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Assessment of the Yemeni Herbal Formulations containing *Azadirachta indica* Leaves Extract as a Wound Healing

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ABSTRACT

Background: Azadirachta indica, resemble as the neem plant, which used as alternative therapy in Asia as well as Africa, known for its antibacterial, antifungal, and anti-inflammatory properties. as its rich in biologically active compounds can be used as wound healing. *objective:* to assess and compare the wound healing activity of neem leaves obtained from two different regions. *Methodology*: Neem leaves were collected, dried, and extracted using ethanol via a rotary evaporator. Phytochemical screening and microbial testing were conducted. Cream and gel formulations were prepared and evaluated for physical properties. The wound healing activity was assessed by applying these formulations to rabbits. *Results: Azadirachta indica* shows the availability of phenols, alkaloids, flavonoids, tannins, saponins, and glycosides. The extract exhibited antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, as well as antifungal activity against Candida albicans. The cream formulations had appropriate pH and homogeneity. The application of the formulations on rabbits yielded positive and excellent wound healing results, surpassing those of standard and control treatments. *Conclusion:* The ethanolic extract of *Azadirachta indica* leaves exhibits powerful bactericidal and fungicidal properties, making it a useful agent for treating wounds and accelerating the healing process.

Key words: *Azadirachta indica*, Wound Healing, Antibacterial, Yemeni Herbal Medicine

1. INTRODUCTION

Historically, ethnomedicine has extensively utilized the plant's entire spectrum of parts, including its bark, fruit, leaves, stem, and roots, all of which possess therapeutic qualities. People recognise the use of herbal medicine and botanical extracts as a substitute for synthetic or pharmaceutical medications, often due to their reduced side effects. The evidence indicates that the application of herbal medicine

techniques aligns with a resurgence in natural remedies that typically have fewer or no side effects (Haque et al., 2022; Khadka et al., 2022). Since ancient times, human beings have unquestionably recognised the importance of medicinal plants. It can be inferred that early humans were somewhat aware of the properties of the plants they encountered and used for fuel, clothing, shelter, and sustenance prior to the onset of recorded history. Plants were frequently used as medicinal agents in ancient Persia (Hamilton et al., 2004). The designation of medicinal plants encompasses a diverse array of flora that possess therapeutic attributes. These plants provide a significant reservoir of compounds for the synthesis of pharmaceuticals (Hassan, 2012).

For thousands of years, this plant has been utilized not only as a preventative and curative measure for various ailments but also as a vegetable with high nutritional value. Over 50,000 plant species, which account for more than a tenth of all species, are utilised in pharmaceutical and cosmetic products. Nonetheless, the global distribution of medicinal plants is characterised by significant variability (Huang, 2011; Rafieian-Kopaei, 2012). It is extensively described in the Vedic literature for the treatment of various diseases (Al-Shami et al., 2022). The neem, scientifically known as *Azadirachta indica* has species of evergreen tree native to tropical regions of Indian subcontinent. This substance has been used as antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer, and antifungal properties. The material holds significant promise in the areas of pest management, environmental protection, and pharmaceutical drugs. Environmentally friendly insecticides, pesticides, and agrochemicals can be made naturally from neem. This tree, considered the most promising species of the twenty-first century, has been the subject of numerous studies. The tree has a remarkable ability to adapt to many soil-related, topographic, and climatic situations.

2. MATERIALS AND METHODS

Study design

The study was designed to compare the wound-healing and antimicrobial activities of *Azadirachta indica* leaf extract from two different regions (Taiz and Hudaydah) against microbial strains and for wound healing in rabbits.

Plant Extraction

The leaves of *Azadirachta indica* were collected, dried, and ground into powder. The powdered leaves were mixed with 95% ethanol in a 1:4 ratio (1000g of powder in 4 litters of ethanol) and left to macerate for five days (Bhat et al., 2007). The solution was passed through via a Buchner funnel with a mesh and the solvent was eliminated using a rotary vacuum evaporator to concentrate the extract. The concentrated extract was subsequently dried at room temperature and then in an oven, yielding a viscous green residue.

Preparation of sample

We prepared the samples by diluting 0.5g of the extract in 50ml of distilled water. We heated the mixture on a hot plate at 50°C. in addition, the pH of the samples that had been diluted was tested.

Antimicrobial assay

On the basis of the extract's antibacterial properties, tests were conducted against selected bacterial strains *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and a fungal strain *Candida albicans*. Agar plates were used to propagate the germs, and wells were made. The wells were filled with:

- 100µl of ethanol (negative control)
- 100µl and 200µl of *Azadirachta indica* extract
- Antibiotic discs (positive control)

An incubator set at 40°C was used to keep the plates covered for 24 hours, and the inhibition zones were quantified to assess antimicrobial activity.

