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ANTIOXIDANT ACTIVITY OF *Amburana cearensis* ETHANOLIC EXTRACT AND ITS IMPACT ON THE OXIDATIVE STABILITY OF SOYBEAN BIODIESEL

ATIVIDADE ANTIOXIDANTE DO EXTRATO ETANÓLICO DE *Amburana cearensis* E SEU IMPACTO NA ESTABILIDADE OXIDATIVA DO BIODIESEL DE SOJA

ACTIVIDAD ANTIOXIDANTE DEL EXTRACTO ETANÓLICO DE *Amburana cearensis* Y SU IMPACTO EN LA ESTABILIDAD OXIDATIVA DEL BIODIÉSEL DE SOJA

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ABSTRACT

This article addresses the study of biodiesel oxidative stability by, for the first time, employing the ethanolic extract obtained from the exudate of *Amburana cearensis* as a natural antioxidant additive. The incorporation of antioxidants is a widely used strategy to mitigate the oxidative degradation of biodiesel; however, synthetic antioxidants are often associated with high cost, potential toxicity, and low biodegradability. In this context, the discovery of new chemical substances has become an area of significant scientific advancement, and the ethanolic extract emerges as a promising and sustainable product. The extract exhibited high antioxidant capacity in a time-dependent manner, as determined by the ABTS•⁺ radical scavenging assay. Thermogravimetric and differential thermal analyses (TG/DTA) demonstrated its thermal stability at elevated temperatures, supporting its applicability under biodiesel processing and storage conditions. Chemical characterization by HPLC and DI-ESI-IT-MSn identified the compounds responsible for the antioxidant activity, with the predominant metabolites associated with coumarins, flavonoids, and phenolic acids. Fragments of five compounds were identified as butein, calycosin, cirsimaritin, 3,5,3′-trihydroxy-7,4′-dimethoxyflavone, and nevadensin. The effectiveness of the extract as an antioxidant in biodiesel was evaluated using the Rancimat method (EN 14112). The treated

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sample exhibited an induction time of 11.03 h, coming very close to limits established by the Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP). Overall, the phytochemicals present in the exudate of *A. cearensis* demonstrate significant scientific, environmental, and socioeconomic potential, supporting its use as a green and regional alternative to synthetic antioxidants.

Keywords: Biodiesel. Natural Antioxidant. *Amburana cearensis*. Oxidative Stability. Ethanolic Extract.

RESUMO

Este artigo aborda o estudo da estabilidade oxidativa do biodiesel, empregando, pela primeira vez, o extrato etanólico obtido do exsudato de *Amburana cearensis* como aditivo antioxidante natural. A incorporação de antioxidantes é uma estratégia amplamente utilizada para mitigar a degradação oxidativa do biodiesel; no entanto, os antioxidantes sintéticos são frequentemente associados a alto custo, potencial toxicidade e baixa biodegradabilidade. Nesse contexto, a descoberta de novas substâncias químicas tornou-se uma área de significativo avanço científico, e o extrato etanólico surge como um produto promissor e sustentável. O extrato exibiu alta capacidade antioxidante de forma dependente do tempo, conforme determinado pelo ensaio de sequestro do radical $ABTS^{\bullet+}$. As análises termogravimétrica e térmica diferencial (TG/DTA) demonstraram sua estabilidade térmica em temperaturas elevadas, corroborando sua aplicabilidade nas condições de processamento e armazenamento do biodiesel. A caracterização química por HPLC e DI-ESI-IT-MSn identificou os compostos responsáveis pela atividade antioxidante, com os metabólitos predominantes associados a cumarinas, flavonoides e ácidos fenólicos. Fragmentos de cinco compostos foram identificados como buteína, calicosina, cirsimaritina, 3,5,3′-triidroxi-7,4′-dimetoxiflavona e nevadensina. A eficácia do extrato como antioxidante em biodiesel foi avaliada pelo método Rancimat (EN 14112). A amostra tratada apresentou um tempo de indução de 11,03 h, aproximando-se bastante dos limites estabelecidos pela Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP). De modo geral, os fitoquímicos presentes no exsudato de A. cearensis demonstram significativo potencial científico, ambiental e socioeconômico, corroborando seu uso como uma alternativa verde e regional aos antioxidantes sintéticos.

Palavras-chave: Biodiesel. Antioxidante Natural. *Amburana cearensis*. Estabilidade Oxidativa. Extrato Etanólico.

RESUMEN

Este artículo aborda el estudio de la estabilidad oxidativa del biodiesel mediante el uso, por primera vez, del extracto etanólico obtenido del exudado de *Amburana cearensis* como aditivo antioxidante natural. La incorporación de antioxidantes es una estrategia ampliamente utilizada para mitigar la degradación oxidativa del biodiesel; sin embargo, los antioxidantes sintéticos suelen estar asociados con un alto costo, toxicidad potencial y baja biodegradabilidad. En este contexto, el descubrimiento de nuevas sustancias químicas se ha convertido en un área de importante avance científico, y el extracto etanólico emerge como un producto prometedor y sostenible. El extracto exhibió una alta capacidad antioxidante de manera dependiente del tiempo, según lo determinado por el ensayo de eliminación de radicales $ABTS^{\bullet+}$. Los análisis termogravimétricos y térmicos diferenciales (TG/DTA) demostraron su estabilidad térmica a temperaturas elevadas, lo que respalda su aplicabilidad en las condiciones de procesamiento y almacenamiento del biodiesel. La

caracterización química por HPLC y DI-ESI-IT-MSn identificó los compuestos responsables de la actividad antioxidante, con los metabolitos predominantes asociados con cumarinas, flavonoides y ácidos fenólicos. Se identificaron fragmentos de cinco compuestos como buteína, calicosina, cirsimaritina, 3,5,3′-trihidroxi-7,4′-dimetoxiflavona y nevadensina. Se evaluó la eficacia del extracto como antioxidante en biodiésel mediante el método Rancimat (EN 14112). La muestra tratada presentó un tiempo de inducción de 11,03 h, muy cercano a los límites establecidos por la Agencia Nacional de Petróleo, Gas Natural y Biocombustibles (ANP) de Brasil. En general, los fitoquímicos presentes en el exudado de A. cearensis demuestran un importante potencial científico, ambiental y socioeconómico, lo que respalda su uso como una alternativa ecológica y regional a los antioxidantes sintéticos.

Palabras clave: Biodiésel. Antioxidante Natural. *Amburana cearensis*. Estabilidad Oxidativa. Extracto Etanólico.

1 INTRODUCTION

Biodiesel is a biofuel obtained through the transesterification of oils and fats and is widely recognized for its renewable origin and its contribution to reducing greenhouse gas emissions. However, oxidative instability remains one of the main technical and commercial challenges for its production and efficient use. This limitation arises from the susceptibility of fatty acid methyl esters to oxidation during storage and transportation, which can lead to undesirable changes in the physicochemical properties of the fuel, such as increased acidity and viscosity, as well as sediment formation, thereby compromising the performance of diesel engines (David & Kopac, 2023).

In order to ensure the quality and competitiveness of Brazilian biodiesel, Resolution ANP No. 920, issued on April 4, 2023, established the technical specifications for biodiesel marketed in the country, including requirements related to oxidative stability. This regulation defines an antioxidant additive as a substance capable of delaying the lipid oxidation of oils and fats, and it mandates that biodiesel in circulation contain additives with this function. Furthermore, the type and concentration of the antioxidant used must be stated in the product's quality certificate (Brazil, 2023).

