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Research Article

The Impact of *Hypericum perforatum L.* as an Organic Free-Radical Scavenger in Biodiesel-Diesel Blends

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ABSTRACT

The extraction of *Hypericum perforatum* L. (HP) was performed using the Soxhlet extraction method to evaluate its potential as an organic free-radical scavenger in biodiesel-diesel blends. Experimental blends—B100, B20D80, B20D80BHT, and B20D80HP—were prepared, incorporating *Hypericum perforatum* L. extract at a concentration of 3000 ppm, and compared with butylhydroxytoluene (BHT). The antioxidant properties were assessed using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), Fourier-transform infrared spectroscopy (FT-IR), high-performance liquid chromatography (HPLC), and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. DSC analysis ranked the antioxidant efficiency as D100 < B20D80 < B20D80BHT < B20D80HP, demonstrating the superior stabilization effect of *Hypericum perforatum* L. extract. TGA and FT-IR results confirmed enhanced thermal stability, while HPLC identified key phenolic compounds such as rutin, ellagic acid, and kaempferol, which contribute to antioxidant activity. DPPH assays further confirmed the extract's superior free-radical scavenging efficiency compared to BHT. These findings highlight *Hypericum perforatum* L. as a promising natural antioxidant for improving biodiesel oxidative stability.

1. Introduction

The proportion of energy requirements that are now being covered by fossil fuels is expected to grow. Due to the detrimental impact of fossil resource utilization on environmental contamination, there has been a growing body of research focused on exploring alternate fuel sources [1]. Biodiesel, a sustainable energy source, is widely considered essential for maintaining a healthy ecosystem [2]. The use of biodiesel has several advantages. One noteworthy advantage of this specific feature lies in its non-toxic nature, which guarantees safety and cost-effectiveness [3].

The biodiesel production process entails a sequential progression of chemical reactions, resulting in the formation of free radicals that are prone to oxidation within the surrounding atmosphere [4]. This oxidation process has been shown to reduce fuel efficiency and engine performance [5]. The maintenance of storage stability is a crucial aspect of assessing the quality of biodiesel. Consequently, the significance of standards and fuel quality assurance in Europe cannot be overstated [6].

Free-radical scavengers are a class of molecules that play a crucial role in terminating the oxidation process. They achieve this by either inhibiting the formation of free radicals or scavenging existing radicals, thereby effectively managing the oxidation of biodiesel even at low concentrations [7]. Typically, free-radical scavengers possess phenolic functional groups within their chemical structure [8]. Antioxidant defenses in organisms depend on the intricate interaction between tiny molecules and enzymes to control the levels of potentially hazardous oxidizing species within physiological

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boundaries. Chain reactions fueled by peroxyl radicals (ROO·) from uncontrolled oxygen and nitrogen centered radicals lead to enhanced toxic effects [9].

Recent research has shown that organic and synthetic free-radical scavengers must be included in biodiesel in order to improve and optimize its oxidative stability. The prominence of lowering the quantity of free radicals in biodiesel and prolonging oxidation is underscored by the use of free-radical scavengers [10]. This study investigates the phenolic compounds acquired by the extraction process from readily accessible, ecologically sustainable sources [11]. Plant-based biodiesel-diesel fuel blends involving radical reactions were quenched using the *Hypericum perforatum L.* plant from soxhlet extraction.

The free-radical scavenger capacity of the phenolic compounds employed has been validated by a comparative analysis with butylhydroxytoluene (BHT). 3,5-di-tert-butyl-4-hydroxybenzoic acid is a significant metabolite of BHT that can be produced from the corresponding alcohol and aldehyde (BHT-CHO) [12]. This synthetic free-radical scavenger variant is believed to possess hazardous and carcinogenic properties. BHT is a derivative of toluene utilized as an antioxidant. BHT has been found to have both positive and negative effects on cancer growth in many tissues and organs, suggesting that it can serve as either a prooxidant or an antioxidant [13].
However, the specific antioxidant activity kinetics of BHT remain uncertain [14]. Table 1 lists the properties of BHT antioxidation. To achieve the desired objective, blends of biodiesel and diesel were created in certain proportions, namely B100, B20D80, B20D80BHT, and B20D80HP.

The extraction of *Hypericum perforatum L.* plant extract was performed using soxhlet equipment. After adding extract, the mixtures were supplemented with a concentration of 3000 parts per million (ppm) [15].

