Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 7
Open Access

From Antioxidants to Enzyme Inhibitors: The Multifaceted Antioxidant and Anti-Aging Properties of Methanolic extract of Salvia officinalis and Rosmarinus officinalis

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Cite this paper as: Ashok Kumar Gupta , Dr. Gaurav Jain (2024). From Antioxidants to Enzyme Inhibitors: The Multifaceted Antioxidant and Anti-Aging Properties of Methanolic extract of Salvia officinalis and Rosmarinus officinalis. *Frontiers in Health Informatics*, *13 (7) 603-615*

ABSTRACT
The methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) were evaluated for their antioxidant, radical scavenging, and enzyme inhibitory activities to explore their potential as natural anti-aging agents. Antioxidant activities were assessed using reducing power, total antioxidant activity and metal chelating activity. MES and MER demonstrated significant antioxidant potential, with IC_{50} values comparable to or slightly lower than standard antioxidants such as quercetin, ascorbic acid, and EDTA. The total antioxidant activity of both extracts was superior to α-tocopherol and showed a concentration-dependent increase across assays. In enzyme inhibition studies, MES and MER exhibited strong inhibitory activities against elastase, collagenase, and hyaluronidase, enzymes responsible for extracellular matrix degradation. MES showed slightly higher inhibitory effects, with elastase inhibition at 95.72%, collagenase inhibition at 88.72%, and hyaluronidase inhibition at 80.75%, comparable to standard inhibitors. These results highlight the potential of MES and MER to preserve skin elasticity, hydration, and structural integrity. This study demonstrates the therapeutic promise of *Salvia officinalis* and *Rosmarinus officinalis* extracts as natural antioxidants and anti-aging agents, suggesting their potential applications in dermatological formulations and cosmeceutical products.
Keywords: Anti-ageing, antioxidant, rosemary, salvia, reducing power, oxidative stress

INTRODUCTION

Oxidative stress plays a central role in the aging process, influencing cellular and molecular mechanisms that contribute to age-related physiological decline and the onset of chronic diseases. It arises from an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the body's ability to neutralize them through antioxidant defense mechanisms. ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, are byproducts of normal cellular metabolism, particularly in the mitochondria. When their levels exceed the capacity of enzymatic and non-enzymatic antioxidants, oxidative damage to DNA, proteins, lipids, and other cellular components ensues [1]. The accumulation of oxidative damage is a hallmark of aging. It affects essential cellular functions, including energy production, gene expression, and intercellular signaling. Oxidatively damaged DNA can lead to mutations, compromised cellular repair mechanisms, and cellular senescence, a state of irreversible cell cycle arrest. Similarly, oxidation of proteins results in structural and functional impairments, while lipid peroxidation compromises membrane integrity and disrupts cellular homeostasis. These effects collectively impair tissue function, promote inflammation, and increase susceptibility to age-associated disorders such as cardiovascular diseases, neurodegenerative conditions, and cancer [2, 3].

In skin aging, oxidative stress accelerates the degradation of extracellular matrix components, such as collagen and

elastin, by activating enzymes like collagenase and elastase. This leads to the loss of skin elasticity, hydration, and firmness, manifesting as wrinkles, sagging, and dryness. Additionally, oxidative stress contributes to photoaging, driven by ultraviolet (UV) radiation. The body relies on endogenous antioxidants (e.g., superoxide dismutase, catalase, and glutathione) and dietary antioxidants (e.g., vitamins C and E, flavonoids, and polyphenols) to combat oxidative stress. Enhancing antioxidant defenses through natural or synthetic compounds has become a therapeutic strategy to mitigate oxidative damage and delay aging-related changes, promoting overall health and longevity [4, 5]

In this study, *Rosmarinus officinalis* (rosemary) and *Salvia officinalis* (sage) were selected for their extensive historical use in traditional medicine and their well-documented antioxidant properties. Both plants are rich in polyphenolic compounds, such as rosmarinic acid, carnosic acid, and flavonoids, known for their ability to scavenge free radicals and inhibit oxidative damage. Additionally, rosemary and sage have shown potential in modulating enzymatic activities involved in extracellular matrix degradation, such as elastase, collagenase, and hyaluronidase. These properties make them ideal candidates for investigating natural anti-aging solutions that address oxidative stress and its impact on skin and cellular health [6, 7]. The body relies on endogenous antioxidants (e.g., superoxide dismutase, catalase, and glutathione) and dietary antioxidants (e.g., vitamins C and E, flavonoids, and polyphenols) to combat oxidative stress. Enhancing antioxidant defenses through natural or synthetic compounds has become a therapeutic strategy to mitigate oxidative damage and delay aging-related changes, promoting overall health and longevity.

The present research aimed to evaluate the anti-radical, antioxidant, and anti-aging properties of methanolic extracts obtained from the leaves and flowers of *Salvia officinalis* L. and *Rosmarinus officinalis* Linn. This study explored the phytochemical composition of the extracts through preliminary screening and assessed their potential to combat oxidative stress and inhibit aging-related enzymatic activities. Various in vitro techniques, including radical scavenging assays and enzyme inhibition studies, were employed to comprehensively determine their bioactivity and therapeutic potential.

