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Therapeutic potential of *Lawsonia inermis* leaves' ethanolic extract: *In-vitro* antioxidant, anti-inflammatory and antimicrobial activities and efficacy in severe burn wound healing in Wistar rats

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Abstract: **Background**: The effective management of third-degree burns remains challenging due to high risks of infection, oxidative stress and prolonged inflammation. While several medicinal plants are traditionally used in wound care, scientific evidence supporting their efficacy is often lacking. **Objectives**: This study aimed to evaluate the therapeutic effect of an ointment formulated with the ethanolic extract of *Lawsonia inermis* leaves on the healing of severe burns. **Methods**: Twenty-one adult male Wistar rats were randomly assigned to three groups: Negative control, positive control (standard drug) and test group (*L. inermis*). Standardized third-degree burns (200 °C for 10 seconds) were induced on the animals' backs and treated topically for 27 days. Wound contraction was monitored throughout the study and histological analysis was conducted at the end. Additionally, phytochemical screening and *in-vitro* assays were performed to assess the antioxidant (DPPH, FRAP and FIC), anti-inflammatory and antibacterial activities of the extract. **Results**: The results demonstrated that *Lawsonia inermis* exhibits significant antioxidant, anti-inflammatory and antimicrobial activities. Furthermore, its topical application promoted wound healing in a manner comparable to conventional treatments. **Conclusion**: These findings highlight the therapeutic potential of *L. inermis* as a promising natural alternative for the management of severe burn wounds.

Keywords: Antioxidant; Anti-inflammatory; Antimicrobial; Healing; *Lawsonia inermis*; Severe burns

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INTRODUCTION

Burns are traumatic injuries primarily caused by thermal sources, though they can also result from chemical, electrical, or radiation exposure (Jenkins & Johnson, 2024). According to the World Health Organization (WHO, 2023), approximately 11 million people each year require medical care for burns, with an estimated 180,000 burn-related deaths reported globally.

Severe burns lead to increased capillary permeability in the affected area, allowing fluids and large molecules like albumin to leak out of the circulatory system. This results in major fluid loss, particularly when large surface areas are involved. The leakage of macromolecules further worsens the condition by creating an oncotic gradient that draws more fluid into surrounding tissues. If untreated, this can cause severe dehydration and ultimately cardiovascular collapse. Additionally, loss of the skin's thermoregulatory function can result in significant hypothermia, while destruction of the protective barrier, combined with immunosuppression from elevated endogenous steroid levels, significantly increases the risk of sepsis (Jenkins & Johnson, 2024).

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Burn injuries often require prolonged hospitalization, costly treatment and lengthy rehabilitation. Yet, nearly 90% of burn cases occur in low- and middle-income countries, where access to specialized care is limited (WHO, 2023).

Preventing infection is a major goal in burn treatment. However, topical antibiotics may cause allergic reactions that delay healing (Boekema *et al*., 2024). Advanced therapies such as recombinant growth factors and bioengineered dressings, though effective, remain prohibitively expensive (Langer & Rogowski, 2009). These challenges underscore the need for affordable and effective wound-healing alternatives.

Medicinal plants have emerged as promising candidates in this context. Rich in bioactive compounds with antioxidant, anti-inflammatory and immunomodulatory effects, they have gained considerable scientific interest (Mssillou *et al*., 2022). Notably, nearly one-third of herbal remedies are used to treat skin conditions and wounds (Budovsky *et al*., 2015).

Lawsonia inermis, commonly known as Henna, has been traditionally used to dye textiles and body surfaces. It is a deciduous shrub reaching 2.4-5 meters in height, with distinctive morphological features characteristic of the

Lythraceae family (Salma *et al*., 2024). The plant's various parts exhibit documented pharmacological properties, including antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, antioxidant and hepatoprotective activities (Al-Snafi, 2019; Moutawalli *et al*., 2023; Youl *et al*., 2024).

Although a few studies have explored its effects on burn healing (Hadisi *et al*., 2008; Wandira *et al*., 2022), none have employed a severe (third-degree) burn model. Therefore, it has been hypothesized that *L. inermis* can effectively promote healing in such cases. This study aims to evaluate the antioxidant, anti-inflammatory and antibacterial activities of *L. inermis* leaf extract and investigate its wound-healing efficacy when formulated as a 15% ointment in a third-degree burn animal model.

MATERIALS AND METHODS

Plant material collection and identification

The collection of the plant was done in April 2023, from Biskra province (34°52′N 05°45′E) in Southeastern Algeria. Dr. Aissi Djalil (Institute of Veterinary and Agronomic Sciences, Batna 1 University) has authenticated the plant and a voucher specimen was prepared and deposited in the university's herbarium under the reference number BAT-Li-2023-01.

Chemicals and standards

All chemicals and standards used in the present study were of analytical grade. They have been purchased from Sigma-Aldrich (Laborchemikalien GmbH, Germany).

Preparation of the extract

The plant's leaves have been gently washed to remove dirt and then left to shadow dry in the laboratory at room temperature for 3 weeks. Once dried, the plant material was ground into a fine powder with a mechanical grinder. 200 g of the obtained powder was then extracted with ethanol (w/v 98%) for 48 hours. The filtrate was subsequently evaporated in a rotary evaporator (Laborota 4003, Heidolph, Germany), lyophilized (Alpha 1-2 LDplus, Martin Christ, France) and stored at 4°C.

Phytochemical screening

Phytochemical screening was conducted to identify the various secondary metabolites found in the ethanolic extract of *L. inermis*. This screening was based on observing the formation of precipitate or color changes in the reaction mixture (Mouffouk *et al*., 2020).

