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Antioxidant and anti-tyrosinase activities of the methanolic extract of *Origanum vulgare* L

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SUMMARY

Introduction: Sunlight-induced free radicals can contribute to pigmentation disorders, such as hyperpigmentation. Tyrosinase is a crucial enzyme in melanogenesis, and its natural inhibitors are gaining popularity as safer hypopigmenting agents in the cosmetics industry to prevent hyperpigmentation and skin disorders by reducing melanin production. **Objective:** This study evaluates the Total Phenolic Content (TPC), antioxidant activity, and anti-tyrosinase activity of a methanolic extract from *Origanum vulgare* L. for potential cosmetic applications. **Methodology:** The TPC was measured using the Folin-Ciocalteu method. Antioxidant activity was assessed through the DPPH radical inhibition assay, while anti-tyrosinase activity was determined using a mushroom tyrosinase kit with kojic acid as a standard. **Results:** The TPC was found to be 71.37 ± 2.09 mg of gallic acid equivalents (GAE) per gram. The extract demonstrated moderate antioxidant and anti-tyrosinase activity, with IC_{50} values of 310.12 μg/mL and 351.43 μg/mL, respectively. **Conclusion:** Oregano extract showed moderate antioxidant and tyrosinase inhibition, suggesting possible cosmetic use. While less effective than standards, further study is needed to isolate active compounds, develop formulations in association with other extracts, and evaluate efficacy and safety in relevant biological models.

Keywords: methanolic extract; skin pigmentation; free radicals; natural ingredient; whitening products; tyrosinase inhibitor.

RESUMEN

Actividades antioxidante y anti-tirosinasa del extracto metanólico de *Origanum vulgare* L

Introducción: Los radicales libres inducidos por la luz solar pueden contribuir a trastornos de la pigmentación, como la hiperpigmentación. La tirosinasa es una enzima crucial en la melanogénesis, y sus inhibidores naturales están ganando popularidad como agentes hipopigmentantes más seguros en la industria cosmética para prevenir la hiperpigmentación y los trastornos de la piel mediante la reducción de la producción de melanina. **Objetivo:** Este estudio evalúa el contenido fenólico total (CFT), la actividad antioxidante y la actividad antitirosinasa de un extracto metanólico de *Origanum vulgare* L. para

posibles aplicaciones cosméticas. **Metodología:** El CFT se midió utilizando el método Folin-Ciocalteu. La actividad antioxidante se evaluó mediante el ensayo de inhibición de radicales DPPH, mientras que la actividad antitirosinasa se determinó utilizando un kit de tirosinasa de hongos usando ácido kójico como estándar. **Resultados:** Se encontró que el CFT era de 71,37 ± 2,09 mg de equivalentes de ácido gálico (GAE) por gramo. El extracto demostró una actividad antioxidante y antitirosinasa moderada, con valores de CI_{50} de 310,12 μg/mL y 351,43 μg/mL, respectivamente. **Conclusión:** El extracto de orégano mostró una moderada inhibición antioxidante y de la tirosinasa, lo que sugiere su posible uso cosmético. Si bien es menos eficaz que los estándares, se requieren más estudios para aislar los compuestos activos, desarrollar formulaciones en asociación con otros extractos y evaluar la eficacia y la seguridad en modelos biológicos relevantes.

Palabras clave: extracto metanólico; pigmentación de la piel; radicales libres; ingrediente natural; producto blanqueador; inhibidor de la tirosinasa.

RESUMO

Atividades antioxidante e anti-tirosinase de um extrato metanólico de *Origanum vulgare* L.