Preparation of Animals

Animal Selection: Eight adult male rabbits with an average weight of 1kg were selected. They were housed in a sterilized environment and divided into three groups:

- Group A: 6 rabbits treated with *Azadirachta indica* formulations (cream and gel)

- Group B: 1 rabbit treated with MEBO (standard control)
- Group C: 1 rabbit treated with normal saline (negative control)

Wound Creation: The rabbits were anesthetized, and a 2cm area of skin was shaved and cleaned with 70% ethanol. A wound was created using a sterile razor.

Treatment Application: The wounds were dressed with gauze soaked in the respective treatment (cream, gel, MEBO, or normal saline) and secured with zinc oxide plaster. The treatments were applied daily for 10 days.

Macroscopic evaluation: The wound area and closure were measured at intervals (3rd, 6th, and 9th days using the formula: (Agren et al., 1997).

% of wound closure = (Wound Area on Day '0' − Wound Area on Day 'n') / Wound Area on Day '0' × 100

n = total periods

We used the pace of wound contraction and the achievement of full wound closure to measure the activity of wound healing.

3. RESULTS

The physical characteristics of the two locations' extracts are similar, indicating consistency in the quality of the plant material as shown in Table 1. The formulations are suitable for topical application, as their pH is compatible with the skin's natural pH, as shown in Table 2. Table 3 shows that the presence of these bioactive compounds explains the antimicrobial and wound healing properties of Neem, as these compounds are known for their therapeutic effects.

Table 1: The physical properties of *Azadiracta indica* leave extract.

	Taiz extract	**Hudaydah extract**
Color	Dark green	Dark green
Odor	Aromatic	Aromatic
Taste	Bitter	Bitter
Texture	Gummy	Gummy
Solubility	Slightly soluble in water	Slightly soluble in water

Table 2: The pH values of *Azadiracta indica* formulas from (Hudaydah and Taiz)

Regions	**Cream**	**pH value**	**Temp.**	**Gel**	**pH value**	**Temp.**
Hudaydah city	3% 5% 10%	6.87 7.84 7.84	29.6 31.4 31.4	3% 5% 10%	6.41 6.74 6.95	27.7 27.5 27.4
Taiz city	3% 5% 10%	7.59 7.05 6.20	35.1 34.1 34.8	3% 5% 10%	7.29 6.74 7.30	27.7 27.8 27.8

Temp. means temperature.

Table 3: Phytochemical Components

Phytochemical components	**Extracts *Azadirachta indica***
Tannins	++
Terpenoids	--
Saponin	++
Phenols	++
Alkaloids	++
Glycosides	++
Flavonoids	++
Steroids	--

(+) = presence, (-) = absence

Table 4 and 5 displays anti-microbial results of the Taiz, Hudaydah Neem extract against selected bacterial strains Pseudomonas aeruginosa, *E. coli, Staphylococcus aureus* and a fungal strain *Candida albicans*. The Taiz Neem extract has strong antibacterial and antifungal properties. While Table 5 shows the Hudaydah extract has good antimicrobial activity, though slightly less potent than the Taiz extract. Figures 1-4 confirm the results from Tables 4 and 5, demonstrating the antimicrobial efficacy of Neem extracts.

Table 4: Show the results of *Azadirachta indica* as antibacterial of Taiz extract

Name of Bacteria	***P. Aeruginosa***	***E. coli***	***Staph. Aureus***	***Candida albicans***
Extract 200 µl	30 mm	20 mm	23 mm	15 mm
Extract 100 µl	13 mm	15 mm	18 mm	1 mm
Ethanol 96%	_	_	_	_
Amoxic Clave	_	_	20 mm	_
Gentamycin	32 mm	25 mm	38 mm	_
Nystatin	_	_	_	25 mm

Table 5: Show the result of *Azadirachta indica* as antibacterial of Hudaydah extract

Name of Bacteria	***P. Aeruginosa***	***E. coli***	***Staph. Aureus***	***Candida albicans***
Extract 200 µl	25 mm	15 mm	37 mm	20 mm
Extract 100 µl	15 mm	10 mm	30 mm	10 mm
Ethanol 96%	_	_	_	_
Amoxic Clave	_	_	20 mm	_
Gentamycin	30 mm	23 mm	39 mm	_
Nystatin	_	_	_	24 mm