In-vitro studies

Antioxidant activity assays

DPPH radical scavenging activity

A solution of DPPH was prepared at a concentration of 0.1 mM in methanol. Then, 1900 µL of the DPPH solution was mixed with 100 µL of different concentrations of the plant extract or reference antioxidant. The mixtures were incubated in the dark at room temperature for 30 minutes to allow for the reaction to occur. After incubation, the absorbance of each sample was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1700, Japan). The percentage of DPPH radical inhibition was calculated using the following formula, as described by Mouffouk *et al*. (2020):

$$Inhibition\ \% = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100 \quad (1)$$

Where A_{blank} is the absorbance of the blank and A_{sample} is the absorbance of the sample.

Ferric reducing antioxidant activity

The ferric reducing antioxidant activity of the samples was evaluated following a previously described method (Mouffouk *et al*. 2020). Briefly, 100 µL of each sample were mixed with 500 µL of phosphate buffer (0.2 M, pH 6.6) and 500 µL of 1% potassium ferrocyanide. The mixtures were incubated at 50 °C for 20 minutes in a thermostatically controlled water bath (Memmert WNB14, Germany). After incubation, 500 µL of 10% trichloroacetic acid was added and the tubes were centrifuged at 3000 rpm for 10 minutes. Subsequently, 500 µL of the supernatant was mixed with 500 µL of double-distilled water, followed by the addition of 125 µL of 0.1% ferric chloride solution. The absorbance of each reaction mixture was measured at 700 nm using a UV-Visible spectrophotometer (Shimadzu UV-1700, Japan). The reducing power was quantified using a standard calibration curve of ascorbic acid and expressed as ascorbic acid equivalents (AAE).

Chelation of ferrous iron

The ferrous ion chelating activity was assessed using a previously described method. Briefly, 500 µL of each test sample was mixed with 900 µL of methanol and 100 µL of 0.6 mM $FeCl_2$ solution. After incubation for 5 minutes at room temperature, 100 µL of 5 mM ferrozine solution was added to initiate the reaction. The mixtures were incubated for an additional 10 minutes in the dark and the absorbance was measured at 560 nm using a UV-Visible spectrophotometer (e.g., Shimadzu UV-1700, Japan) (Gulcin, 2025). The ferrous ion chelating activity was calculated using the following formula:

$$Inhibition\ \% = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (2)$$

Where $A_{Control}$ is the absorbance at 560 nm of the control and A_{sample} is the absorbance of positive control or sample.

Anti-inflammatory activity

The anti-inflammatory activity of the crude extracts prepared from *L. inermis* and was determined using the bovine serum albumin assay. 500 µL of sample solutions or standard drug (ketoprofen) prepared at different concentrations (125, 250, 500 and 1000 µg/mL) were added to 500 µL of BSA solution (0.2%) prepared in Tris

Buffer Saline (pH 6.6). A control tube containing a volume of 0.5 mL of BSA and 0.5 mL of ethanol was also prepared. The tested tubes were incubated at 37°C for 10 min, then heated at 72°C for 5 min. After cooling, the absorbances of these solutions were read at 660 nm (Gunathilake *et al.,* 2018). Each experiment was performed in triplicate and the average absorbance was recorded. The percentage of inhibition of denaturation was determined using the following equation:

$$Inhibition\ \% = \frac{A_{Control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

Where, $A_{Control}$ is the absorbance at 660 nm of the control and A_{sample} is the absorbance of positive control or sample.

Antimicrobial activity

The antimicrobial activity of the ethanolic extract prepared from *L. inermis* was tested *in-vitro* using the solid medium diffusion assay (CLSI, 2012), against a yeast (*Candida albicans* ATCC 90029) and ten bacterial strains of different genera and Gram. Among these bacteria, four are reference strains of the American Type Culture Collection: *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Klebsiella pneumoniae* ATCC70063, *Pseudomonas aeruginosa* ATCC 27853 and five pathological isolates (*Streptococcus D, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa and Enterococci sp*). All these strains were kindly supplied by the Laboratory of Bacteriology, University Hospital Center-Batna (CHU) and the Institute of Pasteur Algiers, Algeria respectively.

Burn healing assay

Preparation of topical formulation

The ointment base was prepared according to the method described in the United States Pharmacopeia (USP-NF, 2024). Briefly, 85 g of white paraffin, 5 g of hard paraffin, 5 g of lanolin and 5 g of stearic acid were placed in a beaker and heated to 65 °C in a water bath until completely melted. The mixture was then allowed to cool and homogenized by homogenizer at 1500 rpm for 10 minutes. Ointment formulation was obtained by incorporating 15 % of the plant lyophilizate into 100 g of the ointment base and stored in glass bottles.

Animals

All experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals. 21 adult healthy male Wistar albino rats (270 – 300 g) were used in this experiment. Animals have been acclimatized for 10 days prior to any procedure. They were kept in clean polypropylene cages with stainless steel top grill at standard conditions (Temperature: 25 °C ± 2; relative humidity: 65% and 12/12 h dark/light cycle). Sawdust was used as bedding material. Animals were fed with rat pellets and water was provided *ad libitum* in polypropylene bottles with stainless steel sipper tubes. Cleaning of cages and water bottles was performed daily.

Burn wound induction

Before burns induction, routine preparations for a surgical procedure have been carried out. The rats were tranquilized with intramuscular injection of Acepromazine (1 mg/kg) and the back was largely clipped and carefully shaved using a blade, taking care to avoid skin lesions. Animals were anesthetized with an intramuscular injection of Ketamine (60 mg/kg) and Xylazine (10 mg/kg). This protocol causes loss of consciousness, muscle relaxation and analgesia within 5 to 10 minutes following the injection and lasts for 45 minutes. Evaluation of anesthesia depth was made based on the animal's different reflexes such as voluntary movements and responses to painful stimulation (Flecknell, 2023). Once anesthetized, the animals were placed in sternal recumbency and the skin of the dorsal region was placed in contact for 10 seconds with a copper rod (2.5 cm), heated to 200 °C using a soldering iron (Ko *et al*., 2013). The created third-degree skin burn was then dressed with sterile gauze soaked in normal saline. Animals were housed separately until complete recovery from anesthesia and the induced lesion was left uncovered throughout the experimental period.