Table 1. Properties of BHT antioxidation

Property	**Butyl hydroxytoluene (BHT)**
Molecular formula	$C_{15}H_{24}O$
Molecular mass (g/mol)	220.35
Density (g/cm^3)	1.05
Boiling temperature (°C)	265
Flash point temperature (°C)	127

An evaluation was carried out on the plant extract from *Hypericum perforatum L.* to determine its free-radical scavenger activity. Various characterization techniques were utilized, such as thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), fourier-transform infrared spectroscopy (FT-IR), high-performance liquid chromatography (HPLC), and the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) assay. This study's results suggest that incorporating phenolic chemicals, which are recognized for their ability to scavenge free radicals organically, led to a significant reduction in the oxidation of biodiesel-diesel blends [16].

Phenolic free-radical scavengers (AH) are compounds found in plant extracts that can terminate free radicals. These options are typically favored due to their ecologically conscious nature, efficacy, natural composition, affordability, and widespread accessibility [17]. Resonance delocalization in phenolic free-radical scavengers prevents the formation of reactive oxygen species (ROS) and allows for the formation of stable radical intermediates [18]. Phenolic free-radical scavengers demonstrate advantageous attributes in their capacity as hydrogen donors. The stability of the phenoxy radical is attained by the mechanism of electron delocalization across the aromatic ring, as indicated by the presence of valence bond isomers [19].

Oxidation is the most prevalent reaction that produces hydroperoxides as the primary by-product. The rate of the hydroperoxide formation reaction determines the rate of the oxidation reaction. The degree of autooxidation sensitivity is determined by the ease with which allylic hydrogens and peroxyl radicals (ROO·) react with oil production chain forces with weak ties [9]. The hybrid radical is produced when the peroxide radical reacts with the allylic system.

Oxygen attack at both extremities of the allylic system generates a combination of 1- and 3-hydroperoxides [20].

Hypericum perforatum L. is a plant species characterized by its yellow blossoms. Originally indigenous to Europe, this botanical specimen has also been seen to thrive in natural habitat throughout several regions, including North America, Asia, India, Australia, South Africa, and several islands [21] and [22]. The plant in issue is a member of *the Hypericaceae family*, formerly included in the *Clusiaceae family*. The spread of this phenomenon spans across the globe [23]. The pharmacological properties of *Hypericum perforatum L.* have been proven to include its potential for reducing depression and its antiviral and antibacterial activity. These findings support the traditional usage of this plant The potential therapeutic implications of the antidepressant effect within the framework of diabetes have been demonstrated in animal studies [24].

2. General Methods

All compounds were utilized in their as-received state without additional purification and were procured from reputable suppliers such as Merck, Sigma, or Aldrich Chemical Company. The solvent employed in the experiment was of spectroscopic quality. Aves Energy Oil and Food Industry provided Aspire biodiesel, while OPET supplied diesel fuel in Turkiye. The *Hypericum perforatum L.* plant specimens were hand-gathered from the Nebiyan district of Atakum, Samsun, Turkiye. Surface contaminants were removed using distilled water.
Subsequently, the sample was subjected to a drying process in an oven set at a temperature of 60 °C for 72 hours, resulting in the production of a final powdered substance. A grinding apparatus was employed to compact and separate the desiccated substances. The sample was appropriately preserved by being stored at a temperature of 4 °C while ensuring it was shielded from light and humidity until the following extraction protocols were carried out [25-26].

2.1. Soxhlet extraction

Hypericum perforatum L. plant powder (30 g) was put in a filter paper cellulose cartridge and then extracted using 300 mL of analytical grade n-hexane over 8 hours on a soxhlet system. After the completion of the extraction procedure, the residual solvent was efficiently isolated from the solid sample using a rotary evaporator. Following this, the materials within the glass flask with a round bottom were securely centrifuged at 2000 rpm for 7 minute and stored at a temperature of 4 °C, anticipating the upcoming experimental steps [27].

2.2. Preparations of biodiesel-diesel blends

Diesel and biodiesel blends were typically formulated with a biodiesel-to-diesel ratio ranging from 20% to 80%. The B20D80 formula provided the composition information. The plant extracts were combined at a concentration of 3000 ppm [28].

2.3. Differential scanning calorimetry (DSC)

DSC methods can be employed to characterize, quantify, and infer. The present study aimed to examine the initiation temperatures of crystallization for the materials D100, B20D80, B20D80BHT, and B20D80HP. This investigation was conducted using a TA Q-2000 model calorimeter with an RCS90 fitted with a cooling system. Aluminum pans were employed to conduct the analysis. In this experimental procedure, a sample weighing 5 ± 0.5 mg was meticulously placed into the pan. Within the temperature range of 25 °C to -90 °C, the cooling rate was established at 10 °C, accompanied by a nitrogen flow of 50 mL per minute [28].

