ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR | Vol.15 | Issue 1 | Jan - Mar -2026

www.ijpar.com

DOI: https://doi.org/10.61096/ijpar.v15.iss1.2026.527-540

Development of Dermal Cream for the Treatment of Vitiligo and Study Their Efficiency by in-Vitro Assay Methods

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Website: https://www.keitrichy.com

Published by:

04.04.2026

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Abstract: This study aimed to develop and evaluate a Nigella sativa (NS) dermal cream for its anti-inflammatory potential and safety in topical applications. NS seeds were collected, ground, and subjected to hydrothermal extraction. The filtered extract was concentrated at 100 °C and used to formulate a sterile dermal cream, which was stored under refrigeration for further analysis. The anti-inflammatory activity was assessed using the albumin denaturation assay, showing a clear concentration-dependent effect, with the highest inhibition of 68% at 500 µg/ml and an IC50 value of 71.02 µg/ml. Protease inhibition assays further demonstrated moderate inhibitory activity (IC50 = 90.38 µg/ml), indicating potential to protect skin proteins and tissues from inflammatory damage. The cream exhibited a pH of 6, within the ideal range for dermal formulations, and no irritation or adverse effects were observed during skin testing. Solubility studies revealed partial solubility in ethanol and chloroform, providing insights for formulation optimization. Collectively, these findings suggest that NS dermal cream is a safe, effective, and biocompatible natural formulation with anti-inflammatory and protease-inhibitory properties. This cream holds promise as a complementary therapeutic approach for inflammatory skin conditions, including vitiligo, and warrants further clinical evaluation to explore its efficacy, dosing, and potential synergy with conventional treatments.

Keywords: Nigella sativa, dermal cream, Anti-inflammatory activity, Protease inhibition, Topical application

1. INTRODUCTION

Vitiligo is an autoimmune disorder in which the immune system targets and destroys melanocytes, the pigment-producing cells of the skin [1]. The destruction of these cells leads to the development of depigmented patches on the skin. Initially, vitiligo appears as small white macules, which gradually enlarges over time. This condition can affect individuals of all skin types and may involve any part of the body, including the scalp. Since vitiligo is a chronic disease, achieving repigmentation becomes difficult once melanocytes are lost. This disorder affects approximately 0.5–2% of the global population and is more noticeable in individuals with darker skin tones [2][3]. Current treatment options for vitiligo include topical and oral immuno-modulatory agents, phototherapy, and recently introduced topical JAK inhibitors [2]. However, due to the variable severity of the disease and inconsistent treatment outcomes, both patients and clinicians are increasingly interested in

adjuvant and alternative treatment approaches [5]. It is a chronic, acquired Depigmentary disorder of the skin characterized by the appearance of well-defined white macules and patches resulting from the selective loss of melanocytes, the pigment-producing cells of the skin. The condition can affect any part of the body and may also involve hair, leading to premature whitening (leukotrichia), as well as mucous membranes such as the oral cavity[4] . Under normal physiological conditions, skin, hair, and eye color are determined by melanin, a pigment synthesized by melanocytes located in the basal layer of the epidermis. In vitiligo, the destruction or functional impairment of these melanocytes leads to the absence of melanin in affected areas, giving rise to depigmented lesions.

Vitiligo can affect people of any age, gender, or background. It is often more noticeable in individuals with darker skin because of the contrast between normal and depigmented areas. Globally, about 0.5–2% of people have vitiligo, with an average of around 1%. In some countries like India, Egypt, and Japan, the rate is slightly higher (about 1.25% to 6%).[6][7][8]

The condition affects males and females equally and can start at any age, though nearly half of cases begin before age 20. The most common type is non-segmental (generalized) vitiligo, which appears as symmetrical white patches that spread over time. Other types include segmental vitiligo, which follows a specific skin pattern, and localized forms that affect smaller areas. Vitiligo can strongly affect mental and social well-being [10][11]. People with vitiligo may feel anxious, depressed, or socially withdrawn, especially if it starts at a young age, impacting education, work, and relationships.

Treating vitiligo is challenging. Common options like topical steroids, phototherapy, and skin grafts can be costly, slow, and sometimes ineffective, with relapses common. Recent research shows that oxidative stress, auto-immunity, and inflammation play key roles in the disease [13]. This has led to interest in natural treatments, such as medicinal plants, which may be safer and provide antioxidant, immune-supporting, and skin-protective benefits.