Wounds treatment

Animals were randomly assigned to three groups of 7 each: Negative control (Nc): left untreated, Positive control (Pc): treated with Trolamine (Biafine, France) and *L. inermis* group (Li): treated with the plant ointment. Treatment started 24 h after burn induction and was applied daily for 27 days.

Evaluation of burn healing activity

Determination of burn healing percentage

Burn wounds have been photographed on days 0, 3, 9, 15, 21 and 27 using an HD camera. The obtained pictures were then analyzed with ImageJ software to measure burn wound area and determine wound closure percentage according to the following formula:

$$\text{Wound closure } \% = \frac{\text{Wound size at induction day} - \text{Wound size on the specific day}}{\text{Wound size at induction day}} \times 100 \quad (4)$$

Histological evaluation of burn healing

At the end of the experiment, animals were sacrificed with intracardiac injection of Propofol (Provive, Elkendi, Algeria) and skin tissues from the burnt area were collected. Specimens have been stored in 10% formalin for histological evaluation. After routine histological procedure to prepare sections and Hematoxylin & Eosin staining, histological evaluation of burn wound healing was performed under an optical microscope (Leica, Germany). Microscopic observations were transformed into scores following the histological score for dermis and epidermis presented in table 1 and 2 (Guo *et al.,* 2020).

Table 1: Histological score grade for the epidermis.

Score	Crust	Epithelialization	Rete ridges
0	Loosely attached	no	no
1	Tightly attached	minimal	no
2	Tightly attached	mild	no
3	Tightly attached	moderate	no
4	No crust attached	moderate	no
5	No crust attached	severe	no
6	No crust attached	complete	no
7	No crust attached	complete	yes

Table 2: Histological score grade for the dermis.

Score	Adipose cell	Inflammatory cell	Fibroblast	Collagen deposition	Hair follicle
0	+++	+	-	-	-
1	++	++	+	-	-
2	+	++	+	-	-
3	+	+++	+	-	-
4	+	+++	++	+	-
5	-	++	+++	++	-
6	-	+	+++	++	-
7	-	+	++	+++	+

(-): absent, (+): mild, (++): moderate, (+++): severe.

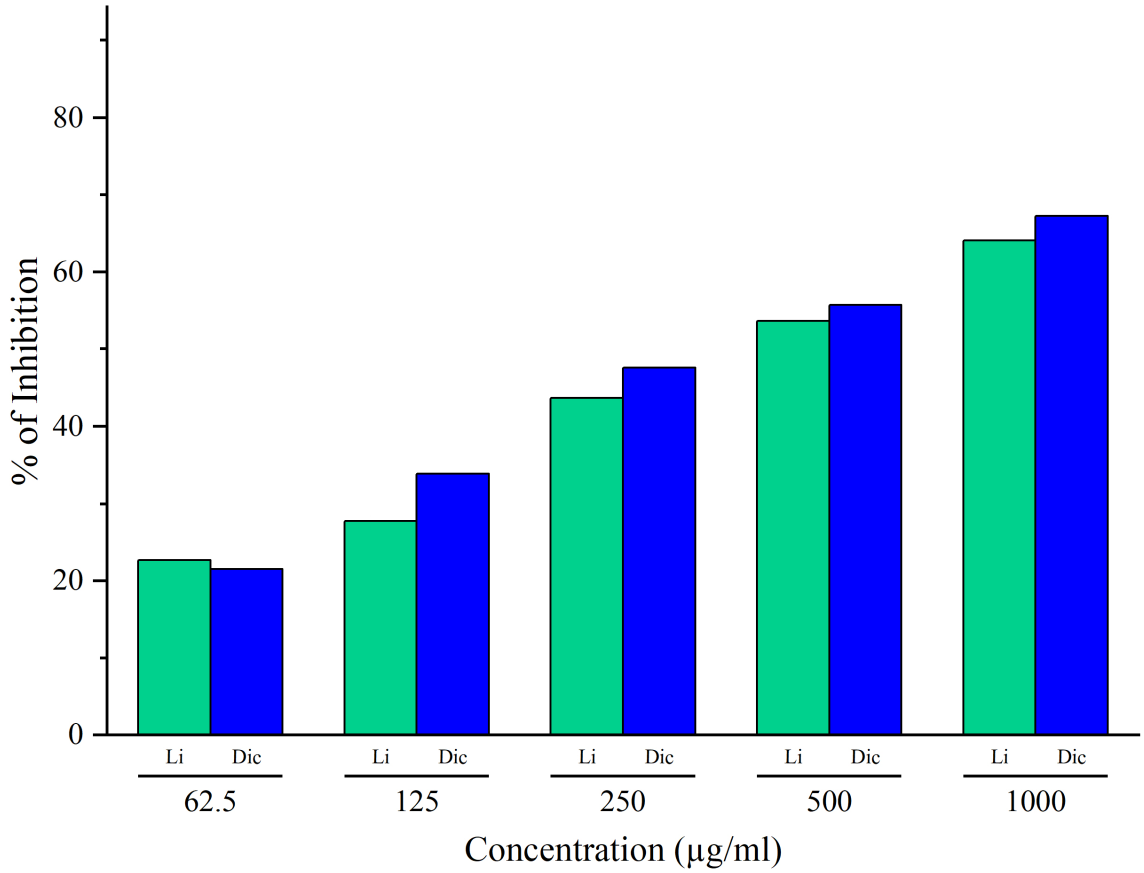

Fig. 1: Comparative anti-inflammatory activity of *Lawsonia inermis* ethanolic extract and diclofenac sodium at varying concentrations using the protein denaturation assay. Li: *Lawsonia inermis*, Dic: Diclofenac.

