STUDY OF THE APPLICATION OF LEMONGRASS, LEMON BALM, AND MALLOW EXTRACTS AND TBHQ AS ANTIOXIDANTS IN SOYBEAN BIODIESEL

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Abstract

Oxidative stability of biodiesel is a crucial factor for its commercial and environmental viability, as it directly influences its durability, performance, and safety during storage and use. In this context, there has been growing interest in natural alternatives to synthetic antioxidants, especially plant extracts rich in secondary metabolites such as flavonoids, tannins, and phenols. In this study, we present an investigation on the application of plant extracts and the synthetic antioxidant TBHQ in soybean biodiesel. The extracts were tested at three different concentrations (500 ppm, 1500 ppm and 3000 ppm) and characterized by total phenolic content, determination of antioxidant activity by the ABTS method, infrared spectroscopy, thermogravimetry and by the Rancimat® accelerated oxidation technique (110 °C). Among the extracts evaluated, the highest total phenolic content was observed in mallow extract (MLE) with 74.24 ± 1.9 mg GAE/g, followed by lemon balm extract (LBE) which presented 41.77 ± 2.8 mg GAE/g and lemongrass extract (LGE) with 25.93 ± 0.8 mg GAE/g. Regarding the antioxidant activity against the ABTS•+ radical, the same decreasing order was observed: MLE (991.62 ± 11.18 mM Trolox/g) > LBE (428.30 ±

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27.0 mM Trolox/g) > LGE (414.01 ± 24.4 mM Trolox/g). Thermal analysis indicated that TBHQ and mallow extract were the most stable, followed by lemongrass extract and lemon balm extract. At a concentration of 3000 ppm, an increase in the induction period of 68.18%, 62.65%, and 50.0% was observed in comparison to the control sample for lemongrass, lemon balm, and mallow extracts, respectively. The antioxidants increased the induction period in the following decreasing order: TBHQ > lemongrass extract > lemon balm extract > mallow extract. However, only TBHQ extended the biodiesel induction period beyond 13 hours. The studies highlighted the importance of using plant extracts and synthetic antioxidants in controlling the oxidative degradation of soybean biodiesel, significantly increasing its stability.

Keywords: Antioxidants, Biodiesel, Lemongrass, Lemon balm, Mallow.

INTRODUCTION

Biodiesel is a biodegradable fuel that is less polluting than fossil diesel and is obtained from renewable sources, such as vegetable oils and animal fats, through a transesterification reaction (Devi; Das; Deka, 2018; Amran; Bello; Hazwan Ruslan, 2022). This chemical process, considered the most viable method for biodiesel production, converts triglycerides into fatty acid esters (Souza et al., 2021).

Although biodiesel offers many advantages over conventional diesel, it also has certain limitations that cannot be ignored, the most concerning of which is oxidative degradation (Shi et al., 2025). The chemical composition of the fatty acid esters present in the feedstocks directly affects the physicochemical properties of biodiesel (Oleinik et al., 2024). The higher the concentration of unsaturated fatty acid esters, the more susceptible biodiesel is to auto-oxidation during storage, generating compounds such as peroxides, aldehydes, and organic acids that cause deposits and corrode engine components (M. Nambiraj; K. Suresh Kumar, 2024).

Biodiesel oxidation can be delayed through the use of antioxidants, reduced exposure to light and air, and the selection of feedstocks with lower unsaturated fatty acid contents (Sonnemberg et al., 2024).

Among these strategies, the application of natural or synthetic antioxidants is the simplest and most effective because it deactivates free radicals and reduces the propagation of oxidative reactions (Hosseinzadeh-Bandbafha, 2022).

This study aimed to evaluate the antioxidant activity of extracts of lemongrass (Cymbopogon citratus (DC.) Stapf), lemon balm (Lippia alba (Mill.) N.E.Br. ex Britton & P.Wilson), and mallow (Plectranthus amboinicus (Lour.) Spreng.), as well as the synthetic antioxidant tert-butylhydroquinone (TBHQ), in reducing the oxidation of soybean biodiesel using the accelerated Rancimat method. It also sought to characterize these antioxidants structurally through infrared spectroscopy and thermogravimetry, quantify the total phenolic content of the extracts using the Folin–Ciocalteu method, and determine their antioxidant activity using the ABTS method. The physicochemical properties of soybean biodiesel were also investigated.

One of the most widely used synthetic antioxidants is tert-butylhydroquinone (TBHQ) (Sousa; Moura; Moura, 2021). Its proposed mechanism of action results from the delocalization of unpaired electrons in the additive's phenolic ring, thereby forming a resonance-stabilized hybrid within the ring and producing inert antioxidant radicals that slow the oxidation rate (Beker, 2014).

Natural antioxidants extracted from plants are rich in phenolic compounds, have minimal harmful effects, and perform comparably to synthetic antioxidants (Sousa; Moura; Moura, 2021). The advantages of using plant matrices as antioxidant additives include renewability, biodegradability, low toxicity, and lower cost (Ramos et al., 2021; Devi; Das; Deka, 2018).

Native to India, Cymbopogon citratus (DC.) Stapf is popularly known in Brazil as capim-cidreira, capim-santo, and capim-limão. Its leaves are used to prepare teas because of their antimicrobial, anti-inflammatory, and antiproliferative properties, as well as their antipyretic, antispasmodic, diuretic, and antitussive effects (Paiva; Domingues, 2021). The chemical composition of lemongrass has been extensively investigated and includes volatile oils, flavonoids, phenolic acids, phenylpropanoids, alcohols, esters, aldehydes, and alkaloids (Du et al., 2023).

Lippia alba (Mill.) N.E. Brown (Verbenaceae) is a medicinal plant native to South America and is popularly known in Brazil by several names, including erva-cidreira-de-arbusto, erva-cidreira-do-campo, erva-cidreira-brasileira, alecrim-do-campo, alecrim-selvagem, cidreira-brava, falsa-melissa, cidró, and cidrão. Preparations made from L. alba are used in traditional medicine to treat various health conditions, including gastric disorders such as cramps, indigestion, pain, nausea, and spasms (Heinzmann; De Barros, 2007).

