



Determination of the chemical composition of the essential oil from Arazá (*Eugenia stipitata*) leaf residues and possible agroindustrial applications

Determinación de la composición química del aceite esencial de residuos de hojas de Arazá (*Eugenia stipitata*) y posibles aplicaciones agroindustriales

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Suggested citation: Tasambay Salazar, A. M., Hidalgo Olmedo, B. G., Rivera Barreto, J. L., Luna Fox, S. B. y Rivera Barreto, J. L. (2025). Determination of the chemical composition of the essential oil from Arazá (*Eugenia stipitata*) leaf residues and possible agroindustrial applications. *La Técnica*, 15(1), 9-20. DOI: <https://doi.org/10.33936/latecnica.v15i1.7000>

Received: September 20th, 2024

Accepted: November 27th, 2024

Published: January 26th, 2025

Abstract

The use of plant waste is a topic of current interest due to the environmental effects it causes on the environment. The waste from the leaves of Arazá (*Eugenia stipitata* Mc Vaugh) is not used in Ecuador and in the Amazonian Experimental Research and Production Center, located in the canton of Santa Clara, province of Pastaza, a significant amount of waste is obtained from this type of crop, therefore the objective of the study was to determine the chemical composition of its essential oil, using three extraction methods, to evaluate the effectiveness of these methods in terms of performance and establish their possible applications in the agroindustrial area. Three methods were used to extract the essential oil: steam drag, maceration and ultrasound-assisted extraction; to determine the chemical components, gas chromatography coupled to mass spectrometry was used. The results determined that ultrasound-assisted extraction turned out to be more efficient, with a yield of 2.17%, phenols (47.75%), hydrocarbons (35.51%), acids and esters (6.10%) were identified as major components; In steam distillation, although less efficient in terms of 0.01% yield, terpenes (57.57%), alcohols and other compounds (36.57%), sesquiterpenes (5.85%) were identified; and in the case of maceration it gave a 1.5% yield, phenols (75.84%), esters and acids (12.91%) and amides (6.17%) were identified. This rich composition of bioactive components highlights its importance as waste due to its applications in the food, pharmaceutical, cosmetic and phytosanitary industries.

Keywords: extraction processes, yield; essential oils, 'Arazá', chemical composition.

Resumen

El aprovechamiento de residuos vegetales es un tema de interés actual debido a las afectaciones que provocan en el ambiente. Los residuos de las hojas de Arazá (*Eugenia stipitata* Mc Vaugh) no son aprovechados en Ecuador y en el Centro Experimental de Investigación y Producción Amazónica, ubicado en el cantón Santa Clara, provincia de Pastaza se obtiene una cantidad significativa de residuos de este tipo de cultivo, por lo cual el objetivo del estudio fue determinar la composición química de su aceite esencial, utilizando tres métodos de extracción, para evaluar la efectividad de estos métodos en términos de rendimiento y establecer sus posibles aplicaciones en el área agroindustrial. Se utilizó para la extracción del aceite esencial tres métodos: arrastre con vapor, maceración y extracción asistida por ultrasonido; para determinar los componentes químicos se empleó la cromatografía de gases acoplada a espectrometría de masas. Los resultados determinaron que la extracción asistida por ultrasonido resultó ser más eficiente, con un rendimiento del 2,17%, se identificaron como componentes mayoritarios fenoles (47,75%), hidrocarburos (35,51%), ácidos y ésteres (6,10%); en la destilación por arrastre con vapor aunque menos eficiente en términos de rendimiento 0,01%, se identificaron terpenos (57,57%), alcoholes y otros compuestos (36,57%), sesquiterpenos (5,85%); y en el caso de la maceración dio un 1,5% de rendimiento, se identificaron fenoles (75,84%), ésteres y ácidos (12,91%) y amidas (6,17%). Esta composición rica de componentes bioactivos destaca su importancia como residuo por sus aplicaciones a nivel de la industria alimentaria, farmacéutica, cosmética y fitosanitaria.

Palabras clave: procesos de extracción, rendimiento, aceites esenciales, arazá, composición química.



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La Técnica: Revista de las Agrociencias

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Introduction

Environmental care is a global priority, making it essential to make use of waste generated by agri-food activities involving fruits, which currently have no use (González-Díaz and Véliz-Jaime, 2020). The waste produced after harvesting different fruits or pruning trees can cause serious problems when left on the same land, as it hinders pest control for the crops. On the other hand, disposing of this waste in sanitary landfills leads to additional high costs (Orrego et al., 2020). An example of this can be seen in the production chain of the Arazá fruit (*Eugenia stipitata* Mc Vaugh) in Ecuador, where leaf pruning generates waste that is not utilized, despite its potential as a valuable raw material for obtaining by-products with added value, such as essential oils that can have various industrial applications (González-Díaz & Véliz-Jaime, 2020).