Table 3: Phytochemical screening of *L. inermis* ethanolic extract.

Metabolites	Flavonoids	Polyphenols	Tannins	Coumarins	Phytosterols	Triterpenoids	Quinones	Anthocyanins	Alkaloids	Saponins
Presence / Absence	+	+	+	+	+	+	+	-	-	-

(+): presence of phytochemical, (-): absence of phytochemical.

Table 4: Antioxidant activities of *L. inermis* and the standards

Extract and standards	DPPH assay IC_{50} (µg/mL)	Chelation of ferrous iron assay EC_{50} (µg/mL)	FRAP assay µg EAA/mg ex
L. inermis extract	39.96 ± 0.15^{a}	117.23 ± 0.95^{d}	48.66 ± 0.23
EDTA	NT	0.63 ± 0.13^{e}	NT
BHT	22.32 ± 0.02^{b}	NT	NT
α-Tocopherol	13.02 ± 0.17^{b}	NT	NT
Ascorbic acid	03.10 ± 0.002^{c}	NT	NT

NT: not tested, EAA: Equivalent Ascorbic Acid. Colum's that don't share a letter are significantly different.

Table 5: Results of the antibacterial activity of *L. inermis* extract on Gram negative strains.

	Dilutions	*E. coli* ATCC25922	*E. coli*	*P. aeruginosa* ATCC 27853	*P. aeruginosa*	*K. pneumoniae* ATCC 70063	*K. pneumoniae*
L. inermis extract (mm)	1/1	22.67±1.5	20.17±0.70	12.67±0.58	13.33±1.15	12.00±0.00	13.50±2.10
	1/2	17.50±2.1	15.50±0.70	10.00±0.00	10.00±0.00	07.50±0.70	11.50±2.10
	1/4	11.50±0.7	13.00±1.40	08.33±0.58	09.67±2.08	07.00±0.50	09.00±1.40
	1/8	9.00±1.4	11.00±0.00	08.00±0.00	11.67±3.51	-	07.50±0.70
	1/16	7.50±0.7	08.50±0.70	07.33±0.58	08.33±1.53	-	07.00±0.70
	1/32	-	-	-	-	-	-
MIC (µg/mL)	-	62.5	62.5	62.5	62.5	250	62.5
Antibiotic (penicillin) (mm)	-	22.50±0.70	21.00±1.41	25.00±0.00	24.50±0.69	23.50±0.70	24.50±0.70

Table 6: Results of the antimicrobial activity of *L. inermis* extract on Gram positive and fungal strains.

	Dilutions	*Streptocoque D*	*Staphylococcus aureus ATCC 25923*	*Staphylococcus aureus*	*Candida Albicans* 90029
L. inermis extract (mm)	1/1	12.67±1.10	14.00 ± 0.70	15.00±0.01	14.83±0.20
	1/2	10.33±1.50	09.17±0.70	10.50±0.70	11.67±0.50
	1/4	07.67±0.50	07.50±1.30	09.00±0.01	09.83±1.40
	1/8	07.33±0.50	07.17±0.20	07.50±0.70	08.00±1.00
	1/16	07.67±0.5	-	-	7.00±0.01
	1/32	-	-	-	-
MIC (µg/mL)	-	62.5	125	125	62.5
Antibiotic (penicillin) (mm)	-	21.00±1.41	26.00±1.40	20.50± 0.70	22.00

Statistical analysis

The data were statistically analyzed using OriginPro 2024. For the *in-vitro* assays, the Student's *t*-test was employed to compare the activity of the extract with that of the standards. In the burn healing assay, one-way ANOVA followed by Tukey's post hoc multiple comparison test was applied. Results are expressed as mean ± standard deviation and differences were considered statistically significant at $p < 0.05$.

RESULTS

Phytochemical screening

Results of the phytochemical screening of the ethanolic extract obtained from *L. inermis* leaves are summarized in

table 3. The data show that a wide variety of biomolecule classes, including flavonoids, polyphenols, tannins, coumarins, phytosterols, triterpenoids and quinones, are abundant in the extract. However, anthocyanins, alkaloids and saponins are absent.

Antioxidant activity

Ethanolic extract of *L. inermis* demonstrated highly significant antioxidant power compared to standards in all the examined techniques (Table 4). The observed percentage of inhibition was 90.94% at 0.4 mg/mL and an IC_{50} value of 39.96 µg/mL in the DPPH test. In chelation of ferrous iron and FRAP methods, the tested extract displayed a good antioxidant potential with an EC_{50} at 117.23 µg/mL and 48.66 µg EAA/mg ex, respectively.

Anti-inflammatory activity

The anti-inflammatory potential of the ethanolic extract of *L. inermis* leaves, evaluated through the inhibition of bovine serum albumin denaturation, is presented in Fig. 1.

The extract demonstrated a concentration-dependent inhibitory effect, with an IC_{50} value of 419.55 ± 0.12 µg/mL. This effect was comparable to that of the standard anti-inflammatory drug diclofenac sodium, which exhibited an IC_{50} of 369.11 ± 0.22 µg/mL across all tested concentrations.

Antimicrobial effect

The antimicrobial activity of ethanolic extract of *L. inermis* leaves was assessed using disc agar diffusion assay against eleven microbial strains from different genera and Gram. The results were represented as the diameter of inhibition zones (Table 5 and 6).

