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Research article

Sage and Rosemary Extract Gel: Anti-Aging Efficacy in D-galactose-Induced Skin Aging Model

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Abstract: This study aimed to develop a topical herbal gel incorporating Salvia officinalis Linn and Rosmarinus officinalis L. extracts to evaluate its anti-aging effects in a mouse model of skin aging. *Salvia officinalis* and *Rosmarinus officinalis* are rich in polyphenols and antioxidants, protecting against oxidative stress and inflammation, common factors in premature aging. The study involved formulating and characterizing twelve herbal gel variants containing methanol extracts from these plants. These gels exhibited desirable qualities, such as stability and viscosity, and HF4, containing carbopol 934, displayed superior release properties. HF4 demonstrated excellent extrudability, 103±1.82% w/w of net content, and 97.65 ± 1.63% cumulative release at 5 hours. In a d-galactose-induced skin aging mouse model, HF4 exhibited significant anti-aging effects, with increased dermal and epidermal layer thickness, elevated glutathione levels, and reduced malondialdehyde levels compared to the untreated group. In conclusion, this study successfully created a topical herbal gel with the potential to combat skin aging by enhancing skin structure and reducing oxidative stress. These findings suggest the promising anti-aging properties of *Salvia officinalis* and *Rosmarinus officinalis* herbal gel formulations.

Keywords: Anti-aging; Antioxidant; Rosemary; Salvia; Oxidative stress; Galactose

1. Introduction

With age and economic growth, people's life expectancies are rising, making them more concerned with their health and skincare because having healthy skin will boost their confidence. Much research has been done on creating skincare products, particularly for inhibiting and delaying skin aging. The natural and predictable aging process affects all living things, including humans. The part of the human body most obviously impacted is the skin. Skin function and aesthetic changes caused by intrinsic and extrinsic factors accelerate skin aging. Age-related intrinsic aging happens naturally, whereas extrinsic aging is brought on by outside factors, like solar ultraviolet B (UVB) radiation. Two types of skin aging can be distinguished: photoaging, which causes premature aging, and chronological aging, which is caused by aging [1]. The effects of photoaging, caused by environmental or external factors, include changes in pigmentation, a leathery appearance, and cavernous furrows [2, 3]. Several intrinsic and extrinsic factors, as well as UVB radiation, all play a role in the complex and multifactorial photoaging process. The most harmful radiation

is UVB, a high-frequency radiation associated with photocarcinogenesis [4]. Erythema, sunburn, edema, hyperplasia, melanoma, carcinogenesis, and hyperplasia can also result from prolonged skin exposure to UVB rays [4, 5].

The skin starts to wrinkle naturally as we get older. The dermis, epidermis, and subcutaneous tissue are the three separate layers of skin. Fibroblasts, collagen, elastin, and other proteins comprise the extracellular matrix (ECM), the top layer of skin. The ECM provides a structural foundation for the skin's elasticity and expansion and is crucial for maintaining the body's physiological processes. Skin wrinkles result from UVB radiation-induced premature aging, also known as photoaging, which reduces collagen biosynthesis and directly degrades collagen in the extracellular matrix (ECM). A few enzymes involved in the skin-aging process, such as collagenase, hyaluronidase, and elastase, have been linked to increased activity due to the degradation of the ECM, which is directly related to skin aging [3, 6].

UV radiation absorption by the skin can increase the production of ROS and oxidative stress. One type of oxidative damage that can happen is gene and protein modification, which can alter the structure and function of proteins [7, 8]. DNA and mitochondrial damage are other potential consequences. Increased ROS levels can activate the enzymes hyaluronidase, collagenase, and elastase, which can speed up the aging process of skin tissue [9]. In radiation-induced oxidative stress, UVB radiation can produce reactive nitrogen species (RNS) and reactive oxygen species (ROS), which can oxidize DNA proteins and peroxide lipids, indirectly damaging biomolecules.

According to studies, the Lamiaceae plant species *Salvia officinalis* Linn and *Rosmarinus officinalis* L. have high antioxidant concentrations and may be helpful in photoprotection and anti-aging. In our earlier studies, the anti-aging and antioxidant properties of sage and rosemary were extracted and evaluated. These plants were chosen based on their conventional, historical, and traditional use in cosmetics and previous reports of their antioxidant activity in research studies or the researchers' lab, even though these results have not yet been published. It has been demonstrated that these two plants have the power to scavenge free radicals and shield cellular components like proteins, lipids, and DNA macromolecules. They have also been shown to have the ability to change a variety of signaling pathways, including those involved in cell growth, apoptosis, and inflammation. Despite these well-known biological properties of *Salvia officinalis* Linn and *Rosmarinus officinalis* L., there are currently no studies or reports on its application in topical formulations to prevent UVB radiation-induced oxidative damage [10-16].

Therefore, in this present study, a topical herbal gel was developed using leaves & flowers methanol extract of *Salvia officinalis* Linn (MES) and *Rosmarinus officinalis* L. (MER), and anti-aging properties of both MES and MER in a topical gel are not reported in animal/preclinical studies, these plants were appraised for anti-aging action to discover the systematic evidence for their usage in the management, treatment, and deferring of aging and associated problems. MES and MER were fabricated as a gel because it is simple to administer, have a localized effect, don't hurt or irritate when applied, don't have a first-pass effect, don't initiate gastro-intestinal metabolic degradation, and can be applied right to the affected zone. The leaves & flowers of MES and MER were used to create a topical gel formulation because, although all parts of the plants are used as medicines, leaves and flowers are primarily rich in phenolic and flavonoid compounds. As evident from a plethora of literature, a higher dietary intake of carbohydrates, especially glucose and galactose, has been linked to skin aging. In fact, through nonenzymatic glycation, the covalent attachment of sugar to a protein, and subsequent production of advanced glycation end products (AGEs), carbohydrates can harm the essential components of the skin. Therefore, to evaluate the anti-aging potential of the topical gel fabricated to contain MES and MER, a d-galactose-induced skin-aging animal model was utilized.

