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ANTIOXIDANT ACTIVITY OF *THUJA OCCIDENTALIS* LEAVES

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Abstract. *Medicinal plants contain bioactive natural compounds that demonstrate considerable therapeutic potential. Their significance arises from the variety of bioactive molecules they possess, such as phenolic compounds, carotenoids, tocopherols, and vitamins, all of which positively impact health. There is growing interest in these bioactive compounds, particularly their antioxidant properties and beneficial effects on chronic conditions like obesity, diabetes, and cancer. Thuja occidentalis (Cupressaceae) originated in Eastern North America and is grown in Europe and Brazil as an ornamental tree, commonly referred to as the "tree of life" or "white cedar." This study assesses the phytochemical profile and antioxidant activity of three Thuja occidentalis leaf varieties. We measured total polyphenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and* IC_{50}*. Furthermore, we analysed how using fresh versus dried plant material for extract preparation affects the phytochemical profile and antioxidant activity. Our findings revealed that TPC ranged from 5.15 to 13.73 mg GAE/g for fresh leaves and 11.12 to 17.52 mg GAE/g for dried leaves. TFC varied between 3.06 and 11.23 mg QUE/g for fresh leaves and 5.73 to 14.52 mg QUE/g for dried leaves. The DPPH radical scavenging activity spanned from 39.12% to 57.33% for fresh material compared to 30.21% to 57.69% for dried material. These results suggest that drying the plant affects the phytochemical compounds and antioxidant activity.*

Keywords*: Cupressaceae, total polyphenolic content, total flavonoid content, DPPH scavenging,* IC_{50}

INTRODUCTION

Medicinal plants offer bioactive natural compounds with significant therapeutic potential. Their importance lies in the diverse bioactive molecules present, including phenolic compounds, carotenoids, tocopherols, and vitamins, all of which contribute positively to health. Increasing attention is given to these bioactive compounds, especially their antioxidant properties and beneficial effects on chronic diseases such as obesity, diabetes, and cancer [Alves et al., 2014, Liu et al., 2023, Gajos et al., 2024, Marquez – Campos et al., 2020]. Some bioactive compounds exhibit slow reactions, requiring extended periods to reveal their antioxidant advantages. Moreover, the favourable effects of biomolecules in mixtures depend not only on their concentrations but also on the interactions between antioxidants [Olszowy et al., 2019, Wang et al., 2011, Saha et al., 2014]. Recently, there has been a surge in preclinical and clinical studies exploring the benefits of plant extracts and biologically active compounds on human health [Domingo et al., 2024].

Thuja occidentalis (*Cupressaceae*), commonly referred to as the "tree of life" or "white cedar," originates from Eastern North America and is widely cultivated in Europe and Brazil as an ornamental tree [Caruntu et al., 2020]. Renowned for its bioactive compounds, such as polyphenols, flavonoids, and essential oils, it has long been utilised in traditional medicine. Research has highlighted its potential health benefits, particularly its antioxidant, antimicrobial, and anti-inflammatory properties. The plant's substantial polyphenolic content and antioxidant capacity are pivotal to its therapeutic applications, providing a natural source for promoting health and preventing diseases.

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This study aims to evaluate the phytochemical profile and antioxidant activity of three varieties of *Thuja occidentalis* leaves. For this purpose, we determined the total polyphenolic content, total flavonoid content, DPPH radical scavenging activity, and IC_{50}. Additionally, we studied the impact of using fresh or dried plant material for extract preparation on the phytochemical profile and antioxidant activity.

MATERIAL AND METHODS

Chemicals

Sigma–Aldrich Chemie GmbH (München, Germany) supplied the reagents Folin–Ciocalteu, gallic acid, quercetin standard, and 1,1-diphenyl-2-picrylhydrazyl (DPPH), while Geyer GmbH (Renningen, Germany) provided sodium carbonate, sodium nitrite, and aluminium chloride. All reagents used for the chemical analysis were of analytical quality. Ascorbic acid was obtained from Lach-Ner Company (Neratovice, Czech Republic), and ethanol was procured from Chimreactiv SRL (Bucharest, Romania).

