



Integrated In Vitro Evaluation of Antioxidant and Anti-inflammatory Properties - *Santalum album*

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ABSTRACT :

Santalum album (*S. album*) has long been utilized in traditional medicine and cosmetic formulations; however, systematic scientific evidence supporting its antioxidant efficacy, enzyme inhibitory effects, and cellular safety remains limited. This study aimed to conduct an integrated in vitro evaluation of the antioxidant properties, enzyme inhibition potential, and cytocompatibility of an aqueous extract of *S. album*. Phytochemical screening revealed the presence of flavonoids (3+), tannins (1+), and saponins (1+), while alkaloids, steroids, and triterpenes were absent. Antioxidant activity was assessed using DPPH, FRAP, total phenolic content (TPC), and malondialdehyde (MDA) assays. The extract exhibited strong DPPH radical scavenging activity ($76.53 \pm 5.57\%$ at 1 mg/mL) and concentration-dependent ferric reducing power, with the highest FRAP value observed at 10 mg/mL, comparable to ascorbic acid. TPC analysis demonstrated a dose-dependent increase, confirming the contribution of phenolic compounds to antioxidant capacity. In an oxidative stress model, *S. album* extract (10 mg/mL) significantly reduced MDA levels compared to the negative control, indicating effective inhibition of lipid peroxidation. Enzyme inhibition assays showed mild to moderate inhibitory effects against collagenase ($26.66 \pm 2.72\%$), lipoxigenase ($16.81 \pm 1.85\%$), hyaluronidase ($11.44 \pm 0.84\%$), elastase ($8.45 \pm 1.60\%$), and xanthine oxidase ($7.73 \pm 0.91\%$), suggesting supportive anti-inflammatory and anti-aging potential. Cytotoxicity evaluation using Vero, WRL-68, and BALB/3T3 cell lines demonstrated no significant toxicity, with IC_{50} values exceeding 500 μ g/mL for all tested cells. Overall, the findings indicate that *S. album* aqueous extract possesses strong antioxidant activity, mild enzyme inhibitory effects, and excellent cellular safety, supporting its potential application as a safe and functional ingredient in cosmeceutical and skin-protective formulations.

Keywords: *Santalum album*; antioxidant activity; enzyme inhibition; cytocompatibility; cosmeceutical applications

Introduction

Oxidative stress plays a critical role in the pathogenesis of skin aging, inflammation, and various degenerative disorders through the excessive generation of reactive oxygen species (ROS) (Leyane *et al.*, 2022). Prolonged oxidative damage accelerates lipid peroxidation, protein degradation, extracellular matrix breakdown, and cellular dysfunction, ultimately contributing to premature aging and compromised skin barrier integrity (Rinnerthaler *et al.*, 2015). Consequently, there is increasing scientific and industrial interest in identifying safe, naturally derived antioxidants capable of mitigating oxidative stress while maintaining high biocompatibility for long-term use in topical and cosmeceutical applications (Shahidi *et al.*, 2025).

Plant-derived phenolic compounds, particularly flavonoids and tannins, are widely recognized for their strong antioxidant properties and their ability to modulate key enzymes involved in inflammation and skin aging (Tungmunthum *et al.*, 2018). These compounds exert their protective effects through free radical scavenging, metal ion chelation, and inhibition of oxidative enzyme pathways (Zahra *et al.*, 2024). In addition to antioxidant activity, the inhibition of matrix-degrading enzymes such as collagenase, elastase, and hyaluronidase has become an important strategy in the development of anti-aging and skin-protective formulations, as these enzymes are directly associated with collagen breakdown, loss of elasticity, and tissue degradation (Michalak, 2022).

Santalum album is a medicinal plant traditionally valued for its soothing, anti-inflammatory, and skin-conditioning properties (Moy *et al.*, 2017). While *S. album* essential oil has been extensively studied, scientific evidence supporting the biological activities of its aqueous extract particularly in relation to antioxidant capacity, enzyme inhibition, and cellular safety remains limited (Chen *et al.*, 2023). Traditional usage suggests that water-based preparations of *S. album* may offer therapeutic benefits with reduced irritation risk, making aqueous extracts especially relevant for sensitive-skin and dermatological applications (Gürgen *et al.*, 2025). However, comprehensive in vitro validation of these claims is still lacking.