MATERIAL AND METHODS

Collection and identification and extraction of the plants

The flowers and leaves of *Salvia officinalis* and *Rosmarinus officinalis* were collected from the Dehradun region during the late spring season of the year, a period considered optimal for harvesting due to the maximum accumulation of bioactive compounds in the plant parts. The collected plant materials were promptly transported to the laboratory for processing and analysis. The plants were identified and authenticated by a botanist to ensure accurate taxonomical classification and botanical integrity. To preserve the documentation for future reference or research purposes, two voucher specimens were prepared and labelled as AKG/SO/2022/11 for Salvia officinalis and AKG/RO/2022/12 for Rosmarinus officinalis. These voucher specimens were meticulously stored in an herbarium under appropriate conditions to maintain their quality and authenticity for potential use in subsequent studies or verification processes. In the laboratory, the flowers and leaves were carefully separated, thoroughly washed to remove debris and contaminants, and allowed to air-dry in a controlled environment to prevent degradation of sensitive phytochemicals. The dried plant materials were then finely chopped and subjected to methanol extraction using a standardized maceration technique. Methanol was chosen as the solvent due to its high efficacy in extracting a broad spectrum of bioactive compounds, including phenolic acids, flavonoids, and essential oils, which are characteristic of these plants. The extraction process was carried out by soaking the plant material in methanol for an extended period, followed by filtration and solvent recovery using a rotary evaporator under reduced pressure. This step ensured that the extracts were free of impurities and concentrated for subsequent use. The resulting methanolic extracts of *Salvia officinalis* leaves and flowers (labelled as MES) and *Rosmarinus officinalis* leaves and flowers (labelled as MER) appeared as dark green to brownish viscous residues. These concentrated extracts were indicative of the rich phytochemical content typical of these plants. To preserve their stability and prevent degradation, the methanolic extracts were stored in amber-coloured airtight containers

at a temperature of 4°C. The low-temperature storage minimized exposure to light, oxygen, and moisture, thereby maintaining the integrity and efficacy of the extracts until they were utilized for further analysis or formulation development. This meticulous approach ensured the extracts retained their bioactive properties for use in anti-aging, antioxidant, or other pharmacological evaluations.

Drugs and chemicals

The study utilized various chemicals and reagents, including collagenase; FALGPA [N-[3-(2-Furyl)acryloyl]-L-leucyl-glycyl-L-prolyl-L-alanine]; HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); N-Methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide; p-Dimethylaminobenzaldehyde (DMAB; also known as Ehrlich's Reagent); 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH); and 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), all procured from Sigma-Aldrich, St. Louis, MO, USA. Quercetin was generously provided as a gift sample from a reliable source. All other chemicals and reagents utilized were of reagent-grade quality and were sourced from reputable suppliers, including SRL Mumbai and E. Merck India

Preliminary phytochemical screening

The extracts were analyzed for their phytochemical constituents using a series of established chemical tests to determine the presence of bioactive compounds. These standard qualitative assays were employed to identify various classes of phytochemicals, including alkaloids, flavonoids, saponins, tannins, phenols, glycosides, terpenoids, and steroids. Each test was carefully conducted under controlled laboratory conditions to ensure accurate identification. For alkaloid detection, reagents such as Dragendorff's and Wagner's were used to observe the formation of characteristic precipitates. Flavonoids were identified using the Shinoda test, which involves the addition of magnesium turnings and concentrated hydrochloric acid to observe the development of a pink or red color, indicating their presence. Saponins were tested using the froth formation test, where persistent foam upon shaking indicated their presence. Tannins and phenols were detected using ferric chloride tests, where the appearance of a blue-green or black coloration signified their presence. Glycosides were screened using Keller-Kiliani and Borntrager's tests, revealing characteristic color changes upon reaction with specific reagents. The Liebermann-Burchard test was used to detect steroids and terpenoids, with the development of a reddish-brown ring confirming their presence. These preliminary tests provided a qualitative overview of the chemical profile of the extracts, serving as a foundation for further quantitative and analytical investigations. The results from this screening highlighted the potential bioactive compounds present, laying the groundwork for understanding the pharmacological activities associated with the extracts [8].

Determination of the total phenolic contents (TPC)

The quantification of soluble phenolic content, expressed as total phenolic compounds (TPC), in the methanolic extracts was conducted using the well-established Folin-Ciocalteu method [9]. This method is widely recognized for its accuracy in estimating the phenolic content of plant-based extracts. Quercetin was employed as the reference standard phenolic compound due to its well-characterized phenolic structure and reliable response in this assay, as outlined in the method described by Slinkard and Singleton (1977). During the assay, a defined volume of the extract solution was mixed with the Folin-Ciocalteu reagent, a phosphomolybdic-phosphotungstic acid complex that reacts with phenolic hydroxyl groups. This reaction produces a blue-colored complex with an absorbance measurable at a specific wavelength, typically 765 nm, using a spectrophotometer. The intensity of the color is directly proportional to the phenolic content in the sample. To determine the TPC, a standard calibration curve was prepared using a series of quercetin solutions of known concentrations. The linear regression equation derived from the calibration curve, expressed as Y=mx+cY = mx + cY=mx+c, was used to calculate the TPC of the methanol extracts. Here, YYY represents the absorbance measured at 765 nm, mmm is the slope of the regression line, xxx denotes the concentration of the phenolic compounds, and ccc is the y-intercept. The coefficient of determination (r2r^2r2) was calculated to ensure the linearity and accuracy of the standard curve.

