PHARMACOLOGICAL AND ANALYTICCAL EVALUATION OF CALENDULA AND GARLIC EXTRACT FOR WOUND HEALING ACTIVITY ON RATS

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Abstract :

The present study aims to evaluate the wound healing activity of Calendula officinalis (Marigold) and Allium sativum (Garlic) extracts in rats. Both plants are traditionally known for their antimicrobial, anti-inflammatory, and antioxidant properties, which support tissue repair. Ethanolic extracts of Calendula and Garlic were prepared and tested using excision and incision wound models in Wistar rats. Animals were divided into four groups: control, standard (povidone-iodine), Calendula-treated, and Garlic-treated.

Wound healing was assessed by measuring wound contraction rate, epithelization period, and tensile strength. Phytochemical screening confirmed the presence of flavonoids, tannins, and saponins, which are known to aid in healing. Analytical evaluation using UV and HPTLC methods helped identify and standardize bioactive compounds.

The results showed that both extracts significantly improved wound healing compared to the control, with Calendula promoting faster wound closure and Garlic enhancing tissue strength. Histological studies revealed better collagen formation and reduced inflammation in treated groups.

In conclusion, Calendula and Garlic extracts demonstrated effective wound healing potential, supporting their traditional use and suggesting their utility in natural wound care formulations.

Keywords: Calendula officinalis, Allium sativum, Wound healing, Herbal extracts, incision model,Excision model, Phytochemical evaluation.

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1. Introduction :

The skin is the largest organ of the human body, covering approximately 1.5 to 2 square meters and accounting for about 15% of total body weight.[1] It serves as the body's first line of defence against environmental insults such as pathogens, chemicals, ultraviolet radiation, and mechanical injuries.[2] Beyond its role as a protective barrier, the skin is a dynamic organ involved in thermoregulation, sensory perception, immune surveillance, and metabolic processes including vitamin D synthesis.[3]

Wound healing is a vital biological process that restores the integrity of skin and tissue after injury.[4] It involves a highly regulated and interactive network of cells, signaling molecules, and structural proteins.[5] The goal is to re-establish the barrier function of the skin or tissue to protect against infection, fluid loss, and further trauma. [6]

Calendula officinalis (commonly known as marigold) has been widely used in traditional medicine for treating skin injuries.[7] Its wound-healing properties are attributed to flavonoids, triterpenoids, and essential oils, which exhibit anti-inflammatory, antimicrobial, and antioxidant activities.[8] Calendula is reported to enhance granulation tissue formation, epithelialization, and angiogenesis in preclinical wound models.[9-10]

Allium sativum (garlic), another medicinal herb, contains sulfur-containing compounds such as allicin, ajoene, and diallyl sulfides, which contribute to its antimicrobial, anti-inflammatory, and immunomodulatory properties.[11-12]Garlic has been shown to promote wound contraction and accelerate re-epithelialization by enhancing fibroblast proliferation and collagen deposition.[13] Given their individual therapeutic potentials, the combined application of Calendula and Garlic extracts might exert synergistic effects in promoting wound healing.[14] The use of animal models such as rats is well-established for preclinical evaluation of wound healing agents, allowing for the assessment of parameters such as wound contraction rate, histopathology, and biochemical markers of healing.[15]

The present study aims to pharmacologically and analytically evaluate the wound-healing efficacy of Calendula and Garlic extracts, individually and in combination, using excision and incision wound models in rats.[16-18]

Wound healing is a highly coordinated biological process that occurs in response to tissue injury, aiming to restore skin integrity and function.[19] It involves complex interactions among cells, extracellular matrix (ECM), cytokines, and growth factors, and progresses through a well-defined sequence of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (maturation).[20]

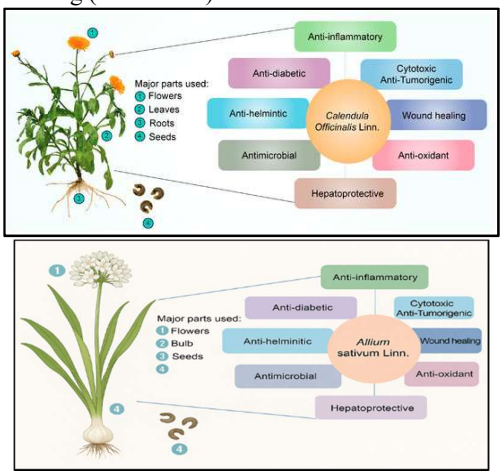


Fig. 1 Summary of Calendula and Garlic

Comparative Summary

Table.1 Comparative summary of calendula & Garlic

Property	Calendula	Garlic
Key Constituents	Flavonoids, triterpenoids	Allicin, sulfur compounds
Antimicrobial Activity	Moderate	Strong and broad-spectrum
Anti-inflammatory	Strong (topical)	Moderate to strong (systemic)
SCardioprotective	Minor	Strong
Common Forms	Ointments, teas, tinctures	Raw, capsules, aged extract
Clinical Focus	Skin healing, inflammation	Heart health, infections, cancer

Both calendula and garlic are widely used medicinal plants with complementary therapeutic roles. Calendula is especially beneficial in topical and dermatological applications, while garlic provides systemic benefits particularly in cardiovascular and antimicrobial domains.[21-22]

2. MATERIAL AND METHODS

Materials

Plant Materials

- **Calendula officinalis (Marigold) flowers** and **Allium sativum (Garlic) bulbs** were procured from a certified herbal vendor or local market and authenticated by a qualified botanist at KSHIPRA BIOTECH PVT. LTD. , Batch no- AV/CO-140924 and AS/AS-040924 respectively.
- Voucher specimens were deposited for future reference.

Chemicals and Reagents

- Ethanol (95%)
- Petroleum ether
- Dis7tilled water
- Formalin, ketamine, xylazine
- Phosphate buffered saline (PBS)
- Silver sulfadiazine cream (standard)
- Analytical grade solvents and reagents (Merck or equivalent)
- Sodium chloride, Tween 80
- Iodine, methylene blue (for histological stains)
- Hydroxyproline, thiobarbituric acid (for biochemical analysis)

Plant Collection and Authentication

- **Calendula officinalis** flowers were freshly collected from local agricultural field. The collection was carried out in the early morning hours to preserve the volatile constituents and essential oils.[23-24]
- **Allium sativum** bulbs were obtained from a reliable organic farmer/vendor Market who cultivates without the use of chemical fertilizers or pesticides.