Arazá is a bush known for its nice aroma and its potential for use in medicinal and food products. This plant, when grown extensively for its fruit, is subjected to constant pruning, resulting in residual foliage to be used in essential oil extracts with many benefits and uses (Pardo et al., 2023; Rodilla et al., 2024). Arazá (*E. stipitata*) is native to the western Amazon, it belongs to the Myrtaceae family, and is grown in Ecuador, Peru, Brazil, Bolivia, Costa Rica and Colombia. In Ecuador, Arazá farms are found in all three regions of mainland territory (Reyes-Álvarez y Lanari, 2020).

Essential oils are obtained from the fruits or other parts of the plants. These derivative have nice aromas and are exploited for various uses in the food, cosmetic and pharmaceutical industries (Tekin et al., 2015). They are bioactive with antimicrobial, antioxidant, antifungal and antiviral properties (Ferrari et al., 2019; Rodilla et al., 2024). Presently, in the agricultural industry, the extraction of plant-based essential oils is highly important due to its high added value, not only for their organoleptic properties, but also because they can be used in other industries (Paucarchuco et al., 2023).

It would be of great interest to extract oil from Arazá (*E. stipitata*) produced in the Amazonian Research and Production Experimental Center (Centro Experimental de Investigación y Producción Amazónica) (CEIPA). Then, by analyzing its chemical composition, consider the use of pruning residue, which, until now, has had only negative impact.

If the arazá leaves were adequate to produce essential oil extracts with high added value, it would then be possible to present an innovative solution that contributes to fulfill the current needs of the population (Chen et al., 2022). Arazá production may involve three types of pruning: training and shaping pruning, phytosanitary and cleaning pruning, and renewal pruning;

depending on physiological conditions (Hernández et al., 2006). The lack of research on the exploitation of pruning residue of arazá leaves presents a significant challenge for defining its potential applications. With this conditions in mind, two objectives were established:

1. To evaluate the extraction methods of essential oils from arazá leaves using steam distillation (SD), maceration (MC), and ultrasound-assisted extraction (UAE), with the aim of identifying the most efficient method based on yield.

To determine the composition of the extracts according to the extraction method used, through gas chromatography coupled with mass spectrometry, in order to identify the potential agro-industrial uses or applications of the major compounds.

Materials and methods

Study location: The research was conducted in the Ecuadorian Amazon, at the Amazonian Research and Production Experimental Center (CEIPA), located in the Santa Clara municipality in the province of Pastaza, Ecuador. The site is situated at an altitude of 940 meters above sea level, with geographic coordinates GCS WGS 84; 1°14'19.46" S and 77°53'8.25" W. The analyses of the extraction methods and chemical composition were carried out in the Chemistry and Food Science laboratories of the Amazon State University (Universidad Estatal de la Amazonía).

Preparing the plant material for extraction: Samples of arazá leaf residues were collected from the Amazonian Research and Production Experimental Center (CEIPA) during April and May 2024. Leaves selected for extraction were free of impurities, clean, and showed no signs of insect damage or any other type of contaminating material. The plant material was weighed prior to extraction in order to calculate the yield of essential oil obtained per 100 g of fresh material (Stegmayer et al., 2021).

Steam distillation extraction of essential oil: Eight kilograms of arazá leaf residues were used along with 10 liters of water in a handcrafted, industrial-scale steam extractor made of steel, with a capacity of 200 liters. The base of the extractor contains a mesh that separates the water from the leaves, generating a flow of steam through the plant material. This steam softens the cells and allows the essential oils to escape in vapor form. The ascending vapors were cooled and condensed, resulting in a liquid distillate composed of two layers: an aqueous phase and an organic phase. The extraction lasted three hours, yielding an essential oil of high purity according to the conditions recommended by Božović et al. (2017) and Wedamulla et al. (2022). A 500 mL separatory funnel was used to isolate the organic phase corresponding to the

essential oil. The biomass associated with the extracted essential oil was then determined in order to calculate the yield (Cedeño et al., 2023).

Essential oil yield: To calculate the extraction yield, Equation (1) was used and the result was expressed as a percentage (w/w) of extracted oil (Nolazco et al., 2020). The essential oil was stored in an amber-colored container under refrigeration to preserve it, protected from light (Melo-Guerrero et al., 2020).

$$\% \text{Yield} = \frac{\text{Essential oil extract weight}}{\text{Initial leaf residue weight}} \times 100 \quad (1)$$

Essential oil extraction by maceration: Arazá leaf residues were first dried in an oven (BINDER, ED series) at 30 °C for 72 hours, then ground in a mill (Thomas Wiley USA, Model 4). A sample of 25.01 g was weighed and placed in a 600 mL glass flask, to which 500 mL of hexane was added as a solvent. The flask was sealed tightly and left to rest for 48 hours, with occasional stirring to allow the solvent to diffuse into the cell walls and solubilize the constituents present in the plant material. The liquid was then filtered to separate the solid plant material from the soluble extract, and the solid residue was compressed to recover the maximum amount of trapped solution, following the conditions recommended by Rasul (2018), Melo-Guerrero et al. (2020), and Wedamulla et al. (2022). The extract was concentrated to obtain the essential oil using a rotary evaporator (Yamato RE-200) at 60 °C. Finally, the oil was weighed to determine its yield. The essential oil was stored in an amber-colored bottle under refrigeration, protected from light (Cedeño et al., 2023).

