DISCIPLINARUM SCIENTIA

Disciplinarum Scientia. **Série: Naturais e Tecnológicas**
Santa Maria, v. 26, n. 2, p. 181-194, 2025

Recebido em: 27.04.2025. Aprovado em: 16.06.2025.
ISSN 2176-462X | DOI: doi.org/10.37779/nt.v26i2.5357

ANALYSIS OF THE YIELD OF ROSEWOOD ESSENTIAL OIL UNDER EXPERIMENTAL CONDITIONS[1]

ANÁLISE DO RENDIMENTO DO ÓLEO ESSENCIAL DE PAU-ROSA EM CONDIÇÕES EXPERIMENTAIS

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ABSTRACT

Rosewood (*Aniba roseodora* Ducke), a species native to much of Latin America and once very common in Brazil, is widely used for the extraction of linalool. The high demand for its oil led to a disalignment between the extraction of its essential oil and the time taken for the natural regeneration of the species, almost leading to its extinction in the last century. In this context, the objective of this work was to develop a sustainable protocol for the extraction and yield of rosewood essential oil, from branches and leaves, on a laboratory scale. Three main variables that were correlated with each other were used: type of sample (branch or leaf), amount of water used in the hydrodistillation (1 L or 1.5 L) and extraction time (1 h, 1.5 h or 2 h). The data obtained were submitted to analysis of variance and, for comparison of the means of the treatments, the Tukey test was used at the level of 5% probability. We observed that there was a significant difference between the treatments tested. The treatment with 100 g of rosewood leaves when associated with 1 L of distilled water and submitted to 1 hour of extraction resulted in the highest average yield rate (0.70935 mL), in addition to an average linalool content that was greater among all the samples analyzed, being equal to 83.11% when compared with the other treatments. We conclude that the protocol developed on the extraction and yield of rosewood oil on a laboratory scale is efficient for the study in question; however, we suggest further studies for the optimization of the protocol in order to ensure the full sustainable development of this species of economic interest.

Keywords: Chromatography; Analysis; Hydrodistillation; Production chain; Extraction.

RESUMO

O Pau-Rosa (Aniba roseodora Ducke), espécie nativa de grande parte da América Latina e outrora tão presente no Brasil, é muito utilizado para a extração do linalol. A alta demanda levou a um desarranjo entre a remoção de óleo essencial e o tempo de regeneração natural da espécie, quase levando-a à extinção no século passado. Neste contexto, o objetivo desse trabalho foi desenvolver um protocolo sustentável de extração e rendimento do óleo essencial de Pau-Rosa, a partir de galhos e folhas, em escala laboratorial.

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Foram utilizadas 3 variáveis principais correlacionadas entre si: tipo de amostra (galho e folha), quantidade de água utilizada na hidrodestilação (1L - 1,5L) e tempo de extração (1h - 1,5h - 2h). Os dados obtidos foram submetidos à análise de variância e, para comparação das médias dos tratamentos foi utilizado o teste de Tukey ao nível de 5% de probabilidade. Observamos que houve diferença significativa entre os tratamentos testados. O tratamento com 100 g de Folhas de Pau-Rosa quando associado a 1 Litro de água destilada submetida à 1 hora de extração, resultou na maior taxa de rendimento médio (0,70935 ml) além de um teor de linalol médio, mais alto dentro todas as amostras analisadas, sendo igual a 83,11% quando comparados com os demais tratamentos. Concluímos que, o protocolo desenvolvido sobre a extração e o rendimento do óleo de pau-rosa em escala laboratorial é eficiente para o estudo em questão, no entanto, sugerimos novos estudos para a otimização do protocolo a fim de garantir o pleno desenvolvimento sustentável desta espécie de interesse econômico.

Palavras-chave: *Cromatografia; Análise ; Hidrodestilação; Cadeia produtiva; Extração.*

1 INTRODUCTION

In general, essential oils are volatile liquid substances, with a strong and pleasant aroma, that are present in many plant species and be extracted through specific processes, the most commonly used being water vapor drag and hydrodistillation. In nature, essential oils play an important role in protecting plants by acting as antibacterial, antiviral, antifungal, insecticidal and herbivorous agents. In addition, they can attract some insects to favor the dispersion of pollen and seeds, or repel undesirable ones (Lima Junior *et al*., 2024)..