2.4. Thermogravimetric analysis (TGA)

TGA is mainly utilized for the purpose of ascertaining the relationship between mass loss and increasing or constant temperature under regulated atmospheric conditions. This analytical technique is employed to measure vapors, assess combustion reactions, evaluate degradation processes, and determine leftover substances in products. The breakdown points of numerous organic compounds and the enhancement of their

components may be seen using the TGA [29]. TGA was conducted with the help of an SDT Q-600 (TA Instrument-Waters, USA). The samples were obtained by heating five 5 ± 0.5 mg powder samples to a temperature of 10 °C/min in an alumina pan with an oxygen gas flow of 50 mL/min up to 400 °C [30].

2.5. Fourier transform infrared spectroscopy (FT- IR)

The chemical functional groups present in the plant extract of *Hypericum perforatum L.* were examined using FT-IR spectroscopy with a Perkin Elmer Spectrum-Two equipment from the USA. The spectral range of 650 to 4000 cm^{-1} was utilized for analyzing the surface of the sample. The ATR FT-IR spectra were collected at a consistent temperature under normal environmental conditions. Background subtraction methods, baseline correction, and data fine-tuning were applied as described in reference [31-32].

2.6. High performance liquid chromatography (HPLC)

HPLC is a separation method that involves the transfer of mass between stationary and mobile phases. The primary usage of this is for analytical purposes, where a liquid mobile phase is mechanically pushed through a column holding a stationary phase This setup is illustrated in Figure 1 of an HPLC equipment.

HPLC analysis was performed on the samples using a Shimadzu LC20-A Prominence device. A mass of 0.1 mg was taken from the model and subsequently dissolved in 10 mL of methyl alcohol. 100 µL of extractant was acquired from a filter with a pore diameter of 0.45 µm. The extractant was then combined with 900 µL of methanol and subsequently analyzed [33].

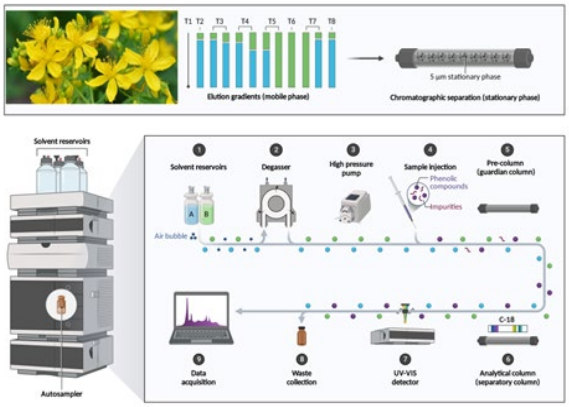

Figure 1. Components and steps of HPLC analysis

2.7. Free-radical scavenger activity

DPPH· is a stable radical with an unpaired electron. It reacts with antioxidant chemicals to produce 1,1-diphenyl-2-picrylhydrazine, leading to discoloration. DPPH· was dissolved in ethanol at 100 mM. The novel compounds were dissolved in dimethyl sulfoxide (DMSO) to create stock solutions at a concentration of 1024 µg/mL. A uniform mixture was created by mixing 150 µL of material with quantities varying from 2 to 1024 µg/mL, along with a reference component (BHT). The mixture was uniformly combined with 50 µL of 0.1 mM DPPH· and dissolved in ethanol. The combination was prepared on a 96-well plate. The combinations were kept in a dark area at room temperature for 30 minutes. Absorbance measurements were taken for each combination at a wavelength of 517 nm, with a blank used as the reference point. The IC50 value in grams per milliliter was determined using the calibration curve. The IC50 value is calculated by measuring the concentration of chemicals needed to cause a 50% inhibition, where a lower IC50 value signifies a higher level of free-radical scavenging activity [34- 35].

2.8. Statistical analysis

Information was examined using SPSS 20.0, an application developed by IBM for the Statistical Package for the Social Sciences. Because the data were normally distributed and the two independent groups' means were similar, an analysis of variance (ANOVA) was used in the study. Using the data gathered, the Tukey honestly significant difference (HSD) test was used for multiple comparisons. The significance

threshold of the values was set at $p < 0.05$, and their statistical significance was ascertained by comparing them to the set of results from the activity analysis. Statistical significance was determined for these values [36-37].

3. Results and Discussion

3.1. Differential scanning calorimetry (DSC)

DSC readings of mixed fuel with organic free-radical scavengers and unbleached fuel were obtained in a nitrogen environment, as shown in Figure 2. The crystallization temperature of the organic free-radical scavenger mix fuel rose from -8.06 °C to -12.67 °C upon comparison of the samples, as indicated in Table 2.