Plant material and extraction method

Thuja occidentalis plants were sourced from Liebling, Timis County, Romania (45°34′00″N 21°19′54″E). Three varieties were utilised in the experiment: smaragd (TOS), golden smaragd (TOGS), and fastigiata (TOF). After discarding all unsuitable parts, the fresh leaves were divided into two samples, preserving the superior plant material. The first sample was ground and immediately subjected to the extraction process. The second sample was allowed to air dry in ambient conditions and was regularly turned until it achieved a constant mass. The dried samples were stored in paper bags at 18–20°C, protected from light.

The extraction was conducted using a conventional solvent method: 1 g of the sample was combined with 10 ml of 70% ethyl alcohol and allowed to extract for 24 hours on a Holt plate stirrer at room temperature. The samples were filtered through filter paper and stored at 4°C until they were prepared for chemical analysis.

Determination of total phenolic content (TPC)

To assess the total polyphenol content, 0.5 mL of the extract was combined with 1.25 mL of Folin-Ciocalteu reagent and allowed to sit at room temperature for 5 minutes. Following this, 1 mL of 6% Na_2CO_3 was added, the mixture was vigorously mixed, and then allowed to rest at room temperature for 2 hours. Absorbance was measured at 750 nm with a Specord 205 UV-VIS spectrophotometer (Analytik Jena AG, Jena, Germany). Each sample was analysed in triplicate, and the results were reported as mean ± standard deviation. A calibration curve was created using gallic acid as the standard, expressing results in mg GAE/g.

To compare the TPC content obtained from the two samples, dried and fresh, the following indicator was employed:

$$\text{Increasing TPC}_{\text{DRIED/FRESH}}\ (\%) = \left[\frac{TPC\ D - TPC\ F}{TPC\ F}\right] \times 100 \qquad (1)$$

Where:

TPC_D - TPC of the dried sample (mg GAE/g)

TPC_F - TPC of the fresh sample (mg GAE/g)

Determination of flavonoid content (TFC)

To assess the flavonoid content, we combined 1 mL of extract with 0.3 mL of 10% $AlCl_3$ and 0.3 mL of 5% $NaNO_2$, allowing the mixture to sit at room temperature for 6 minutes. Then, 2 mL of 1M NaOH and 6.4 mL of 70% ethanol were added. The mixture was shaken vigorously and left at room temperature for 30 minutes. Absorbance was then measured at 415

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nm using a Specord 205 UV-VIS spectrophotometer (Analytik Jena AG, Jena, Germany). Each sample was analysed in triplicate, and the results were presented as mean ± standard deviation. A calibration curve was plotted using quercetin as the standard, with values reported in mg QUE/g.

To compare the TFC content obtained from the two samples, dried and fresh, the following indicator was employed:

$$\text{Increasing } TFC_{DRIED/FRESH}\ (\%) = \left[\frac{TFC\ D - TFC\ F}{TFC\ F}\right] \times 100 \qquad (2)$$

Where:
TFC_D - TFC of the dried sample (mg QUE/g)
TFC_F - TFC of the fresh sample (mg QUE/g)

DPPH assay antioxidant capacity

Dilutions of various samples were prepared to establish five different concentrations for assessing the capacity for free radicals inhibition. A volume of 1 mL from each diluted sample was combined with 2.5 mL of 0.3 mM DPPH reagent, mixed, and allowed to stand in darkness for 30 minutes. One sample was created for the control by substituting the extract volume with 70% ethanol. Additionally, analyses were conducted using five ascorbic acid concentrations (0.02–0.1 mg/mL) as a positive control. The absorbance was measured at 518 nm using a Specord 205 UV-VIS (Analytik Jena AG, Jena, Germany) spectrophotometer. Each sample underwent triplicate analysis, presenting results as mean ± standard deviation. The outcomes were calculated based on the following formula:

$$\text{RSA } (\%) = \left(\frac{A\,c - A\,s}{A\,c}\right) \text{ x } 100$$

Where: A_c = absorbance value of the control sample
A_s = absorbance value of the extract sample

The IC_{50} value, representing the antioxidant capacity, was compared to ascorbic acid.

Statistical analysis

The statistical analysis was conducted using JASP 0.19.3.0. Descriptive statistics, including the mean and standard deviation, were calculated to evaluate the study data. Group differences were analysed through analysis of variance (ANOVA), with the Tukey test applied for post hoc comparisons. Statistical significance was determined at a threshold of $p < 0.05$.

