ANTIOXIDANT ACTIVITY AND ENZYME-ASSISTED HYDRODISTILLATION OF *MATOUREA AZUREA* ESSENTIAL OIL

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ABSTRACT

The plant species *Matourea azurea* possesses essential oil rich in β-copaen-4a-ol, along with molecules exhibiting antioxidant, antitumoral, and antimicrobial activities. Treatments with enzymatic digestion have proven to be efficient in increasing the yield of essential oils. In this study, the commercial enzyme Celluclast 1.5 L was used as a pretreatment at concentrations of 1%, 1.5%, and 2%, and the yield was compared with conventional hydrodistillation. Gas Chromatography/Mass Spectrometry was performed to describe the chemical composition of the oil and investigate qualitative and quantitative variations based on enzyme concentration. The analysis of variance (ANOVA) was done with 95% level of confidence. Additionally, antioxidant activity was assessed through DPPH scavenging. According to statistical analysis, the concentrations of 1% and 1.5% showed significant differences with an increase in yield of 32% and 26%, respectively. The chemical qualitative profile changed according to the different cellulase concentration. The essential oil contains compounds with pharmaceutical potential such as myrtenal and viridiflorol, but it exhibits low antioxidant activity with an IC50 of 225.41 µL/mL.

Keywords: DPPH scavenging. cellulase. terpenes.

1 INTRODUCTION

In recent decades the antitumoral, antimicrobial, and antioxidant effects of phytochemicals from various categories such as phenolic compounds, flavonoids, and terpenoids has been studied aiming the isolation and synthesis of these molecules, which are of great value to the pharmaceutical and food industries, as well as to produce biofilms, fuels, and polymers.

Essential oils are products of plant secondary metabolism mainly consisting of mono- and sesquiterpenes and are classified according to their functional group into hydrocarbons, alcohols, aldehydes, ketones, esters, and ethers. [1] Due to the high demand for this raw material, there is an imperative need to increase the extraction yield, and methods using enzymolysis during the process stand out for being eco-friendly, as they do not use environmentally harmful solvents like hexane.

The species *Matourea azurea* (Plantaginaceae) is used as an anti-inflammatory medicine in traditional communities of the Amazon basin. Its essential oil is rich in β-copaen-4α-ol which has antifungal properties and inhibits the development *of Leishmania amazonensis*. [2] Therefore, this study has as objective to analyze the chemical composition of the essential oil and its antioxidant effect and compare the yield obtained from conventional hydrodistillation against enzymatic assisted hydrodistillation.

2 MATERIAL & METHODS

The extraction of the essential oil was carried out through the hydrodistillation process using a Clevenger apparatus for 1.5 hours. The leaves were reduced to approximately 2 cm, the mass/volume ratio of 10% was used in distilled water. The yield was calculated based on the ratio of the volume of extracted oil (mL) over the weight of the plant material (g).

Gas Chromatography/Mass Spectrometry (GC/MS) was performed with adaptations for the Shimadzu QP Plus-2010 system, with the oil solubilized in dichloromethane. The compounds identification was based on the fragmentation pattern observed in mass spectra, comparing these values with those existing in the NIST (National Institute of Standards and Technology) database.

For the antioxidant activity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition method was used. The solution was prepared at a concentration of 0.06 mol/L, with the sample weighed and made up to volume with methanol P.A. to a final volume of 1000 mL. 50 µL of each concentration of the test sample were transferred and added to 1950 µL of the DPPH solution (0.06 mol/L). Concentrations of 50, 100, 200, and 300 µL/mL were used to plot the inhibition curve.

For enzymatic pre-treatment, Celluclast 1.5L (cellulase) in the concentration of 1%, 1.5%, and 2% were used. The experiment was conducted in duplicates, and enzymatic digestion lasted 1 hour in a water bath at 50°C.

The analysis of variance (ANOVA), post-hoc tests and graph plots were done in the Origin 2018 software.