Previous studies on *S. album* have largely focused on its volatile constituents and antimicrobial effects, with comparatively less emphasis on its non-volatile, water-soluble phytochemicals such as phenolics and flavonoids. Furthermore, existing reports often evaluate antioxidant or enzyme inhibitory activities in isolation, without integrating safety assessments at the cellular level. This represents a critical knowledge gap, as the development of functional cosmetic and pharmaceutical ingredients requires not only bioactivity but also demonstrated cytocompatibility.

Therefore, the present study aimed to conduct an integrated in vitro evaluation of the antioxidant properties, enzyme inhibition potential, and cellular safety of *S. album* aqueous extract. Antioxidant capacity was assessed using multiple complementary assays, including DPPH, FRAP, total phenolic content, and lipid peroxidation (MDA). Enzyme inhibitory effects against key inflammatory and skin-aging-related enzymes were evaluated, while

cytotoxicity was determined using normal kidney, liver, and fibroblast cell lines. By combining phytochemical characterization, functional bioactivity assays, and safety evaluation, this study provides a comprehensive scientific basis supporting the potential use of *S. album* aqueous extract as a safe and effective ingredient for cosmeceutical and skin-protective applications.

Materials and Methods

Plant Material and Extract Preparation

The authenticated plant material of *S. album* was obtained from a verified source and assigned the laboratory sample code (AI 077 0525 / TS0080525). The plant material was cleaned, air-dried at room temperature, and ground into a fine powder. The aqueous extract was prepared by macerating the powdered material in distilled water at a solid-to-solvent ratio of 1:10 (w/v) under continuous stirring for 24 h at room temperature. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator. The resulting extract was freeze-dried and stored at -20°C until further analysis.

Phytochemical Screening

Preliminary phytochemical screening of the aqueous extract was conducted using standard qualitative methods to detect the presence of major phytochemical groups, including flavonoids, tannins, saponins, triterpenes, steroids, and alkaloids. The intensity of phytochemical presence was semi-quantitatively graded as weak (+1), moderate (+2), or strong (+3) based on color intensity and precipitate formation.

High-Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was performed to obtain the chemical fingerprint profile of the *S. album* extract. The extract was prepared by methanol extraction and filtered through a $0.45\ \mu\text{m}$ membrane filter prior to analysis. Chromatographic separation was achieved using a reverse-phase C18 column with a mobile phase consisting of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), applied under gradient elution conditions. The flow rate was maintained at 1.0 mL/min, and detection was carried out using a UV detector. Retention times and UV spectra were recorded to characterize the chemical constituents present in the extract.

Total Phenolic Content (TPC) Determination

Total phenolic content was determined using the Folin–Ciocalteu method. The extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution and incubated at room temperature for 30 min. Absorbance was measured at 765 nm. Gallic acid was used as the standard, and results were expressed as milligrams of gallic acid equivalents (mg GAE/g extract).

DPPH Radical Scavenging Assay

The antioxidant activity of the extract was evaluated using the DPPH radical scavenging assay. Various concentrations of *S. album* extract were prepared and mixed with DPPH solution (0.1 mM in methanol). The reaction mixture was incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm using a microplate reader. Ascorbic acid was used as the positive control. The percentage of radical scavenging activity was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted to assess the reducing power of the extract. The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and ferric chloride (20 mM) in a ratio of 10:1:1. The extract was mixed with the FRAP reagent and incubated at 37°C for 30 min. Absorbance was measured at 593 nm, and results were expressed as $\mu\text{mol Fe}^{2+}$ equivalents per milliliter of extract.

Malondialdehyde (MDA) Assay

The lipid peroxidation inhibitory effect of the extract was evaluated by measuring malondialdehyde (MDA) levels using a thiobarbituric acid reactive substances (TBARS) assay. Samples were reacted with thiobarbituric acid under acidic conditions and heated at 95°C . After cooling, absorbance was measured at 532 nm. MDA levels were calculated and compared among normal, negative control, positive control, and *S. album*-treated groups.

Enzyme Inhibition Assays

The inhibitory effects of the extract against selected enzymes were evaluated using established in vitro methods. Lipoxxygenase, xanthine oxidase, hyaluronidase, elastase, and collagenase inhibition assays were performed at specified extract concentrations. Enzyme activity was monitored

spectrophotometrically, and percentage inhibition was calculated relative to enzyme controls without extract.

Cytotoxicity Assay

Cellular safety of the *S. album* aqueous extract was evaluated using the MTT assay. Normal cell lines, including Vero (kidney), WRL-68 (liver), and BALB/3T3 clone A31 (fibroblast), were cultured under standard conditions. Cells were treated with varying concentrations of the extract for 24 h, followed by incubation with MTT solution. The resulting formazan crystals were dissolved, and absorbance was measured at 570 nm. Cell viability was expressed as a percentage of untreated control cells, and IC₅₀ values were determined.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using appropriate software, and differences between groups were considered statistically significant at *p* < 0.05.

