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Research Article

Natural Protective Power: Prevention of Biodiesel Oxidation with *Tilia platyphyllos*

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Abstract

Traditional energy sources are incompatible with contemporary environmental sustainability goals, hence renewable and eco-friendly alternatives are more popular. Due to its polyunsaturated fatty acid make up, biodiesel is prone to oxidative breakdown despite its low toxicity, biodegradability, and emissions. This reduces gasoline storage life and engine performance. *Tilia platyphyllos* (ıhlamur) extract, a natural phenolic, improved biodiesel-diesel (50% biodiesel + 50% diesel) blends' oxidation resistance in this research. The soxhlet extract was added to the gasoline at 3000 ppm and compared to TBHQ, a synthetic antioxidant. Thermal and chemical characterization methods FT-IR, TGA, and DSC were used to assess antioxidant activity. We tested the extract's ability to eliminate free radicals using DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays. The results show that *Tilia platyphyllos* is a powerful antioxidant that prevents biodiesel-diesel blend oxidative degradation. Biofuel technology might benefit from *Tilia platyphyllos*.

Keywords: *Tilia platyphyllos,* Biodiesel, Natural antioxidant, Oxidative stability, DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays

Doğal Koruyucu Güç: *Tilia platyphyllos* ile Biyodizel Oksidasyonunun Önlenmesi

Öz

Geleneksel enerji kaynakları, günümüz çevresel sürdürülebilirlik hedefleriyle uyumsuzdur; bu nedenle yenilenebilir ve çevre dostu alternatifler giderek daha fazla ilgi görmektedir. Biyodizel; düşük toksisitesi, biyolojik olarak kolayca parçalanabilirliği ve düşük emisyon seviyelerine rağmen, çoklu doymamış yağ asidi içeriği nedeniyle oksidatif bozulmaya karşı hassastır. Bu durum, yakıtın depolama ömrünü kısaltmakta ve motor performansını olumsuz etkilemektedir. Bu araştırmada, doğal bir fenolik bileşik olan *Tilia platyphyllos* (ıhlamur) ekstresi, biyodizel-dizel (yüzde 50 biyodizel + yüzde 50 dizel) karışımlarının oksidatif direncini artırmak amacıyla kullanılmıştır. Soxhlet yöntemiyle elde edilen ekstre, yakıta 3000 ppm oranında eklenmiş ve sentetik bir antioksidan olan TBHQ ile karşılaştırılmıştır. Antioksidan aktivite, FT-IR (Fourier Dönüşümlü Kızılötesi Spektroskopisi), TGA (Termogravimetrik Analiz) ve DSC (Diferansiyel Taramalı Kalorimetri) gibi termal ve kimyasal karakterizasyon yöntemleriyle değerlendirilmiştir. Ekstrenin serbest radikalleri uzaklaştırma kapasitesi DPPH$^{\bullet}$ ve ABTS$^{\bullet+}$ analizleriyle test edilmiştir. Sonuçlar, *Tilia platyphyllos*'un biyodizel-dizel karışımının oksidatif bozulmasını önlemede güçlü bir antioksidan olduğunu göstermektedir. *Tilia platyphyllos,* biyoyakıt teknolojisinde faydalı bir doğal katkı maddesi olarak değerlendirilebilir.

Anahtar Kelimeler: *Tilia platyphyllos,* Biyodizel, Doğal antioksidan, Oksidatif kararlılık, DPPH• ve ABTS$^{\bullet+}$ analizleri

