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Research article

Characterization of Melaleuca armillaris essential oil in Brazil

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Abstract

The objective of this study was to propose metrics that could be used in the quality control of *Melaleuca armillaris* oil, extracted from plants cultivated in Brazil, to differentiate it from *Melaleuca alternifolia* oil. The oil was obtained by hydro-distillation using steam stripping. The physicochemical characteristics of the *M. armillaris* essential oil were evaluated, obtaining the following results: density 0.9146 g/mL, pH4.36, specific rotation 2.38, refractive index 1.45948, viscosity of 3 centipoises, 35901 particles ≥ 10 µm and 1743 particles between 10 and 25 µm/5 mL, moisture content 0.84% and decomposition temperature of 115.19 ºC. Osmolarity was not detected. The components of the *M. armillaris* oil were identified using the following techniques: gas chromatography (GC), high performance liquid chromatography (HPLC) with a mass detector, Raman and near infrared (NIR) spectroscopy. A total of 24 compounds were identified by GC, the main one being 1,8 cineole, and 145 substances were identified by HPLC. In the identification by Raman the transmittance of the oil was found to be 785 nm, and using the NIR technique, the greatest absorbance was at 2350 nm. In this way the *M. armillaris* oil was characterized by physicochemical analyses and its components identified.

Introduction

Australia was the pioneering country to work with the essential oil (EO) of the Melaleuca plant [1]. The different species of this genus are cultivated in various parts of the world, including in Brazil [2], where the area cultivated is still small due to a series of factors such as the absence of technical and handling information concerning the species, amongst others [3].

The first literature reports concerning the extraction of EO from the genus Melaleuca occurred in Brazil at the beginning of the 21st century [3, 4]. Other important reports were published resulting from the studies of Oliveira *et al.* (2007) [5] and Pereira *et al.* (2010) [6], who researched the effect of the oil in controlling a fungus that was attacking strawberry cultures in the south of Minas Gerais State, Brazil. Silva *et al.* (2010) [7] carried out research with the species *M. hypericifolia* and *M. thymifolia* at the Federal University of Viçosa, and a couple of years later, Pereira & Teixeira (2012) [8], also working in the south of Minas Gerais State (MG), working with the oil of *Melaleuca* sp., cultivated as an ornamental plant in the town of Pouso Alegre (MG), aimed to verify the efficacy of the oil in the control of the bacteria *Staphylococcus* sp. *in vitro*. Subsequent research aimed to verify the efficiency of the oil extracted from these plants, cultivated in the south of Minas

Gerais State, in controlling hospital pathogens [9, 10] as well as other properties such as anti-histaminic activity [11]. The chromatographic identification of the EO was also carried out by Falci *et al.,* 2015, which demonstrated a chemodiversity of the oil content, such as Eucaliptol, Limonene, isomers of Terpineol, and others [9].

Interest in the use of Melaleuca EO is increasing in Brazil, and thus the quality of these oils is important to maintain the control of microorganisms in the areas of health, agriculture and in industries. The studies in these areas have been focused on the antimicrobial actions of the oil in the control of diverse types of fungi, bacteria, and virus [9, 12, 13].

In Brazil, the Melaleuca EO commercialized is produced on small agricultural farms, or imported from various other countries, and for this reason, divergencies in the quality and efficacy of the oil in the combat of microorganisms has been constantly observed [14]. Essential oils can present quality problems due to environmental conditions that intervene in the composition and percentage of the principle active constituents [15]. In addition, the product may be adulterated by the addition of synthetic compounds or of less valuable EO or even the complete falsification of the oil by mixing with synthetic substances dissolved in an inert vehicle [15]. Also, the sale of *M. armillaris* oil as *M. alternifolia* oil is common in Brazil, due to errors in species identification.

Thus, this study aimed to propose some metrics that could be used in the quality control of *Melaleuca armillaris* EO, extracted from plants cultivated in Brazil, to differentiate it from *Melaleuca alternifolia* oil.