Plectranthus amboinicus (Lour.) Spreng. occurs naturally in tropical and warm regions of Africa, Asia, and Australia, as well as throughout tropical America, from the Antilles to southern Brazil (Arumugam; Swamy; Sinniah, 2016; Oliveira et al., 2007). It has numerous pharmacological properties, including activity related to urolithiasis, as well as antiepileptic, antitumorigenic, antimutagenic, radioprotective, antiviral, antifungal, and neuropharmacological properties (Bhatt; Negi, 2012).

METHODOLOGY

REAGENTS

All reagents used in this study were of analytical grade (Sigma-Aldrich, Vetec, Merck, and Dinâmica) and were used without prior purification. The vegetable oils were Concórdia-brand products manufactured by ADM® Brasil.

PREPARATION OF THE EXTRACTS

Leaves of lemongrass (Cymbopogon citratus (DC.) Stapf), lemon balm (Lippia alba (Mill.) N.E.Br. ex Britton & P.Wilson), and mallow (Plectranthus amboinicus) were obtained from a community garden in the city of Timon, Maranhão State (LAT: 5°12'26.2"S and LON: 42°50'55.5"W). They were cleaned and dried in a forced-air oven at 40 °C for five days and were subsequently ground manually into a solid with reduced particle size (Macêdo et al., 2018). The leaves were then immersed in ethanol, kept at room temperature, manually stirred every 24 hours for five days, and protected from light. They were

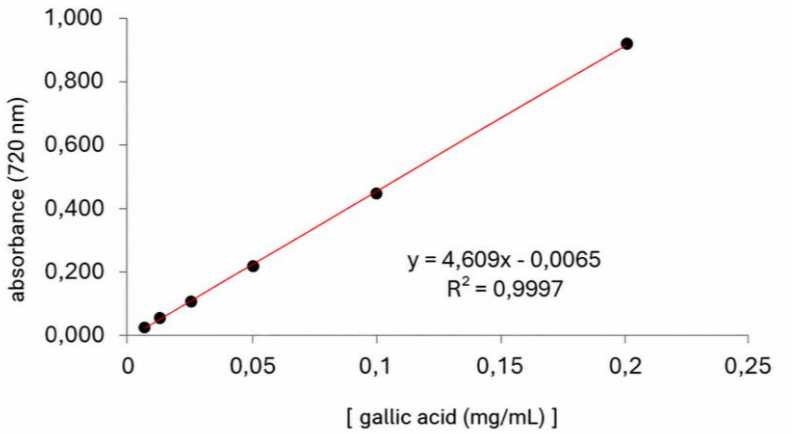
subsequently filtered through quantitative filter paper, and the resulting solution was concentrated in a rotary evaporator at 40 °C (Sousa, 2019; Batista et al., 2016; Kasper et al., 2018). The ethanolic extracts were then stored in refrigerated plastic containers protected from light.

DETERMINATION OF THE TOTAL PHENOLIC COMPOUND (POLYPHENOL) CONTENT OF THE EXTRACTS

Total phenolic content was determined according to the method described by Swain and Hills (1959). The extracts were solubilized in absolute ethanol at a concentration of 1000 $\mu g \cdot mL^{-1}$. A 0.5 mL aliquot of each prepared solution was transferred to a test tube, after which 8 mL of distilled water and 0.5 mL of 20% (v/v) Folin–Ciocalteu reagent were added. The solution was then homogenized using a vortex mixer, and after 3 minutes, 1 mL of a 20% (m/v) sodium carbonate (Na_2CO_3) solution was added. After 1 hour in a water bath at 37 °C, optical density readings were obtained using a spectrophotometer at 720 nm. Sigma® gallic acid was used as the reference standard to construct the calibration curve. The equation obtained from the calibration curve was used to calculate total phenolic content, expressed as mg GAE (gallic acid equivalent)$\cdot g^{-1}$ of sample (Figure 1). All analyses were performed in triplicate. GraphPad Prism 8.0® was used to construct the graph and conduct the statistical analyses.

Figure 1

Calibration curve for the gallic acid reference standard.



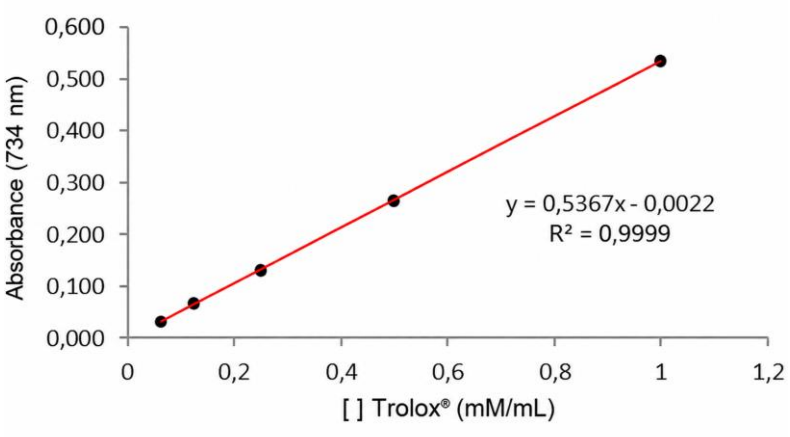
Source: Authors, 2025.

DETERMINATION OF ANTIOXIDANT ACTIVITY USING THE ABTS ASSAY

The ABTS•+ radical was generated through the reaction of 7 mM ABTS with 2.45 mM potassium persulfate and was kept at room temperature in the absence of light for 12–16 hours. After this period, the solution was diluted in absolute ethanol until an absorbance of 0.70 (± 0.05) was obtained (Rufino et al., 2007). A volume of 40 µL of the samples diluted in ethanol was added to 1960 µL of the radical-containing solution. After 30 minutes of reaction, absorbance was determined using a spectrophotometer at 734 nm (Re et al., 1999, as adapted by Lima, 2008). Trolox®, a synthetic antioxidant and vitamin E analogue, was used as the standard solution to construct a calibration curve. GraphPad Prism 8.0® was used to construct the graph and conduct the statistical analyses (Figure 2). All readings were performed in triplicate, and the results were expressed as Trolox® Equivalent Antioxidant Capacity (TEAC), in mM Trolox per gram of sample.