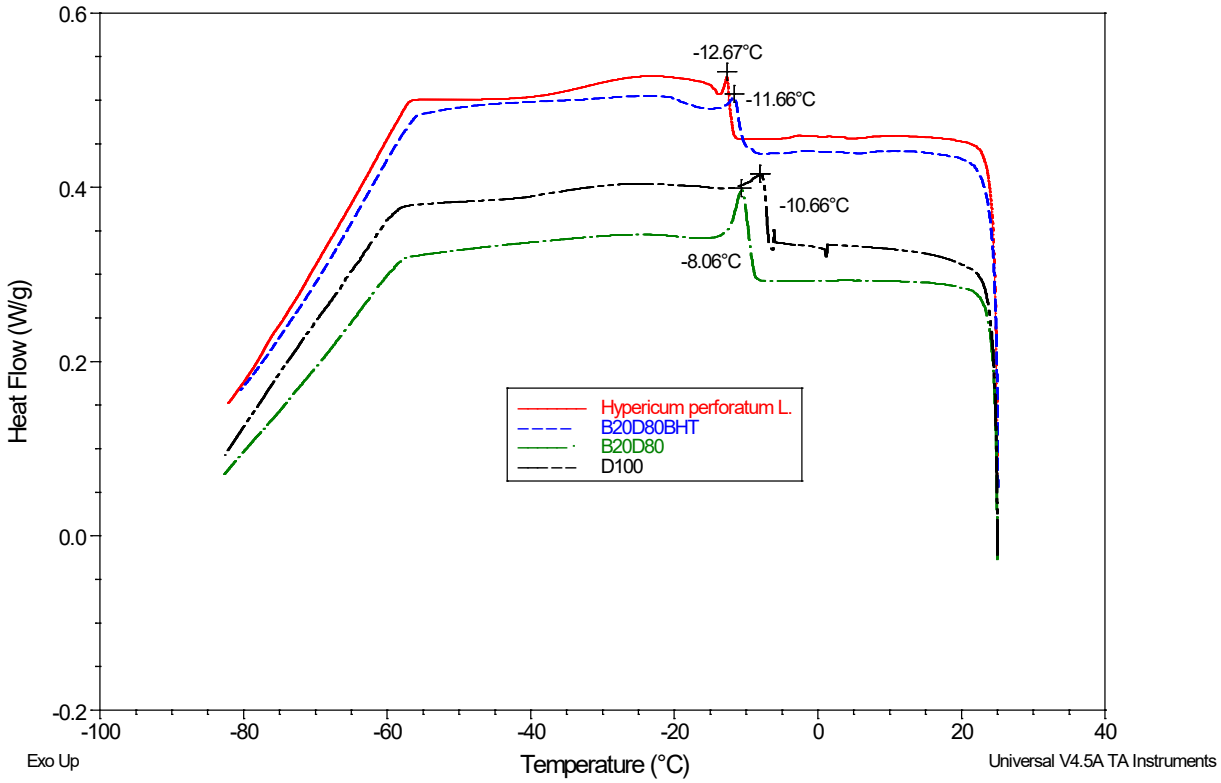

Figure 2. DSC thermograms of D100, B20D80, B20D80BHT, B20D80HP under N_2 atmosphere

Table 2. D100, B20D80, B20D80BHT, and B20D80HP crystallization onset temperatures (°C) in a N2 atmosphere and biodiesel-diesel mix amounts

Sample	Crystallization onset temperature (°C)	Biodiesel (%)	Diesel (%)
D100	|-8.06|	-	100
B20D80	|-10.66|	20	80
B20D80 BHT	|-11.66|	20	80
B20D80 HP	|-12.67|	20	80

When comparing the regular solution model's predictions to the data from the DSC, it is evident that the amount of precipitated crystal is rather close to what was seen in the experiment.

The objective of this research was to determine the critical sites of crystallization for biodiesel-diesel blends, including extracts from the herb *Hypericum perforatum L.*, which neutralize toxic free radicals. A higher crystallization point was found in the *Hypericum perforatum L.* extract, according to the study's results [36].

The temperatures at which the crystallization process begins for D100, B20D80, B20D80BHT, and B20D80HP are different from one another. The experimental findings indicate that the crystallization starting temperatures for the samples labeled as D100, B20D80, B20D80BHT, and B20D80HP were determined to be $|-8.06|$ °C, $|-10.66|$ °C, $|-11.66|$ °C, and $|-12.67|$ °C, respectively.

Due to the fact that the incorporation of organic free-radical scavengers improves oxidation stability in the same sequence, B20D80, B20D80BHT, and B20D80HP would all crystallize at a temperature that is lower than that of D100.
Considering all these factors, it can be concluded that the chemical exhibits traits like quick oxidation, premature crystallization, and a heightened susceptibility to oxidation. This decline results in decreased stability of the properties. The items are B20D80HP, B20D80BHT, B20D80, and D100.