Several studies report that synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) are traditionally employed to enhance the oxidative stability of biodiesel by interrupting the chain reactions responsible for fuel degradation. Nevertheless, these compounds present relevant limitations, including negative environmental impacts, performance restrictions at low temperatures, and low biodegradability, which has stimulated the search for more sustainable alternatives aligned with the principles of Green Chemistry (David & Kopac, 2023).

In this context, natural antioxidants extracted from plant sources have emerged as promising alternatives due to their lower toxicity, higher biodegradability, and broad availability of phenolic compounds with recognized antioxidant activity. The main natural sources of polysaccharides include fungi, cellulose, plant exudates, and algae (Paula et al., 2018; de Oliveira Farias et al., 2025). Studies involving plant extracts demonstrate that natural phenolic compounds can act as electron or hydrogen donors to free radicals, thereby interrupting oxidative chain reactions in a manner similar to that observed in lipid systems such as biodiesel.

The gum of Amburana cearensis (GAmb) is a polyelectrolyte polysaccharide obtained from the trunk exudate of this species and has been widely investigated due to its

phytochemical composition rich in coumarins and other phenolic compounds with relevant antioxidant activity. Ethnobotanical and phytochemical studies indicate that ethanolic extracts and fractions of A. cearensis exhibit significant antioxidant activity in free radical assays, mainly attributed to the presence of flavonoids and phenols, highlighting its potential as a source of natural antioxidants for technological applications (de Oliveira Farias et al., 2025).

Considering the need to add antioxidants to biodiesel in order to enhance its oxidative stability due to the presence of reactive sites associated with methylenic carbons adjacent to unsaturations, which are susceptible to auto-oxidation it becomes relevant to develop alternatives to the synthetic antioxidants currently in use. These conventional additives exhibit high production costs, elevated toxicity levels, and low biodegradability. In this sense, this study evaluates the ethanolic extract obtained from the gum of Amburana cearensis as a natural antioxidant, whose results demonstrated compliance with the standards established by the Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP). The phytochemicals present in A. cearensis gum reveal high scientific, social, and environmental relevance, contributing to the development of sustainable solutions based on the use of regional resources, in line with the principles of Green Chemistry. Thus, this study presents an innovative approach by highlighting the scientific, environmental, and socioeconomic potential of natural antioxidants applied to the oxidative stabilization of biodiesel.

2 METHODOLOGY

2.1 COLLECTION OF CRUDE EXUDATE FROM AMBURANA CEARENSIS

The exudate of Amburana cearensis (Sis-Gen with number A5A1D05) was collected in July and September 2022 in the region of Cocal da Estação, Piauí, Brazil (Latitude: 3°28′33″ S; Longitude: 41°33′28″ W). After collection, the plant material was sun-dried for two weeks, or until complete resin solidification. Subsequently, the crude exudate was obtained by removing macroscopic residues such as fragments of trunk bark and branches. The material was then ground and sieved using a 425 μm mesh sieve.

2.2 PREPARATION OF THE ETHANOLIC EXTRACT OF AMBURANA CEARENSIS

The sieved material was subjected to ethanolic extraction following the methodology described by Samrot et al. (2019). For this purpose, 10.0 g of the exudate were weighed using an analytical balance (Sartorius ED124s Extend) and transferred to a 150 mL beaker, to which 50.0 mL of ethanol (ACS Dinâmica, analytical grade, 95%) were added. The mixture

was maintained under magnetic stirring at room temperature for 2 h. After this period, the system was allowed to stand for decantation, and the liquid phase was carefully separated and stored for subsequent use.

The obtained extract was concentrated by solvent evaporation through dropwise heating in a beaker placed on a heating plate. After complete drying of the material in a desiccator, the resulting solid was weighed on an analytical balance, thus obtaining the ethanolic extract of the Amburana cearensis exudate.

2.3 ANTIOXIDANT ACTIVITY

The antioxidant activity of the ethanolic extract of Amburana cearensis exudate was determined by the ABTS•⁺ radical cation scavenging assay [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], according to the methodology described by Gião et al. (2007), with adaptations. Statistical analysis was performed using one-way analysis of variance (one-way ANOVA), followed by Tukey's multiple comparison test (Gião et al., 2007).

The experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation, with a significance level of $p < 0.05$. The extract was dissolved in ethanol and prepared in a series of dilutions, with concentrations ranging from 31.25 to 0.24 μg mL^{-1}. The ABTS•⁺ radical solution was prepared in advance and stored in the dark. Aliquots of the extract solutions were added to the radical solution, and the mixture was incubated in the dark for 6 min.

The ABTS•⁺ radical scavenging capacity of the extract was monitored at three reaction times (6, 30, and 60 min) using an ELISA-type microplate reader (SpectraMax, Molecular Devices, USA). Antioxidant activity was expressed as the percentage of radical inhibition relative to the control, and the half-maximal inhibitory concentration (IC_{50}) values were determined from inhibition curves as a function of extract concentration.

2.4 THERMOGRAVIMETRIC ANALYSIS (TGA)

Thermogravimetric (TG/DTG) analyses were performed using a Shimadzu DTG-60H thermal analyzer. Approximately 5–10 mg of the sample was placed in alumina (or platinum) crucibles, which had been previously cleaned and dried. Two atmospheric conditions were applied: an inert argon atmosphere and an oxidative oxygen atmosphere, both with a flow rate of 100 mL min^{-1} and a heating rate of 20 °C min^{-1} from room temperature to 600 °C. The

data were acquired using an SDT Q600 V20.9 Build 20 Module: standard DSC-TGA thermal analyzer.

2.5 HPLC-DAD ANALYSIS OF THE ETHANOLIC EXTRACT

The ethanolic extract of Amburana was analyzed by high-performance liquid chromatography (HPLC) using an analytical system equipped with a degasser (DGU-20A5R), pump (LC-20AT), LPGE valve kit, autosampler (SIL-20AHT), column oven (CTO-20A), diode array detector (DAD, SPD-M20A), and controller (CBM-20A), all from Shimadzu. Instrument control and data acquisition were performed using LabSolutions software (Shimadzu).

Chromatographic separation was carried out on a C18 column (Luna®, Phenomenex; 250.0 × 4.6 mm; 5 µm). Prior to analysis, the sample was subjected to a clean-up procedure by solid-phase extraction (SPE). An aliquot of 10 mg of the extract was dissolved in 2.0 mL of methanol (MeOH) and applied to a C18 cartridge previously conditioned with the same solvent. Elution was performed with 20.0 mL of MeOH. After drying, 5 mg of the eluted material were redissolved in 2.0 mL of a MeOH/H_2O mixture (1:1, v/v) and filtered through a nylon membrane (47 mm diameter, 0.22 µm pore size, Chromafil® Xtra).

Chromatographic analysis of the Amburana ethanolic extract (2.5 mg mL^{-1}) was performed in gradient elution mode according to the following program: 0–50 min, 5–100% solvent B; 50–60 min, 100% solvent B. The mobile phase consisted of ultrapure water (solvent A) and methanol (solvent B), with a flow rate of 1.0 mL min^{-1}, an injection volume of 10 µL, and the column temperature maintained at 40 °C. Detection was carried out over a spectral range of 200–800 nm.