Introdução: Os radicais livres induzidos pela luz solar podem contribuir para distúrbios de pigmentação, como a hiperpigmentação. A tirosinase é uma enzima crucial na melanogênese, e seus inibidores naturais estão ganhando popularidade como agentes hipopigmentantes mais seguros na indústria cosmética para prevenir a hiperpigmentação e distúrbios da pele, reduzindo a produção de melanina. **Objetivo:** Este estudo avalia o teor de fenólicos totais (TPC), a atividade antioxidante e a atividade anti-tirosinase de um extrato metanólico de Origanum vulgare L. para potenciais aplicações cosméticas. **Metodologia:** O TPC foi medido pelo método de Folin-Ciocalteu. A atividade antioxidante foi avaliada pelo ensaio de inibição do radical DPPH, enquanto a atividade anti-tirosinase foi determinada utilizando um kit de tirosinase fúngica com ácido kójico como padrão. **Resultados:** A concentração funcional total (CFT) foi de 71,37 ± 2,09 mg de equivalentes de ácido gálico (EAG) por grama. O extrato demonstrou atividade antioxidante e antitirosinase moderadas, com valores de IC50 de 310,12 μg/mL e 351,43 μg/mL, respectivamente. **Conclusão:** O extrato de orégano apresentou atividade antioxidante e inibição da tirosinase moderadas, sugerindo seu potencial para uso cosmético. Embora menos eficaz que os padrões, estudos adicionais são necessários para isolar os compostos ativos, desenvolver formulações em combinação com outros extratos e avaliar a eficácia e segurança em modelos biológicos relevantes.

Palavras-chave: extrato metanólico; pigmentação da pele; radicais livres; ingrediente natural; produto clareador; inibidor de tirosinase.

1. INTRODUCTION

Melanin is a pigment that determines skin color and provides protection against ultraviolet (UV) damage. Excessive melanin production in melanocytes leads to hyperpigmentation disorders, such as age spots, melasma, freckles, and chloasma. Therefore, melanin is a significant target for anti-melanogenic agents designed to prevent these hyperpigmentation issues. Various skin disorders are associated with melanin synthesis, including lentigines, acanthosis nigricans, melanoma, incontinentia pigmenti, melasma, periorbital hyperpigmentation, cervical poikiloderma, progressive pigmentary purpura, pityriasis alba, and neurodegeneration related to Parkinson's disease [1]. As a result, various melanogenesis inhibitors from synthetic and natural sources have been developed to date; however, many have been reported to have serious side effects.

Free radicals produced by the sun are believed to significantly contribute to various pigmentation disorders, especially hyperpigmentation [2]. Melanogenesis is a process in melanocytes regulated by the microphthalmia-associated transcription factor (MITF), tyrosinase, and tyrosinase-related proteins (TRPs). UV radiation and alpha-melanocyte-stimulating hormone (α-MSH) often trigger this process [3]. Repeated short-term exposure to ultraviolet radiation (UVR) causes erythema, resulting in skin redness. This activates melanocytes, increasing melanin production and leading to tanning or hyperpigmentation. However, excessive long-term UVR exposure can cause premature skin aging, immune system suppression, and a higher risk of skin cancers, including melanoma [4].

UV radiation exposure increases the production of hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS) during melanogenesis, leading to oxidative stress in melanocytes [5]. Melanin production increases due to ROS formation, which signals further melanogenesis [6]. Thus, ROS scavengers and tyrosinase inhibitors may effectively reduce melanogenesis [2].

Tyrosinase is a crucial enzyme in the melanin biosynthesis pathway [7]. It catalyzes the conversion of L-tyrosine to L-DOPA, which is then oxidized to dopaquinone, leading to melanin production [8, 9]. Excessive melanin accumulation in the skin can result in spots and moles and may even contribute to the development of skin cancer [10]. Therefore, inhibiting tyrosinase activity can effectively promote skin hypopigmentation, as it catalyzes the key steps in melanogenesis [2].

Several compounds with antioxidant and anti-tyrosinase properties, such as hydroquinone, kojic acid, and arbutin, are commonly used in the cosmetic industry. However, their use has been limited due to potential adverse effects on the skin and concerns regarding cytotoxicity. It is essential to identify other natural inhibitors of tyrosinase [11]. Recent efforts have concentrated on developing natural skin-lightening products derived from plant extracts. These products are designed to be less toxic and offer beneficial properties in response to market demand. Consequently, one of the most popular types of cosmetic products now includes ingredients that inhibit melanin formation.

Numerous studies have demonstrated that natural components of plants, such as phenolic compounds and flavonoids, have the potential to be developed as skin-whitening agents [12]. This effectiveness is primarily due to the strong correlation between polyphenolic compounds and their roles in antimelanogenesis, anti-tyrosinase activity, and antioxidant properties. However, it's important to note that the methods used for extraction and the types of solvents employed play a crucial role in determining the biological activities of these plants [13].