The distillation of organic substances with the use of water vapor is a unitary operation that is technically based on the difference in volatility that the various compounds may present in the raw material. This process uses a boiler or thermal blanket for heat generation, an extractor or distiller, into which the raw material to be extracted is placed, a condenser and a collection flask. The steam that forms due to the heating of water to high temperatures is passed through the material subjected to extraction and deposited in the extractor vessel, dragging the essential oil contained therein. The water vapor-essential oil mixture then moves to the condenser where the phase change occurs due to cooling by indirect contact with cold water. The condensate (oil + hydrolate) is collected in the collection bottle, where phase separation occurs, due to polarity differences, since essential oils are non-polar or slightly polar (Krainovic *et al*., 2017).

Brazil stands out in global production of essential oils, but due to structural problems that have dragged on over the years, this prominence does not translate into satisfactory gains. These problems can include maintaining quality, which is due to lack of public investment. The economic factors command the sector, since the mastery of these conversions is important to make cultivation of plants producing essential oils a more profitable enterprise, thereby adding value to a primary product through chemical technology (Araújo *et al*., 2022).

The bioindustry, which is focused on the cosmetics industry, in addition to bringing money from abroad to the region, offers an opportunity to generate employment in the production chain, not only in the urban area, but especially in the rural area, contributing to the deconcentration of income and, consequently, to the internalization of development. In fact, the enterprises that use natural raw materials usually have as suppliers the rural population that necessarily needs to be aware that the extraction or cultivation of these products has to be associated with the conservation care of these resources. In this way, they will be guaranteed continuity of more than one income option for their families for many years (Lara, 2024).

Rosewood (*Aniba roseodora Ducke*), a species native in Latin America and once very common in Brazil, is widely used for the extraction of linalool. However, due to the high demand, there was a disalignment between the extraction of its essential oil and the length of time for natural regeneration of the species, thus bringing the species to the brink of extinction in the last century. With its essential oil being widely applied to various areas of cosmetics and perfumery, at the height of its extractivism, around the 60s, it reached the impressive number of more than 500 tons of rosewood essential oil extracted and exported annually in the state of Amazonas alone. Linalool is used as a starting compound for several important syntheses, such as linalyl acetate, and was been tested as an acaricide, bactericide and fungicide. In medicine, it has been successfully applied as a sedative, and its anticonvulsant properties are being analyzed. Thus, linalool has a wide application in various areas of human knowledge, and its production in ever-increasing quantities is required. A unique characteristic of linalool in the essential oil is the presence of the asymmetric carbon in its structure, which is a determining factor in its properties (Silva *et al.*, 2001).

The intense exploitation of the species since the last century has placed it in near extinction and, currently, according to Ordinance No. 148 of the Brazilian Ministry of the Environment, published on June 7th, 2022, the situation of the rosewood tree is classified as “in danger”. In addition, previously, on August 25th, 2011, Regulation No. 9 was instituted by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA), in order to regularize and implement procedures for the management and use of rosewood with a view to proposing sustainable management that guarantees the perpetuation of the species. However, the available information is still insufficient to attract investors because it does not give them any assurance that these ventures are economically profitable, environmentally sustainable and socially fair (Araújo *et al.*, 2022).

Seasonal factors, insufficient knowledge of sustainable protocols for plant populations by producers and reduced public investment in the sector undermine the profits of this activity, which without the proper support of the public sector, can lead to the loss of a large economic market. The relevance of this research is evidenced by the history of systematic deforestation over the years, which makes the development of extraction protocols that promote sustainable practices fundamental.

Considering the above, the objective of this work was to develop a sustainable protocol for the extraction of rosewood essential oil on a laboratory scale and obtain the best oil yield and linalool content in relation to branches and leaves of individuals, thus allowing the maintenance of essential oil extraction activities without the risk of extinction of the species.

2 MATERIALS AND METHODS

The research was developed at the Laboratory of Tropical Silviculture and Digital Technologies (LASTED), as well as at the Thematic Laboratory of Chemistry and Natural Products (LTQPN), both linked to the National Institute for Amazonian Research (INPA), in addition to the agile collaboration of the Chromatography Laboratory and Mass Spectrometry (LABCEM) linked to the Analytical Center of the Federal University of Amazonas (UFAM).

