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Research Article

A comparative analysis of the effects of different drying and storage techniques on the phenolic content of *Pistacia atlantica* leaves

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Abstract: This study presents a comparative analysis of the effects of different drying and storage techniques on the phenolic content of *Pistacia atlantica* leaves in both whole and ground states. Four drying methods were evaluated: direct sunlight, a ventilated drying chamber, shade, and obscurity. Additionally, three storage conditions were assessed: direct sunlight, shade, and freezing. The concentrations of total phenolics, total flavonoids, and tannins were quantified, and antioxidant activity was evaluated using the DPPH radical scavenging assay. Our findings indicate that drying in obscurity and shade yielded the highest levels of total phenolics (369.45±2.12 and 362.78±1.36 mg GAE/g DM), total flavonoids (54.34±0.95 and 55.68±1.25 mg QE/g DM), and tannins (36.35±0.91 and 33.80±0.79 mg CE/g DM), correlated with strong antioxidant activity (1.85±0.01 and 1.92±0.01 µg/mL, respectively), particularly when the leaves were stored whole. The results emphasize that controlled drying methods (ventilated chambers and darkness), along with storing the leaves whole and under freezing conditions, are optimal for preserving the antioxidant activity and phenolic content of *P. atlantica* leaves. Freezing proved to be the most effective storage condition for preserving phenolic compound concentrations and their associated antioxidant properties. Overall, the study highlights that the strategic selection of drying techniques and storage conditions is critical for optimizing the preservation of phenolic compounds and antioxidant activity in plant materials.

1. INTRODUCTION

Medicinal plants have been utilized across various fields for centuries, including traditional medicine, pharmaceuticals, cosmetics, nutrition, agriculture, and environmental restoration. They yield active compounds for pharmaceutical applications, natural ingredients for skincare, essential nutrients for well-being, biopesticides for crop protection, and contribute to ecological balance. Medicinal plants are critical subjects of scientific inquiry, leading to the discovery of new therapeutic compounds and expanding our understanding of their mechanisms of action. Medicinal plants are often used in their dried form rather than fresh. Drying the plants helps to enhance their shelf life, improve their storage stability, and concentrate their active compounds. The quality and effectiveness of dried medicinal plants heavily rely on the precise drying and

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storage conditions employed. It is crucial to employ suitable drying techniques, along with implementing proper storage conditions such as controlled temperature, humidity, and protection from light. By ensuring these optimal conditions, the integrity of the plant's constituents can be preserved, guaranteeing the long-term potency and efficacy of the dried medicinal plants.

Several studies have been conducted on the effects of drying techniques like air-drying, freeze-drying, and shade-drying, as well as on storage conditions such as freezing, canning, and other methods (Bettaieb Rebey *et al*., 2020; Czarniecka-Skubina, 2002; Kumar *et al*., 2022; Lin *et al*., 2020; Roshanak *et al*., 2016; Vega-Gálvez *et al*., 2011).

The main objective of this study was to assess the impact of various drying techniques, including direct sunlight, ventilated drying chamber, shade, and obscurity, as well as different storage methods such as direct sunlight, shade, and freezing, on the phenolic content and antioxidant activity. The chosen example was the leaves of *P. atlatica*, which is a species that has been extensively studied (Amel *et al*., 2016; Belyagoubi-Benhammou *et al*., 2015; Belyagoubi *et al*., 2016; Benhammou *et al*., 2008; Benmahieddine *et al*., 2021, 2023; Toul *et al*., 2017, 2022), focusing on various aspects. However, the specific effects of different drying and storage techniques on the phenolic content of these leaves have not been previously investigated. This study aims to fill this research gap and provide insights into the impact of drying and storage conditions on the phenolic composition of *P. atlantica* leaves.

2. MATERIAL and METHODS

2.1. Plant Material

The leaves of *P.atlantica* were randomly harvested, without regard to exposure, from the municipality of Igli, wilaya of Beni Abbass, located in the southwest of Algeria, in March 2021. Botanical identification was performed in the Laboratory of Valorization of Plant Resources and Food Security in Semi-Arid Areas, Department of Biology, Faculty of Nature and Life Sciences, Tahri Mohammed University of Bechar.