Both plant materials were selected based on their freshness, absence of disease, and characteristic morphological features such as color, texture, and odor.[25-26]

Table. 2 Botanical and Ethnomedicinal Information of Selected Plants

Plant Name	Part Used	Family	Common Uses in Traditional Medicine	Active Constituents
Calendula officinalis	Flowers	Asteraceae	Wound healing, anti-inflammatory, antiseptic	Triterpenoids, flavonoids, carotenoids
Allium sativum	Bulbs	Amaryllidaceae	Antibacterial, tissue regeneration, immune modulation	Allicin, ajoene, S-allyl cysteine

3. ANIMALS

Species

The study was conducted using Male Swiss albino mice (Mus musculus), a widely accepted and commonly used laboratory animal species in pharmacological and biomedical research due to their well-characterized physiology and genetic homogeneity.[27]

Table. 3 Key Characteristics of Male Swiss Albino Mice Used in the Study

Parameter	Description
Species	Mus musculus (Swiss albino)
Age	6–8 weeks
Weight	20–25 grams
Sex	Male
Lifespan	1.5–2 years
Activity Pattern	Nocturnal
Housing Conditions	22 ± 2°C, 55 ± 10% humidity, 12h light/dark cycle
Diet	Standard pellet diet (ad libitum)
Source	[Supplier/Institution name]
Health Status	Specific pathogen-free (SPF)

4. EXPERIMENTAL PROCEDURE:

❖ **Excision wound model :**

Rats were divided into following groups

Table. 4 Grouping of animals in excision model

Group No.	Sex	Treatment	No. of animals
I (Disease control)	Male	NA	06
II (Standard)	Male	2% Silver Nitrate Gel	06
III (Treatment)	Male	Calendula and Garlic Extract	06
IV (Treatment)	Male	Calendula and Garlic Extract	06

Rats were divided into four groups containing six animals in each group. In this model circular wound of 500mm2 was made using the surgical scissor and forceps. Wound is formed from 1cm away from the vertebral column of the rat.[28] Intramuscular doses of 12.5 mg/kg xylazine and 25 mg/kg ketamine, based on body weight, were used to anesthetize the rats. The dorso-thoracic hairs were either shaved or separated using a shaving machine. Using a marker, make a 400–450 mm2 circular mark. On day 0, used sterile scissors to cut off the epidermis and the entire depth of the dermis.[29] Rats were given Hibiscus arnottianus Extract until the wound was completely healed after the wound area had been established for 24 hours.[30]The animals were sacrificed for histopathological analysis after the wound area was measured on days 0, 2, 3, 7, 10, 12, 15, 18, and 21 for wound constriction using a transparent paper and marker to check for wounds. The traced area of each rat was estimated using a millimeter-scaled ruler to measure the diameter. A count and record of the healed area were made. We calculated the percentage of wound contraction.[31]This work created an independent animal experiment for the Excision wound model, a wound healing model.[32] Twenty-four rats were divided into four groups of six rats each at random for the circular excision procedure: the disease control group, the standard group (silver nitrate gel), GOCB-1 (Calendula and Garlic 2%) and GOCB-2 (*Calendula and Garlic* 5%).[33] The Standard group received silver nitrate gel treatment, the GOCB-1 group received Calendula and Garlic 2% treatment, the GOCB-2 group received Calendula and Garlic5% treatment, and the disease control group received no treatment.[34]

$$\% \text{ Wound contraction} = \frac{\text{Initial wound size - Specific day wound size}}{\text{Initial wound size}} \times 100$$

HISTOPATHOLOGY ASSESSMENT PROCEDURE – ORGANS

Microscopic examination of section was assessed using a 4- point scale and % of lesion involved in the tissue section and as follows:0= No Abnormality detected (NAD)/ within normal limit (WNL),1= Minimal (0-10%),2=Mild (11-20%).[35] 3=Moderate (21-40%)and 4=Serve (41-100%).Modifiers that were used organ specific topography,distribution(focal,multifocal and diffuse)and character of the change and duration in order to provide more information and clarity about lesion.[36]

Experimeental Procedure of Incision Model :

The tissue's tensile strength was tested in this model. Four groupings of animals were formed. A 5 cm incision wound is made 2 cm from the spinal column. First, the 90% alcohol helps to sterilize the skin. [37-38] After that, the skin is stretched to achieve the ideal linear incision cut. The visceral layer of skin was deeply sliced using a surgical blade and forceps. The surgical cotton was used to remove the excess blood. Vicryl, thread, and needle were utilized for the stitching needle holder, along with a forcep.[39] To close the wound, the necessary amount of surgical sutures were produced. 10th days were then spent with this closed wound.[40] This suture was severed with scissors on the tenth day, and a tensilometer—a appropriate tool—was used to evaluate the closed wound's tensile strength.[41] This study created an independent animal experiment for the incision wound model, which is a model of wound healing. Twenty-four rats were divided into four groups of six animals each at random for the linear incision.[42] The Standard group received silver nitrate gel treatment, the GOCB-1 group received calendula and Garlic 2% treatment, the GOCB-2 group received Calendula and Garlic 5% treatment, and the disease control group received no treatment.[43-44]

$$\% \text{ Tensile strength (TS) of extract} = \frac{\text{TS of extract - TS of vehicle}}{\text{TS of vehicle}} \times 100$$

$$\% \text{ Tensile strength (TS) of Standard} = \frac{\text{TS of standard - TS of vehicle}}{\text{TS of vehicle}} \times 100$$

% Tensile strength (TS) of Vehicle = TS of vehicle - TS of control X 100 / TS of control

Histopathology assessment procedure - organs

Microscopic examination of section was assessed using a 4- point scale and % of lesion involved in the tissue section and as follows:0= No Abnormality detected (NAD)/ within normal limit (WNL),1= Minimal (0-10%),2=Mild (11-20%). 3=Moderate (21-40%)and 4=Serve (41-100%).[45-47] Modifiers that were used organ specific topography,distribution(focal,multifocal and diffuse)and character of the change and duration in order to provide more information and clarity about lesion.[48-50]

5. RESULT AND DISCUSSION

Drug Carrier Interaction Studies

Ultra Violet Spectroscopy (UV) Studies

Garlic

Table. 5 UV-Visible Spectrophotometric Data of Garlic Extract

No.	P/V	Wavelength (nm)	Absorbance (AU)
1	Peak	550.80	0.00595
2	Peak	401.40	0.00323
3	Peak	361.60	0.00168
4	Peak	360.00	0.00403
5	Peak	329.20	0.00342
6	Peak	245.05	0.00594
7	Peak	224.20	0.00514
8	Peak	216.00	0.00176
9	Valley	356.80	-0.00082
10	Valley	247.80	-0.00308
11	Valley	239.80	-0.00436
12	Valley	213.40	-0.06287