Using this equation, the TPC in the methanol extracts was expressed in grams of quercetin equivalents (QE) per gram of

extract. This standardization enabled the comparison of phenolic content across different extracts. The Folin-Ciocalteu method provided a reliable estimation of the phenolic compounds, which are key contributors to the antioxidant activity of the extracts. This quantitative assessment formed the basis for further exploring the pharmacological potential of the methanolic extracts.

Determination of the reducing power

The reducing power of the methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) was evaluated using a previously established method, designed to measure the electron-donating ability of the extracts. This method involved the reduction of ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, indicated by the development of a blue-green color complex when potassium ferricyanide was used as the reagent. The intensity of the color, measured spectrophotometrically at a specific wavelength, provided a quantitative estimate of the reducing power. The greater the absorbance, the higher the reducing power, indicating the potential antioxidant efficacy of the extracts.

Determination of the total antioxidant activity

Additionally, the total antioxidant activity of MES and MER was determined using the thiocyanate technique as described by Mitsuda et al. (1996). This method measures the ability of the extracts to inhibit lipid peroxidation in a linoleic acid emulsion system. In this assay, the extracts were incubated with linoleic acid, and the peroxides formed during oxidation were allowed to react with ferric chloride and thiocyanate. The resulting ferric thiocyanate complex, measured spectrophotometrically at 500 nm, reflected the extent of lipid peroxidation. A lower absorbance value in this assay corresponded to a higher antioxidant activity, as it indicated effective inhibition of lipid peroxidation by the extracts. These complementary methods provided a comprehensive evaluation of the antioxidant potential of MES and MER, highlighting their ability to donate electrons and suppress oxidative stress-related damage [10].

Evaluating the metal chelating activity

The metal chelating activity of the methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) was assessed based on their ability to chelate ferrous ions (Fe^{2+}), following the method described by Kumaran and Joel Karunakaran (2006). This method measures the inhibition of the formation of the ferrous-ferrozine complex, a purple-colored compound formed when ferrous ions react with ferrozine [11]. In this assay, MES and MER were incubated with ferrous sulfate and ferrozine under specific conditions. The extracts, acting as chelating agents, competed with ferrozine for ferrous ions, reducing the formation of the ferrous-ferrozine complex. The decrease in color intensity of the complex was measured spectrophotometrically at 562 nm. The extent of color reduction corresponded to the metal chelating ability of the extracts. The percentage of inhibition of ferrous-ferrozine complex formation was calculated using the following formula:

$$\% \text{ Inhibition } = \frac{(A_0 - A_t)}{(A_0)}$$

In the above formula, Where, A0 = Control absorbance (without extract) and At = optical density/absorbance in the existence of the extract/standard. As the typical chelating compound, Ethylenediaminetetraacetic acid (EDTA) had been utilised. Higher inhibition percentages indicated a stronger chelating ability of MES and MER, signifying their potential to bind and neutralize ferrous ions. This activity is critical in reducing oxidative stress caused by metal-catalyzed free radical generation, particularly through the Fenton reaction. These findings further underscored the antioxidant capabilities of the extracts, highlighting their possible applications in preventing oxidative damage mediated by metal ions.

Evaluating the Anti-ageing activity of MES and MER

Anti-elastase activity.

The elastase inhibitory activity of the test samples, methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER), was evaluated using a modified method based on the procedure described by Kraunsoe et al. (1996). Elastase, a serine protease, plays a significant role in the degradation of elastin, an essential protein responsible for skin

elasticity [12]. The inhibition of elastase activity is a key indicator of anti-aging potential, as it helps to maintain skin integrity and delay signs of aging such as sagging and wrinkles. In this assay, elastase activity was monitored using a specific synthetic substrate, N-Succinyl-Ala-Ala-Ala-p-nitroanilide, which is hydrolyzed by elastase to release p-nitroaniline, a chromophore. The reaction mixture contained the elastase enzyme, the synthetic substrate, and the test sample (MES or MER) in a suitable buffer system. The mixture was incubated under controlled conditions, and the release of p-nitroaniline was measured spectrophotometrically at 410 nm. The test samples inhibited elastase activity by interfering with the enzyme-substrate interaction, thereby reducing the amount of p-nitroaniline released. The degree of inhibition was calculated as a percentage using the following formula:

$$\% \text{ Inhibition } = \frac{(A_0 - A_t)}{(A_0)}$$

In the above formula, Where, A0 = Control absorbance (without extract) and At = optical density/absorbance in the existence of the extract/standard. Higher inhibition percentages indicated stronger anti-elastase activity of MES and MER, suggesting their potential in protecting elastin fibers and mitigating the enzymatic processes that contribute to skin aging. This assay provided a crucial metric for assessing the anti-aging properties of the extracts and their applicability in dermatological or cosmetic formulations.