2.2. Drying Conditions

As shown in Figure 1, the harvested leaves were divided into four distinct groups, with each group subjected to a different drying method. The leaves were deemed dry when they became brittle or partially brittle, depending on the drying technique, and their color transitioned from green to yellow-green.

The first group of leaves was dried under direct sunlight (S), exposing them to natural environmental conditions, including variable temperatures and humidity levels, for approximately 3 days (they were moved to a well-ventilated indoor area during the night to prevent moisture reabsorption. The drying process resumed the following day under direct sunlight until the desired dryness was achieved). The second group was dried inside a ventilated chamber (laboratory oven) set at a constant temperature of 30°C (Dc), ensuring a controlled drying environment with minimal exposure to external factors, over 2 days. The third group was shade-dried at room temperature (Sh), allowing for a slower drying process under indirect light, which took about 5 days to complete. The fourth group was dried in a completely dark place (obscurity) at room temperature (O), minimizing light exposure to prevent degradation of light-sensitive compounds, for 7 days. Once the drying process was complete, a portion of each group was analyzed to assess their phenolic content and antioxidant activity.

2.3. Storage Conditions

The four groups of dried leaves were divided into three subgroups, and each subgroup from each group was stored for 30 days under different conditions: in the freezer at -18°C (F), under shade at room temperature and relative humidity (Sh), or exposed to direct sunlight (S). Additionally, each leaf subgroup was further stored in two different forms: entire leaves (E) and ground leaves (G), as depicted in Figure 1.

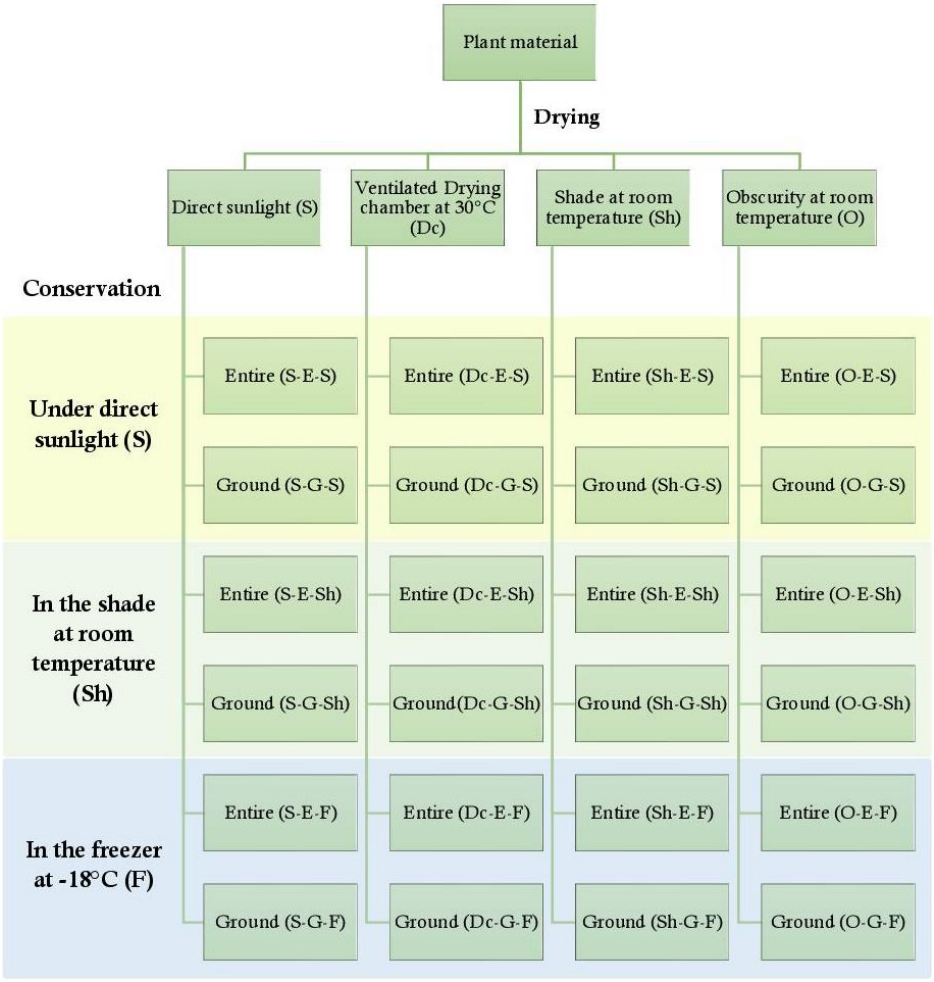

Figure 1. Drying and storage protocol.