Plants from various species and ecotypes of *Origanum* are extensively utilized in agriculture, as well as in the pharmaceutical and cosmetic industries. They are particularly valued for their aromatic properties, which enhance the flavors of culinary herbs, food products, alcoholic beverages, and perfumes. Antioxidant studies have shown that the methanolic extract of *O. vulgare*, a member of the Lamiaceae family, acts as a powerful free radical scavenger [14, 15].

Although many studies have investigated tyrosinase inhibition and related enzyme activities in oregano extracts, most focus on essential oils or chemically variable extracts, limiting result comparability. For exa*mple, Origanum compactum* essential oils show in vitro tyrosinase inhibition, but effects depend on volatile compounds like thymol and carvacrol, which differ by plant origin [16]. High-Andean Peruvian oregano is particularly interesting due to its unique environment affecting phytochemical composition. While antioxidant activity has been noted for methanolic extrac*ts of Origanum vulgare* [15] and other species like *Origanum dictam-*

nus have confirmed tyrosinase inhibitory effects in enzymatic assays, underscoring the importance of the chemical makeup of each specific plant material [17] remains a critical gap in the literature. To date no indexed journal has systematically studied high-Andean oregano extracts using a static method for heat- and light-sensitive polyphenols with methanol as the solvent. Thus, the knowledge gap concerns comprehensive evaluation of specific oregano varieties under controlled extraction conditions. This study addresses this gap by linking the botanical and geographical origins, selective extraction, total phenolic content, antioxidant activity, and tyrosinase inhibition of the methanolic extract of *Origanum vulgare* L. of Peruvian origin.

2. MATERIALS AND METHODS

2.1. Plant material collection

Aerial parts of *Origanum vulgare* L. were collected during the flowering stage from the community of Hanccohocca, Canchis province, at Cusco, Perú (Figure 1). Botanical identification was carried out at the Vargas CUZ Herbarium of the National University of San Antonio Abad of Cusco.

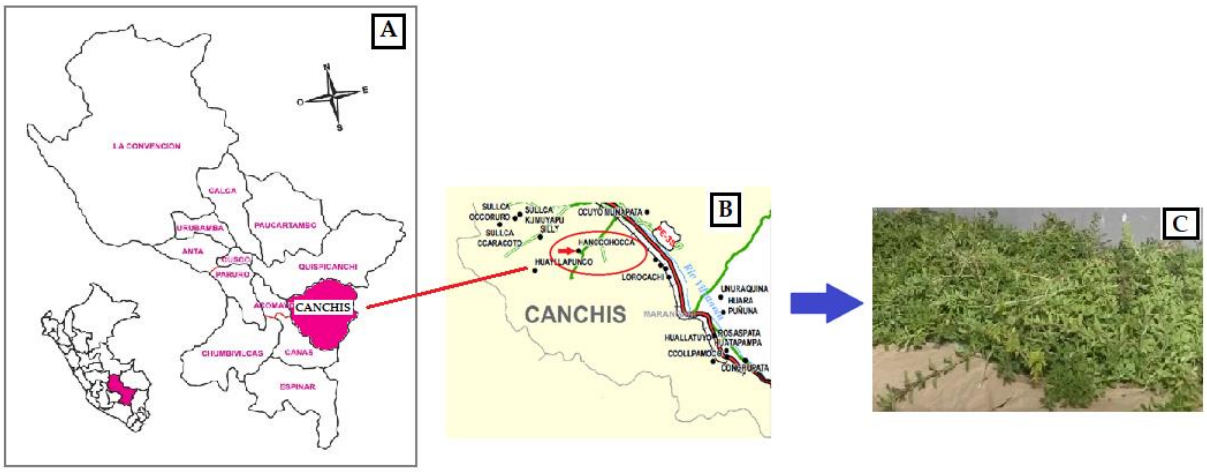


Figure 1. (A) Geographical location of Canchis province in Cusco, Perú. (B) Plant collection location: Hanccohocca. (C) Aerial parts of *Origanum vulgare* L.

2.2. Methanol extraction

Air-dried aerial parts of *Origanum vulgare* (500 g) were finely ground and subjected to cold maceration in 1 L of methanol at ambient temperature for 15 days with intermittent agitation. To reduce oxidation risk, the procedure was performed under controlled conditions, protected from light, using hermetically sealed bottles, anhydrous solvent, and kept at a low temperature (4–8°C) to limit degradation of sensitive compounds. The resulting mixture was filtered using Whatman No. 1 paper, and the methanol was subsequently removed under reduced pressure at 40–45 °C to yield the crude extract. Conventional maceration was selected to maintain the integrity of thermolabile phenolic compounds [18].