2. Materials and Methods

2.1 Drugs and chemicals

Carbopol 934, carbopol 940, and propylene glycol were purchased from Sigma-Aldrich (St. Louis, MO, USA. DTNB solution (Ellman's reagent), thiobarbituric acid, TCA (trichloroacetic acid), disodium edetate, and triethanolamine were purchased from Himedia Laboratories in Mumbai, India. All other chemicals and reagents used in the study were of reagent grade and were commercially available (SRL Mumbai, E. Merck India).

2.2 Authentication and preparation of extracts

Salvia officinalis and *Rosmarinus officinalis* were collected in the late spring from the Dehradun region. A botanist authenticated the collected flowers and leaves, and voucher specimens (AKG/SO/2022/11 and AKG/RO/2022/12) were preserved for future reference. The flowers and leaves were dried in the shade and cut into small pieces. The powdered flowers and leaves (2000 gm) underwent maceration using petroleum ether at room temperature for three cycles of 48 hours each to remove fats. The resulting extracts were air-dried and further subjected to methanol extraction. The methanol extracts were collected and concentrated under reduced pressure at 40 to 50°C. This process yielded dark greenish-brown concentrated methanol extracts of Salvia officinalis (MES) and Rosmarinus officinalis (MER) leaves and flowers. The final extracts were stored at 4°C until they were used.

2.3 Fabrication of the gel base

To avoid agglomeration, carbopol 934 was gradually dissolved with stirring for an hour in 60 mL of demineralized water. Disodium edetate and triethanolamine were dissolved in 10 mL of demineralized water and stirred for 10 minutes. An aliquot of 4.83 mL of propylene glycol was added to 12 mL of demineralized water and stirred for 10 minutes. After adding the disodium edetate and triethanolamine solutions, the pH of the Carbopol solution was raised to 7.4 by stirring the mixture for ten minutes. A clear and uniform gel base was then achieved by adding the propylene glycol solution and stirring for 10 minutes [17].

Table 1. The composition for the herbal gel formulations.

	Gel Formulation code											
Ingredients	**HF1**	**HF2**	**HF3**	**HF4**	**HF5**	**HF6**	**HF7**	**HF8**	**HF9**	**HF10**	**HF11**	**HF12**
MES (g)	0.5	1	1.5	2	2.5	3	0.5	1	1.5	2	2.5	3
MER (g)	0.5	1	1.5	2	2.5	3	0.5	1	1.5	2	2.5	3
Carbopol 934 (g)	1.5	1.5	1.5	1.5	1.5	1.5	---	---	----	----	----	---
Carbopol 940(g)	---	----	----	----	---	----	1.5	1.5	1.5	1.5	1.5	1.5
Triethanol amine (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Disodium EDTA (g)	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Propylene Glycol (g)	5	5	5	5	5	5	5	5	5	5	5	5
D.M. water (100 g)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

2.4 Preparation of gel formulation

Twelve topical gel formulations were prepared following the drug formulation manual [18]. For formulations HF1 to HF6, 1.5% of the carbopol 934 as gel base was used, while for formulations HF7 to HF12, 1.5% of carbopol 940 as gel base was utilized. MES and MER were separately weighed and slowly introduced into the Carbopol dispersion (the gel base), with continuous stirring to ensure thorough mixing and homogeneity. The specific composition details for each formulation can be found in Table I. Among the formulations, HF4 prepared using carbopol 934 exhibited superior quality characteristics and was selected for further evaluation of its anti-aging activity.

2.5 Characterization

2.5.1 Assessment of active constituents

The individual gel formulation was prepared by adding the required amount of methanol to 1 g of the formulation in a 50 mL volumetric flask. The flask was vigorously shaken to ensure the active ingredients in the methanol were dissolved. After that, Whatman filter paper was used to filter the resulting solution. The filtrate was carefully separated into an aliquot of 0.1 mL diluted with methanol to a final volume of 10 mL. The concentration of the active constituents in the solution was determined spectrophotometrically by utilizing a standard curve constructed at 280 nm, corresponding to the maximum absorbance (λmax) of the active constituents in the extracts [19].

2.5.2 Extrudability and measurement of the pH

The gel weighed approximately 20 g and was contained in a closed collapsible tube. A clamp was used to stop any rollback while applying firm pressure at the crimped end to extract the gel. After removing the tube's cap, the extruded gel was gathered and weighed. The weight of the extruded gel was then used to calculate the percentage of gel successfully extruded from the tube [20]. Using a digital pH meter, the gel's pH was determined. The pH meter's glass electrode was completely submerged in the gel system to ensure it was completely covered. The pH measurement was performed three times to obtain triplicate readings, and the average value of the three measurements was recorded [21].

2.5.3 Appearance, homogeneity and viscosity

The homogeneity and physical attributes of the prepared gels were evaluated visually. The viscosity of the gel was determined using a Brookfield viscometer. The measurement was conducted at 25°C, with the viscometer's spindle speed set at 12 rpm. This allowed the gel's viscosity to be determined [22].

