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RESEARCH ARTICLE

Exploring the Potential Effect of Methanolic Extract of *Salvia officinalis* Against UV Exposed Skin Aging: *In vivo* and *In vitro* Model

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ARTICLE HISTORY

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Abstract: ***Background*:** The medicinal plants have enormous pharmacological properties with fewer side effects. Today, there is an increasing demand of medicinal plants as an anti-aging and anti-wrinkle agent.

***Objective*:** The aim of this study is to evaluate the antioxidant, anti-aging and anti-wrinkle potential of *Salvia officinalis.*

***Materials and Methods*:** *Salvia officinalis (Lamiaceae)* is folk medicine of Asia and Latin America. Powdered crude drug 100 g was successively extracted in a soxhlet apparatus with petroleum ether (60-80ºC), chloroform and methanol. After successive solvents, extraction methanolic extract was used for testing of antioxidant potential using DPPH assay. Further, the antiaging potential of the extract was investigated by the inhibitory effect of various enzymatic estimations *i.e*. Col-I, Ela-I and Hya-I inhibitory assays on early aging human skin fibroblasts. The antiwrinkle potential of plant *Salvia officinalis* was done by using a UV light-induced photoaging model.

***Results*:** Phytochemical analysis showed the presence of glycosides, alkaloids flavonoids, and triterpenoids, saponins and Phenolic Compounds at high level. The extract showed inhibitory concentration (IC_{50}: 24.65) and ascorbic acid. The standard antioxidant showed inhibitory concentration (IC_{50}: 20.10). In enzymatic estimations assay, the Col-I, Ela-I and Hya-I of extract were assessed showing inhibitory concentration as Col-I (IC_{50}:21.36), Ela-I (IC_{50}:35.05) and Hya-I (IC_{50}:23.44), respectively. Thus, MeOH extract of *Salvia officinalis* can inhibit 50% of the activity of aging-related enzymes Col-I, Ela-I and Hya-I. The wrinkle score of negative control *i.e.* UV treated group was 2.83 ± 0.408, and MeOH extract of *Salvia officinalis* treated group is 1.83 ± 0.753.

***Conclusion*:** This study concluded that MeOH extract of *Salvia officinalis* has confirmed the high antioxidant potential and *in vitro* and *in vivo* inhibitory potential of antiaging enzymes assessed, thus they could be used for further development of cosmetic products and nutraceuticals.

Keywords: Salvia officinalis, sage, photoaging, fibroblast, wrinkle score, nutraceuticals.

1. INTRODUCTION

Skin is a very sensitive part of the human body damaged by extrinsic factors such as stress, chemicals, and ultraviolet (UV) radiation. Daily exposure to UV light causes photoaging when the thickness of skin increases, the roughness of skin, coarse wrinkles and mottled pigmentation occurs. It may cause histological alterations, including Extra Cellular Matrix (ECM) degradation and increased Stratum Corneum (SC) thickness [1, 2].

Aging is an inevitable process for all living organisms. Two types of skin aging exist age-dependent/chronological aging and premature aging/photoaging [3]. The latter is caused by extrinsic factors and includes signs such as a leathery appearance, dark/light pigmentation and deep furrows [4, 5].

ECM, the outer most part of the skin is composed of fibroblasts, proteins, collagen and elastin as they build a fiber network to hold tensile strength of skin [6]. The ECM provides a structural framework which is essential for growth and elasticity of the skin and plays an important role in the maintenance of physiological functions of the body [7].

Collagen is a natural structural protein and it provides strength, flexibility and resistance to the skin. In other words, the presence of collagen gives skin its firmness**.** Elastin is also a protein and it provides the skin with the flexibility and elasticity necessary to regain the form when stretched or compressed. Collagen and Elastin work together to keep the skin smooth, supple and flexible. Hyaluronic Acid is a carbohydrate, which helps in keeping the skin moisturized and

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hydrated**.** Degradation of the ECM has directly been linked to skin aging and is correlated with an increase in the activity of certain enzymes involved in skin aging, which includes hyaluronidase, elastase and collagenase [8, 9]. These connective tissue proteins (like collagen, elastin and fibronectin) are constantly attacked by several enzymes like collagenases, elastases and matrix metalloproteinase, which lead to decrease in thickness of skin and it becomes dry and wrinkled. In the skin, fibroblasts are the cells which synthesize collagen along with other GAGs [4]. With aging, the level of collagen, elastin and hyaluronic acid decreases, leading to a loss of strength and flexibility in the skin which results in visible wrinkles. Reactive Oxygen Species (ROS) play an important role in many cellular mechanisms [10]. When UV radiation is absorbed by the skin, it leads to increased ROS generation and induction of oxidative stress. High levels of ROS lead to the activation of hyaluronidase, collagenase and elastase, which can further contribute to skin aging [11, 12]. The global market value of anti-aging products , which help the body fight off the damage caused by aging, is continuously increasing. The largest groups of compounds in anti-aging products were having antioxidants properties.

