their efficacy and natural origin. However, their successful integration into final products requires compliance with specific criteria, such as efficacy at low concentrations, compatibility with ingredients in formulations, and stability against pH variations and processing conditions. They must also maintain sensory acceptability, lacking undesirable colors, odors, or irritant properties. In addition, these antioxidants must be safe for consumers, supported by toxicological data, and economically viable for industrial applications [111,122–124].

In summary, the chemical composition of *Pentacalia vaccinioides* EOs directly influences their antioxidant capacity. EOs with a higher concentration of phenolic compounds and oxygenated terpenes, such as those obtained by HD, have higher antioxidant activity. On the other hand, oils rich in monoterpenes, extracted mainly by SDE, have a lower antioxidant capacity, which is reflected in their higher IC_{50}. This highlights the importance of selecting the appropriate extraction method to optimize the antioxidant activity of EOs according to their chemical composition.

4.6. Multifunctional Potential and Study Limitations of Pentacalia vaccinioides Essential Oils

This study highlights the potential of *Pentacalia vaccinioides* EOs as multifunctional agents with antioxidant and antimicrobial properties. These activities rely on bioactive compounds such as phenols and oxygenated terpenes, which neutralize free radicals and disrupt bacterial membranes, making EOs valuable resources for the food, cosmetic, and pharmaceutical industries. They help prevent oxidation and microbial growth in food, protect the skin from oxidative damage and infections in cosmetics, and offer promising solutions to antimicrobial resistance in the pharmaceutical field.

This work is the first to explore these properties in *Pentacalia vaccinioides*, using methodologies that include various extraction techniques and standardized methods, ensuring reliable results. However, a significant limitation is the absence of studies on the hydrolates generated during extraction, which could represent a valuable source of bioactive compounds. This unexplored area presents an opportunity for future research aimed at expanding knowledge and applications of this natural resource. Additionally, the study did not address the molecular mechanisms of action or evaluate a broad spectrum of microorganisms, aspects that could further enhance the understanding and utilization of EOs. Future investigations should address these gaps to fully harness the therapeutic and industrial potential of these essential oils.

5. Conclusions

This study represents the first work on *Pentacalia vaccinioides* in which its EOs are evaluated in terms of biological activities as antioxidants and antimicrobials. The results show that the extraction method and the state of the plant organs (fresh or dried) significantly influence the chemical composition and thus the biological effectiveness of the oils. HD-derived EOs, especially fresh leaves, showed higher antioxidant activity, reflected in lower IC_{50} values. This is due to the higher concentration of oxygenated phenols and terpenes in these samples. On the other hand, SD and SDE were less efficient in the extraction of antioxidant compounds, reflected in their higher IC_{50}. Regarding antimicrobial activity, HD and SD were more effective against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*. However, both *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-negative bacteria, showed significant resistance to the evaluated oils, which may be due to the structure of their cell membranes, which act as a protective barrier against antimicrobial compounds. Future research should investigate how microclimatic conditions and plant development affect the chemical composition and elucidate the molecular mechanisms behind the observed bioactivities to better understand their cellular actions. Furthermore, the evaluation of the applications of EOs and hydrolates in the pharmaceutical and food industries, along with the exploration of the bioactive potential of extraction-derived hydrolates, offers a promising avenue to maximize plant utility.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations12010009/s1, Figure S1: Chromatogram (TIC) of the essential oil of *Pentacalia vaccinioides* (Asteraceae). (A) HP-5MS column (60 m × 0.25 mm, 0.25 mm). (B) HP-Innowax column (60 m × 0.25 mm, 0.25 mm). Split 1:50.

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