Materials and Methods

The *M. armillaris* used in this study was cultivated in a green area at the Botany Laboratory of UNIVAS, Pouso Alegre-MG, Brazil (register nº 003, Herbário UNIVAS). The oil was extracted from leaves of the *M. armillaris* by hydro-distillation, a technique standardized for this type of essential oil [9]. A total of 5 kg of dry *M. armillaris* leaves were used to obtain 150mL of EO. The following tests were used to characterize the EO and identify its components:

Density

The density of the EO was evaluated using a 30 PX® densimeter (Mettler Toledo, Brazil), which uses the oscillating body method. The equipment was calibrated at the reference temperature and duly cleaned and dried.

pH value

The pH value of the *M. armillaris* oil was evaluated using a model 913 pH-meter (Metrohm, USA). The equipment was calibrated with buffers at pH values of 4, 7 and 9 to increase the reliability, and the results obtained for the asymmetric pH value and the slope parameters used to measure the sensitivity and efficiency of the electrode, were satisfactory.

Specific rotation

This test was carried out using an Alemmar® ADP220 polarimeter (Bellingham & Stanley, USA) belonging to the Physicochemical Quality Control Laboratory of the *União Química Farmacêutica Nacional S/A*, Brazil. The reading was made directly with no sample preparation, with the sample at 20 ºC. The equipment was autozeroed using air before starting the test, and the sample then inserted into a glass tube belonging to the equipment and placed in the reading chamber. After a few seconds the value of the rotation angle appeared in the viewfinder, allowing for the measurement of the value of polarized light.

Refractive index

This test was carried out in the Physicochemical Quality Control Laboratory of the *União Química Farmacêutica Nacional S/A*, Brazil, and the refractometer verified before carrying out the test using water at 25 ºC. The temperature was carefully adjusted and maintained, since the refractive index varies significantly with temperature [16]. After filling the whole orifice and adjusting the temperature in the viewfinder, the refractometer was verified, and the sample read.

Osmolality

A Vapro model 5520 (Wescor, USA) osmometer was used, calibrated with 290mmol/kg, 1000mmol/kg and 100mmol/kg standards to guarantee the precision of the result. A volume of 10µL of *M. armillaris* EO was aspirated with the aid of an automatic pipette and dispensed in the sample reading chamber of the equipment. After dispersion of the sample on the paper membrane destined for this purpose, the collection key of the equipment was switched on and the reading taken.

Viscosity

The viscosity was determined in the Physicochemical Quality Control Laboratory of the *União Química Farmacêutica Nacional S/A*, Brazil, using a Brookfield model LVDV viscosimeter (BrasEq, Brazil), which allows for the velocity to be adjusted in rotations per minute using the display. The equipment was adjusted to a velocity of 10 rotations per minute (RPM), an auto-adjustment carried out to certify there was no type of interference, and spindle nº 34 selected for the determination [17].

Particle count

A model 8000A HIAC counter was used (Beckman Coultre, Brazil), coupled to a sampler which sucked in 5 mL three times, ignoring the first reading, and using the other two for the calculations. This particle counter works based on the light blocking principle, allowing for the determination of the particle sizes and their numbers according to the dimensions. One of the reading channels provided results for particles smaller than 10µm and another for particles larger than 25µm.

Determination of moisture content

The moisture content was determined in the laboratory of the company *Metrohm*, using a *Karl Fischer* 915 KF Ti-Touch® equipment (Metrohm, Switzerland). Before starting the analysis the equipment was conditioned with *Karl Fischer* solution to equilibrate it and remove any water. The analysis was carried out in duplicate to minimize possible analytical errors. The first sample weighed 1.3831 grams and the second 1.3743 grams. When titration started a graph of volume by time was provided by the software coupled to the equipment, and after a few minutes an inflection was formed on the curve followed by the constant volume, and hence the results were provided by the program.

Thermogravimetric analysis

This analysis was carried out using the TGA, DSC® equipment (Mettler Toledo, USA) in the laboratory of the company Mettler Toledo. The sample was placed in the small platinum dish and the equipment temperature programmed. Subsequently 80.3182 mg of sample were inserted into the equipment, which was programmed for a temperature range of from 30 ºC to 600 ºC, with a heating rate of 10ºC per minute.

