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Bioactivity Profile of *Datura stramonium* L. Seed Extracts: Collagenase and Tyrosinase Inhibition and Antioxidant Activity

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Abstract

Datura stramonium L., a member of the Solanaceae family, contains diverse phytochemicals with potential pharmacological applications, yet its enzyme inhibitory and antioxidant properties remain insufficiently characterized. In this study, ethanol and aqueous extracts of *D. stramonium* seeds collected from Belmebük village (Amasya, Turkey) were evaluated for collagenase and tyrosinase inhibition, as well as antioxidant capacity. Enzyme inhibition assays conducted at 20 µg/mL revealed that the ethanol extract inhibited collagenase and tyrosinase by 26.8 ± 0.099% and 59.5 ± 0.025%, respectively, while the aqueous extract showed higher activity with 52.1 ± 0.09% and 70.5 ± 0.012% inhibition. Compared to positive controls (epigallocatechin gallate: 88.09% collagenase inhibition; kojic acid: 98.13% tyrosinase inhibition), the extracts demonstrated moderate but significant activities. Antioxidant evaluations using DPPH and ABTS assays indicated IC_{50} values of 0.0962 ± 0.0299 and 0.241 ± 0.052 mg/mL for the ethanol extract, and 0.0522 ± 0.0254 and 0.1974 ± 0.0156 mg/mL for the aqueous extract, respectively. Although less potent than standard antioxidants (BHT: 0.0148 ± 0.001 mg/mL; Trolox: 0.0153 ± 0.001 mg/mL), both extracts displayed notable radical scavenging effects, with the aqueous extract consistently outperforming the ethanol extract. These findings highlight that *D. stramonium* seed extracts possess dual bioactivity by inhibiting collagenase and tyrosinase while exerting antioxidant effects, suggesting their potential in preventing collagen degradation, hyperpigmentation, and oxidative damage. To the best of our knowledge, this is the first report on enzyme inhibitory activities of *D. stramonium* seed extracts, providing novel evidence for their potential utilization in pharmaceutical and dermocosmetic formulations.

Introduction

Datura stramonium L., a member of the Solanaceae family, is a widely distributed annual plant recognized for both its medicinal and toxic properties. Typically, it grows up to 1–1.5 m in height, with erect, branched stems and trumpet-shaped flowers. It thrives in nitrogen-rich, disturbed soils, particularly in temperate and subtropical regions. The Solanaceae family comprises approximately 100 genera and 2,500 species, many of which have significant nutritional, pharmaceutical, and ethnobotanical relevance [1].

The phytochemical composition of *D. stramonium* is diverse and complex. The plant is particularly rich in tropane alkaloids, notably atropine (hyoscyamine) and scopolamine (hyoscine), which are considered the most abundant and biologically active metabolites. In addition, carbohydrates, cardiac glycosides, tannins, flavonoids, phenolic compounds, amino acids, and saponins have also been identified in different plant parts. The accumulation of tropane alkaloids varies according to environmental, developmental, and organ-specific factors, with seeds and leaves reported to contain the highest concentrations [1,2].

The phytochemical profile of *Datura stramonium* is predominantly characterized by tropane alkaloids, which are considered its most pharmacologically relevant constituents. Among these, atropine (hyoscyamine) and scopolamine (hyoscine) are the major bioactive compounds, reported in varying concentrations across plant parts, with seeds and leaves being particularly rich sources [2,3]. Quantitative phytochemical evaluation of *Datura stramonium* revealed that seeds contain significantly higher alkaloid content than leaves (71.28 vs. 32.10 mg/g), underscoring a potentially greater pharmacological and toxicological significance of the seed matrix [4]. Moreover, various studies consistently report the presence of tropane alkaloids, flavonoids, saponins, tannins, amino acids, steroidal glycosides, and phenolics, highlighting the complex metabolic profile of *D. stramonium* [5]. The accumulation of these alkaloids is influenced by

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environmental factors, developmental stage, and the specific organ analyzed, with maximum concentrations typically observed in roots and seeds during the vegetative phase [6]. The high alkaloid content of *D. stramonium* underpins both its traditional medicinal applications and its toxicological risks, making it a plant of considerable pharmacological and toxicological interest.

From an ethnobotanical perspective, *D. stramonium* has been traditionally used across different regions. In Turkey, it is locally known as "Tatala," particularly in the Sakarya province, where seeds have been applied in folk medicine for acne treatment and bronchitis relief [7]. Furthermore, seeds and capsules have been employed in dermatological disorders and cosmetic practices. However, due to its potent anticholinergic activity, the plant is also associated with severe intoxications when misused, which has been highlighted in toxicological reports [8].