Ultrasound-assisted essential oil extraction: Arazá leaf residues were processed in the same way as in the preliminary preparation phase for maceration extraction. The material was ground, and 25.15 g were weighed and placed in a 600 mL glass flask with 500 mL of hexane as the solvent. The flask with the mixture was placed in the ultrasound-assisted extraction equipment (Branson 3800, model BGK0515659205) at a temperature of 30 °C. Ultrasound was applied at a power of 110 W for one hour, following the conditions recommended by Paucarchuco et al. (2023) and Tekin et al. (2015).

Ultrasound-assisted extraction uses high-frequency sound waves to enhance the extraction of compounds. When applied to a liquid, ultrasound generates sonochemical effects arising from acoustic cavitation. This process causes the rupture of the cell walls, facilitating the solvent's penetration into the plant material through a mechanism of ultrasonic cavitation (Ashaq et al., 2024).

After one hour, vacuum filtration was performed to separate the liquid mixture from the solid plant material. After filtration, the extract was concentrated by removing the solvent using a rotary evaporator (Yamato RE 200) at 60 °C, and the essential oil was obtained. The oil was then weighed to

determine its yield and stored in an amber-colored bottle under refrigeration (Melo-Guerrero et al., 2020; Tekin et al., 2015).

The three extraction methods for obtaining essential oil from arazá leaves were compared, and the method with the highest yield was identified, taking into account the factors influencing each type of extraction.

Determination of the chemical composition of the essential oil from the arazá leaf:

The analysis was carried out independently with the essential oils extracted using three different methods. A gas chromatograph (Shimadzu, model GC-MS QP2020NX) equipped with a single quadrupole mass spectrometer detector was used. A capillary column with a stationary phase of 5% diphenyl and 95% dimethylpolysiloxane (low polarity/low bleed), 30 m in length, 0.25 mm internal diameter, and 0.25 µm film thickness (RTx-5 MS) was employed. The programmed temperature for the column was as follows: initial temperature of 50 °C held for 2 minutes; increased at a rate of 15 °C·min⁻¹ up to 140 °C and held for 2 minutes; then increased at 5 °C·min⁻¹ to 180 °C and held for 3 minutes; followed by an increase of 10 °C·min⁻¹ up to 270 °C and held for 10 minutes. The optimal operating conditions were: injection volume of 1 µL, injector temperature at 250 °C, carrier gas flow (He) at 1.25 mL·min⁻¹, and a Split/Splitless injector (splitless time of 1 minute).

The mass spectrometer parameters were: interface temperature at 270 °C, ion source temperature at 280 °C, mass scan range from 15 to 400 m/z, scan speed of 1428 scans·s⁻¹, and a total run time of 40 minutes. For the essential oil analysis, a 0.1 mL volume of essential oil from each extraction method was diluted to volume with hexane in a 5 mL volumetric flask, filtered through a 0.45 µm membrane, and then injected into the equipment. To identify and characterize the main chemical compounds for each extraction method, the retention times and mass spectral fragmentation patterns of the most abundant experimental peaks were compared with those in the instrument's database, the NIST Mass Spectral Library. A subsequent classification was carried out by grouping the identified volatile compounds, and the constituents obtained by each extraction method were compared.

Determination of the potential applications of the essential oil extracted from arazá leaves:

Once the major components were classified and identified for each extraction method, an assessment of potential agro-industrial applications was carried out. This evaluation was based on a literature review of previous studies on essential oils extracted from various plant sources with known or potential applications.

Results and discussion

Evaluation of essential oil extraction methods from the arazá leaf: Table 1 shows the gross and adjusted yields obtained by extracting essential oils from arazá leaf residue. The adjusted yields belong to the chemical compounds that were excluded



from the lipidic profile in each method. It was observed that the method with the highest yield was ultrasound-assisted extraction with 2.17%, higher than conventional methods, and more-eco-friendly, as reported by Ashaq et al. (2024). The steam distillation method had the lowest yield, a similar case to other plant residue processes such as with orange peel (epicarp; Briones-Sornoza et al., 2019).

Table 1. Yields obtained from arazá leaf residue oil extraction (*Eugenia stipitata* Mc Vaugh).

Extraction methods	Arazá leaf (g)	Gross yield (%)	Adjusted yield (%)
Steam distillation (AV)	8.000	0.01	0.01
Maceration (MC)	25.01	2.72	1.51
Ultrasound-assisted(UAE)	25.15	3.34	2.17

Considering the work conditions for each extraction maceration took the longest time, with 48 hours due to the need for longer contact time between the plant material and the solvent. The yield value was average relative to other methods (1.51%). In maceration, a favorable factor was the risk reduction of the decomposing of compounds extracted by using cold solvents; nonetheless, there were some unfavorable aspects when compared to ultrasound assisted method, such as a decrease in the extraction method, and significant consumption of health-adverse solvents. The steam distillation method was the quickest (3 hour process) compared to maceration (table 2); nonetheless, the yield was the lowest of the three.