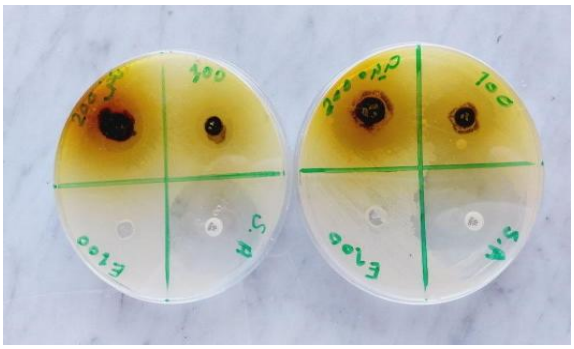


Figure 1: The effect of neem leaves extract on *staph. Aureus*

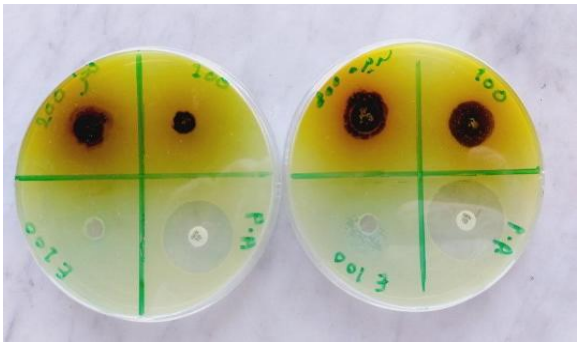


Figure 2: The effect of neem leaves extract on *Pseudo. Aeruginosa*

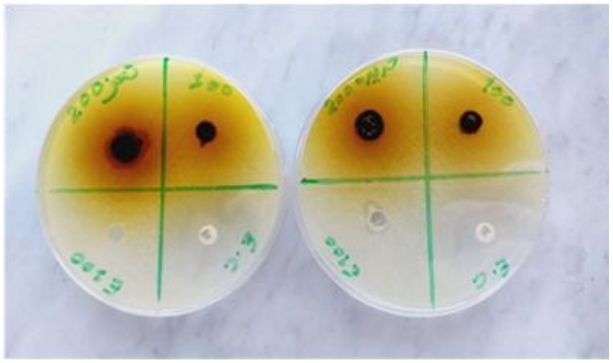


Figure 3: The effect of neem leaves extract on *E.coli*

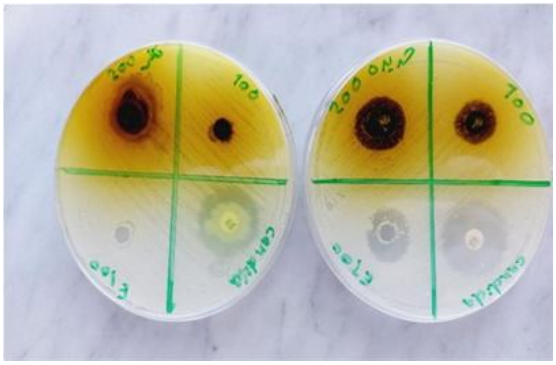


Figure 4: The effect of neem leaves extract on *Candida albicans*

Table 6 shows the percentage of wound contraction (healing) over 9 days for different formulations of Neem creams (3%, 5%, and 10%) from Taiz and Hudaydah, compared to control (normal saline) and standard treatment (MEBO). All Neem cream formulations demonstrate faster wound healing compared to the control and standard treatments. The Taiz 10% cream shows the highest wound contraction rate (40% on day 3, 75% on day 6, and 100% on day 9). Neem creams, especially those from Taiz, have been shown to significantly accelerate wound healing. Figure 5 shows that the graph reinforces the findings from Table 6, showing that Neem creams are highly effective in wound healing.

Table 6: Shows the presence of wound healing activity of herbal creams.

Groups	Day 3 (%)	Day 6 (%)	Day 9 (%)
Control (N/S)	0	25	75
Standard (MEBO)	5	30	85
Hudaydah 3%	5	50	100
Hudaydah 5%	15	60	100
Hudaydah 10%	25	65	100
Taiz 3%	10	55	100
Taiz 5%	20	60	100
Taiz 10%	40	75	100

N/S= Normal saline.