Acquisition of plant material

Rosewood leaves and branches were collected from intermediate regions of adult individuals located in the municipality of Maués, Amazonas state, Brazil, in March 2023. More specifically in the areas of the processing plant owned by the Magalgi family (3°24'21.0" S 57°42'25.2" W).

After collecting the material, the leaves and branches were separated and weighed, totaling 8,500 kg of plant material. Then, they were dried in the shade for 4 days, ground, separated and placed in vacuum polyethylene bags and stored in refrigeration at -15 °C until the moment of extraction.

Methodological design and acquisition of the essential oil

In the methodology for extraction of the oil, we used the method of hydrodistillation via a Clevenger-type apparatus, which was coupled to a volumetric flask of 2,000 mL and a thermal blanket for heating.

For this research, three variables were essentially considered: type of sample, amount of distilled water used in extraction and extraction times.

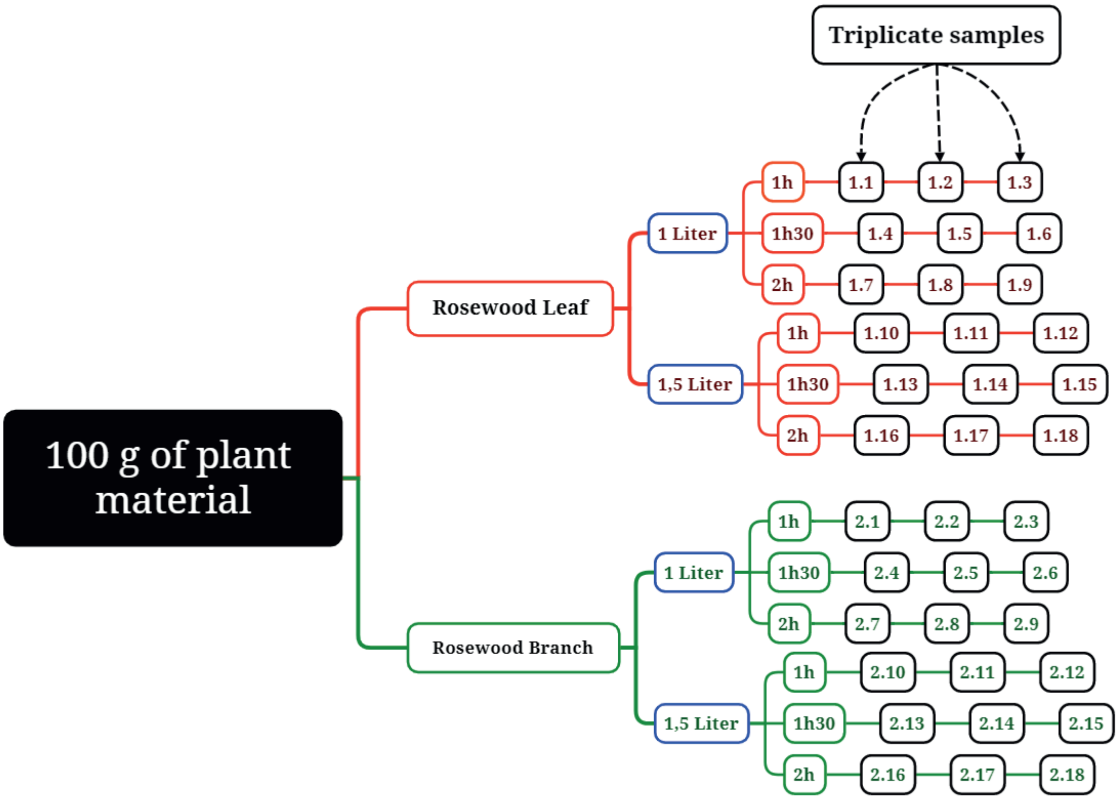
From this, these variables were correlated according to Table 1.

Table 1 - Parameters used in the experiment.

Variables	Parameters
Amount of distilled water	1 L, 1.5 L, 2 L
Extraction time	1 h, 1.5 h and 2 h
Type of sample	rosewood leaves; rosewood branches

For the quantity of distilled water, 1 L and 1.5 L were tested. For the extraction time, 1 h, 1.5 h and 2 h was tested. Finally, the extractions were carried out using leaves and branches of rosewood. When correlating these variables, a total of 36 samples were used, 18 of which were branch samples and 18 were of rosewood leaves, according to figure 1:

Figure 1 - Organizational chart showing how the extraction periods were organized during the research.