Tabla 2. Extraction time for essential oils from the arazá leaf (*Eugenia stipitata* Mc Vaugh) per employed method.

Parameter	Extraction method		
	Steam distillation	Maceration	Ultrasound assisted
Extraction time	Average	Long	Short
(h)	3	48	1
Solvent (mL)	Water	Hexane	Hexane
	10	0.5	0.5

On the other hand, the ultrasound assisted method had the shortest time, only 1 hour, and was the most efficient. The yield was higher than those of other studies from Portugal that used hydrodistillation (0,35%) (Medeiros et al., 2003). The yield was also higher than a study carried in Brazil, with 0,2% (Jerônimo et

al., 2021). The isolated ultrasound extraction method allowed for a better solid-liquid extraction; by using hexane, the process was more complete and resulted in extracted oil yields, compared to the two more conventional methods.

There was correlation between the extraction time and the yields; to obtain a high extraction yield, a better biomass retention is required in the hexane that was obtained through the ultrasound assisted method. The essential oil extraction methods can be selected according to the production objectives and the uses of the arazá leaves, independent of the extraction times (Cedeño et al., 2023).

Identification of the chemical compounds: the identification of the volatile chemical components was done through gas chromatography attached to a mass spectrometer detector. The chemical compositional variability was evaluated according to their chemical properties, as well as the higher concentration components (Cedeño et al., 2023).

Steam distillation extraction: forty-three compounds were identified in a 40 minute interval in a chromatographic run (table 3). The most abundant chemical compounds were the terpenes with 57.57%, followed by alcohols and other components with 36.57%, and lastly, sesquiterpenes with 5.85%. This composition had variations related to the chemical compounds that were found; in other studies from Peru, also involving arazá leaf, high percentages of phenols followed by triterpenes, tannins, flavonoids, saponins and proteins (Tenorio and Muñoz, 2021). These differences in composition with other studies can be attributed to geographical conditions, stage of plant maturation, cultivation method and even climate conditions, as mentioned by other essential oil studies (Ruiz et al., 2015). Other studies involving the arazá fruit in Brazil identified a complex pattern of sesquiterpenes, which were more predominant in amazonian fruits, with germacrene D as the main compound with 38%, a higher value than the arazá leaf's 3.76% (Franco and Shibamoto, 2000).

The resulting chemical composition suggests that the essential oil obtained from arazá leaf residue has a rich and complex aromatic profile, with high concentrations of bioactive compounds, with the most prominent being Propane, 2,2-dimethyl- (19.93%), a hydrocarbon, then germacrene B (8.11%), caryophyllene (6.75%), δ -Cadinene (5.21%) and guaiol (4.70%), copaene (4.35%), γ -Elemene (2.90%), β -Nerolidol (2.10%), α -Elemene (2.66%). If these are compared to the arazá leaf studies from Portugal, there is a difference in composition and concentration of the compounds, in which it was detected that the E-caryophyllene (22.7%) and caryophyllene oxide (15.4%), and other different

compounds such as germacrene D (11.8%) and Z- α -bisabolene (8.38%) had high concentration and some other common compounds such as δ -Cadinene (5.7%) Caryophyllene oxide (2.6%), δ -Elemene (1.9), α -Copaene (1.7%), β -Elemene (0.6%), γ -Elemene (0.2%), Germacrene B (0.3%), E-Nerolidol (0.8%) in Jerônimo et al. (2021) whose concentrations were lower than those obtained from arazá leaf residue at the CEIPA in Ecuador.

This composition of the chemical compounds is valuable and can have applications in various industries for many purposes, as determined by other studies involving natural products (Eddin et al., 2021; Rodilla et al., 2024). The terpenes being the major compounds, they are responsible for the plant's characteristic aroma and taste, which can be used in dyes, colorants, flavorings, aromatics, antioxidants, antibiotics, antitumor agents, and even insecticides (Schrader y Bohlmann, 2015).

Table 3. Chemical compounds identifies in arazá leaf residue essential oils obtained through steam distillation.