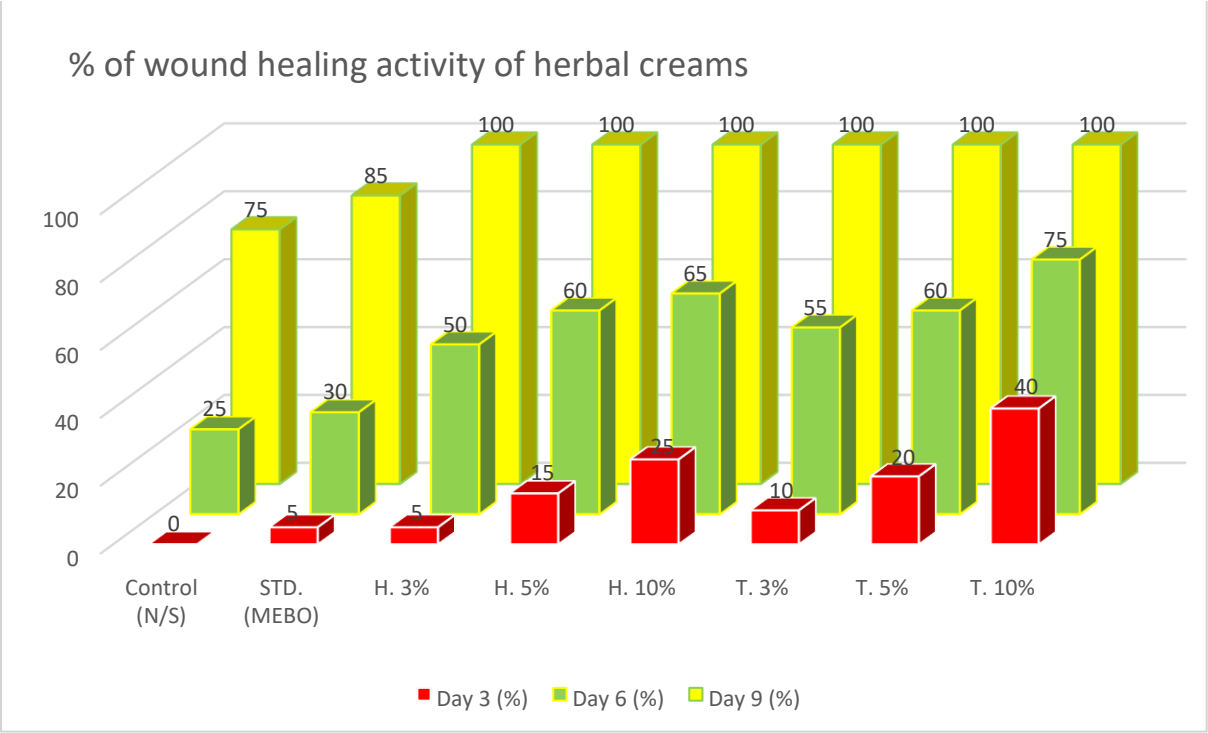


Figure 5: Shows the activity of herbal creams as wound healing.

Figures 6–8 show the Wound healing progression (cream therapy), the photographs of wound healing progression on rabbits treated with Neem creams on days 3, 6, and 9. The photographs provide visual evidence of the accelerated wound healing achieved with Neem creams.

3rd day of wound healing.