According to the results, the plant extract exhibited a broad spectrum of antimicrobial effects against all the tested strains with inhibition zones ranging from 12.67 to 22.67 mm. This significant microbial inhibitory activity was comparable to that of penicillin, which was used as a reference antibiotic. On the other hand, low minimum inhibitory concentrations were recorded against all the tested microbial strains (from 31.25 to 250 µg/ml). The most sensitive strains were *Candida albicans* 90029*, E. coli ATCC* 25922*, Staphylococcus aureus 25923 and* their clinical isolates. However, moderate antibacterial activity was observed against all the rest of the tested bacteria.

Burn healing

Wound closure

Results of wound closure percentage are summarized in Fig. 2 while gross appearances of wounds are illustrated in Fig. 3.

On day 3, *L. inermis* group (25.83 ± 7.93) showed a significantly higher wound closure percentage compared to negative control (14.12 ± 1.91) (p<0.01) and the positive control group showed an intermediate result (22.14 ± 7.44). From days 9 to 21, both *Lawsonia inermis* and positive control groups have shown nearly identical wound closure percentages with a highly significant difference compared to negative control (p<0.01). At the end of the experiment, *L. inermis* (99.68 ± 0.07) and positive control (99.32 ± 0.18) groups achieved almost complete wound closure which was markedly outperforming the negative control group (79.41 ± 1.24) who presented an unhealed open wound (Fig. 2). These results demonstrate that *L. inermis* ointment is as effective as trolamine, the standard treatment used in promoting burn wound healing and accelerate significantly wound closure.

Histological results

In terms of histological results, the groups Pc and Li have shown similar epidermis (6.57 ± 0.53 and 6.71 ± 0.48) and dermis (6.57 ± 0.53 and 6.85 ± 0.37) scores (Fig. 4 A and B), with complete epithelialization, obvious rete ridges, mild inflammatory reaction, important collagen deposition and immature hair follicle and sweat glands (Fig. 5 A and B). These scores are significantly different from Nc scores (3.85 ± 0.69 for dermis and 4.42 ± 0.78 for epidermis) (Fig. 4), with no crust attached and severe epithelialization, mild adipose cells, severe inflammatory cells, moderate fibroblasts and mild collagen deposition (Fig. 5 C).

DISCUSSION

Burn injuries, especially those of third-degree severity, represent a major medical challenge due to their high risk of infection, extended healing duration and the potential for permanent scarring and disability. Standard treatments, such as silver sulfadiazine and trolamine-based ointments, are often limited by cytotoxicity, slow wound closure and insufficient antimicrobial coverage. Consequently, there is a growing need to explore plant-based therapies that offer multiple biological benefits, including antioxidant, anti-inflammatory and antimicrobial effects, all critical for effective burn wound management (Rowan *et al*., 2015).

In this study, the potential of *Lawsonia inermis* ethanolic leaf extract, a plant traditionally used for dermatological conditions, to enhance wound healing in a third-degree burn model has been investigated. The used approach addressed key pathological features of burns, including oxidative stress, inflammation, microbial invasion and tissue degeneration.

The phytochemical analysis revealed a rich profile of secondary metabolites including flavonoids, polyphenols, tannins, coumarins, phytosterols, triterpenoids and quinones, while alkaloids, anthocyanins and saponins were absent. This metabolite diversity is consistent with previous reports and highlights the influence of geographic origin, climatic conditions, soil composition and extraction techniques on phytochemical content (Badoni Semwal *et al*., 2014; Rahmany *et al*., 2015). In particular, the abundance of phenolic compounds observed may reflect adaptive responses to the plant's semi-arid habitat, where the biosynthesis of antioxidant molecules is ecologically advantageous.

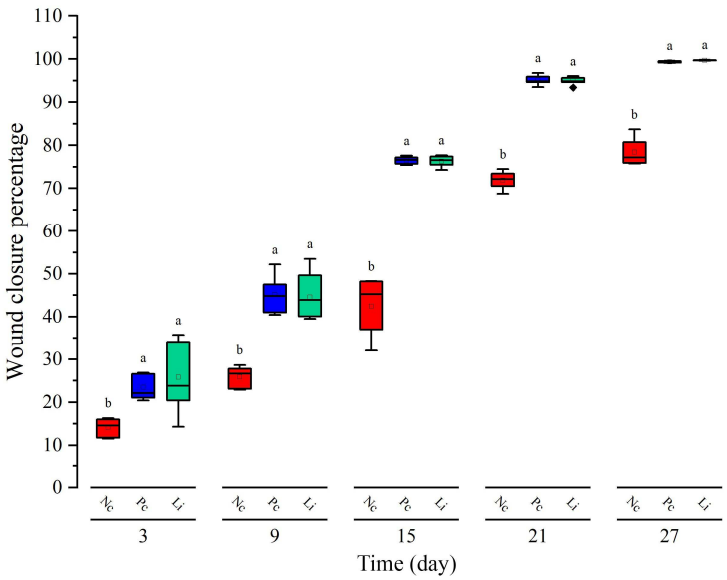

Fig. 2: Time-dependent evaluation of wound healing efficacy of *Lawsonia inermis* ointment compared to positive and negative controls in a rat model of third-degree burns. Groups that do not share a letter are significantly different. Nc: Negative control, Pc: Positive control, Li: *Lawsonia inermis*.

