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FORMULATION AND EVALUATION OF AN HERBAL WOUND HEALING OINTMENT USING *SUNFLOWER SEED CAKE* AND *MARIGOLD WASTE*

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ABSTRACT

This study focuses on the formulation and evaluation of an herbal wound healing ointment using sunflower seed cake and marigold waste as natural active ingredients. Sunflower seed cake, a by-product of oil extraction, is rich in proteins and antioxidants, while marigold waste contains flavonoids and triterpenoids known for their anti-inflammatory and antimicrobial properties. The ointment was prepared using suitable bases such as white soft paraffin, beeswax, and lanolin to ensure proper consistency and stability. The formulated batches were evaluated for physicochemical parameters including pH, spread ability, consistency, washability, and skin irritation. Results indicated that the optimized formulation showed good stability, acceptable pH, and excellent spread ability with no signs of irritation. The herbal ointment demonstrated promising wound healing potential due to synergistic effects of its natural components. This study highlights the effective utilization of agro-waste materials in developing cost-effective and eco-friendly pharmaceutical formulations.

KEYWORDS: Wound healing, Marigold, Sunflower seed cake, Agro-waste utilization, Natural products, Eco-friendly formulation.

❖ INTRODUCTION

Definition of Wound

A wound is defined as any tissue injury that disrupts anatomical integrity and leads to functional loss. The ability of an organism to repair or regenerate tissues is a definite advantage for survival.[1]

Fig. no. 01 Skin wound.

Any violation of live tissue integrity may be regarded as a wound. Skin is the largest organ of the human body and one of its key functions is to protect water-rich internal organs from the dry external environment.[2] Maintaining skin integrity and possessing a robust wound healing capacity are key prerequisites for healthy survival. Furthermore, wound healing can also present a significant challenge and burden on health care systems. Medicare cost estimates for acute and chronic wound treatments ranged from $28.1 billion to $96.8 billion during 2014.[3] The highest wound-related expenses were attributed to surgical wounds followed by diabetic foot ulcers.[4]

Phases of Wound Healing

Skin epithelial cells are labile elements that are continuously eliminated in the stratum corneum through the keratinocyte desquamation process and are replaced, in the basal layer, by differentiated elements derived from stem cell proliferation and differentiation.

Cell renewal varies according to different factors, such as trauma, hormonal influences, skin conditions, and individual wellbeing. However, the cutaneous regenerative process, in reference to a wound lesion, is inversely proportional to the evolution of the considered species.[5] In Image no. 3, a schematic representation of the wound healing process is presented, with the cells involved in each phase.

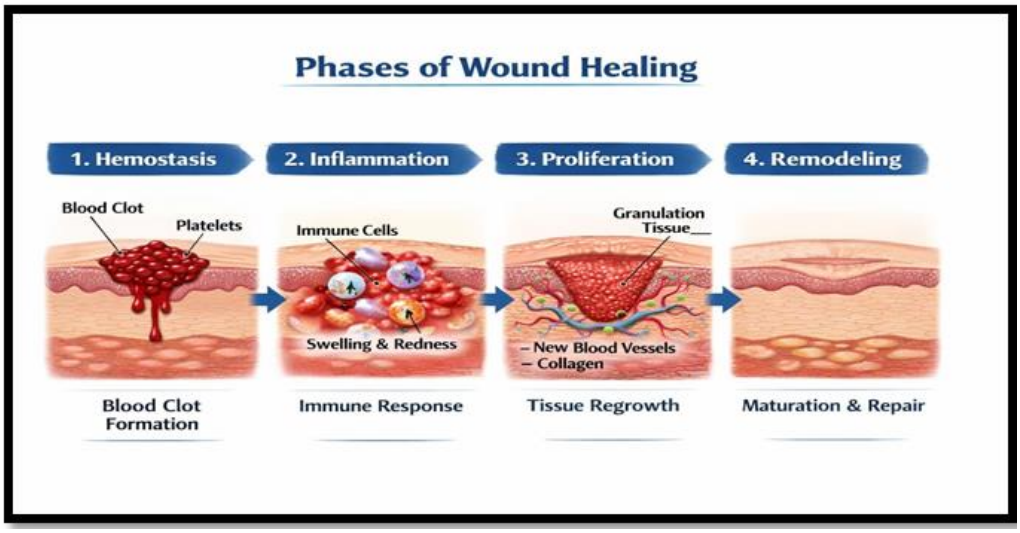


Fig.no: 02 Phases of Wound Healing.