Gas chromatography

A new method to identify *M. armillaris* oil by gas chromatography was elaborated in the present study, and compared with that developed by Falci *et al.*, 2015 [9]. The present method presented a chromatographic profile similar to that found in the literature but with reduced time and reduced carry-over risk. The method with which it was compared used a mass selection detector whereas the present study used a flame ionization detector.

The oil components were identified in the laboratory of the company Agilent Technology using a method that did not use the headspace but used the model 7693 sampler (Agilent®, USA). The solvent dichloromethane (Merck batch: K51006944911) was used to extract the components, the use of 2 mL vials with no cuts on the septum being imperative so that neither the solvent nor the analytes suffered any significant component losses.

High performance liquid chromatography, HPLC

Liquid chromatography was carried out in the application laboratory of the company Waters Corporation using the ACQUITY H-class UPLC, XEVO® equipment (Waters, USA). The HPLC chromatogram obtained was compared with the equipment library of 6000 compounds, thus aiding identification of the substances.

Raman test

This test was carried out in the laboratory of the company Metrohm using the model Mira M3 Raman equipment (Metrohm, USA). The sample was introduced into the equipment vial and then placed in the reading compartment.

Near infrared spectroscopy (NIR)

This test was carried out in the laboratory of the company Metrohm using the model NIRS XDS® infrared spectroscope (Metrohm, USA). A tip specific for liquid samples was introduced onto the probe of the laser, using a ring according to the viscosity of the sample, and the spectral reading of the *M. armillaris* oil subsequently made.

Results and Discussion

The value found for the density of the *M. armillaris* oil (Table 1) was close to that proposed by the monograph for *Melaleuca alternifolia*, which shows a specification between 0.885 and 0.906 g/mL. Small variations can occur due to the glassware or instrument used, and on an industrial scale the density is very important to determine the filling volume. The result observed was different from that suggested for *M. alternifolia* oil.

Table 1. Characteristics of *Melaleuca armillaris* essential oil.

Parameter	Result
Density	0.9146g/mL
pH value	4.36
Specific rotation	2.38º
Refractive index	1.45958
Osmolarity	Not detected
Viscosity	3 centipoises
Number of particles ≥ 10µm	35901
Number of particles between 10 and 25µm	1743
Moisture content	0.84%
Decomposition temperature	115.19ºC
Identification by GC	24 compounds[a]
Identification by HPLC	145 molecules[b]
Identification RAMAN	Identified (Absorption at 785nm)
Identification by NIR	Identified (Absorption at 2350nm)

GC: Gas Chromatography
HPLC: High performance liquid chromatography
NIR: Near infrared
[a] = described in Table 2
[b] = described in Table 3

The *M. armillaris* EO is more acid than that of *M. alternifolia,* which has a pH value of 5.03 [18]. Thus, the correct identification of the EO and measurement of the pH value is extremely important, since this is the main factor influencing the decomposition of drugs. The association of drugs with distinct pH values can lead to instability, increasing the risk of incompatibility [2]. In addition, if the EO is used in a product destined for topical application the control of the cutaneous pH value must be controlled due to

the aggressivity of inadequate topical products [19]. The acid pH value found in the present study (Table 1) favors its antimicrobial action for agricultural formulations and for most microorganisms, both for those that deteriorate foods and for human bacteria such as *Staphylococcus aureus* [9].

The specific rotation test or polarimetry as used in the identification of *M. armillaris* EO, demonstrated a polarized light deviation of the value described in Table 1. The specific rotation for *M. alternifolia* is between 5-15º and the result can be influenced by various factors such as temperature and distinct chemical constituents [17]. This is an important parameter that differentiates the oil of *M. armillaris* from that of *M. alternifólia*.

The refractive index is employed mainly to characterize fats, fatty oils, waxes, sugars, and organic solvents, and some drugs. It is used to determine the purity of volatile oils. The value obtained for *M. armillaris* oil (Table 1) was below the values for *M. alternifolia* oil (1.475 – 1.482), possibly due to chemical differences between them [17]. Thus, this technique is important in the differentiation between the oils produced by the two species.