Pharmacological studies have revealed a wide spectrum of biological activities for *D. stramonium*. Extracts of its seeds and leaves have demonstrated anti-asthmatic, antiepileptic, analgesic, antioxidant, antimicrobial, insecticidal, and repellent properties [8,9,10]. These bioactivities are primarily attributed to the presence of tropane alkaloids and associated phenolic constituents.

The Collagenase is a metalloproteinase enzyme responsible for degrading collagen, a structural protein crucial for maintaining the integrity of the skin's extracellular matrix; thus, inhibition of this enzyme is regarded as a promising strategy for preventing dermal matrix degradation and delaying skin aging [11]. Likewise, tyrosinase is the rate-limiting enzyme in melanin biosynthesis, and its inhibition is associated with reducing hyperpigmentation and achieving skin-lightening effects [12]. Plant-derived polyphenols and flavonoids are known to exert strong antioxidant properties, and through their ability to scavenge free radicals, they also synergistically contribute to the inhibition of collagenase and tyrosinase. Consequently, the interplay between antioxidant activity and enzyme inhibition offers valuable perspectives for the development of anti-aging and depigmenting agents [11,13].

The present study aims to investigate the biological potential of *Datura stramonium* L. seed extracts naturally grown in Belmebük village of Amasya province, Turkey. Specifically, collagenase and tyrosinase enzyme inhibitory activities will be assessed using spectrophotometric methods, while antioxidant capacities will be evaluated through DPPH free radical scavenging and ABTS cation radical decolorization assays. The findings are expected not only to enrich the existing scientific literature but also to provide a foundation for the development of novel pharmaceutical and plant-based dermocosmetic formulations. In addition, exploring the biological activities of *D. stramonium* seed extracts for the first time will contribute to the valorization of Turkey's natural flora and may create new opportunities for applications in the pharmaceutical and cosmetic industries, thereby supporting both scientific progress and national economic development.

Materials and Methods

- **Plant material**

The *Datura stramonium* L. plant used in this study was collected in the summer of 2023 from the village of Belmebük in Amasya province. The taxonomic identification of the collected specimens was made by Prof. Dr. Yavuz Bülent KÖSE, a faculty member at Anadolu University's Faculty of Pharmacy. A specimen from the collected plant was labeled with an herbarium number (16194) and recorded and preserved at the Anadolu University Faculty of Pharmacy Herbarium.

- **Extraction of plants**

In our study, plant extracts prepared from the seed portions of *Datura stramonium* L. were used. The ripe black seeds in the fruit capsules were removed and allowed to dry in the shade for 7–10 days. The dried seed portions of the plant material were ground into a coarse powder. 5 g of ground plant material was weighed, 100 mL of ethanol was added, and the lid was closed and macerated for 24 hours at room temperature on a shaker at 150 rpm. At the end of 24 hours, the macerate was filtered, and another 100 mL of ethanol was added to the plant material. After three repeated processes, the collected extracts were freed from their solvent using a rotavapor vacuum at low temperature (40°C). The resulting extract was transferred to a dark glass bottle and stored at -20°C for use in experimental procedures [14]. For the water extract, infusion was performed. 5 g of ground plant material was weighed, 100 mL of hot distilled water was added, and the mixture was left to stand at room temperature, covered, for 10-15 minutes, shaking occasionally. At the end of this period, the extract was filtered, and another 100 mL of hot distilled water was added to the plant material. The extracts were then placed in a lyophilizer to completely remove any water. At the end of the four-day period, the remaining extract in the lyophilizer was transferred to a dark-colored, lidded glass container and stored in a refrigerator at -20°C until used in experimental studies.

- **Anti-Collagenase activity studies**

Enzyme inhibition assays were performed according to the manufacturer's instructions for the "Collagenase Activity Assay Kit (Colorimetric, Abcam 19699). To determine activity, samples were first dissolved in ethanol. A 96-well plate was filled with the specified amounts of substances according to the accompanying instructions. Immediately after the substrate was added, absorbance values were measured at 345 nm in kinetic mode at 37°C using a multi-mode microplate reader (SpectraMax i3) and compared to the standards provided in the kit. Percentage inhibition values were calculated as the mean values for all samples. Epigallocatechin gallate was used as the positive control. Studies were performed in triplicate [15].

Collagenase enzyme percent inhibition values:

% inhibition = [(A345 control – A345 sample) / [A345 control] x 100 was calculated with the help of the formula.