N.	Compounds	TR (min)	%A
1	Propane, 2,2-dimethyl-	2.145	19.93
2	Cyclopentane, methyl	2.710	0.63
3	Limonene	6.699	1.89
4	γ -Elemene	11.071	2.90
5	Cubebene α-> (11.321 min)	11.321	2.47
6	Copaene	11.872	4.35
7	β -Elemene	12.059	2.66
8	Gurjunene α->	12.510	1.47
9	Caryophyllene	12.723	6.75
10	Elemene γ->	12.833	1.84
11	cis- α -Bergamotene	12.927	0.86
12	α -Guaiene	12.991	1.20
13	Aromadendrene	13.063	2.40
14	Cadina-3,5-diene	13.205	0.45
15	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	13.316	2.45
16	Caryophyllene ϵ->	13.451	0.94
17	Ishwarane	13.560	0.47
18	Cadinene γ->	13.695	1.96
19	(-)-Germacrene D	13.839	3.76
20	(+)- β -Selinene	13.955	1.53
21	Isoledene	14.050	1.32
22	Germacrene B	14.216	8.11
23	Cyperene	14.390	0.61
24	Cadinene γ->	14.445	0.61
25	Cadinene δ->	14.654	5.21
26	Aristolene	14.753	0.63
27	Cadina-1,4-diene trans->	14.827	0.87
28	Maaliene β->	14.917	0.92
29	β - Nerolidol	15.299	2.10
30	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)- (E,E)	15.367	0.67

Table 3. Chemical compounds identifies in arazá leaf residue essential oils obtained through steam distillation.

31	β -Maaliene	15.444	1.23
32	Sphatulenol	15.632	1.42
33	Caryophyllene oxide	15.764	0.75
34	(-)-Globulol	15.863	1.83
35	Guaiol	16.114	4.57
36	Rosifoliol	16.208	0.52
37	Viridiflorol	16.280	0.47
38	Selina-4,7 diol	16.616	0.62
39	Murolol α->	16.947	1.07
40	Cadin-4-en-10-ol	17.208	1.10
41	trans-sesquisabinene hydrate	17.547	2.73
42	Henicos-1-ene	18.041	0.79
43	trans-Farnesyl acetato	21.694	0.93

RT: retention time; A: %area.

Maceration extraction: nine compounds were identified in the 40 minute chromatography run, the most significant area percentage shown by the compound n.7, Bisalkofen BP con 75.84%, followed by compound n.8, 2-Palmitoylglycerol con 9.06%, and n.6 N,N-Dimethylpalmitamide with 6.16%, as shown in table 3. The major compounds were classified in three groups: phenolic compounds (75.84%), esters and acids (12.91%), and amides (6.17%); and in lower proportions, nitrogenated compounds (3.48%) and hydrocarbons (1.62%). Thus, the importance of phenolic compounds in the arazá leaf is highlighted. Studies on the fruit and seeds from *E. stipitata* also evidenced a high phenolic compound content, especially in the seeds, which shows great anti-oxidant capacity, a characteristic of great interest, despite the fact that these parts of the plants are often discarded.

The phenolic compounds are synthesized by the plant's secondary metabolism, they are classified as non-nutritional compounds, found in small quantities of fruits and vegetables, and play a promising role in the prevention of illnesses (de Araújo et al., 2021). Other studies have shown a direct relation between daily diet and chronic illnesses, thus, it is important to look for sources of plant phenolic compounds, which are currently increasing, due to their potential bioactive compounds and their antioxidants, anti-inflammatory, antimicrobials, anti-obesity, anti-tumor properties, among others (de Araújo et al., 2019).

Table 4. Chemical compounds found in the arazá leaf essential oil obtained through maceration by CG-EM.

N.	Compounds	TR (min)	%A
1	Diethyl hexylmalonate	5.841	0.29
2	Oxalic acid, di(cyclohexylmethyl) ester	7.351	0.92
3	cis-3-hexyl tiglate	9.379	0.58
4	3',7'-Dimethyloct-6'-enyl-3-methyl-2-oxopentadecanoate	22.671	2.06



Table 4. Chemical compounds found in the araza leaf essential oil obtained through maceration by CG-EM.

No.	Componentes	TR (min)	%A
5	Phytane	22.964	1.62
6	N,N-Dimethylpalmitamide	28.288	6.17
7	Bisalkofen BP; Antioxidant 2246	29.855	75.84
8	2-Palmitoylglycerol	30.850	9.06
9	5-Amino-1-methyl-1H-pyrazole-4-carboxamide, 3TMS	30.974	3.48

TR: retention time; A: % de área.

Ultra-sound assisted extraction: forty-eight chemical components were identified in a 40-minute chromatographic run as indicated in (table 4), the component that predominates significantly corresponds to Bisalkofen BP (47.75%), followed by other important major components, which correspond to 2-Palmitoylglycerol (4.54%), Dodecane (4.52%), n-Heptatriacontane (4.02%), Tetracosyl acetate 1.88%, Tridecane 1.85%, Cyclopentasiloxane decamethyl- (1.78%).

Table 5. Chemical components identified in the essential oil of Arazá leaf residues obtained by the UAE method by GC-MS.