Among various medicinal plants, Nigella sativa, also called black seed or black cumin, has been used for centuries in traditional medicine systems like Unani, Ayurveda, and Siddha. Its seeds contain active compounds, especially thymoquinone, which have strong antioxidant, anti-inflammatory, and immune-supporting effects. These qualities make Nigella sativa a promising option for Vitiligo

2. VITILIGO

Vitiligo is a complex and multifactorial skin disorder with a polygenic genetic background, and its exact cause is still not fully understood [13]. It affects a considerable portion of the world population, with an estimated prevalence ranging from 0.5% to 2% [14][15]. The disease commonly begins during childhood or early adulthood, and the highest incidence is observed between 10 and 30 years of age [16]. Vitiligo occurs in people of all ethnic groups and affects both males and females, but several studies report a slightly higher prevalence among females [16].

Despite extensive research, the exact mechanism responsible for the selective loss of melanocytes in vitiligo remains unclear. Under normal conditions, melanocytes and keratino-cytes function together as a structural and functional unit in the epidermis. Direct inter-action between these cells supports melanocyte survival, growth, and melanin production. In addition, keratinocytes release various growth factors that regulate melanocyte proliferation and differentiation. In vitiligo-affected skin, increased apoptosis of keratinocytes has been reported. This leads to a reduced release of important keratinocyte-derived growth factors, such as stem cell factor, which are essential for melanocyte

maintenance. As a result, the skin environment becomes unfavourable for melanocytes, leading to their indirect or passive death and the development of depigmented patches [13].

Several pathogenic mechanisms have been proposed to explain the development of vitiligo. These include autoimmune destruction of melanocytes, biochemical abnormalities during melanin synthesis, oxidative stress due to an imbalance between oxidants and antioxidants, neural factors involving neurochemical mediators, and possible viral influences. Among these, autoimmune mechanisms and oxidative stress are considered to play major roles. Genetic susceptibility also significantly contributes to disease onset and progression, particularly in individuals with a family history of vitiligo or other autoimmune disorders [16].

2.1. CLASSIFICATION OF VITILIGO:

According to the Vitiligo Global Issues Consensus Conference (2011–2012), vitiligo is clinically classified into three main types: segmental, non-segmental, and mixed or unclassified forms [17]. These types differ in age of onset, pattern and extent of skin involvement, disease progression, and association with autoimmune disorders [24]. Vitiligo commonly affects areas prone to pigmentation changes such as the face (around the mouth and eyes), hands, nipples, axillae, umbilicus, groin, and genital region. It also frequently involves sites exposed to repeated pressure or trauma, including elbows, knees, fingers, and wrists [25].

2.1.1 Segmental Vitiligo

Segmental vitiligo is characterized by unilateral depigmented patches that often follow a dermatomal pattern. It usually begins at a younger age and is less common than non-segmental vitiligo. Early involvement of hair follicles is common, leading to leukotrichia (premature whitening of hair). The face is most frequently affected, followed by the trunk and limbs. Disease progression is usually limited to a short period and then becomes stable. Segmental vitiligo often responds poorly to medical treatment due to early follicular melanocyte loss [18][20][22][26].

2.1.2. Non-Segmental Vitiligo

Non-segmental vitiligo is the most common form, accounting for about 80–90% of cases. It presents as bilateral and often symmetrical depigmented patches that gradually increase in size. This type involves a significant loss of epidermal melanocytes and partial loss of follicular melanocytes. Clinical variants include focal, mucosal, acrofacial, generalized, universal, and mixed forms. When depigmentation affects more than 80% of the body surface area, it is termed universal vitiligo [19][20][21][23].

2.2.3 Unclassified / Mixed Vitiligo

Unclassified vitiligo includes focal vitiligo and isolated mucosal vitiligo affecting a single mucosal site. Mixed vitiligo occurs when both segmental and non-segmental types are present in the same patient. It often begins as segmental vitiligo and later progresses to a generalized form. The presence of leukotrichia and halo nevi increases the risk of developing mixed vitiligo. Halonevi are pigmented moles surrounded by depigmented skin and indicate an autoimmune response against melanocytes [20][27][28].

