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# Isolation of Geranyl Acetate and Chemical Analysis of the Essential Oil from *Melaleuca armillaris* (Sol. ex Gaertn.) Sm.

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**Abstract:** A method was developed for the isolation of geranyl acetate from the crude essential oil (EO) of *Melaleuca armillaris* (Sol. ex Gaertn.) Sm. leaves, and the purity of the isolated compound was analyzed by GC/MS spectral and NMR analysis and was found to have high purity (98.9%). In addition, the EO isolated presented 0.907 g/cm$^3$, 1.474 and $[\alpha]_D^{20} = -17.6$ of density, refraction index and optical rotation, respectively. The chemical composition of the EO obtained for steam distillation from *M. armillaris* was analyzed by gas chromatographic and spectroscopic techniques (GC/MS and GC/FID). Thirty-eight compounds were identified, representing 99.92% of the total EO analyzed on a DB-5 ms (5% phenylmethylpolysiloxane) capillary column. This analysis showed that the EO consisted mainly of oxygenated monoterpenes (77.01%), followed by monoterpene hydrocarbons (21.31%) and sesquiterpene hydrocarbons (1.31%). Furthermore, the essential oil of *M. armillaris* was rich in 1,8-cineol (67 ± 2%), followed by limonene (10 ± 1%), α-Terpineol (9 ± 1%) and α-Pinene (5 ± 1%). Finally, the results suggest that the geranyl acetate isolated with high purity from crude essential oil is recommended to be explored as a component in medicinal or industrial use.

**Keywords:** essential oil; geranyl acetate; *Melaleuca armillaris*; isolation; chemical composition



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## 1. Introduction

Medicinal plants have been a valuable source of therapeutic agents, and still, many of today's drugs are plant-derived natural products or their derivatives [1]. The medicinal properties of these plants can be attributed to the presence of high amounts of chemical compounds such as polysaccharides, polyphenols, hydrolyzable tannins and other secondary metabolites [2,3]. Plants containing essential oils (EOs) are a great resource for alternative therapy. They also have properties and act against different types of microorganisms, providing a variety of antimicrobial compounds produced by aromatic plants [4].

The *Myrtaceae* family, with more than 3500 species distributed worldwide, especially in the humid tropics of South America, Australia and tropical Asia [5], is one of the most diverse and widespread plant kingdoms and contains many plant species that produce EOs [6]. In general, EOs from species of this family contain substances from the terpene class, including the monoterpene α-pinene and the sesquiterpene β-caryophyllene. In addition, they contain phenylpropanoids such as eugenol and flavonoids [7]. The C-methylation of flavonoids is a typical feature of secondary metabolites found in *Myrtaceae*, and therefore it has been suggested that these natural products can be used as markers for chemotaxonomy [8]. Due to the diversity of this family, several compounds are used in folk medicine, mainly as antidiarrheal, antimicrobial, antioxidant, cleanser, antirheumatic, anti-inflammatory and for the reduction of blood cholesterol [9,10]. The family includes genera such as *Eucalyptus*, *Eugenia*, *Leptospermum*, *Melaleuca*, *Myrtus*, *Plinia*, *Psidium*, *Pseudocaryophyllus* and *Syzygium* [10]. The species of the *Melaleuca* genus include 290 species [11]. *Melaleuca armillaris* (Sol. ex Gaertn.) Sm. is one of the most cultivated and is commonly

known as bracelet myrtle [12]. It grows naturally in the Moluku Islands of Indonesia and is grown in many parts of the world, such as Tanzania, Madagascar, Sri Lanka, India, China, Indonesia, Malaysia, Brazil, the Malagasy Republic, Jamaica and Guinea [13]. In Ecuador, there are 83 species, of which 9 are endemic [14]. Its species are described in the Ecuadorian Andean region, especially in the provinces of southern Ecuador (Azuay and Loja) [15]. They grow as small trees or large shrubs between 2500 and 2900 m above sea level (Figure 1). These native species are mainly cultivated for their edible fruits and wood and are also used as medicinal plants for their biological properties [16]. Various studies and investigations of these species reported the chemical composition of EOs using gas chromatography coupled to mass spectrometry, which revealed the presence of 1,8-cineole (72.3%), limonene (7.8%) and $\alpha$-pinene (6%) as the most abundant components [17]. The chemotype 1,8-cineole has shown potential virucidal effects against *Herpes simplex* (virus type 1). It is used in traditional medicine for the treatment of acute and chronic bronchitis, sinusitis and respiratory infections [10,13,18]. This genus is known for its antibacterial, fungicidal, insecticidal, anticancer, antiviral and hypotensive properties. The other species of the *Melaleuca* genus are valuable sources of biologically active secondary metabolites for the treatment of bacterial and fungal infections [13].