2.5.4 Spreadability

Two glass slides of standard dimensions were utilized for this experiment. The herbal gel formulation was applied onto one of the slides, and the other slide was placed on top of the gel, creating a sandwich structure where the gel was enclosed between the two slides. The gel occupied a 7.3 cm area along the slides. A weight of 100 g was then placed on the upper slide to ensure uniform pressure, forming a thin layer of gel. The weight was subsequently removed, and any excess gel adhering to the slides was carefully removed. The two slides, now in position, were securely fixed to a stand without any disturbances. Only the upper slide was allowed to slide off freely due to the force exerted by the weight attached. A 20 g weight was carefully tied to the upper slide, and the time taken for the upper slide to travel the distance of 7.3 cm and separate from the lower slide under the influence of the weight was recorded. This experiment was repeated thrice, and the mean time was calculated for further analysis [23]. Spreadability was computed employing the succeeding formula: $S = m \times l/t$, and in the formula, S denoted spreadability, m denoted weight knotted to upper top slides (20 g), l denoted the length of the glass slide (7.3 cm), and t denotes the time taken in sec.

2.5.6 Diffusion study In vitro: permeation using rat skin

In vitro diffusion tests were carried out on all formulations using a Franz diffusion cell. The diffusion area was 3.9 cm2 in the diffusion cell apparatus, which had an open-ended cylindrical tube design and was made locally. Its area was 3.8983 cm^2, and its height was 100 mm. A phosphate buffer solution with a pH of 7.4 was used as the receptor media. As the dialysis membrane, rat abdominal skin (dorsal skin of the rat and 0.6 mm in thickness) was used. To ensure that the rat skin's stratum corneum side was in direct contact with the release surface of the formulation, it was carefully attached to the diffusion cell, specifically the donor cell. An isotonic phosphate buffer solution with a volume of 100 mL and a pH of 7.4 was added to the donor compartment before mounting the diffusion cell. The rat skin was applied with a weighed amount of the formulation that was 1 g of gel thick, slightly submerged in 100 mL of constantly stirring receptor medium. A constant temperature of 37±1 ^oC was maintained throughout the entire system. A 5 mL aliquot was taken at predetermined intervals up to 8 hours and spectrophotometrically estimated at 280 nm. The diffusion medium was replaced with an equal volume of fresh diffusion medium following each withdrawal. To evaluate the release of the active ingredients from the formulation, the cumulative percent release was calculated for each period (in hours) [23].

2.5.7 Release kinetics

The obtained data were fitted to various mathematical models to determine the release pattern of the active constituent from the herbal gel [24]. The concentration-dependent first-order kinetics model and the concentration-independent zero-order kinetics model were considered when analyzing the release behavior. Additionally, the possibility of drug release occurring through mechanisms such as swelling, erosion, or simple diffusion was taken into account [24]. Higuchi's model was employed to confirm the nature of the release reaction and validate the data.

2.5.8 Stability study

The main objective of stability testing or studies is to show how a drug product's quality changes over time, primarily due to temperature and humidity. A six-month stability study was carried out on a topical herbal gel formulation following the standards established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The selected formulation, consisting of 2% MES and MER, was subjected to stability testing in a dedicated stability chamber. The stability chamber used for the study was a floor-standing model with three units, each equipped with an individual humidity and temperature controller. The chamber dimensions were 300 x 300 x 300 mm, and the temperature and humidity conditions were set as follows: 40°C ± 2°C/75% RH ± 5% RH, 32°C ± 2°C/60% RH ± 5% RH, and 25°C ± 2°C/60% RH ± 5% RH. During the stability study, samples were withdrawn at specific intervals, including the initial time point, and at the first, second, third, and sixth months. These samples were then evaluated for any changes in homogeneity, color, pH, odor, viscosity, microbial load, and net content and subjected to a sterility test. These evaluations aimed to assess the stability and quality of the herbal gel formulation over the designated period [17].

2.6 Anti-aging activity

2.6.1 Evaluation of the anti-aging activity of gel formulations

The anti-aging properties of the formulation were assessed using a galactose-induced aging model in albino mice, as described previously [25]. The study protocols were approved by the Institutional Animal Ethics Committee (2210/PO/Re/S/23/CCSEA). Male Swiss albino mice, aged 6-8 weeks, weighing between 20g and 30g, were used for the experiments. The mice were housed under standard conditions with a 12-hour light-dark cycle and provided with pellet food and water ad libitum. Except for the control group, all the mice were administered galactose (100 mg/kg body weight subcutaneously) once daily for 42 days, as reported earlier [25]. The normal control group received a daily subcutaneous saline injection (1 mL/kg body weight) for 42 days. In addition to galactose administration, the test group was also treated with topical applications of formulations (HF4) on a 2 mm × 2 mm square area located on the lower abdomen region of the mice. This treatment regimen was followed for 42 days. On the 43rd day, the mice were euthanized, and their skin was excised for further analysis of biochemical parameters, including glutathione (GSH) and malondialdehyde (MDA) levels, as well as histopathological examinations.

2.6.2 Histopathological studies

The collected skin samples were preserved and fixed in a 10% v/v formalin solution in neutral phosphate buffer to facilitate histopathological analysis, following the standard procedures outlined previously [26]. The formalin-fixed skin tissues were then embedded in paraffin using conventional techniques. Subsequently, a microtome cut sections of approximately 5 μm thickness from the embedded tissues. These sections were stained with haematoxylin and eosin (H&E) for histopathological examination. Microphotographs of the stained skin specimens were captured using a digital camera mounted on an Olympus BX51 microscope with a 2x objective lens. The obtained microphotographs were used to analyze the histopathological results. The Image J program, an image analysis system, was employed to calculate the thickness of the epidermal and dermal layers in micrometers (μm) based on the captured images.