3.2.Thermogravimetric analysis (TGA)

In TGA, the mass of a sample is measured against time and a temperature that is linearly changing in a certain environment. TG is carried out with the use of a thermobalance or a thermogravimetric analyzer. Monitoring the change in weight while maintaining a steady heating rate is how this is accomplished. The outcome is a graph that shows the relationship between mass and either time or temperature. TGA has developed as an alternative technology in the biofuels business that is less costly, faster, and easier to manage [37].

There is just one deterioration degree vs. temperature when all TGA-DTG graphs of the mixes are compared to one another. The onset temperature, which represents the early deterioration temperature, provides insight into the thermal stability and the first boiling point [38].

There is a positive correlation between the stability of the samples and the Tonset values, indicating that as the stability of the samples increases, the Tonset values also increase [39].

The curves depicting the TGA and derivative thermogravimetric analysis (DrTGA) are presented in Figure 3.

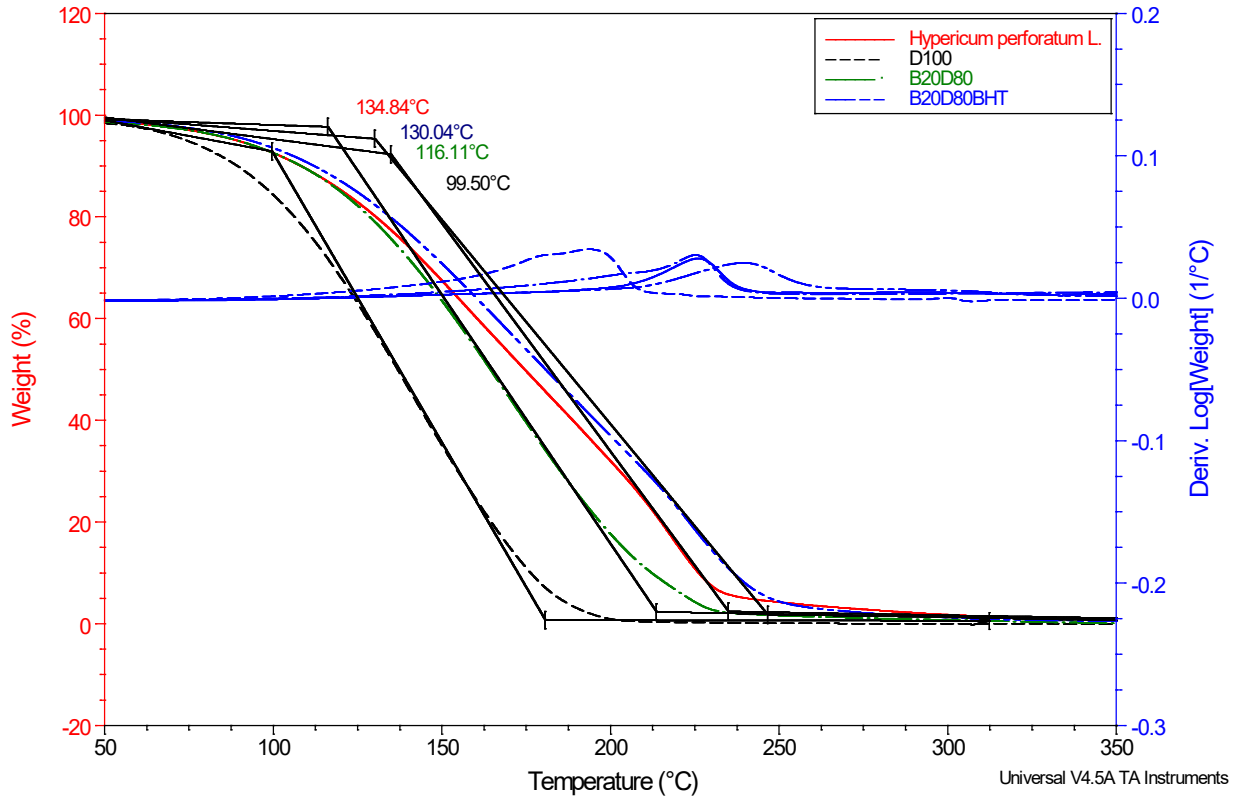

Figure 3. TGA and DrTGA curves of D100, B20D80, B20D80BHT, B20D80HP under O_2 atmosphere

The curves show a significant similarity when observed. Between these temperatures, there is a sample mass loss ranging from 99.28% to 99.59%. The thermometer values obtained from the thermograms are outlined in Table 3.