2.6 DI-ESI-IT-MSN ANALYSIS OF THE ETHANOLIC EXTRACT

The ethanolic extract was dissolved in methanol at a concentration of 0.1 mg mL^{-1} and filtered through a polytetrafluoroethylene (PTFE) membrane with a pore size of 0.22 µm prior to spectrometric analysis. Mass spectra were acquired by direct infusion (DI) into a mass spectrometer equipped with an ion trap analyzer (IT/MS, AmaZon X, Bruker Daltonics, Bremen, Germany) and an electrospray ionization (ESI) source, operating in the DI-ESI-IT-MSn mode.

Analyses were performed in the negative ion mode [ESI(−)], with a mass scan range of m/z 100–1500 Da, a syringe flow rate set to 3.0 µL min^{-1}, a capillary voltage of 4.5 kV, a drying gas (N_2) flow rate of 5.0 L min^{-1}, a nebulizer pressure of 8.0 psi, and a source

temperature maintained at 250 °C. For the MS^n experiments, the collision energy, signal amplitude, acquisition time, and radiofrequency parameters were individually optimized for each precursor ion in order to maximize the quality of the spectral information obtained.

Data acquisition and processing were carried out using Bruker Compass Data Analysis software (version 4.3), as provided by the manufacturer.

2.7 RANCIMAT METHOD

The analytical method complies with international specifications for biodiesel commercialization and is accepted in Europe, the United States, and Brazil, where it is included in the standards EN 14112 (2003) and the Technical Regulation ANP No. 920/2023. According to the EN 14112 standardized method, oxidation is induced by passing a stream of air through the biodiesel sample at a flow rate of 10 L h^{-1} while maintaining a constant temperature of 110 °C.

The conductivity of the measuring solution is continuously monitored, and an oxidation curve is obtained, whose inflection point can be determined by the intersection of two tangents. The induction period was determined as the point of intersection of the two tangents of the conductivity curve (Jain & Sharma, 2011).

3 RESULTS AND DISCUSSION

3.1 PROCESSING OF THE EXUDATE AND OBTAINING THE ETHANOLIC EXTRACT OF AMBURANA CEARENSIS

The sequence shown in Figure 1 shows the visual and physical evolution of the exudate from Amburana cearensis throughout the process of obtaining the ethanolic extract, allowing us to infer the efficiency of the steps applied. The raw exudate initially appears in the form of irregular solid aggregates, with characteristic coloration and high rigidity, aspects that reflect the natural process of exudation and solidification of the plant material (Figure 1A). After physical processing, a significant reduction in particle size and greater homogeneity of the material is observed, which favors the release of bioactive compounds present in the polysaccharide matrix (Figures 1B and 1C).

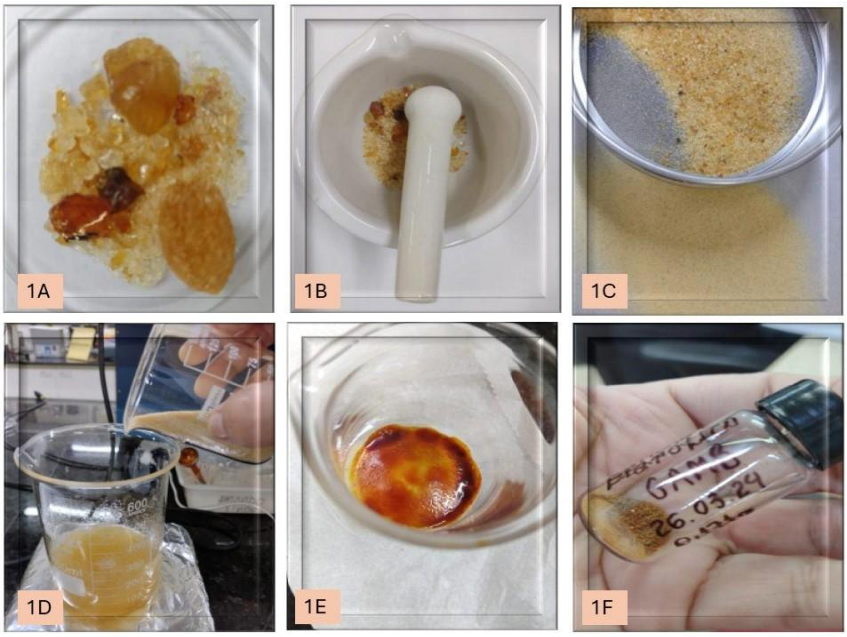
The interaction of the exudate with the ethanolic solvent results in visible changes in the system, notably the coloration of the liquid phase, indicative of the solubilization of organic compounds, possibly associated with phenols, flavonoids, and coumarins previously reported in the literature for the species (Figure 1D). This behavior suggests efficient extraction of the

constituents of interest, corroborating studies that point to ethanol as a suitable solvent for obtaining natural antioxidants from plant matrices.

Thus, the material shown in Figure 1F corresponds to the dry ethanolic extract of Amburana cearensis exudate, characterized by its homogeneous appearance and physical stability, demonstrating the efficiency of the extraction process and its suitability for subsequent analyses and technological applications proposed in this study.

Figure 1

Extraction and purification of the ethanolic extract from the exudate of Amburana cearensis.



3.2 ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACT OF AMBURANA CEARENSIS EXUDATE

The antioxidant activity of the ethanolic extract of Amburana cearensis exudate was evaluated using the ABTS•+ radical scavenging assay, with statistical analysis performed by one-way ANOVA, followed by Tukey's multiple comparison test. The results showed high antioxidant capacity of the extract, with behavior dependent on the reaction time between the radical and the bioactive compounds present in the sample (Sanches et al., 2025).

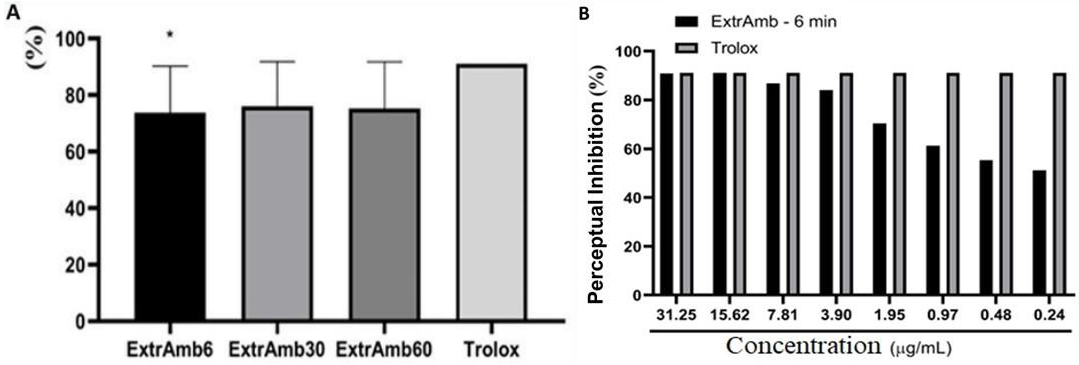
The mean inhibitory concentration (IC_{50}) values decreased progressively over time, indicating increased antioxidant efficiency with prolonged reaction. IC_{50} values of 1.562 µg/mL after 6 minutes, 1.176 µg/mL after 30 minutes, and 0.9961 µg/mL after 60 minutes

were observed. This behavior suggests favorable reaction kinetics, in which the antioxidant compounds in the extract continue to neutralize the ABTS•+ radical efficiently over time.