2.6.3 Evaluation of antioxidant parameters

2.6.3.1 Estimation of the content of glutathione (GSH)

The skin samples obtained from the treated animals were processed following the procedure outlined elsewhere [27, 28]. Glutathione (GSH) content was measured in nanomoles (nM). To prepare the stock standard solution, 0.0307 g of GSH was dissolved in a final volume of 100 mL of 0.2 M EDTA solution, resulting in a concentration of 0.001 M. After the final treatment, all the animals in the different groups were euthanized 1 hour later. The freshly collected skin samples were weighed (Precisely weighed out around 0.5 to 1 gram of the excised skin tissue) and homogenized using a mixer with 1 mL of 0.2 M EDTA for 10 minutes. The homogenate was then centrifuged, and 0.5 mL of the supernatant was mixed with 2 mL of tris buffer. To each tube, 50 μL of DTNB solution (Ellman's reagent) was added and thoroughly mixed using a vortex mixer. The

absorbance of the samples was read at 405 nm within 2-3 minutes after the addition of DTNB. The results were expressed as nanomoles per milliliter (nmol/mL).

2.6.3.2 Estimation of the content of malondialdehyde (MDA)

For the determination of malondialdehyde (MDA), the lipid peroxidation method was employed [28, 29]. The experiment utilized an analytical kit from Himedia Laboratories in Mumbai, India. Briefly, fresh skin samples were weighed, homogenized, and centrifuged. The resulting supernatant (0.5 mL) was mixed with 2 mL of a reagent consisting of 0.5% TBA (thiobarbituric acid), 20% TCA (trichloroacetic acid), and 0.25 M HCl in a ratio of 1:1:1 (v/v). The mixture was then heated and cooled in a water bath for 15 minutes. The absorbance of the solution was measured at 532 nm using a spectrophotometer. The results were expressed as micromoles per milliliter (μmol/mL).

2.7 Statistical analysis

Data analysis was performed using the GraphPad Prism Software package. The results are presented as mean ± SD (standard deviation). A one-way analysis of variance (ANOVA) was conducted to compare multiple groups, followed by Dunnett's test as *post hoc* to determine the statistical significance. A *p*-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

Among various topical semisolid preparations, gel formulations are generally preferred for several reasons. They offer numerous advantages such as prolonged residence time on the skin, high viscosity, moisturizing effects for flaky skin due to their occlusive properties, enhanced bioadhesives, reduced irritation, independence from the water solubility of active ingredients, ease of application, and improved release characteristics [30, 31]. Numerous studies have highlighted the anti-inflammatory, antioxidant, protective, and anti-aging activities of flavonoids and phenolic compounds found in herbs. Moreover, these polyphenolic flavonoids, including rosmarinic acid and apigenin, have demonstrated the ability to penetrate the human skin [32-35]. Based on these findings, a topical herbal gel formulation was developed to incorporate these flavonoid and phenolic compound extracts to prevent and treat skin aging and associated conditions.

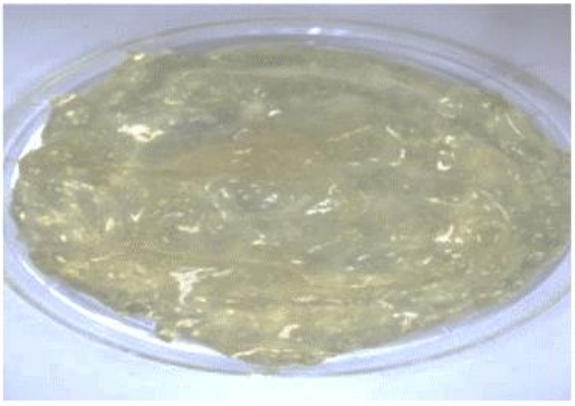

Figure 1. The gel formulation

3.1 Fabrication and evaluation of topical herbal gel

Diverse concentrations (0.5 to 3% w/w) of the extract (MES and MER) were used to fabricate a total of twelve gel formulations (HF1 to HF12). The gels were formulated with either 1.5% Carbopol 934 or 1.5% Carbopol 940 polymer. These polymers were chosen as gelling agents due to their desirable properties, such as biodegradability, bioadhesives, biocompatibility, lack of irritation, and inability to be absorbed into the body. Previous studies reported that Carbopol 934 exhibits superior gelling properties compared to Carbopol 940 (Blanco-Fuente et al., 1996), which aligns with our findings. In the gel formulation, the carbopol 934 polymer demonstrated promising potential as a carrier for the controlled release of active phytoconstituents.

The polymer's concentration was optimized by creating gels with various concentrations ranging from 0.5% to 2.5%. This optimization process determined that a gel formulation containing 1.5% of either Carbopol 934 or Carbopol 940 fulfilled the requirements for gel formulations and demonstrated compatibility with the desired characteristics.