**Figure 1.** *Melaleuca armillaris* used as an ornamental plant in Ecuador. Photo sourced from one of the authors (J.C.).

Geranyl acetate, an acyclic isoprenoid monoterpene biosynthesized by many aromatic plants, is considered a secondary metabolite and an important component of the EO of the species *M. armillaris* [19]. The medicinally active components of these EOs, like geranyl acetate, show antimicrobial and anticancer properties [20]. Among its most important properties, it has been shown to exhibit various biological activities, including anti-tumor, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, cardioprotective and neuroprotective effects [21,22]. Geranyl acetate is mainly used as a component of perfumes for creams and soaps and as a flavoring agent. Recognized as safe by the Food and Drug Administration (FDA), it is primarily used in rose, lavender and geranium fragrance formulations where a sweet fruit or citrus aroma is desired.

In Ecuador, the aromatic species *M. armillaris* has not been studied chemically, and to date, a potential industrial use is unknown. This study is the first report of the volatile components of EO from Ecuador, as well as the isolation of the secondary metabolite geranyl acetate. The result presented here will be useful for new studies aimed at the

isolation of pure molecules of essential oils from aromatic plants in Ecuador, with possible industrial or pharmaceutical use.

## 2. Materials and Methods

### *2.1. Collection of Plant Material*

*M. armillaris* leaves were collected from the Ecuadorian province of Loja, Saraguro, sector Acanana hill, at an altitude of 2840 m a.s.l. (latitude, 3°42′17.4″ S; longitude, 79°14′48.2″ W). A voucher specimen was prepared and deposited in the herbarium of the Universidad Técnica Particular de Loja (HUTPL). The plant collection was carried out under the authorization of the Ministerio de Ambiente, Agua y Transición Ecológica del Ecuador (MAE-DBN-2016-048).

### *2.2. Extraction of Essential Oil*

Essential oil from fresh leaves (4.5 kg) was extracted by steam distillation using a Clevenger glass apparatus (Figure 2) and the procedure described by Maldonado et al. (2023) [23], with a difference of 3 h in the process and at atmospheric pressure, c.a. A total of 1 g of anhydrous sodium sulfate was added to remove moisture, and the EO was stored at −7 °C for further experiments. Yields were expressed as the means and standard deviations of the three distillations and reported as $w/w$.