Figure 2

Calibration curve for the inhibition response of the ABTS•+ free radical to the Trolox® antioxidant standard.



Source: Authors, 2025.

FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Infrared vibrational spectroscopy analyses were performed using a PerkinElmer Spectrum 100 FTIR spectrometer equipped with a GTX ATR cell containing a ZnSe crystal at an angle of 45°. The transmission technique was employed using a KBr pellet for TBHQ and a NaCl window for the plant extracts and biodiesel, together with attenuated total reflectance over the spectral range of 4000–400 cm^{-1}.

THERMOGRAVIMETRIC ANALYSIS (TG/DTG)

The TG and DTG curves of the antioxidants were obtained using a TG-DTA SDT Q600-SHIMADZU simultaneous thermal analyzer. Analyses were conducted at a heating rate of 10 °C·min^{-1} over a temperature range of 25–600 °C under a nitrogen atmosphere, using a platinum cell.

DETERMINATION OF OXIDATIVE STABILITY USING THE RANCIMAT® METHOD

The accelerated oxidation method was performed in accordance with EN 14112. A Metrohm Rancimat model 873 was used. Approximately 3 g of sample was added to each test tube and subjected to a constant airflow of 10 L·h^{-1} at a constant temperature of 110 °C. Each electrical-conductivity measuring vessel contained 50 mL of deionized water for determination of the induction period.

BIODIESEL ADDITIVATION PROCEDURE

The samples were supplemented as shown in Table 1, using antioxidant concentrations of 500 ppm, 1500 ppm, and 3000 ppm (mg antioxidant·kg^{-1} biodiesel). All analyses were performed in triplicate. GraphPad Prism 8.0® was used to construct the graph and conduct the statistical analyses.

Table 1

Concentrations of the antioxidants used

Sample identification	Additive content
B100	Biodiesel without antioxidant
BCL500	Biodiesel + 500 ppm lemongrass
BEC500	Biodiesel + 500 ppm lemon balm
BMR500	Biodiesel + 500 ppm mallow
BT500	Biodiesel + 500 ppm TBHQ
BCL1500	Biodiesel + 1500 ppm lemongrass
BEC1500	Biodiesel + 1500 ppm lemon balm
BMR1500	Biodiesel + 1500 ppm mallow
BT1500	Biodiesel + 1500 ppm TBHQ
BCL 3000	Biodiesel + 3000 ppm lemongrass
BEC 3000	Biodiesel + 3000 ppm lemon balm
BMR 3000	Biodiesel + 3000 ppm mallow
BT 3000	Biodiesel + 3000 ppm TBHQ

Source: Authors, 2025.

RANCIMAT

The method standardized by EN 14112 and adopted in Brazil by the National Agency of Petroleum, Natural Gas and Biofuels (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis – ANP) was used to evaluate the oxidative stability of the biodiesel. A Metrohm Rancimat model 743 was used in the assays. In this method, 3 g of sample was aged under an airflow of 10 $L \cdot h^{-1}$ at 110 °C in a measuring cell supplied with deionized water. The induction time was determined by measuring conductivity. In Brazil, the adopted minimum limit is 13 hours of testing.

STABILIZATION FACTOR

Antioxidant efficiency was evaluated using the stabilization factor, also known as the antioxidant effectiveness factor, F, according to the following equation:

$$F = \frac{PIx}{PI0}$$

Where PI_x is the induction period in the presence of antioxidants and PI_0 is the induction period of pure biodiesel, that is, the control sample (Sonnemberg et al., 2024).

RESULTS AND DISCUSSION

TOTAL PHENOLICS (POLYPHENOLS)

Phenolic compounds are products of plant secondary metabolism through the pentose phosphate, shikimate, and phenylpropanoid pathways in response to stress, UV radiation, and pests. Acting as antioxidants, they protect plants from stressors and biomolecule oxidation (De Vargas; Bellaver, 2022). Structurally, they contain an aromatic ring and hydroxyl groups and may be simple or complex (Arnoso; Da Costa; Schmidt, 2019). They include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans, and lignins (Sousa; Moura; Moura, 2021).

The phenolic compounds present in these plant extracts are notable for their multifunctionality as antioxidants, acting primarily by neutralizing free radicals (Gonçalves; Santos; Morais, 2015). This activity occurs through the donation of hydrogen atoms from the hydroxyl groups attached to the aromatic ring, enabling them to stabilize unpaired electrons (Sousa et al., 2014).

As shown in Table 2, MLE had the highest total phenolic content among the plant extracts used. Based on the values obtained, the total phenolic content of the compounds investigated followed this descending order: MLE > LBE > LGE.

The values obtained for LGE were higher than those reported by Pereira et al. (2024), who obtained a phenolic content of 1538.92 ± 30.88 mg GAE/100 g of sample using the same method and solvent.

Table 2

Total phenolics (polyphenols)

SAMPLES	mg GAE/g of sample ± SDM*
LGE	25,93 ± 0,8
LBE	41,77 ± 2,8
MLE	74,24 ± 1,9

Source: Authors, 2025. *SDM: Standard deviation of the mean. n = 3.

Sacramento et al. (2024) and Pavanelli and Povh (2024) found higher phenolic-content values for LBE, at 58.66 ± 0.98 mg GAE/g and 51.2 ± 4.23 mg GAE·g^{-1}, respectively.

Studies conducted by Nguyen et al. (2020) indicated that the total phenol concentration in MLE was 26.84 ± 0.91 mg GAE/g, which was lower than the value obtained in this study. Shubha and Bhatt (2015) found an even higher phenolic content of 313 mg GAE/g.

Differences between the values obtained in this study and those reported in the literature may result from several factors, including climate, extraction method and temperature, solvent type, season, maturity stage, harvesting, cultivation conditions, and postharvest storage (Arnoso; Da Costa; Schmidt, 2019; Spacino et al., 2015; Gonçalves; Santos; Morais, 2015).

EVALUATION OF ANTIOXIDANT ACTIVITY USING ABTS

In this study, the ABTS•+ radical cation was generated using potassium persulfate, and the samples were evaluated at a wavelength of 734 nm. Table 3 presents the antioxidant-activity results obtained using the ABTS•+ method, expressed as mM Trolox/g of sample.