2.4. Preparation of Extracts

Entire leaves were ground using a basic cutter mill (IKA A10, IKA-Werke, Germany) into a fine powder just before extraction, without considering milling time and speed. Only the necessary quantity was processed to avoid browning and potential degradation of active compounds. From each subgroup, 1 g of powdered leaves was macerated in 20 mL of methanol for 24 hours at room temperature. The methanolic solution was filtered and then evaporated to dryness at 60°C using a standard rotary evaporator. The dry residue was resolved in some milliliters of methanol and stored in the refrigerator for later use (Toul *et al*., 2017).

2.5. Total Phenolic Content (TPC)

A volume of 200 µl of each crude extract was added to 1 mL of 10 times diluted Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (Na_2CO_3) at 7.5%. The mixture was incubated at room temperature for 30 minutes, and the absorbance was recorded at 765 nm of wavelength against a blank (Singleton & Rossi, 1965). The results were expressed in milligram equivalents of gallic acid per gram of dry matter.

2.6. Total Flavonoid Content (TFC)

A volume of 500 µL of different concentrations of extract was mixed with 1500 µL of distilled water. At time zero, 150 µL of 5% $NaNO_2$ was added to the mixture. After 5 min, 150 µL of 10% $AlCl_3$ were introduced. After being incubated for 6 min at room temperature, 500 µL of NaOH (1M) were added. Immediately, the mixture was thoroughly shaken, and the absorbance was recorded at 510 nm against the blank. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry matter (Zhishen *et al*., 1999).

2.7. Tannin Content (TC)

An aliquot of 50 µl extract was added to 1500 µl of a 4% (m/v) vanillin/methanol solution. After being stirred, 750 µl of concentrated hydrochloric acid (HCl) was added. The absorbance was measured at 550 nm after 20 minutes of incubation (Julkunen-Tiitto, 1985). Tannin content was expressed as milligram equivalents of catechin per gram of dry matter.

2.8. DPPH Radical Scavenging Assay

The antioxidant activity of the different extracts was assessed through their scavenging potential against the DPPH radical. Fifty microliters of extract were mixed with 1950 µL of 0.025 g/L DPPH methanolic solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The reaction of the DPPH radical was estimated by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the following equation:

$$RSA(\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where A_C is the control's absorbance and A_S is the absorbance of the tested extract. Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotting inhibition percentages against extract concentrations. BHA and quercetine were used as reference compounds.

2.9. Statistical Analysis

Experimental data were statistically analyzed using one-way analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), and Pearson correlation to evaluate the impact of drying methods, conservation and storage conditions on the antioxidant activity and the concentrations of bioactive compounds in *P. atlantica* leaf extracts. One-way ANOVA was employed to assess the significance of differences in IC50 values for each individual parameter. MANOVA was utilized to evaluate the combined effects of drying techniques, conservation and storage conditions on IC50, total phenolic content (TPC), total flavonoid content (TFC), and tannin content (TC). Pearson correlation analysis was conducted to explore the relationships between IC50 values and levels of TPC, TFC, and TC. Data are presented as means ± standard deviation (*SD*). All statistical analyses were performed using SPSS version 25.0, with significance determined at $p \leq 0.05$, and graphical representations were generated using Microsoft Excel 2021.

3. FINDINGS and DISCUSSION

The results of this study are presented using a pseudo-Pareto chart, which clearly illustrates the relative significance of each drying technique, drying state, and storage condition. This visual representation, organized in descending order of impact, allows for the identification of the most influential factors affecting the phenolic composition and antioxidant activity of *P. atlantica* leaf extracts. This approach provides a comprehensive overview, enhancing the understanding of how various external factors influence the bioactive properties of the plant material.