Table 3. Thermogravimetric analysis (TGA) of samples

Sample name	Temp. range (°C) (From 25 °C)	Max, degradation temp. (°C) (Tonset)	Mass loss (%)
D100	180.55	99.50	99.42
B20D80	213.58	116.11	99.28
B20D80BHT	246.64	130.04	99.33
B20D80HP	234.94	134.84	99.59

3.3. Fourier transform infrared spectroscopy (FT-IR)

The FTIR spectra of a material can be analyzed to determine the presence of ether or ester functional groups by looking for the characteristic ν(CO) and ν(C=O) vibrations. The valence-stretching vibration of an unbounded hydroxyl group, denoted as ν(O−H), is reported to have a frequency of 3392 cm^{-1}. Furthermore, the infrared spectra of the functional biodiesel samples demonstrate a reduction in the magnitude of the vibrations at ν(C−H) (2700−3000 cm^{-1}). Esters exhibit two distinct absorptions resulting from the ν(C=O) and ν(C−O) functional groups.

The presence of an adjacent oxygen atom significantly elevates the carbonyl frequency compared to typical ketones, hence facilitating the distinction between the two. Nevertheless, there exists a degree of overlap among unsaturated esters, resulting in a reduction in the CO frequency [40].

The spectra as a whole exhibit significant absorption bands that are characteristic of the important ester carbonyl functional group ν(CO–O). Because of this, the absence of any neighboring bands indicates the absence of carboxylic acids. The durability of the examined biodiesel samples during storage and oxidation is a result of the extremely low oxidation levels across the board [41]. The FT-IR spectra of D100, B20D80, B20D80BHT, and B20D80HP are depicted in Figures 4, 5 and 6. The impact of including both organic and synthetic free-radical scavengers in biodiesel samples at a concentration of 3000 ppm is documented in Table 4.

Table 4. Frequencies of the functional groups for the fuel samples

Wavenumber, cm^{-1}	Types of vibration	Functional Groups
3392	Stretching	O−H of alcohols functional group
2955	Asymmetrical stretching	=C−H of alkenes functional group
2923	Asymmetrical stretching	C−H of alkanes functional group
2850	Symmetrical stretching	C−H of methylene functional group
1748	Stretching	C=O of ester carbonyl functional group
1462−1380	Stretching	C−O of alkoxy esters, ethers and C−O−C functional groups
725	Bending of alkenes and overlapping of rocking vibration of methylene	=C−H and $-(CH_2)_n$ methylene functional groups of cis disubstituted alkenes and aromatic functional groups

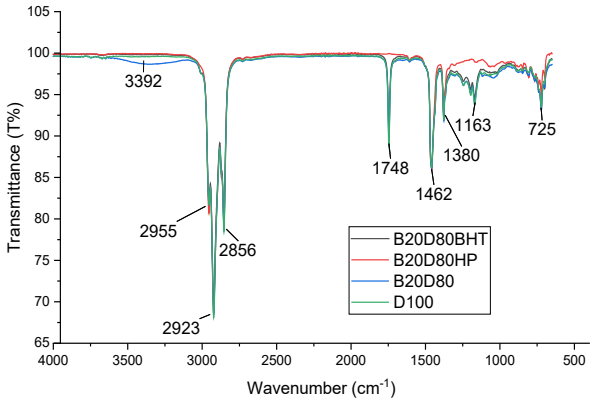

Figure 4. FT-IR spectra of D100, B20D80, B20D80BHT, and B20D80HP at 4000-500 cm^{-1}

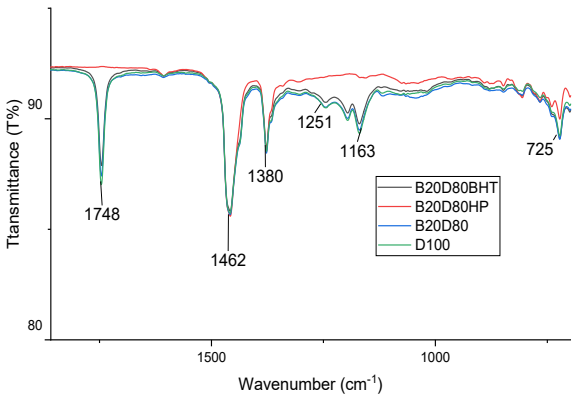

Figure 5. FT-IR spectra of D100, B20D80, B20D80BHT, and B20D80HP at 1875-750 cm^{-1}