Evaluation of antioxidant capacity against the ABTS•+ radical cation showed values ranging from 414.01 to 991.62 mM Trolox/g among the extract samples analyzed. MLE had the highest antioxidant activity among the extracts evaluated, at 991.62 mM Trolox/g of sample. This result was attributed to the presence of phenolic compounds derived from the secondary metabolism of this species (Freitas et al., 2014). LBE had the second-highest antioxidant activity, at 428.30 mM Trolox/g of sample, followed by LGE, at 414.01 mM Trolox/g of sample.

Table 3

Trolox® Equivalent Antioxidant Capacity (TEAC)

SAMPLES	mM Trolox/g of sample
LGE	414,01 ± 24,4
LBE	428,30 ± 27,0
MLE	991,62 ± 11,18

Source: Authors, 2025. *SDM: Standard deviation of the mean. n = 3.

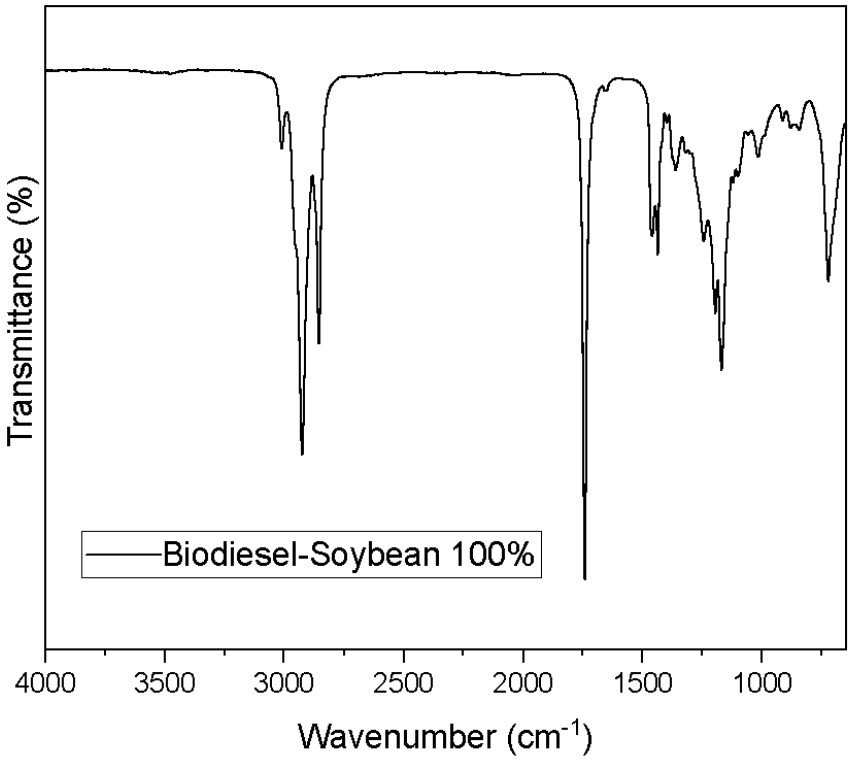
The antioxidant activity of the extracts showed a strong positive correlation with their total phenolic-compound content (Table 2), suggesting that the phenolic compounds present in the extracts have a high free-radical-scavenging capacity and are therefore important sources of primary antioxidant agents, which contributed to the higher antioxidant activity observed (Freitas et al., 2014; Sousa; Moura; Moura, 2021).

FTIR

Infrared absorption spectroscopy was used to identify the constituents of soybean biodiesel (Figure 3). Aliphatic C–H stretching vibrations were observed between 3011 and 2853 cm^{-1}. The band at 3011 cm^{-1} corresponds to symmetric and asymmetric C–H stretching vibrations (C-sp^2), the band at approximately 2925 cm^{-1} corresponds to an asymmetric C–H stretching vibration (C-sp^3), and the band at 2853 cm^{-1} corresponds to a symmetric stretching vibration (C-sp^3) (Pavia, 2015). The intense band at approximately 1740 cm^{-1} indicates carbonyl (C=O) stretching characteristic of esters, whereas the bands at 1171 cm^{-1} and 1000 cm^{-1} confirm the presence of stretching characteristic of C–O bonds, further supporting ester formation. The vibrations at 1461 cm^{-1} in the biodiesel are characteristic of CH_3. The signal at 717 cm^{-1} corresponds to CH_2 bending in long fatty-acid chains (Sousa et al., 2014; Silverstein; Webster; Kiemle, 2015; Pavia, 2015).

Figure 3

FTIR spectrum of soybean biodiesel.



Source: Author, 2025.

Analysis of the FTIR spectra also made it possible to identify the principal functional groups present in LGE, LBE, and MLE by comparing them with the synthetic antioxidant TBHQ (Figure 4).