Results

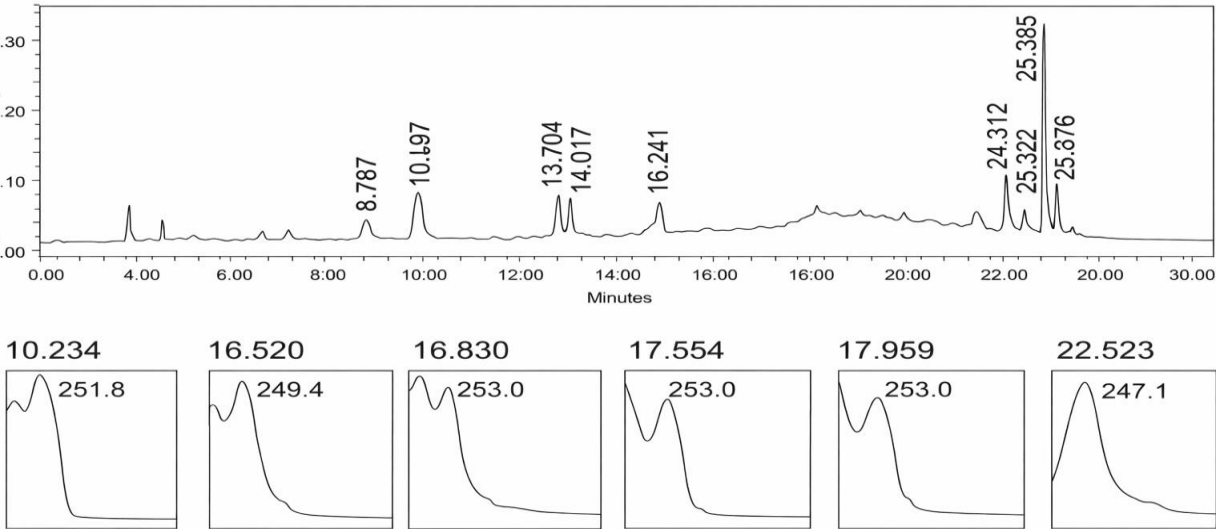
Table 1. Qualitative phytochemical profile of the aqueous extract of *S. album*. The presence of phytochemical constituents was determined using standard qualitative tests and graded semi-quantitatively as weak (+1), moderate (+2), or strong (+3), while “–” indicates absence or levels below the detectable limit.

Tests	<i>S. album</i>
Saponin	+1
Tannin	+1
Triterpene	-neg
Steroid	-neg
Flavonoid	+3
Alkaloid	-neg

Qualitative phytochemical screening of the aqueous extract of *S. album* revealed the presence of several bioactive secondary metabolites. The extract showed a strong presence of flavonoids (3+), indicating that flavonoids are the dominant phytochemical constituents in the aqueous fraction. Tannins and saponins were detected at low levels (1+), suggesting a minor but contributory presence of these compounds. In contrast, triterpenes, steroids, and alkaloids were not detected, indicating that these classes of compounds are either absent or present below the detectable limit in the aqueous extract.

The predominance of flavonoids is consistent with the strong antioxidant activity observed in subsequent assays, as flavonoids are well known for their free radical scavenging and metal-chelating properties. The absence of alkaloids and steroids further supports the favorable safety profile of the extract, as these compounds are often associated with irritation or cytotoxicity in topical applications. Overall, the phytochemical profile suggests that the aqueous extract of *S. album* is enriched with polar phenolic compounds, which may contribute to its antioxidant and skin-protective properties.

Figure 1. High-performance liquid chromatography (HPLC) chromatogram of *S. album* aqueous extract showing multiple prominent peaks detected under UV monitoring. The chromatogram illustrates the retention times and relative abundance of major constituents present in the extract, indicating a chemically diverse profile dominated by polar compounds.



The HPLC chromatogram of the *S. album* aqueous extract revealed a complex chemical profile characterized by several distinct peaks at retention times of approximately 8.787, 10.087, 13.704, 14.017, 16.241, 24.312, 24.802, 25.325, and 25.676 minutes. These peaks indicate the presence of multiple phytochemical constituents that are detectable under the applied chromatographic conditions.