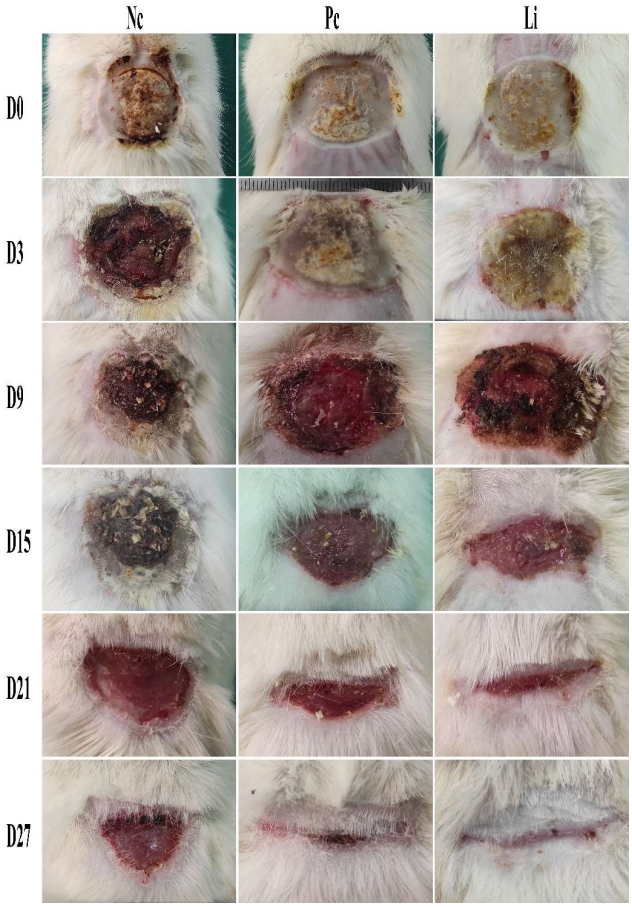

Fig. 3: Macroscopic progression of wound healing over 27 days in rats treated with *Lawsonia inermis* ointment compared to positive and negative controls following third-degree burns, showing almost healed wounds in *L. inermis* and positive control groups compared to incomplete healing in negative control group. Nc: Negative control, Pc: Positive control, Li: *Lawsonia inermis*.

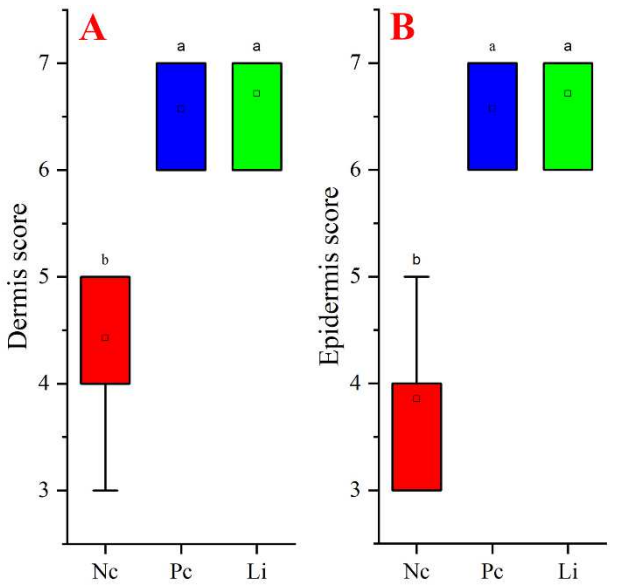

Fig. 4: Effects of *Lawsonia inermis* on (A) dermis and (B) epidermis scores compared to control groups. Groups that do not share a letter differ significantly. Nc: Negative control, Pc: Positive control, Li: *Lawsonia inermis*.

Evaluation of the extract's antioxidant activity confirmed its strong radical-scavenging and iron-chelating capacities. Compared to literature values, the obtained results align closely with those of *L. inermis* samples collected from Sudan and extracted using Soxhlet methods in 75% ethanol, which demonstrated DPPH and metal chelation inhibition rates above 90% and 32%, respectively (Taha *et al*., 2019). Variability among IC_{50} values across studies (Philip *et al*., 2011; Mir *et al*., 2019) is likely attributable to differences in solvents, extraction efficiency and plant chemotypes. Nevertheless, the consistent antioxidant potential detected by FRAP and other assays (Moutawalli *et al*., 2024) affirms the extract's role in neutralizing reactive oxygen species, a critical factor in mitigating burn-induced tissue damage.

Inflammation, an unavoidable consequence of burn injury, often leads to extended healing times and secondary complications. The ethanolic extract of *L. inermis* significantly inhibited protein denaturation *in-vitro*, a key marker of anti-inflammatory efficacy. These findings mirror those of Vinchurkar *et al*. (2014), Bouhlali *et al*. (2016) and Batool *et al*. (2024), who found that *L. inermis* extracts prevented both egg and bovine serum albumin denaturation. Additional anti-inflammatory mechanisms reported in the literature include nitric oxide inhibition in activated macrophages (Khantamat *et al*., 2021), membrane stabilization (Khatun *et al*., 2022) and suppression of inflammatory mediators such as TNF-α and IL-1β (Al-Snafi *et al*., 2022; Kalusalingam and Roshan 2023). These effects are likely mediated by the plant's rich content in flavonoids and coumarins, known for their capacity to modulate immune responses.