RESULTS AND DISCUSSIONS

Determination of total phenolic content (TPC)

Phenolic compounds are secondary metabolites plants produce, primarily synthesised as defence mechanisms against biotic and abiotic stresses, including predation and UV radiation exposure. Their unique chemical structures grant these compounds natural antioxidant properties, allowing them to protect cellular components from oxidative damage [Rao et al., 2025].

Figure 1 presents the TPC values expressed in mg GAE/g. Figure 2 illustrates the percentage increase in TPC following various sample preparations, calculated using Equation (1).

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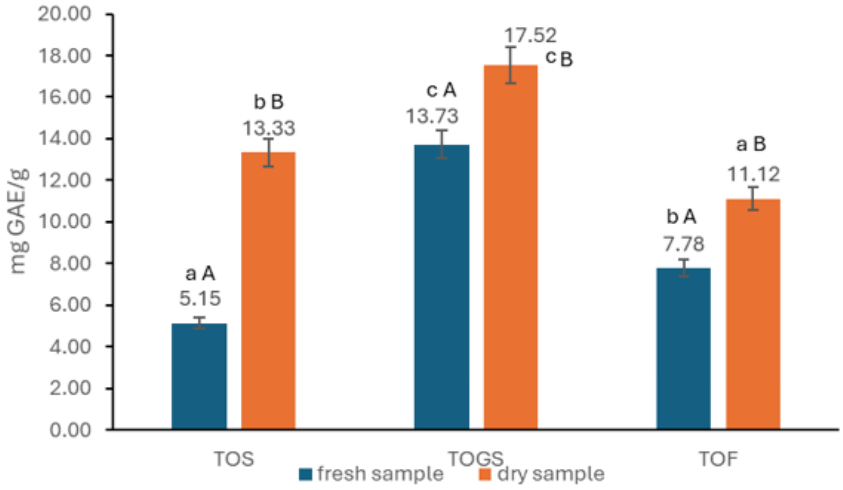

Figure 1. TPC content samples

According to the ANOVA, the different lower-case letters (a–c) indicate significant differences ($p < 0.05$) between samples processed using the same method. The different capital letters (A, B) indicate significant differences ($p < 0.05$) between samples obtained through different processing methods of the material.

Figure 1 shows that the TPC value ranges from 5.15 mg GAE/g to 13.73 mg GAE/g for the samples obtained through the extraction of the fresh plant, with values increasing in the order TOS < TOF < TOGS, indicating statistically significant differences. For the samples derived from the extraction of the dried plant, the TPC values range from 11.12 mg GAE/g to 17.52 mg GAE/g, demonstrating statistically significant differences and increasing in the order TOF < TOS < TOGS.

Analysing each sample individually, statistically significant differences can be noted between the two forms examined: fresh and dried.

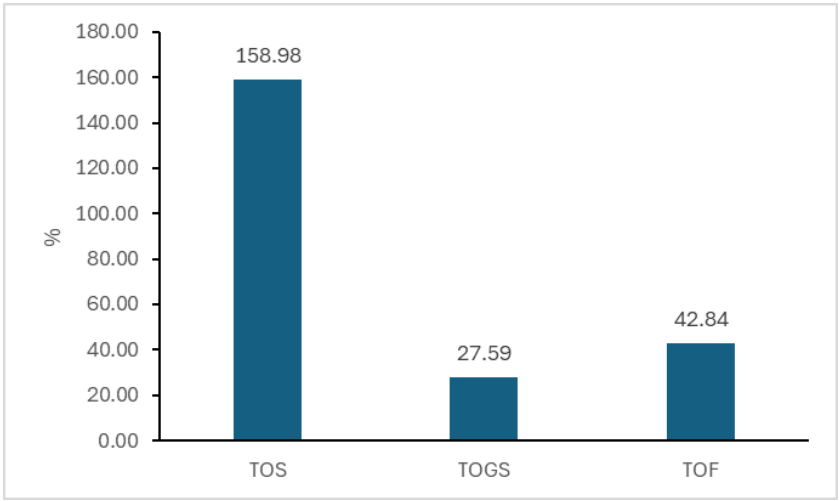

Figure 2. Increasing TPC using dried/fresh leaves

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From Figure 2, it is evident that there is a remarkable increase in TPC values between the two forms analysed. The highest increase is noted in the TOS sample (158.98%), while the lowest increase is recorded in the TOGS sample (27.59%).