Estimation of collagenase inhibitory activity

The collagenase inhibitory activity of the methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) was evaluated using a modified and improved procedure based on the methods described by Moore and Stein (1948) and Mandl et al. (1953). Collagenase, a matrix metalloproteinase, degrades collagen, which is a critical structural protein in connective tissues. Inhibiting collagenase activity is a key factor in preventing collagen degradation, thereby maintaining skin integrity and combating aging [13, 14]. In this assay, collagenase activity was monitored using the synthetic substrate FALGPA (N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala), which is hydrolyzed by collagenase to release measurable chromophores. The reaction mixture included the collagenase enzyme, the synthetic substrate, and the test sample (MES or MER) in a suitable buffer system. The mixture was incubated under optimized conditions to facilitate enzyme-substrate interaction. The presence of MES or MER inhibited collagenase activity by binding to the enzyme or interfering with its active site, thereby reducing the hydrolysis of the substrate. The decrease in absorbance of the hydrolyzed substrate was measured spectrophotometrically at 335 nm. The percentage of collagenase inhibition was calculated using the formula:

$$\% \text{ Inhibition } = \frac{(A_0 - A_t)}{(A_0)}$$

In the above formula, Where, A0 = Control absorbance (without extract) and At = optical density/absorbance in the existence of the extract/standard. Higher inhibition percentages indicated a stronger anti-collagenase activity of the extracts, suggesting their potential to protect collagen fibers and delay aging-related structural degradation in the skin. This assay is pivotal in evaluating the anti-aging efficacy of MES and MER and their suitability for therapeutic or cosmetic applications aimed at preserving skin health and appearance.

Hyaluronidase inhibitory activity

The hyaluronidase inhibitory activity of the methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) was evaluated using the Morgan-Elson fluorometric assay method, as originally developed by Reissig et al. (1955) and further refined by Takahashi et al. (2003). Hyaluronidase is an enzyme responsible for the degradation of hyaluronic acid, a vital component of the extracellular matrix that maintains skin hydration, elasticity, and structural integrity. Inhibiting hyaluronidase activity is essential for preserving hyaluronic acid levels and counteracting skin aging processes. In this assay, hyaluronidase activity was measured by its ability to degrade hyaluronic acid into smaller oligosaccharides. The reaction mixture included hyaluronidase enzyme, hyaluronic acid as the substrate, and the test sample (MES or MER) in a suitable buffer system. The mixture was incubated under optimized conditions, and the

enzymatic reaction was terminated by the addition of an alkaline solution. The degradation products of hyaluronic acid were subjected to the Morgan-Elson reaction, which involves the formation of a chromophore or fluorescent compound detectable at specific wavelengths. The fluorescence intensity or absorbance of the reaction mixture was measured spectrophotometrically or fluorometrically, respectively [15, 16]. The inhibitory activity of the extracts was calculated as a percentage using the formula:

$$\% \text{ Inhibition } = \frac{(A_0 - A_t)}{(A_0)}$$

In the above formula, Where, A0 = Control absorbance (without extract) and At = optical density/absorbance in the existence of the extract/standard. Higher inhibition percentages indicated a stronger ability of MES and MER to inhibit hyaluronidase activity, demonstrating their potential to preserve hyaluronic acid levels and promote skin hydration and elasticity. This assay provided critical insights into the anti-aging properties of the extracts and their potential applications in dermatological or cosmetic formulations targeting skin health.

Statistical analysis

The study's results were presented as the mean ± standard deviation (SD), with n=6 or n=3, depending on the specific experiment. Statistical analysis was conducted to assess the significance of the observed differences among the groups. One-way analysis of variance (ANOVA) was employed as the primary statistical test to evaluate variations across multiple groups. Following this, Dunnett's Multiple Comparison Test was used as a post hoc analysis to compare each experimental group against the control group, enabling precise identification of statistically significant differences. The statistical calculations were performed using GraphPad Prism software, a widely used tool for biological and pharmacological data analysis. A p-value (p) of 0.05 or less was considered statistically significant, indicating a less than 5% probability that the observed differences occurred by chance. This rigorous approach ensured the reliability and validity of the findings, allowing for robust conclusions regarding the effects of the tested extracts in the various assays.

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening

The qualitative preliminary phytochemical screening of the methanolic extracts of *Salvia officinalis* and *Rosmarinus officinalis* revealed the presence of a diverse range of phytoconstituents. These included flavonoids, alkaloids, phenols, phytosterols, and saponins, which are known for their pharmacological activities. The presence of these bioactive compounds suggests the potential of the extracts for therapeutic applications. Additionally, Borntrager's test, a specific assay for detecting the presence of anthraquinones, yielded positive results for both extracts. This further highlights the richness of the extracts in bioactive components, reinforcing their suitability for anti-aging, antioxidant, and other pharmacological evaluations. The findings provided a strong basis for further quantitative and analytical studies to explore the pharmacological properties of these plant extracts.