❖ **PLANT PROFILE**

SUNFLOWER SEED CAKE

Fig. no. 03 Sunflower seed cake.

Over the years this seed cake have been used as animal feed, organic fertilizer, and soil compost but from past years, considering the nutritional potential of sunflower seedcake as it remains with multifarious nutrients, various researches are being conducted for its human consumption. In the circumstances of hunger, nutritional crisis, and deprivation of food sources, if the appropriate and innovative use of this underutilized seed cake, in developing the food products would be done, then due the nutritional quality and health benefits of SSC, an encouraging solution can be achieved.[1] Helianthus annuus occurs as a wild, multi-headed annual plant and a domesticated form with a single large flower head on an unbranched stem.

Sunflowers typically grow up to about 3 m tall, though the tallest recorded reached 9.17 m[2] The leaves of sunflower are expanded, coarsely toothed, rigid, and usually disjunctive. The outer flowers known as 'Ray flowers' generally harmonize to petals and the flowers at the Centre of the head are known as 'Disk flowers' that further mature into the fruit of the plant called as 'Sunflower seeds'[3] Sunflower oil is widely used for cooking and margarine production, leaving sunflower seed cake as a by-product. This cake is rich in protein and crude Fiber, and due to its high amino acid content and bioavailability, it serves as a valuable plant-based protein source.[4]

Taxonomical Classification[32]

Table no.01

Rank	Classification
Kingdom	Plantae
Subkingdom	Tracheobionta (Vascular plants)
Division	Magnoliophyta (Angiosperms)
Family	*Asteraceae*
Genus	Helianthus
Species	*Helianthus annuus*

Chemical constituents.[5,6,7,8]

Table no.02

Constituent	Description	Role in Wound Healing
Proteins	Rich in essential amino acids (arginine, glutamine)	Supports collagen synthesis, tissue regeneration, and cell proliferation
Carbohydrates	Cellulose, hemicellulose	Provides structural support; may aid in bioactive formulations
Crude Fiber	Indigestible Fibers	Can support scaffold structure in wound dressings
Residual Oils/Fats	Small amounts of sunflower oil	Moisturizes wound area; may aid bioactive compound delivery
Phenolic Compounds	Chlorogenic acid, caffeic acid	Antioxidant and anti-inflammatory effects

Pharmacological activity[33,34,35,36,37]

- Antioxidant activity
- Anti-inflammatory activity
- Wound healing / Tissue repair
- Immunomodulatory effect
- Antimicrobial potential
- Antimicrobial potential

MARIGOLD

Fig. no. 04 Marigold.

INTRODUCTION

Marigold is a globally recognized ornamental and medicinal plant belonging to the family *Asteraceae*, widely cultivated for its bright flowers, pharmacological value, and industrial applications. The most prominent species include *Calendula officinalis* (commonly known as Pot marigold) and *Tagetes erecta* (African marigold), both of which hold significant ethnobotanical and commercial importance.[9] In terms of industrial use, lutein extracted from marigold accounts for over 85% of the global natural lutein supply, valued at approximately USD 360–400 million in 2023.[10] Medicinally, marigold has been recognized in traditional systems such as Ayurveda, Unani, and European folk medicine. Clinical studies indicate that *Calendula* extracts promote faster wound closure by 41–48% compared to controls, while essential oils from Tagetes species demonstrate significant antibacterial activity against Staphylococcus aureus (MIC 50–100 µg/mL).[11]

Taxonomical classification[38]

Table no.03

Rank	Classification
Common names	Pot Marigold, Garden Marigold.
Kingdom	Plantae
Clade	Angiosperms
Order	Asterales
Family	*Asteraceae*
Genus	Calendula
Species	*Calendula officinalis*

Chemical constituents[12,13,14,15,16,17,18]