The early- to mid-eluting peaks (8–16 min) suggest the predominance of polar to moderately polar compounds, which is consistent with the aqueous extraction method used. In addition, a cluster of peaks observed at later retention times (24–26 min) indicates the presence of compounds with relatively lower polarity. The UV spectral patterns recorded for the selected peaks further support the presence of phenolic-type compounds, which typically exhibit characteristic UV absorption profiles.

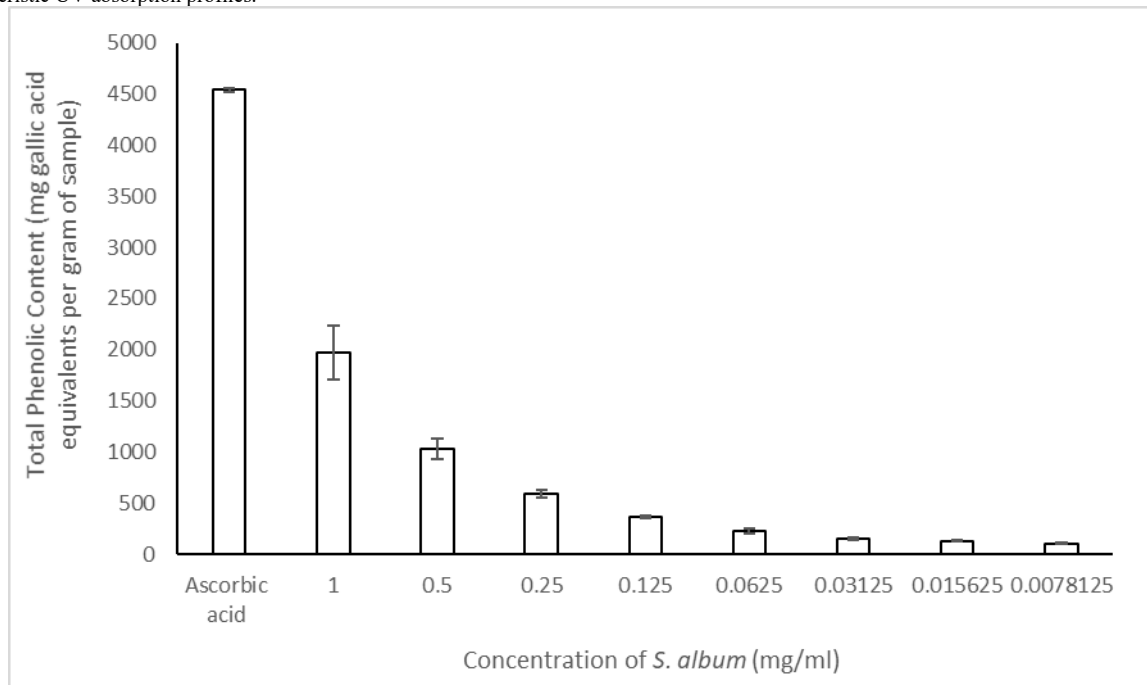


Figure 2. Total phenolic content (TPC) of *S. album* aqueous extract at different concentrations, expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g). Ascorbic acid was used as a reference standard. Results are presented as mean $\pm$ SD (n = 3).

The total phenolic content of *S. album* aqueous extract showed a clear concentration-dependent trend. At the highest concentration tested (1 mg/mL), the extract exhibited a substantial phenolic content, which progressively decreased with decreasing extract concentration. Moderate TPC values were observed at 0.5 and 0.25 mg/mL, while markedly lower phenolic levels were detected at concentrations below 0.125 mg/mL.

Ascorbic acid, used as a reference compound, displayed the highest phenolic-equivalent value, serving as a benchmark for comparison. The gradual decline in TPC values across concentrations indicates that phenolic compounds are present in appreciable amounts in the aqueous extract and are diluted proportionally with decreasing concentration.