Source: Authors.

Thus, all samples were tested in triplicate so that yields could be verified and analyzed statistically.

Chromatographic analysis

For chemical characterization, gas-liquid chromatography (GLC) or gas chromatography is the most widely used methodology for the separation of the constituents of essential oils. The use of capillary columns allows the fractionation of volatile complex mixtures and, in most cases, the individualization of most of its components.

Given its characteristics, this methodology is separative and also analytical because it allows the acquisition of qualitative information. In addition, today, it is considered the most efficient chromatographic modality for the fractionation of volatile complex mixtures and underlies the derived methodologies, such as multidimensional modalities (Marriot *et al.*, 2001). However, the GLC chromatographic record allows one to obtain important information for the qualitative analysis of complex mixtures by the interpretation and retention behavior of the eluted compounds.

The gas-liquid chromatographic analyses were conducted in a chromatograph (Agilent HP 6890) with an automatic injection system with split/splitless injector, in addition to a system with two columns, one non-polar (HP-5) and another polar (Innowax-20), with identical dimensions of 30 m in length, internal diameter of 0.32 mm and films of 0.32 micrometers in thickness. The oven was initially set at 60 °C with a maximum temperature of 150 ºC and a ramp rate of 2 ºC/minute. The period for the analysis of each sample was 45 minutes, and a total of 24 analyses were performed. The drag gas used was hydrogen (H_2), regulated at a continuous flow of 6 mL/minute and each sample was prepared in the proportion of 1 mg/mL - 1 uL of solvent. The injection nozzles were set at a temperature of 240 ºC at a constant pressure of 12.7 atm. Two flame ionization detectors were used at 280 ºC (30/300/24 H_2/AR/N2), this methodology being adapted from Souza *et al.* (2007).

Thus, from a total of 36 samples obtained through extractions, two samples were selected based on the lowest standard deviation observed in each treatment, with the aim of ensuring greater uniformity and precision in the results. Therefore, a total of 24 samples were analyzed via chromatography, which are specified according to Table 2, below:

Table 2 - Details of the samples analyzed via chromatography, highlighting their yield and standard deviation and demonstrating their greater numerical proximity.

Sample	Yield (mL)	Standard Deviation
1.1	0.697	0.017
1.2	0.721	
1.5	0.659	0.217
1.6	0.759	
1.8	0.661	0.023
1.9	0.627	
1.11	0.822	0.032
1.12	0.776	
1.14	0.839	0.044
1.15	0.901	
1.17	0.910	0.102
1.18	0.765	
2.1	0.191	0.003
2.2	0.186	
2.4	0.186	0.015
2.6	0.208	
2.8	0.155	0.019
2.9	0.128	
2.10	0.150	0.010
2.12	0.165	
2.13	0.225	0.011
2.15	0.209	
2.16	0.228	0.020
2.18	0.256	

Additionally, mass spectrometry of the GC-MS type was used to identify the majority compound in the analyzed samples, since this technique is widely used in the detection of organic, volatile and semi-volatile compounds, such as those found in essential oils. The procedure was conducted using a gas chromatograph (Thermo Scientific, TRACE GC Ultra) coupled to a mass spectrometer (Thermo Scientific, ISQ).

Statistical analysis

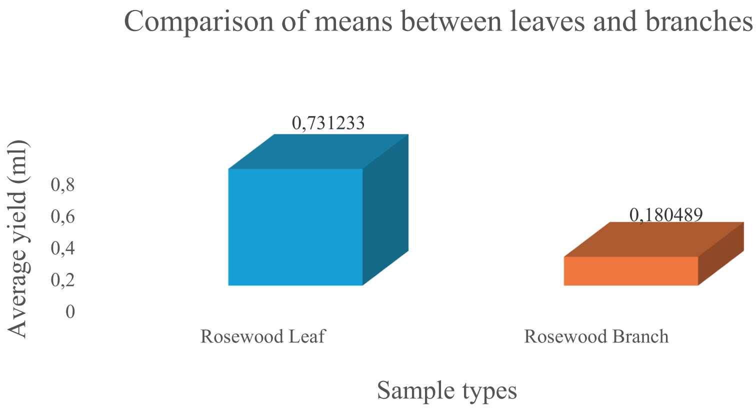
The experimental design adopted in the entire experiment mentioned above was completely randomized. Three replications were used for all the experiments. For all the phases of the experiment, when comparing the groups, the analysis of variance (ANOVA) F-test was used, followed by Fisher's test when a significant difference was detected. A significance level of $p<0.05$ was used in all the analyses. The Minitab version 18 program was used for the analyses.