According to the outcomes of the quality control tests, it was clear that Carbopol 934 (HF1 to HF6), the gelling agent used to create the gel formulations, exhibited superior qualities to Carbopol 940 (HF7 to HF12), except for the Spreadability parameters, where Carbopol 940 performed better. Therefore, the six herbal topical gel formulations (HF1 to HF6) made with Carbopol 934 were the only ones used in the in vitro diffusion studies. Furthermore, studies on in vitro release and stability were conducted specifically for the best herbal gel formulation, HF4. Studies have suggested that dimethylsulfoxide (DMSO) and propylene glycol are effective permeation enhancers in permeation enhancement [36]. However, due to reports of skin erosion caused by DMSO, propylene glycol was chosen as the permeation enhancer in preparing the gel formulation [37, 38]. Disodium edetate and triethanolamine were included in the formulation to adjust the pH. Overall, the selection of Carbopol 934 as the gelling agent, the use of propylene glycol as the permeation enhancer, and the inclusion of triethanolamine and disodium edetate to adjust pH contributed to the development of a superior herbal gel formulation with desirable characteristics for further in vitro study for release profile and stability.

3.2 Characterisation of the formulated topical gel

Using carbopol polymers, twelve gel formulations designated as HF1 to HF12 were fabricated, and their physical characteristics were evaluated. The evaluations included assessments of viscosity, pH, physical appearance, spreadability, extrudability, in vitro diffusion profile, and net content. The outcomes obtained from these evaluations were compared against the acceptable limits specified in the ICH guidelines, and the detailed findings are presented in Table 2. The prepared gel formulations exhibited homogeneity and a favorable appearance, demonstrating good consistency. Since the pH range for all of the formulations was between 7.33 and 7.69, they were unlikely to irritate the skin, a conclusion further supported by the skin irritation study conducted. The inclusion of polymers in the designed topical formulations was aimed to ensure the prompt release of the drug and maintain the drug concentration within the therapeutically effective range. No discernible difference in viscosity was seen by maintaining the polymer concentration at 1.5% in all gel formulations. Notably, a viscosity value between 0.38 and 0.39 poise has been reported as ideal for topical gel formulations developed using carbopol polymers [39]. The Spreadibility values obtained for the gel formulations demonstrated their ease of Spreadibility. More than 90% of the contents of the HF1 to HF6 gel formulations could be extruded, indicating excellent extrudability. However, in the case of HF1 and HF3, 80% of the contents were extrudable, which still falls within the excellent range (>80% extrudability: good). Overall, the gel formulations exhibited favorable extrudability characteristics, with the majority surpassing the 80% extrudability threshold.

3.3 In vitro diffusion profile and release kinetics

Table 3 presents the in vitro diffusion profiles of formulations HF1 to HF6. To carry out the gel formulations' in vitro release studies, a phosphate buffer saline solution with a pH of 7.4 was selected as the medium, considering that the pH range of the membrane used was 5 to 7.8. Notably, the drug release *in vitro* for all 6 formulations formulated with carbopol 934 demonstrated an impressive release performance, with nearly 100% of the formulation contents being released within 5 hours.

Table 2. Characterizations for the topical gel formulations fabricated with Carbopol 934 at 1.5% concentration.

Code	Conc (%)	pH*	Viscosity* (poise)	Spreadability* g cm/sec	Net content* % w/w	Extrudability*	Physical appearance
HF1	0.5	7.48 ± 0.98	0.3841 ± 0.0011	33.08 ± 1.30	99.82 ± 1.96	Good	Homogenous, translucent and dark-greenish
HF 2	1.0	7.48 ± 0.97	0.3853 ± 0.0022	46.14 ± 1.11	104.54 ± 2.79	Excellent	Homogenous, translucent and dark-greenish
HF 3	1.5	7.68 ± 0.94	0.3863 ± 0.0021	57.48 ± 1.33	104.23 ± 1.91	Good	Homogenous, translucent and dark-greenish
HF 4	2.0	7.51 ± 0.91	0.3874 ± 0.0022	65.12 ± 1.08	104 ± 1.992	Excellent	Homogenous, translucent and dark-greenish
HF 5	2.5	7.69 ± 0.98	0.3882 ± 0.0013	72.47 ± 1.42	103 ± 1.82	Excellent	Homogenous, translucent and dark-greenish
HF 6	3.0	7.33 ± 0.99	0.3902 ± 0.0020	76.83 ± 1.21	102 ± 1.92	Excellent	Homogenous, translucent and dark-greenish

Data are preseneted as Mean ± SD for n =3.

Table 3. *In vitro* diffusion profile of HF4

TIME (Hr)	Gel Formulations					
	% % Cumulative Release					
	HF1	**HF2**	**HF3**	**HF4**	**HF5**	**HF6**
1	7.45 ± 0.98	16.91 ± 0.99	9.63 ± 0.99	16.59 ± 1.15	10.46 ± 0.98	12.87 ± 1.08
2	15.52 ± 0.99	33.38 ± 1.08	23.84 ± 0.99	32.57 ± 1.27	25.63 ± 1.11	26.79 ± 1.32
3	31.61 ± 1.03	68.77 ± 1.29	46.82 ± 1.82	51.77 ± 1.42	49.68 ± 1.49	54.36 ± 1.77
4	40.12 ± 1.01	88.75 ± 1.14	62.54 ± 1.56	68.63 ± 1.57	66.94 ± 1.39	64.44 ± 1.59
5	64.91 ± 1.24	78.69 ± 1.52	77.79 ± 1.87	97.65 ± 1.63	82.91 ± 1.59	81.28 ± 1.85