2.3. Total phenolic content

The Folin-Ciocalteu method [19] with slight modifications was used to determine the total phenolic content. A sample weighing 1 mg was diluted in 1 mL of methanol. From this solu-

tion, 100 µL was mixed with 200 µL of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich Production GmbH, Switzerland) and allowed to react for 5 minutes. After the reaction, 3.9 mL of distilled water was added, followed by 800 µL of a 75 g/L sodium carbonate (Na_2CO_3, Merck) solution. The mixture was allowed to stand for 2 hours at room temperature, then its absorbance was measured at 765 nm using methanol as a blank. Absorbance readings were taken with a UV/VIS PG Instrument T80+ Spectrophotometer (Woodway Lane, Leicestershire, UK). Gallic acid (Sigma-Aldrich Production GmbH, Switzerland) was used as the standard for creating a calibration curve with concentrations ranging from 50 to 150 µg/mL. The total phenolic content was expressed in micrograms of gallic acid equivalents (GAE) per milligram of the dry weight of the methanolic extract.

2.4. Antioxidant activity

The antioxidant activity was evaluated using the free DPPH radical inhibition method, following the procedure described by Chou *et al*. [20], with some modifications. A total of 0.1 milliliters of each extract, at varying concentrations (31.5 - 500 µg/mL), was mixed with 2.9 milliliters of DPPH (1,1-diphenyl-2-picrylhydrazyl) purchased from Sigma Chemicals, USA. The mixture was vortexed and incubated at room temperature for 60 minutes, protected from light. The absorbance was measured at 517 nm using a PG Instrument T80 UV-Vis spectrophotometer. Trolox ((+/-)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97%) from Aldrich (Milwaukee, WI) was utilized as the standard antioxidant, tested at concentrations ranging from 25 to 125 µg/mL. 0.1 mL of methanol was carefully measured and added to the control, followed by 2.9 mL of the DPPH solution. Antioxidant activity was expressed as a percentage of inhibition and calculated using the following equation:

$$\boldsymbol{Inhibition}\ (\%) = \frac{\boldsymbol{Abs.\ control - Abs.\ sample}}{\boldsymbol{Abs.\ control}} \times \mathbf{100} \qquad \textbf{Formula (1)}$$

The IC_{50} value, indicating the extract concentration required for 50% inhibition, was determined from a graph that plots inhibition percentage against extract concentration.

2.5. Anti-tyrosinase activity

The anti-tyrosinase activity of the samples was assessed using the method described by Tan *et al*. [21], which employed a tyrosinase inhibitor screening kit from Sigma Aldrich (MAK257). A 20 µL methanolic extract at concentrations of 25 - 500 µg/mL (S), tyrosinase assay buffer (EC), positive control, and DMSO were added in duplicate to a 96-well plate. 50 µL of the tyrosinase enzyme solution (composed of 2 µL of tyrosinase and 48 µL of tyrosinase assay buffer) were added to each well. The samples were thoroughly mixed and incubated for 10 minutes at 25 °C. Then, 30 µL of the tyrosinase substrate solution (consisting of 2 µL of tyrosinase substrate, 5 µL of tyrosinase enhancer, and 23 µL of tyrosinase assay buffer) were added to each well. The mixture was thoroughly mixed again and incubated for a further 15 minutes. Absorbances were measured at 510 nm using a microplate reader (Absorbance Microplate Reader EPOCH 2, Biotek, Germany). Kojic acid served as a positive control. The slope for all samples was calculated by dividing net absorbance values (Abs2 – Abs1) by the time interval (T2 – T1). Inhibitory activity was measured by kinetic assay, calculating enzyme activity from the absorbance–time slope (ΔAbs/min). The results are expressed as a percentage of relative inhibition, calculated as follows:

$$\textbf{\textit{Relative inhibition}}\ (\%) = \frac{[\textbf{\textit{slope}}\ (\textbf{\textit{EC}}) - \textbf{\textit{slope}}\ (\textbf{\textit{S}})]}{\textbf{\textit{slope}}\ (\textbf{\textit{EC}})} \times \mathbf{100} \qquad \textbf{Formula (2)}$$

Where: *EC* = Tyrosinase assay buffer; *S* = methanolic extract of *O. vulgare*

2.6. Statistical analysis

Experiments were performed in duplicate (n = 2), so statistical analyses are exploratory. Results are mainly shown as descriptive statistics, including means and standard deviations to best represent the findings.