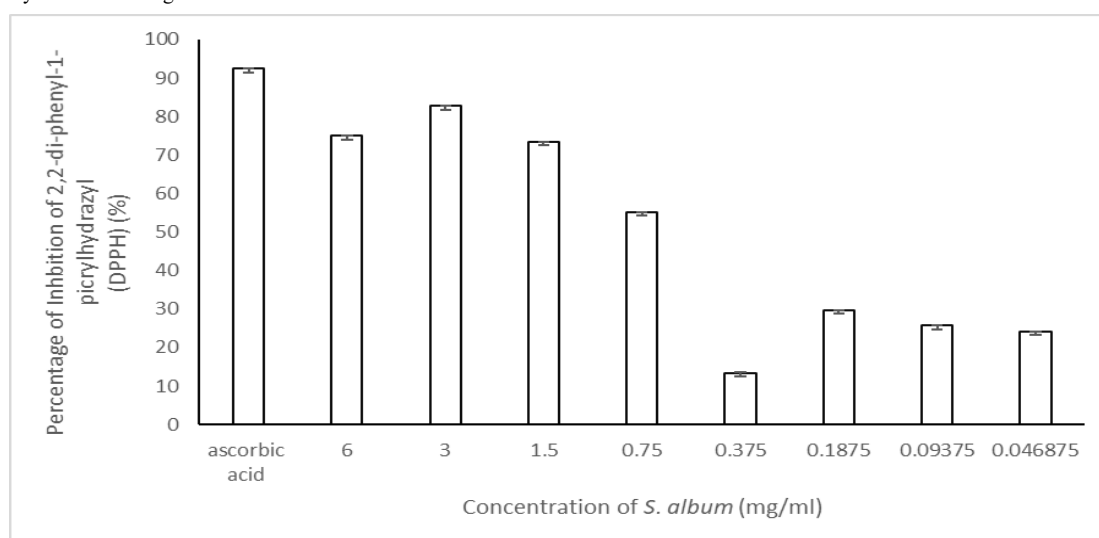


Figure 3. DPPH radical scavenging activity of *S. album* aqueous extract at various concentrations. Ascorbic acid was used as the positive control. Results are expressed as percentage inhibition of DPPH radicals and presented as mean $\pm$ SD (n = 3).

The DPPH radical scavenging activity of *S. album* aqueous extract exhibited a clear concentration-dependent pattern. At higher concentrations, the extract demonstrated strong antioxidant activity, with percentage inhibition values exceeding 70% at concentrations of 6, 3, and 1.5 mg/mL. The highest concentration tested (6 mg/mL) showed radical scavenging activity comparable to that of ascorbic acid, the positive control.

As the concentration decreased, a gradual reduction in DPPH inhibition was observed. Moderate antioxidant activity was recorded at 0.75 mg/mL, while substantially lower scavenging activity was detected at concentrations below 0.375 mg/mL. The lowest concentration tested (0.0469 mg/mL) exhibited minimal radical scavenging capacity, indicating reduced antioxidant effectiveness at very low extract concentrations.

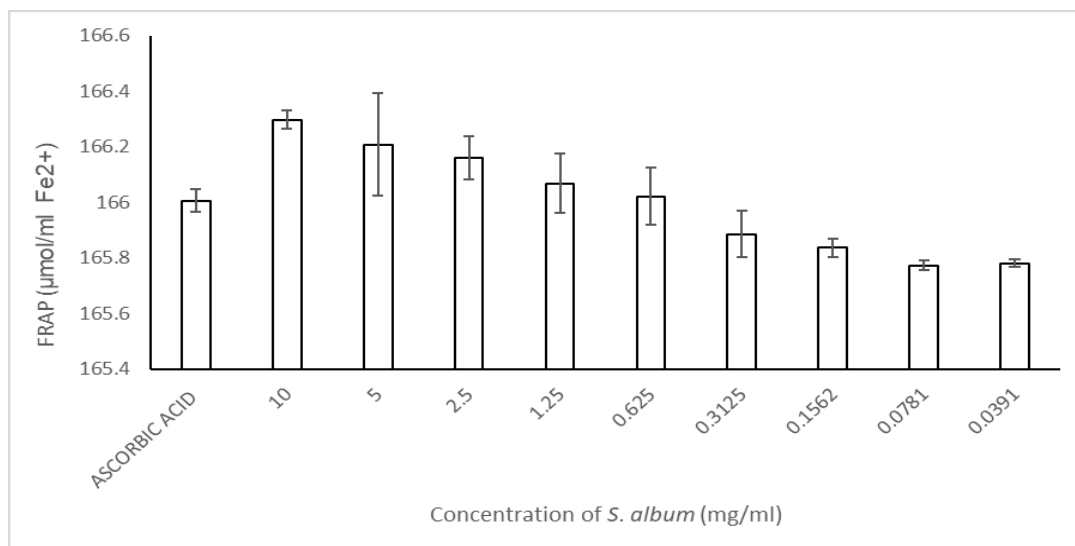


Figure 4. Ferric reducing antioxidant power (FRAP) of *S. album* aqueous extract at various concentrations, expressed as $\mu\text{mol/mL Fe}^{2+}$ equivalents. Ascorbic acid was used as the positive control. Data are presented as mean $\pm$ SD ($n = 3$).