Table no.04

Class of Compound	Description	Activity
Flavonoids	Quercetin, isorhamnetin, kaempferol	Antioxidant, anti-inflammatory
Triterpenoids / Triterpenes	Faradiol, ursolic acid, oleanolic acid	Anti-inflammatory, wound healing
Carotenoids	Lutein, lycopene, beta-carotene	Antioxidant, skin protection
Phenolic acids	Chlorogenic acid, caffeic acid	Antioxidant, antimicrobial
Essential oils	Alpha-cadinol, delta-cadinene, terpinen-4-ol	Antimicrobial, aromatic properties
Saponins	Glycosides of triterpenes	Anti-inflammatory, immune-modulatory
Polysaccharides	Heteropolysaccharides from petals	Promote wound healing, immune stimulation
Resins & Volatile compounds	Sesquiterpenes, coumarins	Anti-inflammatory, antimicrobial

Pharmacological activity[39,40]

- Anti-inflammatory
- Wound-healing
- Antioxidant
- Antimicrobial
- Antifungal

❖ MATERIALS AND METHODS

Plant Materials

- Sunflower seed cake powder obtained from oil extraction of *Helianthus annuus.*
- Marigold flower waste powder (dried petals) of *Tagetes erecta* collected from local flower markets.

Chemicals and Reagents

- Marigold Powder
- Sunflower seed cake Powder
- White soft Paraffin
- Bees wax
- Lanolin
- Mineral Oil
- Vitamin E
- Methyl Paraben

Equipment

- Water bath
- Mortar and pestle
- Glass beakers and measuring cylinders, etc.

Method

Collection of Sample

- Take the Sunflower seed cake powder and Marigold Powder passed through #80, #100 and #120 mesh sieve to obtain uniform particle size.

- **Authentication of Sample**

  Sample is Authenticated from science college Nanded under the supervision of Dr. Marathe sir.

❖ **PHARMACOGNOSTIC STUDY**[41,42,43,44]

➢ **Marigold powder**

a) Shinoda Test

- Take a small quantity of powder + Add 5ml ethanol + Add Mg. pieces + Add few drops of conc. Hcl
- Pink or Red colour is obtained
- Flavonoid is present

b) Keller-Kilani test

- Take a small quantity of powder + Add acetic acid + $Fecl_3$ + Add conc.H_2SO_4
- Brown ring is obtained
- Glycoside is present

c) Ferric Chloride test

- Take a small quantity of powder + Add few drops of $Fecl_3$
- Green or black blue colour is obtained
- Tannin is Present

d) Salkowski test

- Take a small quantity of powder +Add chloride + Add conc. H_2SO_4
- Reddish brown ring is obtained

- Steroid is present

➢ **Sunflower seed cake powder**

a) Ninhydrin test

- Take a small quantity of powder + Heat with Ninhydrin sol^{n}
- Purple or Blue colour is obtained
- Amino acid is present

b) Biuret test

- Take a small quantity of powder + NaOH sol^{n} +Add drops of $CuSO_4$
- Violet colour is obtained
- Proteins are present

c) Molisch test

- Take a small quantity of powder +Add Molisch reagent + conc. H_2SO_4 along the side
- Violet ring is obtained
- Carbohydrate is present

d) Shinoda test

- Take a small quantity of powder + Add 5ml ethanol + Add Mg. pieces + Add few drops of conc. Hcl
- Pink or Red colour is obtained
- Flavonoid is present

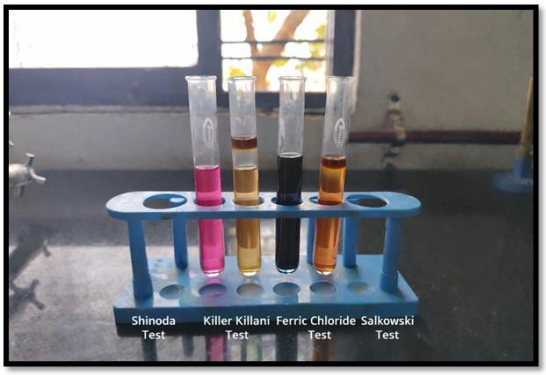


Fig no.06 Pharmacognostic tests of Marigold powder.