3 RESULTS

For better organization and visualization, the results are divided according to the comparisons below:

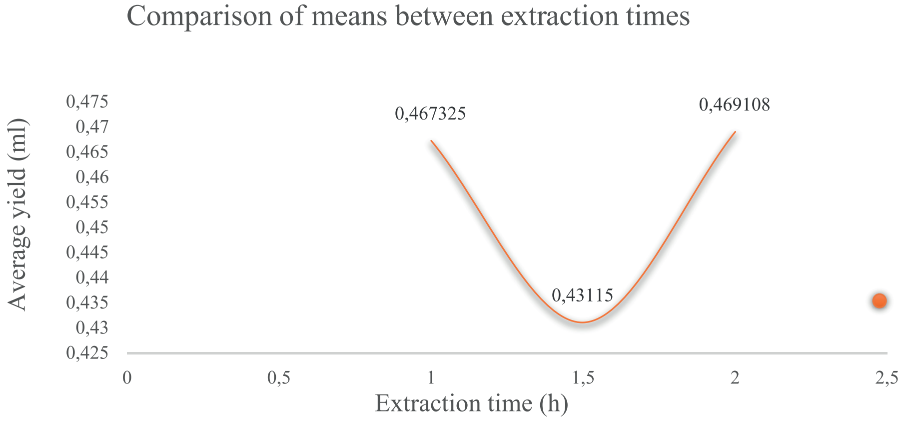
Yield of essential oil by sample type

According to Figure 2, the analysis revealed a significant difference between the two types of samples tested. The data indicate that the rosewood leaves showed an average yield 400% higher than that of the branches, confirming their superiority in terms of yield.



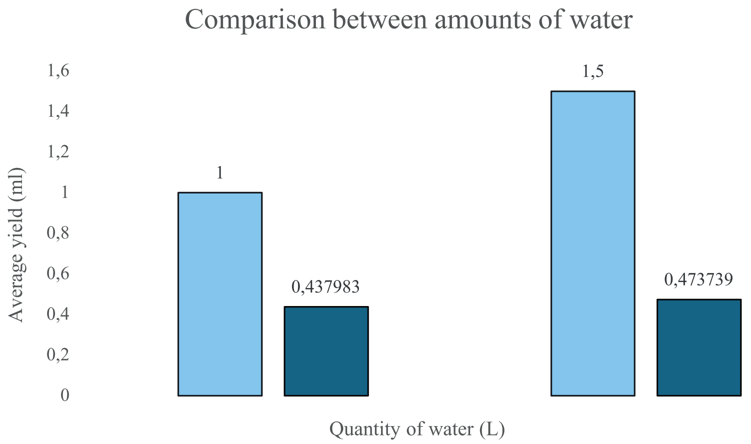
Yield of essential oil per extraction time

According to Figure 3, we observed that the results obtained for yield for the extraction times of 1 h and 2 h are almost identical. However, the extraction time of 1 h is considered preferable, since it presents results extremely close to the maximum yield observed with a much shorter time and a minimum difference of 0.001783 mL between the respective means, with no significant difference between their yields.



Yield of essential oil per quantity of water

The amount of distilled water used during the extractions, when analyzed in isolation, did not show a great influence on the final yield of the essential oil in the samples and, according to Figure 4, there were no significant differences between the yields of the observed tests.



When analyzing the variables involved in the extraction process, it was observed that the combination of rosewood leaves with 1 L of distilled water and an extraction time of 1 h provided the highest average yield of essential oil, achieving 0.792900 mL (Table 3). This result stood out as the highest among the treatments tested, evidencing the potential of this methodology for optimizing process efficiency and increasing productivity, which makes it promising for future applications.