Comparison with the standard antioxidant Trolox revealed a statistically significant difference ($p < 0.05$) only at the initial time of 6 minutes. At 30 and 60 minutes, no statistically significant differences were observed, indicating that the average percentage of ABTS•+ radical inhibition by the extract was equivalent to that of Trolox. These results are illustrated in Figure 2A, which demonstrates the similarity of antioxidant activity between the extract and the standard at longer reaction times, while Figure 2B shows the antioxidant response at the initial time of 6 minutes as a function of extract concentration.

Figure 2

(A) Percentage inhibition of antioxidant activity similar to the Trolox standard at 30 and 60 minutes. (B) Percentage inhibition of antioxidant activity compared to the Trolox standard considering the 6-minute time period during which the substance was left to react in the dark and its concentration in (µg/mL).



3.3 TGA ANALYSIS OF THE ETHANOLIC EXTRACT OF AMBURANA CEARENSIS

The experimental tests were carried out using the ethanolic extract of Amburana cearensis exudate, as well as purified gum (GAmb), obtained from the same plant material. It was found that GAmb had high water solubility, a characteristic that imposed significant limitations on its dispersion and solubilization in biodiesel, a predominantly lipophilic system. This incompatibility compromised the homogeneity of the formulations and made their direct application as an antioxidant additive unfeasible, leading to the discontinuation of tests with this fraction. In contrast, the ethanolic extract showed greater affinity with the lipid matrix of biodiesel, demonstrating greater potential for technological application. Furthermore, this

extract is obtained from a previously discarded stage of the purification process, which reinforces its use from a technological, economic, and environmental perspective.

Thermogravimetric analysis (TGA), associated with the derivative mass loss curves (DTG) of the ethanolic extract and GAmb (Figures 4A and 4B), revealed similar thermal profiles, characterized by the occurrence of two main degradation events during heating. The first event was observed between 40 and 120 °C for the ethanolic extract and between 45 and 151 °C for GAmb, with mass losses greater than 8%. This stage is predominantly attributed to the elimination of residual moisture and the volatilization of low molar mass compounds. The DTG curves showed the presence of multiple peaks in this temperature range, indicating that mass loss occurs in stages. The broadening and smoothing of the peaks was also observed, especially for GAmb, suggesting a reduction in the content of volatile and hydrophilic constituents as a result of the exudate purification process (Mothé; de Freitas, 2014; Sampaio et al., 2016).

The second mass loss event occurred at higher temperatures, between 281 and 600 °C for the ethanolic extract and between 250 and 600 °C for GAmb, with average losses of approximately 50.0% and 54.7%, respectively. This stage is associated with the thermal degradation of the polymer structure, involving mechanisms of depolymerization, decarbonylation, and pyrolytic decomposition, with the consequent formation and release of gaseous products such as water, carbon monoxide, and light hydrocarbons. The initial degradation temperature indicates that the ethanolic extract has thermal stability up to approximately 281 °C, while GAmb maintains stability up to about 250 °C. These values are significantly higher than the usual temperatures for storage, handling, and application of biodiesel, which highlights the thermal suitability of the ethanolic extract for use as an antioxidant additive (Mothé; de Freitas, 2014; Sampaio et al., 2016).

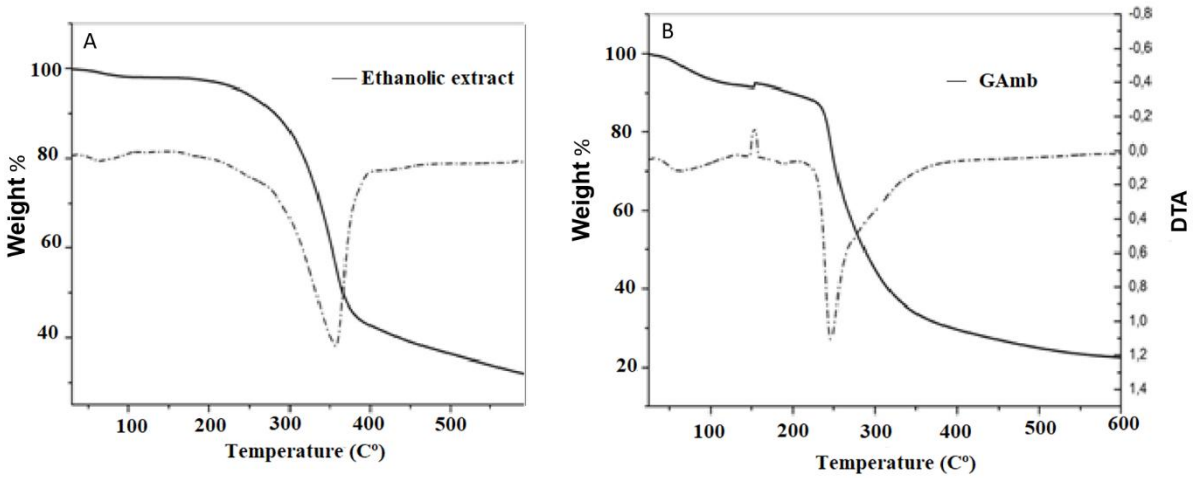
Overall, the results show that although the ethanolic extract and purified gum from Amburana cearensis exhibit similar thermal behavior, differences in solubility and compatibility with the lipid matrix are decisive for their application in biodiesel. The ethanolic extract stood out as the most promising fraction, not only because of its greater affinity with biodiesel, but also because of its high thermal stability and antioxidant potential associated with the presence of phenolic compounds, consolidating its viability as a natural, efficient, and sustainable alternative to synthetic antioxidants used in the oxidative stabilization of biodiesel.

Thermogravimetric analysis (TGA) is used to investigate processes related to thermal stability, decomposition, dehydration, and oxidation, by measuring changes in a sample's mass as a function of temperature and time during heating (TENGKU-ROZAINA, BIRCH, 2019; XIAO et al., 2014).

Among the studies reviewed that included an experimental thermogravimetric analysis, we can cite analyses of other substances containing antioxidant elements, such as the analysis of microparticles contained in pequi oil (Caryocar coriaceum). According to the study published by Castelo et al., 2020, pequi oil degraded at 424°C. This temperature is higher than the degradation temperature of the ethanolic extract of Amburana cearensis, highlighting the difference between the two: the extract studied here was obtained by evaporation on a hot plate and is in solid form, whereas pequi oil, despite undergoing several processes, It was not an ethanolic extract, and its final form is liquid.

Figure 3

Graphs showing the results of thermal analysis of the ethanolic extract (3A) and GAmb (3B), showing mass loss in relation to temperature increase.



3.4 HPLC ANALYSIS OF THE ETHANOLIC EXTRACT OF AMBURANA CEARENSIS

The HPLC-DAD chromatogram of the ethanolic extract of Amburana recorded at 254 nm on a C18 column revealed a profile dominated by compounds of intermediate to low polarity, with most signals eluting between approximately 28 and 45 min. A major peak was observed around 36–37 min, indicating the presence of a predominant metabolite, possibly associated with coumarin derivatives, widely described as chemical markers of the species

(coumarins, flavonoids, phenolic acids). Secondary peaks in the regions of 30–34 min and 40–45 min suggest the presence of flavonoids and other less polar phenolic compounds. The stable baseline and good resolution between the main peaks indicate adequate chromatographic efficiency and sample preparation (Dantas et al., 2015; Maia et al., 2020).