Table no.05

Sr.no.	Test name	Observation	Inference
a)	Shinoda Test	Pink/red colour	Flavonoid is present
b)	Keller-Kilani test	Brown ring	Glycoside is present
c)	Ferric Chloride test	Black blue colour	Tannins are present
d)	Salkowski test	Reddish-brown ring	Steroid is present

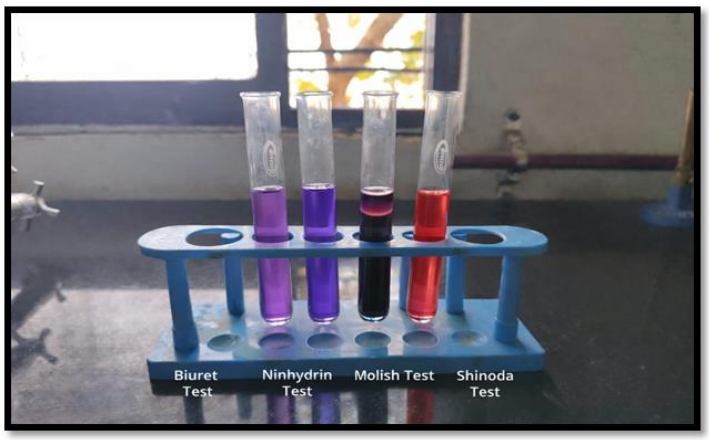


Fig. no.07 Pharmacognostic tests of Sunflower seed cake powder.

Table no.06

Sr.no.	Test name	Observation	Inference
a)	Ninhydrin test	Purple/blue colour	Amino acid is present
b)	Biuret test	Violet colour	Proteins are present
c)	Molisch test	Violet ring	Carbohydrate is present
d)	Shinoda Test	Pink/red colour	Flavonoid is present

PREFOMULATION TEST[45,42,46,47,48,49]

1. Bulk density
2. Tapped density
3. Carr's index
4. Hausner's ratio
5. Angle of repose
6. Ash value
7. Solubility

1. Bulk density

Bulk Density is the ratio between the given mass of a powder and its bulk volume. Required amount of the powder is dried and filled in a 50 ml measuring cylinder up to 50 ml mark. Then the cylinder is dropped onto a hard wood surface from a height of 1 inch at 2 second intervals. The volume of the powder is measured. Then the powder is weighed. This is

repeated to get average values. The Bulk Density is calculated by using the below given formula.

$$\text{Bulk Density} = \frac{mass}{bulkvolume}$$

2. Tapped density

Tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped for 1 min and volume or mass readings are taken until little further volume or mass change was observed.

$$\text{Tapped density} = \frac{Mass}{Tapped\ volume}$$

Fig no.08 Sunflower seed cake powder.

Fig. no.09 Marigold Powder.

3. Carr's index

Carr's Index is a pharmaceutical and industrial measure of powder compressibility and flowability, calculated as 100. Carr's index is calculated by using following formula

$$\text{Carr's Index} = \frac{(Tapped\ Density - Bulk\ Density)}{Tapped\ Density}$$

4. Hausner's ratio

It is the ratio between tapped density and bulk density. The major function of the Hausner's ratio is to check the powder's flow ability since it is the primary phenomenon of flow property. if the Hausner ratio is falling the powder has great properties, if it is increasing, the powder`s flow ability decreases. It`s also reported that the flow is good.

It is calculated by using following formula

$$\text{Hausner's ratio} = \frac{Tapped\ Density}{Bulk\ Density}$$

Carr's index	Flow character	Hausner's ratio
≤ 10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	V. very poor	>1.60

Fig. no. 09.

5. Angle of repose

The angle of repose is the steepest possible angle at which a granular material will remain stable and at rest on an inclined plane, without any external support. This is the largest angle that can exist between the powder pile`s surface and the horizontal flow. This is the most crucial approach since it provides the precise result of whether your powder flows well or poorly. This approach mostly depends on the powder`s angle, as the pile`s angle grows, the flow properly decreases. The following formula is provided to determine the angle of repose.

$$\emptyset = tan - 1\frac{h}{r}$$

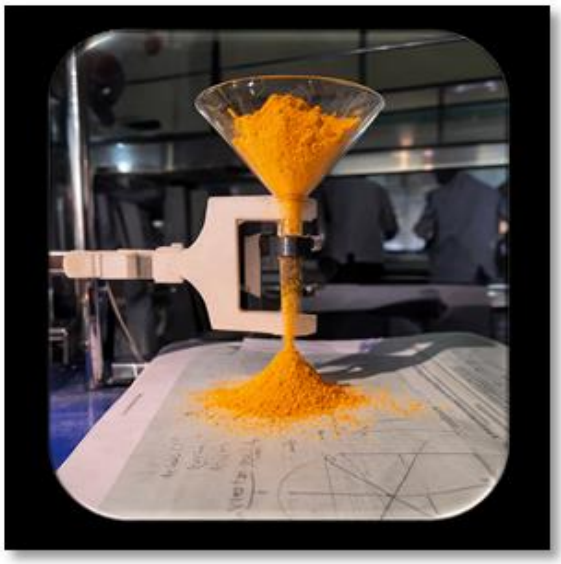


Fig. no. 10 Marigold powder.