Plants have long been used in the cosmetic industry as skin lighteners and sun-screen agents and antiaging. The medicinal plants have enormous commercial potential throughout the globe. In the herbal boom worldwide, it is estimated that high-quality phytomedicinals will provide safe and effective medication. Medicinal plants have served as rich sources of pharmacologically active substances. Herbs have been used in a diverse array of purposes, including medicine, nutrition, flavorings, beverages, dying, repellents, fragrances, cosmetics, charms, smoking and industrial uses. Today, herbs are still found in 40% of prescription drugs [13].

Traditional herbs provide interesting and largely unexplored sources for the development of potential new cosmetic and pharmaceutical products. In this regard, one such plant is *Salvia officinalis* L (sage), known from Greeks and Romans ancient times used in medicament, cooking, beauty products and production of essential oils [14].

Salvia officinalis is *a* plant of family *Lamiaceae* native of Asia and Latin America. Traditionally *Salvia officinalis* is used as antiseptic, anti-scabies, antisyphilitic, and anti-inflammatory, used against the skin and eye diseases and also in pleurisy local anesthetic for the skin and hair tonic [15, 16]. Several literatures revealed that it contains active components flavonoids, alkaloids and essential oils [17]. The major phytochemical constituents of *Salvia officinalis* include alkaloids, carbohydrate, fatty acids, glycosidic derivatives (*e.g*., cardiac glycosides, flavonoids glycosides, saponins), phenolic compounds (*e.g*., coumarins, flavonoids, tannins), polyacetylenes, steroids, terpenes/terpenoids (*e.g*., monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids), and waxes [18-24].

Rutin, a polyphenolic bioflavonoid, has been studied extensively due to its promising pharmacological actions, such as antitumor, antibacterial, antiviral, and antiallergic effects [25, 26]. Rutin and its partners can fortify blood vessels against the inflammatory response, which can prevent the skin from getting its fair share of nutrients. Rutin also has the capacity to regenerate vitamin C after it neutralizes a free radical, thus helping to restore its antioxidant potential [27]. As vitamin C plays a critical role in the manufacture of collagen, an important component of capillary walls as well as the sustaining framework of the epidermis, this could have a dramatic impact on the health of your skin.

No scientific report is available till date to validate the antiaging and antiwrinkle properties of MeOH extract of *Salvia officinalis* because of flavonoids, which are present in the plant.

2. MATERIAL AND METHODS

2.1. Selection, Collection and Authentication of Plant

The plant was selected on the basis of the literature survey, information collected from standard books and internet and also from traditional medicine system (Ayurveda, Unani) practitioners. The leaves of *Salvia officinalis* plant was collected from the nearby area of Bhopal, Madhya Pradesh, India. The plant was identified and authenticated by Dr. Zia Ul Hasan, Professor & Head-Department of Botany, Safia College of Science, Bhopal, India. A voucher specimen number 124/Bot/Saf/17was kept in the Department of Botany, Safia College of Science, Bhopal for future reference.

2.2. Processing of Plant Material

Plants was shade dried and ground (1 mm) using a blender. Ground plant material (100 g) was extracted with Petroleum ether, chloroform and MeOH using a soxhlet apparatus. Resultant extracts were evaporated under vacuum at 40°C and stored at 4°C until use. The extract yields were determined gravimetrically (Table **1**).

Table 1. Percentage yield.

Scientific Name	Plant Part	Percentage Yield
Salvia officinalis	Leaves	4.13

2.3. Chemicals

All the chemicals used were of analytical grade and were obtained from Merck, Sigma and S.D. Fine Chemicals.

2.4. Qualitative Phytochemical Screening

The extracts obtained by successive solvent extraction were subjected to various qualitative phytochemical analysis to detect the presence of Phytoconstituents as glycosides, phenols, steroid/ triterpenoid, Saponins, tannins, flavonoids, and alkaloids [28, 29].

2.5. Quantitative Phytochemical Screening

Estimation of Total Flavonoids Content (TFC) [30]:

Aluminum chloride colorimetric technique was used for total flavonoids estimation. Flavonoids are capable of forming complexes with metal ions and act as antioxidants.

2.5.1. Procedure

In this method, rutin was used to make the calibration curve. 10 mg of rutin was dissolved in methanol and then diluted to 20,40,60,80, and 100 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 510nm (λmax of rutin) with a Shimadzu UV-1800 spectrophotometer. Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared.

2.5.2. Stock Solution of Extracts

100 mg of the plant extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

2.5.3. Preparation of Test Solutions

0.5ml of plant extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in a similar way by replacing aluminium chloride with distilled water. Sample and sample blank of the extract was prepared and their absorbance was measured at 510 nm. All prepared solutions were filtered through Whatman filter paper before measuring.

2.6 Antioxidant Activity

2.6.1. DPPH (2, 2-Diphenyl-1-picrylhydrazil) Radical Scavenging Assay

The best-known antioxidants are β-carotinoids, ascorbic acids, and tocopherols as well as Phenolic compounds, therefore, ascorbic acid has been used.