3.1. Total Phenolic, Flavonoid, and Tannin Content

As shown in Figure 2, total phenolic content across the main drying techniques decreased in the following order: O>Sh>Dc>S. Specifically, leaves dried under direct sunlight exhibited a loss in total phenolics ranging between 22% and 37% compared to the other techniques. Figure 2 also reveals that the use of direct sunlight (S) as a drying or preservation technique consistently reduced total phenolic content. In contrast, leaves stored whole and frozen maintained the highest phenolic content, followed by those stored whole in the shade.

These findings are consistent with those reported by Bettaieb Rebey *et al*. (2020), where shade drying yielded a total phenolic content 1.4 times higher than that observed with ventilated

drying chamber. Additionally, other studies have indicated that drying leaves in Dc leads to significantly higher total phenolic content compared to sun drying and shade drying, where the total phenolic content being 1.6 times higher for leaves dried in Dc and 1.9 times higher compared to shade-dried leaves (Roshanak *et al*., 2016). The losses in total phenolic content (TPC) observed during the drying processe may result from several factors, including binding of polyphenols with other compounds or alterations in their chemical structure, which may reduce their extractability and quantification by available methods. However, it is important to note that the impact of drying on TPC can vary, and in some cases, drying may even lead to an increase in TPC (Vega-Gálvez *et al*., 2011).

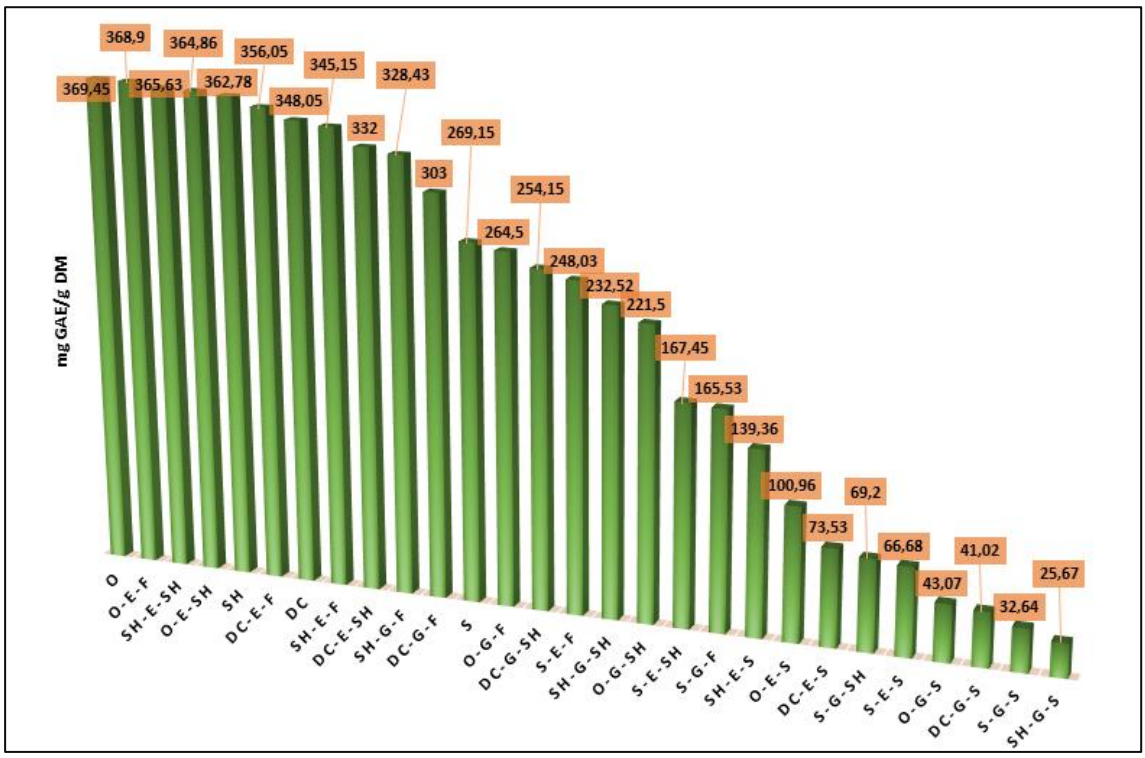

Figure 2. Decreasing total phenolic content of *P. atlantica* leaf extracts.