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1. Introduction

The reliance on fossil fuels for energy generation persists, leading to considerable environmental issues stemming from emissions and ecological harm [1,2]. As a result, the rise of renewable energy sources, especially biodiesel, has become increasingly significant as a viable and sustainable option. Biodiesel is characterized by its non-toxic and biodegradable nature, along with its potential to lower greenhouse gas emissions. Nevertheless, a significant challenge lies in its oxidative instability, which is attributed to elevated levels of unsaturated fatty acid esters, impacting both storage and engine performance [3,4]. Biodiesel breaks down through oxidation, which includes chain reactions mainly driven by peroxyl radicals ($ROO^{\cdot}$). These radicals play a crucial role in initiating lipid peroxidation and the polymerization of fuel. To address this concern, the incorporation of antioxidant substances that can neutralize free radicals and disrupt oxidation processes is frequently advised. Antioxidants are usually identified by their phenolic hydroxyl groups, which are important for giving away hydrogen or electrons to fight against harmful radicals [5,6]. Synthetic antioxidants like *tert*-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT) are effective; however, they face growing scrutiny due to concerns about their potential toxic and carcinogenic effects [7,8]. Additionally, limitations imposed by regulations on synthetic additives in the food and fuel sectors have intensified the quest for safe and natural substitutes. Lately, more attention has been given to using natural antioxidants from plants because they are safe, good for the environment, and may have health benefits. *Tilia platyphyllos* Scop. is a deciduous tree species from the Malvaceae family, commonly found across Europe [9]. This species, often referred to as “linden,” has been utilized in traditional medicine for a considerable time, addressing issues like colds, insomnia, and nervous tension [10,11]. Recently, *Tilia platyphyllos* has captured the attention of researchers because of its abundant array of bioactive compounds. Research into plant chemicals has shown that the flowers, leaves, and seeds contain important phenolic compounds like rutin, tiliroside, chlorogenic acid, protocatechuic acid, and catechin. These compounds play a crucial role in establishing its strong antioxidant properties [12,13] Researchers acknowledge tiliroside's significant ability to scavenge radicals. Additionally, it has demonstrated that using infrared-assisted drying improves the overall phenolic and flavonoid content in the plant material [14,15]. Additionally, the seed oils from *Tilia* species contain a lot of fat-soluble antioxidants, such as *β*-sitosterol, *γ*-tocopherol, and *δ*-tocopherol. These compounds are linked to the postponement of lipid oxidation and enhanced oxidative stability [16]. These findings show that *Tilia platyphyllos* is a strong candidate for use in natural antioxidants, which are important for both food and fuel stability. Even though we know that *Tilia* species have antioxidant benefits for food and medicine [17], we haven't looked closely at how they could help stabilize biodiesel. The similarity in structure between the fats in biodiesel and those in biological membranes means that looking into plant-based antioxidants for fuel systems is both important and makes sense scientifically. This study looks at how well *Tilia platyphyllos* extract can neutralize free radicals, showing its promise as a natural antioxidant in biodiesel–diesel blends. The extract was acquired through Soxhlet extraction and added at a concentration of 3000 ppm into B50D50 fuel mixtures. The antioxidant ability was tested using several methods: Fourier-transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and two tests that measure how well it can neutralize free radicals: DPPH$^{\cdot}$ and ABTS$^{\bullet+}$. Results were compared to TBHQ, a widely utilized synthetic antioxidant. This comprehensive method allows for the concurrent assessment of chemical reactivity, thermal stability, and oxidative inhibition potential. We aim to demonstrate that *Tilia platyphyllos*, which has a lot of phenolic compounds, is a safer and similarly effective alternative to synthetic antioxidants for improving the stability of biofuels against oxidation and heat. This introduction explains an important scientific reason, pointing out how biodiesel is prone to oxidation, the drawbacks of synthetic antioxidants like TBHQ, and the hopeful benefits of *Tilia platyphyllos*. The graphical abstract below (Figure 1) visually encapsulates these concepts, providing an overview of the plant's role in improving the performance and longevity of biodiesel–diesel fuel systems.

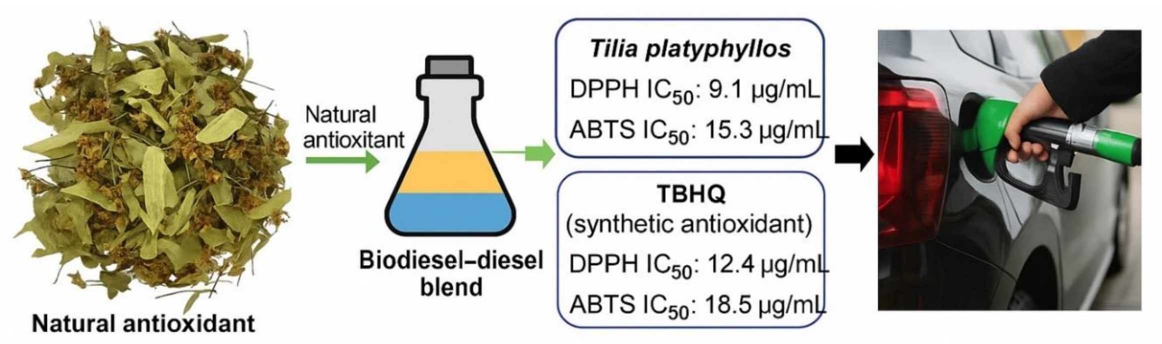


Figure 1. The antioxidant efficiency of *Tilia platyphyllos* inflorescence extract, as compared to TBHQ, in enhancing the oxidative and thermal stability of B50D50 fuel systems.

2. Materials and Methods

2.1. Measurement and Reagents

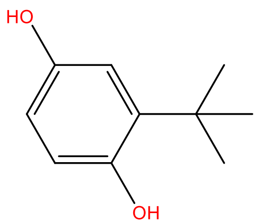
Analytical grade ethanol, methanol, and DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) were procured from Sigma-Aldrich (St. Louis, MO, USA). We acquired ABTS$^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate, and TBHQ (*tert*-butylhydroquinone) from Sigma-Aldrich. All solvents were of high-performance liquid chromatography quality. We synthesized biodiesel (B100) by trans esterifying sunflower oil. From a nearby gas station, commercial-grade diesel (D100) was purchased. All other compounds employed were of analytical quality and utilized without additional purification.

2.2. Plant Material and Extraction

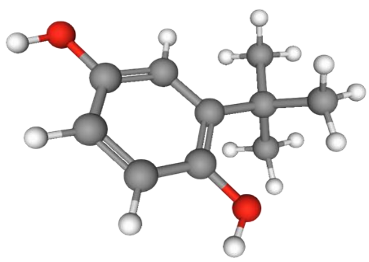
Tilia platyphyllos specimens were procured from the Samsun/Atakum region of Turkiye in May 2024, air-dried at ambient temperature (25 ± 2 °C) for seven days, and subsequently pulverized into a fine powder Fifty (50) grams of powdered material were extracted using Soxhlet apparatus with 500 mL of 70% ethanol for 6 to 8 hours. The extract was then concentrated under reduced pressure with a rotary evaporator (Buchi, Switzerland) and stored at 4 °C in amber vials. The final yield was 15.66% (w/w), corresponding to 7.83 grams of crude extract obtained from the initial plant powder. (Buchi, Switzerland) and kept at 4 °C in amber vials for future use. The ultimate yield was documented as % (w/w).