3. RESULTS

3.1. Percentage of extraction

The extraction percentage of the aerial parts of *O. vulgare* L. using a methanolic solution was 8.65%. This result demonstrates the efficiency of the extraction process, highlighting the yield obtained from the plant material under the specified conditions, as shown in Table 1.

Table 1. Percentage of extraction from the dried samples of *O. vulgare*

Solvent	Extraction %
Methanol	8.65

In our study, the aerial parts of *O. vulgare* L. yielded an extraction percentage of 8.65% when using a methanolic solution. Ličina *et al.* [22] conducted a study to evaluate the extraction percentages of various solvents, yielding the following results: water had an extraction percentage of 21.6 %, ethanol 6.36 %, acetone 3.66 %, ethyl acetate 3.28 %, and diethyl ether 2.8 %. Previous research has shown that methanol is an effective solvent for extracting phenolic and flavonoid compounds, with methanol extracts demonstrating the highest antioxidant activity [23]. This effectiveness is likely due to the polarity of methanol. These findings align with earlier studies that suggest solvent polarity significantly enhances the extraction of phenolic compounds from plant materials [24].

3.2. Total phenolic content

Table 2 shows a 71.37 ± 2.09 mg GAE/g of total phenolic contents in the methanolic extract of *O. vulgare* L. The calibration curve shows the relationship between gallic acid concentration and absorbance with the equation Y = 0.0051X + 0.018 and an R^2 value of 0.9903, indicating a strong correlation.

Table 2. Polyphenol content in the methanolic extract of *O. vulgare* L

Extract	Polyphenols* (mg GAE/g)	R^2	Equation
Methanolic	71.37 ± 2.09	0.9903	y = 0.0051x + 0.018

*The results are shown as the mean ± SD of two determinations.

The methanolic extract of the aerial parts of *O. vulgare* showed a total phenolic content of 71.37 ± 2.09 mg GAE/g, as shown in Table 2. The findings of this study are consistent with those of Mohamed *et al.* [14], who reported that methanol yielded the highest phenolic content, measuring 75.3 ± 0.5 mg GAE/g of dry weight. Their research demonstrated that methanol is a more effective solvent for extracting phenolic compounds compared to other solvents. For

example, acetone produced only 6.93 ± 0.05 mg GAE/g, while dichloromethane resulted in even lower levels at 4.28 ± 0.04 mg GAE/g.

In a previous study, the highest total phenol content reported for an *O. vulgare* water extract was 235 mg GAE/g, while the acetone extract contained 84.5 mg GAE/g. Conversely, Weerakkody *et al.* [25] found lower concentrations, with 49.69 mg GAE/g in the water extract and 26.16 mg GAE/g in the ethanol extract. In contrast, Barros *et al.* [26] reported an exceptionally high concentration of 369.18 mg GAE/g. Chun *et al.* [27] identified key phenolic compounds in both water and ethanol extracts, including rosmarinic acid and quercetin. Additionally, other polyphenolic compounds were identified, including apigenin, acacetin, herbacetin, diosmetin, luteolin, hispidulin, and naringin [28] that have multiple biological effects. Jiang *et al.* [29] conducted qualitative phytochemical assays, revealing that *O. vulgare* samples contain flavonoids, tannins, glycosides, and terpenoids in all extracts (aqueous and organic solvents). In *O. vulgare*, notable variability in total polyphenol content (TPC) was noted among various extracts [30]. Previous studies have identified key phenolic components in the methanolic extract of *O. vulgare*, including p-coumaric acid, rosmarinic acid, quercetin, chlorogenic acid, and ellagic acid, all of which demonstrated the ability to inhibit tyrosinase [14, 15].

3.3. Antioxidant activity

Figure 2 illustrates that the percentage of DPPH radical inhibition is directly proportional to the concentration of the methanolic extract of *O. vulgare*. This relationship is described by the equation Y = 0.1578x + 1.0625. The R^2 value of 0.9974 indicates a strong confirmation of dose-dependent scavenging activity in our study.