The values observed in the present study follow those documented in previous research published within the scientific literature. In their study, Stan et al. investigated the antioxidant and anti-inflammatory properties of a mother tincture (MT) obtained from fresh, young, non-woody branches with leaves of Thuja occidentalis L. using distillation-based techniques for the treatment of ulcerative colitis. They found a total phenolic content (TPC) value of 3.9±0.09 mg GAE/g [Stan et al., 2019].

In another study, Nazir et al. investigated the phytochemical composition and antioxidant potential of methanolic extract (leaves) of Thuja occidentalis from India, and they reported a TPC value of 135.32 mg GAE/g; this value was higher than the value found in our study [Nazir et al., 2016]. Tekaday et al. evaluated the antimicrobial, antioxidant, and phytochemical properties of Thuja occidentalis (Arbor vitae) leaf extract and reported a TPC value of 0.125 mg GAE/g. This value was smaller than the value found in our study [Tekaday et al., 2020].

Polyphenol levels exhibit measurable differences influenced by biotic and abiotic factors affecting their biosynthesis pathways. The total phenolic content (TPC) varies according to geographical location, altitude, seasonal variations, and soil nutrient availability. For example, greater exposure to UV light and drought stress typically enhance phenolic accumulation by activating biosynthetic pathways, particularly the phenylpropanoid pathway. Furthermore, biotic stressors such as fungal infections or insect herbivory can induce increased phenolic production as a protective response.

Determination of flavonoid content (TFC)

The antioxidant capacity of flavonoids relies on their molecular structure, especially the number and position of hydroxyl (–OH) groups, the effects of conjugation and resonance, the environmental factors that affect the chosen antioxidant site, and the distinct antioxidant mechanisms of each compound [Ullah et al., 2020].

Figure 3 displays TFC values in mg QUE/g. Figure 4 depicts the percentage increase in TFC after different sample preparations, calculated using Equation (2).

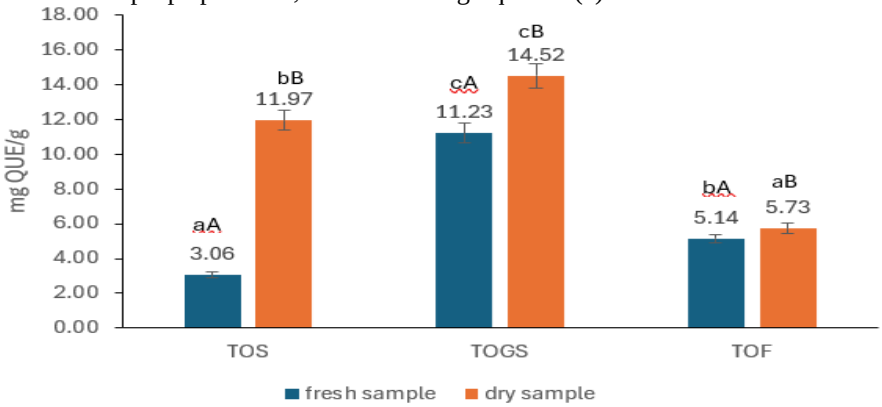

Figure 3. TFC content samples

According to the ANOVA, the different lower-case letters (a–c) indicate significant differences ($p < 0.05$) between samples processed using the same method. The different capital letters (A, B) indicate significant differences ($p < 0.05$) between samples obtained through different processing methods of the material.

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According to Figure 3, TFC values for samples extracted from fresh plants range from 3.06 mg QUE/g to 11.23 mg QUE/g, with values trending in the order of TOS < TOF < TOGS, indicating statistically significant differences. For samples extracted from dried plants, TFC values range from 5.73 mg QUE/g to 14.52 mg QUE/g, showing statistically significant differences and increasing in the order of TOF < TOS < TOGS. When analysing each sample individually, considerable differences are evident between the fresh and dried forms.

Figure 4 reveals a notable increase in TFC values between the two examined forms, with the highest increase observed in the TOS sample at 291.43%, while the lowest increase is noted in the TOF sample (11.38%).