2.3. Preparation of Antioxidant-Enhanced Fuel Blends

Biodiesel-diesel blends (50% biodiesel and 50% diesel, v/v) were formulated as the base fuel. The desiccated *Tilia platyphyllos* extract was solubilized in ethanol and included in the mixtures at a final concentration of 3000 ppm. For comparison, TBHQ (Figure 2) was similarly included in distinct B50D50 blends at an equivalent concentration. All mixtures were homogenized with a magnetic stirrer for 30 minutes and thereafter kept in sealed glass containers at ambient temperature in the absence of light until analysis.



Properties



(3DModel)

Molecular formula	$C_{10}H_{14}O_2$
Molecular mass (g/mol)	166.22
Density (g/cm^3)	1.050
Boiling temperature (°C)	273
Flash point temperature (°C)	171
m/z	166.10 (100.0%), 167.10 (10.8%)
Elemental Analysis	C, 72.26; H, 8.49; O, 19.25

Figure 2. The structural formula and properties of TBHQ antioxidant.

2.4. DPPH Radical Scavenging Assay

The $DPPH^{•}$ radical scavenging activity of *Tilia platyphyllos* extract was evaluated using the method described by Brand-Williams et al. (1995), with minor modifications. A 0.1 mM $DPPH^{•}$ solution was prepared in methanol, and 1.0 mL of extract solution at five different concentrations (5, 10, 25, 50, and 100 µg/mL) was mixed with 2.0 mL of the $DPPH^{•}$ solution. The mixture was incubated in the dark at room temperature (25 ± 2 °C) for 30 minutes. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). The extract exhibited concentration-dependent scavenging activity, with inhibition percentages of 22.1%, 38.4%, 61.7%, 79.3%, and 91.2%, respectively. Based on these values, the IC_{50} of *Tilia platyphyllos* extract was calculated to be 9.1 ± 0.7 µg/mL [18].

2.5. $ABTS^{•+}$ Radical Cation Decolorization Assay

The $ABTS^{•+}$ radical scavenging activity of *Tilia platyphyllos* extract was assessed using the method of Re et al. (1999), with minor modifications. $ABTS^{•+}$ solution was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate, then incubating the mixture in the dark for 16 hours. Before use, the solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Extract solutions at five different concentrations (5, 10, 25, 50, and 100 µg/mL) were mixed with the $ABTS^{•+}$ solution, and absorbance was measured at 734 nm after 6 minutes. The percentage inhibitions recorded at these concentrations were 18.3%, 35.2%, 59.6%, 76.5%, and 89.7%, respectively. Ethanol was used as a blank, and TBHQ served as a positive control. Based on these results, the IC_{50} value of *Tilia platyphyllos* extract was calculated to be 15.3 ± 1.0 µg/mL. All measurements were carried out in triplicate [19].

2.6. Spectroscopic and Thermal Analyses

FT-IR samples were conducted using the Perkin Elmer Spectrum Two, USA. The sample surface was analyzed within the 500–4000 cm^{-1} spectrum. The ATR FT-IR spectra were obtained at ambient temperature. Thermogravimetric analysis (TGA) was conducted using the SDTQ 600 TA Instrument-Waters, USA, while differential scanning calorimetry (DSC) examination of materials was executed with the DSC Q-2000 TA Instrument-Waters, USA.

2.7. GC-MS Analysis

Conduct gas chromatography-mass spectrometry (GC-MS) analysis following the techniques set out by Aytar (Can et al., 2024). A GCMS-QP2010 mass spectrometer with an Rxi-5MS column was utilized to examine the samples.

3. Results and Discussion

3.1. FT-IR Spectral Analysis

Fourier-transform infrared (FT-IR) spectroscopy was utilized to ascertain the functional groups in the B50D50 fuel matrix augmented with *Tilia platyphyllos* extract. The recorded spectra displayed vibrational bands indicative of both the fatty acid methyl ester (FAME) framework of biodiesel and the phenolic components derived from the plant extract. An extensive absorption band seen between 3752–3479 cm^{-1} is ascribed to O–H stretching vibrations, signifying the existence of alcohol and phenolic hydroxyl groups. This section is notably important as it validates the inclusion of polyphenolic chemicals, including tiliroside, rutin, and chlorogenic acid, derived from the *Tilia platyphyllos* extract, recognized for their antioxidant properties [12,13].