The osmotic pressure or osmolarity plays a critical role in all biological processes that involve solute diffusion of fluid transfer through membranes [16]. The equipment did not detect a reading for the osmolarity of *M. armillaris* oil (Table 1), signifying no evaporation of the components of this oil at room temperature.

The value obtained in the viscosity test showed low resistance of the fluid to flow. A low viscosity directly influences the characteristics of creams and ointments, guaranteeing stability and quality to the herbal medicine and aiding in consumer acceptance, since this depends on the appearance, sensation on the first contact with the skin, spreadability and residual oiliness after application [20]. The low viscosity of 3 centipoises of the *M. armillaris* oil (Table 1) provides these results and facilitates the filling process of the oil using industrial scale machinery.

Membrane filtration showed large amounts of particles ≥ 10µm and between 10 and 25µm (Table1). The large number of particles found in the particulate material test could be an indication of pollutants coming from the environment, collection and other factors that lead to undesirable effects in the formulation [21]. Thus, the filtration of the EO before incorporating it into a formulation is imperative. Considering the literature review carried out, it appears that this was the first time this test was applied to *M. armillaris* oil in Brazil, and no papers were found concerning this type of information for *M. alternifolia* oil.

The *M. armillaris* oil showed a low moisture content, favoring its use in formulations. The moisture of essential oils interferes in the quality of the formulation, and on its shelf life and stability. An excess of water in vegetable raw materials makes enzyme action possible and allows for the development of fungi and bacteria [22].

The thermogravimetric analysis demonstrated the initial decomposition temperature of the oil, providing values that allowed for its non-refrigerated storage (Table 1), thus avoiding increases in moisture content, hydrolysis and other phenomena. Similar storage properties are recommended for *Melaleuca alternifolia* [17]. The test also allowed for the determination of the ash content, which evaluates the quantity of inorganic impurities present in the raw material, such as, for example, metal ions. This may have been the first time this type of thermogravimetric analysis was applied to *M. armillaris* oil in Brazil and in addition, no papers providing this type of information were found for *M. alternifolia*.

A new gas chromatographic method was developed in the present study in which the run time was reduced from 39 to 26 minutes, when compared with Falci *et al.,* 2015 [9]. Another advantage of the new method was the adjustment of the parameterized injector syringe such that it only collected the gas formed by the boiling of dichloromethane (solvent), which could significantly decrease contamination phenomena. Using gas chromatography, 24 chemical compounds were identified in the *M. armillaris* EO, the main one being 1,8 cineole (Table 2), as already reported in other papers [1, 23-26], the active principal of *Melaleuca alternifolia* being terpinene-4-ol.

The identification test using HPLC with mass spectrometry allowed for the identification of 145 compounds in the *M. armillaris* oil (Table 3; Figure 1). Since this equipment recognizes isomers, some of the compounds appear to be repeated. This test was very interesting since it showed a much greater number of compounds than that found by gas chromatography.

In the identification of *M. armillaris* EO by Raman, the spectrum showed a single identity (wavelength of 785nm), as shown in Table 1 and Figure 2, any spectrum obtained using the same technique and presenting different bands possibly being an indication of adulteration, problems in obtaining the oil, or cross contamination due to inadequate cleaning of the extraction apparatus. Similarly, using NIR spectrophotometry, a single identity was found for the *M. armillaris* oil (absorption at 2350nm) (Table 1; Figure 3).

Due to the characterization of *M. armillaris* oil using two identification techniques, NIR and Raman, the test using ATR-IR was not carried out. Infrared and Raman spectroscopy are constantly used in the identification, characterization and elucidation of structures of molecules and compounds, as well as in the monitoring and control of chemical reactions. Both techniques measure the levels of vibrational energy associated with chemical bonds in the sample through the interaction of electromagnetic radiation with the vibrational movement of the nuclei, which generates the vibrational spectrum. Such spectra display a greater wealth of details about the analyzed material to the detriment electronic spectra, which are made up of broad, deformed bands, while vibrational spectra are unique spectra of each material and the molecules that make it up [27].