3. Etiology and Pathogenesis

Vitiligo occurs due to the destruction of melanocytes, leading to depigmented skin patches. The disease develops through multiple mechanisms, including genetic factors, autoimmune reactions, oxidative stress, neural influences, and biochemical abnormalities

3.1 Autoimmune Theory

The autoimmune theory is the most accepted explanation for vitiligo. It suggests that the immune system mistakenly attacks melanocytes through cytotoxic T cells or autoantibodies. Vitiligo is frequently associated with autoimmune diseases such as thyroid disorders, diabetes mellitus, Addison's disease, alopecia areata, and pernicious anemia [24]

3.2 Genetic Theory

Vitiligo often runs in families, indicating a genetic role. However, low concordance rates in identical twins show that environmental factors are also important. Vitiligo is a polygenic disorder involving immune-related genes such as HLA, CTLA-4, CAT, ACE, and IL2RA [29]

3.3 Oxidative Stress Theory

Oxidative stress plays a major role in vitiligo development. Increased hydrogen peroxide levels damage melanocytes and impair their survival. Patients show reduced antioxidant

enzymes such as catalase and glutathione peroxidase, along with decreased vitamins C and E[30]

3.4 Neural Theory

The neural theory suggests that chemicals released from nerve endings may damage melanocytes or disturb melanin production. Reduced catalase activity further increases oxidative damage in vitiligo patients [21].

3.5 Biochemical Theory

According to the biochemical theory, toxic by-products formed during melanin synthesis and poor antioxidant defense lead to melanocyte destruction. Although vitiligo is mainly considered an autoimmune disease, no single theory fully explains its development. Environmental factors play an important role in triggering the disease in genetically susceptible individuals [21][22][23].

Signs and Symptoms

- Flat white patches on the skin, often with darker edges
- Patches may increase in size and change shape
- Depigmentation commonly appears around eyes and mouth
- Can cause emotional distress, depression, or mood changes

4. Diagnosis

- **Nevus depigmentosus:** Localized hypo pigmented or depigmented patches present from birth.
- **Pityriasis alba:** Common in children; causes pale, scaly patches mainly on the face and sun-exposed areas.
- **Idiopathic guttate hypomelanosis:** Small white spots seen mostly in older people on sun-exposed skin.
- **Tinea versicolor:** A superficial fungal infection causing pale, scaly patches on the chest and back; shows yellow fluorescence under Wood's lamp.
- **Halo nevus:** A mole surrounded by a ring of depigmented skin.
- **Progressive macular hypomelanosis:** Light-colored patches on the trunk in young adults.
- **Drug-induced leukoderma:** Loss of skin pigment caused by certain medications, especially corticosteroids.

5. Treatment for Vitiligo Using Black Cumin Seed Cream

5.1 Primary Treatment Approach

Vitiligo is an autoimmune skin disorder characterized by the destruction of melanocytes, leading to loss of pigmentation. Treatment mainly focuses on reducing inflammation, controlling immune responses, and promoting melanocyte regeneration. Black cumin seed (Nigella sativa) contains the bioactive compound thymoquinone, which exhibits strong anti-inflammatory, antioxidant, and immunomodulatory properties. These effects help protect melanocytes from immune-mediated damage and support the restoration of skin pigmentation.

Topical application of black cumin seed cream may assist in reducing oxidative stress, enhancing melanocyte survival, and improving re-pigmentation, particularly in mild to moderate and stable vitiligo cases. The cream can be used alone or as an adjunct to phototherapy, which may further enhance treatment outcomes. Being a natural and well-tolerated formulation, black cumin seed cream offers a safer alternative with fewer side effects compared to conventional therapies.

5.2 Topical Anti-Inflammatory Role of Black Cumin Seed Cream

Black cumin seed cream acts as a topical anti-inflammatory and immune-regulating agent, helping to reduce skin inflammation and limit autoimmune activity. Its antioxidant properties protect skin cells from oxidative damage and encourage melanocyte recovery and pigment restoration. Regular topical use may support skin healing, improve pigmentation, and enhance overall skin appearance. When combined with supportive treatments such as controlled sun exposure or phototherapy, the effectiveness of re-pigmentation may be improved.