The spectra displayed a faint shoulder at 3009 cm^{-1}, commonly linked to alkenic =C–H stretching. This band, however small, indicates the presence of trace unsaturated structures, maybe originating from residual plant metabolites or unsaturated fatty acids in the biodiesel matrix [20,21]. The prominent bands at 2961 cm^{-1} and 2925 cm^{-1} correspond to asymmetric C–H stretching vibrations of alkenes and alkanes, respectively. The symmetric stretching band at 2856 cm^{-1} corroborates the existence of methylene ($–CH_2–$) groups, essential to the aliphatic ester chains of biodiesel [3]. A pronounced signal at 1747 cm^{-1} signifies C=O stretching vibrations in ester carbonyl groups, typical of biodiesel and confirming efficient transesterification. This area further coincides with potential contributions from esterified phenolics found in the extract. The band at 1460 cm^{-1} and the significant area at 1253–1024 cm^{-1} correspond to C–O stretching vibrations of alkoxy esters, ethers, and C–O–C couplings. These findings indicate both the biodiesel composition and the polyphenolic profile of *Tilia platyphyllos*, especially owing to its high concentration of glycosylated flavonoids [14,22]. These fingerprint area bands are commonly employed to identify plant-derived antioxidant chemicals in functional formulations. Finally, the absorption band at 729 cm^{-1} is attributed to out-of-plane bending and rocking vibrations of methylene and =C–H groups, linked to long-chain hydrocarbons and aromatic ring structures. The existence of these signals further substantiates the aromatic nature and structural intricacy imparted by the plant extract. The FTIR spectrum conclusively demonstrated the presence of biodiesel-associated functional groups and phytochemicals abundant in antioxidants. These structural insights corroborate earlier findings on *Tilia platyphyllos* as a natural source of phenolic antioxidants and confirm its chemical compatibility within the biodiesel–diesel matrix. The related vibrational frequencies of the identified functional groups are listed in Table 1, and the FT-IR spectral profiles of the fuel samples are clearly shown in Figures 3, 4, and 5. [23–26].

Table 1. Frequencies of the functional groups for the fuel samples.

Wavenumber, cm^{-1}	**Types of vibration**	**Functional Groups**
3752-3479	Stretching	O−H of alcohols functional group
3009	Shoulder	Shoulder
2961	Asymmericalstretching	=C−H of alkenes functional group
2925	Asymmetricalstretching	C−H of alkanes functional group
2856	Symmetricalstretching	C−H of methylene functional group
1747	Stretching	C=O of ester carbonyl functional group
1460	Stretching	C−O of alkoxy esters, ethers and C−O−C functional groups
1253-1024	Stretching	C−O of alkoxy esters, ethers and C−O−C
729	Bending of Alkenes andoverlapping of rocking vibration ofmethylene	=C−H and $-(CH_2)_n$ methylene functional groupsof cis disubstituted alkenes and aromatic functional groups

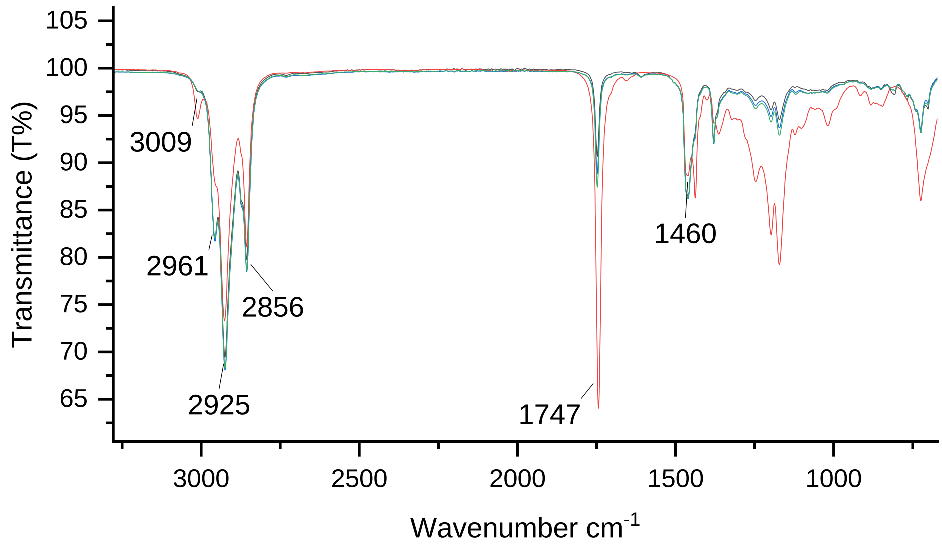


Figure 3. FT-IR spectra of D100, B50D50, B50D50TBHQ, B50D50Tp at 3250-500 cm^{-1}. (Purple: B50D50Tp, blue: B50D50TBHQ, green: B50D50, red: D100)

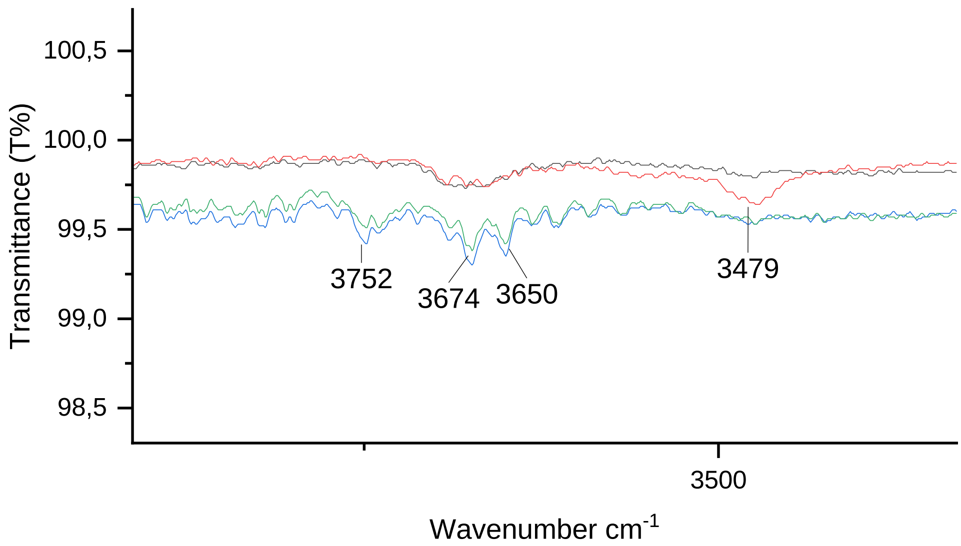


Figure 4. FT-IR spectra of D100, B50D50, B50D50TBHQ, B50D50Tp at 4000-3250 cm^{-1}. (Purple: B50D50Tp, blue: B50D50TBHQ, green: B50D50, red: D100)