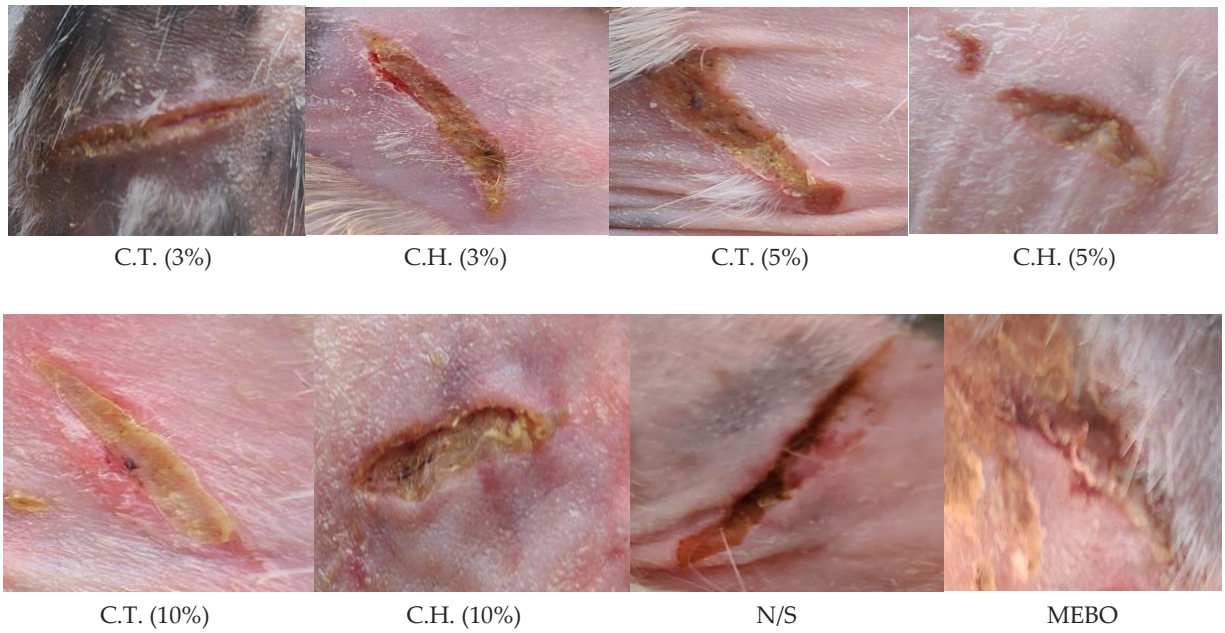
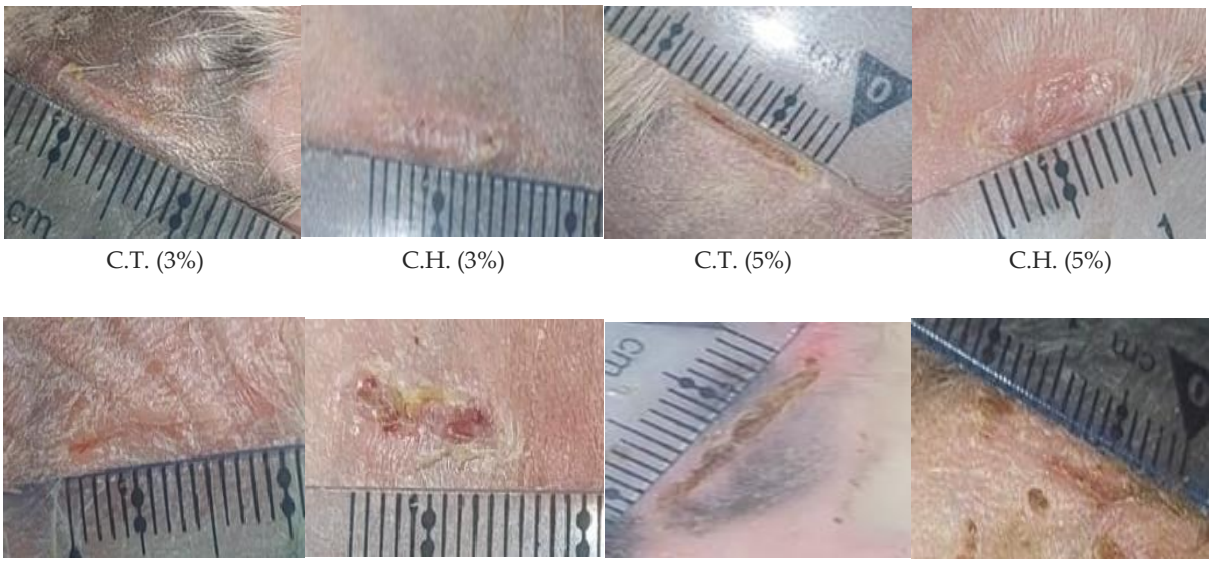


Figure 6: Shows pictures of wounds for cream therapy at 3rd day (C. means cream, T. means Taiz, H. means Hudaydah, N/S. means normal saline)

6th day of wound healing.