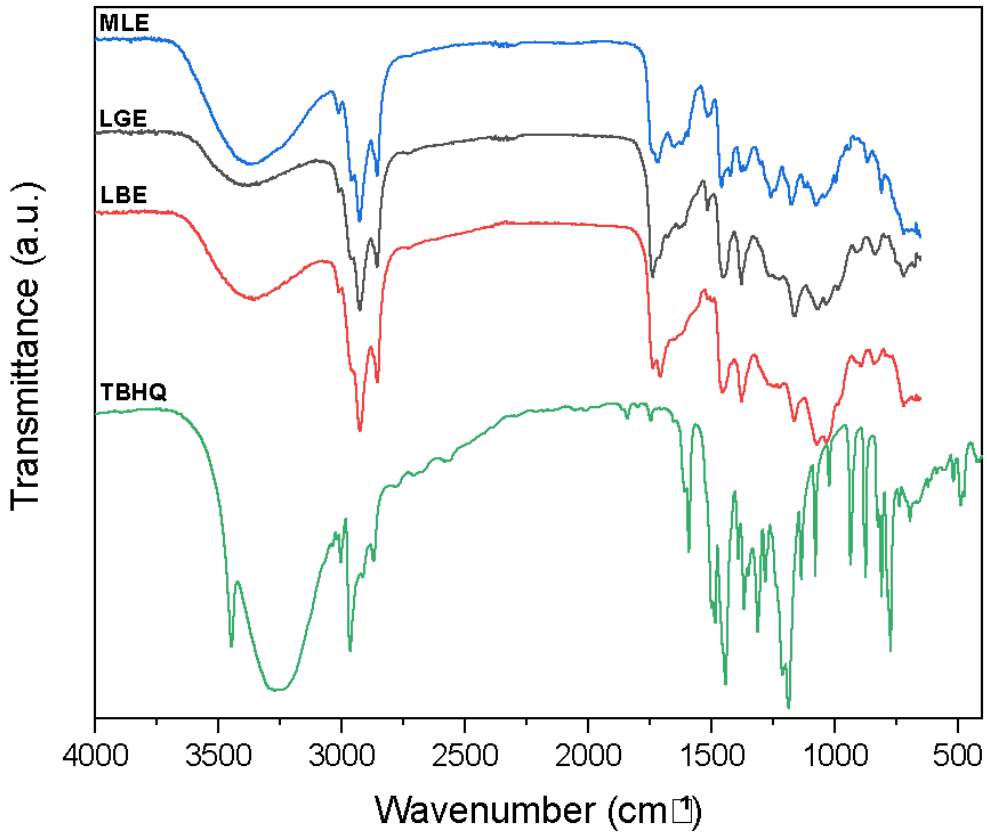
As shown in Figure 4, a broad band between 3400 and 3200 cm^{-1}, characteristic of O–H stretching in phenols and alcohols, was common to all samples (Silverstein; Webster; Kiemle, 2015; Pavia, 2015; Ammawath, 2004; Mekala; Banu; Mathammal, 2025). Aliphatic C–H stretching bands were observed in the region of 2924 and 2862 cm^{-1} (Ammawath, 2004; De Jesus et al., 2020).

The region from 1740 to 1709 cm^{-1} showed C=O vibrations from carboxylic acids, aldehydes, and esters present only in the extracts, suggesting that they contain bioactive carbonyl compounds. Between 1845 and 1746 cm^{-1}, a set of C=C bands corresponding to a trisubstituted aromatic system (1,2,4-trisubstituted) was detected (Ammawath, 2004). The bands between 1590 and 1450 cm^{-1} correspond to

aromatic C=C stretching vibrations and C–H deformations, whereas the band at 1375 cm^{-1} was attributed to the symmetric deformation of the CH_3 groups in the tert-butyl group of TBHQ (Silverstein; Webster; Kiemle, 2015).

Figure 4

FTIR spectra of the plant extracts and TBHQ.



Source: Authors, 2025.

Intense absorptions were recorded in all samples in the region from 1260 to 1000 cm^{-1} and were attributed to C–O stretching in phenols, alcohols, and esters. Finally, below 900 cm^{-1}, bands associated with out-of-plane aromatic-ring C–H deformations appear and are particularly pronounced in TBHQ (Ammawath, 2004; De Jesus et al., 2020). The functional groups present in the extracts and TBHQ are shown in Table 4.

Table 4

Bands (cm^{-1}) corresponding to the functional groups present in the extracts and TBHQ analyzed by FTIR

LBE	MLE	LGE	TBHQ	Type of vibration	Functional group
3387	3395	3260	3250	O–H stretching	Phenol, alcohol
2924	2924	2924	2962	C–H stretching	Alkanes
1709	1709	1746	-	C=O vibrations	Carboxylic acids, aldehydes, and esters
-	-	-	1845	C=C stretching	Aromatic
-	-	-	1802	C=C stretching	Aromatic
-	-	-	1746	C=C stretching	Aromatic
1454	1454	1454	1454	C=C stretching	Aromatic
1168	1168	1168	1168	C–O stretching	Phenols, alcohols, and esters

Source: Silverstein; Webster; Kiemle, 2015; Pavia, 2015.

Overall, the spectra of the plant extracts showed significant similarities to that of TBHQ, suggesting that these extracts contain bioactive compounds with antioxidant potential.

TG/DTG

Studies using thermogravimetric analysis have shown that the stability of several antioxidants decreases as temperature increases (Santos, 2012). Thermogravimetric analysis by TG/DTG (Table 5 and Figure 5) of the antioxidants MLE, LBE, LGE, and TBHQ showed that MLE and LBE underwent mass loss in three decomposition stages, LGE underwent two stages, and TBHQ underwent only one mass-loss stage.

Table 5

Thermal-analysis data for mallow extract (MLE), lemon balm extract (LBE), lemongrass extract (LGE), and TBHQ

Samples	First event (°C)	Mass loss (%)	Second event (°C)	Mass loss (%)	Third event (°C)	Mass loss (%)
MLE	26-88	8	103-250	27	250-296	10
LBE	31-67	7	95-252	24	269-300	6,71
LGE	27-78	3,56	100-297	34,91	-	-
TBHQ	132-205	98	-	-	-	-

Source: Authors, 2025.

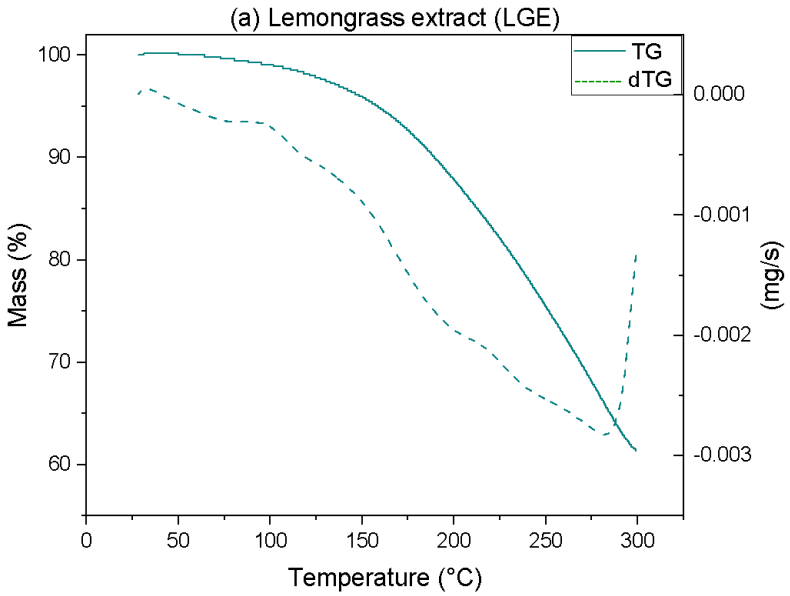
Table 5 presents the thermal-event data obtained by interpreting the TG/DTG curves. According to the thermogravimetric analyses of the extracts, the first mass-loss event occurred at approximately 26–88 °C for mallow extract, with an 8% mass loss; at 31–67 °C for lemon balm extract, with a 7% mass loss; and at 27–78 °C for lemongrass extract, with a 3.56% mass loss. The reduction in mass during this first event was probably attributable to moisture and traces of ethanol, since ethanol was the solvent used to obtain the extracts (Chissico, 2019; Laureanti et al., 2023).