Estimation of TPC (Total Phenolic Compounds)

The total phenolic content (TPC) of the methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) was quantified using the Folin-Ciocalteu method. The TPC was expressed in terms of quercetin equivalents per gram of extract, highlighting the richness of phenolic compounds in these plants. The TPC for MES was calculated to be 183.09±2.36 mg QE/g extract, while MER exhibited a TPC of 167.87±2.57 mg QE/g extract. These values indicate a significant presence of phenolic compounds, which are well-known for their potent antioxidant and free radical scavenging activities. The results, represented in Figure 1, underscore the potential of these extracts as sources of natural antioxidants.

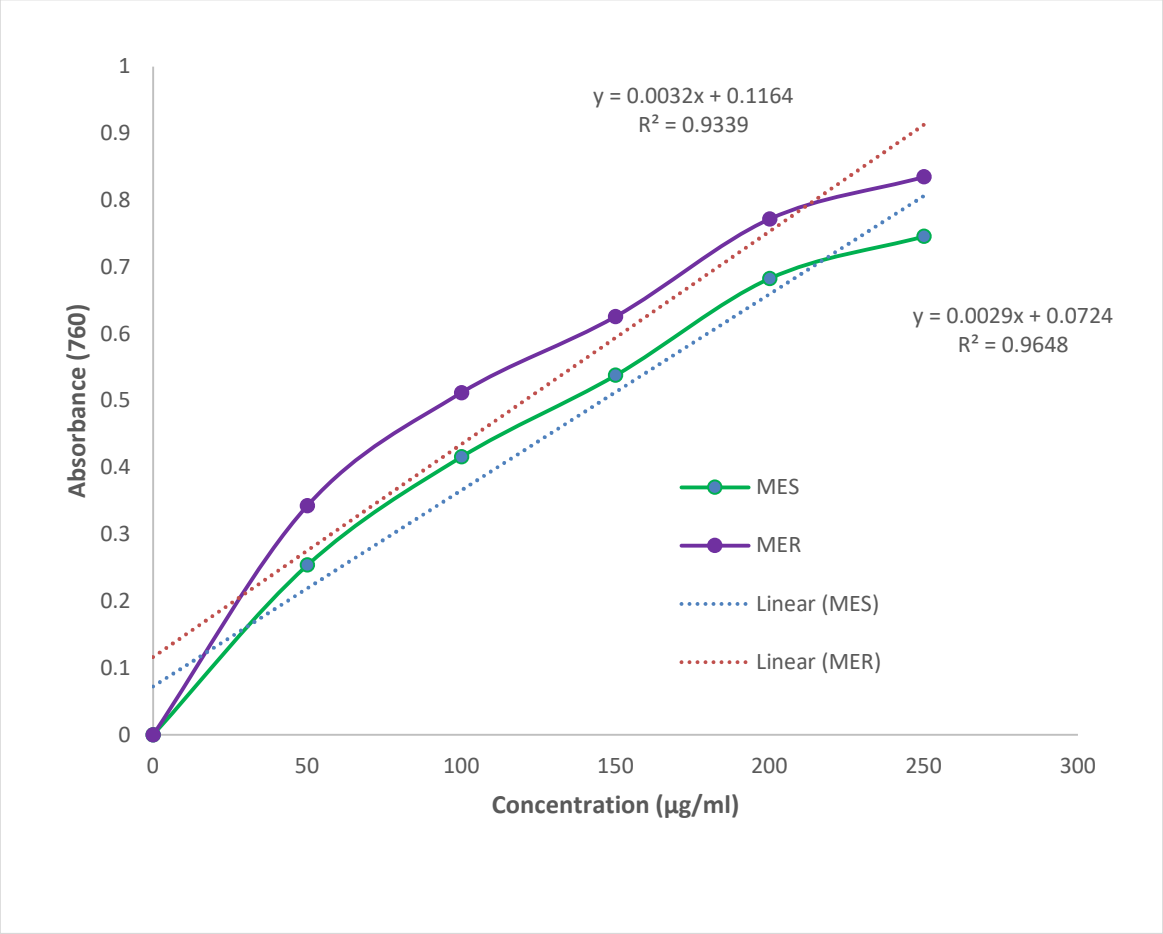

Figure 1. Total phenolic compounds in MES and MER are displayed.

Comprehensive Evaluation of Antioxidant and Radical Scavenging Activities

The antioxidant potential of the methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) was evaluated through various assays, each highlighting their ability to combat oxidative stress and neutralize free radicals.

Reducing Power Assay

The reducing power assay demonstrated that both MES and MER exhibited significant antioxidant potential when compared to standards such as quercetin, sodium metabisulfite, and ascorbic acid (Figure 2). The reducing power of MES and MER was found to be concentration-dependent, with higher concentrations resulting in increased reducing ability. This activity underscores the extracts' electron-donating capacity, which is crucial for neutralizing oxidative agents.