Table 2. Identification of *Melaleuca armillaris* essential oil compounds by gas chromatography.

Peak	Retention Time (minutes)	Relative Time	Compound name	%A
1	5.64	0.75	Trimethyl bicyclic heptane	11.63
2	6.47	0.86	Dimethyl methylene bicyclic heptane	2.58
3	6.64	0.88	Myrcene	0.04
4	6.79	0.90	Methylene methylethenyl cyclohexane	3.28
5	7.02	0.93	Methyl methylethylidene cyclohexane	0.22
6	7.28	0.96	Tetramethyl benzene	0.23
7	7.51	0.99	Limonene	9.42
8	7.56	1.00	Eucalyptol	69.70
9	8.12	1.07	Terpinene	0.41
10	8.70	1.15	Isopropylidene methyl bicyclic hexane	0.08
11	10.28	1.36	Terpineol isomer	0.02
12	10.45	1.38	Terpineol isomer	0.09
13	10.70	1.42	Terpineol isomer	0.45
14	17.43	2.31	Decahydro trimethyl methylene	0.12
15	19.81	2.62	Cyclopropuzulene	0.06
16	21.17	2.80	Aloaromadendrene	0.05
17	22.20	2.94	Octahydro tetramethyl cyclopropazulene	0.10
18	22.47	2.97	Hexahydro Dimethyl methyl ethyl naphthalene	0.66
19	23.50	3.11	Unknown	0.08
20	24.00	3.17	Unknown	0.05
21	24.32	3.22	Globulol	0.09
22	24.53	3.24	Octahydro Dimethyl methyl ethenyl azulene	0.38
23	24.85	3.29	Eudesmol	0.12
24	25.12	3.32	Hexahydro Dimethyl methylethyl naphthalene	0.09

RT = Retention time
Relative RT = Retention time in relation to 1,8 cineole
%A = percentage of component in the sample

Table 3. Identification of *Melalecua armillaris* essential oil compounds by high performance liquid chromatography coupled to a mass spectrometer.

	Component identification	Observed mass (Dalton)		Component identification	Observed mass (Dalton)
1	trans-Carvyl acetate	194.1309	37	Debilone	234.1624
2	Esculentoside A	826.4318	38	3ß-Hudrosantamarine-1-0-ß-D-glucopyranoside	428.2040
3	Gomisin S	418.1985	39	1-methyl-3-(1methyl-ethyl)-benzene	166.0995
4	Oxyphyllenodiol B	238.1571	40	Dictamnol	178.1359
5	Cimidahuside H	618.3742	41	Vellerdiol	236.1777
6	Benzyl benzoate	212.0840	42	Furanodiene	216.1516
7	B Myrcene	136.1255	43	Shizonol	168.1152
8	Vellerdiol	236.1779	44	Nardosinone	250.1573
9	Deoxypaeonisuffrone	182.0945	45	Debilone	234.1622
10	Dihydroeugenol	166.0996	46	3ß Hydrohyatractylon	232.1466
11	Oxyphyllenodiol B	238.1570	47	m-Isopropyltolueno	134.1098
12	Δ1(10)-Aristolen-9-ol	220.1827	48	Nardosine	250.1573