Figure 4

Chromatogram of the ethanolic extract of Amburona. l=254 nm. C-18 column, mobile phase: MeOH-H2O, exploratory gradient: 0-50 min, 5-100% MeOH; 50-60 min, 100% MeOH, mobile phase flow rate of 1.0 mL min-1.

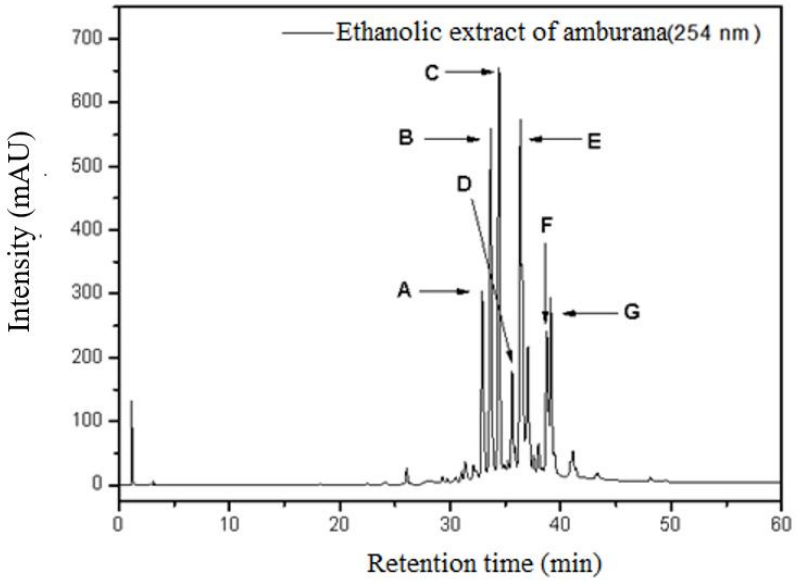
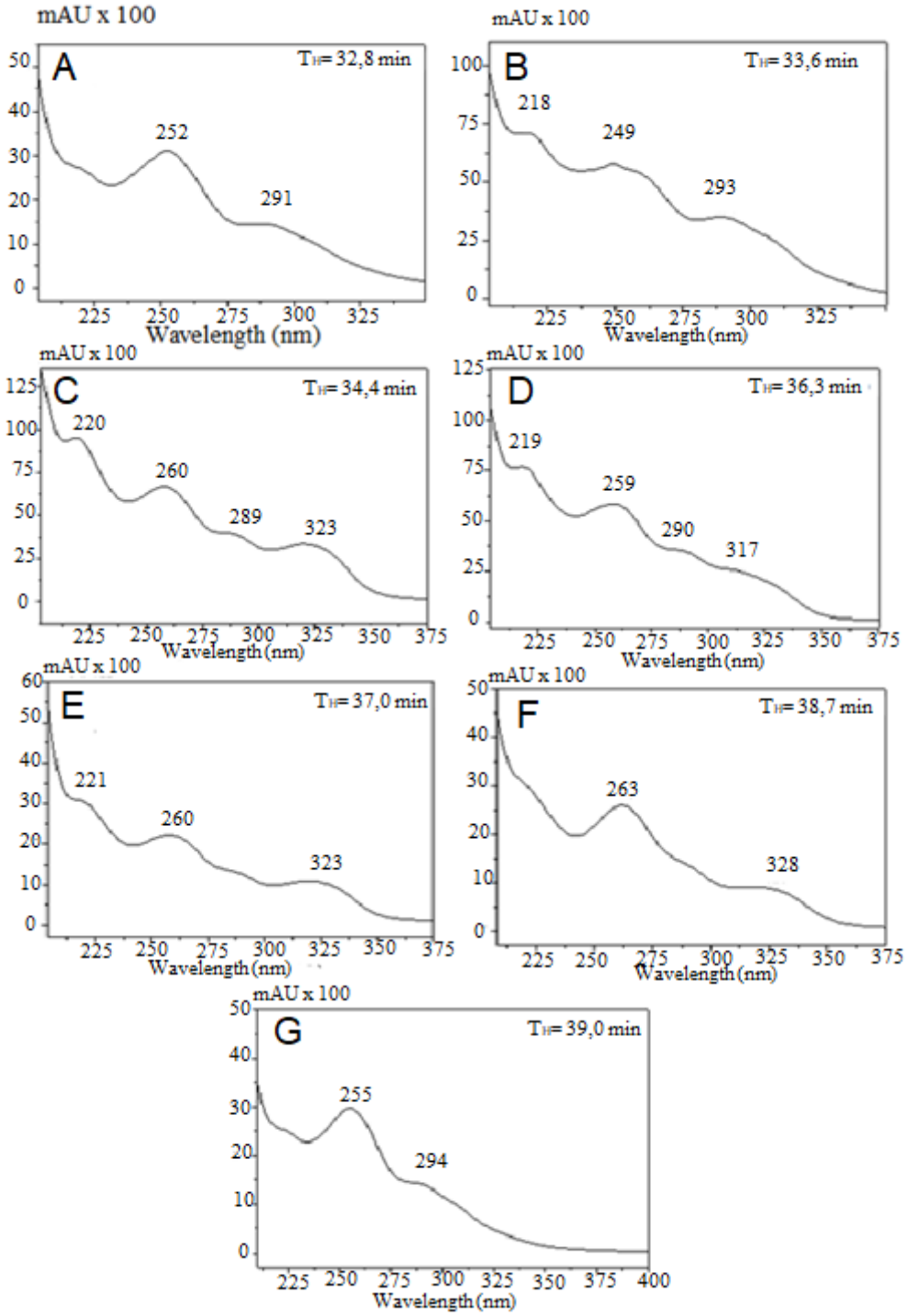


Figure 5 shows the UV-DAD spectra (200-400 nm) of the compounds that eluted from the ethanolic extract of Amburana between 30 and 40 minutes of retention time (RT). Peaks A-G correspond to a different chromatographic peak with specific retention times for each compound. Bands between 250-280 nm indicate the presence of aromatic rings and phenolic compounds, and absorption between 300-350 nm indicates flavonoids and coumarins (Benheim et al., 2011; Canuto et al., 2014).

Figure 5

UV-DAD spectra (200-400 nm, A-G) of the ethanolic extract of Amburana at retention times between 30-40 min.



In the analysis by DI-ESI-IT-MS/MSn in negative ion mode, a total of 5 compounds with retention times of 32.8–34.4 min may indicate more polar phenolic compounds. The predominant peak at 36.3–37.0 min shows the major product of the extract, probably

coumarin or a methoxylated derivative. Compounds such as flavonoids were found in the ethanolic extract of Amburana: chacona (1), isoflavone (2), flavones (3 and 5), and flavonol (4) (Figures 6–7), corroborating the HPLC-DAD data. In Figure 7, we can observe the fragments of the compounds found in the antioxidants provided by the extract (Silveira et al., 2022).

Figure 6

Chemical profile of the ethanolic extract of Amburana by DI-ESI-IT-MS/MSn in negative ion mode.