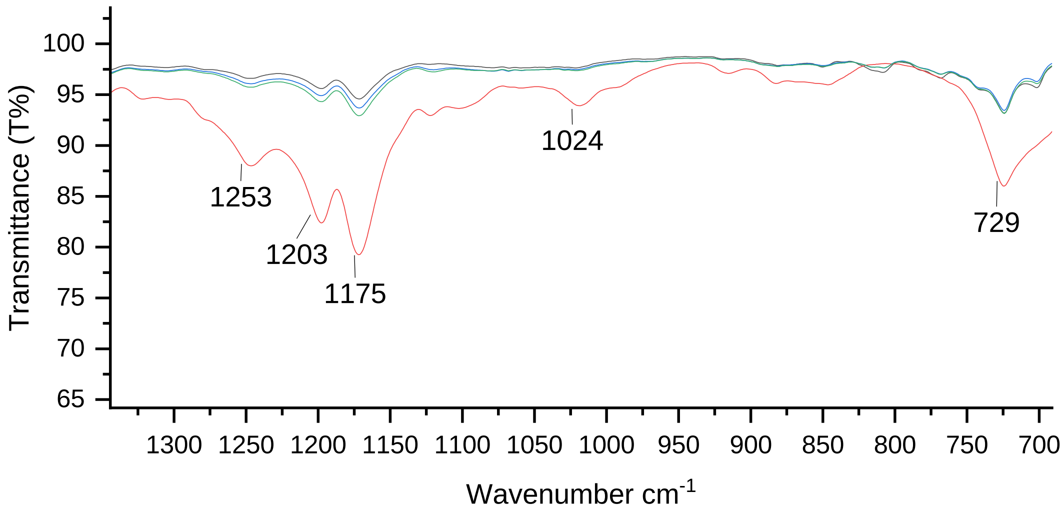


Figure 5. FT-IR spectra of D100, B50D50, B50D50TBHQ, B50D50Tp at 1350-700 cm^{-1}. (Purple: B50D50Tp, blue: B50D50TBHQ, green: B50D50, red: D100)

3.2. TGA Spectral Interpretation

The primary use of TGA is to evaluate mass loss as a function of increasing or constant temperature in a controlled environment. Approximately 10 mg of the sample was deposited in the alumina pan and thereafter put into the apparatus. The analysis was conducted at a flow rate of 50 mL/min in an inert nitrogen environment. The temperature was further elevated to 600 °C at a heating rate of 10 °C/min. The TGA test required approximately 58 minutes to complete one cycle. Mass losses of samples were documented on the computer in relation to temperature and time. The TGA graphs (Figure 6) indicate that heat breakdown transpired in a singular, continuous phase throughout the temperature range of 30–250 °C for all samples. The onset degradation temperature (Tonset) indicates the initial boiling point of the samples as well as their thermal stability. The reduced polyunsaturation and enhanced oxidation stability of the samples demonstrate a higher Tonset value. Conversely, the oxidative stability increases as the proportion of biodiesel in the mix rises [27,28].