❖ Anti-Tyrosinase activity studies

In vitro anti-tyrosinase activity of the samples was performed according to the method designed by Hearing and Jiménez [16]. First, the inhibition of diphenolase function of the compounds was evaluated, and L-DOPA was used as the substrate. Tyrosinase (E.C. 1.14.18.1) (30 U, 28 nM) from mushrooms was dissolved in Na-phosphate buffer (pH=6.8, 50 nM), and the compounds were added to the solution for pre-incubation at room temperature for 10 minutes. 0.5 mM L-DOPA was added to the mixture to initiate the enzymatic reaction, and the change in absorbance at 475 nm was measured at 37°C. Kojic acid was used as a positive control [15].

Tyrosinase enzyme % inhibition values:

% inhibition = [(A475 control - A475 sample)/ A475 control] x 100 was calculated with the help of the formula.

❖ Antioxidant activity tests

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity test:

The total antioxidant capacity of *Datura stramonium* L. ethanol and water extracts was determined using the DPPH method described by Blois and colleagues [17]. The reaction mixture contained 100 µM DPPH and formulations in methanol. After 30 min, absorbance was read at 517 nm using a UV spectrophotometer (UV-1800, Shimadzu, Japan) at 25 ± 2 °C [18]. Butyl hydroxy toluene (BHT) was used as a positive control. Studies were performed in triplicate. Results were calculated as IC50 (µg/mL).

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) antioxidant activity test:

The antioxidant capacity of the ethanol and water extract of *Datura stramonium* L. was measured using the ABTS radical cation decolorization protocol described by [19]. ABTS dissolved in 7 mM water and 2.45 mM potassium persulfate were mixed to form ABTS. The mixture was left in a dark room at 25°C for 16 hours before use. Ethanol was added to the mixture, and absorbance was measured at 734 nm at 25°C [17]. Trolox was used as a positive control. Studies were performed in triplicate. Results were calculated as IC50 (µg/mL).

Analyses were performed in triplicate. Results are averaged with standard deviation.

Results

❖ Collagenase and tyrosinase inhibition

The inhibitory effects of *Datura stramonium* L. seed extracts on collagenase and tyrosinase were evaluated at a concentration of 20 µg/mL (Table 1). Both ethanol and aqueous extracts exhibited significant enzyme inhibitory activities when compared to the positive controls. The ethanol extracts inhibited collagenase and tyrosinase by 26.8 ± 0.099% and 59.5 ± 0.025%, respectively. In contrast, the aqueous extract demonstrated stronger inhibition with 52.1 ± 0.09% against collagenase and 70.5 ± 0.012% against tyrosinase. Although the inhibition rates were lower than those of the positive controls (epigallocatechin gallate for collagenase, 88.09 ± 0.03%, and kojic acid for tyrosinase, 98.13 ± 0.01%), both extracts displayed noteworthy enzyme inhibitory potential.

Table 1. Inhibitory activity of Datura stramonium seed extracts against collagenase and tyrosinase:

Sample	Collagenase (% inhibition)	Tyrosinase (% inhibition)
Ethanol extract	26.8 ± 0.099	59.5 ± 0.025
Aqueous extract	52.1 ± 0.09	70.5 ± 0.012
Positive control	88.09 ± 0.03 (EGCG)	98.13 ± 0.01 (Kojic acid)

❖ Antioxidant activities (DPPH and ABTS assays)

The antioxidant activities of ethanol and aqueous extracts were determined using DPPH and ABTS radical scavenging assays (Table 2). The ethanol extract exhibited IC_{50} values of 0.0962 ± 0.0299 mg/mL (DPPH) and 0.241 ± 0.052 mg/mL (ABTS). The aqueous extract showed stronger antioxidant activity with IC_{50} values of 0.0522 ± 0.0254 mg/mL (DPPH) and 0.1974 ± 0.0156 mg/mL (ABTS). Compared to the positive controls (BHT for DPPH: 0.0148 ± 0.001 mg/mL; Trolox for ABTS: 0.0153 ± 0.001 mg/mL), the extracts demonstrated moderate yet significant radical scavenging activities.

Table 2. Antioxidant activity of *Datura stramonium* seed extracts determined by DPPH and ABTS assays:

Sample	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)
Ethanol extract	0.0962 ± 0.0299	0.241 ± 0.052
Aqueous extract	0.0522 ± 0.0254	0.1974 ± 0.0156
Positive control	0.0148 ± 0.001 (BHT)	0.0153 ± 0.001 (Trolox)

Discussion

The present findings revealed that *Datura stramonium* seed extracts exhibit significant inhibitory effects on collagenase and tyrosinase, alongside notable antioxidant capacities. The aqueous extract demonstrated consistently superior activity compared to the ethanol extract, suggesting that water-soluble constituents play a pivotal role in these bioactivities.