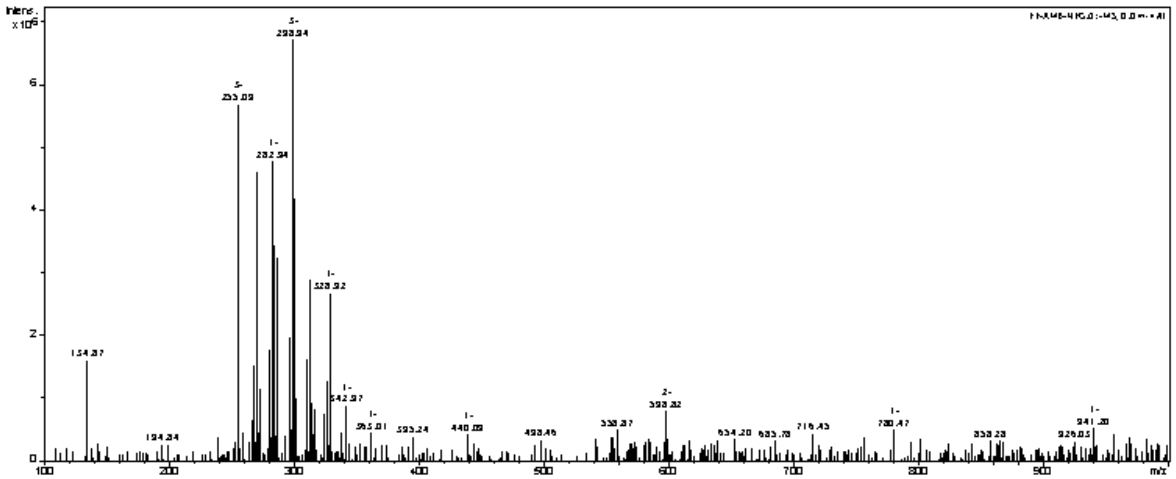
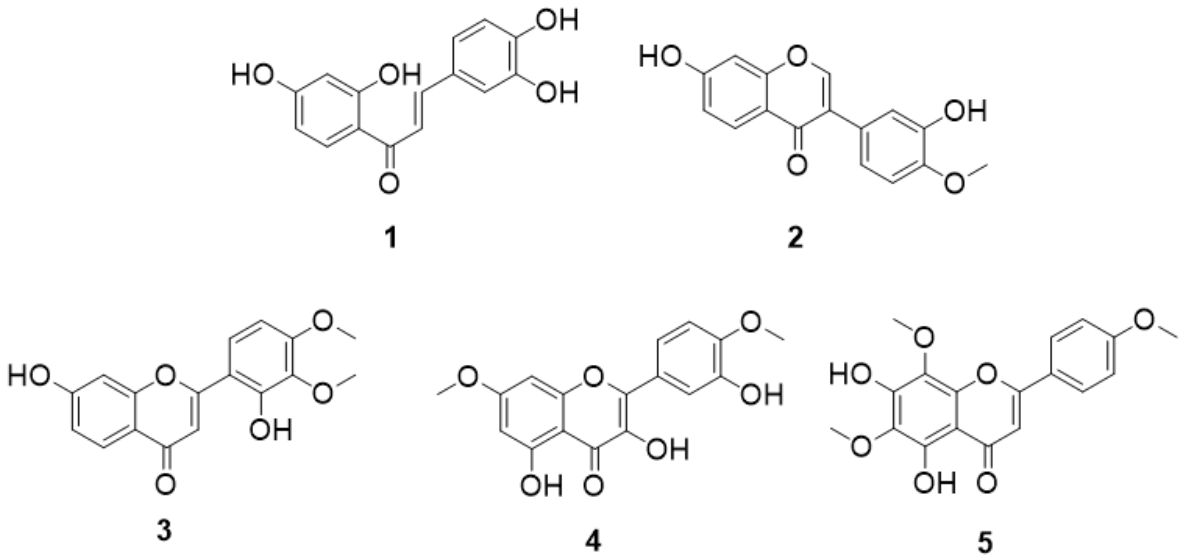


Figure 7

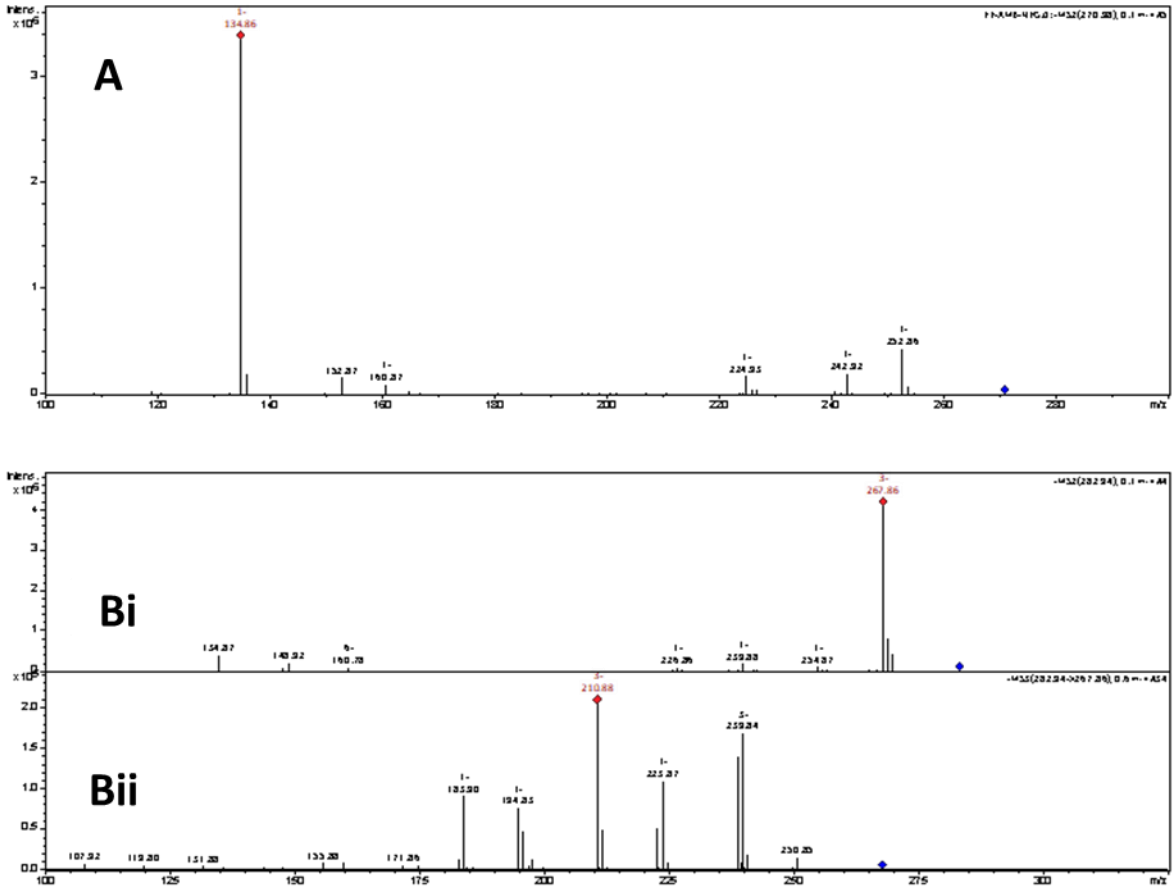
Chemical constituents annotated in the ethanolic extract of Amburana by DI-ESI-IT-MSn.