6. NIGELLA SATIVA

Nigella sativa, commonly referred to as blackseed or black cumin, is native to regions of Eastern Europe and Western Asia and is widely recognized for its diverse medicinal benefits. The medicinal use of N. sativa dates to the first century, when it was traditionally employed to treat several conditions such as influenza, dental pain, and headaches [6]. Although the exact mechanisms responsible for its therapeutic effects are not completely clear, these effects are largely attributed to the complex chemical makeup of the seeds [7]. The constituents of N. sativa can be broadly grouped into fixed oils, proteins, alkaloids, saponins, and essential oils, among which essential oils exhibit the most notable therapeutic activity. Some lesser-known components such as Nigel lone and p-cymene, which belong to the essential oil fraction, possess important medicinal properties due to their broncho dilatory and analgesic actions, respectively [8]. Among all these compounds, thymoquinone is considered the most biologically active constituent. From a biochemical point of view, thymoquinone is a monoterpene composed of a benzene ring with hydroxyl groups and alkyl side chains [9]. This unique molecular structure is responsible for its strong antioxidant activity, which supports the body's natural defense systems against cellular injury. Various internal and external stressors, including toxins, environmental factors, and metabolic waste products, lead to the generation of reactive oxygen species (ROS) and free radicals. Under normal conditions, these harmful molecules are neutralized by antioxidant pathways such as the glutathione reductase system. However, during severe metabolic imbalance or localized stress, excessive production of free radicals can overwhelm the body's defense mechanisms, resulting in accelerated cellular aging, suppression of immune function, and an increased risk of DNA mutations

The high thymoquinone content in N. sativa helps preserve and enhance the activity of important antioxidant enzymes, including catalase, glutathione-S-transferase, and glutathione reductase, thereby improving the body's ability to counter oxidative stress [10]. This protective effect, together with the additional nutraceutical benefits provided by other components of N. sativa, makes it a plant of considerable therapeutic importance. At present, N. sativa is widely used in both traditional and modern medicine for its cardio protective, gastro protective, and neuroprotective effects. It is also considered useful in disease prevention due to its anti-cancer and anti-metabolic properties [7]. Studies have shown that supplementation with 1 gram of N. sativa oil can significantly improve several cardiometabolic risk parameters, including reductions in HbA1c, total cholesterol, LDL cholesterol, triglycerides, blood pressure, and C-reactive protein, while simultaneously increasing HDL cholesterol levels [11]. In addition, this nutraceutical has demonstrated beneficial effects in the treatment of skin disorders such as atopic dermatitis because of its immunomodulatory action, which suppresses inflammatory cytokines including IL-4, IL-5, and IFN-gamma [12]. These findings suggest that N. sativa may also have a broad therapeutic potential in the management of other inflammatory skin conditions such as psoriasis, chronic urticaria, and contact dermatitis, which involve similar immune dysregulation.

7. AIM

To develop a dermal cream for the treatment of Vitiligo and study their efficiency by in-vitro methods.

8. OBJECTIVE

1. Collection of raw material
2. Preparation of dermal cream
3. Anti – inflammatory effect of dermal cram by Albumin denaturation method
4. Skin irritation test
5. Optimization of pH
6. Protease inhibition assay method
7. Solubility of dermal cream
8. Statistical analysis of results by Graph pad prism software

9. MONOGRAPH OF NIGELLA SATIVA SEEDS

9.1. Name

- Biological name: *Nigella sativa*
- Family: Ranunculaceae
- Common names: Black seed, Black cumin, Kalonji

9.2. Biological Source

Nigella sativa seeds are obtained from the dried ripe seeds of the plant ***Nigella sativa*** **Linn.**

9.3 Geographical Source

Native to Southwest Asia, the Middle East, India, and Mediterranean regions.

9.4. Macroscopical Characters

- Seeds are small, black, angular, and rough
- Shape: Triangular or oval
- Odor: Aromatic
- Taste: Bitter and slightly pungent

9.5 Microscopical Characters

- Seed coat contains thick-walled cells
- Presence of oil globules
- Endosperm rich in fixed oils and proteins

9.6. Chemical Constituents

- Thymoquinone (major active compound)
- Fixed oils (linoleic acid, oleic acid), volatile oils, alkaloids.

9.7. Pharmacological Activities

- Antioxidant
- Anti-inflammatory
- Antimicrobial
- Antidiabetic
- Hepatoprotective

9.8. Uses

- Treatment of asthma, diabetes, and hypertension
- Used for digestive disorders
- Supports immune system
- Applied in skin diseases and wound healing
- Traditional use in Ayurveda and Unani medicine

10. MATERIALS AND METHODS

10.1 Collection of raw material

Nigella sativa seeds were bought and ground into a powder. 10 g of the ground powder was added in 100 ml of distilled water, and hydrothermal extraction was done to collect the seed extract.