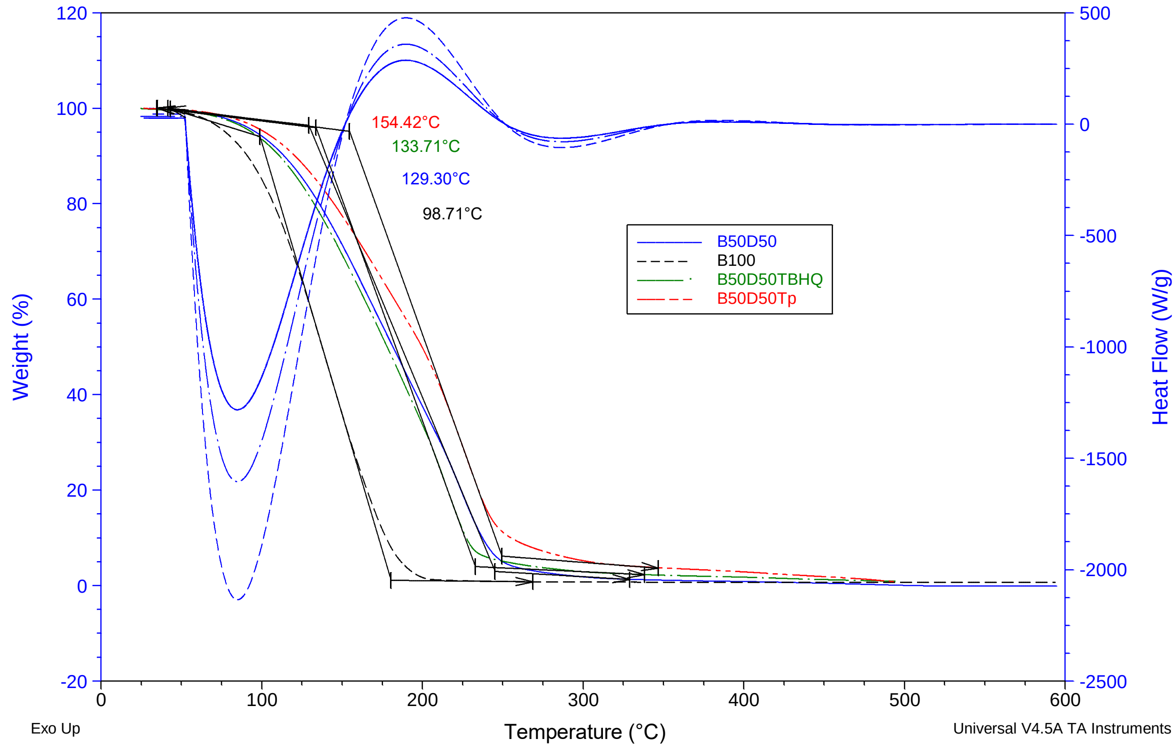


Figure 6. TGA thermograms of D100, B50D50, B50D50TBHQ, B50D50Tp.

3.3. DSC Spectral Interpretation

DSC measurements were conducted in an aluminum crucible with a little fluctuation in sample mass, roughly 10 mg, positioned in the DSC module beside a comparable empty pan as a reference. The cooling rate was 10 °C/min from −90 °C to 25 °C, with a flow rate of 50 mL/min. The apparatus was purged with nitrogen. Each DSC test required around 17.5 minutes to analyze a single sample. DSC curves are seen in Figure 7. The crystallization onset temperatures (Tc) for D100, B50D50, B50D50TBHQ, and B50D50Tp are -11.83 °C, -12.47 °C, -12.86 °C, and -12.92 °C, respectively. We observed that the extract of *Tilia platyphyllos* elevated the crystallization point compared to other samples. The use of natural antioxidants enhances oxidation stability in a corresponding manner [29,30]. The crystallization onset temperature (Tc) is a significant thermal parameter that directly affects the operability of fuels at low temperatures. A reduced Tc signifies a postponed development of crystalline structures, including waxes and esters, thereby aiding in the preservation of fuel fluidity in cold environments. This characteristic is essential for preventing fuel line obstruction and facilitating smooth engine start-up and operation in sub-ambient conditions. The observed decrease in Tc following the addition of Tilia platyphyllos extract indicates improved oxidative stability and enhanced cold flow performance of the B50D50 blend.

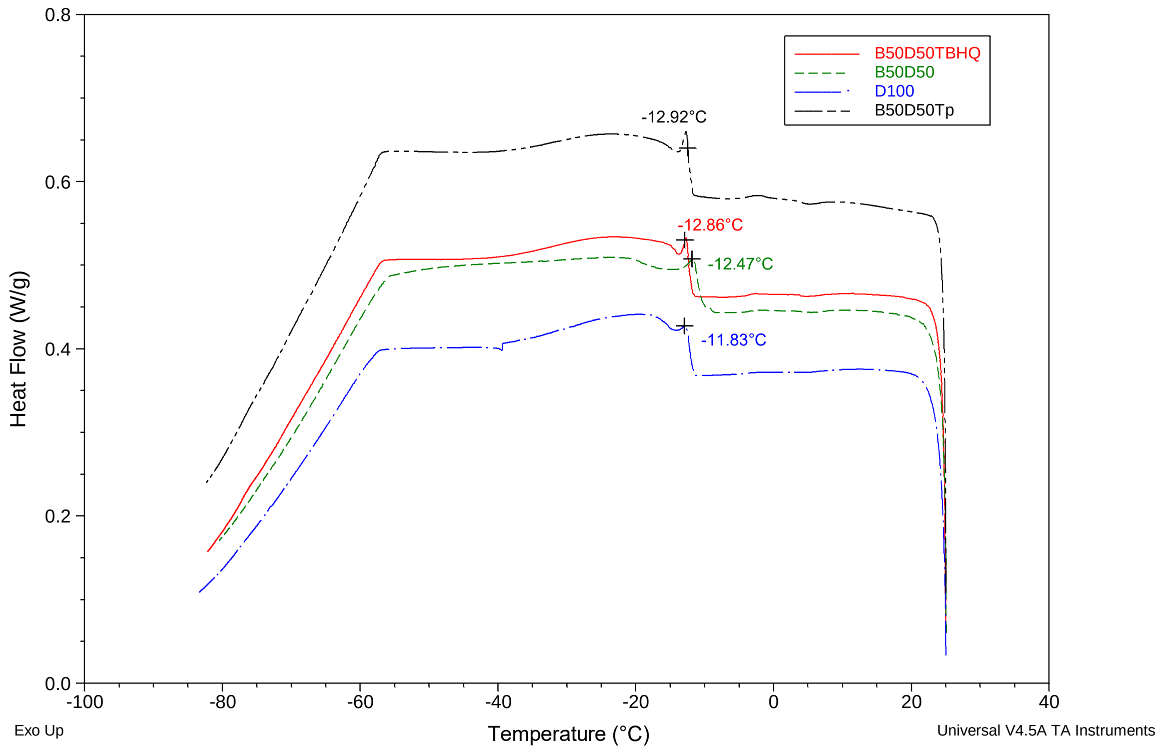


Figure 7. DSC thermograms of D100, B50D50, B50D50TBHQ, B50D50Tp.