DPPH is a stable organic radical which has the capacity to scavenge biological reagents. Its solution is deep purple in color with an absorption peak at 517 nm, which disappears with the presence of the radical scavenger in the reactive system, when odd electrons of nitrogen in DPPH molecule are paired. The reactive rate and the ability of the radical scavenger depend on the rate and the peak value of disappearance of the DPPH [31, 32]. 2 ml of DPPH radical solution (75 µM) and 2 ml solution of plant extract of various concentrations (20 µg/ml - 100 µg/ml) were prepared in methanol and ascorbic acid was used as standard. The reaction mixtures were shaken thoroughly and kept at dark for 30 min. The control solution was prepared by adding 2 ml of methanol with 2 ml of DPPH solution. The absorbance of all the reaction mixtures and control solution was measured at 517 nm. The % inhibition was calculated using the following formula:

% Inhibition = [(AC 517 nm- AS 517 nm/ AC 517 nm) x 100]

Where,

AC is absorbance of Control and AS is the absorbance of Sample.

The graph was plotted between % inhibition and different concentrations of plant extract and ascorbic acid and IC_{50} value was determined.

2.7. *In vitro* Enzymatic Estimation

2.7.1. Determination of Hyaluronidase Inhibitory Activity Assay

Hyaluronidase inhibitory activity was determined according to the method of Tu and Twata, 2015. A mixture of 25 µL of *Salvia officinalis* extract(20-100µg/ mL) were prepared in methanol and 3 µL hyaluronidase from bovine serum albumin [Sigma A4503] was pre-incubated for 10 min at 37°C and then added 12 µL phosphate buffer (300mM, pH 5.35) incubated for 10 min at 37°C. Afterward, 10 µL hyaluronic acid substrate [Sigma H5388, USA] was added and incubated for 45 min at 37°C. Decomposition reaction of hyaluronic acid was stopped by adding 100 µL acidic albumin acids. Mixed solution 25 µL incubated at room temperature for 10 min, and then absorbance was measured at 600 nm wavelengths. The absorbance in the absence of enzyme is used as a control group. Rutin was prepared in methanol used as a positive control group [33].

% Inhibition = [(AC 600 nm- AS 600 nm/ AC 600 nm) x 100]

Where,

AC is absorbance of Control and AS is the absorbance of sample *Salvia officinalis* extract.

2.7.2. Determination of Collagenase Inhibitory Activity

The method of Kim *et al*., 2004 with modifications was used to determine a Collagenase Inhibitory Activity. 25 µl Collagenase from *Clostridium histolyticum* was dissolved in the 25 µl 50 mM Tricine buffer (400 mM NaCl and 10 mM $CaCl_2$, pH 7.5). The synthetic substrate, FALGPA- (N-(3-[2-Furyl] acryloyl)-Leu-Gly-Pro-Ala) (50 µl 2mM) was dissolved in the Tricine buffer added to 2 mM. *Salvia officinalis* extract was incubated with the enzyme for 15 min before adding substrate to start the reaction. The final reaction mixture (75 µl total volume) contained 25 µl of 50 mM Tricine buffer, 25 µl of extract (20–100 µg/ml), and 25 µl of 0.1 units of enzyme Collagenase. Controls performed with 50 mM Tricine buffer as extract was dissolved in Tricine buffer (50 mM), while rutin was used as a positive control. After adding 50 µl of 2 mM FALGPA substrate, collagenase activity was measured immediately at 340 nm. Control was 50 mM tricine buffer as extract was dissolved in tricin buffer. Rutin was used as a positive control group [34].

% Inhibition = [(AC 340 nm- AS 340 nm/ AC 340 nm) x 100]

2.7.3. Determination of Elastase Inhibitory Activity

Elastase inhibitory activity was determined as per the procedure Thring *et al*., 2009. This assay was performed in 0.2 mM Tris-HCL buffer (pH 8.0). Porcine pancreatic elastase (PE - E.C. 3.4.21.36) was dissolved to make a 1 mg/ml stock solution in 0.2 mM Tris-HCL buffer. The substrate 0.8 mM N-Succinyl-Ala-Ala-Ala-p-nitro anilide (SANA) was dissolved in buffer. The *Salvia officinalis* extract (20-100µg/ml) dissolved in Tris-HCL and incubated with the enzyme for 20 min before adding substrate to begin the reaction. The final reaction mixture (Total 250 µl) contained 50 µl plant extract, 160 µl buffer, 20 µl enzymes, and 20 µl substrate. Rutin was used as a positive control. Negative con-

trols were performed using Tris-HCL buffer. Absorbance was measured immediately at 410 nm and then continuously for 20 min using a 96 well microplate reader [35].

% Inhibition = [(AC 410 nm- AS 410 nm/ AC 410 nm) x 100]

2.8. *In vivo* Assay

2.8.1. Selection of Animals

The study was carried out after obtaining the Institutional Animal Ethics Committee approval number PBRI/IAEC/PN-17037 as of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) from Pinnacle Biomedical Research Institute. Swiss albino mice were used in this experiment. Animals were housed in polyacrylic cages maintained under standard conditions of 18°C ± 2°C and 12 h light/dark cycle. Animals had free access to standard pellet diet and water, *ad libitum.*

2.8.2. Skin Irritation Study

Female Swiss albino mice, weighing 15-25 g, were used. 24h before the test (dose application), the hair on demarcated areas of approximately 4 cm^2 on the dorsal surface of each mouse was removed using a Rose Anne French hair- removing cream. The mice were observed for 48 h and those showing any abnormal hair growth or any reaction to the cream were excluded. Hair removing cream was preferred to shaving blade in order to minimize free radical production due to trauma from the blade.