3 RESULTS & DISCUSSION

The plant species *M. azurea* stands out for its essential oils rich in bioactive compounds with high potential for the food, cosmetic, and pharmaceutical markets. The substances identified in the analysis were also found in previous studies. [3] The main compounds are: β-Copaen-4α-ol, pinocarveol, myrtenal, copaene and humulene. The average essential oil yield of the control was 0.43% ± 0.01. For the assays treated previously with cellulase, the yields according to the concentrations were: 1% (0.57% ± 0.01), 1.5% (0.55% ± 0.01), and 2% (0.49 ± 0.03). The figure 1a below shows the volume of the extractions, there is an increase in the absolute values of the essential oil of *M. azurea*. The result of the analysis of variance (ANOVA) in table 1 shows that the p-value is less than 0.05, indicating that at least one of the treatments is statistically different. In figure 1b it is possible to observe that the statistical difference lies between the treatments with 1% and 1.5% of cellulase compared to the control according to the Tukey test, with an increase of 32% and 26%, respectively.

Table 1 ANOVA One-Way for essencial oil enzyme-assisted yield

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Prob>F	R^2
Treatment	3	0.01294	0.00431	13.8	0.014	0.91
Error	4	0.00125	0.000313			
Total	7	0.01419				

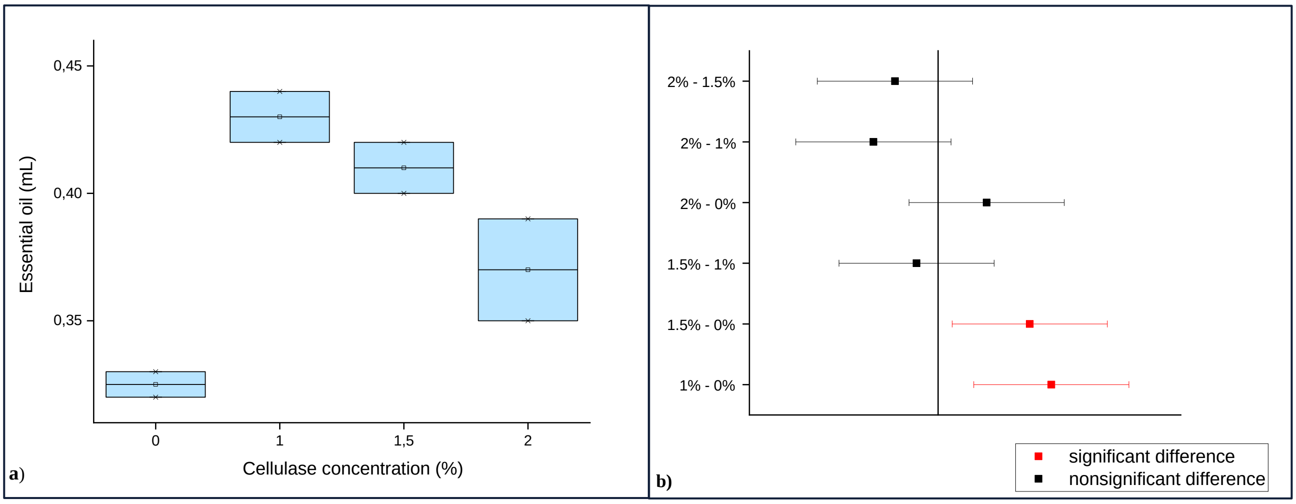

Figure 1 *M. azurea* essential oil extraction assited by cellulase and post-hoc Tukey test.

Regarding antioxidant activity, the percentage of DPPH inhibition by the essential oil increases proportionally with concentration, and by R^2 value it can be stated that the reduction occurs in a dependent and linear nature as shown in figure 2. The highest percentage was observed at a concentration of 300 µL/mL with 62.60%, and the IC50 calculated from the linear equation was 225.41 µL/mL.

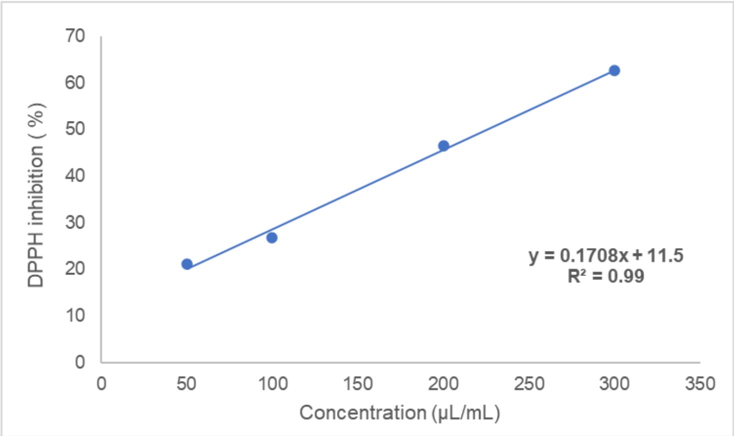

Figure 2 *M. azurea* essential oil DPPH inhibition curve.