Table 4. Kinetic modeling of *In vitro* release data of HF4

Code for Formulations	Zero-order	First Order	Higuchi diffusion model	Best fitted model
	Qt=Q0+k0t	lnQt=lnQ0+k1t	Qt=kHt	
	R^2	R^2	R^2	
HF1	0.918 ± 0.001	0.834 ± 0.001	0.639 ± 0.000	Zero-order
HF2	0.843 ± 0.001	0.834 ± 0.001	0.737 ± 0.001	Zero-order
HF3	0.973 ± 0.002	0.876 ± 0.001	0.721 ± 0.000	Zero-order
HF4	0.978 ± 0.002	0.827 ± 0.002	0.733 ± 0.001	Zero-order
HF5	0.975 ± 0.001	0.864 ± 0.002	0.723 ± 0.000	Zero-order
HF6	0.976 ± 0.001	0.889 ± 0.001	0.754 ± 0.001	Zero-order

Note: Qt = amount of drug released at time t, Q0 = initial amount of drug in the solution, k0 = zero-order release constant, k1 = first-order release constant and kH = Higuchi dissolution constant

The drug release *in vitro* of the fabricated topical gel exhibited promising characteristics comparable to the commercially available gel. Among the different formulations (HF1 to HF6), HF4 demonstrated superior release performance (97.65±1.63%) compared to HF1, HF2, HF3, HF5, and HF6 (as depicted table 3). According to our kinetic release study results, the HF4 formulation had zero order release kinetics, which is good for prolonged release. Therefore, the gel formulation with 2% of each MES and MER was chosen for additional in vivo research.

In contrast to the commercial gel formulation, which released roughly 90% of its content in 3 hours, the HF4 formulation with 2% of MES and MER showed a prolonged release of active ingredients for up to 5 hours (almost 100% release). Because of its prolonged release profile, the HF4 formulation can be used for sustained release, improving patient compliance. The gel formulation containing 2% of each MES and MER followed zero-order release kinetics, according to the release data obtained using various mathematical models (Table 4). For subsequent *in vivo* studies, the HF4 gel formulation was chosen.

3.4 Stability study

To ensure consistent quality, safety, and efficacy over its shelf life, a stability study was conducted per the guidelines established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The HF4 formulation prepared using carbopol 934 demonstrated superior quality characteristics and was subjected to the stability study. Throughout the stability testing period of 0, 1, 2, 3, and 6 months, the topical herbal gel formulation exhibited no noticeable changes in color, odor, homogeneity, pH, viscosity, or net content. These parameters are crucial indicators of the formulation's

stability. Based on the results obtained from the study (refer to Table 5), it is evident that the formulated topical gel HF4 remains stable over time.

3.5 Anti-Aging activity

3.5.1 Appraisal of anti-aging activity: Histopathological assessment

The subcutaneous administration of galactose caused the aging of the skin for 42 days at a dose of 100 mg/kg body weight (bwsc). After 42 days, the disease control group saw a significant reduction ($p < 0.01$) in the thickness of the epidermal and dermal layers. The thickness of the epidermal layer decreased from 52.55 μm to 40.16 μm. The thickness of the dermal layer decreased from 656.9 μm to 587.0 μm when compared to the control group (Group 1) (Fig. 2). In Treatment Group 3, galactose was administered at a dosage of 100 mg/kg bwsc for 42 days, along with the topical application of the gel. The topical application of the gel formulations showed a significant increase ($p < 0.05$) in the thickness of the epidermal layer and a significant increase ($p < 0.05$) in the thickness of the dermal layer compared to the disease control group (Group 2) (Fig. 3). The epidermal thickness increased from 40.16 μm (in the disease control group) to 41.64 μm due to the topical application of the gel formulation. When the gel formulation was applied topically, the epidermal thickness increased from 40.16 μm (in the disease control group) to 41.64 μm. Due to the topical gel formulation, the dermal thickness increased from 587.0 μm (in the disease control group) to 862 μm.

Table 5. Stability study data of HF4

Storage condition 1						
S. N.	**Evaluation Parameters**	**Topical herbal gel formulation (HF4) (2% w/v of each MES and MER)**				
		25 °C ± 2 °C/60% RH ± 5% RH				
		Months				
		0	1	2	3	6
1	Color	No colour change				
2	Odor	No Odour change				
3	Homogeneity	Homogeneous				
4	pH	7.35 ± 0.98	7.33 ± 0.97	7.29 ± 0.98	7.28 ± 0.96	7.25 ± 0.921
5	Viscosity (poise)	0.384 ± 0.0020	0.384 ± 0.0021	0.382 ± 0.0022	0.374 ± 0.0011	0.372 ± 0.0020
6	Net content (%)	99.78 ± 1.91	99.84 ± 1.94	99.14 ± 1.69	98.82 ± 1.89	97.91 ± 1.98
7	Microbial load (Bacteria & Fungi)	Observed no microbial growth Time points: 24 h, 48 h and 72 h				
8	Sterility test	Observed no microbial growth Time points: 24 h, 48 h and 72 h				