2.8.3. UV Light Exposure Conditions and Development of Photo-Ageing [36, 37]

The mice were divided into 4 groups, each containg 6 animals. Group I served as control. Saline solution used as a vehicle. Group II animals received 5 min UV exposure twice a day and served as irradiated control. The test groups III received both UV radiations and 5% *Salvia officinalis* extract topically, simultaneously (extract was dissolved in distilled water). The group IV was treated as standard received both UV radiations and 1% Rutin [38]. The treatment was given 4 h prior to UV exposure as per the protocol. All the animals were kept inside a solar simulator (designed in the laboratory and fitted with UV lamp) at a distance of 40 cm from the UV light source (Ultra Vitalux 300 W Waton® bulb, Germany). The bulb gave the full spectrum of UV radiation, i.e., 260 - 400 nm, simulating the full solar spectrum. UV exposure was controlled by the time of exposure. Exposures were given twice daily for 5 min. For the mice receiving topical formulation treatment, the dorsal skin was treated with formulation 4 h prior to each UV radiation exposure. The standard rutin (1%) was administered topically at the dose of 30mg/kg body weight [39]. The extract (5%) was delivered as topically at a dose of 100 µl/cm2 area of the skin with a micropipette. The animals were treated for 30 days following which the skin was excised and used for biochemical estimation and histological study. Skin Slides of mice were obtained after termination of the experiment and fixed in 4% paraformaldehyde for 24 h. Then skin specimens were embedded in paraffin and sectioned. Hematoxylin and Eosin (H&E) staining was used to observe epidermal thickness. H&E staining was successively conducted by deparaffination, hydration, Hematoxylin staining, Eosin staining, and dehydration. Later, it was observed by microscopy. The wrinkle score was observed at the termination of the study [36, 37].

2.8.4. Scoring of Wrinkles Produced

Skin wrinkling in hairless mice was scored as follows: grade 0, no coarse wrinkles; grade 1, a few shallow coarse wrinkles; grade 2, some coarse wrinkles; grade 3, several deep coarse wrinkles. The scale ranged from 0 for normal animals to 3 for the heavily wrinkled skin.

2.9. Method for Wrinkle Measurement

After 30 days, wrinkles were produced on the dorsal surface of the mice skin. EXAFINE hydrophilic vinyl polysiloxane impression material (GC Corp. Tokyo, Japan) was used for the impression of wrinkles. We set the impression of wrinkles on the sample stand so that the measurement surface was horizontal and produced wrinkle shadows by illumination with light of a fixed intensity at 30°, using a fiber-optic light source (Nikkon). The shadow images were photographed with a still video camera (MS-C1100) and a digital image recorder (M SR1100, Minolta) with a macro 50 lens system and were input into an image analyzer (LA555 personal image analysis system, PIASS Co Ltd., Japan). We measured the shadow area for all shadows in one image, using the image analyzer and calculated the ratio of wrinkle area (%), defined as the ratio of the sum of the shadow area to the measured area. The wrinkle grading score and wrinkle area (%) were expressed as mean + standard deviation. Differences between means were checked for significance using Student's t-test.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening of *Salvia officinalis*

Phytochemical screening of the plants showed the presence of carbohydrates, proteins, glycosides, phenols, flavonoids, terpenoids, saponins, and alkaloids. The result of *Salvia officinalis* phytochemical screening can be seen in Table **2**.

Table 2. The result of qualitative phytochemical screening of *Salvia officinalis.*

Phytochemical Content	*Salvia officinalis*
Glycosides	+
Triterpenoids	+
Flavonoids	+
Phenols	+
Saponins	+
Alkaloids	+
Tannins	-

3.2. Quantitative Phytochemical Screening

3.2.1. Estimation of Total Flavonoids Content

To perform the calculations of total flavonoids content in the study, a standard curve is needed which is obtained from a series of different rutin concentrations.

y =0.001x+0.092; (R2 = 0,979).

All analyses were carried out aseptically in triplicate.

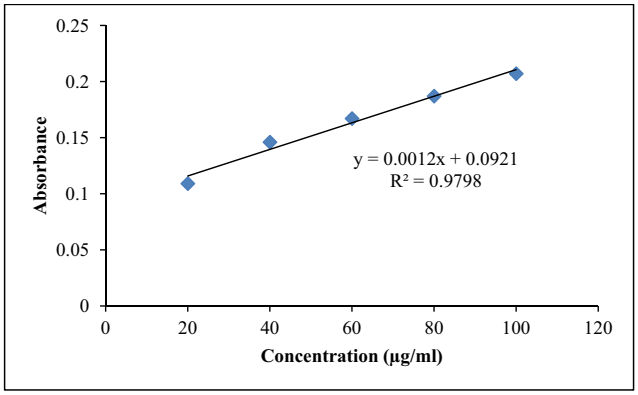
Standard curve of flavonoids has regression coefficient which was 0.979 with regression line y =0.001x+0.092 and the total amount of flavonoids present in *Salvia officinalis* as shown in Graph **1**, in 100 gms of *Salvia officinalis extract* contains 31.667 of flavonoids as shown in Tables **3** and **4**.