13	1β-Hydroxycolartin	268.1680	49	Δ1(10)- Aristolen-9-ol	220.1832
14	Dihydroactinidiolide	180.1154	50	Debilone	234.1624
15	Dihydroeugenol	166.0995	51	Epi-α- Bisabolol	222.1986
16	3-Phenyl-2-butanone	148.0893	52	Fureanodiene	261.1518
17	(25R)-Spirostan-4-ene-3,12-dione	426.2751	53	Debilone	243.1625
18	Nardosinone	250.1572	54	Δ1(10)- Aristolen-9-ol	220.1828
19	Δ1(10)-Aristolen-9-ol	220.1829	55	Debilone	234.1622
20	Dihydroeugenol	166.0996	56	Debilone	234.1623
21	Thymol isobutyrate	220.1462	57	Δ1(10)- Aristolen-9-ol	220.1831
22	Δ1(10)-Aristolen-9-ol	220.1830	58	Chenodeoxycholic acid	392.2914
23	M- Isopropyltoluene	134.1099	59	(-) Calamenene	202.1722
24	3-Phenyl-2-butanone	252.1730	60	Methyllisoeugenol	178.0993
25	14(R)-Hydroxy-7ß-isovaleroyloxyplop-8(10)en-2-0ne	336.2304	61	Velledio	236.1779
26	Nardosinone	250.1575	62	(-) Calamenene	202.1724
27	Dihydroeugenol	166.0996	63	1 methyl-3-(1methyl-ethyl)-benzene	148.1256
28	Orientalol E	254.1887	64	Bufalin	386.2463
29	Nardosinone	250.1572	65	Orientalol E	254.1889
30	Cavacrol	150.1047	66	Velledio	236.1779
31	Nardosinone	250.1575	67	Spinasterone	410.3555
32	Nardosinonediol	252.1729	68	Calamenene	202.1726
33	Cumene	120.0944	69	Tetramethoxy-trans-stilbene	300.1347
34	25-O-Acetyl-cimigenol	530.3582	70	Piperitone	152.1202
35	Progesterone	314.2253	71	Debilone	234.1625
36	Diethylamine hydrochloride	108.0579	72	Flavenochromane	440.1818
73	Apocynin	166.0633	110	Piperitone	152.1204
74	Turmerone	218.1674	111	ß-Myrcene	136.1257
75	Campest-7,24(28)-dien-3ß-ol	398.3544	112	Nardosinone	250.1573
76	Turmerone	218.1873	113	Dehydrololiolide	194.0946
77	Debilone	234.1624	114	14(R) Hydroxy-7ß-isovaleroyloxyoplop-8(10)-en-2-one	218,1677
78	1-Methyl-3(1-methyl –ethyl)-benzene	148.1257	115	Turmerone	218.1677
79	1-Methyl-3(1-methyl –ethyl)-benzene	148.1256	116	Chenodeoxycholic acid	392,2908
80	Nardosinonediol	252.1730	117	2α-Acetoxycostic acid	292.1683
81	Δ7-Stigmasterol	410.3553	118	Diisobutyl phatel	278.1527
82	2-octyphenol	206.1675	119	Sinenofuranol	238.1939
83	Debilone	234.1624	120	3-Butanone	72.0575
84	Turmerone	218.1677	121	Guaiene	204.1884
85	m-Isopropyltoluene	134.1099	122	Vellerdiol	236.1781
86	Δ(10)-Aristolen-9-ol	220.1830	123	Turmerone	218.1676
87	Dendronobilin B	284.1611	124	Carvacrol	150.1049
88	Ergot-7,22-diene-3ß,5α,6ß-triol	430.3445	125	Guaiene	204.1881
89	Allyl Disulfide	146.0228	126	Chenodeoxycholic acid	392.2908
90	Carvacrol	150.1048	127	Turmerone	218.1678
91	1,3-cyclooctadiene	108.0942	128	Calamenene	202.1726
92	m-Isopropyltolueno	134.1100	129	1-Methyl-3-(1 methyl-ethyl)-benzeno	148.1258
93	Epi-α-Bisabolol	222.1988	130	Debilone	234.1627
94	Δ(10)-Aristolen-9-ol	220.1832	131	Dictamnol	178.1361

95	Calamenene	202.1728	132	Guanina	204.1884
96	Chenodeoxycholic acid	392.2908	133	Chenodeooxycholic acid	392.2908
97	Turmerone	218.1677	134	Epi-α-Bisabolol	222.1989
98	Turmerone	218.1674	135	Calamenene	202.1728
99	Tetratriacontanamin	493.5580	136	Atractylodin	182.0736
100	Guaiene	204.1881	137	Guaiene	204.1885
101	Calamenene	202.1725	138	Calamenene	202.1728
102	Turmerone	218.1675	139	Vellerdiol	236.1782
103	Vellerdiol	236.1780	140	Olibanumols I	428.3654
104	Cavacrol	150.1048	141	Calamenene	202.1727
105	Guaiene	204.1882	142	Δ 7- Stigmasterol	410.3552
106	Methyl arteannate	248.1784	143	Guaiene	204.1886
107	1-Methyl-3-(1 methyl-ethyl)-benzene	148.1257	144	Guaiene	204.1886
108	Turmerone	218.1677	145	Stigmast-4-en-6ß-ol-30ne	428.3653
109	Calamenene	202.1728			