Table 5. Stability study data of FH4

Storage condition 2						
				32 °C ± 2 °C/60% RH ± 5% RH		
				Months		
		0	1	2	3	6
1	Color			No colour change		
2	Odor			No Odour change		
3	Homogeneity			Homogeneous		
4	pH	7.35 ± 0.98	7.33 ± 0.97	7.34 ± 0.98	7.28 ± 0.96	7.25 ± 0.921
5	Viscosity (poise)	0.384 ± 0.0020	0.384 ± 0.0021	0.383 ± 0.0021	0.374 ± 0.0011	0.372 ± 0.0020
6	Net content (%)	99.78 ± 1.91	99.84 ± 1.94	99.36 ± 1.99	98.82 ± 1.89	97.91 ± 1.98
7	Microbial load (Bacteria & Fungi)			Observed no microbial growth Time points: 24 h, 48 h and 72 h		
8	Sterility test			Observed no microbial growth Time points: 24 h, 48 h and 72 h		
Storage condition 3						
				40 °C ± 2 °C/75% RH ± 5% RH		
				Months		
		0	1	2	3	6
1	Color			No colour change		
2	Odor			No Odour change		
3	Homogeneity			Homogeneous		
4	pH	7.33 ± 0.98	7.33 ± 0.98	7.33 ± 0.98	7.33 ± 0.98	7.33 ± 0.98
5	Viscosity (poise)	0.386 ± 0.0012	0.386 ± 0.0012	0.386 ± 0.0012	0.386 ± 0.0012	0.386 ± 0.0012
6	Net content (%)	99.89 ± 1.67	99.89 ± 1.67	99.89 ± 1.67	99.89 ± 1.67	99.89 ± 1.67
7	Microbial load (Bacteria & Fungi)			Observed no microbial growth Time points: 24 h, 48 h and 72 h		
8	Sterility test			Observed no microbial growth Time points: 24 h, 48 h and 72 h		

3.5.2 Evaluation of antioxidant parameters

In the disease control group, there was a significant reduction ($p < 0.05$) in the levels of glutathione (GSH) in the skin, measuring 88.72 µmol/mL, compared to the control group (Group 1) with levels of 114.67 µmol/mL. Nevertheless, topical application of the HF4 gel during treatment restored the skin's GSH levels. The GSH levels increased from 88.72 µmol/mL (in the disease control group) to 94.76 µmol/mL in Group 3 (Fig. 5). The induction of oxidative stress in the skin tissues as shown by an increase in malondialdehyde (MDA) levels occurred as a result of the administration of galactose at a dosage of 100 mg/kg bwsc. MDA levels rose to 0.088 mol/mL in the diseased group as opposed to 0.052 mol/mL in the control group. However, the levels of MDA were reduced after treatment with the gel formulations. The MDA levels decreased from 0.088 µmol/mL (in the disease control group) to 0.074 µmol/mL in Group 3 (Fig. 6).

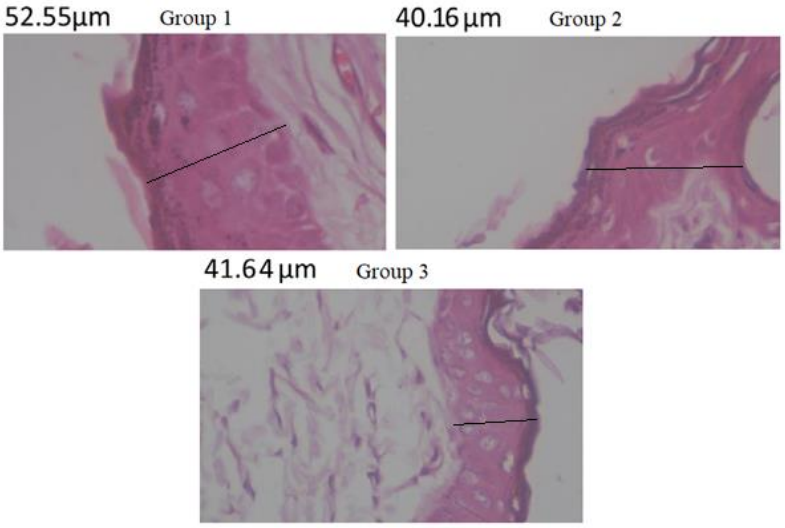

Figure 2. Effect of topical application of gel formulation containing MES and MER on galactose (100 mg/kg bwsc.), induced aging in mice skin epidermal layer thickness Group 1 served as normal control and received saline solution, Group 2 served as positive (disease) control received galactose, group 3 served as test group received formulation (HF4).

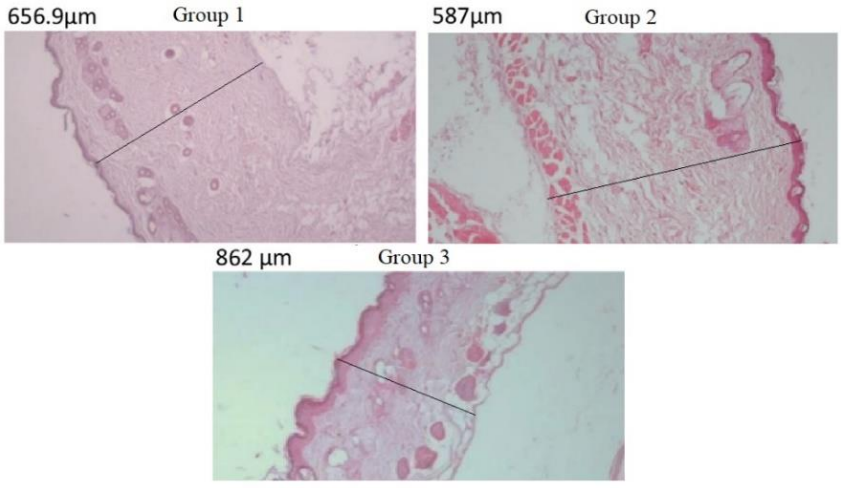

Figure 3. Effect of topical application of gel formulation containing MES and MER on galactose (100 mg/kg bwsc.), induced aging in mice skin dermal layer thickness Group 1 served as normal control and received saline solution, Group 2 served as positive (disease) control received galactose, group 3 served as test group received formulation (HF4).