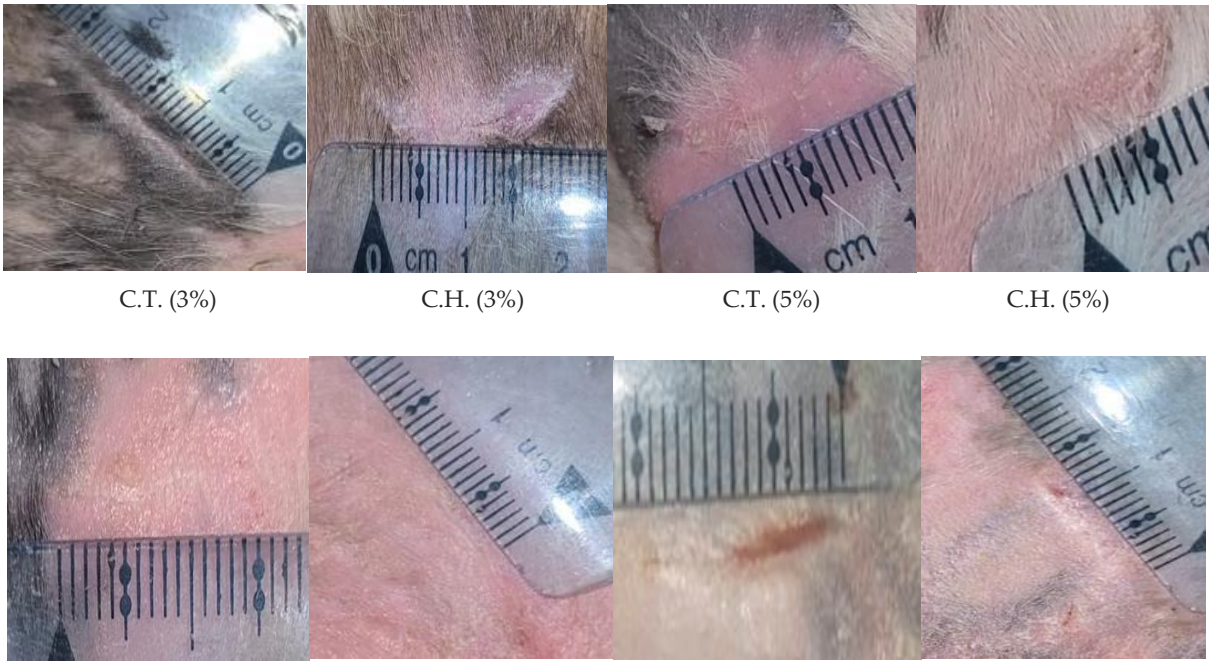


C.T. (3%) C.H. (3%) C.T. (5%) C.H. (5%)

C.T. (10%) C.H. (10%) N/S MEBO

Figure 7: Shows pictures of wounds for cream therapy at 6th day (C. means cream, T. means Taiz, H. means Hudaydah, N/S. means normal saline)

9th day of wound healing.



C.T. (3%) C.H. (3%) C.T. (5%) C.H. (5%)

C.T. (10%) C.H. (10%) N/S MEBO

Figure 8: Shows pictures of wounds for cream therapy at 9th day (C. means cream, T. means Taiz, H. means Hudaydah, N/S. means normal saline)

Table 7 shows the percentage of wound contraction over 9 days for Neem gel formulations from Taiz and Hudaydah, compared to control and standard treatment. The Taiz 10% gel shows the highest wound contraction rate (40% on day 3, 85% on day 6, and 100% on day 9). Neem gels, especially those from Taiz, are highly effective in promoting wound healing.

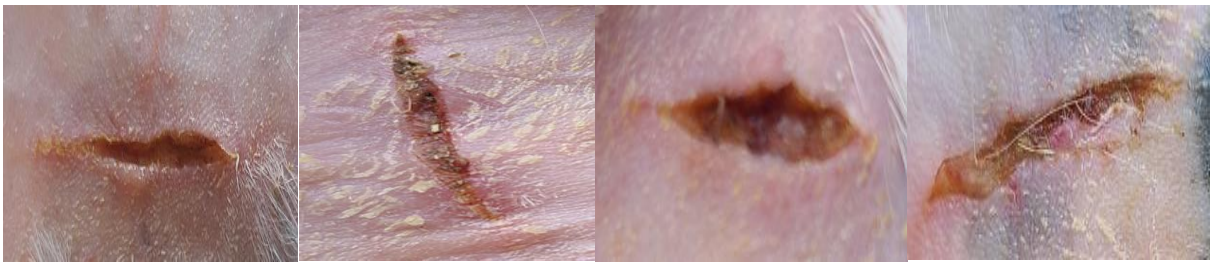
Table 7: Shows the present of wound healing activity of herbal gels.

Groups	Day 3 (%)	Day 6 (%)	Day 9 (%)
Control (N/S)	0	25	75
Standard (MEBO)	5	30	85
Hudaydah 3%	15	40	100
Hudaydah 5%	20	55	100
Hudaydah 10%	30	75	100
Taiz 3%	15	75	100
Taiz 5%	35	80	100
Taiz 10%	40	85	100

N/S= Normal saline.

Figures 9-11 show photographs of wound healing progression on rabbits treated with Neem gels on days 3, 6, and 9. The photographs provide visual evidence of the accelerated wound healing achieved with Neem gels.

3rd day of wound healing.