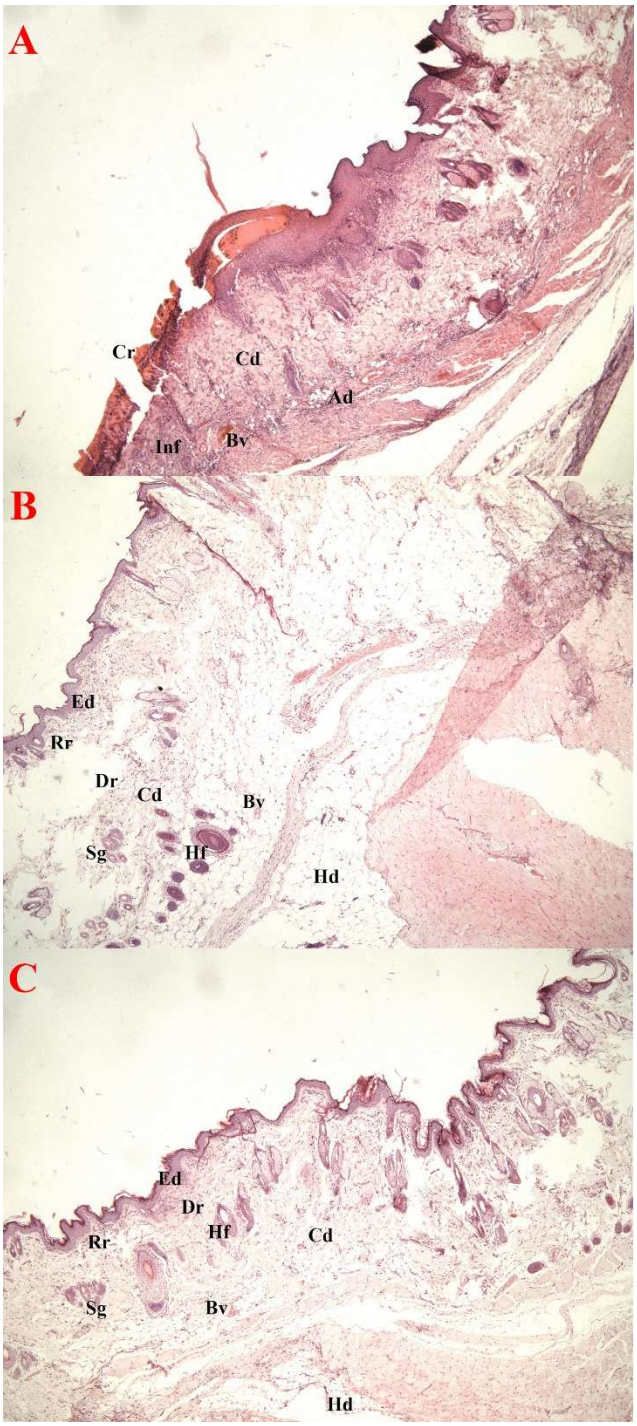

Fig. 5: Histological sections of scar tissue formed in (A) negative control (B) positive control and (C) *Lawsonia inermis* after 27 days of treatment. H&E, X40.
Ad: Adipocytes, Bv: Blood vessel, Cd: Collagen deposition, Cr: Crust, Dr: Dermis, Ed: Epidermis, Hd: Hypodermis, Hf: Hair follicle, Inf: Inflammatory cells, Rr: Rete ridges, Sg: Sweat glands.

The antimicrobial evaluation demonstrated a broad-spectrum effect of the ethanolic extract against both Gram-positive and Gram-negative bacteria as well as fungi.

Notably, the inhibition zones were comparable to those of penicillin and minimal inhibitory concentrations were low across all tested strains. These findings surpass those of earlier investigations (Elmanama *et al*., 2011; Habbal *et al*., 2011; Yusuf 2016), which may reflect differences in extraction procedures and plant origin. The antimicrobial effects are attributed primarily to phenolic compounds such as apigenin, rutin and p-coumaric acid derivatives (Elansary *et al*., 2020), though other components like ß-asarones, lawson and diacylheptenoids may also contribute (Hindi *et al*., 2017; Fatahi Bafghi *et al*., 2022). Given that microbial infections often delay or complicate burn healing, these antimicrobial properties represent a critical therapeutic advantage.

The application of the *L. inermis* ointment to burn-induced lesions led to a marked and accelerated wound contraction. A 25% reduction in wound surface was observed within three days and nearly complete closure occurred by day 27. This rapid recovery suggests that the plant's multifaceted bioactivity may mitigate burn progression, reduce oxidative stress and enhance tissue regeneration. The role of flavonoids and terpenes in dampening inflammation and modulating wound mediators supports this interpretation (Salma *et al*., 2024). Similar enhancements in healing have been reported in several experimental studies, where *L.* inermis-based treatments enhanced wound contraction and accelerated tissue repair in different wound models (Nayak *et al.,* 2007; Rekik *et al.,* 2019). In burn-specific models, topical application of *L. inermis* preparations has also been associated with significant reductions in healing time and increased wound contraction rates (Wandira *et al.,* 2022; El Massoudi *et al.,* 2023).

Histopathological analysis provided further confirmation of the extract's regenerative effects. In the treated groups, complete epithelialization, dermal thickening and significant collagen deposition were observed. These histological patterns are consistent with previous reports, which also noted enhanced fibroblast proliferation and well-organized collagen networks (Nayak *et al.,* 2007; Towfik *et al.,* 2015). Furthermore, the integration of *L. inermis* into biomaterial scaffolds has been shown to amplify these effects (Hadisi *et al*., 2018) and enhanced angiogenesis, collagen synthesis and epithelial restoration have been described in *L. inermis*-based dressings (Khan *et al*., 2021).

CONCLUSION

In this study, *Lawsonia inermis* ethanolic extract was screened to determine its phytochemical profile. Its antioxidant, anti-inflammatory and antibacterial *in-vitro* activities have been evaluated and severe wound healing was evaluated *in-vivo* after its use in the form of a 15% ointment. The results showed that *L. inermis* leaves' ethanolic extract contains several bioactive compounds and exhibits high antioxidant activity in DPPH, FRAP and FIC assays. It has similar anti-inflammatory effect to Diclofenac sodium and is active against a yeast and eleven microbial strains from different genera and Gram. In the *in-vivo* assay, the prepared ointment obtained similar results to Trolamine, making it a better alternative to treat severe burn wounds.