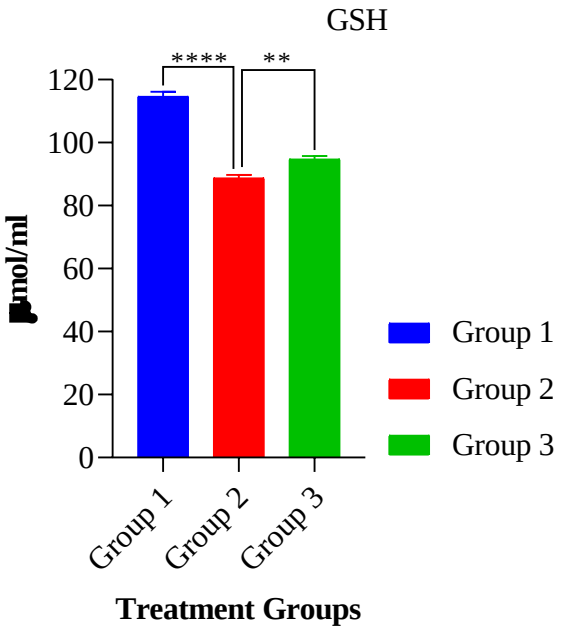

Figure 4. Effect of topical application of formulation (HF4) containing MES and MER on GSH levels in galactose-induced aging in mice skin: Group 1 served as normal control and received saline solution, Group 2 served as positive (disease) control received galactose, group 3 served as test group received formulation (HF4). Data are expressed as the mean ± SD for each group of mice (n = 6). **** $P < 0.05$ compared to normal control (Group 1) and ** $P < 0.05$, Comparison with Group 2 (Disease control).

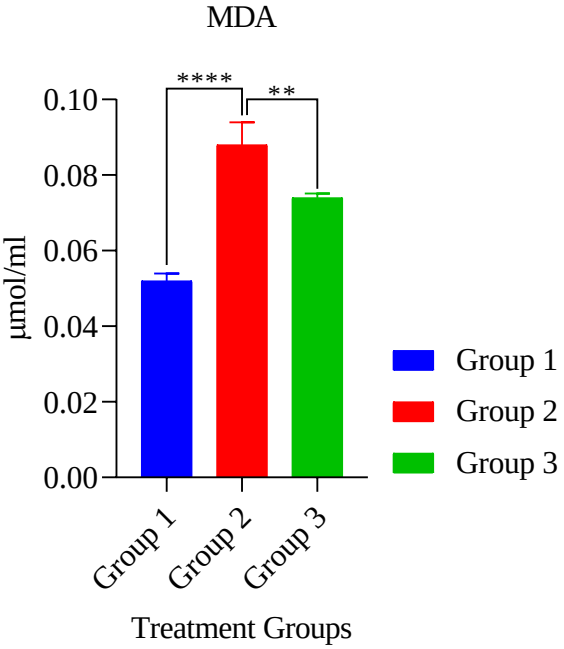

Figure 5. Effect of topical application of formulation (HF4) containing MES and MER on MDA levels in d-galactose induced aging in mice skin, Group 1 served as normal control and received saline solution, Group 2 served as positive (disease) control received galactose, group 3 served as test group received formulation (HF4). Data are expressed as the mean ± SD for each group of mice (n = 6). **** $P < 0.05$ compared to normal control (Group 1) and ** $P < 0.05$, Comparison with Group 2 (Disease control).

3.6 Summary

The findings of the study suggested that the herbal gel formulation code-named HF4, which is composed of 2% of methanol extracts from *Salvia officinalis* Linn. (MES) and *Rosmarinus officinalis* Linn. (MER) each, demonstrated a significant slowing down of skin aging by reducing oxidative stress in the skin. The anti-aging action of the fabricated topical gel might be attributed to the presence of several phytochemical compounds, including carnosic acid and rosmarinic acid, found in the methanol extracts of the leaves and flowers *of Salvia officinalis* and *Rosmarinus officinalis*. The developed formulation HF4 is demonstrated as a potential topical herbal gel for the treatment and prophylactic prevention of skin aging, capable of significantly reversing skin aging. The formulation contained 2% MES and MER, fabricated with 1.5% Carbopol polymers. Rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) are well known for their anti-aging properties due to their rich content of polyphenols and antioxidants. These compounds help reduce oxidative stress and enhance skin elasticity, thereby reducing signs of aging. Studies have shown that extracts from these herbs, viz. Rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) can increase collagen production and reduce wrinkle depth [40-42]. Additionally, their anti-inflammatory effects contribute to overall skin health and vitality. The application of rosemary and sage in skincare formulations is supported by their ability to protect against UV-induced damage and improve skin hydration, making them valuable in anti-aging treatments [40-42]. Additional research in clinical settings can support the use of this formulation in patients with skin aging and related problems, potentially validating its efficacy and safety for broader dermatological applications.

4. Conclusions

The study's findings suggest that the herbal gel formulation code HF4, which contains 2% of MES and MER each, significantly slowed skin aging by reducing oxidative stress in the skin. Anti-aging action of the fabricated topical gel might be owing to the occurrence of several phytochemical compounds, including carnosic acid, rosmarinic acid, etc, in methanol extracts of the leaves and flowers of *Salvia officinalis* Linn. (MES) and *Rosmarinus officinalis* Linn. (MER). The developed formulation HF4 was demonstrated as a potential topical gel (herbal) for the treatment and prophylactic prevention of skin aging. It contained 2% MES and MER fabricated with 1.5% carbopol 934. Additional research in the clinical setting can support using this formulation in patients with skin aging and related problems.

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