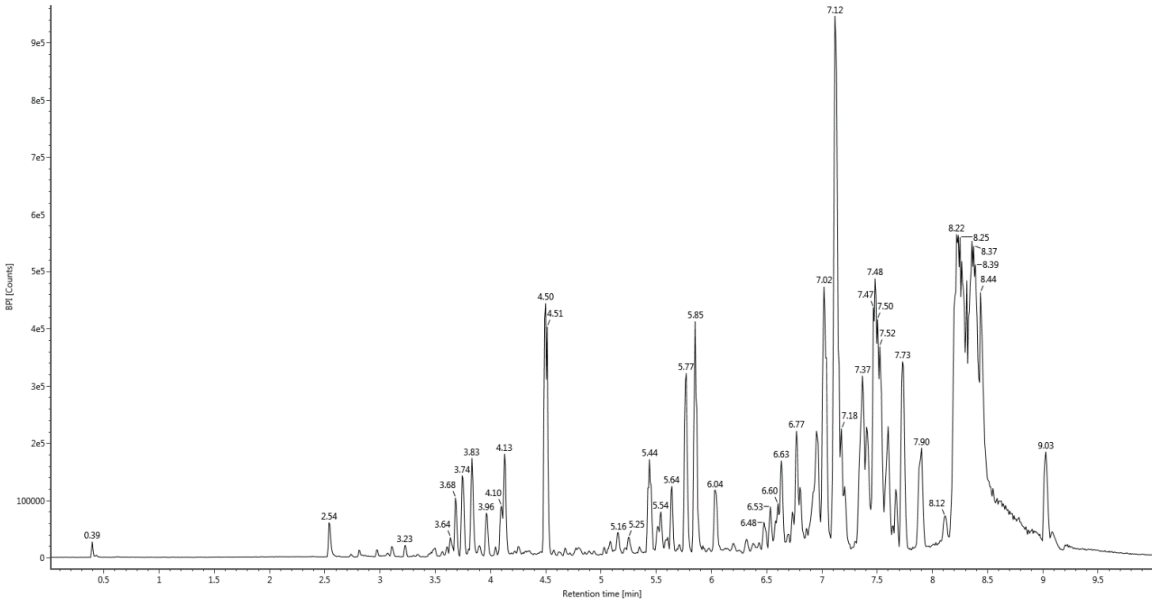

Figure 1. Chromatogram (HPLC) of *Melalecua armillaris* essential oil.