Figure 3 illustrates the variation in total flavonoid content (TFC) and tannin content (TC) in *P. atlantica* leaf extracts subjected to different drying techniques, storage conditions, and preservation methods. Flavonoids and tannins exhibited different responses to the drying techniques employed. Total flavonoid content decreased in the following order: Sh > O > Dc > S. Leaves dried under direct sunlight (S) showed a significant decrease in flavonoid levels, with losses ranging from 46% to 56%. These results are consistent with the findings of Roshanak *et al*. (2016), who reported that sun and oven drying caused respective losses of 20% and 25%, compared to shade drying. In contrast, tannins exhibited a different response, following the order: O > Sh > S > Dc. Interestingly, leaves dried under direct sunlight displayed a smaller decrease in tannin levels ranging from 9% to 25%. This can be attributed to the heat stability of tannins, as suggested by Chung *et al*. (1998), and further confirmed by Kumar *et al*. (2022). Previous studies have shown that ventilated drying chambers may lead to losses in both flavonoids and tannins due to increased heat exchange and air circulation, which can enhance oxidation and degradation processes (Hamrouni-Sellami *et al*., 2013; Hu *et al*., 2021; Mediani *et al*., 2014).

Additionally, while the effects of drying techniques on phenolic compounds are well-documented, there is limited information regarding the impact of preservation methods, such as freezing, on phenolic content in vegetables and fruits. Freezing is widely recognized as an effective preservation method, capable of maintaining the active constituents of raw plant materials over extended periods. This approach has demonstrated efficacy in preserving bioactive compounds, with potential applications in pharmaceuticals, cosmeceuticals, and

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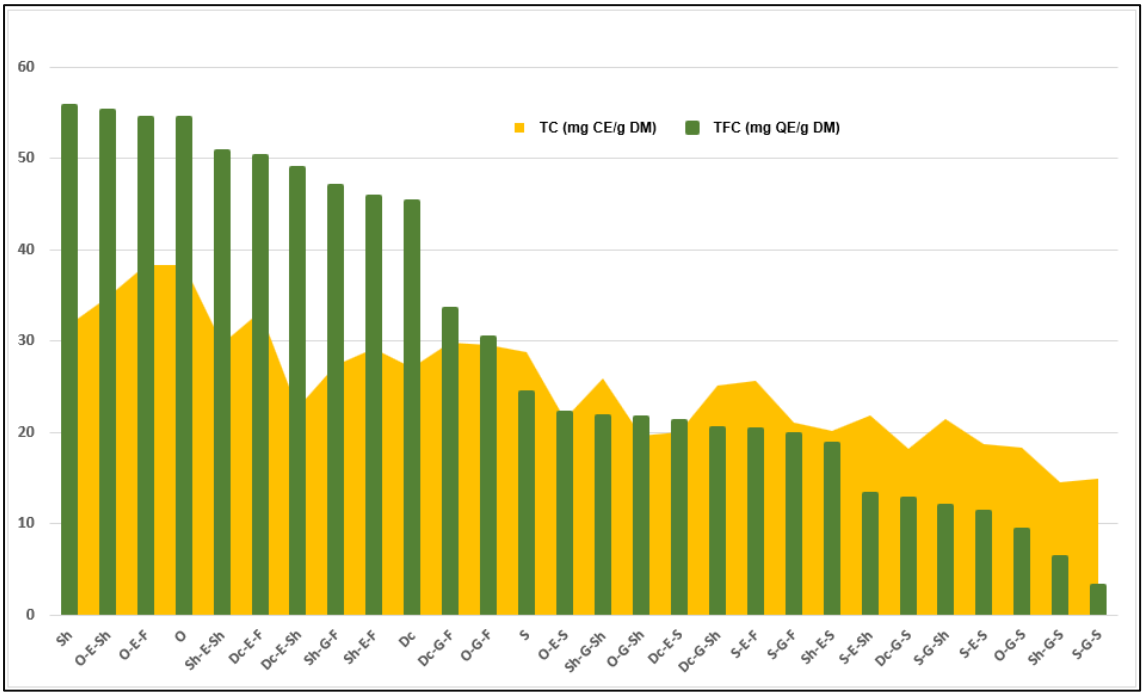

Figure 3. Combined total flavonoid and tannin content of *P. atlantica* leaf extracts.