No. pico	Componentes	TR (min)	%A
1	Cyclohexane, (2-methylpropyl)-	5.489	0.32
2	Cyclohexane, 1-ethyl-2-propyl-	5.796	0.42
3	Cyclohexane, 1-ethyl-2,4-dimethyl-	5.923	0.35
4	(2E)-5-Methyl-2-undecene	6.060	0.53
5	Decano	6.421	1.68
6	Oxalic acid, 2-ethylhexyl pentyl ester	6.724	0.63
7	Cyclohexane, undecyl-	6.800	0.52
8	1-Stearoyl-1H-imidazole	6.867	1.12
9	Naphthalene, decahydro-, trans-	7.076	0.73
10	Heptane, 2,5,5-trimethyl-	7.176	0.38
11	2-Methyltetracosane	7.219	0.80
12	5-Isobutylnonano	7.296	0.47
13	Cyclohexane, 1-ethyl-1-methyl-(2,4-Dimethylcyclohexyl)methanol	7.458	0.40
14	(isomer 3)	7.521	0.61
15	Dodecane	7.635	4.32
16	Trans-Decalin, 2-methyl-	7.766	0.89
17	Dodecano	7.860	0.56
18	9-Eicosyne	7.963	1.10

Table 5. Chemical components identified in the essential oil of Arazá leaf residues obtained by the UAE method by GC-MS.

19	Undecane, 2-cyclohexyl-	8.039	0.91
20	1-Hexadecanol, 3,7,11,15-tetramethyl-	8.162	0.45
21	9-Octadecen-1-ol, (Z)-	8.216	0.58
22	Tridecano, 6 methyl-	8.284	1.07
23	Sulfurous acid, octadecyl pentyl ester	8.341	0.93
24	Undecane, 2-methyl-	8.394	1.11
25	Undecane, 3-methyl-	8.477	1.03
26	Naphthalene, decahydro-1,6-dimethyl-	8.631	0.52
27	1,3-Dimethylcyclohexane	8.692	0.62
28	Cyclohexano, 1,1'-(1,2-dimethyl-1,2-ethanediy)bis-, (R*,R*)-(./-./-)	8.739	0.40
29	Dodecane	8.841	4.52
30	Undecane,2,5-dimethyl-	9.056	1.16
31	Dodecane, 4-methyl-	9.165	0.34
32	Tridecane, 6 methyl-	9.595	0.40
33	Dodecane, 2-methyl-	9.770	0.58
34	Dodecane, 4,6-dimethyl-	10.113	0.62
35	Tridecane	10.356	1.85
36	1-Tetradecene	11.973	0.65
37	1-Decanol, 2-hexyl-	12.199	1.25
38	Hentriacontane <n>	14.282	0.80
39	11-Methyltricosane	24.234	0.43
40	Cyclotetrasiloxane, octamethyl-	27.551	1.37
41	Tetracosyl acetato	27.966	1.88
42	N,N-Dimethylpalmitamide	28.294	0.95
43	Cyclopentasiloxane, decamethyl-	29.265	1.78
44	Bisalkofen BP; Antioxidant 2246	29.858	47.75
45	1-Docosanol, acetate	30.097	0.97
46	N,N-Dimethylpalmitamide	30.457	0.70
47	2-Palmitoylglycerol	30.852	4.54
48	Heptatriacontane <n>	37.336	4.02

TR: tiempo de retención; A: % de área.

The identified chemical components were classified into seven groups: phenols (47.75%), hydrocarbons (35.51%), acids and esters (6.1%), alcohols and other compounds (3.66%), siloxanes (3.15%), amides (2.79%), and imidazoles (1.07%). Among these, the compounds with the highest

concentration were phenols, followed by hydrocarbons, and then acids and esters. This composition stood out for its phenol content, similar to what was observed in the maceration method. However, it differed from other studies on the essential oil of arazá leaves obtained by hydrodistillation, which identified sesquiterpenes and monoterpenes as the most significant compounds—specifically E-caryophyllene (22.7%), caryophyllene oxide (15.4%), α -pinene (14.1%), 4,8 α -epoxy caryophyllene (10.9%), and germacrene D (5.64%).

This compositional profile exhibited antibacterial properties against *Staphylococcus aureus*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* (Medeiros et al., 2003). Similarly, the chemical composition of the oil from arazá seeds in Colombia also showed significant differences, where limonene was identified as the main compound. Most of the volatile compounds in that oil were hydrocarbon terpenes (90.4%), followed by aliphatic aldehydes (7.1%). These compositional differences can be attributed to the plant part used, the extraction method, and environmental conditions (da Costa et al., 2020). The presence of terpenes such as limonene contributed to an acidic aroma, while aldehydes such as 9,17-octadecadienal, octanal, β -sinensal, α -sinensal, nonanal, decanal, undecanal, and dodecanal were responsible for a pungent, woody odor during steam distillation (Fajardo et al., 2022).

Comparison of essential oil composition:

The results obtained from the analysis of essential oil extracted from arazá leaf residues using the three methods—steam distillation, maceration, and ultrasound-assisted extraction—clearly revealed differences in chemical composition, as confirmed in each of the analyses (Figure 1). Each extraction method had a specific influence on the composition of the volatile compounds in the essential oil from arazá leaf residues, resulting in a chemical profile suited to different applications (Rasul, 2018). The presence of bioactive components was related to factors such as species type, edaphoclimatic conditions, plant part used, solvents employed, and others (Acosta-Vega et al., 2024).