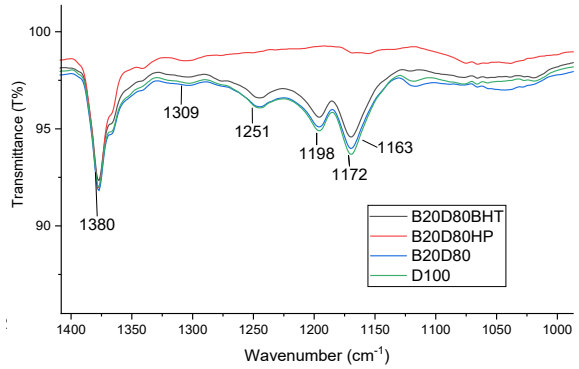

Figure 6. FT-IR spectra of D100, B20D80, B20D80BHT, and B20D80HP at 1400-1000 cm^{-1}

3.4. High performance liquid chromatography (HPLC)

Research has related the ability to scavenge free radicals to the concentration of phenolic components. Phenolic chemicals generated from plants are commonly found in nature and have been noted for their ability to scavenge free radicals. Therefore, it is crucial to identify the phenolic compounds found in plant extracts [42].

Hypericum perforatum L. is known to possess a diverse array of chemical constituents, encompassing volatile oils, flavonoids, anthraquinone derivatives (such as naphthodianthrones), prenylated phloroglucinols, tannins, xanthones, and several other odd substances. The medicinal chemicals found in *Hypericum perforatum L.* species are of significant importance. These compounds include phloroglucinols, including hyperforin, naphthodianthrones like hypericin and pseudohypericin, and flavonoids such as quercetin, quercitrin, rutin, and hyperoside [43].

The HPLC examination of extracts obtained from *Hypericum perforatum L.* revealed that rutin was present at a concentration of 195.243 mg/L, while ellagic acid was shown to be the major component among the phenolic compounds, with a concentration of 173.492 mg/L. The findings of the study indicate that the secondary metabolites present in the *Hypericum perforatum L.* extract are butein (45.989 mg/L), 2,5-dihydroxy benzoic acid (36.902 mg/L), kaempherol (31.050 mg/L), catechin (20.693 mg/L), and myricetin (18.325 mg/L). Naringenin (11.629 mg/L), ferulic acid (5.817 mg/L), chrysin (3.572 mg/L), taxifolin (2.059 mg/L), and coumaric acid (0.576 mg/L) are small components found in the extract as well.

Table 5 shows the findings from high-performance liquid chromatography (HPLC) tests on certain phenolic parts of *Hypericum perforatum L.* extracts. When it comes to the existence of phenolic and flavonoid compounds, our study's results are in line with previous studies [46-48]

3.5. Antioxidant activity evaluation with DPPH· free radical scavenger effect

As seen in Table 6, in comparison to BHT, it was observed that the *Hypericum perforatum L.* extract exhibited a greater numerical value. This finding suggests that the phenolic compounds included in the composition of *Hypericum perforatum L.* extracts exhibit greater efficacy compared to BHT. This implies that the biological activity and free radical-scavenging capabilities of *Hypericum perforatum L.* extracts are superior to those of BHT.

Table 5. HPLC determination of particular phenolic compounds in *Hypericum perforatum L.* extracts

Phenolic Compound	***Hypericum perforatum L.***		
	t_R (min.)	**Conc. (mg/L)**	**λ (nm)**
Catechin	26.688	20.693	280
Taxifolin	47.664	2.059	280
Ellagic acid	71.227	173.492	280
Caffeic acid	0.000	0.000	320
Coumaric acid	43.125	0.576	320
Myricetin	75.086	18.325	360
Kaempherol	79.744	31.050	360
Naringenin	67.671	11.629	280
Chrysin	81.456	3.572	280
Triacetin	0.000	0.000	280
2,5-Dihydroxy Benzoic acid	26.654	36.902	320
Ferulic acid	48.487	5.817	320
Rutin	70.595	195.243	360
Butein	77.534	45.989	360

Table 6. DPPH· free radical scavenging activity of compounds

Sample	**IC50**
Hypericum perforatum L.	15.63±0.95a
BHT	24.42±0.39a

4. Conclusion

In order to carry out this investigation, four samples were created, namely D100, B20D80, B20D80BHT, and B20D80HP. The samples underwent characterization by the utilization of several analytical methods, including DSC, TGA, FT-IR, HPLC, and assessment of the DPPH· free radical scavenging action. The subsequent section presents a concise overview of the outcomes that were obtained.

The use of organic or synthetic antioxidants leads to an elevation in the crystallization temperature (Tc). As a result of contrasting several samples, the crystallization temperatures were found to range from |-8.06| °C to |-12.67| °C, with lower temperatures indicating more purity. The relative ranking of antioxidant potency was determined to be as follows: D100 < B20D80 < B20D80BHT < B20D80HP. The findings derived from the DSC research revealed that the biodiesel samples B100, B20D80, B20D80BHT, and B20D80HP exhibited crystallization onset temperatures of |-8.06| °C, |-10.66| °C, |-11.66| °C, and |-12.67| °C, respectively.