The ferric reducing antioxidant power of *S. album* aqueous extract exhibited a concentration-dependent pattern. At the highest concentration tested (10 mg/mL), the extract demonstrated the greatest reducing capacity, with FRAP values slightly higher than that of ascorbic acid. A gradual decline in FRAP values was observed with decreasing extract concentration, although measurable reducing power was retained even at lower concentrations.

The results indicate that the aqueous extract possesses a notable electron-donating ability, which is a key mechanism underlying antioxidant activity. The relatively small error bars observed across concentrations suggest good experimental reproducibility.

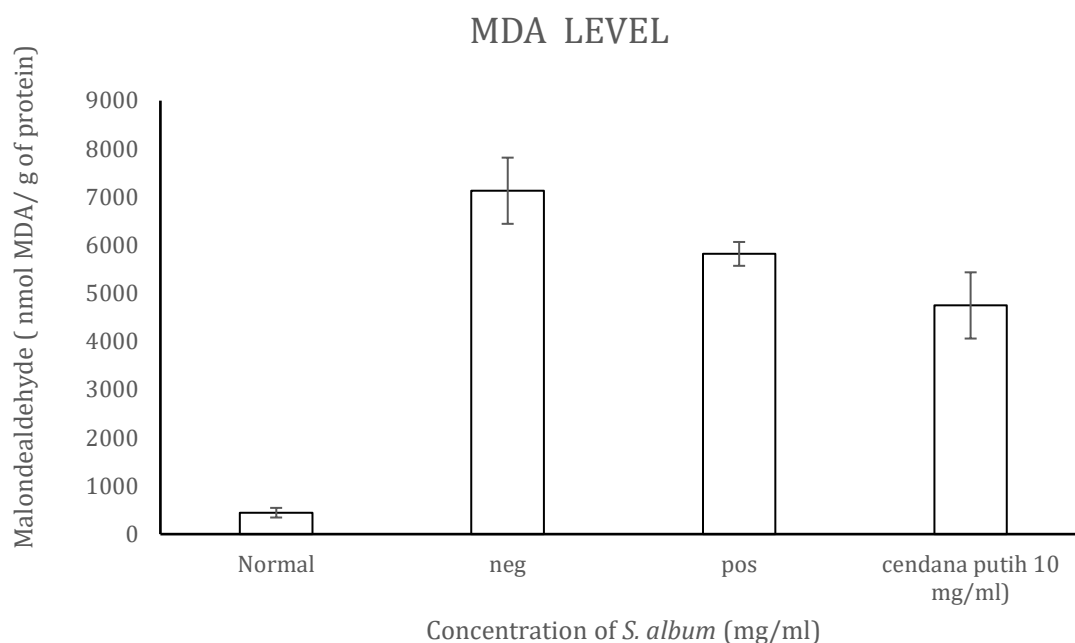


Figure 5. Effect of *S. album* aqueous extract on malondialdehyde (MDA) levels, expressed as nmol MDA/g protein. Normal, negative (neg), and positive (ascorbic acid) controls were included for comparison. Data are presented as mean $\pm$ SD ($n = 3$).

The malondialdehyde (MDA) levels differed markedly among the experimental groups. The normal control group exhibited a very low MDA level, approximately 300 nmol MDA/g protein, indicating minimal lipid peroxidation under physiological conditions. In contrast, the negative control group showed a pronounced increase in MDA levels, reaching approximately 7,200 nmol MDA/g protein, reflecting severe oxidative stress and extensive lipid peroxidation.

Treatment with the positive control significantly reduced MDA levels to approximately 5,800 nmol MDA/g protein compared to the negative control, although the value remained substantially higher than that of the normal group. Notably, treatment with *S. album* aqueous extract at 10 mg/mL resulted in a further reduction of MDA levels to approximately 4,600 nmol MDA/g protein, representing a marked attenuation of lipid peroxidation relative to both the negative and positive control groups.

Table 2. Inhibitory effects of *S. album* aqueous extract against inflammatory- and skin-aging-related enzymes. Results are expressed as mean percentage inhibition (%) $\pm$ SEM from three independent experiments, with standard inhibitors used as positive controls.