Fig. no.11 Sunflower seed cake powder.

6. Ash value

Ash value or ash content, is the inorganic residue remaining after a substance is incinerated. It serves as a measure of the total mineral content and inorganic impurities in a material and is

used as a key quality control parameter to assess the purity, authenticity, and quality of raw materials and finished products, especially in herbal medicines and pharmaceuticals.

$$\text{Ash Content (\%)} = \frac{(Weight\ of\ Ash\ Residue\ .}{Weight\ of\ Original\ Sample)} \times 100$$

Fig. no. 12 Ash value.

7. Solubility

Solubility is the maximum amount of a solute that can dissolve in each amount of solvent at a specific temperature and pressure to form a saturated solution. It is a physical property that indicates a substance's ability to dissolve, and it depends on the type of solute and solvent, the temperature, and the pressure.

Fig. no.13: Solubility.

FORMULATION OF OINTMENT[50,51,52,53,54,55]

Weigh Ingredients

Accurately weigh all required ingredients.

Prepare Ointment Base

Melt white soft paraffin, beeswax, lanolin, and mineral oil together in a water bath at about 65–70°C.

Add Preservatives

Add methyl paraben and propyl paraben to the melted base and stir until completely dissolved.

Add Active Powders

Gradually add marigold powder and sunflower seed cake powder while stirring continuously to obtain uniform mixing.

Add Antioxidant

Add Vitamin E to improve stability and support wound healing.

Cooling

Stir the mixture continuously while cooling until a smooth and homogeneous ointment is formed.

Packaging

Transfer the prepared ointment into sterile ointment containers and store in a cool, dry place.

❖ **EVALUATION STUDY**[56,42,57,58]

1. Organoleptic Evaluation

- Colour
- Odor
- Appearance
- Texture

2. pH Determination
3. Spread ability Test

4. Consistency Test
5. Washability Test
6. Skin Irritation Test

1) Organoleptic Evaluation

Organoleptic evaluation is a qualitative method used to assess the physical and sensory characteristics of an ointment. It includes color, odor, texture, and appearance, which are critical for patient acceptability and quality control. The evaluation helps identify any inconsistencies in formulation, presence of foreign particles, or undesirable changes due to instability. Uniform color, characteristic odor, smooth and non-gritty texture, and consistent appearance indicate a well-prepared, stable ointment suitable for topical application.

2) pH determination

pH determination of an ointment measures the acidity or alkalinity of the formulation. It is a crucial parameter for skin compatibility, stability, and efficacy. A pH close to the natural skin pH (around 5.5) minimizes irritation, ensures better patient compliance, and helps maintain the integrity of active ingredients. The pH can be measured by dispersing a known amount of ointment in distilled water and using a calibrated pH meter or pH indicator paper. Changes in pH may indicate chemical degradation or microbial contamination.

Fig. no.14 pH determination test.

3) Spreadability test

Spread ability measures the ease with which an ointment spreads on the skin, reflecting patient compliance, uniform application, and therapeutic efficiency. It depends on the viscosity of the ointment base and the proportion of active and excipient ingredients.

$$S=\frac{M\times L}{T}$$

- S = Spreadability (g·cm/s)
- M = Weight tied to glass slide (g)
- L = Length of spread (cm)
- T = Time taken to spread the ointment (s)