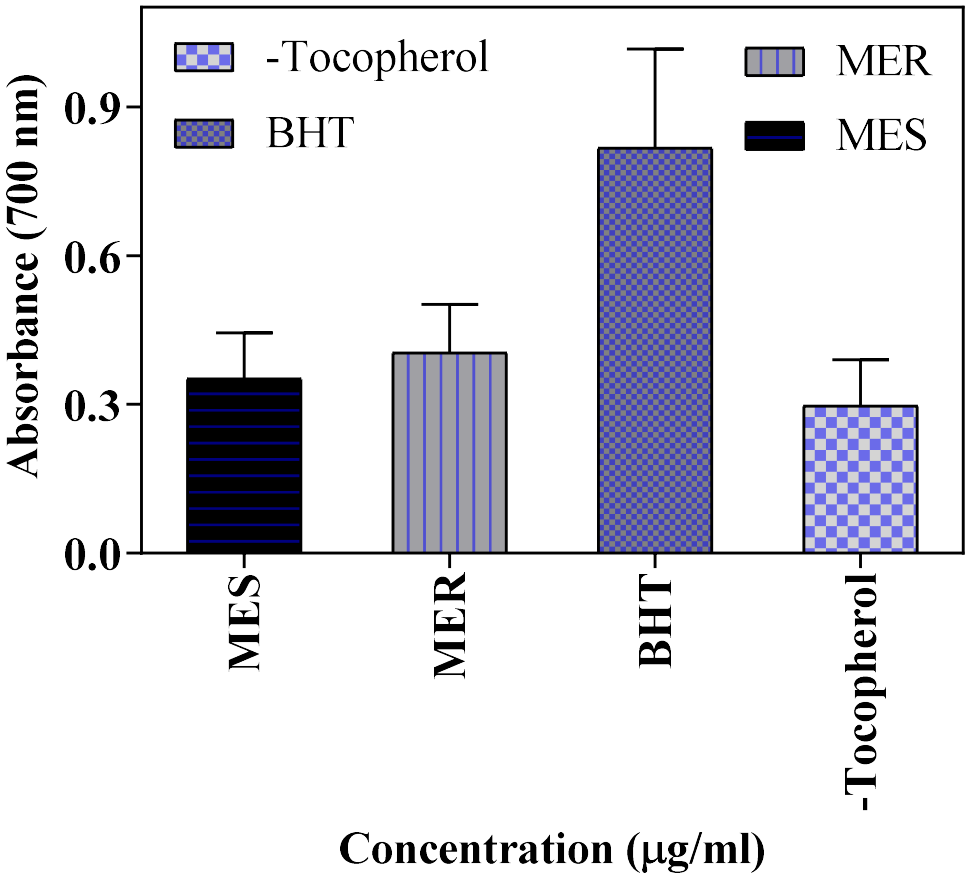

Figure 2. Demonstrating the MES and MER reducing power assay

Total Antioxidant Activity

The total antioxidant activity of MES and MER was assessed over prolonged incubation periods (up to 36 hours). Both extracts exhibited significant antioxidant activity, although their efficacy was slightly inferior to butylated hydroxyanisole (BHA) but superior to α-tocopherol at equivalent concentrations. The percentage inhibitions for MES, MER, BHA, and α-tocopherol were 63.88 ± 0.69%, 62.77 ± 0.65%, 87.76 ± 0.54%, and 44.68 ± 0.56%, respectively (Figure 3). These results highlight the potential of MES and MER as natural antioxidants capable of long-lasting effects