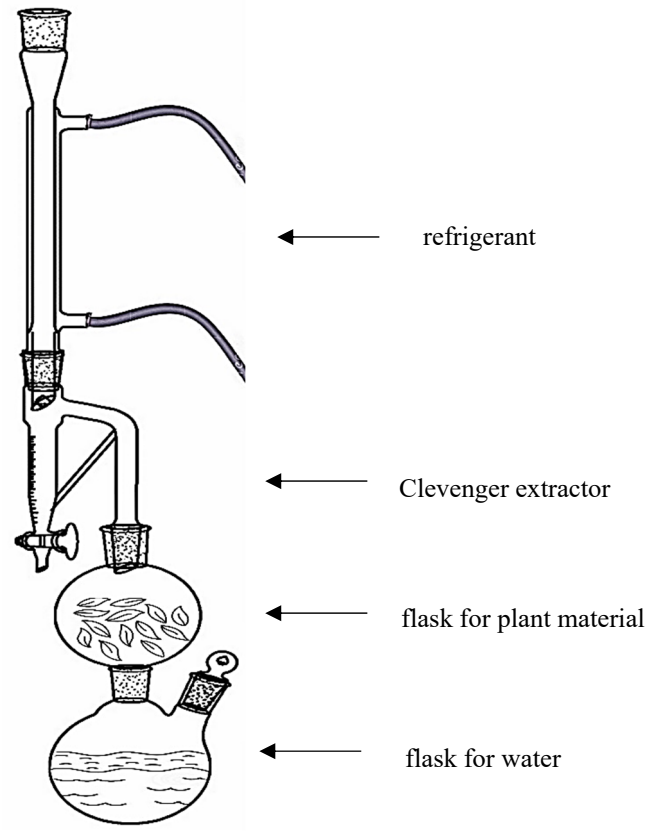


**Figure 2.** Distillation apparatus used to obtain the *Melaleuca armillaris* EO.

### *2.3. Physical Properties Determination*

The EO's density was determined according to the international standard AFNOR NF T 75-111 [24] (ISO 279:1998). The refraction index ($RD_{20}$) was measured according to the international standard AFNOR NF T 75-112 [25] (ISO 280:1998) by the Abbe's refractometer method. The specific optical rotation was determined with a Hanon P 810 automatic polarimeter according to the standard [26] ISO 592:1998. Each analysis was performed three times.

### 2.4. EO Composition Analysis and Sample Preparation

For each 10 µL of the EO sample, 990 µL of $CH_2Cl_2$ grade HPLC (Thermo Fisher Scientific, Waltham, MA, USA) was added. Gas chromatography/mass spectrometry (GC/MS) was used for identification, and gas chromatography with a flame ionization detector (GC/FID) was used for quantification.

For qualitative analysis, the samples were analyzed using a Thermo Fisher Scientific GC/MS (model Trace 3000 series) coupled to a mass spectrometry detector (ISQ 7000 series) operating at 70 eV with a scan rate of 2 scans/s and a mass range of $m/z$ 40–350 using a non-polar column DB-5ms (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) with helium ultra-pure as carrier gas (1 mL/min). The GC oven temperature was kept at 60 °C for 5 min, then increased by 2 °C/min to 100 °C, 3 °C/min to 150 °C, 5 °C/min to 200 °C and 15 °C/min to 250 °C for 5 min. The split ratio was adjusted to 40:1.

The quantitative analysis was carried out using the same Thermo Fisher system. The flame ionization detector (FID) temperature was 250 °C. The relative amounts of individual components were determined on the basis of their GC peak areas, without corrections for FID response factors. The analytical parameters were the same as for the GC/MS analysis. A calibration curve was established (0.6, 1.8, 4.3, 8.3, 16.8 and 34.3 mg isopropyl caproate in 10 mL cyclohexane) and n-nonane (7 mg) were used as calibration standards to generate internal standards and establish the calibration curve, respectively. The LOD (0.4 µg/mL) and LOQ (1.2 µg/mL) were measured, and a correlation coefficient of 0.995 was obtained from the calibration curve.

### 2.5. Identification of the EO Components

The identification of the compounds was achieved on the basis of retention time, compared with the corresponding GC-EIMS mass spectra library search NIST [27] and the calculated linear retention index (LRI) with data reported in the literature [28]. By comparing each LRI calculated in accordance with Van den Dool and Kratz [29], a range of ±20 units was considered reasonable for the comparison of the LRI. They were determined using a homologous series of n-alkanes C9–C25 (ChemService, West Chester, PA, USA).

### 2.6. Isolation and Identification of Geranyl Acetate

Fractionation and purification of geranyl acetates were carried out on *M. armillaris* EO (4.05 g), which was separated by column chromatography on silica gel 60 (0.25 mm; $GF_{254}$, Merck, Darmstadt, Germany) (40 g). Isocratic elution with a hexane–EtOAc (90:10) obtained a total of 50 fractions (C1–C50), approximately 8 mL each. The fractions were combined based on their TLC profiles, and the solvent was evaporated at reduced pressure to give 34 main fractions (CS2–CS36). The fraction CS-28 yielded 3 mg of geranyl acetate, which was detected on a TLC plate as a purple spot by the vanillin spraying reagent (0.5%). The compound was identified by $^{1}$H-NMR and $^{13}$C-NMR spectroscopy.