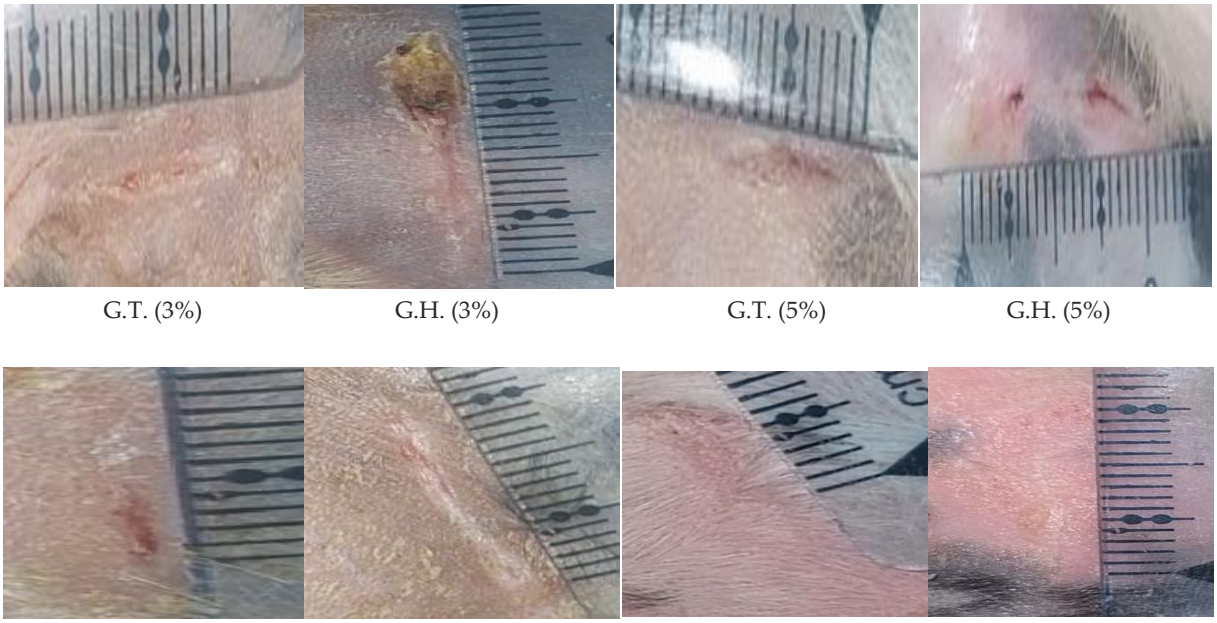


G.T. (3%) G.H. (3%) G.T. (5%) G.H. (5%)

G.T. (10%) G.H. (10%) N/S MEBO

Figure 9: Shows pictures of wounds for gel therapy at 3rd day (G. means gel, T. means Taiz, H. means Hudaydah, N/S. means normal saline)

6thday of wound healing.