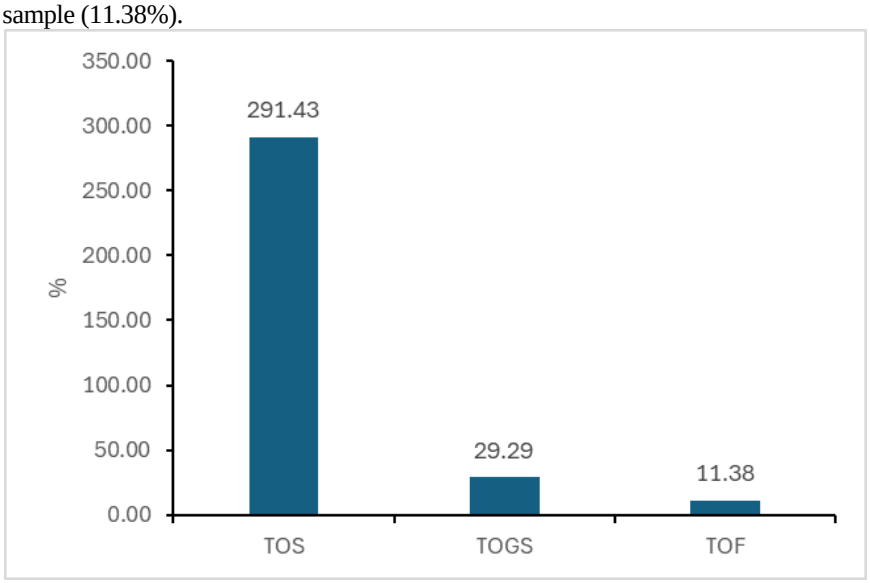

Figure 4. Increasing TFC using dried/fresh leaves

The values observed in the current study align with those recorded in earlier research published in the scientific literature.

The total flavonoid content in the methanolic extracts of *Thuja occidentalis* was 3.46 mg QE/g, as the Nazir et al. study reported [Nazir et al., 2016]. Yogesh et al. reported a TFC value of 7.48 mg CE/gdw in their research on the antioxidant activity of thuja cone extract in raw chicken mince during refrigerated storage [Yogesh et al., 2014].

Dried leaves typically exhibit a higher TPC and TFC per gram due to moisture loss and enhanced extraction efficiency. The TPC and TFC of *Thuja occidentalis* leaves vary considerably between fresh and dried samples, influenced by moisture content, enzymatic activity, and chemical stability. Active enzymes like polyphenol oxidase (PPO) and peroxidase can degrade polyphenols through oxidation in fresh leaves. Drying, particularly at elevated temperatures, reduces enzyme activity and helps preserve polyphenols. Additionally, the drying process converts bound polyphenols into free forms, improving their extractability. Research indicates that drying can sometimes increase TPC by breaking down complex phenolic compounds into more straightforward, more easily extractable forms [Pradhan et al., 2021].

Antioxidant capacity by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

Antioxidant activity is a crucial biological property that significantly impacts different industries, such as cosmetics, food, and beverages. Because antioxidant potential is essential for assessing the therapeutic benefits of plants, this study utilised antioxidant assays,

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specifically the radical scavenging (DPPH•) method. This particular assay is often recommended because of its simplicity, speed, reproducibility, and cost-effectiveness, making it an ideal tool for measuring the antioxidant activity of plants.

The values presented in Figure 5 show that the maximum radical scavenging activity was recorded at the highest concentrations of 10 mg/mL, 4 mg/mL, and 2.5 mg/mL for all samples.