Table 3. Total flavonoids content in *Salvia officinalis.*

S. No.	*Salvia officinalis* (MeOH)
1	0.124
2	0.123
3	0.124
Mean	0.124
SD	0.001
TFC Value	31.667

Table 4. Results of the calibration curve.

S. No.	Concentration (µg/ml)	Absorbance
1	20	0.109
2	40	0.146
3	60	0.167
4	80	0.187
5	100	0.207

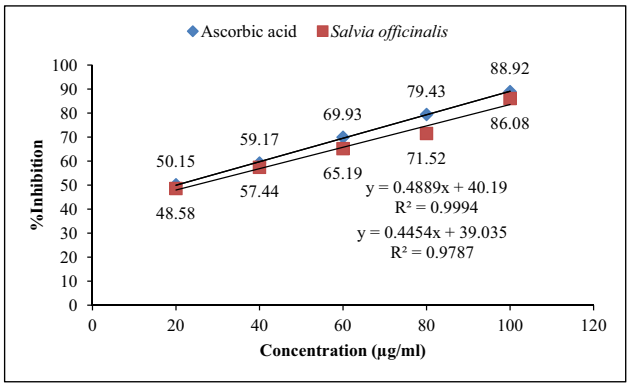


Graph 1. Total flavonoids content in *Salvia officinalis.* (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

3.3. DPPH (2, 2-Diphenyl-1-picrylhydrazil) Radical Scavenging Assay

The *In vitro* antioxidant and enzymatic activities of the *Salvia officinalis* was studied. Free radicals can damage the skin by altering the lipid contents in cellular membranes and by affecting the passage of nutrients and other active components in cell structure. The major protein content of healthy functional collagen and elastin fibers also g*et al*tered thus resulting in the formation of wrinkles, sagging and loss of skin tone. The enzymes produced by collagen are attacked by free radicals. Reactive oxygen species play a complex role in the inflammatory cascade [40].

Graph **2** shows the percent inhibition values for DPPH scavenging activity assay. Thus, the extract of *Salvia officinalis* exhibited antioxidant activity when compared to standard antioxidant ascorbic acid. The IC_{50} value of ascorbic acid for DPPH is 20.10 and extract of *Salvia officinalis* showed the higher IC_{50} 24.65. Lower the IC_{50}, stronger the antioxidant activity means the activity of plant is less but close to the standard ascorbic acid.



Graph 2. Percentage Inhibition of ascorbic acid and *Salvia officinalis* by DPPH assay. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 5. IC_{50} value DPPH scavenging activity of Ascorbic acid and *Salvia officinalis.*

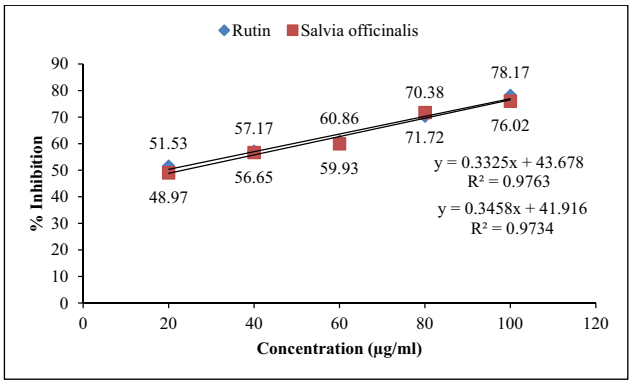
Concentration	Control	% Inhibition	
		Ascorbic Acid	*Salvia officinalis*
20 µg/ml	0.632	50.15	48.58
40 µg/ml	0.632	59.17	57.44
60 µg/ml	0.632	69.93	65.19
80 µg/ml	0.632	79.43	71.52
100 µg/ml	0.632	88.92	86.08
IC_{50}	-	20.10	24.65

3.4. Hyaluronidase Inhibitory Activity Assay

The polysaccharide hyaluronan (Hyaluronic Acid, HA) was isolated from bovine vitreous humor by Meyer and Palmer, 1934. Hyaluronidase assay based on the precipita-

tion of HA with cetylpyridinium chloride, which is used for high throughput screening for hyaluronidase inhibitors. This method can be used to evaluate the anti-aging activity of various herbal formulations [41].

Based on Graph **3** extract of *Salvia officinalis* showed the IC_{50} 23.44 which is almost near about the value observed in standard drug rutin with IC_{50}19.06 but less than the rutin. The anti-hyaluronidase activity of *Salvia officinalis* has not been reported to date.



Graph 3. Percentage inhibition of rutin and *Salvia officinalis* by hyaluronidase assay. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

3.5. Elastase Inhibitory Activity Assay

Elastin is a protein found in connective tissue which is responsible for the elasticity of the skin and lungs. This protein is catalyzed by the enzyme elastase. Degradation of elastin by intracellular elastase increases with age and/or repeated UV-radiation, leading to skin aging [42, 43].