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Phytochemical studies on the genus *Datura* indicate that tropane alkaloids such as atropine, hyoscyamine, and scopolamine dominate its chemical profile [2]. Although generally considered lipophilic, these alkaloids display moderate water solubility due to protonation of their tertiary amine groups, which facilitates their extraction into aqueous solvents [6]. The higher collagenase (52.1%) and tyrosinase (70.5%) inhibition in our aqueous extract may thus be attributed not only to phenolics and flavonoids but also to enhanced solubilization of alkaloids. The observed superior activity of the aqueous extract in our study aligns with the higher alkaloid yield in seeds (71.28 mg/g) compared to leaves (32.10 mg/g), as reported by phytochemical quantitation of *D. stramonium* [4]. This suggests that seed-derived phytochemicals, including tropane alkaloids and phenolics, are likely concentrated in aqueous fractions. The diversity of phytochemical classes—including steroid glycosides and phenolics—also supports multifaceted bioactivity [5].

Comparable evidence exists across the genus. For example, *Datura metel* leaf extracts demonstrated measurable tyrosinase inhibition (~20.9%) in a comparative screen of Thai medicinal plants, albeit weaker than kojic acid, confirming the enzyme-modulatory capacity of *Datura* phytochemicals [20]. Similarly, mushroom tyrosinase inhibition was reported for organic extracts of *D. metel* leaves [21]. Our results showing ~70% inhibition therefore highlight a comparatively strong response within the genus.

Moreover, in the context of plant-derived anti-aging agents, have shown that phenolic-rich extracts can inhibit collagenase and elastase—key enzymes in skin aging—thereby validating the biological relevance of such activity in cosmetic applications of botanical extracts [11]. In line with this, *D. metel* leaf supercritical CO_2 extracts reduced MMP-2 expression in fibroblasts, correlating with protection against collagen degradation and oxidative stress [22]. Our aqueous extract's moderate collagenase inhibition (52.1%) aligns well with these prior findings, suggesting potential relevance for skin-aging interventions.

Antioxidant assays further support the role of polar extracts. A methanolic D. stramonium seed extract exhibited a DPPH IC_{50} of ~94.9 µg/mL, which is highly concordant with our ethanol extract (0.0962 mg/mL) [23]. Similarly, *D. innoxia* leaves yielded ABTS IC_{50} values around 149 µg/mL, and phenolic-rich aqueous fractions showed superior activity compared to organic extracts [24]. This pattern is consistent with our observation that the aqueous extract was more potent than the ethanol extract in both DPPH and ABTS assays.

Taken together, these results emphasize that the superior bioactivity of the aqueous extract arises from a dual contribution: phenolic/flavonoid enrichment and solubilized tropane alkaloids. This synergy likely underpins the simultaneous inhibition of collagenase and tyrosinase and the strong antioxidant effects observed. By aligning with literature on *D. metel*, *D. innoxia*, and *D. stramonium*, our study provides novel evidence that aqueous preparations of *Datura* seeds may serve as promising candidates for pharmaceutical and dermocosmetic applications aimed at preventing collagen degradation, hyperpigmentation, and oxidative damage.

Conclusions

The present study demonstrated that seed extracts of D. stramonium L. possess notable biological activities, including collagenase and tyrosinase inhibition as well as antioxidant capacity. Both ethanol and aqueous extracts exhibited significant inhibitory effects, with the aqueous extract consistently outperforming the ethanol extract across all tested assays. These findings suggest that water-soluble phytochemicals may play a central role in the observed bioactivities.

The dual ability of *D. stramonium* extracts to inhibit enzymes associated with skin aging and pigmentation, combined with their antioxidant potential, highlights their relevance as promising natural sources for pharmaceutical and dermocosmetic applications. This study provides the first evidence of enzyme inhibitory properties of *D. stramonium* seed extracts, thereby contributing novel insights into the pharmacological potential of this species. Future research focusing on phytochemical profiling and isolation of active constituents will be essential to fully elucidate the mechanisms underlying these activities and to advance their possible applications in drug discovery and cosmetic formulations.

Conflict of Interest: NIL

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Declarations:

Authors' Contribution:

- All authors contributed to study design, data collection, analysis, and interpretation of results.
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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