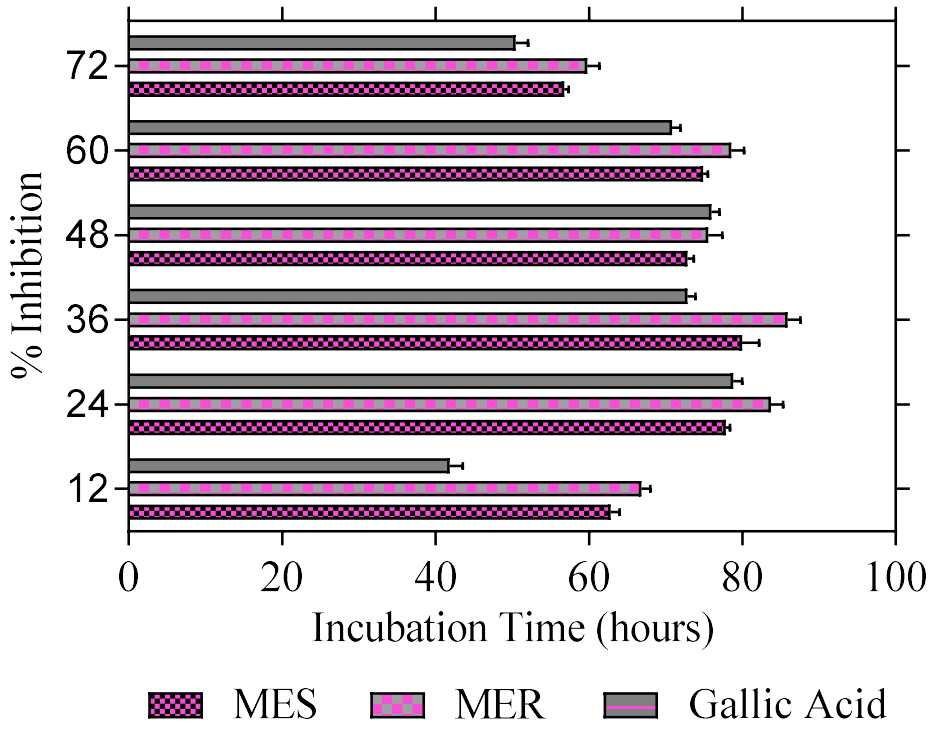

Figure 3. Showing MES and MER's total antioxidant activity

Metal Chelating Activity

The ability of MES and MER to chelate ferrous ions was assessed, showing that higher concentrations of the extracts resulted in increased metal chelating activity. Although their activity was lower than that of EDTA, MES and MER still demonstrated significant chelating potential. The IC50 values were 349.53 ± 0.68 µg/mL for MES, 113.48 ± 1.03 µg/mL for MER, and 67.21 ± 0.54 µg/mL for EDTA (Table 1). This chelating ability highlights their potential in mitigating oxidative damage caused by metal-catalyzed free radical production.

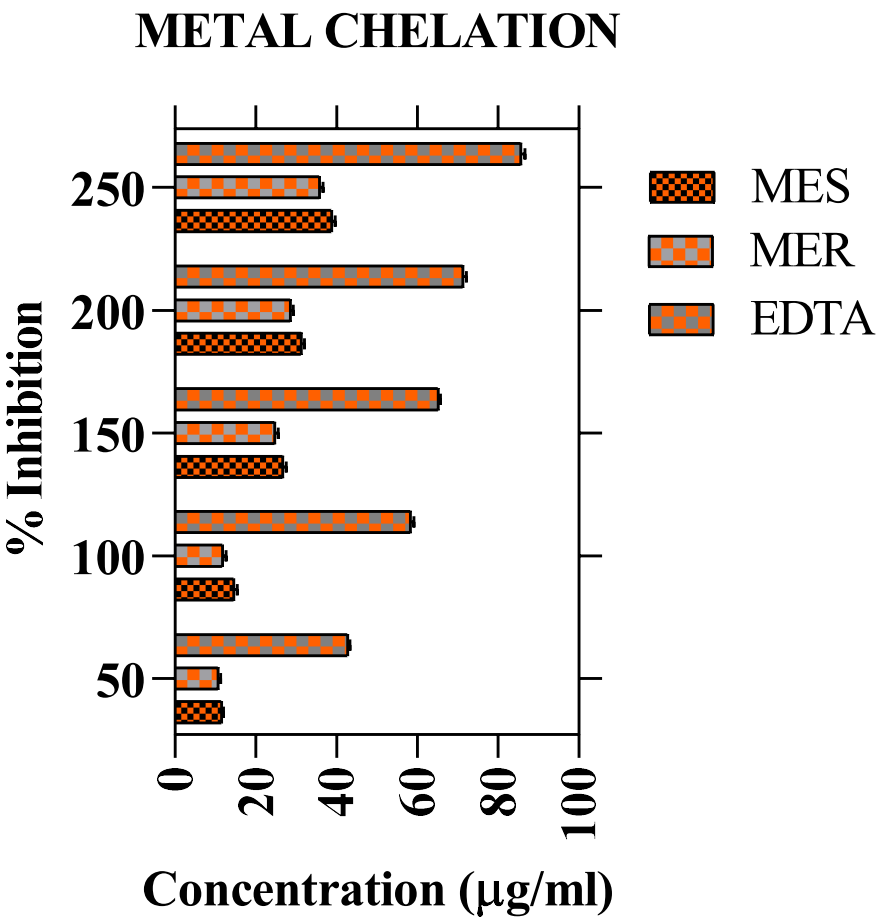

Figure 4. Antioxidant activity of MES and MER at 250 µg/ml concentration (Metal chelating activity)

These findings collectively indicate that MES and MER possess robust antioxidant activities, as evidenced by their performance across multiple assays. Their ability to scavenge various radicals, inhibit oxidative stress, and chelate metal ions demonstrates their potential as natural antioxidant agents. These results support the therapeutic and cosmetic applications of these extracts in mitigating oxidative damage and promoting overall health.

Table 1. Using linear regression analysis, the antioxidant activity potential of MES and MER in comparison to reference substances is expressed as IC50 (µg/ml) values.

Assay	MES (µg/mL)	MER (µg/mL)	Quercetin (µg/mL)	Ascorbic Acid (µg/mL)	Standard (Other)	Standard Name
DPPH Radical Scavenging	81.72 ± 0.00	80.13 ± 0.00	77.25 ± 0.00	85.08 ± 0.00	-	-
ABTS Radical Scavenging	104.94 ± 0.00	106.58 ± 0.00	76.28 ± 0.00	125.18 ± 0.00	-	-
Superoxide Radical Scavenging	140.68 ± 0.68	149.94 ± 0.91	-	89.35 ± 0.89	-	-
Nitric Oxide Scavenging	142.07 ± 0.93	167.25 ± 0.98	-	107.11 ± 0.71	-	-

Hydrogen Peroxide Scavenging	107.68 ± 0.98	113.34 ± 0.59	-	105.96 ± 0.54	-	-
Hydroxyl Radical Scavenging	132.67 ± 0.65	140.42 ± 0.85	75.35 ± 0.92	110.89 ± 0.62	-	-
Metal Chelating Activity	349.53 ± 0.68	113.48 ± 1.03	-	-	67.21 ± 0.54	EDTA

Anti-Ageing activity

The study evaluated the inhibitory effects of methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) on key enzymes associated with extracellular matrix degradation, specifically elastase, collagenase, and hyaluronidase, at a concentration of 250 µg/mL. The findings indicate that both MES and MER exhibit substantial enzyme inhibitory activities, suggesting their potential as natural anti-aging agents.