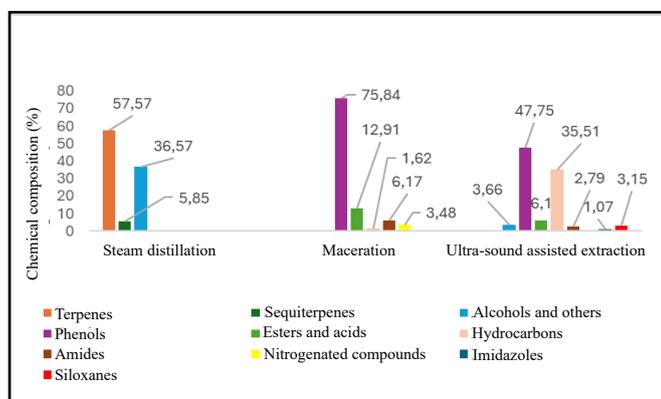


Figure 1. Comparison of chemical compounds found in essential oil.

The steam distillation method, a conventional technique, provided high levels of terpenes (57.57%), while maceration and ultrasound-assisted extraction showed a high phenol content (Figure 1), highlighting their importance for potential applications in the agro-food, pharmaceutical, cosmetic, and chemical industries, among others. Terpenes can be used as key components in nutraceutical products and functional foods (González-López et al., 2016).

In the composition of the essential oil obtained by steam distillation, the presence of Germanene B (8.11%) was observed—this compound was not reported in the study by Medeiros et al. (2003), although it appeared in lower concentrations in arazá leaf residues compared to the 38.3% reported in studies with arazá fruits (Franco and Shibamoto, 2000).

In the maceration method, another conventional technique, a lower extraction quality was evident due to the use of hexane as a solvent in the extraction of essential oil from arazá leaves. Many key compounds found in steam distillation—such as limonene and caryophyllene—were not detected.

When compared to steam distillation, these variations may be related to the limitations inherent to certain extraction methods, including the loss of volatile compounds, the presence of thermosensitive substances, and the potential for solvent residues in the essential oil, all of which can significantly affect the final quality and purity. In this regard, steam distillation more closely resembled the composition reported in studies of arazá leaf oil conducted in Portugal and Brazil, which are among the few investigations on arazá leaves to date (Medeiros et al., 2003; Putnik et al., 2018).

Despite the limited number of studies in other countries, some similarities were reported. However, the chemical composition of arazá cultivated in the Ecuadorian Amazon region exhibited distinct characteristics that should be considered for local utilization, making this study a novel contribution. In the ultrasound-assisted essential oil extraction method, a greater number of chemical components were identified compared to steam distillation and maceration methods. The most abundant compounds were phenols (47.75%), although this percentage was lower than that obtained through maceration. These were followed by hydrocarbons (35.51%), which were higher than those obtained by maceration; esters and acids (6.1%), also higher than those from maceration; followed by alcohols and other compounds (3.66%), which were lower than in maceration; siloxanes (3.15%), which were not identified in steam distillation or maceration; amides (2.79%), present in a lower percentage than in steam distillation; and imidazoles (1.07%), which were not identified in either steam distillation or maceration.

In addition, ultrasound-assisted extraction is a clean process that yielded the highest amount of essential oil in a shorter extraction

time, preventing oil degradation and using the smallest volume of solvent. Therefore, it is a cost-effective extraction method (Tekin et al., 2015; Dzah et al., 2020). It is a modern, non-conventional technique that offers additional benefits over conventional methods, particularly in terms of environmental sustainability (Sabat et al., 2024).

The three essential oil extraction methods—steam distillation (SD), maceration (MC), and ultrasound-assisted extraction (UAE)—showed distinctive chemical profiles, each with its own advantages and limitations. The choice of extraction method will depend on the desired chemical profile of the arazá essential oil.

Table 6. Major chemical components of the essential oil from arazá leaf residues obtained through the steam distillation method (SD) and their uses in the agro-industry.

Chemical compounds	RT (min) A (%)	Industrial applications	Referencias
Limoneno	6 1,89	Due to its aromatic properties, it is used in the food industry, as well as in the production of cosmetics and cleaning products.	(Sánchez et al., 2021).
γ- Elemene	12 1,84	Due to its pleasant woody aroma, it is used in cosmetics for the formulation of moisturizing creams and perfumery products.	(Gonzales et al., 2016).
Caryophyllene	12 6,75	Thanks to its antimicrobial and antioxidant properties, it is used in hair conditioners and cosmetic skin creams.	(Ruiz Quiroz et al., 2021)
Germacrene D	13 3,76	Its anti-inflammatory, antioxidant, and antimicrobial properties allow for its use in the pharmaceutical industry as well as in the cosmetic industry for the production of skincare products such as creams and lotions.	(Xu y Dickschat, 2023)
δ-Cadinene	14 5,21	The anti-inflammatory effects, along with its soothing and sedative properties, make it valuable for the pharmaceutical industry.	(Qin et al., 2024).