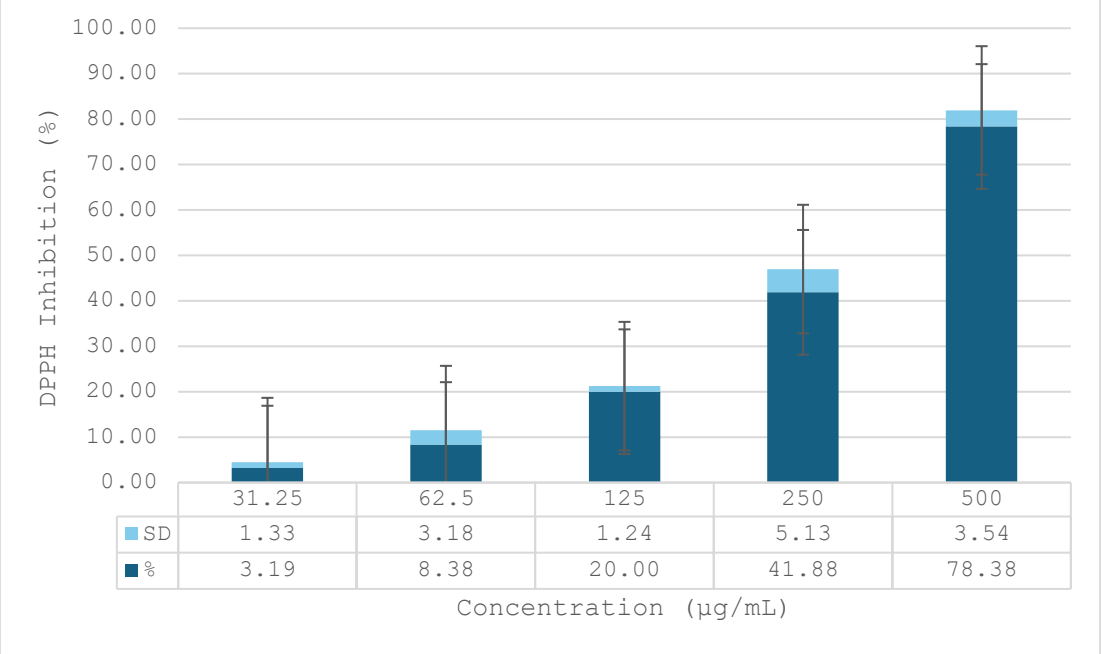


Figure 2. DPPH inhibition by different concentrations of *O. vulgare* methanolic extract is shown as the mean ± SD.

In Figure 2, a concentration of 500 µg/mL of the methanolic extract of *O. vulgare* demonstrated a DPPH inhibition percentage of 78.38%. This result is comparable to the findings of Antoniadou *et al.* [30], who reported an inhibition of 77.33 ± 0.11% for 40% ethanolic extracts at the same concentration. In the study by Ličina *et al.* [22], five *O. vulgare* extracts were

examined for DPPH radical scavenging activity, with the ethanolic extract demonstrating the strongest activity. Previous research has demonstrated that oregano extracts possess a strong ability to scavenge free radicals. For instance, Kaurinovic *et al.* [31] found that various extracts (ethyl acetate, n-butanol, and water) from wild-grown oregano in Serbia effectively inhibited hydroxyl (–OH) radicals. Among these, the ethyl acetate extract, which is rich in phenolic compounds and flavonoids, exhibited the most significant inhibitory activity. Additionally, Bešta-Gajević *et al.* [32] reported that methanolic extracts from oregano leaves displayed intense antioxidant activity, while aqueous extracts were less effective.

Variability in antioxidant activity as measured by the DPPH assay among extracts of *Origanum vulgare* is influenced by various intrinsic and extrinsic factors. Geographic origin and environmental parameters such as climate, soil composition, and plant stress responses impact the biosynthesis and accumulation of phenolic compounds. This results in notable differences in radical scavenging capacity across accessions and cultivars of *O. vulgare*, even under similar cultivation conditions [33]. Additionally, the extract type and solvent polarity play a critical role in determining both the yield and diversity of extracted phenolics [15]. The extraction methodology includes variables such as extraction duration, temperature, solvent concentration, and physical processes (e.g., cold maceration versus assisted extraction) that substantially influence the quality and quantity of recovered bioactive phytochemicals [34]. Furthermore, factors like plant genotype, harvest timing, developmental stage, and post-harvest handling contribute to the observed heterogeneity in antioxidant properties [35].