Fig 3.1 Nigella sativa seeds

10.2 Preparation of dermal cream

Composition of dermal cream:

1. Plant extract - 0.1 g
2. Calcium chloride - 0.5 g
3. Glycerine - 1 g
4. Petroleum wax - 1 g
5. C. M. cellulose - 1 g
6. Gar gum - 0.5 g
7. D. H_2O - 10 ml

The above compositions were added and mixed together to prepare the dermal cream. The prepared cream was autoclaved and stored in the refrigerator.

10.3 Anti – inflammatory effect of dermal cram by Albumin denaturation method

Denaturation of proteins is the main cause of inflammation. Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Saka*et al*. with slight modification. 500 L of 1% bovine serum albumin was added to NS – Dermal cream Sample (500, 250, 100, 50 and 10 μg/mL) of test sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\% \text{ Inhibition} = 100 - ((A1-A2)/A0)*100)$$

Where A1 is the absorbance of the control, A2 is the absorbance of the test sample and A0 is the absorbance of the positive control.

10.4 Skin irritation test

The primary skin irritability test and can be considered as the first clinical stage of safety assessment. The dermal cream was applied on the left hand for 1h. It was performed to investigate irritation signs.

10.5 Optimization of pH

The pH of the sample was measured by an electronic portable pH meter. The pH meter was calibrated with phosphate buffer of known pH. At a constant. Temperature, a pH change was observed in the electrical property of the solution. This change was read by the electrode and the accuracy was obtained in the middle pH ranges.

10.6 Protease inhibition assay method

The proteinase inhibitory assay was performed following the method modified by Oyedepo and Femurewa. The reaction mixture (2

ml) contained 0.06 mg trypsin, 1 ml Tris-HCl buffer (20 mM, pH 7.4) and test sample (SP-C) at different concentrations (500, 250, 100, 50 and 10 µg/ml). The reaction mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (*w/v*) casein was added. The mixture was incubated for an additional 20 min. Per chloric acid (2 ml of 70%) was added to stop the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was measured at 210 nm against Tris-HCl buffer as blank. The experiment was performed in triplicate.

10.7 Solubility of dermal cream

Solubility test was done by dissolving 50mg of the dermal cream in 1 ml of a known solvent and solubility was observed.

11. RESULTS AND DISCUSSION

In this study, *Nigella sativa* seeds were successfully used to prepare a dermal cream and evaluate its anti-inflammatory potential. The collected seeds were finely powdered and subjected to hydrothermal extraction. The extract was filtered and concentrated by heating at 100 °C, resulting in a thick plant extract suitable for formulation. This extract was then used to prepare a dermal cream, which was autoclaved to ensure sterility and stored under refrigeration for further studies.

The anti-inflammatory activity of the *Nigella sativa* cream was evaluated using the albumin denaturation assay. Protein denaturation is a common cause of inflammation, and inhibition of this process indicates anti-inflammatory potential. The results showed a clear concentration-dependent inhibition of albumin denaturation. At a concentration of 500 µg/ml, the cream showed a mean inhibition of about 68%, while lower concentrations showed gradually reduced activity. The standard drug, acetyl salicylic acid, showed higher inhibition, confirming the validity of the method. The IC_{50} value of the test sample was found to be 71.02 µg/ml, indicating good anti-inflammatory activity.

The skin irritation test revealed that the dermal cream did not cause redness, itching, or irritation even after one hour of application, proving it to be safe for topical use. The pH of the cream was found to be 6, which lies within the ideal range for skin applications and supports skin compatibility.

Protease inhibition assay results further supported the anti-inflammatory potential of the cream. The extract showed moderate inhibition of protease activity, with an IC_{50} value of 90.38 µg/ml. Solubility studies showed that the cream was insoluble in water and ACETONE, partially soluble in ethanol and chloroform, and insoluble in ethyl acetate. Overall, the results confirm that *Nigella sativa* dermal cream is safe and possesses promising anti-inflammatory properties

11.1 Collection of raw material and extraction

The collected Nigella sativa seeds were ground and hydrothermal extraction was performed. The filtered extract was then heated at 100 ºC to concentrate the extract. Then, the concentrated extract was collected in a tube.z

Fig 11.1 Nigella sativa

Fig 11.2: NS concentrated extract

11.2 Preparation of dermal cream

The NS extract was used to prepare the dermal cream, and then it was autoclaved and stored in the refrigerator.