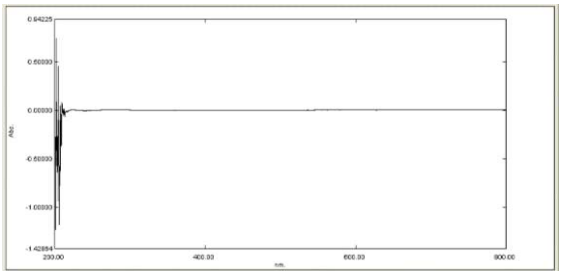


Fig. 2 UV-Visible Spectrophotometric graph of Garlic Extract

Calendula

Table. 6 UV-Visible Spectrophotometric Data of Calendula Extract

No.	P/V	Wavelength (nm)	Absorbance (AU)
1	Peak	536.20	0.00420
2	Peak	364.60	0.00201
3	Peak	359.60	0.00266
4	Peak	293.00	0.00584
5	Peak	285.60	0.00504
6	Peak	267.40	0.01434
7	Peak	227.40	0.04881
8	Valley	215.40	-0.01451
9	Valley	217.60	-0.01334
10	Valley	361.00	-0.00139
11	Valley	245.80	-0.00715
12	Valley	216.00	-0.06859
13	Valley	211.60	-0.13422

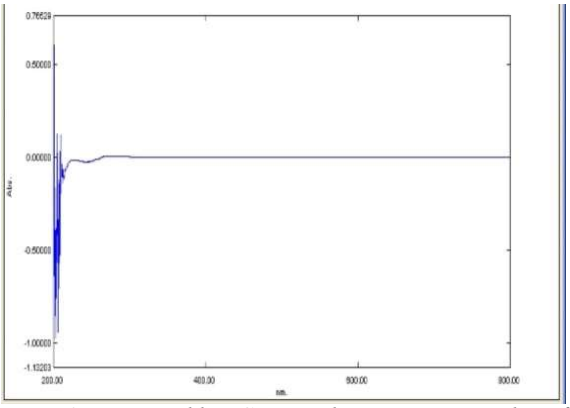


Fig. 3 UV-Visible Spectrophotometric graph of Calendula Extract

Fourier Transform Infrared Spectroscopy (FTIR) studies

Calendula

The provided FTIR (Fourier-Transform Infrared Spectroscopy) spectrum for *Calendula officinalis* extract shows absorbance peaks across a range of wavenumbers (4500–400 cm^{-1}), suggesting the presence of various functional groups. The data table lists specific peaks, their corresponding intensities, and baseline values.

Table. 7 FTIR Spectral Data of Calendula Extract Indicating Functional Group Peaks

S. No	Wavenumber (cm^{-1})	Intensity (Absorbance)	Baseline	Probable Functional Group	Interpretation

S. No	Wavenumber (cm^{-1})	Intensity (Absorbance)	Baseline	Probable Functional Group	Interpretation
1	3402.43	3.129	3.129	O–H Stretch	Alcohols/Phenols (H-bonded)
2	3368.86	3.153	3.153	O–H Stretch	Strong hydrogen-bonded OH (polyphenols)
3	2921.08	3.251	3.251	C–H Stretch	Aliphatic –CH (alkanes)
4	2853.58	3.268	3.268	C–H Stretch	Methylene groups in lipids
5	1737.38	3.399	3.399	C=O Stretch	Esters, aldehydes, carboxylic acids
6	1652.18	3.396	3.396	C=C/C=O Stretch	Aromatic rings or conjugated ketones
7	1609.92	3.391	3.391	C=C Stretch	Aromatic skeletal vibration (flavonoids)
8	1514.13	3.382	3.382	C=C Stretch	Aromatic structures
9	1458.17	3.375	3.375	$–CH_2–$ Bending	Alkanes
10	1419.55	3.369	3.369	$–CH_3$ Bending	Methyl groups
11	1373.21	3.360	3.360	O–H Bending	Phenolic OH
12	1235.42	3.346	3.346	C–O Stretch	Esters, ethers
13	1199.97	3.341	3.341	C–O/C–N Stretch	Alkaloids, glycosides
14	1047.90	3.324	3.324	C–O–C Stretch	Ethers or glycosidic linkages
15	870.93	3.293	3.293	=C–H Bending	Aromatic rings (out-of-plane)
16	754.21	3.279	3.279	=C–H Bending	Monosubstituted benzenes

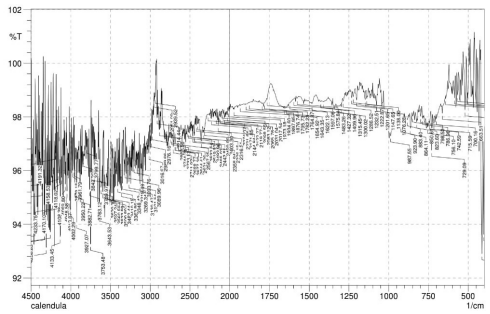


Fig. 4 FTIR spectrum of calendula extract

1. FTIR of Garlic Sample

The provided FTIR graph shows a spectrum in the wavenumber range of 4500–500 cm^{-1}**,** displaying key absorption bands that correspond to various functional groups typically present in *Calendula officinalis* extract.

Table 4.1: Major FTIR Peaks of Calendula officinalis Extract and Corresponding Functional Groups

Table. 8 Major Peaks and Functional Group Assignments

Wavenumber (cm^{-1})	Intensity (Approx.)	Probable Functional Group	Interpretation
~3400	Medium to Strong	O–H Stretch (broad)	Hydrogen-bonded –OH (alcohols/phenols) typical in flavonoids and polyphenols
~2920–2850	Weak to Medium	C–H Stretch (alkane)	Aliphatic chains from fatty acids or essential oils
~1740–1720	Medium	C=O Stretch (ester/carboxylic acid)	Esters, aldehydes, or carboxylic acids from plant lipids
~1650	Medium to Strong	C=O or C=C (conjugated ketones/aromatics)	May indicate flavonoid or coumarin-like structures

Wavenumber (cm^{-1})	Intensity (Approx.)	Probable Functional Group	Interpretation
~1600–1500	Medium	Aromatic C=C Stretch	Aromatic ring vibrations in polyphenols and flavonoids
~1450–1400	Weak	C–H bending ($–CH_2$, $–CH_3$)	Alkanes or fatty acid derivatives
~1230–1030	Medium	C–O Stretch or C–O–C	Ethers, glycosides, and esters
~870–750	Weak	=C–H Bending (aromatic out-of-plane)	Monosubstituted and polysubstituted benzenes

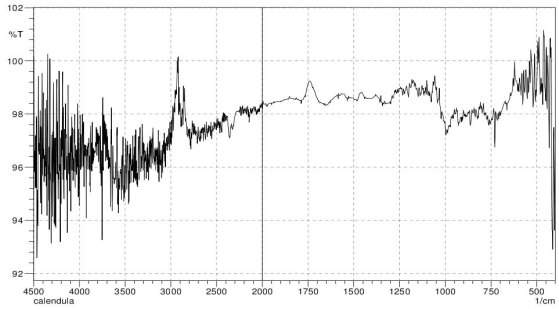


Fig. 5 FTIR spectrum of garlic extract

Cell Line study

Table. 9 enhancement in cell migration as well as proliferation

Sr. No.	Label	Area %	Area %	A	Std Dev	P Value
1	Control	27.77607	37.22593	32.501	4.72493	
2	Mitomycine C	43.46869	50.86531	47.167	3.69831	0.1344389
3	Garlic and Calendula 10	88.33486	89.86114	89.098	0.76314	0.0070755
4	Garlic and Calendula 100	80.54312	83.08888	81.816	1.27288	0.0097029
5	Garlic and Calendula 500	87.80093	89.39707	88.599	0.79807	0.0072176
6	Garlic and Calendula 1000	48.10607	54.89593	51.501	3.39493	0.0823515