The elastase inhibitory activity of *Salvia officinalis* extract and Rutin were measured and showed in Graph **4**. Elastase inhibitory activity of *Salvia officinalis* extract and Rutin showed the highest inhibition percentage at the highest concentration (61.49 and 62.20 respectively). However, Rutin showed the highest activity in elastase inhibition with IC_{50} value 25.11µg/mL. *Salvia officinalis* have IC_{50} value 35.05. The result showed that *Salvia officinalis* extract possess low elastase inhibition compared to Rutin (Tables **5** and **6**).

Table 6. IC_{50} value of elastase inhibitory activity of Rutin and *Salvia officinalis.*

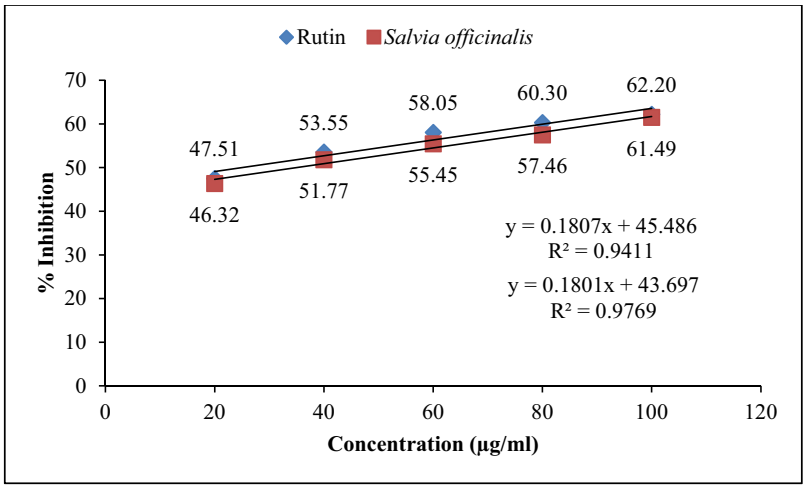
Concentration	Control	% Inhibition	
		Rutin	*Salvia officinalis*
20 µg/ml	0.844	47.51	46.32
40 µg/ml	0.844	53.55	51.77
60 µg/ml	0.844	58.05	55.45
80 µg/ml	0.844	60.30	57.46
100 µg/ml	0.844	62.20	61.49
IC_{50}	-	25.11	35.05

3.6. Collagenase Inhibitory Activity Assay

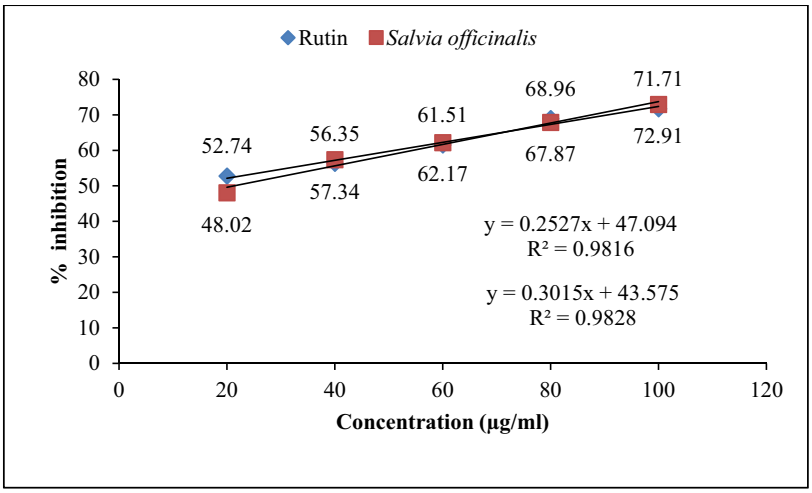
Collagen, the major component of the skin, is degraded by the enzyme collagenase Table **7**. Inhibition of collagenase activity delays the process of forming pre-collagen fibers and subsequently the wrinkling process [44].

Table 7. IC_{50} value of collagenase inhibitory activity of rutin and *Salvia officinalis.*

Concentration	Control	% Inhibition	
		Rutin	*Salvia officinalis*
20 µg/ml	0.912	52.74	48.02
40 µg/ml	0.912	56.35	57.34
60 µg/ml	0.912	61.51	62.17
80 µg/ml	0.912	68.96	67.87
100 µg/ml	0.912	71.71	72.91
IC_{50}		11.54	21.36



Graph 4. Percentage inhibition of rutin and *Salvia officinalis* by anti-elastase assay. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).



Graph 5. Percentage inhibition of rutin and *Salvia officinalis* by anti-collegenase assay. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 8. Estimation of wrinkle score.

Group	Treatment	Wrinkle Score
1	Control	0 ± 0
2	UV treated	2.83 ± 0.408
3	UV + Methanolic extract of *Salvia officinalis*	1.83 ± 0.753**
4	UV+ Rutin	0.83 ± 0.753**

Values are means ± SDs (n = 6), significantly different at ***p<0.01, **p<0.1, *p<0.5 in comparison to control calculated by Multiple Comparison Procedures (Bonferroni t-test). **Group 1**: Normal: control with pellet diet only; **Group 2**: UV irradiated + standard diet; **Group 3**: UV irradiated + standard diet + methanolic extract of *Salvia officinalis*, **Group 4**: UV irradiated + standard Rutin.