Fig 11.3: NS Dermal cream

11.3 Anti –inflammatory effect by Albumin denaturation assay

The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\% \text{ Inhibition} = 100 - ((A1-A2)/A0)*100)$$

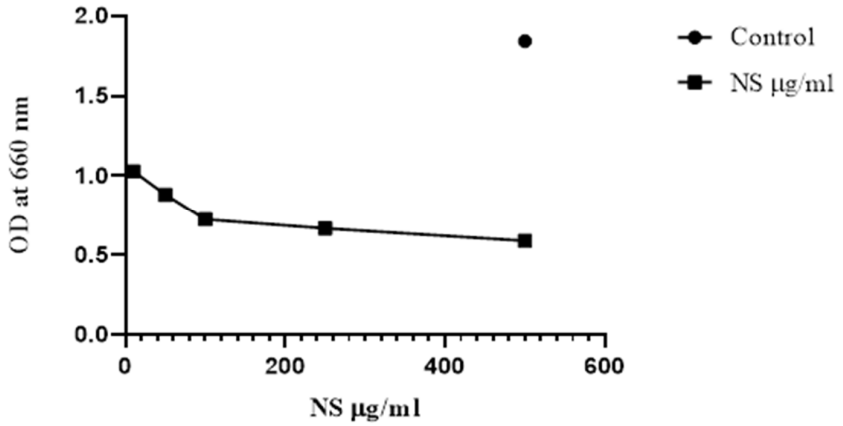
Where A1 is the absorbance of the control, A2 is the absorbance of the test sample and A0 is the absorbance of the positive control.

A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

A. OD Value at 660 nm

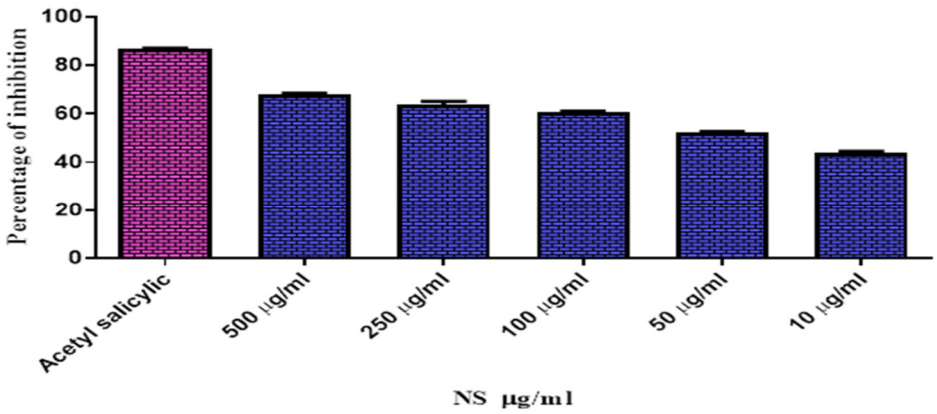
Control Mean OD value: 1.842

S. No	Tested sample concentration (µg/ml)	OD Value at 660 nm (in triplicates)		
1.	Control	1.875	1.826	1.827
2.	500 µg/ml	0.594	0.578	0.596
3.	250 µg/ml	0.687	0.639	0.678
4.	100 µg/ml	0.727	0.730	0.717
5.	50 µg/ml	0.875	0.894	0.878
6.	10 µg/ml	1.029	1.022	1.038
7.	Acetyl salicylic	0.257	0.246	0.237



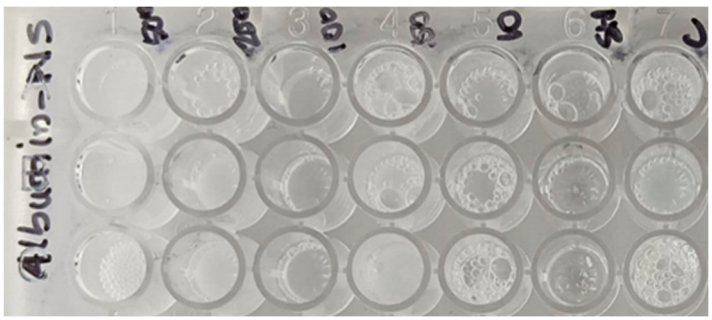
B. Inhibition percentage of albumin denaturation (%)

S. No	Tested sample concentration (µg/ml)	Inhibition percentage albumin denaturation (%) (in triplicates)			Mean Value (%)
1.	Acetyl salicylic acid	86.052822	86.64978292	87.1382055	86.6136035
2.	500 µg/ml	67.76411	68.63241679	67.6555716	68.0173661
3.	250 µg/ml	62.7170767	65.32199711	63.2054993	63.748191
4.	100 µg/ml	60.5463097	60.38350217	61.0890014	60.6729378
5.	50 µg/ml	52.5144718	51.48335745	52.3516643	52.1164978
6.	10 µg/ml	44.1570188	44.53690304	43.6685962	44.1208394