Table 3 - Fisher comparisons of the three variables correlated with each other, at a 95% confidence interval.

Type of material	Time (h)	Water (L)	Average (mL)	Grouping (Fisher)
Leaves	1	1	0.792	A
Leaves	2	1.5	0.780	A
Leaves	1.5	1.5	0.769	A B
Leaves	1	1.5	0.767	A B
Leaves	2	1	0.687	A B
Leaves	1.5	1	0.590	B
Branches	2	1.5	0.212	C
Branches	1.5	1.5	0.198	C
Branches	2	1	0.196	C
Branches	1	1	0.194	C
Branches	1.5	1	0.166	C
Branches	1	1.5	0.114	C

* Means that do not share a letter are significantly different.

Chromatographic analysis and mass spectrometry

However, similar to the results for essential oil yield, in the quantification of linalool using gas chromatography, the best linalool content was found in rosewood leaf samples, extracted with 1 L of distilled water during 1 h of extraction, with an average content of 83.11%. On the other hand, the treatment that obtained the lowest mean content (75.09%) used the following configuration:

- Rosewood branches
- 2 h extraction
- 1.5 L of water

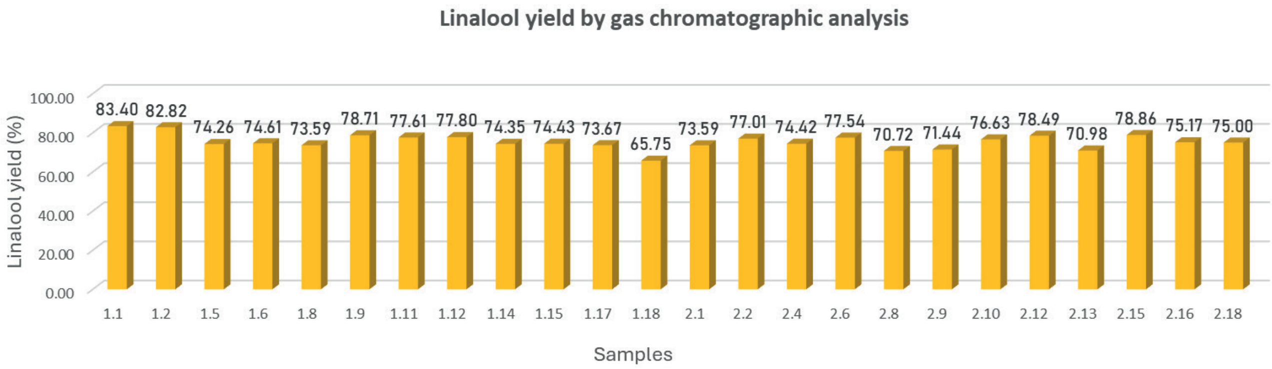
To validate the effectiveness of the protocol that presented the best performance throughout the analysis and considering the superior performance of the rosewood leaves compared to the branches, a comparison was made using the same protocol with the rosewood branches. Thus, Table 4 presents the following data:

Table 4 - Comparison of the same parameter for different types of samples, highlighting their respective linalool contents.

Sample	Type	Time (h)	Water (L)	Weight (g)	Linalool content (%)	Yield (mL)
1.1	Leaves	1	1	100	83.40	0.822
1.2	Leaves	1	1	100	82.82	0.776
2.10	Branches	1	1.5	100	76.63	0.150
2.12	Branches	1	1.5	100	78.49	0.165

The results obtained indicate that the rosewood leaves surpass the branches both in the yield of essential oil and in the amount of linalool, the target compound of the extractions. To facilitate the interpretation of the analysis data, which quantified linalool in the samples, a bar graph was prepared, shown in Figure 5.

Figure 5 - Bar graph demonstrating the linalool yield according to the chromatographic analysis.



Source: Authors.

With regard to the gas chromatography coupled to mass spectrometry, the identification of the chromatograms was performed via comparison with the data available in the NIST library. The identification of compounds whose similarity with the library data exceeded 90% was considered correct. Thus, the result of 98.55% similarity for the majority compound linalool (3,7-dimethylocta-1,6-dien-3-ol, according to the IUPAC nomenclature) was accepted as correct.