The *Salvia officinalis* extract possess Collagenase inhibitory activity Shown in Graph **5**. Collagenase activity of *Salvia officinalis* extract at 100 micro /ml is 72.91 and Rutin is 71.71. However, the IC_{50} value of *Salvia officinalis* extract was 21.36 and Rutin was 11.54. *Salvia officinalis* extract exhibits low antiaging antiwrinkle activity as compared to standard rutin Table **8**.

3.7. Skin Irritation Study

In skin irritation test, erythema and edema scores for a regular interval of time *i.e*. 0, 24, 48 and 72 h were observed. The plant extract showed no redness, edema, inflammation and irritation after application on the skin. Thus, it was found safe to use over the skin.

3.8. Inhibition of UV-induced Wrinkle Formation by Methanolic Extract of *Salvia officinalis*

The number of wrinkles was measured in the vehicle, UV irradiated and treated mice to determine whether treatment with 5% methanolic extract inhibit wrinkle formation induced by UV radiation or not. After UV radiation, deeper and wider wrinkles were formed and number of wrinkles were significantly (P<0.001) higher in the UV treated group than in the non-irradiation group. In particular, the more decrease was detected in the methanolic extract of *Salvia officinalis* treated group when compared with UV treated group. Therefore, the topical application of methanolic extract of *Salvia officinalis* can effectively inhibit wrinkle formation on the dorsal skin of an animal. The effectiveness of standard drug Rutin was highest in the inhibition of wrinkle formation.

3.9. Histopathological Study

The changes in the histological structures of mouse skins were observed in the normal, UV irradiated, standard treated and extract-treated groups. UV treated group showed thicker epidermis and dermis layer as compared to a normal control group. Adipose tissues were also found in higher level in the subcutaneous region as compared to the *Salvia offinalis* extract-treated group. From these results it is found that the topical application of methanolic extract of *Salvia officinalis* can induce a decrease in the thickness of epidermis and dermis, and the number of adipose tissues in skin.

3.10. Statistical Analysis

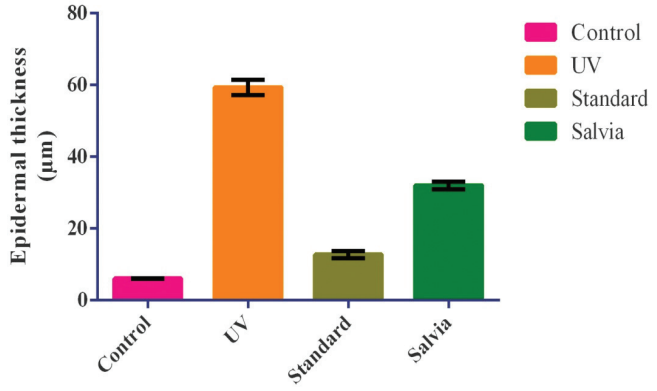
Values are expressed as Mean ± SEM, n=6, p≤0.05 analysed by Two-way ANOVA followed by Tukey's Post Hoc test for multiple comparison. Extract group was found to be significant when compared to control, UV treated and STD rutin group.

Epidermal thickness was measured in different cases as shown in Table **9**. In the control, the mean of epidermal thickness was equal to 6.028 ± 0.073, while epidermal thickness in UV exposure group indicated an increase and the mean was equal to 59.318 ± 2.148; this meant that chronic UV exposure led to an increase in the epidermal thickness 9.84 times more than normal epidermal thickness Fig. (**1**). In

Table 9. Epidermal thickness measurement for each individual in different groups.

-	Control	UV Treated	Standard	*Salvia officinalis*
Epidermal Thickness (µm)	5.82	54.62	11.6	28.24
	5.95	54.84	10.25	32.12
	6.34	58.88	9.64	34.61
	6.18	55.92	12.43	31.44
	6.00	69.54	16.37	29.17
	5.88	62.11	15.72	36.05
Mean ± SEM	6.028 ± 0.073	59.318 ± 2.148	12.668 ± 1.044	31.938 ± 1.126

the standard treatment group, epidermal thickness was reduced in comparison to the exposure group, its mean became 12.668 ± 1.044; and plant extract-treated group also effectively reduces the skin thickness and the mean of the group became 31.938 ± 1.126; it meant that *Salvia officinalis* 1.857 times reduced the epidermal thickness when compare to UV exposed group shown in Graph **6**.



Graph 6. Comparison of epidermal thickness (no. of sample n=6). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

CONCLUSION

This is the novel study in which we investigated the anti-aging and antiwrinkle effects of *Salvia officinalis*. From the study, an extract of *Salvia officinalis* showed potential anti-oxidant activity by DPPH radical scavenging assay.