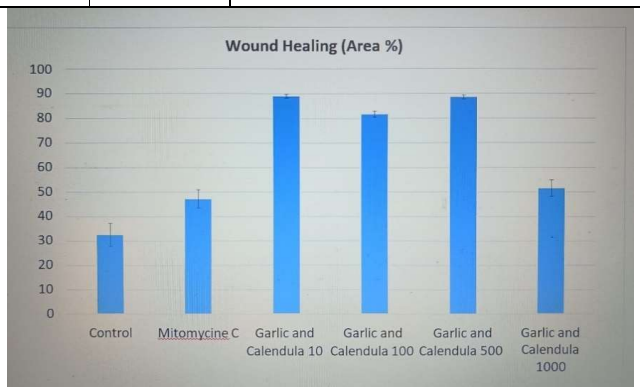
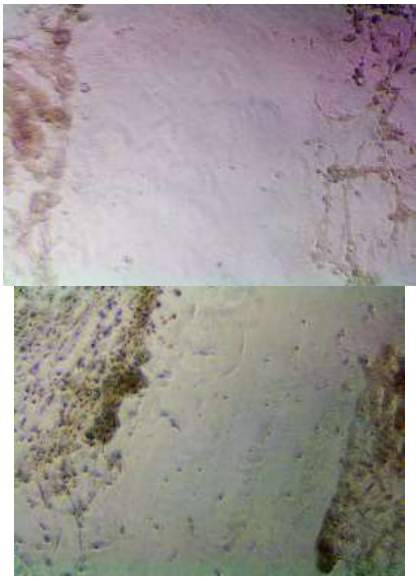
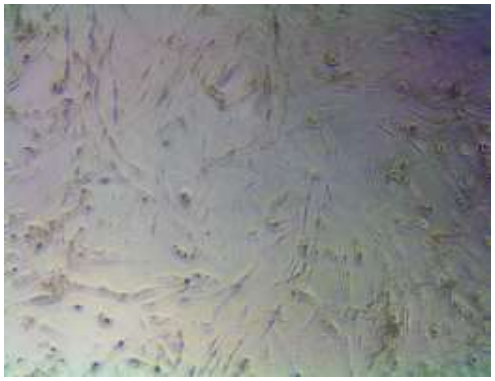