3.4. GC-MS Analysis

The gas chromatography–mass spectrometry (GC–MS) analysis of the hexane extract from *Tilia platyphyllos* flowers identified a unique volatile profile primarily consisting of nonpolar diterpenoids, fatty acid esters, and long-chain hydrocarbons. Neophytadiene was identified as a major constituent in the hexane extract of *Tilia platyphyllos* flowers. It appeared in three distinct peaks at retention times 34.276, 34.802, and 35.189 minutes, which are likely attributable to its geometric isomers. The combined relative abundance of these peaks was calculated to be 21.16% of the total volatile content (Table 5).

Currently, there are no reports in the literature regarding the presence of neophytadiene in *Tilia platyphyllos* based on conventional studies utilizing hydrodistillation or headspace solid-phase microextraction (HS-SPME) methods [31–34]. Earlier reports predominantly emphasized lighter monoterpenes, including limonene, *β*-ocimene, linalool, and benzyl alcohol, as the principal components [35].

The identification of neophytadiene, a diterpenoid hydrocarbon synthesized from phytol, is significant and likely due to hexane's selective extraction ability for highly hydrophobic, higher molecular weight compounds. This finding is consistent with earlier studies on neophytadiene presence in other members of the Malvaceae family, including Abutilon pannosum [36], Grewia bulot [37], and Sida cordata [38], where nonpolar extraction techniques demonstrated comparable profiles abundant in diterpenoids.

The biochemical affinity of the Malvaceae family for producing phytol-derived diterpenes, including neophytadiene, supports the hypothesis that *Tilia platyphyllos*, as a member of this family, possesses a conserved metabolic capability for synthesizing these compounds. This study presents the first report of neophytadiene in *Tilia platyphyllos* flower extracts obtained through hexane extraction.

Neophytadiene exhibits antioxidant, anti-inflammatory, and antimicrobial properties [39]. Its high abundance may enhance the bioactivity and protective effects associated with *Tilia platyphyllos* in both traditional medicine and contemporary phytotherapy.

Phytol was similarly identified at 4.77% in the hexane extract. Phytol, a diterpenoid alcohol derived from chlorophyll breakdown, is well-known for its antioxidant, anti-inflammatory, antibacterial, and cytoprotective properties. While previous publications mainly linked phytol to the unsaponifiable components of *Tilia* seed oils, its presence in the floral extract underscores the extensive phytochemical potential of *Tilia* species. The presence of phytol, in conjunction with neophytadiene, underscores the role of phytol-derived diterpenoids in the observed bioactivity and highlights the therapeutic significance of hexane-extracted *Tilia platyphyllos* preparations [40].

Methyl palmitate was also detected at 8.50% in the extract. Methyl palmitate, the methyl ester of palmitic acid, is linked to significant antioxidant and antibacterial properties [41]. Its presence indicates a dual role in the chemical composition of *Tilia platyphyllos* by improving oxidative stability and offering possible protective benefits against microbial destruction. The discovery of methyl palmitate further substantiates the multifunctional biological potential of the hexane extract.

Carvacrol was identified at a concentration of 2.87% in the hexane extract. Carvacrol is a monoterpenoid phenol recognized for its potent antibacterial, antioxidant, and anti-inflammatory characteristics [42]. Despite being a tiny element in the total volatile profile, its existence corresponds with prior findings on *Tilia platyphyllos* and *Tilia cordata*, in which carvacrol was recognized as a natural component of the volatile oil fraction. The presence of this compound in the extract enhances its antibacterial efficacy and substantiates the traditional application of *Tilia* flowers for respiratory infections and inflammatory disorders.

Pentacosane was identified at 4.83% in the hexane extract. Pentacosane, a high molecular weight aliphatic hydrocarbon, has been identified as a significant component in the essential oil profile of *Tilia platyphyllos* inflorescences [43]. Its presence enhances the hydrophobic nature of the extract and may bolster barrier activities, providing protection against environmental oxidative stress. The presence of pentacosane underscores the significance of long-chain hydrocarbons in the phytochemical profile of Malvaceae family plants. Docosane was also identified as a prominent long-chain hydrocarbon, with a total relative abundance of 11.00% in the hexane extract (Table 5). This compound, previously reported in the essential oil profiles of *Tilia* species, contributes to the hydrophobic and film-forming characteristics of floral surfaces. Docosane's abundance may enhance the barrier functions of the extract, reducing moisture loss and improving protection against oxidative degradation and microbial colonization [44][45]. A detailed summary of the principal volatile constituents identified in the hexane extract of *Tilia platyphyllos* through GC–MS analysis is presented in Table 2. Figure 8 illustrates a graph of the principal volatile molecules, highlighting that neophytadiene is the most prevalent, with other significant bioactive chemicals

Table 2. Principal volatile chemicals identified in the hexane extract of *Tilia platyphyllos* using GC–MS analysis.