NMR experiments were performed on an NMR Varian spectrometer (500 MHz for $^{1}$H NMR and 125 MHz for $^{13}$C NMR). NMR spectra were recorded using deuterated chloroform CDCl3 (Sigma Aldrich, Saint Louis, MO, USA). A Fourier transform (FT) NMR spectrometer was equipped with a 5 mm multinuclear inverse probe head with a Z-shielded gradient. A 1D processing was performed with additional zero filling at 64k to perform a baseline correction on the FID prior to Fourier transformation.

## 3. Results

### 3.1. Physical Properties of EO

The essential oil from the fresh leaves of *M. armillaris* was obtained by steam distillation for 3 h, obtaining 44.5 mL of pure EO yielding an average of 1.0 ± 0.0003% ($w/w$). The physical properties at 20 °C, as the mean of three analyses, were a relative density of 0.907 ± 0.004 g/cm$^3$, a refractive index of 1.474 ± 0.004 g/cm$^3$ and an average specific optical rotation of $[\alpha]_D^{20} = -17.6$ ($CHCl_3$; c:11.4).

### 3.2. Chemical Composition

Thirty-eight components were identified in the *M. armillaris* EO using GC/MS and GC/FID techniques, representing 99.92% of the total EO analyzed on a non-polar DB-5ms capillary column. The analysis showed that the EO consisted mainly of oxygenated monoterpene (77.01%), followed by monoterpene hydrocarbons (21.31%) and sesquiterpene hydrocarbons (1.31%). Oxygenated sesquiterpene occurs in the oil as a minor component. The chemical composition expressed in percentage is presented in Table 1, and the major compounds were 1,8-cineole (66.51%), Limonene (10.34%), α-Terpineol (8.83%) and α-Pinene (5.23%); the gas chromatogram is shown in Figure 3.

**Table 1.** Chemical composition of *Melaleuca armillaris* EO through a 5% phenyl-methylpolysiloxane column.

| N° | Compound | LRI [a] | LRI [b] | % | ±SD |
|---|---|---|---|---|---|
| 1 | α-Thujene | 933 | 924 | 0.09 | 0.05 |
| 2 | α-Pinene | 938 | 932 | 5.23 | 0.52 |
| 3 | Camphene | 952 | 946 | Trace | - |
| 4 | Sabinene | 973 | 969 | 0.59 | 0.03 |
| 5 | β-Pinene | 977 | 974 | 1.55 | 0.47 |
| 6 | Myrcene | 990 | 988 | 2.22 | 0.16 |
| 7 | ρ-Mentha-1(7),8-diene | 1002 | 1003 | Trace | - |
| 8 | δ-3-Carene | 1005 | 1008 | Trace | - |
| 9 | α-Terpinene | 1015 | 1014 | 0.19 | 0.03 |
| 10 | Limonene | 1016 | 1024 | 10.34 | 1.58 |
| 11 | 1,8-cineole | 1035 | 1026 | 66.51 | 1.33 |
| 12 | (*E*)-β-Ocimene | 1046 | 1044 | 0.39 | 0.06 |
| 13 | γ-Terpinene | 1056 | 1054 | 0.56 | 0.09 |
| 14 | *cis*-Sabinene hydrate | 1070 | 1065 | Trace | - |
| 15 | Terpinolene | 1083 | 1086 | 0.08 | 0.04 |
| 16 | *trans*-Sabinene hydrate (IPP vs. OH) | 1101 | 1098 | Trace | - |
| 17 | *n*-Nonanal | 1105 | 1100 | Trace | - |
| 18 | *cis*-ρ-Menth-2-en-1-ol | 1124 | 1118 | Trace | - |
| 19 | δ-Terpineol | 1170 | 1162 | 0.16 | 0.09 |
| 20 | Terpinen-4-ol | 1180 | 1174 | 0.66 | 0.18 |
| 21 | α-Terpineol | 1196 | 1186 | 8.83 | 0.79 |
| 22 | Thymol | 1293 | 1289 | 0.11 | 0.09 |
| 23 | Geranyl acetate | 1379 | 1379 | 0.68 | 0.06 |
| 24 | (*E*)-Caryophyllene | 1415 | 1417 | 0.69 | 0.10 |
| 25 | Aromadendrene | 1434 | 1439 | Trace | - |
| 26 | *cis*-Muurola-3,5-diene | 1445 | 1448 | Trace | - |
| 27 | α-Humulene | 1451 | 1452 | 0.09 | 0.03 |
| 28 | Allo-Aromadendrene | 1455 | 1458 | Trace | - |
| 29 | Dauca-5,8-diene | 1455 | 1471 | Trace | - |
| 30 | γ-Muurolene | 1487 | 1478 | 0.09 | 0.04 |
| 31 | Neryl isobutanoate | 1491 | 1490 | 0.13 | 0.03 |
| 32 | Bicyclogermacrene | 1495 | 1500 | 0.07 | 0.10 |
| 33 | β-Bisabolene | 1508 | 1505 | Trace | - |
| 34 | δ-Amorphene | 1515 | 1511 | 0.20 | 0.02 |
| 35 | Zonarene | 1519 | 1528 | Trace | - |
| 36 | *trans*-Cadina-1,4-diene | 1528 | 1533 | Trace | - |
| 37 | Globulol | 1582 | 1590 | 0.10 | 0.07 |
| 38 | 1-epi-Cubenol | 1624 | 1627 | 0.06 | 0.02 |
| Monoterpene hydrocarbons (%) | | | | 21.31% | |
| Oxygenated monoterpenoids (%) | | | | 77.01% | |
| Sesquiterpene hydrocarbons (%) | | | | 1.31% | |
| Oxygenated sesquiterpenes (%) | | | | 0.16% | |
| Others (%) | | | | 0.13% | |
| Total identified (%) | | | | 99.92% | |