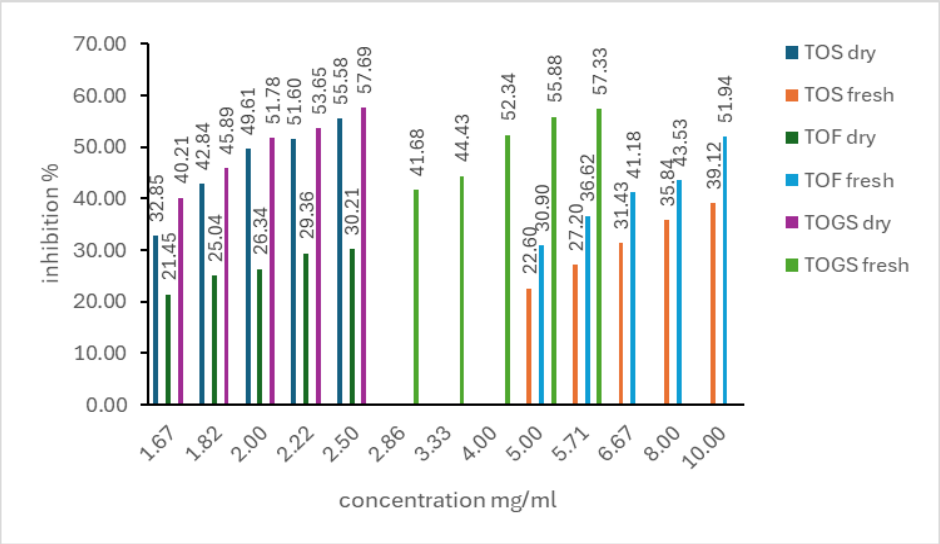

Figure 5. The DPPH radical scavenging activity (% inhibition)

The TOS values for DPPH radical scavenging were 39.12% in fresh samples compared to 55.58% in dried samples. For TOGS, the scavenging values were 57.33% for fresh samples and 57.69% for dried samples. In the case of TOF, the DPPH radical scavenging values stood at 51.94% for fresh samples and 30.24% for dried samples.

Other authors reported similar values for *Thuja occidentalis* extracts. Stan et al. reported a DPPH free radical scavenging activity of 88.3%, which exceeds our value [Stan et al. 2019]. In contrast, Yogesh et al. found a scavenging activity of 25.52%, which is lower than the value reported in our study [Yogesh et al., 2014].

Table 1 presents the values obtained for IC_{50} compared to those obtained for the control sample, ascorbic acid.

Table 1

The IC_{50} value of sample extracts vs. ascorbic acid

Sample	IC_{50} (mg/mL)
TOS_F	7.50 ± 0.23^{c}
TOS_D	3.65 ± 0.51^{b}
$TOGS_F$	2.95 ± 0.23^{ab}
$TOGS_D$	2.05 ± 0.22^{a}
TOF_F	9.43 ± 0.67^{d}
TOF_D	7.98 ± 0.53^{c}
Ascorbic acid	2.47^{a}

$_F$ - fresh leaves, $_D$ – dry leaves

According to the ANOVA, the different lower-case letters (a–d) indicate significant differences ($p < 0.05$) between samples processed using different processing methods of material.

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Table 1 demonstrates that the maximum antioxidant activity was attained for all the examined dried samples, followed by the fresh sample. IC_{50} values ranged from the $TOGS_D$ 2.05 mg/mL (highest antioxidant capacity) to the TOF_F 9.43 mg/mL (lowest antioxidant capacity).
Most samples exhibit statistically significant differences ($p < 0.05$), except for TOS_F and TOF_D, as well as $TOGS_D$ and ascorbic acid, which do not show significant differences ($p > 0.05$).
The $TOGS_D$ sample exhibited a lower IC_{50} value than the ascorbic acid control, indicating it may offer enhanced protection against oxidation.

CONCLUSIONS

This study highlights the significant influence of plant processing methods on the phytochemical profile and antioxidant activity of *Thuja occidentalis leaf* extracts. The findings demonstrate that dried leaves generally have higher total polyphenolic content (TPC) and total flavonoid content (TFC) than fresh leaves, indicating that the drying process may concentrate certain bioactive compounds. However, as measured by DPPH radical scavenging activity, antioxidant activity remained relatively stable between fresh and dried material, suggesting that the antioxidant potential is preserved despite the changes in phytochemical composition. TOGS exhibited the highest enhanced protection against oxidation among the tested samples, highlighting its superior antioxidant capacity. These results emphasise the importance of selecting appropriate plant processing methods to maximise the therapeutic potential of *Thuja occidentalis* extracts. The enhanced phytochemical content in dried leaves could offer advantages for developing natural antioxidant products with potential health benefits. Further research is needed to explore the specific mechanisms underlying these changes and to evaluate the bioavailability and efficacy of these compounds in vivo.

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