3.2. Antioxidant Activity

The results of the assessment of antioxidant activity, measured through DPPH scavenging potential, are presented in descending order of IC50 values (µg/mL) and supplemented by graphs illustrating the levels of total polyphenols, flavonoids, and tannins. This integrated representation effectively highlights the most influential factors, enabling a clear determination of their relative significance. A lower IC50 value in the DPPH assay indicates stronger antioxidant activity, reflecting the compound's ability to scavenge free radicals at lower concentrations (Amel *et al*., 2016; Toul *et al*., 2022).

Figure 4 clearly shows a dose-dependent relationship between antioxidant activity and total phenolic content. The results emphasize the impact of different drying methods on this relationship. Specifically, the leaves dried in obscurity or under shade exhibited the lowest IC50 values, 1.85±0.03 and 1.92±0.05 µg/mL, respectively, indicating the highest antioxidant activity, even when compared to the reference compounds, BHA and quercetine (2.09±0.02 and 5.21±0.15 µg/mL, respectively). These leaves also exhibited the highest phenolic contents. This correlation highlights the influence of drying methods on both the antioxidant activity and phenolic content of the extracts, as evidenced by the graph progression with the exposure of other factors: ventilation, grinding, and direct sunlight, where the IC50 values increased from 13.64±1.66 to 225.64±7.48 µg/mL.

Regarding the storage conditions, samples stored in the freezer exhibited slightly higher antioxidant activity than those stored under shade, and significantly higher activity those stored under direct sunlight. Lin *et al*. (2020) reported a 97.41% decrease in DPPH scavenging ability when stored at 37°C for 3 days, a 54.36% decrease at 25°C, and only a 5.15% decrease when stored at 4°C.

The statistical analysis revealed significant effects of drying methods, conservation and storage conditions on the antioxidant activity and bioactive compound content of *P. atlantica* leaf extracts. One-way ANOVA showed significant differences in IC50 values across various

conditions ($F = 16.71$, $p < 4.86\times10^{-9}$). Drying in a ventilated chamber (Dc) and in obscurity (O) preserved the highest levels of TPC, TFC, and TC, resulting in the lowest IC50 values, which indicate high antioxidant activity. In contrast, drying under direct sunlight (S) caused significant losses in bioactive compounds, resulting in higher IC50 values.