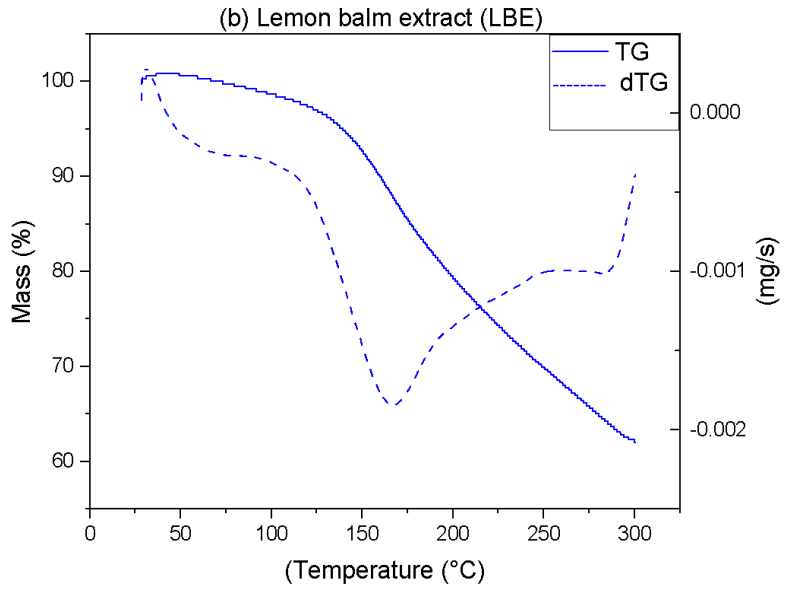
The second event occurred at approximately 103–250 °C for MLE, with a 27% mass loss; at approximately 95–252 °C for LBE, with a 24% mass loss; and at 100–297 °C for LGE, with a 34.91% mass loss and 65.09% residual mass. In general, decomposition during the second event was attributed to the degradation of secondary metabolites, including polysaccharides such as cellulose and hemicellulose, phenolic compounds such as tannins and flavonoids, fixed oils, and medium-volatility compounds present in these samples (De Sousa et al., 2019).

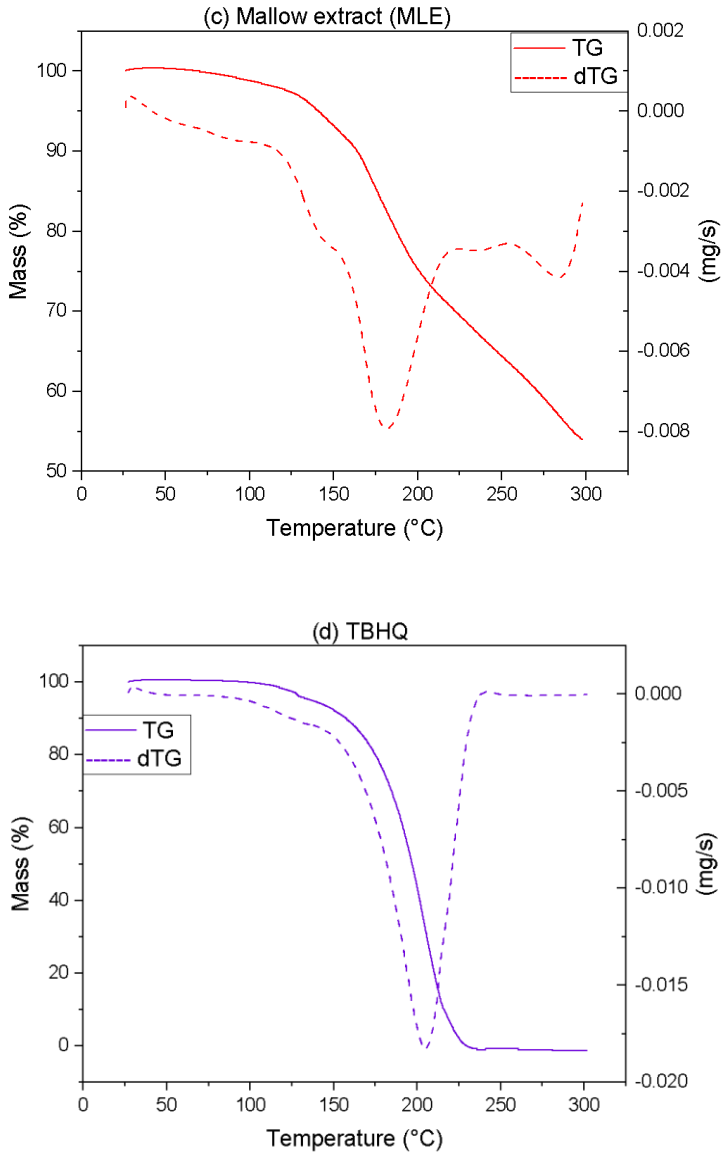
The third event occurred at approximately 250–296 °C for MLE, with a 10% mass loss and 55% residual mass, and at approximately 269–300 °C for LBE, with a 6.71% mass loss and 65.09% residual mass. This third and final event indicates the complete thermal degradation of the bioactive compounds and the decomposition of complex compounds such as lignin (Rahman et al., 2024).

Figure 5

Thermogravimetric analysis (TG) and its derivative (DTG) for lemongrass extract (a), lemon balm extract (b), mallow extract (c), and TBHQ (d).