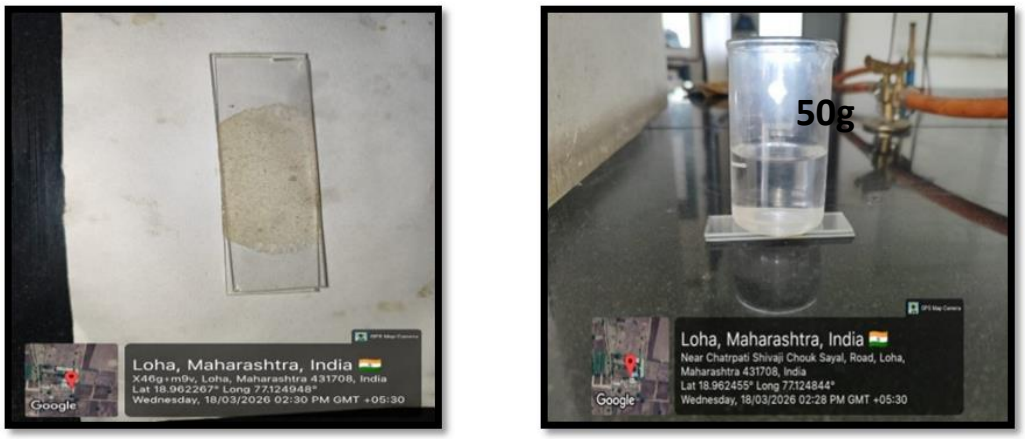


Fig.15, 16 Spreadability test.

4) Consistency tests

The finger press method is a simple and quick technique to evaluate the consistency or firmness of an ointment. It determines how resistant the ointment is to deformation under light pressure, which reflects its ease of application and patient acceptability. A properly formulated ointment should offer slight resistance without being too hard or too soft.

Fig. no. 17 Consistency tests.

5) Washability test

The washability test evaluates how easily an ointment can be removed from the skin with water or mild cleansing agents. This parameter is important for topical formulations where ease of removal is desired, such as cosmetic or herbal ointments. Ointments that are too greasy or poorly water-dispersible may be difficult to wash off, affecting patient comfort and compliance.

Fig. no. 17, 18 Washability test.

6) Skin Irritation Test

The skin irritation test is performed to evaluate the safety and compatibility of an ointment when applied to the skin. It determines whether the formulation causes redness, itching, swelling, or inflammation**.** A small amount of ointment is applied to a specific area of skin (usually on animals or human volunteers under controlled conditions) and observed for a defined period (24–72 hours). Absence of irritation indicates that the formulation is safe for topical use**,** while any adverse reaction suggests the need for reformulation.

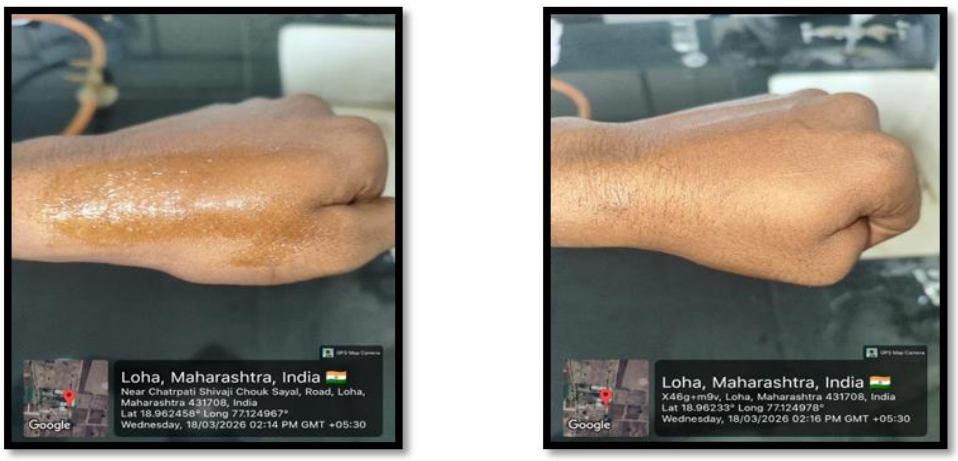


Fig. no. 19, 20 Skin Irritation Test.