C. IC50 Value of tested sample: 71.02 µg/ml

Log (inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	1.851
HillSlope	-1.746
IC50	71.02
Std. Error	
LogIC50	0.02453
HillSlope	0.1846
95% CI (profile likelihood)	
LogIC50	1.798 to 1.903
HillSlope	-2.239 to -1.405
IC50	62.80 to 80.07
Goodness of Fit	
Degrees of Freedom	13
R squared	0.9782
Sum of Squares	422.2
Sy.x	5.699
Number of points	
# of X values	15
# Y values analyzed	15



11.4 Skin irritation test

The skin irritation test showed that the dermal cream does not cause any irritation and is safe for topical application.

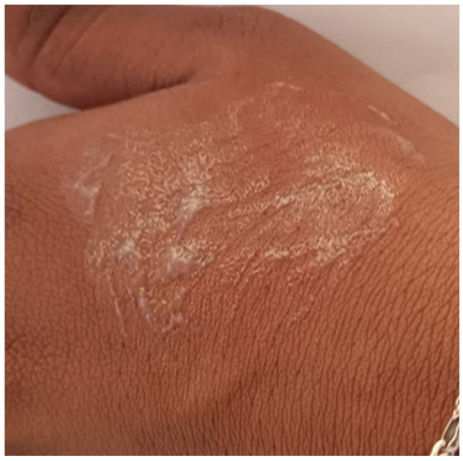


Fig 11.5: After application on the skin

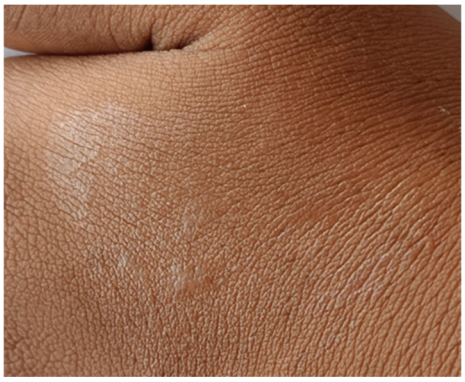


Fig 11.6 1 hour after application on the skin

11.5 Optimization of pH

The pH of the dermal cream was found to be **6**, which is in the ideal pH range for dermal creams.

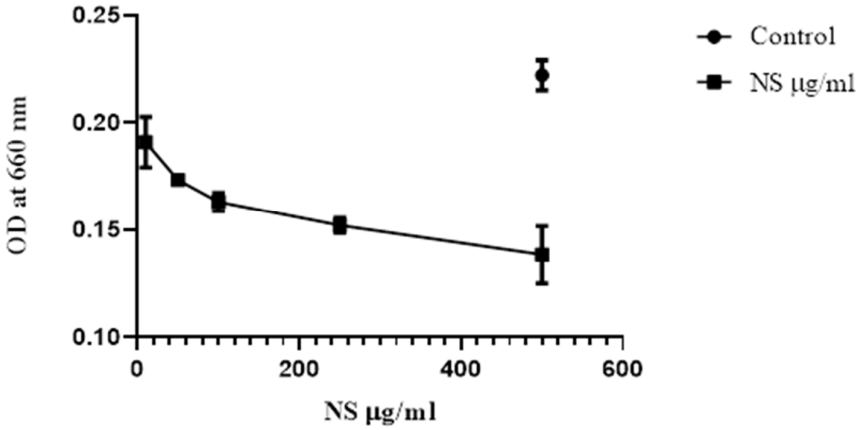
11.6 Protease inhibition assay method

The proteinase inhibition assay was performed in triplicate, and the readings are as follows:

A. OD Value at 210 nm

Control Mean OD value: 0.222

S. No	Tested sample concentration (µg/ml)	OD Value at 210 nm (in triplicates)		
1.	Control	0.222	0.229	0.215
2.	500 µg/ml	0.145	0.123	0.147
3.	250 µg/ml	0.148	0.155	0.153
4.	100 µg/ml	0.160	0.161	0.168
5.	50 µg/ml	0.171	0.174	0.175
6.	10 µg/ml	0.204	0.181	0.188