Source: Authors, 2025.

Thermogravimetric analysis (TG) and its derivative (DTG) for TBHQ show a thermal profile typical of a high-purity synthetic antioxidant, with decomposition concentrated in a single stage. Initially, the sample remains thermally stable up to approximately 130 °C, with minimal mass variation, indicating the absence of moisture or low-temperature volatile compounds (Laureanti et al., 2023). The principal degradation stage occurs at approximately 130–220 °C, with a mass loss of approximately 98% and 2% residual mass, as shown by the sharp decrease in the TG curve and the pronounced peak in the DTG

curve. The intensity of this peak indicates rapid and extensive decomposition, with the maximum mass-loss rate occurring at approximately 200 °C (De Jesus et al., 2020).

According to the thermogravimetric data, the antioxidants exhibited thermal stability in the following descending order: TBHQ > MLE > LGE > LBE. The extracts may be used in applications involving moderate heating but cannot withstand very high temperatures without a significant loss of bioactive constituents. They are therefore suitable for applications in the pharmaceutical, food, cosmetic, and fuel industries.

PHYSICOCHEMICAL CHARACTERIZATION OF SOYBEAN BIODIESEL

Biodiesel quality was evaluated using the characterization parameters shown in Table 6. The values presented below were within the limits permitted by the current resolution, except for the oxidative stability of soybean biodiesel, which was 3.52 ± 0.02 hours and therefore below the minimum limit of 13 hours established by ANP Resolution No. 920 of April 4, 2023. The low stability index is attributable to the higher linoleate ester content of soybean biodiesel (Aurelio, 2023; ANP, 2023).

Table 6

Physicochemical parameters of soybean methyl biodiesel

Parameters	Unit	Soybean biodiesel	ANP limit
Kinematic viscosity at 40 °C	$mm^2\ s^{-1}$	4,40	3,0-6,0
Maximum acid value	mg KOH g^{-1}	0,26	0, 5
Iodine value	$gI_2/100\ g$	105,4	Report
Peroxide value	meq Kg^{-1}	1,81	*ns
Oxidative stability at 110 °C	h	3,52	13

Source: Authors, 2025. *ns: not specified.

As biodiesel oxidizes, its physicochemical properties change, thereby affecting its quality. These parameters must therefore be carefully evaluated (M. Nambiraj; K. Suresh Kumar, 2024).

Oxidation generates contaminants that affect biodiesel quality and engine operation, increasing viscosity through the formation of hydroperoxides, dimers, and polymers (Ashokan et al., 2025). As

shown in Table 6, the kinematic viscosity of the biodiesel at 40 °C complies with ANP standards, thereby confirming its quality (ANP, 2023).

The acid value of biodiesel is relevant during storage because variations indicate the formation of oxidation products (Ashokan et al., 2025; Saluja; Kumar; Sham, 2016). As shown in Table 6, the biodiesel's acid value complies with ANP standards, thereby confirming its quality (ANP, 2023).

The iodine value indicates the degree of unsaturation, with higher values reflecting a greater number of unsaturated bonds and greater susceptibility to oxidation (M. Nambiraj; K. Suresh Kumar, 2024). Table 6 shows that the value obtained was below the limit of 120 g I_2/100 g established by EN 14111, indicating that the synthesized biodiesel has a high degree of unsaturation despite remaining below the established limit (Ramos et al., 2021).

The peroxide value, measured in milliequivalents of peroxide per kilogram of sample, indicates the quantity of hydroperoxides in the biodiesel. As primary oxidation products, hydroperoxides indicate the onset of biodiesel degradation (M. Nambiraj; K. Suresh Kumar, 2024). Low values indicate greater oxidative stability, as observed in Table 6, which shows a low concentration of these compounds in the biodiesel (Kanwal et al., 2025).

STUDY OF THE OXIDATIVE STABILITY OF SOYBEAN BIODIESEL

As shown in Figure 6, soybean biodiesel without antioxidants (B100) had an induction period of 3.52 ± 0.02 hours in the Rancimat test at 110 °C. This value is below both the 13-hour limit established by the ANP (ANP, 2023) and the European standard of 8 hours. The addition of antioxidants is therefore necessary to reach the minimum oxidative-stability limit required by the standard governing the commercialization of biodiesel (Romola et al., 2021; ANP, 2023).

The addition of natural antioxidants at a concentration of 500 ppm increased the induction period. Biodiesel supplemented with MLE performed best, reaching 4.80 ± 0.03 h, an increase of 36.36% relative to the control sample. It was followed by LGE, with an induction period of 4.46 ± 0.04 h and a 26.70%

increase in IP, and LBE, with an IP of 4.14 ± 0.08 h and a 17.6% increase. TBHQ produced an induction period of 9.65 ± 0.9 h and exhibited the best performance among the antioxidants. Except for BEC500, all results differed statistically from the B100 IP value. Results are expressed as mean ± standard deviation (n = 3).

When the concentration was tripled by adding 1500 ppm of antioxidants to soybean biodiesel, only a very small increase in oxidative stability was observed compared with the results obtained at 500 ppm. Biodiesel containing MLE had an induction period of 5.32 ± 0.02 h, biodiesel containing LBE had an induction period of 5.28 ± 0.08 h, and biodiesel containing LGE had an induction period of 4.72 ± 0.01 h. Biodiesel containing TBHQ showed a substantial increase in induction period, reaching 34.14 ± 0.015 h. All results differed statistically from the B100 IP value, although BEC1500 and BMR1500 were statistically equivalent.