The *in vitro* analysis of antiaging showed that *Salvia officinalis* extract effectively inhibited the activity of Col-I, Ela-I and Hla-I enzymes of skin as during aging process, the level of these enzymes increases which degrade the skin components. From histology of skin, it is clearly demonstrated that *Salvia officinalis* extract reduced the epidermal thickness and helped in restoring skin elasticity and thereby slowing the wrinkling process. As *Salvia officinalis* contains rich Phytoconstituents already reported in previous studies, thus supporting the *in vitro* antioxidant and enzyme inhibitory activity in the present analysis. The study on exact responsible active constituent in the aging process is under pipeline for future study of the mechanistic pathway. Commercially

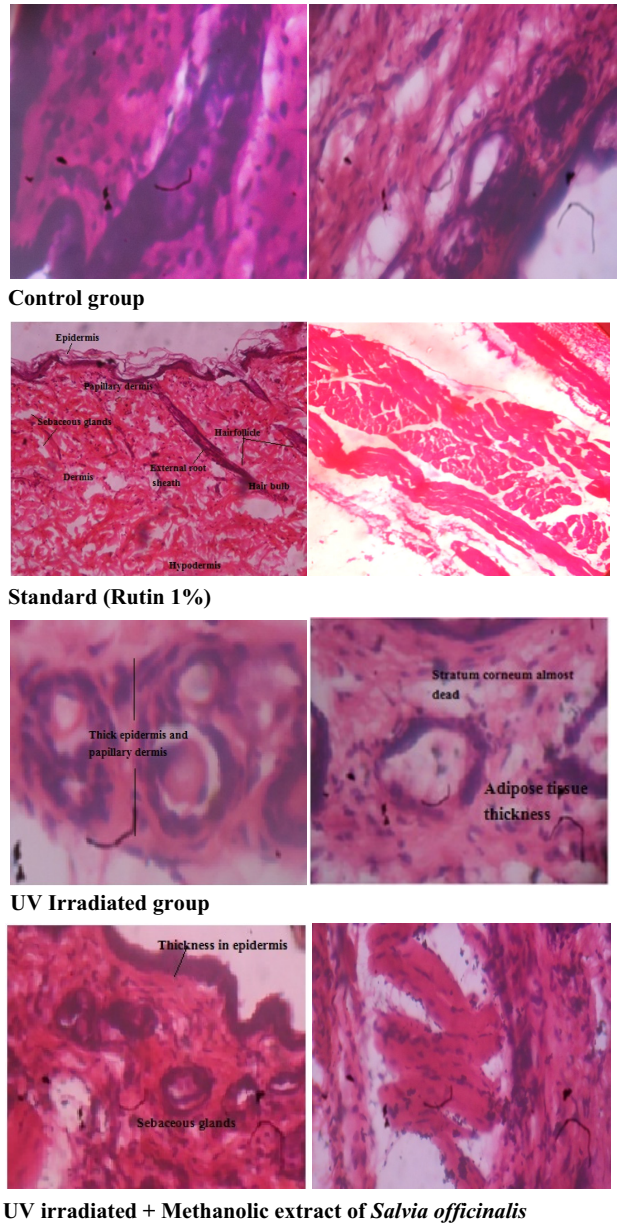


Fig. (1). Photomicrography of skin. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

available chemical-based sunscreens prepared by using titanium dioxide, zinc oxide, dioxybenzone, etc. thus may cause dermatitis and skin irritation by reacting with other molecules. Because Methanolic extract from *Salvia officinalis* effective in UV radiation produced aging means it minimizes the adverse effect of UV radiation, so we can say that the plant extract may serve as a protective sunscreen against UV radiation.

At the same time, extract of *Salvia officinalis* is less toxic as compared to dermal formulation of skin, commercially available. UV radiations damage the skin tone by damaging macromolecules such as proteins and lipids in skin. Therefore, it is the need of today's lifestyle to prepare such herbal preparations inclusive of polyphenols, flavonoids and antioxidants for better tolerability and greater efficacy.

LIST OF ABBREVIATIONS

UV	=	Ultraviolet Radiation
ECM	=	Extracellular Matrix
SC	=	Stratum Corneum
MeOH	=	Methanolic
DPPH-2	=	2-Diphenyl-1-picrylhydrazil
AC	=	Absorbance of Control
AS	=	Absorbance of Sample
Col-I	=	Collagenase Enzyme
Ela-I	=	Elastase Enzyme
Hla-I	=	Hyaluronidase Enzyme
FALGPA	=	(N-(3-[2-Furyl]acryloyl)-Leu-Gly-Pro-Ala)
SANA	=	N-Succinyl-Ala-Ala-Ala-p-nitro Anilide
GAGs	=	Glycos Amino Glycan
MMP	=	Matrix Metalloproteinase
So	=	*Salvia officinalis*
HA	=	Hyaluronic Acid
IC	=	Inhibition Concentration
H&E	=	Hematoxylin and Eosin
CPCSEA	=	Committee for the Purpose of Control and Supervision of Experiments on Animals

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was carried out after obtaining the Institutional Animal Ethics Committee (IAEC) approval for Animal Experimentation for that Approval number is PBRI/IAEC/PN-17037 which is approved by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

HUMAN AND ANIMAL RIGHTS

No humans were used. All expermients on animals were in accordance with Institutional Animal Ethics Committee (IAEC) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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