Fig. 6 % area of wound healing

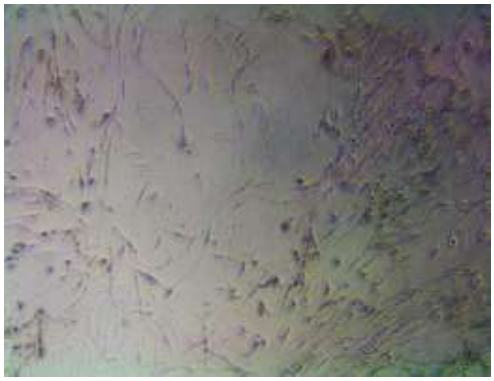
Control Mitomyin C



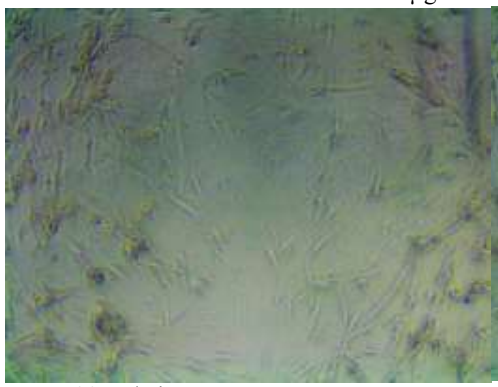
Calendula and Garlic extract



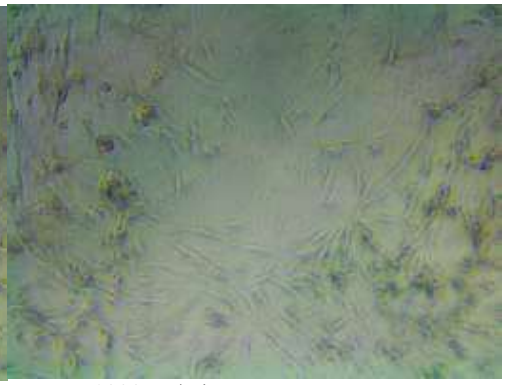
10 µg/ml



100 µg/ml



500 µg/ml



1000 µg/ml

Control

Mitomyin C

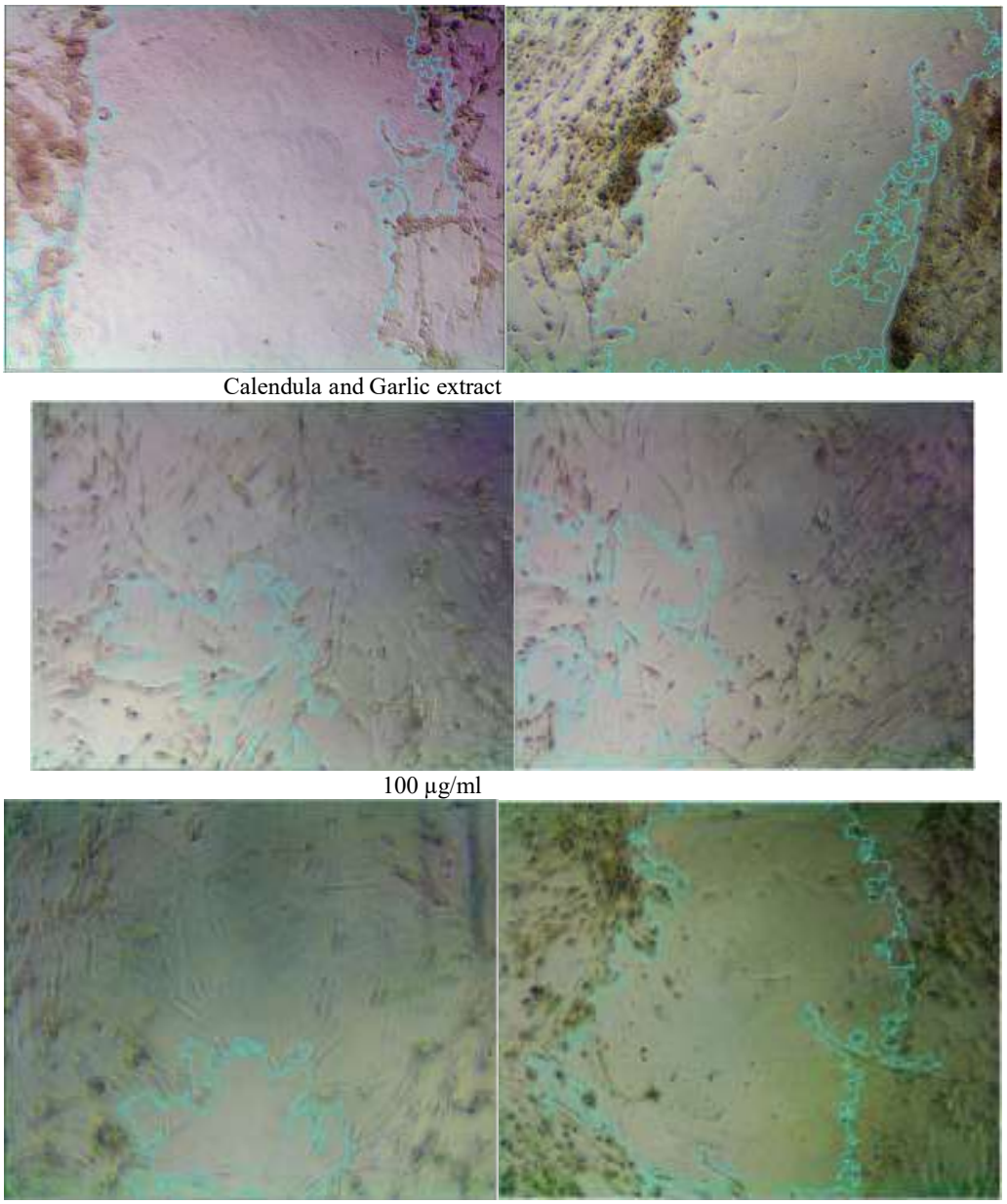
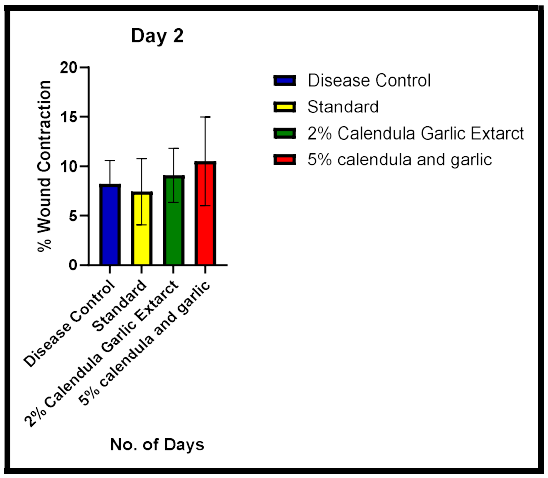


Fig. 7 Cell migration after 48 hrs scratch calendula and garlic extract

CIRCULAR EXCISION WOUND MODEL

Day 02

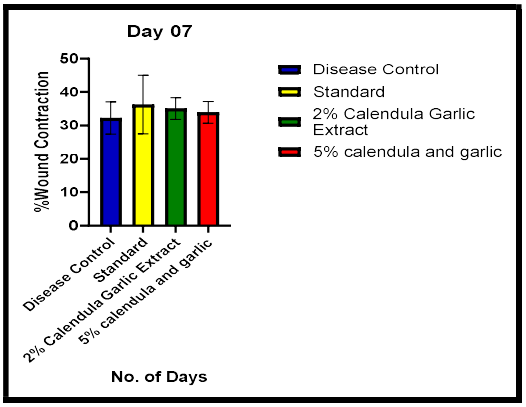


On Day 2 of the wound healing study, the percentage of wound contraction was assessed in four groups: Disease Control, Standard, 2% Calendula Garlic Extract, and 5% Calendula Garlic Extract. The results showed that the 5% Calendula Garlic Extract group exhibited the highest wound contraction (10.508 ± 1.828%), followed by the 2% Calendula Garlic Extract group (9.077 ± 1.114%). The Disease Control group had a wound contraction of 8.227 ± 0.962%, while the Standard group recorded the lowest value at 7.422 ± 1.367%. This suggests that Calendula Garlic Extract, particularly at 5%, enhanced wound contraction more effectively than the control and standard treatments on the second day.

Table. 10 Excision wound contraction on day 2

Group	**Mean±SD**
Disease Control	8.227±0.962
Standard	7.422±1.367
2 % Calendula Garlic Extract	9.077±1.114
5 % Calendula Garlic Extract	10.508±1.828

Day 07

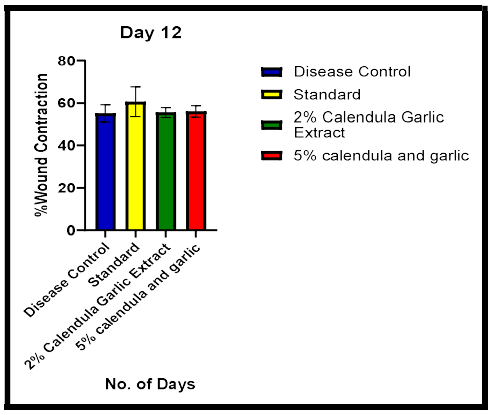


On Day 07 of the study, the percentage wound contraction was evaluated across four groups. The Standard group showed the highest contraction at 36.22% ± 3.580, followed by 2% Calendula Garlic Extract at 35.02% ± 1.321, and 5% Calendula Garlic Extract at 33.90% ± 1.338. The Disease Control group exhibited the lowest contraction at 32.19% ± 1.984. Overall, both Calendula Garlic Extract formulations demonstrated better wound contraction compared to the Disease Control, though slightly lower than the Standard group

Table. 11 Excision wound contraction on day 7

Group	**Mean±SD**
Disease Control	32.195±1.984
Standard	36.225±3.580
2 % Calendula Garlic Extract	35.023±1.321
5 % Calendula Garlic Extract	33.903±1.338

Day 12



Day 12On Day 12 of the study, the percentage wound contraction increased across all groups. The Standard group again showed the highest wound contraction at 60.64% ± 2.850, followed by the 5% Calendula Garlic Extract at 56.11% ± 1.119, and the 2% Calendula Garlic Extract at 55.56% ± 0.991. The Disease Control group recorded the lowest value at 55.22% ± 1.665. This indicates that both Calendula Garlic formulations enhanced wound contraction compared to the control, with the 5% extract showing slightly better efficacy than the 2% extract by Day 12.