Anti-elastase assay

MES showed remarkable elastase inhibitory activity, achieving 95.72 ± 3.82%, closely comparable to the standard elastase inhibitor, elafin (96.18 ± 4.33%). MER also displayed high elastase inhibition at 91.74 ± 3.47%, slightly lower than MES. Elastase is critical in degrading elastin, which contributes to skin elasticity. The ability of these extracts to effectively inhibit elastase suggests their potential in preserving skin firmness and preventing wrinkle formation, making them promising candidates for anti-aging applications.

Collagenase inhibitory activity

In terms of collagenase inhibition, MES and MER achieved 88.72 ± 4.68% and 86.45 ± 3.79%, respectively, demonstrating activities comparable to EDTA (86.77 ± 3.91%), a well-known collagenase inhibitor. Collagenase degrades collagen, the primary structural protein in the skin. The high inhibitory activity of both extracts indicates their ability to protect collagen integrity, which is essential for maintaining the structural framework of the skin and delaying visible signs of aging such as sagging and loss of firmness.

Hyaluronidase inhibitory activity

For hyaluronidase, MES inhibited enzyme activity by 80.75 ± 4.78%, while MER showed slightly lower inhibition at 77.56 ± 4.84%. Although their activities were less potent than the standard sodium salt of aurothiomalate (100 ± 1.07%), both extracts demonstrated significant potential to preserve hyaluronic acid levels. Hyaluronic acid is a key component for maintaining skin hydration and elasticity, and its degradation leads to dryness and loss of skin volume. The observed hyaluronidase inhibition further supports the role of MES and MER in promoting skin health and combating aging.

Comparatively, MES showed slightly higher inhibitory activities across all three enzyme assays, indicating a marginally stronger anti-aging potential than MER. Both extracts, however, exhibited significant enzyme inhibition, comparable to or slightly lower than the respective standard inhibitors. This highlights their potential as natural and safer alternatives to synthetic inhibitors for addressing skin aging concerns. These findings underscore the promise of MES and MER as potent natural inhibitors of enzymes responsible for skin aging. Their ability to inhibit elastase, collagenase, and hyaluronidase suggests a multifaceted approach to preserving the extracellular matrix and maintaining skin integrity. Future studies focusing on the identification of active compounds, mechanisms of action, and validation through in vivo models could pave the way for the development of novel, plant-based anti-aging formulations. These extracts hold potential for incorporation into dermatological and cosmetic products aimed at improving skin health and delaying the onset of aging-related changes

Table 2: The effect of the plant extracts (MES and MER at 250 µg/ml) on enzyme activities

Plants (Code)	Plant parts used	Extract	Inhibitory activity

			Elastase	Collagenase	Hyaluronidase
Salvia (MES)	Leaves & Flowers	Methanol	95.72 ± 3.82	88.72 ± 4.68	80.75 ± 4.78
Rosemary (MER)	Leaves & Flowers	Methanol	91.74 ± 3.47	86.45 ± 3.79	77.56 ± 4.84
Controls (10 µg/ml)					
Elafin			96.18± 4.33	-	-
N-Methoxysuccinyl-Ala-Ala-Pro-Chloro			94.17 ± 4.37	-	-
Ethylenediaminetetraacetic acid (EDTA)			-	86.77 ± 3.91	-
Sodium salt of aurothiomalate			-	-	100 ± 1.07

Data are given as percentage inhibition ± Standard Deviation

CONCLUSIONS

The methanolic extracts of *Salvia officinalis* (MES) and *Rosmarinus officinalis* (MER) demonstrated substantial antioxidant and anti-aging potential, as evidenced by their robust free radical scavenging and enzyme inhibitory activities. The extracts effectively scavenged reactive oxygen and other species, with IC_{50} values comparable to standard antioxidants such as quercetin and ascorbic acid. Their metal chelating activity further highlighted their antioxidant efficacy. In enzyme inhibition assays, MES and MER showed remarkable activity against elastase, collagenase, and hyaluronidase, key enzymes responsible for the degradation of elastin, collagen, and hyaluronic acid. MES demonstrated slightly higher inhibitory effects across all assays, indicating its superior potential in preserving skin elasticity, hydration, and structural integrity. The comparable performance of these extracts to standard synthetic inhibitors underscores their suitability as natural alternatives in anti-aging formulations. These findings provide a strong foundation for the use of *Salvia officinalis* and *Rosmarinus officinalis* extracts in dermatological and cosmetic applications. Further research, including in vivo studies and compound characterization, will be critical to unlocking their full therapeutic potential in combating oxidative stress and skin aging.

FUNDING

Nil.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

DECLARATION Of INTEREST

The authors declare that there is no conflict of interest in this manuscript.

REFERENCE

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