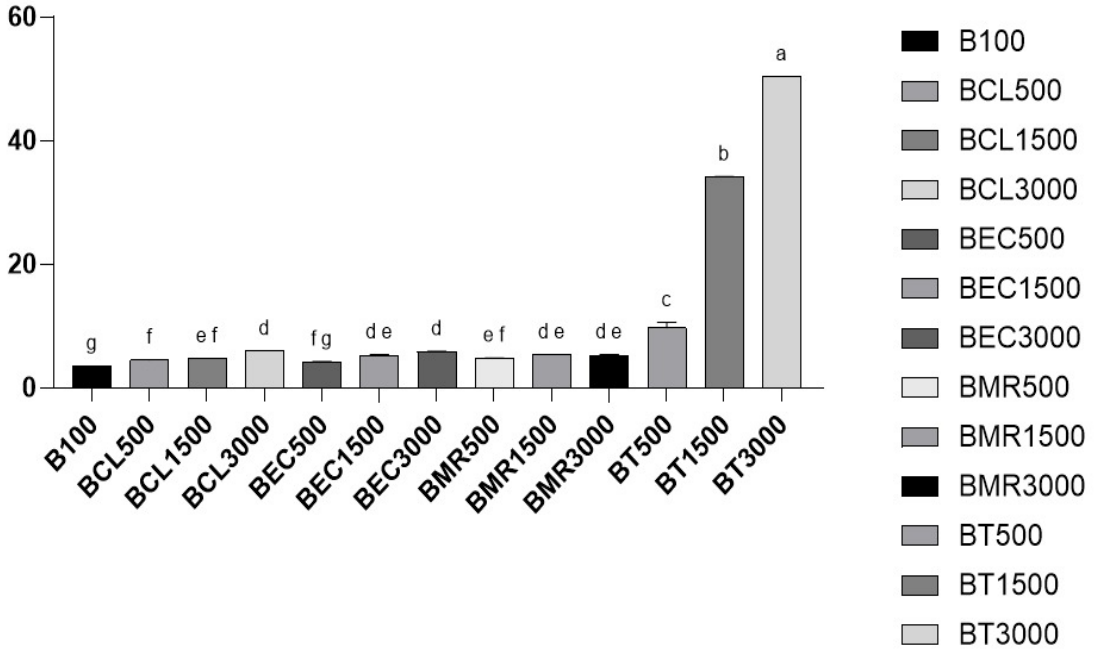
Based on the induction-time values obtained, only biodiesel containing the synthetic antioxidant TBHQ reached a satisfactory induction time, with stability of 50.38 ± 0.01 h at a concentration of 3000 ppm. For the other antioxidants, the induction time remained below 13 hours. Biodiesel supplemented with the natural antioxidants present in lemongrass, lemon balm, and mallow leaf extracts had induction times greater than that of the control (B100), demonstrating the extracts' potential antioxidant activity. The sample supplemented with LGE at the highest concentration, 3000 ppm, had an IP of 5.92 ± 0.02 h, followed by the sample containing LBE, with an IP of 5.83 ± 0.05 h, and the sample containing MLE, with an IP of 5.28 ± 0.07 h. These values differed statistically from the B100 IP but were statistically equivalent to one another. Relative to the control sample, the induction period increased by 68.18%, 62.65%, and 50.0% for LGE, LBE, and MLE, respectively. These increases were nevertheless insufficient to raise the induction period above the 13 hours established by the ANP (ANP, 2023).

For samples containing LGE, there was no statistical difference between BCL500 and BCL1500, demonstrating that LGE was more effective in increasing the control sample's IP only at the highest concentration, 3000 ppm. For LBE, only the lowest concentration used proved effective; the other

concentrations were statistically equivalent. Samples containing MLE were likewise effective in increasing the B100 IP only at the lowest concentration, while the other concentrations tested were statistically equivalent.

Figure 6

Induction period at 110 °C, determined using the Rancimat technique, for biodiesel supplemented with antioxidants at concentrations of 500 ppm (a), 1500 ppm, and 3000 ppm (b).



Source: Author, 2025. a,b,c,d,e,f,g Means followed by the same letters above the columns do not differ statistically from one another according to Tukey's multiple-comparison test at the 5% probability level.

Overall, the BCL, BEC, and BMR samples had relatively similar means, with modest dose-dependent increases, because oxidative stability increases with antioxidant concentration until a saturation point is reached, after which no significant additional benefit is observed (Souza et al., 2021). In contrast, samples containing TBHQ produced substantial and statistically distinct increases at all doses evaluated, demonstrating greater efficiency. This occurs because of the hydroxyl groups at positions 1 and 4 of the benzene ring and the tert-butyl group at position 2, which react with oxidizing species to form a stable TBHQ radical (Domingos et al., 2007).

ANTIOXIDANT EFFECTIVENESS FACTOR

The stabilization factor (F), calculated from the induction-period values, evaluates antioxidant efficiency (Table 8). An F value greater than 1 indicates an increase in IP (Sonnemberg et al., 2024). TBHQ produced significant F values, demonstrating strong protection of the biodiesel, whereas the extracts produced F values close to 1, indicating low effectiveness.

Table 7

Stabilization factor

Sample	F
BCL500	1,27
BEC500	1,18
BMR500	1,36
BT500	2,74
BCL1500	1,34
BEC1500	1,50
BMR1500	1,51
BT1500	9,69
BCL 3000	1,68
BEC 3000	1,66
BMR 3000	1,50
BT 3000	14,31

Source: Authors, 2026.

CONCLUSION

Mallow extract had the highest total phenolic content among the plant extracts analyzed. Based on the values obtained, the total phenolic content of the compounds investigated followed this descending order: mallow extract > lemon balm extract > lemongrass extract.

Analysis of antioxidant capacity using the ABTS•+ method, based on 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), showed that mallow extract had the highest antioxidant activity among the extracts used because of its higher concentration of phenolic compounds.

FTIR analysis confirmed the functional groups present in the secondary metabolites identified in the antioxidants evaluated.

The thermogravimetric data confirmed the potential application of these samples in biodiesel, since the extracts exhibited good thermal stability in the following descending order: TBHQ > MLE > LGE > LBE.

The physicochemical parameters of soybean methyl biodiesel without antioxidants were within the limits permitted by the ANP, except for oxidative stability.

The extracts effectively protected the biodiesel and increased its oxidative stability. LBE and MLE were more efficient when used at a concentration of 500 ppm. Based on the values obtained at the highest concentration used, the antioxidants increased the induction period in the following descending order: TBHQ > lemongrass extract > lemon balm extract > mallow extract.

This study makes a significant contribution to the scientific community by evaluating the application of low-cost, environmentally sustainable extracts from renewable sources and demonstrating the potential of the compounds present in these extracts to protect biodiesel. Future studies may refine the methods used in this study and obtain even better results.

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