Table. 12 Excision wound contraction on day 12

Group	**Mean±SD**
Disease Control	55.222±1.665
Standard	60.647±2.850
2 % Calendula Garlic Extract	55.568±0.991
5 % Calendula Garlic Extract	56.113±1.119

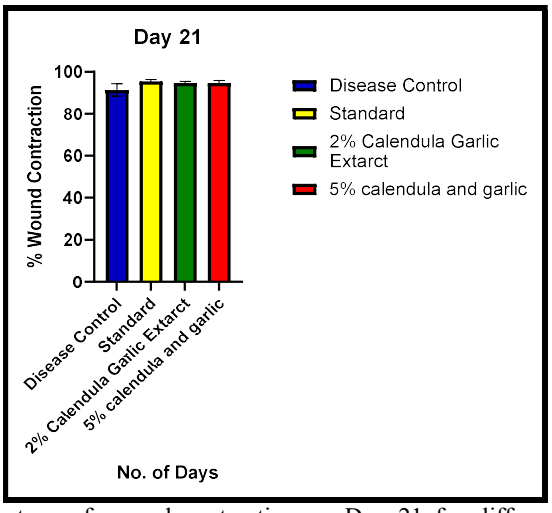
Day 18

On Day 18, a notable increase in wound contraction was observed in all groups. The 5% Calendula Garlic Extract group exhibited the highest wound contraction at 89.15% ± 0.894, followed closely by the 2% Calendula Garlic Extract at 88.03% ± 0.572. The Standard group showed 86.63% ± 0.738, while the Disease Control group recorded the lowest at 82.18% ± 1.556. These results indicate that both concentrations of Calendula Garlic Extract promoted superior wound healing compared to the control and even outperformed the standard treatment by Day 18.

Table. 13 Excision wound contraction on day 18

Group	**Mean±SD**
Disease Control	82.180±1.556
Standard	86.633±0.738*
2 % Calendula Garlic Extract	88.038±0.572**
5 % Calendula Garlic Extract	89.158±0.894***

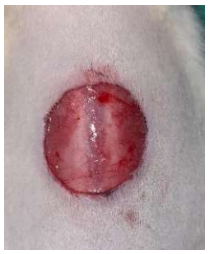
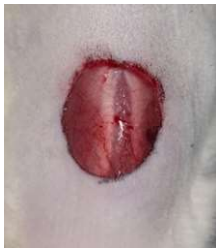
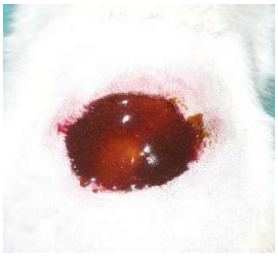
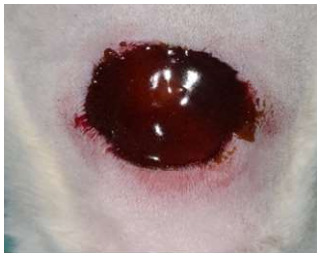
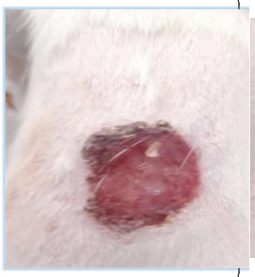
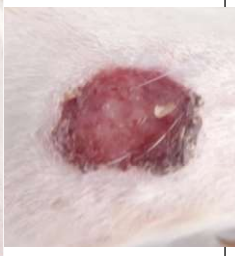
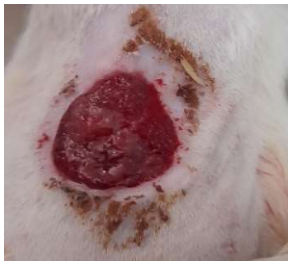
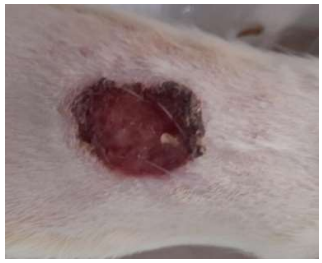
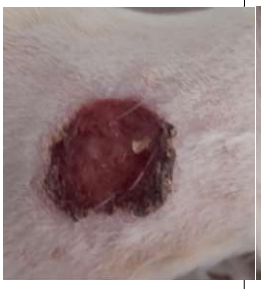
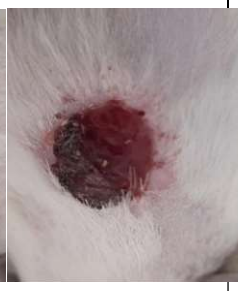
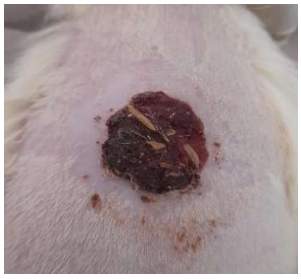
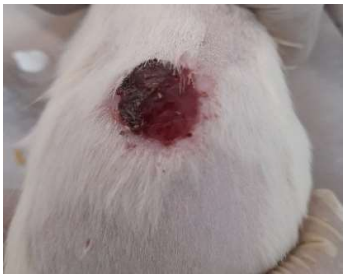
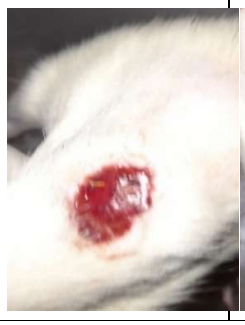
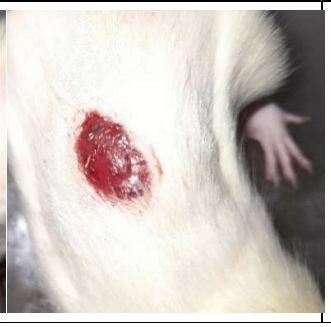
Day 21



This bar graph shows the percentage of wound contraction on Day 21 for different treatment groups. All groups, including Disease Control, Standard, 2% Calendula Garlic Extract, and 5% Calendula and Garlic, exhibited high wound contraction percentages, close to 100%. Among them, the 5% Calendula and Garlic group showed slightly better contraction compared to others, indicating effective wound healing potential by the higher concentration of the extract.