The ion of the deprotonated molecule at m/z 271.0 [M-H]-, presented fragment ions at m/z 252.8 [M-H-H2O]-, 224.9 [M-H-H2O-CO]- and 134.8 [M-H-C6H7O2]-, referring to an alpha cleavage at the carbonyl (Figure 8A). The compound was annotated as butein (1). (Liu et al., 2020; Silveira et al., 2022). Compound 2 showed a deprotonated molecule ion at m/z 282.9 [M-H]- and a fragment ion in the MS2 spectrum at m/z 267.8 [M-H-CH3]-. The MS3 spectrum showed fragment ions at m/z 238.9 [M-H-CH3-CO]-, 223.8 [M-H-CH3-CO2]-, and m/z 210.8 [M-H-CH3-C2O2H]-. (Figure 8Bi-Bii). The compound was annotated as calicosin (2). (Wang et al., 2008).

Figure 8

MS2 mass spectrum of (A) precursor ion m/z 271.0 [M-H]- (compound 1), (Bi) and MS3 (Bii) of precursor ion m/z 282.9 [M-H]- (compound 2).



The ions of the deprotonated molecule m/z 312.9 [M-H]- and 342.9 [M-H]-, were noted as being methoxylated flavones. Compound 3 presented a deprotonated molecular ion m/z 312.9 [M-H]- and fragment ions in the MS2 spectrum at m/z 297.8 [M-H-CH3]-, in the MS3 spectrum at m/z 282.8 [M-H-(CH3)2]- and MS4 m/z 254.8 [M-H-(CH3)2-CO]- (Figure 9Ai-

Aiii), annotated as cirsimaritin (3). (Boukhalkhal et al., 2020). The mass spectrum of compound 4 showed a deprotonated molecule ion at m/z 328.9 [M-H]-, and in the MS2 spectrum, the fragment ions m/z 313.9 [M-H-CH3]-, 298.9 [M-H-(CH3)2]-, 285 [M-H-CO2]-, and 271 [M-H-(CH3)2-CO]- (Figure 9B).

The compound was noted as 3,5,3′-trihydroxy-7,4′-dimethoxyflavone (4) (Falcão et al., 2008). For compound 5, a deprotonated molecule ion was observed at m/z 342.9 [M-H]-, the MS2 spectrum showed a fragment ion m/z 327.9 [M-H-CH3]-, the MS3 m/z 312.9 [M-H-(CH3)2]-, and MS4 m/z 297.8 [M-H-(CH3)3]-, corresponding to successive losses of methyl groups. In addition, fragment ions were observed at m/z 284.8 [M-H-(CH3)2-CO]-, m/z 269.8 [M-H-(CH3)2-CO-CH3]- and m/z 241.8 [M-H-(CH3)2-CO-CH3-CO]- (Figure 9Ci-Ciii), making it possible to identify the compound as nevadensine (5). (Végh et al., 2017).

All these results show which constituents contained in the ethanolic extract of Amburana contributed to the improvement in oxidation resistance. This stability of more than 11.03 hours suggests less formation of compounds in engines and fuel systems. The test was performed on the Rancimat EN 14112 a (B. B. Ferrari et al., 2021). We were able to identify the substances added to biodiesel that are capable of minimizing oxidation reactions, preventing the onset or anticipating the end of oxidation reactions. To verify its effectiveness in biodiesel, we used the Rancimat method shown in Figure 10. Table 1 shows the compounds identified by DI-ESI-IT-MSn.

Figure 9

MS2 (Ai), MS3 (Aii), and MS4 (Aiii) mass spectra of the precursor ion m/z 312.9 [M-H]- (compound 3). (B) Precursor ion m/z 328.9 [M-H]- (compound 4). (Ci) and MS3 (Cii) and MS4 (Ciii) of the precursor ion m/z 342.9 [M-H]- (compound 5).

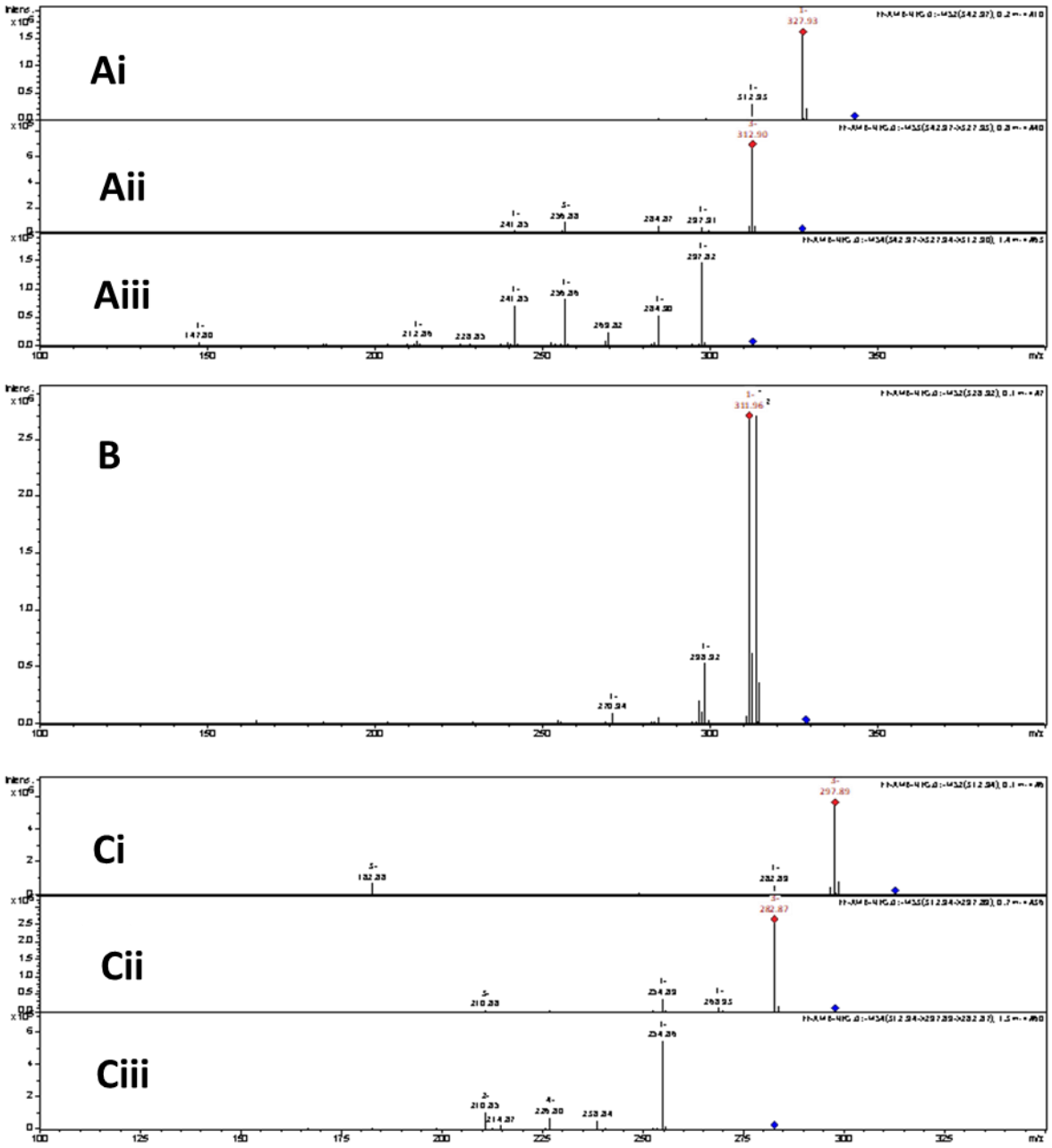


Table 1

Compounds annotated by DI-ESI-IT-MSn in the ethanolic extract of Amburana.