4 DISCUSSION

It is essential to highlight that the extraction of rosewood must be carried out responsibly, considering the conservation of the species and the sustainability of local production chains. As the demand for natural products grows, sustainable management practices become crucial so as to protect biodiversity and ensure the economic viability of communities that depend on these resources (Maia *et al.*, 2007).

The extraction carried out using the leaves showed higher yields, both in terms of essential oil and linalool content, when compared to the samples from the branches. This result indicates that distillation, lasting one hour, favors the most effective release of volatile compounds, thus optimizing the efficiency of the extractive process. This relationship between extraction time and yield is fundamental for the development of more effective protocols.

The results of this study substantially corroborate the data presented by Zellner *et al.* (2006) and highlight the considerable potential of essential oil extracted from rosewood leaves for use in the perfumery, cosmetics and pharmaceutical industries. The evidence obtained in this study highlights the superior performance of the leaves of this plant in relation to other sources of essential oils.

Moreover, our findings are in line with the observations of Araujo *et al.* (1971), who reported a higher proportion of linalool in the leaves during the months of December (71.6%) and

January (85.5%). The average linalool content found in this study, which is approximately 75.9%, reinforces the seasonal trend previously identified in other investigations. This shows the influence of environmental conditions on the chemical composition of the oil.

The main component identified, linalool, presented high concentrations, especially in the extractions using 1 L of water during the process. Linalool is widely recognized for its aromatic and therapeutic properties and is a valuable ingredient in the cosmetics and perfumery industry. The presence of other compounds, such as linalyl acetate and geraniol, enriches the aromatic profile of the oil, suggesting a diversified potential for commercial applications.

Additionally, the results converge with the findings of Lara (2021), who identified a higher concentration of linalool in essential oil samples obtained from individuals from the Maués State Forest, Amazonas, Brazil. This finding strongly suggests the potential of this population to be used in sustainable development programs and in the formulation of products of various natures.

Thus, this study not only validates previous research, but also opens up new perspectives in relation to extraction protocols in an environmentally sustainable and economically viable way, are recommended on a laboratory scale and can be adopted by the essential oil extraction industry. The data obtained not only reinforce the relevance of efficient extraction practices, but also contribute to a deeper understanding of the variability of yield and linalool content present in rosewood leaves and branches, depending on the extraction conditions.

All in all, the evidence from this study offers a practical and valuable protocol for the extraction of rosewood essential oil. More than that, it highlights the relevance of a sustainable approach in the exploitation of natural resources. By prioritizing the conservation of tropical forests, we contribute to a more balanced and sustainable future in the Amazon, benefiting both the essential oil industry and local communities. Thus, it is our role to encourage farmers and producers to adopt these methods, promoting development that respects the environment and values the natural wealth of the region.

5 CONCLUSIONS

The results obtained in this study indicate that the extraction of rosewood essential oil, carried out under controlled experimental conditions, can be effectively carried out in a sustainable manner. The use of branches and leaves of the plant not only maximizes the efficiency of the process, but also contributes to the conservation of the species. The proposed experimental protocol, which covers the extraction of leaves, the use of 1 L of water and an extraction period of 1 h, resulted in a significant yield of approximately 0.73 mL of essential oil, with a linalool content of around 83% in the extracted solution. This approach is considered to be viable and effective for the production of essential oil, without compromising the perpetuation of the species. Given these findings, it is recommended that extraction protocols be adopted by local communities and bioindustries focused on this economic activity in order to promote sustainability and boost the economic development of the region. Their implementation may contribute to the valorization of natural resources, while ensuring environmental preservation and strengthening local economies.

6 ACKNOWLEDGMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the granting of scientific initiation and postdoctoral scholarships, respectively, which made this study possible. We also thank the National Institute for Amazonian Research (INPA) for the use of their infrastructure and the opportunity to develop this research. We thank the Laboratório de Cromatografia e Espectrometria de Massa (LABCEM) of the Universidade Federal do Amazonas (UFAM) for the mass spectrometry analyses, in addition to extending sincere thanks to Dr. Sérgio Nunomura, whose support was essential for the realization of chemical analyses at the Laboratório Temático de Química de Produtos Naturais (LTQPN) at INPA. Finally, we thank the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), under registration No. 01.02.016301.040068/2022-15 PDCA, for funding this research.

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