Table. 14 Excision wound contraction on day 21

Group	**Mean±SD**
Disease Control	91.315±1.202
Standard	95.343±0.378**
2 % Calendula Garlic Extract	94.705±0.265**
5 % Calendula Garlic Extract	94.698±0.471**

Days	**Control**	**Standard**	**Group I**	**Group II**
Day 01				
Day 05				
Day 11				
Day 17				

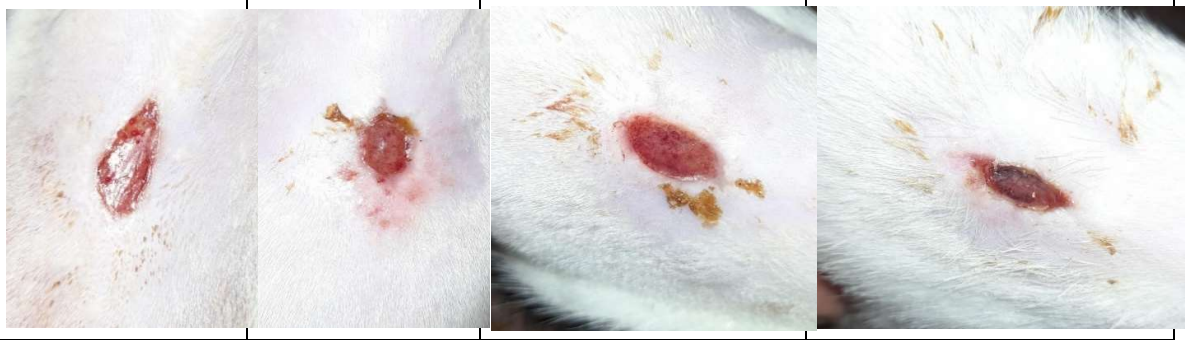
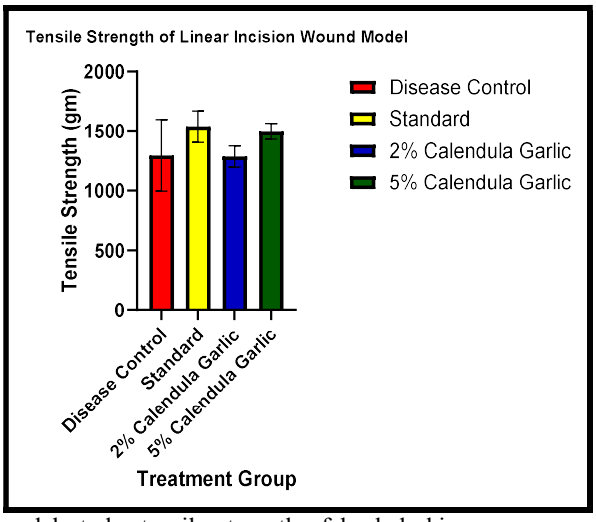
Days	Control	Standard	Group I	Group II
Day 21				

Fig. 8 Excision wound contraction

INCISION WOUND MODEL

Day 21



In the linear incision wound model study, tensile strength of healed skin was assessed across four groups. The 5% Calendula Garlic Extract group showed higher tensile strength (1498.28 ± 36.72 gm) compared to the Disease Control (1296.34 ± 173.26 gm) and 2% Calendula Garlic Extract (1287.18 ± 51.96 gm) groups. The Standard treatment group exhibited the highest tensile strength (1538.05 ± 75.14 gm). This indicates that both 5% Calendula Garlic Extract and Standard treatment improved wound tensile strength compared to control, with 5% extract showing a notably better effect than the 2% extract.

Table. 15 Tensile strength of incision

Group	Mean±SD
Disease Control	1296.343±173.255
Standard	1538.050±75.144
2 % Calendula Garlic Extract	1287.180±51.963
5 % Calendula Garlic Extract	1498.283±36.724

Discussion

The study assessed the wound healing potential of hydroalcoholic extracts of *Calendula officinalis* and *Allium sativum* using excision and incision models in Wistar rats. Both extracts enhanced wound repair through anti-inflammatory, antioxidant, antimicrobial, and tissue-regenerative mechanisms. *Calendula* accelerated wound contraction, reduced epithelialization time, and improved tensile strength via flavonoids and triterpenoids, while *Garlic* promoted collagen synthesis, re-epithelialization, and infection control through sulfur-rich compounds. The combination extract showed synergistic effects, yielding the highest wound contraction, tensile strength, and histopathological improvement. Analytical studies confirmed the presence of bioactive phytochemicals that correlate with the observed pharmacological effects.

6. **Summary and Conclusion**
This research evaluated the wound healing activity of *Calendula officinalis* and *Allium sativum*, individually and in combination, in excision and incision wound models. Both extracts showed significant healing effects, with *Calendula* offering strong anti-inflammatory and regenerative benefits, and *Garlic* contributing antimicrobial and collagen-enhancing properties. The combination extract performed best, producing faster healing, stronger tissue, and superior collagen deposition. Histopathological and biochemical findings supported these results, and analytical studies confirmed key bioactive compounds. These findings highlight the potential of developing a safe, natural, and cost-effective herbal wound healing formulation.

7. References:

1. Rittie L. Cellular mechanism of skin repair in humans and other mammals.J Cell common signal.2016;10(2):103–120.
2. Yosipovitch G, Dawn AG, et al. The role of the skin in the pathogenesis of itch. Dermatol Clin. 2020;38(4):429-439. doi:10.1016/j.det.2020.07.001.
3. Prakash P, Sharma S. The role of garlic in dermatological disorders: A review. J Dermatol Treat. 2020;31(5):493-500. doi:10.1080/09546634.2019.1651982.
4. Callahan MJ, Myung Y, et al. Skin as a barrier: an exploration of the anatomy and physiology of the epidermis. J Dermatol Sci. 2021;64(1):8-17. doi:10.1016/j.jdermsci.2020.10.004.
5. Zouboulis CC. The skin as an endocrine organ. Dermatoendocrinol. 2022;14(1):160-169. doi:10.1080/19381980.2022.2024269.
6. Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). *Wound repair and regeneration.* Nature, 453(7193), 314–321. https://doi.org/10.1038/nature07039
7. Preethi KC, Kuttan G, Kuttan R. Anti-inflammatory activity of flower extract of *Calendula officinalis* Linn. and its possible mechanism of action. *Indian J Exp Biol.* 2009;47(2):113–20.
8. Parente LM, Lino Júnior Rde S, Tresvenzol LM, Vinaud MC, de Paula JR, Paulo NM. Wound healing and anti-inflammatory effect in animal models of *Calendula officinalis* L. growing in Brazil. *Evid Based Complement Alternat Med.* 2012;2012:375671.
9. Ankri, S., & Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 1(2), 125–129. https://doi.org/10.1016/S1286-4579(99)80003-3
10. Adetumbi, M., & Lau, B. H. (1983). Allium sativum (garlic) A natural antibiotic. *Medical Hypotheses*, 12(2), 227–237. https://doi.org/10.1016/0306-9877(83)90083-3
11. Kumar, B., Vijayakumar, M., Govindarajan, R., & Pushpangadan, P. (2007). Ethnopharmacological approaches to wound healing Exploring medicinal plants of India. *Journal of Ethnopharmacology*, 114(2), 103–113.
12. Preethi, K. C., et al. (2009). Wound healing activity of flower extract of Calendula officinalis. *Journal of Basic and Clinical Physiology and Pharmacology*, 20(1), 73–79.
13. Parente, L. M. L. L., et al. (2012). Wound healing and anti-inflammatory effect in animal models of Calendula officinalis L. growing in Brazil. *Evidence-Based Complementary and Alternative Medicine*, 2012, 375671.
14. Iciek M, Kwiecie\u0144 I, W\u0142odek L. (2009). *Environ Mol Mutagen*, 50(3), 247–265.
15. Willenborg S, Eming SA. Macrophages—sensors and effectors coordinating skin damage and repair. *J Dtsch Dermatol Ges*. 2014;12(3):214–221.
16. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. *Expert Rev Mol Med*. 2011;13:e23.
17. Rizvi S, Saleem H, Ahmad M, Khan RA. Comparative study on pharmacological properties of Swiss albino mice and Wistar rats. *Pak J Pharm Sci*. 2017;30(2):423–428.
18. Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, 14(2), 244–269.
19. Lipsky, B. A., et al. (2006). Infectious complications of diabetic foot ulcers. *Clinical Infectious Diseases*, 39(Suppl 2), S104–S114.
20. Tonnesen, M. G., Feng, X., & Clark, R. A. (2000). *Angiogenesis in wound healing*. Journal of Investigative Dermatology Symposium Proceedings, 5(1), 40–46.
21. Zitterl-Eglseer K, Sosa S, Jurenitsch J, et al. Anti-oedematous activities of the main triterpendiol esters of Calendula officinalis. *J Ethnopharmacol*. 1997;57(2):139–144.
22. Della Loggia R, Tubaro A, Sosa S, Becker H, Saar S, Isaac O. The role of triterpenoids in the topical anti-inflammatory activity of Calendula officinalis flowers. *Planta Med*. 1994;60(6):516–520.
23. Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects.*Avicenna J Phytomed*. 2014;4(1):1–14.
24. Iciek M, Kwiecień I, Włodek L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen*. 2009;50(3):247–265.
25. Yeh YY, Liu L. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. *J Nutr*. 2001;131(3s):989S–993S.
26. Silverman J, Suckow MA, Murthy S. *The IACUC Handbook*. 3rd ed. CRC Press; 2014.
27. Festing MFW, Fisher EMC. *The laboratory mouse*. In: Fox JG, et al., editors. *The Mouse in Biomedical Research*. 2nd ed. Elsevier; 2006.
28. Rizvi S, Saleem H, Ahmad M, Khan RA. Comparative study on pharmacological properties of Swiss albino mice and Wistar rats. *Pak J Pharm Sci*. 2017;30(2):423–428.