No.	Sample code	Lipoxygenase inhibition (%)	Xanthine oxidase inhibition (%)	Hyaluronidase inhibition (%)	Elastase inhibition (%)	Collagenase inhibition (%)
1	<i>S. album</i>	16.81 $\pm$ 1.85	7.73 $\pm$ 0.91	11.44 $\pm$ 0.84	8.45 $\pm$ 1.60	8.45 $\pm$ 1.60

Positive control	Concentration	Lipoxygenase (%)	Xanthine oxidase (%)	Hyaluronidase (%)	Elastase inhibition (%)	Collagenase inhibition (%)
NDGA	100 μ g/mL	97.84 $\pm$ 1.06	–	–	–	–
Allopurinol	100 μ g/mL	–	99.85 $\pm$ 0.14	–	–	–
Apigenin	200 μ M	–	–	93.77 $\pm$ 3.26	–	–
EGCG	2.5 mg/mL	–	–	–	87.03 $\pm$ 0.61	–
EGCG	2.5 mg/mL	–	–	–	–	87.03 $\pm$ 0.61

The aqueous extract of *S. album* exhibited low to moderate inhibitory effects against the enzymes evaluated (Table 2). The extract demonstrated 16.81 $\pm$ 1.85% inhibition of lipoxygenase, indicating a mild capacity to interfere with inflammatory pathways mediated by leukotriene biosynthesis. Inhibition of xanthine oxidase was relatively low (7.73 $\pm$ 0.91%), suggesting limited suppression of uric acid production and reactive oxygen species generation via this enzymatic route.

In addition, *S. album* showed 11.44 $\pm$ 0.84% inhibition of hyaluronidase, reflecting a modest protective effect against extracellular matrix degradation. The extract also exhibited 8.45 $\pm$ 1.60% inhibition of elastase and a comparatively higher collagenase inhibition of 26.66 $\pm$ 2.72%, suggesting a greater modulatory effect on collagen-degrading enzymes than on elastase activity. The corresponding positive controls (NDGA, allopurinol, apigenin, and EGCG) displayed strong inhibitory activities (>87%), confirming the validity and sensitivity of the assay system.

Table 3. Cytotoxicity of *S. album* aqueous extract against normal cell lines expressed as median inhibitory concentration (IC₅₀, μ g/mL). Values are presented as mean $\pm$ standard error of the mean (SEM) from three independent experiments. Sodium dodecyl sulfate (SDS) was used as the positive control.

Test sample	Vero cell line	WRL-68 cell line	BALB/3T3 clone A31 cell line
<i>S. album</i>	> 500	> 500	> 500
SDS (positive control)	33.23 $\pm$ 3.40	15.66 $\pm$ 0.17	13.80 $\pm$ 1.60

The cytotoxicity assessment demonstrated that the aqueous extract of *S. album* exhibited no significant cytotoxic effects against all tested normal cell lines, including Vero, WRL-68, and BALB/3T3 clone A31 cells, with IC₅₀ values exceeding 500 μ g/mL. In contrast, the positive control SDS showed pronounced cytotoxicity, with low IC₅₀ values ranging from 13.80 to 33.23 μ g/mL, confirming the sensitivity and reliability of the assay. These findings indicate that *S. album* aqueous extract is biocompatible and safe toward normal cells under the tested conditions.

Discussion

The present study provides an integrated in vitro evaluation of the antioxidant capacity, enzyme inhibitory activities, and cellular safety of *S. album* aqueous extract, offering scientific validation for its traditional use and expanding current knowledge beyond studies focused primarily on *S. album* essential oil. The findings demonstrate that the aqueous extract of *S. album* possesses a multifunctional bioactivity profile characterized by strong antioxidant properties, mild modulation of inflammation- and skin-aging-related enzymes, and excellent cytocompatibility, supporting its potential application as a safe cosmeceutical and skin-protective ingredient.

Qualitative phytochemical analysis revealed that the aqueous extract was dominated by flavonoids (3+), with minor contributions from tannins and saponins (1+), while alkaloids, steroids, and triterpenes were absent. This phytochemical composition is typical of polar, water-based extracts and is consistent with previous reports showing that aqueous extraction preferentially recovers phenolic compounds rather than lipophilic constituents (Thavamoney *et al.*, 2018). The predominance of flavonoids provides a strong mechanistic basis for the biological activities observed in this study, as flavonoids are well recognized for their ability to scavenge free radicals, chelate metal ions, and modulate oxidative pathways (Hassanpour *et al.*, 2023). Importantly, the absence of alkaloids and steroids further supports the favorable safety profile of the extract, particularly for topical and long-term applications (El-Saadony *et al.*, 2025).