[a] calculated linear retention index; [b] linear retention index from reference [27]; % = mean percent content in the EO over three determinations; SD = mean standard deviation over three determinations.

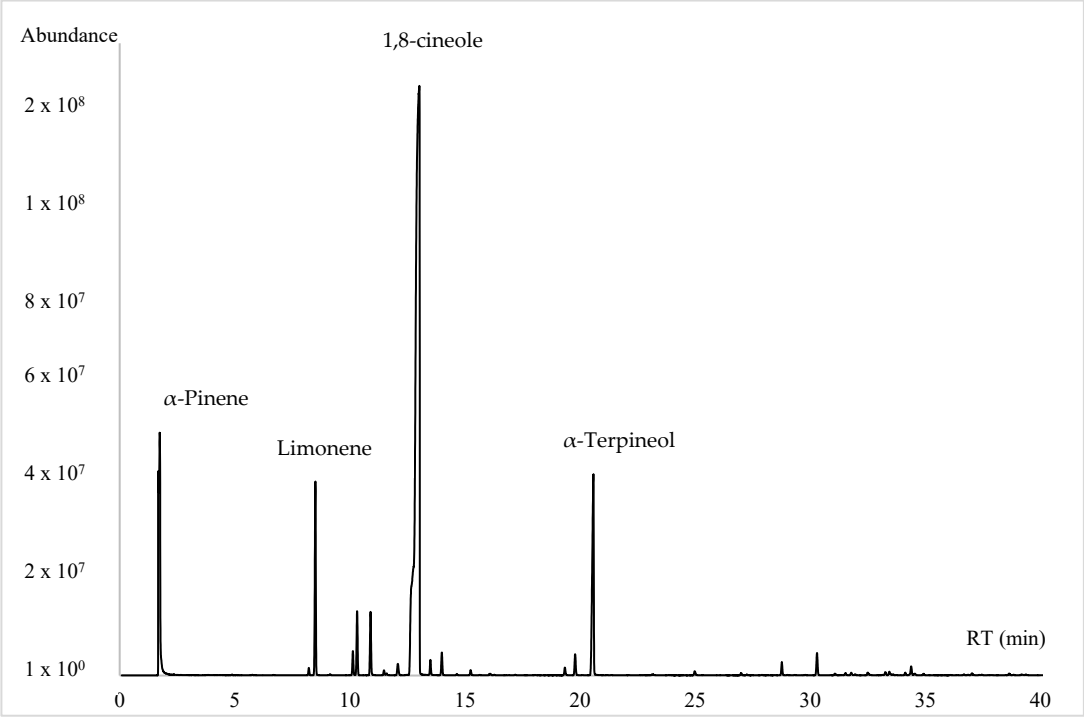


**Figure 3.** GC/MS chromatogram of the EO from the leaves of *Melaleuca armillaris* in a 5% phenylmethylpolysiloxane column.