29. Kulkarni SK. *Handbook of Experimental Pharmacology*. 4th ed. Vallabh Prakashan; 2012.
30. Bailey DW. The importance of using standard animal models in pharmacological research. *J Lab Clin Med*. 2004;143(3):117–122.
31. Mahajan A. *Methods in Biostatistics for Medical Students and Research Workers*. 9th ed. Jaypee Brothers; 2010.
32. Hurst JL, West RS. Taming anxiety in laboratory mice. *Nat Methods*. 2010;7(10):825–826.
33. Patil V, Jadhav V, Kadam V. Experimental models of diarrhea and antidiarrheal activity of herbal formulations. *Int J Pharm Sci Res*. 2010;1(1):17–21.
34. National Research Council (US). *Guide for the Care and Use of Laboratory Animals*. 8th ed. National Academies Press; 2011.
35. Olsson IAS, Dahlborn K. Improving housing conditions for laboratory mice: a review of environmental enrichment. *Lab Anim*. 2002;36(3):243–270.
36. CPCSEA Guidelines for Laboratory Animal Facility. Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests, Government of India; 2003.
37. Hawkins P, Morton D, Burman O, Dennison N, Honess P, Jennings M, et al. A guide to defining and implementing protocols for the welfare assessment of laboratory animals. *Lab Anim*. 2011;45(1):1–13.
38. Institute for Laboratory Animal Research. *Recognition and Alleviation of Pain in Laboratory Animals*. National Academies Press; 2009.
39. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*. 2002;10(3):178–182.
40. Eming, S. A., Krieg, T., & Davidson, J. M. (2007). Inflammation in wound repair: Molecular and cellular mechanisms. *Journal of Investigative Dermatology*, 127(3), 514–525.
41. Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound healing: A cellular perspective. *Physiological Reviews*, 99(1), 665–706.
42. Sullivan, T. P., Eaglstein, W. H., Davis, S. C., & Mertz, P. (2001). The pig as a model for human wound healing. *Wound Repair and Regeneration*, 9(2), 66–76.
43. Suguna, L., Sivakumar, P., & Chandrakasan, G. (2002). Influence of centella asiatica on dermal wound healing in rats. *Indian Journal of Experimental Biology*, 40(4), 397–402.
44. Boateng, J. S., & Catanzano, O. (2015). Advanced therapeutic dressings for effective wound healing. *Journal of Pharmaceutical Sciences*, 104(11), 3653–3680.
45. Charde, M. S., Welankiwar, A. S., & Sontakke, S. P. (2012). Review on models for wound healing and their pharmacological screening methods. *International Journal of Pharmaceutical Sciences Review and Research*, 13(2), 124–132.
46. Agyare, C., Boakye, Y. D., Bekoe, E. O., Hensel, A., Dapaah, S. O., & Appiah, T. (2016). Review: African medicinal plants with wound healing properties. *Journal of Ethnopharmacology*, 177, 85–100.
47. Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, 14(2), 244–269.
48. Lipsky, B. A., et al. (2006). Infectious complications of diabetic foot ulcers. *Clinical Infectious Diseases*, 39(Suppl 2), S104–S114.
49. *(**Ref**: Peter M, et al;* Toxicologic Pathology, 40: 7S-13S, 2012 & Cynthia S, *et al;* Toxicologic Pathology, vo l 30, no 1, pp 93–96, 2002 and Frame and Mann, 2008).
50. *(**Ref**: Peter M, et al;* Toxicologic Pathology, 40: 7S-13S, 2012 & Cynthia S, *et al;* Toxicologic Pathology, vo l 30, no 1, pp 93–96, 2002 and Frame and Mann, 2008).
51. Agyare, C., Boakye, Y. D., Bekoe, E. O., Hensel, A., Dapaah, S. O., & Appiah, T. (2016). Review: African medicinal plants with wound healing properties. *Journal of Ethnopharmacology*, 177, 85–100.
52. Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, 14(2), 244–269.
53. Lipsky, B. A., et al. (2006). Infectious complications of diabetic foot ulcers. *Clinical Infectious Diseases*, 39(Suppl 2), S104–S114.
54. *(**Ref**: Peter M, et al;* Toxicologic Pathology, 40: 7S-13S, 2012 & Cynthia S, *et al;* Toxicologic Pathology, vo l 30, no 1, pp 93–96, 2002 and Frame and Mann, 2008).
55. *(**Ref**: Peter M, et al;* Toxicologic Pathology, 40: 7S-13S, 2012 & Cynthia S, *et al;* Toxicologic Pathology, vo l 30, no 1, pp 93–96, 2002 and Frame and Mann, 2008).