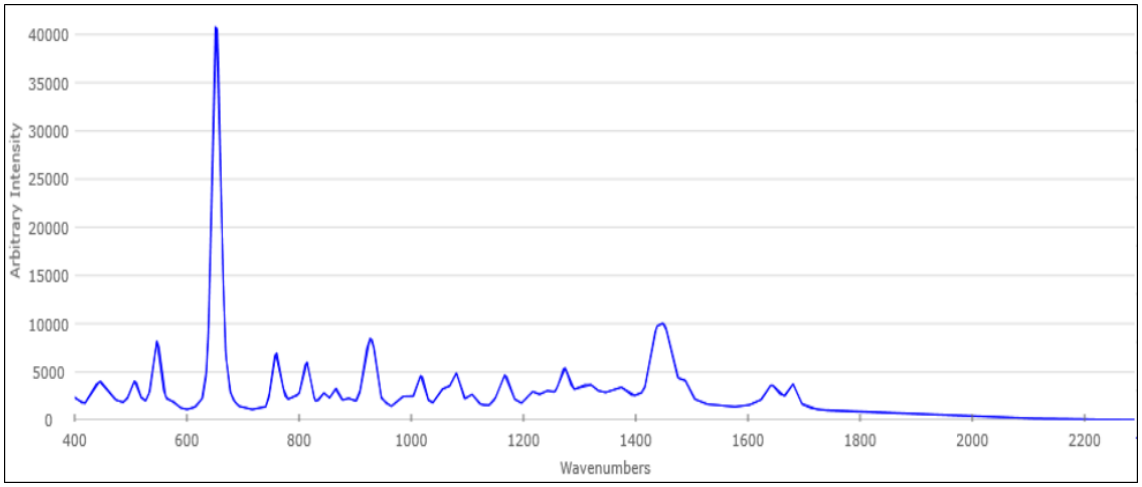

Figure 2. Raman spectrum of the *M. armillaris* essential oil. The spectrum showed a single identity (wavelength of 785nm).

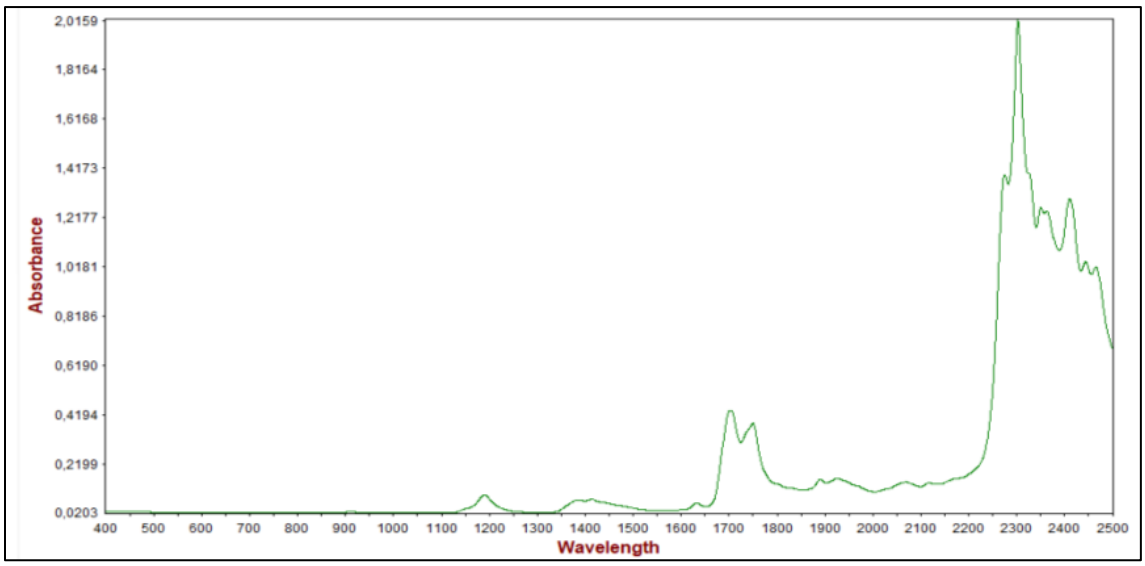

Figure 3. NIR spectrophotometry of the *M. armillaris* essential oil. The single identity was found for the *M. armillaris* oil (absorption at 2350nm).

The characterization of *M. armillaris* oil allowed for standardization of its use and more predictable results for formulations that use it. In addition, the identification of the compounds in the oil helped in the creation of new products and drugs, diversifying the application of *M. armillaris* oil in the health area. Knowledge concerning the biomass of *M. armillaris* oil allows for the identification of possible interactions with other chemical substances which could lead to risks for the patients.

Conclusions

This study established some parameters for *M. armillaris* EO. Amongst these, the main parameters that differentiate this EO from that of *Melaleuca alternifolia* are the principle active constituents (1,8 cineole and terpineol, respectively). The pH value, specific rotation, refractive index and Raman are also important metrics in the differentiation of these oils, since they establish very particular characteristics for the *Melaleuca armillaris* EO.

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Conflict of interests

Author declares no conflict of interest.

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