### *3.3. Isolation and Characterization of Geranyl Acetate*

Geranyl acetate (3.1 mg) was isolated for the first time from *M. armillaris* EO using column chromatography with silica gel G60 (40 g). The mixture was eluted isocratically using 90:10 hexane–ethyl acetate, and the isolated compound was confirmed by NMR analysis using $^{1}$H-NMR and $^{13}$C-NMR and is presented in Figure 4.

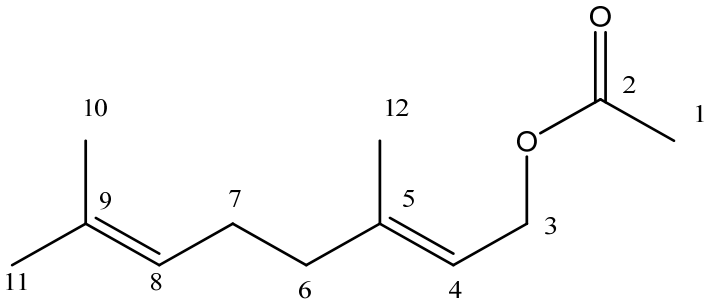


**Figure 4.** Chemical structure of geranyl acetate isolated from *Melaleuca armillaris* essential oil.

$^{1}$H NMR (500 MHz, $CDCl_3$), δ (ppm): 1.60 (3H, s, H11), 1.68 (3H, s, H12), 1.70 (3H, s, H10), 2.05 (5H, s, H1), 2.09-2.16 (4H, m, H7 and H6), 4.59 (2H, d, J = 7 Hz, H3), 5.08 (1H, t, J = 6.7 Hz, H4), 5.34 (1H, td, J = 7.05 Hz, H8).

$^{13}$C NMR (125 MHz, $CDCl_3$), δ (ppm): 171.16 (C2), 142.3 (C5), 131.86 (C9), 123.74 (C8), 118.24 (C4), 61.41 (C3), 39.53 (C7), 26.7 (C6), 25.67 (C10), 21.06 (C1), 17.68 (C11), 16.46 (C12). The resulting data were identical to those found in the literature. (Supplementary Materials, Figures S1 and S2).

## 4. Discussion

The yield of fresh leaves of *M. armillaris* EO was estimated from the amount of oil mass obtained, with an average yield of 1.01 ± 0.0003% (*w*/*w*), a poor yield compared to others reported in the literature for the species studied in Indonesia, which was 3.47% [30].

The yield obtained varies according to different parameters, such as the type of plant material, extraction methods, environmental conditions and geographical distribution, among others [31]. On the other hand, the results of the physical properties are similar to studies with other species of the same genus, such as *M. alternifolia*, which has a density of 0.885 g/mL and a refractive index of 1.482 [17,32,33].

In the chemical analysis of *M. armillaris* EO, more than 99.92% of the total constituents of the sample volatile fraction were identified on the DB-5 ms chromatography column. The most frequently reported major constituents were oxygenated monoterpenes (77.01%), hydrocarbon monoterpenes (21.31%) and hydrocarbon sesquiterpenes (1.31%). Compared to other studies, it has been reported that *M. armillaris,* in some cases, reports constituents as terpene compounds and their corresponding alcoholic derivatives [34]. Another EO of *M. armillaris* presented that the main compounds were 1,8-cineol (66.51%), α-terpineol (8.83%), limonene (10.34%), α-pinene (5.23%), myrcene (2.22%) and β-pinene (1.55%). Other studies reported similar compounds for the same species, indicating that 1,8-cineol is the major component (71.9–72.3%), followed by limonene (7.8–8.2%) and α-pinene (6%) [35,36]. Another study showed that the EO from Pakistan was rich in 1,8-cineole (33.93%), terpinen-4-ol (18.79%), limonene (10.37%) and B-pinene (6.59%) [37]. When compared with the literature results, the results of this study were also consistent in the overall range of percentages of major compounds. In contrast to our study, the study by Kumar et al. [38] on the EO of *Melaleuca leucadendron*, a species of the same family, reported that the main compounds found were β-eudesmol (15.8%), α-eudesmol (11.3%), viridifloral (8.9%) and guaiol (9.0%), respectively. Amri et al. (2012) showed that the concentration of the major compound in *Melaleuca* species was markedly low (3.6%) [39]. Finally, other studies reported that the essential oil of this species contained mainly caryophyllene oxide (43.8%), followed by (−)-spathulenol (9.7%) [40].