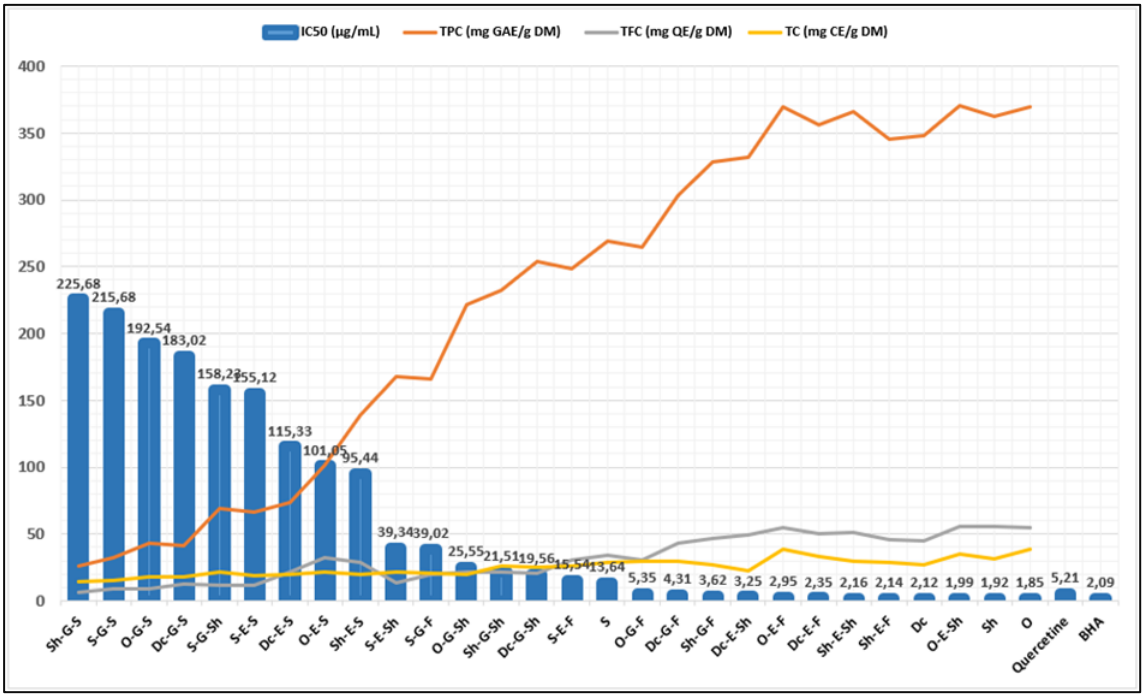

Figure 4. Decreasing IC50 Correlated with TPC, TFC, and TC in *P. atlantica* leaf extracts.

Multivariate analysis of variance (MANOVA) showed significant multivariate effects for drying techniques (Wilks' $\lambda = 0.0012$, $F(48, 83.29) = 3.04$, $p < 0.05$), conservation (Wilks' $\lambda = 0.0021$, $F(32, 66.54) = 2.65$, $p < 0.05$), and storage conditions (Wilks' $\lambda = 0.0033$, $F(16, 54.12) = 2.17$, $p < 0.05$), confirming the combined influence of these factors on IC50, TPC, TFC, and TC. Pearson correlation analysis further revealed strong inverse relationships between IC50 and TPC ($r = -0.865$, $p < 0.05$), IC50 and TFC ($r = -0.864$, $p < 0.05$), and IC50 and TC ($r = -0.869$, $p < 0.05$), indicating that higher levels of these compounds are associated with higher antioxidant activity.

These findings are consistent with previous studies on the preservation of bioactive compounds. Vega-Vega-Gálvez *et al*. (2009) reported that air-drying temperature significantly influences the physicochemical properties and antioxidant capacity of red pepper, with controlled drying conditions better preserving phenolic and flavonoid contents. This is in agreement with the current study, which indicates that drying in a ventilated chamber or in darkness is more effective. Similarly, Ratti (2001) revealed that freeze-drying is highly effective for preserving the quality of high-value foods by minimizing enzymatic and oxidative degradation. This supports the finding in the present study that freezing is the most effective storage method for preserving bioactive compounds.

Chan *et al*. (2009) demonstrated that different drying methods significantly impact the antioxidant properties of ginger species, with controlled environments better preserving bioactive compounds, which aligns with the current findings that drying in controlled conditions (ventilated chamber and darkness) preserves antioxidant properties.

4. CONCLUSION

The study highlights that to preserve the antioxidant activity and bioactive compound integrity in *P. atlantica* leaves, it is critical to employ specific post-harvest handling practices. The optimal preservation involves drying in a ventilated chamber or in darkness, which minimizes photodegradation and oxidative loss of phenolic compounds. Additionally, storing the leaves

intact, rather than ground, helps in retaining cellular integrity and reducing exposure to oxidative processes. Conservation under freezing conditions further prevents enzymatic activity and degradation of sensitive bioactive compounds. Together, these methods preserve the levels of phenolic, flavonoid, and tannin contents, thereby maximizing the leaves' therapeutic and antioxidative potential. Among the techniques assessed, the most effective approach is drying the plant material in a ventilated chamber under darkness, keeping the leaves whole, and storing them in the freezer after complete drying.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Fethi Toul: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. **Marwa Ballou**: Investigation, Methodology. **Marwa Kessou**: Investigation, Methodology.

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