DI-ESI-IT-MSⁿ						
Nº	Formula Molecular	Massa Molecular	[M-H]⁻ (*m/z*)	MS/MSⁿ fragmentos (abundância em porcentagem/%)	Composto	Ref.
1	$C_{15}H_{12}O_5$	272	271,0	MS^2 [270,9]: 252,8 (13); 224,9 (15); **134,8** (100)	Buteina	Liu et al., 2020
2	$C_{16}H_{12}O_5$	284	282,9	MS^2 [282,9]: **267,8** (100); 238,9 (5) MS^3 [267,8]: 238,8 (68); 223,8 (53); **210,8** (100)	Calicosina	Wang et al., 2008
3	$C_{17}H_{14}O_6$	314	312,9	MS^2 [312,9]: **297,8** (100); 282,8 (11) MS^3 [297,8]: **282,8** (100); 254,8 (15) MS^4 [282,8]: **254,8** (100); 226,8 (13)	Cirsimaritina	Boukhalkhal et al., 2020
4	$C_{18}H_{16}O_7$	330	328,9	MS^2 [328,9]: **313,9** (100); 298,9 (19); 285 (1), 271 (3)	3,5,3′-tri-hidroxi-7,4′-dimetoxiflavona	Falcão et al., 2012
5	$C_{17}H_{14}O_7$	344	342,9	MS^2 [342,9]: **327,9** (100); 312,9 (19) MS^3 [327,9]: **312,9** (100); 297,9 (7), 284,8 (4), 256,8 (12) MS^4 [312,9]: **297,9** (100); 284,9 (36), 256,8 (55), 241,8 (24)	Nevadensina	Végh et al., 2017

3.5 ANALYSIS OF THE OXIDATIVE STABILITY OF COMMERCIAL SOYBEAN BIODIESEL IN THE PRESENCE AND ABSENCE OF ETHANOLIC EXTRACT FROM AMBURANA CEARENSIS

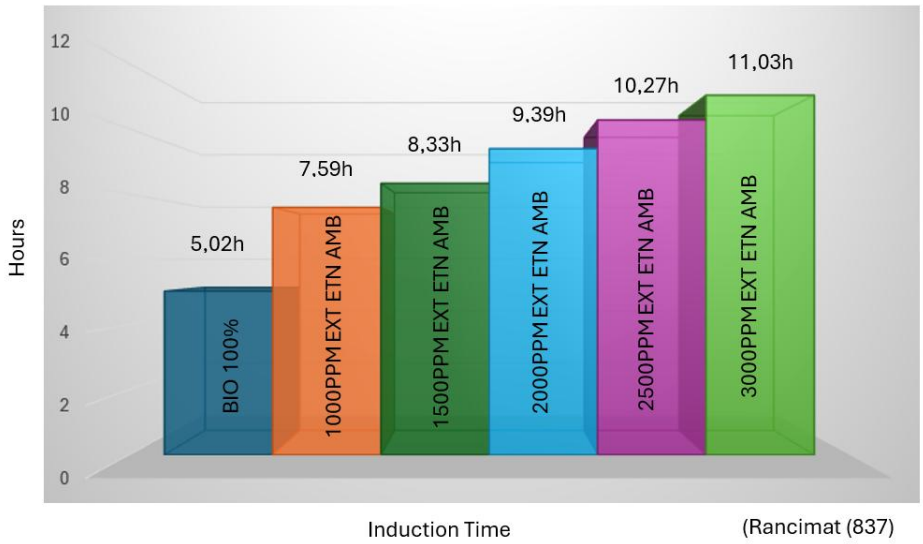
The study of biodiesel oxidative stability is essential to assess its resistance to degradation during storage and use. Oxidation promotes the formation of compounds that alter the physical and chemical properties of the fuel and can compromise the performance and durability of injection systems. Thus, the evaluation of oxidative stability allows predicting the behavior of biodiesel over time, supporting compliance with regulatory standards, and guiding strategies, such as the addition of antioxidants, to ensure the performance and reliability of biofuel.

The oxidation stability test at 110°C, performed using the Rancimat method (EN 14112), determines the induction time until the formation of oxidation products, which is a fundamental parameter for evaluating the resistance of biodiesel to oxidative degradation.

Figure 10 shows the results of the induction times for pure soybean biodiesel, without the addition of antioxidants, as well as biodiesel with 1000, 1500, 2000, 2500, and 3000 ppm of Amburana ethanolic extract used as an antioxidant additive.

Figure 10

Oxidative stability graph for soybean biodiesel/BIOPAR - pure - 1000 to 3000 ppm (ethanolic extract of Amburana Cearensis)



ANP Resolution No. 920/2023 establishes a minimum value of 13 hours for the oxidative stability of biofuels, as shown in Table 2.

Table 2

Minimum values for the induction period. (Lôbo et al., 2009).

Regulation/Standard	**Minimum Induction Period**	**Test method**
Brazil (ANP)	≥ 13 h	EN 14112 (Rancimat) or equivalent
Europe (EN 14214)	≥ 06 h	EN 14112 (Rancimat)
EUA (ASTM D6751)	Unspecified	Tests can be performed according to associated methods, but there are no official minimum requirements in the official minimum in the D6751

The results obtained show that pure soybean biodiesel had low oxidative stability, with an induction period of 5.02 hours, which is lower than the minimum established by European standard EN 14214 (6 h) and, even more significantly, the requirement in force in Brazil, defined by the ANP as 13 h, reinforcing the need to use antioxidants to enable its commercialization in the domestic market.

The addition of Amburana ethanolic extract resulted in a significant and gradual increase in oxidative stability, reaching 7.59 h (1000 ppm), 8.33 h (1500 ppm), 9.39 h (2000 ppm), 10.27 h (2500 ppm), and 11.03 h (3000 ppm), values that, although still not meeting

Brazilian requirements, exceed the European minimum limit, indicating that biodiesel with these concentrations of additives would be suitable for use in international markets with less restrictive specifications.

Thus, although the results indicate that additional formulation adjustments are still necessary to fully meet Brazilian specifications, the use of natural antioxidants proves to be a technically promising alternative, capable of increasing the oxidative stability of biodiesel and enabling its application both in the domestic market and in other international markets, in accordance with different regulatory criteria.

4 CONCLUSION

It was possible to identify the compounds present in the exudate of Amburana cearensis. The natural product used proved to be relevant in combating the oxidation of soybean biodiesel, showing promise in the scientific field in the area of substitutes for synthetic antioxidants. Thermal analyses showed that the product is stable when used at high temperatures, indicating greater fuel resistance to different storage, temperature, and operating conditions.

The oxidation stability test at 110°C, performed using the Rancimat method, showed an induction period of 11.03 hours, using 3000ppm of ethanolic extract from the exudate of Amburana Cearensis as an antioxidant additive, achieving a value close to the minimum required by law. This demonstrated its efficiency in mitigating oxidation compared to biodiesel without additives, showing that further research is promising.

This performance highlights the extract's potential as a future sustainable alternative to traditionally used synthetic antioxidants, or even studies to create blends, meeting the demand for renewable and environmentally safe inputs, showing the extract as a viable and efficient strategy to maintain better oxidative stability of biodiesel, adding value to a natural product that obtained good results when tested in commercial biodiesel.

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