Table 6. Major chemical components of the essential oil from arazá leaf residues obtained through the steam distillation method (SD) and their uses in the agro-industry.

Guaiol	16 4,57	Due to its antimicrobial activity and repellent and insecticidal properties, it is used in agrochemical and phytosanitary products.	(Liu et al., 2013)
Trans-sesquisabinene hydrate	17 2,73	The antimicrobial properties make it useful for the production of intimate hygiene products.	(Noriega et al., 2020)

RT: retention time, A: % of area.

Steam distillation is preferable for obtaining an oil rich in terpenes and sesquiterpenes with high purity. Maceration is ideal for maximizing the extraction of phenols, ethers, and acids, although it carries the risk of solvent contamination—as does ultrasound-assisted extraction. However, UAE provides a more diverse profile of volatile compounds, making it a versatile option for various applications of *Eugenia stipitata* essential oil. It is the most efficient, reliable, and cost-effective extraction methodology, bringing significant benefits to the industry (Shen et al., 2023).

The identification of the predominant chemical constituents in the essential oil extracted from arazá leaf residues enabled the valorization of its characteristics and helped outline its potential uses across different industrial applications

Determination of possible applications of the essential oil: Steam distillation yielded a terpene-dominant profile, indicating potential uses in perfumery and personal care products, while the presence of alcohols and other secondary metabolites may contribute to the stability and versatility of the essential oil in medicinal and food-related applications, as reported in studies on various Peruvian aromatic plants (Pardo et al., 2023). The identification of chemical compounds made it possible to evaluate potential uses based on research on different types of essential oils containing similar chemical components. Applications stand out in the food, cosmetic, cleaning, pharmaceutical, and agrochemical industries, as shown in table 5.

From maceration and ultrasound-assisted extraction, the most significant and abundant chemical components identified in the essential oil of arazá leaf residues were bisalphafene and

2-palmitoylglycerol, which have applications in the chemical, pharmaceutical, rubber, cosmetic, and food industries. In ultrasound-assisted extraction, dodecane was another major compound with potential applications in the cosmetic, food, and cleaning products industries (table 6). N,N-dimethylpalmitamide, found in high concentrations in both methods, has not yet been reported to have any significant application.

Arazá (*E. stipitata*) leaf residues represent a valuable source for obtaining promising secondary metabolites due to their applications in various areas of agroindustry (Pardo et al., 2023). The search for new scents, flavors, and uncommon characteristics is a motivating factor for the development of novel food products using underutilized sources of Amazonian biodiversity. These could be employed in the creation of innovative functional foods with unique exotic characteristics through enrichment with bioactive compounds for the benefit of human health (González-López et al., 2016).

Table 7. Applications of major chemical compounds found in essential oils obtained from arazá residues using through maceration and ultra-sound assisted methods.

Chemical compounds	TR (min) A (%)	Industrial application	References
Dodecane	7 4,32	As a surfactant for the production of cleaning products. In the cosmetic industry, as emollients for the formulation of creams and lotions; in the food industry, to improve textures. As a raw material for the synthesis of other industrial chemical compounds.	(Gonzales, 2024)
Bisalkofen BP o Antioxidante 2246	29 47,75	For the production of adhesives, polymer binders, rubber antioxidants, lubricant antioxidants, fuels, and hydraulic fluids. In the cosmetic industry, as an antioxidant for fragrances and cosmetic products.	(NICNAS, 2016 ; Chai et al., 2023).
2-Palmitoylglycerol	30 9,06	As an antibacterial and antimycobacterial agent; used as an emulsifier in the food industry; in the cosmetic industry for the production of beauty products such as creams, lip balms, and lotions.	(Njeru et al., 2020) (Huang et al., 2011).

TR: tiempo de retención; A: % de área.

Conclusions

The extraction of essential oils from arazá leaf residues was carried out using three methods: two conventional methods—steam distillation and maceration—and one non-conventional method—ultrasound-assisted extraction. The latter stood out for achieving the highest yield and the greatest efficiency in identifying active chemical components with profiles highly valuable to the agro-industry. It is important to consider that the extraction method significantly influences the yield, nature, and stability of the bioactive compounds obtained. The results revealed a composition with a high predominance of phenols, terpenes, sesquiterpenes, esters and acids, hydrocarbons, and other secondary metabolites, suggesting that arazá leaf residues could represent a valuable raw material for obtaining compounds of great interest in the food, pharmaceutical, cosmetic, and plant protection industries.

Acknowledgements

A the Amazon State University (UEA) for the financial support provided for the execution of the research.

Conflict of interest

The authors declare to not have conflict of interest in the present publication or any of its phases.

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