The antioxidant potential of *S. album* aqueous extract was clearly demonstrated across multiple complementary assays. In the DPPH radical scavenging assay, the extract exhibited strong activity, achieving 76.53 $\pm$ 5.57% inhibition at 1 mg/mL and exceeding 70% inhibition at higher concentrations (1.5–6 mg/mL), with activity comparable to that of ascorbic acid at the highest tested concentration. This concentration-dependent scavenging behavior indicates effective hydrogen- or electron-donating capacity, a hallmark of phenolic antioxidants (Jiménez-Ortega *et al.*, 2024). These findings are further supported by the FRAP assay, in which the extract demonstrated substantial ferric reducing power, with the highest activity observed at 10 mg/mL, slightly exceeding that of ascorbic acid. Since FRAP values reflect the electron-donating ability of antioxidants, the results suggest that *S. album* aqueous extract is capable of neutralizing oxidizing agents through redox-based mechanisms (Seleshe *et al.*, 2022).

The contribution of phenolic compounds to the observed antioxidant activity was further confirmed by total phenolic content analysis, which showed a clear dose-dependent trend. The extract exhibited its highest phenolic content at 1 mg/mL, followed by a gradual decline with decreasing concentration. The strong agreement between total phenolic content, DPPH scavenging activity, and FRAP values indicates that phenolic constituents, particularly flavonoids, are the primary drivers of antioxidant efficacy in the aqueous extract. Similar correlations between phenolic content and antioxidant capacity have been widely reported for medicinal and cosmeceutical plants (Muflihah *et al.*, 2021).

Beyond chemical antioxidant assays, the biological relevance of the antioxidant activity was substantiated by the lipid peroxidation (MDA) assay. The negative control group exhibited markedly elevated MDA levels of approximately 7,200 nmol MDA/g protein, confirming severe oxidative damage. Treatment with *S. album* aqueous extract at 10 mg/mL significantly reduced MDA levels to approximately 4,600 nmol/g protein, corresponding to a reduction of around 36–40% relative to the negative control. This reduction was more pronounced than that observed with the positive control (~5,800 nmol/g protein), indicating effective suppression of lipid peroxidation. Given that lipid peroxidation plays a central role in membrane damage, inflammation, and skin aging, the ability of the extract to attenuate MDA formation highlights its functional antioxidant potential at the biological level (Li Pomi *et al.*, 2025).

The enzyme inhibition assays revealed that *S. album* aqueous extract exhibits low to moderate inhibitory effects against key inflammatory- and skin-aging-related enzymes. The extract inhibited lipoxigenase by $16.81 \pm 1.85\%$, xanthine oxidase by $7.73 \pm 0.91\%$, and hyaluronidase by $11.44 \pm 0.84\%$, indicating mild modulation of inflammatory pathways and extracellular matrix degradation. Notably, the extract demonstrated a comparatively higher inhibitory effect against collagenase ($26.66 \pm 2.72\%$) than elastase ($8.45 \pm 1.60\%$), suggesting selective modulation of collagen degradation processes. Although these inhibitory activities are substantially lower than those of the respective positive controls (>87%), such moderate enzyme modulation is biologically meaningful in the context of cosmeceutical applications, where excessive enzyme inhibition may disrupt normal physiological processes. Flavonoids and tannins are known to interact weakly with enzyme active sites through non-covalent interactions, resulting in partial rather than complete inhibition, which may explain the observed enzyme inhibition profile (Šarić Mustapić *et al.*, 2018).

A key strength of this study lies in the comprehensive evaluation of cellular safety. Cytotoxicity assays demonstrated that *S. album* aqueous extract exhibited no significant cytotoxic effects against Vero, WRL-68, and BALB/3T3 clone A31 cell lines, with IC_{50} values exceeding 500 $\mu\text{g/mL}$ for all tested cells. In contrast, the positive control sodium dodecyl sulfate displayed strong cytotoxicity, with IC_{50} values ranging from 13.80 to 33.23 $\mu\text{g/mL}$, confirming the sensitivity and reliability of the assay system. The high cellular tolerance observed is particularly important for ingredients intended for topical use, as repeated exposure requires a wide safety margin to minimize irritation and adverse effects (Bialas *et al.*, 2023).

Taken together, the findings of this study indicate that *S. album* aqueous extract functions as a balanced, multifunctional bioactive rather than a potent pharmacological inhibitor. Its strong antioxidant capacity, effective suppression of lipid peroxidation, mild modulation of inflammation- and skin-aging-related enzymes, and excellent cellular safety collectively support its suitability as a safe and functional ingredient for cosmeceutical and skin-protective formulations. This integrated evaluation provides a robust scientific foundation for the utilization of *S. album* aqueous extract in applications where long-term safety, antioxidant protection, and gentle enzyme modulation are essential.

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