The essential oil of *M. azurea* has low antioxidant activity even at high concentrations, however, there is a record in the literature where a scavenging capacity of 90% and an IC50 of 88.5 µg/mL were obtained in DPPH assay. The high presence of tertiary alcohols such as β-copaen-4a-ol, trans-pinocarveol, and viridiflorol decreases the reducing capacity of the solution.

Among the identified substances comprised in table 2 is myrtenal which is a compound widely studied and possess physiologically active molecules that enhance the antioxidant activity of enzymes in the cortex, improve TNF-α levels, and spatial memory. [4]

Cyclosativene also has antioxidant and anticarcinogenic properties, this sesquiterpene can protect the cortical neurological cells from damage caused by H_2O_2-induced cytotoxicity. [5] Viridiflorol is a lipophilic molecule with a topological polar surface area of 20.23; this parameter has been shown to correlate very well with blood-brain barrier penetration, so it is a target for drugs carrier. In addition, viridiflorol inhibits the growth of various types of cancer. [6]

Table 2 Chemical profile of *M. azurea* essential oil obtained by enzimatic assisted hydrodistillation.

Name	RT	c0(%)	c1(%)	c2(%)	c3(%)
Cyclofenchene	8.574	6.3	5.36	-	5.88
Sabinene	9.800	1.52	1.79	0.73	1.74
(-)-β-Pinene	9.955	3.24	2.98	1.74	3.25
β-Cymene	11.448	0.75	0.73	0.53	0.69
D-Limonene	11.607	0.68	0.57	-	0.78
γ-Terpinene	12.542	0.54	0.56	0.47	0.67
Pinocarveol	15.216	9.49	-	8.17	-
trans-Pinocarveol	15.212	-	-	-	10.22
p-Mentha-2,8-dien-1-ol	15.224	-	10.02	-	-
Pinocarvone	15.870	6.31	6.77	5.11	6.06
(-)-Terpinen-4-ol	16.447	2.41	2.35	2.31	2.86
(1R)-(-)- Myrtenal	16.909	8.74	9.91	8.24	9.42
Cyclosativene	22.075	0.29	0.31	0.25	-
Copaene	22.258	4.95	5.84	5.47	4.93
Humulene	24.466	3.8	-	-	-
1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl- Z,Z,Z-	24.475	-	4.2	4.58	5.22
Alloaromadendrene	24.591	0.32	0.39	-	-
Naphthalene	26.067	1.45	1.54	1.79	1.57
β-Copaen-4α-ol	27.920	20.73	20.17	23.93	19.09
Viridiflorol	28.084	4.6	4.22	5.11	4.28

RT= retention time; c0, c1, c2 and c3 = cellulase concentration at 0, 1%, 1.5% and 2% respectively.

4 CONCLUSION

The essential oil of *M. azurea* has a chemical composition of interest to the pharmaceutical and fragrance sectors due to the presence of molecules such as naphthalene, viridiflorol and β-Copaen-4α-ol. The studied chemotype shows low oxidant activity for DPPH assay because of the high number of tertiary alcohols and aldehydes such as myrtenal. The optimal concentration for hydrodistillation pretreated with cellulase is 1% of the enzyme. In the future, analyses with pectinase and enzymatic mixtures will be incorporated into the experimental design.

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ACKNOWLEDGEMENTS

We would like to thank the company LFN Latino Americana for providing the commercial enzymes, the Chemical Analysis Center of the State University of Amazonas, the Pharmacokinetics Study Center Laboratory (NePK) and Biological Activities Laboratory (Biophar). We also thank the funding from the Foundation for Research Support of the State of Amazonas (FAPEAM) and the National Council for Scientific and Technological Development (CNPq).