The thermal stability of the samples is observed to increase with the addition of organic free-radical scavengers, as indicated by the TGA and DrTGA curves [44].

The TGA method accurately evaluates the thermal and oxidative stability of fuels. We utilized the DSC technique to precisely identify the initiation of crystallization in the fuel samples [45]. The rationale behind this argument is that depositing foreign material, such as salt or sand, onto snowy roadways functions as a means of impeding the process of crystallization and averting frostbite. Based on the concept, the freezing point exhibits a drop, and the lower the temperature, the later the crystallization occurs. The delayed solidification of matter implies that it may maintain its characteristics for an extended duration, directly correlated with its stability. Thus, a product that has undergone late crystallization has the ability to be stored for a long duration. Late crystallization refers to a situation when the freezing point is low and oxidation occurs slowly.

The FT-IR diagrams show that the oxidative stability of the biodiesel-diesel blend with 3000 ppm of *Hypericum perforatum L.* oil extract (designated B20D80D) was improved because the blend contained fewer functional groups than other simples, such as B20D80BHT.

HPLC analysis of *Hypericum perforatum L.* extracts from Turkiye demonstrated a comprehensive profile of its phenolic constituents. The notable presence of rutin and the significant concentration of ellagic acid underscore their importance in the chemical composition of this plant extract. Especially, rutin is a flavonoid with free-radical scavenger properties. It has been studied for its potential to improve blood vessel health, reduce inflammation, and protect against oxidative stress, which would be beneficial in managing conditions such as cardiovascular disease, diabetes, and arthritis. Rutin-rich plants like buckwheat have been used in traditional medicine for their potential to strengthen blood vessels and reduce bleeding. Rutin-containing herbs have also been employed to treat conditions like hemorrhoids and varicose veins.

The second dominant compound, ellagic acid is a polyphenol with potent free-radical scavenger properties. It has been investigated for its potential in cancer prevention, as it may help inhibit the growth of cancer cells and reduce oxidative damage. Ellagic acid is found in various fruits like strawberries, raspberries, and pomegranates. Traditional herbal medicine has used these fruits to treat diarrhoea, inflammation, and wounds. It has also been considered for its potential anticancer properties.

Similarly to rutin and ellagic acid may also have anti-inflammatory and antimicrobial effects. Moreover, our study has identified and quantified a range of secondary metabolites, including butein, 2,5-dihydroxy benzoic acid, kaempherol, catechin, and myricetin. Naringenin, ferulic acid, chrysin, taxifolin, and coumaric acid also have various health-promoting properties. Naringenin, for example, is found in citrus fruits and has been studied for its potential role in reducing cholesterol levels. Overall, our findings align with previous research, confirming the presence of various flavonoid and phenolic compounds in

this extract. This knowledge is crucial for understanding the potential therapeutic benefits associated with *Hypericum perforatum L.* It may pave the way for further investigations into its applications in pharmaceuticals, nutraceuticals, and natural product-based therapies. The detailed composition data provided in this study will serve as a valuable resource for future research endeavors in the fields of phytochemistry and plant-based medicine.

The assessment of in vitro free-radical scavenger activity was conducted using the free radical scavenging approach, employing the DPPH· molecule. The results were presented in relation to IC50 values, which were denoted in micrograms per milliliter (µg/mL). The samples that included free-radical scavengers showed a decrease in values in comparison to the biodiesel sample lacking free-radical scavengers (D100).

The ranking was primarily established by DSC, TGA, FT-IR, and HPLC tests, illustrating the contribution of *Hypericum perforatum L.* extract to improved oxidative stability. The IC50 values from the DPPH study corroborated this ranking, so validating the effectiveness of the organic free radical scavenger.

Mixed samples of Aspire biodiesel and diesel mix benefit from organic free-radical scavengers. Organic free-radical scavengers improve the oxidative stability of diesel and Aspire biodiesel blends at all concentrations.

Incorporating *Hypericum perforatum L.* plant extract as an organic free-radical scavenger improved biodiesel's oxidative stability, especially at a concentration of 3000 ppm. An extract of the plant *Hypericum perforatum L.* has free-radical scavenger properties that are equivalent to those of the synthetic drug BHT.

Article Information Form

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No conflict of interest or common interest has been declared by the author.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The author of the paper declares that she complies with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that she does not make any falsification on the data collected. In addition, she declares that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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