❖ RESULT AND DISCUSSION

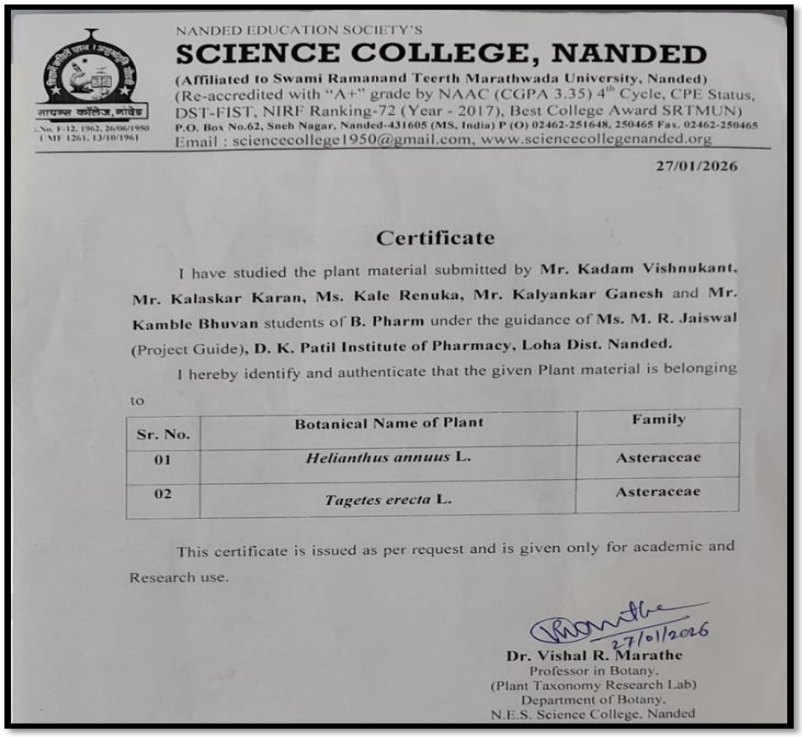
1. Collection of samples

- Sample was collected and authenticated from science collage of Nanded under the supervision of Dr. Marathe sir.

Fig. no.21, 22 Collection of samples.

2. Authentication of Samples

Then preformulation studies was performed and based on result of preformulation studies the batch was conformed according their particle size.



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27/01/2026

Certificate

I have studied the plant material submitted by **Mr. Kadam Vishnukant, Mr. Kalaskar Karan, Ms. Kale Renuka, Mr. Kalyankar Ganesh** and **Mr. Kamble Bhuvan** students of **B. Pharm** under the guidance of **Ms. M. R. Jaiswal** (Project Guide), **D. K. Patil Institute of Pharmacy, Loha Dist. Nanded.**

I hereby identify and authenticate that the given Plant material is belonging to

Sr. No.	Botanical Name of Plant	Family
01	*Helianthus annuus* L.	Asteraceae
02	*Tagetes erecta* L.	Asteraceae

This certificate is issued as per request and is given only for academic and Research use.

27/01/2026

Dr. Vishal R. Marathe
Professor in Botany,
(Plant Taxonomy Research Lab)
Department of Botany,
N.E.S. Science College, Nanded

Fig. no. 23 Authentication Letter.

3. Preformulation of samples

After the authentication of samples, the Preformulation studies are done on the samples of Powders which is given into below table no.07.

Table no. 07 Preformulation of samples.

Sr.no.	Tests	Marigold Powder		Sunflower seedcake Powder		
01.	Seive #	**100#**	120#	80#	100#	**120#**
02.	Bulk Density(gm/ml)	**0.44**	0.40	0.50	0.44	**0.40**
03.	Tapped Density(gm/ml)	**0.57**	0.52	0.66	0.58	**0.52**
04.	Carr's index	**22.80**	23.07	24.2	24.13	**23.07**
05.	Hausner's ratio	**1.29**	1.30	1.32	1.31	**1.30**
06.	Angle Of Repose(θ)	**35.8^{o}**	35^{o}	29^{o}	32.8^{o}	**38.7^{o}**
07.	Ash Value	3.25%		1.41%		
08.	Solubility	Soluble in Ethanol		Soluble in Water		

4. Formulation of Ointment

The Ointment was formulated by using following formulation tables no.08, 09, 10 &11.

Table no.08 Ointment Formulation Batch A (15g).