Regarding the geranyl acetate isolation, the results obtained are in agreement with those presented by Smith et al. (2007) for $^{1}$H NMR ($CDCl_3$; 400 MHz), 1.61 (3H, s), 1.69 (3H, s), 1.71 (3H, s), 2.07 (3H, s), 2.08–2.15 (4H, m), 4.60 (2H, d, J = 7 Hz), 5.09 (1H, t, J = 7 Hz), 5.35 (1H, t, J = 7 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$), δ (ppm): 171.5, 142.6, 132.2, 124.5, 118.6, 61.8, 39.9, 26.7, 26.1, 21.4, 18.1, 16.8 [41].

Previous NMR studies confirmed the presence of geranyl acetate, and the $^{13}$C NMR spectra showed characteristic peaks associated with the presence of carbon. The peak at δ = 170.89 represents the R-CO-OR group. The peaks at δ = 142.00 and δ = 131.62 represent carbons with unsaturated (double) bonds. The peaks at δ = 123.72 and δ = 118.35 have positive signs and represent carbon atoms with double bonds, corresponding to CH groups. The $^{1}$H NMR spectrum showed characteristic peaks of the CH group located at δ = 5.36-5.33, δ = 5.10-5.07, δ = 4.60-4.58 and δ = 2.23 [19]. The results obtained were consistent with the findings of Silverstein et al. (2005). Peak detection bands of the $^{13}$C NMR spectrum: R-CO-OR group (δ = 175-165), double bond carbon (δ = 150-100), $CH_2$-O group (δ = 75-55), R-$CH_3$ groups (δ = 35-8). $^{1}$H NMR spectra of CC=H (δ = 6-5), R-$CH_2$-O (δ = 4-3), O = C-CH3 (δ = 2.1), C=C-$CH_3$ (δ = 1.7) [42].

Studies have shown that geranyl acetate has excellent antifungal activity and significant synergistic activity with fluconazole at concentrations that do not affect the viability of human cells [43], bactericidal activity against *Campylobacter jejuni* and *E. coli* [44], antioxidant, anti-inflammatory and anti-proliferative properties [45], and is mainly used as a component of perfumes for creams and soaps as well as an ingredient in flavorings.

## 5. Conclusions

An efficient method was developed for the isolation and purification of geranyl acetate for the first time from the essential oil of *M. armillaris* and identified by GC/MS and NMR using $^{1}$H-NMR and $^{13}$C-NMR. In addition, the chemical composition of the *M. armillaris* EO from southern Ecuador was determined; thirty-eight components were identified, representing 99.92% of the total oil, and we found that the major components were 1,8-cineol (67 ± 1%) and limonene (10 ± 2%). Furthermore, this study provided an interesting

perspective, despite the complex composition of EO. Finally, it is recommended that the compound geranyl acetate be explored as a component of perfumes for creams and soaps, as a flavoring ingredient and in the pharmaceutical industry by testing and analyzing its antioxidant, anti-inflammatory and anti-proliferative properties.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14051864/s1, Figure S1: $^{1}$H NMR (500 MHz) spectrum of geranyl acetate in $CDCl_3$; Figure S2: $^{13}$C NMR (100 MHz) spectrum of geranyl acetate in $CDCl_3$.

**Author Contributions:** Conceptualization, C.A. and J.C.; methodology, A.J. and J.C.; formal analysis, J.C.; investigation, A.J.; data curation, C.A.; writing—original draft preparation, S.P.J. and J.C.; writing—review and editing, J.C. and C.A.; supervision, C.A. All authors have read and agreed to the published version of the manuscript.

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