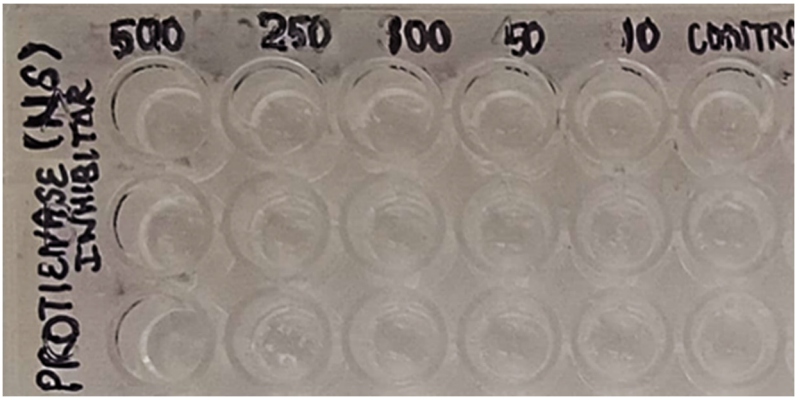


B. Inhibition percentage (%)

S. No	Tested sample concentration (µg/ml)	Inhibition percentage (%) (in triplicates)			Mean Value (%)
1.	500 µg/ml	34.6846847	44.59459459	33.7837838	37.6876877
2.	250 µg/ml	33.3333333	30.18018018	31.0810811	31.5315315
3.	100 µg/ml	27.9279279	27.47747748	24.3243243	26.5765766
4.	50 µg/ml	22.972973	21.62162162	21.1711712	21.9219219
5.	10 µg/ml	8.10810811	18.46846847	15.3153153	13.963964

C. IC50 Value of tested sample: 90.38 µg/ml

log(inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	1.956
HillSlope	-1.406
IC50	90.38
Std. Error	
LogIC50	0.07292
HillSlope	0.3404
95% CI (profile likelihood)	
LogIC50	1.787 to 2.113
HillSlope	-2.336 to -0.8844
IC50	61.29 to 129.8
Goodness of Fit	
Degrees of Freedom	13
R squared	0.8519
Sum of Squares	2969
Sy.x	15.11
Number of points	
# of X values	15
# Y values analyzed	15



11.7. Solubility test

The dermal cream was dissolved in 5 different solvents to investigate their solubility. The results are:

Solubility of the dermal cream in Water, Ethanol, Acetone, Ethyl Acetate, Chloroform

S.NO	Name of the test sample	Result				
		Water solubility	Ethanol solubility	Acetone solubility	Ethyl Acetate solubility	Chloroform solubility
1.	SPL	Insoluble	Partially soluble	Insoluble	Insoluble	Partially soluble

12. CONCLUSION

The present study demonstrates the therapeutic potential of Nigella sativa (NS) dermal cream as a natural, safe, and effective agent for managing inflammatory skin disorders, including vitiligo. The anti-inflammatory activity, evaluated through the albumin denaturation assay, showed significant dose-dependent inhibition of protein denaturation, with a maximum inhibition of 68.02% at 500 µg/ml and an IC50 value of 71.02 µg/ml. This indicates that NS extract can stabilize proteins under stress, potentially mitigating inflammatory responses that contribute to melanocyte damage in vitiligo and other dermatoses.

Protease inhibition assays further confirmed the extract's protective effects, showing a dose-dependent reduction in enzyme activity with an IC50 of 90.38 µg/ml, highlighting its potential to prevent tissue degradation and maintain skin integrity. The cream's physico-chemical properties were favourable, with a pH of 6—ideal for topical application and no observed irritation during skin safety testing, suggesting suitability for prolonged dermal use. Solubility analysis indicated partial solubility in ethanol and chloroform, providing insight for formulation optimization.

These results collectively indicate that NS dermal cream possesses bioactive properties capable of modulating inflammatory and proteolytic pathways, which are key contributors to the pathogenesis of vitiligo, particularly in areas prone to oxidative stress and immune-mediated melanocyte damage. By combining safety, bioactivity, and skin compatibility, this formulation offers a promising complementary therapy for re-pigmentation and inflammation management. Future clinical studies are warranted to assess its efficacy in vitiligo patients, optimize dosing, and explore synergistic effects with conventional treatments such as phototherapy or JAK inhibitors, ultimately contributing to improved therapeutic outcomes and quality of life in individuals affected by vitiligo and related dermatological conditions.

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