Sr.no.	Ingredient	Quantity given	Role
01.	Marigold Powder	1.5g	Wound healing, anti-inflammatory
02.	Sunflower seed cake powder	1.5g	Antioxidant, tissue repair
03.	White soft paraffin	6g	Main ointment base
04.	Bees wax	2g	Stiffening agent
05.	Lanolin	2g	Absorption base, emollient
06.	Mineral oil	1.5g	Levigating agent, smooth texture
07.	Vitamin E	0.3g	Antioxidant, skin healing
08.	Methyal paraben	0.2g	Preservative

Table no. 09 Ointment Formulation Batch B (15g).

Sr.no.	Ingredient	Quantity given	Role
01.	Marigold Powder	1g	Wound healing, anti-inflammatory
02.	Sunflower seed cake powder	1g	Antioxidant, tissue repair
03.	White soft paraffin	6.5g	Main ointment base
04.	Bees wax	1.5g	Stiffening agent
05.	Lanolin	2g	Absorption base, emollient
06.	Mineral oil	2g	Levigating agent, smooth texture
07.	Vitamin E	0.3g	Antioxidant, skin healing
08.	Methyal paraben	0.2g	Preservative

Table no. 10 Ointment Formulation Batch C (15g).

Sr.no.	Ingredient	Quantity given	Role
01.	Marigold Powder	0.75g	Wound healing, anti-inflammatory
02.	Sunflower seed cake powder	0.75g	Antioxidant, tissue repair
03.	White soft paraffin	7.5g	Main ointment base

04.	Bees wax	1g	Stiffening agent
05.	Lanolin	2.5g	Absorption base, emollient
06.	Mineral oil	1.5g	Levigating agent, smooth texture
07.	Vitamin E	0.3g	Antioxidant, skin healing
08.	Methyal paraben	0.2g	Preservative

Table no. 11 Ointment Formulation Batch D (15g)

Sr.no.	Ingredient	Quantity given	Role
01.	Marigold Powder	1g	Wound healing, anti-inflammatory
02.	Sunflower seed cake powder	1g	Antioxidant, tissue repair
03.	White soft paraffin	5.5g	Main ointment base
04.	Bees wax	2.5g	Stiffening agent
05.	Lanolin	2g	Absorption base, emollient
06.	Mineral oil	2g	Levigating agent, smooth texture
07.	Vitamin E	0.3g	Antioxidant, skin healing
08.	Methyl paraben	0.2g	Preservative

Table no.12 Evaluation studies of formulation.

Sr.no.	Batch	A	B	C	D
01.	Appearance	Smooth, light-yellow black	**Smooth, light black**	Smooth, light black	Smooth, dark black
02.	pH	4.41	**5.23**	5.26	6.2
03.	Spreadability (g.cm/sec)	9.5	**10**	10.5	9.8
04	Consistency test	Soft	**Smooth & uniform**	Thick	Very soft
05.	Washability	Good	**Very good**	Good	Good
06.	Skin irritancy test	No irritation	**No irritation**	Mild irritation	Moderate irritation

❖ EVALUATION STUDY

After formulation the evaluation is done on the five batches (A, B, C, &D)

The result of evaluation studies is shown in following table.

Fig. no.24 pH of Formulation.

Fig. no. 25 Consistency test.

Fig. no.26 Organoleptic Evaluation.

Fig. no. 27 Skin irritancy test.

❖ CONCLUSION

The present study successfully formulated and evaluated an herbal wound healing ointment containing powder of Marigold (*Calendula officinalis*) and Sunflower (*Helianthus annuus*) seed cake. Four different batches (A–D) were prepared using varying concentrations of the herbal powders and evaluated for physicochemical properties, stability, spreadability, and wound healing activity. Among all the formulations, Batch B showed the most promising results with superior wound healing activity compared to other batches. The enhanced effectiveness of B may be attributed to the optimum concentration and synergistic action of bioactive constituents such as flavonoids, tannins, and antioxidants present in marigold and sunflower seed cake. These phytochemicals are known to promote anti-inflammatory, antimicrobial, and collagen-stimulating effects, which accelerate tissue regeneration and wound contraction. The evaluation parameters confirmed that the B formulation possessed good consistency, acceptable pH, better spreadability, and making it suitable for topical application. The study demonstrates that herbal ingredients can be effectively incorporated into ointment formulations to produce a safe, natural, and cost-effective wound healing preparation.

Therefore, the B batch formulation can be considered the optimized formulation and has significant potential for further pharmacological evaluation and possible development as an herbal wound healing therapeutic agent.

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