No	Retention Time (min)	Compound Name	Area (%)
1	34.276	Neophytadiene	12.45
2	34.802	Neophytadiene	3.38
3	35.189	Neophytadiene	5.33
4	36.086	Methyl Palmitate	8.50
5	31.419	Pentacosane	4.83
6	39.833	Phytol	4.77
7	20.910	Carvacrol	2.87
8	Multiple	Docosane (Total)	11.00

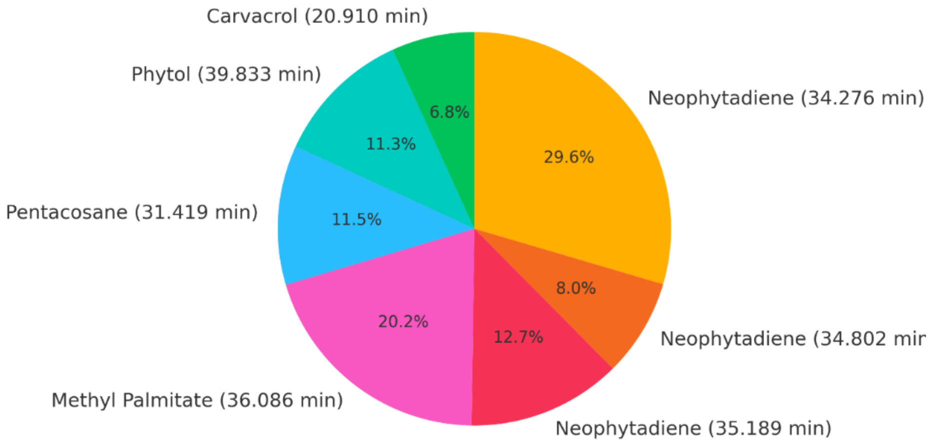


Figure 8. The GC–MS-identified principal volatile chemicals in *Tilia platyphyllos* hexane extract relative abundances (%).

3.5. Antioxidant Activity Evaluation with DPPH$^{•}$ and ABTS$^{•+}$

The antioxidant ability of *Tilia platyphyllos* flower extract was tested using DPPH$^{•}$ and ABTS$^{•+}$ methods and compared to TBHQ, a widely used synthetic antioxidant. Table 3 shows that the IC_{50} value of *Tilia platyphyllos* in the DPPH$^{•}$ test was 9.1 ± 0.7 µg/mL, which is much lower than TBHQ's value of 12.4 ± 0.5 µg/mL, indicating that the natural extract is better at scavenging radicals. In the ABTS$^{•+}$ assay, *Tilia platyphyllos* exhibited an IC_{50} value of 15.3 ± 1.0 µg/mL, surpassing TBHQ, which had an IC_{50} value of 18.5 ± 0.7 µg/mL. The findings are consistent with other research that indicates plant extracts rich in phenolic compounds can have similar or even better antioxidant effects than synthetic products (Floegel et al., 2011; Mishra et al., 2012). The strong effectiveness of *Tilia platyphyllos* is due to its high levels of polyphenolic compounds, like chlorogenic acid, tiliroside, and rutin, which are known to significantly help with antioxidant activity through hydrogen atom transfer (HAT) and single electron transfer (SET) methods. The studies show that *Tilia platyphyllos* is a promising natural antioxidant that could be used in food preservation and fuel stabilization, offering a better and safer option than TBHQ.

Table 3. Comparison of IC_{50} values (µg/mL) for radical scavenging activity.

Sample	**DPPH$^{•}$ IC_{50} (µg/mL)**	**ABTS$^{•+}$ IC_{50} (µg/mL)**
Tilia platyphyllos	9.1 ± 0.7	15.3 ± 1.0
TBHQ	12.4 ± 0.5	18.5 ± 0.7

Note: IC_{50} values were calculated using linear regression of inhibition (%) versus concentration (µg/mL). The R^2 values were 0.9971 for DPPH$^{•}$ and 0.9965 for ABTS$^{•+}$ assays.

4. Conclusion

This research has conclusively demonstrated that *Tilia platyphyllos* flower extract serves as a potent natural antioxidant, enhancing the oxidative and thermal stability of biodiesel–diesel (B50D50) blends. The extract, abundant in phenolic compounds such as tiliroside, rutin, and chlorogenic acid, has shown superior efficacy in combating free radicals compared to the synthetic antioxidant TBHQ, evidenced by lower IC_{50} values in both $DPPH^{\bullet}$ (9.1 ± 0.7 µg/mL) and $ABTS^{\bullet +}$ (15.3 ± 1.0 µg/mL) assays. Spectroscopic (FT-IR) and thermal analyses (TGA and DSC) validated the effective incorporation of phytochemicals into the fuel mixture, enhancing its structural integrity and postponing oxidative degradation. The thermal stability of B50D50 blends was markedly enhanced by the incorporation of *Tilia platyphyllos* extract, evidenced by elevated Tonset values and alterations in crystallization behavior. The thermal stability of B50D50 blends was markedly enhanced by the incorporation of *Tilia platyphyllos* extract, evidenced by elevated Tonset values and alterations in the crystallization behavior of the combination. The GC–MS study identified a distinctive combination of nonpolar diterpenoids (including neophytadiene and phytol), fatty acid esters (such as methyl palmitate), long-chain hydrocarbons (like pentacosane), and trace quantities of monoterpenes (such as carvacrol and limonene). These components collaborate to enhance the extract's efficacy in combating oxidation and bacteria, hence affirming its suitability for biofuel applications.. In conclusion, *Tilia platyphyllos* extract is a very promising and environmentally sustainable component for biodiesel, providing a natural substitute for synthetic antioxidants while enhancing storage stability, augmenting heat resistance, and perhaps reducing environmental effects.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Nalan Türköz Karakullukçu: Contributed to the conceptual design of the study, was responsible for data acquisition and organization, performed the interpretation of the findings, and took the lead in drafting and finalizing the manuscript.

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