As shown in Table 3, the methanolic extract of *O. vulgare* exhibited DPPH inhibition with an IC_{50} value of 310.12 μg/mL ($y = 0.1578x + 1.0625$; $R^2 = 0.9974$). This inhibition activity was compared to that of Trolox, which displayed an IC_{50} value of 84.79 μg/mL ($y = 0.5005x + 7.5625$; $R^2 = 0.9975$). The methanolic extract exhibited moderate in vitro antioxidant activity, which was lower than that of the standard antioxidant. These results suggest further research is needed on compound isolation, formulation in association of other extracts or whitening products, and testing in more relevant biological models.

Table 3. Antioxidant activity of *O. vulgare* methanolic extract expressed as IC_{50}

Antioxidant substance	**IC_{50} ***
***Origanum vulgare* methanolic extract**	310.12 μg/mL
Trolox	84.79 μg/mL

*The results are shown as the mean of two determinations

However, research by Jan *et al.* [36] reported that *O. vulgare* methanolic extracts exhibited significantly higher and dose-dependent antioxidant activity, with an IC_{50} value of 19.9 μg/mL. Additionally, Mrabet *et al.* [37] noted that the methanolic extract of *O. vulgare* acts as a potent free radical scavenger, with an IC_{50} value of 58 μg/mL. Sahin *et al.* [38] found that a methanol extract from Eastern Anatolia, Turkey, had an IC_{50} value of 9.9 mg/mL. Furthermore, a study by Albano and Miguel [39] revealed that the ethyl acetate extract was the most effective scavenger, boasting an IC_{50} value of 1.8 mg/mL, followed by the water extract with an IC_{50} of 2.6 mg/mL, and the diethyl ether extract with an IC_{50} of 3.7 mg/mL.

3.4. Anti-tyrosinase activity

The anti-tyrosinase activity of the samples was evaluated using L-DOPA as the substrate. Figure 3 illustrates the significant concentration-dependent inhibitory effect of the *O. vulgare* methanolic extract. The results indicated that at a concentration of 500 μg/mL, the extract exhibited a tyrosinase inhibitory activity of 72.56%.