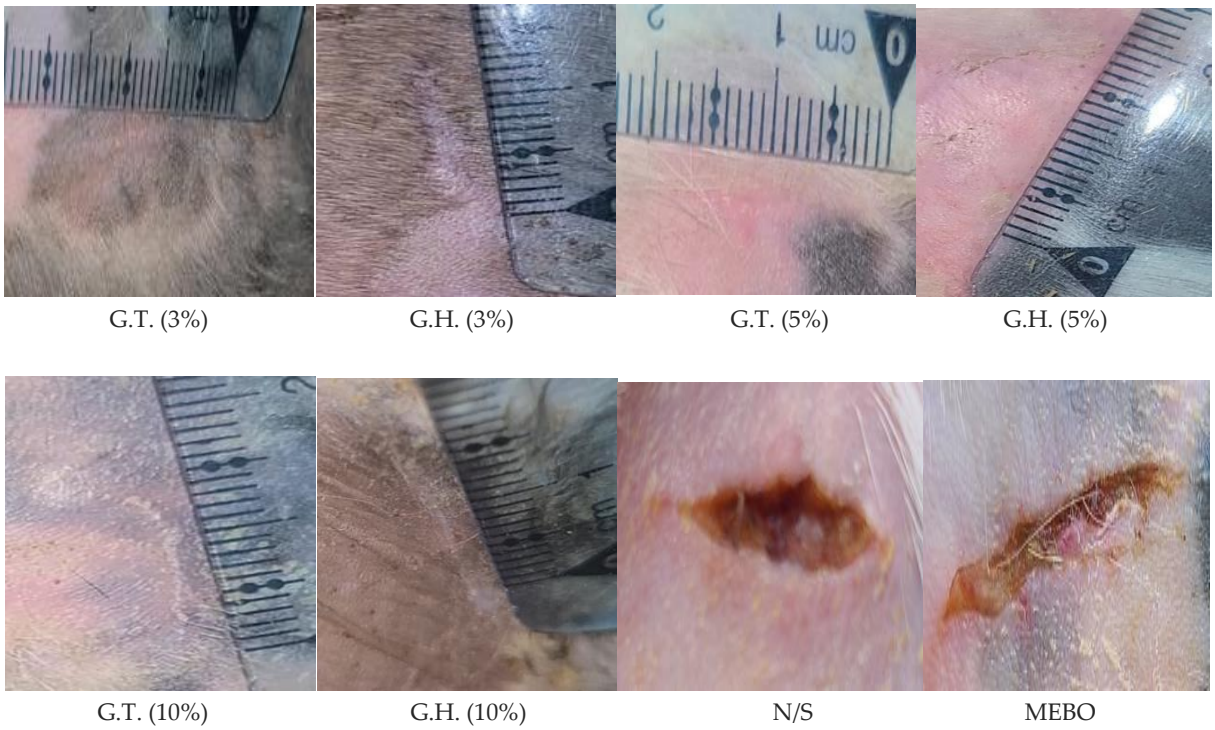


G.T. (3%) G.H. (3%) G.T. (5%) G.H. (5%)

G.T. (10%) G.H. (10%) N/S MEBO

Figure 10: Shows pictures of wounds for gel therapy at 6th day (G. means gel, T. means Taiz, H. means Hudaydah, N/S. means normal saline)

9th Day of wound healing.



G.T. (3%) G.H. (3%) G.T. (5%) G.H. (5%)

G.T. (10%) G.H. (10%) N/S MEBO

Figure 11. illustrate the Images of wounds treated with gel on the ninth day (G. means gel, T. means Taiz, H. means Hudaydah, N/S. means normal saline)

4. DISCUSSION

Herbal medicine has contributed numerous powerful medications to the extensive drug arsenal of contemporary medical research worldwide. both in crude form and as a pure chemical on which modern medicines are structured (Haque et al., 2022). The study evaluated the wound healing and antimicrobial properties of *Azadirachta indica* (neem) leaf extracts from two different regions in Yemen (Taiz and Hudaydah). The findings indicated that ethanolic extracts of neem leaves displayed notable anti-microbial effects.

While the Hudaydah extracts were more effective against *S. aureus* and *C. albicans*, the Taiz extracts showed better antibacterial activity against *P. aeruginosa* and *E. coli*. Variations in the concentration of bioactive chemicals may be the cause of this activity fluctuation. Neem extracts showed high inhibitory zones against *S. aureus, P. aeruginosa, E. coli*, and *C. albicans* in the current study, which supports this conclusion. Neem extracts' ability to accelerate wound healing was evaluated using cream and gel formulations applied to full-thickness excisional wounds in rabbits. The results showed that the neem formulations significantly accelerated wound contraction compared to the standard treatment (MEBO) and the control (normal saline). By the 9th day, wounds treated with neem formulations achieved 100% contraction, whereas the standard treatment achieved 85% and the control achieved only 75%. This suggests that neem extracts possess potent wound healing properties, likely due to their antimicrobial, anti-inflammatory, and immunomodulatory effects.

Osunwoke et al., (2013) found that aqueous extracts of neem leave significantly accelerated wound healing in rats, with complete wound closure observed by the 9th day, similar to the findings of this study. The authors attributed this effect to the presence of bioactive compounds like Nimbidin and Azadirachtin, which have anti-inflammatory and antimicrobial properties. The phytochemical analysis conducted in this investigation identified the existence of tannins, saponins, phenols, alkaloids, and flavonoids, which are known to contribute to the medicinal properties of neem. Rahmani et al., (2018) also highlighted the presence of these compounds in neem and their role in its therapeutic effects, including wound healing and antimicrobial activity.

5. CONCLUSION

The findings of this study support the traditional use of *Azadirachta indica* in wound healing and as an antimicrobial agent. The findings align with prior studies that emphasize the antimicrobial and wound-healing properties of neem, although regional variations in efficacy were observed. The study also underscores the importance of concentration and formulation in maximizing the therapeutic effects of neem extracts.

Acknowledgments

Our sincere appreciation goes out to each and every person who took part in the research.

Authors contribution

Material preparation, data collection performed by A S A, and analysis, review and editing the manuscript was performed by A S A, software and original draft preparing was written by S M H, the result section performed by A A A , resources and conclusion section was done by S M H.

Ethics approvals

This study was authorised by the Ethics Committee (Institutional Review Board) of the Department of Pharmacology, Faculty of Medicine and Health Science, Sanaa University, Yemen, and was carried out in compliance with the Declaration of Helsinki's guidelines (Research code: REC-13-2022). The Animal ethical guidelines are followed in the study for observation, identification & experimentation.

Informed Consent

Not applicable.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

The corresponding author can provide the data that substantiates the findings of this study upon request.

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