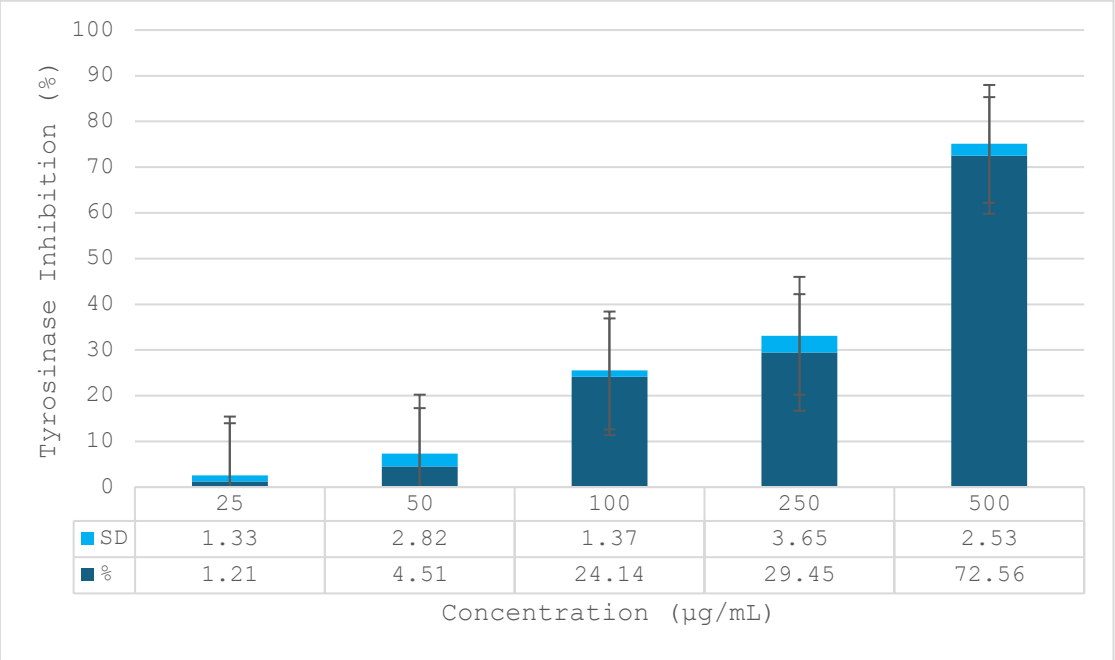


Figure 3. Tyrosinase inhibition by different concentrations of *O. vulgare* methanolic extract is shown as the mean ± SD.

Our results showed that at a concentration of 500 µg/mL, the methanolic extract demonstrated a tyrosinase inhibitory activity of 72.56%, with an IC_{50} value of 351.43 µg/mL. A limitation of this study is the absence of extract-specific blanks; however, the kinetic approach and low extract concentrations minimize the impact of potential optical interference. The absorbance–time profiles were roughly linear over the chosen interval, allowing accurate reaction rate calculations.

Previous studies have indicated that methanolic extracts of cultivated *O. vulgare* L. exhibit higher anti-tyrosinase activity, ranging from 48% to 81.1% at concentrations of 10–20 µg/mL [40]. In contrast, the ethanolic extract of *O. vulgare* was reported to have a tyrosinase inhibitory activity of 6.5 ± 0.2%, as noted by Moghrovyan *et al.* [40]. Additionally, Rao *et al.* [41] reported a tyrosinase inhibition percentage of 45% with an IC_{50} value of 150 µg/mL.

Table 4 shows that the methanolic extract of *O. vulgare* moderately inhibits tyrosinase (IC_{50} = 351.43 µg/mL), likely due to its phenolic compounds, although the extraction may miss fewer polar inhibitors. Flavonoids, phenolic acids, and their interactions also affect activity. In comparison, kojic acid, the standard inhibitor, is much more effective (IC_{50} = 0.35 µg/mL).

Table 4. Tyrosinase inhibition of *O. vulgare* methanolic extract expressed as IC_{50}

Tyrosinase inhibitor	**IC_{50} ***
Origanum vulgare methanolic extract	351.43 µg/mL
Kojic acid	0.35 µg/mL

*The results are shown as the mean of two determinations

Although the methanolic extract of *Origanum vulgare* exhibited a moderate antityrosinase activity, this value is consistent with the complex chemical nature of crude plant extracts and reflects the combined effects of multiple phenolic constituents with variable affinities toward the enzyme rather than the action of a single potent inhibitor [15, 42]. Kojic acid is a low-

molecular-weight compound that strongly inhibits tyrosinase via copper chelation at the enzyme site, yielding IC_{50} values far lower than crude plant extracts [43]. Crude extracts typically show moderate IC_{50} values due to synergistic or antagonistic interactions among their constituents [44]. Their antityrosinase properties are also associated with antioxidant activity, as phenolic compounds capable of scavenging radicals can chemically reduce *o*-quinones (*o*-dopaquinone) and semiquinones, thereby inhibiting melanogenesis [43, 45]. Moderately effective inhibition with antioxidant properties may be ideal for cosmeceuticals, offering balanced performance and safety compared to strong inhibitors like kojic acid [46, 47]. Thus, *O. vulgare* methanolic extract appears multifunctional, suitable as a natural ingredient rather than a direct replacement for conventional inhibitors.

5. CONCLUSIONS

Oregano extract showed moderate antioxidant and tyrosinase inhibitory activities, suggesting its potential use in cosmetics. Although not as potent as reference standards, these results support further research to isolate active compounds, extract optimization, fractionation, evaluation of synergies between compounds, develop formulations in association with other extracts, and assess efficacy and safety testing in systems closer to real world usage conditions. These findings provide a foundation for future research on the functional applications of peruvian oregano extracts.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

STATEMENT ON ARTIFICIAL INTELLIGENCE USE

The authors declare that Microsoft Copilot was used exclusively for languaje editing and stylistic refinement of the manuscript. The content, scientific analysis, interpretation of results, and conclusions were entirely produced by the authors. No artificial intelligence tool was used to generate original scientific content.

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