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Authors' contribution
KAY: Contributed to the design of the study, conducted the *in-vivo* experiments and participated in the writing and revision of the manuscript; MS: Responsible for the evaluation of antioxidant activities; MC: Performed the antimicrobial and anti-inflammatory activity assessments; BMMT: Prepared ointment formulations; HH: Carried out the histopathological investigations.

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Data availability statement
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. All relevant data supporting the findings of this study are included in the article and its supplementary materials. Raw data from the *in-vivo*, antioxidant, antimicrobial and histopathological experiments can be provided by the corresponding author upon justified request.

Ethical approval
All experimental procedures involving animals complied with international ethical standards for laboratory animal care and use. The study protocol was reviewed and approved by the Ethical and Scientific Committee of the Institute of Veterinary and Agronomic Sciences, Batna 1 University (Ethical Approval No. 07/2022). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declare that they have no competing interests.

Supplementary data
https://www.pjps.pk/uploads/2026/05/SUP1778147271.pdf

REFERENCES

Abdul Rahiman F, Mahmad N, Taha R, Elias H and Huyop F (2013). Antimicrobial properties of *Lawsonia inermis* syn. *Lawsonia alba in-vivo* and *in-vitro*. *J. Food Agric. Environ.*, **11**: 502–504.

Al-Snafi AE (2019). A review on *Lawsonia inermis*, a potential medicinal plant. *Int. J. Curr. Pharm. Sci.*, **11**(5): 1–13.

Al-Snafi AE, Talab TA and Alfuraiji N (2022). The analgesic and anti-inflammatory effect of lawsone isolated from *Lawsonia inermis*. *ScienceRise, Pharm. Sci.*, **1**(35): 77–84.

Badoni Semwal R, Semwal DK, Combrinck S, Cartwright-Jones C and Viljoen A (2014). *Lawsonia inermis* L. (henna): Ethnobotanical, phytochemical and pharmacological aspects. *J. Ethnopharmacol.*, **155**: 80–103.

Batool A, Akbar J, Ashiq K, Munir A, Alsubari K and Ashiq S (2024). Phytochemical profiling using GCMS and HPLC and evaluation of anti-oxidant, anti-inflammatory and anti-bacterial activities of *Lawsonia inermis*. *Pak. J. Anal. Environ. Chem.*, **25**: 313–327.

Boekema BKHL, Chrysostomou D, Ciprandi G, Elgersma A, Vlig M, Pokorná A, Peters LJF and Cremers NAJ (2024). Comparing the antibacterial and healing properties of medical-grade honey and silver-based wound care products in burns. *Burns.*, **50**: 597–610.

Bouhlali E, Sellam K, Bammou M, Alem C and Filali-Zehzouti Y (2016). *In-vitro* antioxidant and anti-inflammatory properties of selected Moroccan medicinal plants. *J. Appl. Pharm. Sci.*, **6**(5): 156–162.

Budovsky A, Yarmolinsky L, Ben-Shabat S (2015). Effect of medicinal plants on wound healing. *Wound Repair Regen.*, **23**: 171–183.

Chuku LC, Chinaka NC and Damilola D (2020). Phytochemical screening and anti-inflammatory properties of henna leaves (*Lawsonia inermis*). *Eur. J. Med. Plants.*, **31**(18): 23–28.

CLSI (2012). Performance standards for antimicrobial disk susceptibility tests, approved standard, 7th ed. *CLSI document M02-A11.* Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

El Massoudi S, Zinedine A, Rocha JM, Benidir M, Najjari I, Ghadraoui LE, Benjelloun M and Errachidi F (2023). Phenolic composition and wound healing potential assessment of Moroccan henna (*Lawsonia inermis*) aqueous extracts. *Cosmetics*, **10**(3): 92.

Elansary HO, Szopa A, Kubica P, Ekiert H, Al-Mana FA, Al-Yafrsi MA (2020). Antioxidant and biological activities of *Acacia saligna* and *Lawsonia inermis* natural populations. *Plants.*, **9**: 908.

Elmanama A, Alyazji AA and Abu-Gheneima N (2011). Antibacterial, antifungal and synergistic effect of *Lawsonia inermis*, *Punica granatum* and *Hibiscus sabdariffa*. *Annals of Alquds Medicine.,* **7**: 33–41.

Fatahi Bafghi M, Salary S, Mirzaei F, Mahmoodian H, Meftahizade H and Zareshahi R (2022). Antibacterial and anti-*Trichomonas* characteristics of local landraces of *Lawsonia inermis* L. *BMC Complement. Med. Ther.*, **22**: 203.

Flecknell A (2023). *Laboratory Animal Anaesthesia.*, 4th ed., Academic Press Elsevier. pp.91–124.

Gulcin I (2025). Antioxidants: A comprehensive review. *Arch. Toxicol.,* **99**(5): 1893–1997.

Gull I, Sohail M, Aslam MS and Athar MA (2013). Phytochemical, toxicological and antimicrobial evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. *Ann. Clin. Microbiol. Antimicrob.*, **12**: 36.

Gunathilake KDPP, Ranaweera KKDS and Rupasinghe HPV (2018) *In-vitro* anti-inflammatory properties of selected green leafy vegetables. *Biomedicines,* **6**(4): 107.

Guo HF, Mohd Ali R, Abd Hamid R, Chang SK, Zainal Z and Khaza'ai H (2020). A new histological score grade for deep partial-thickness burn wound healing process. *Int. J. Burns Trauma.*, **10**: 218–224.

Habbal O, Hasson S, El-Hag A, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, Al-Balushi M and Al-Jabri A (2011). Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. *Asian Pac. J. Trop. Biomed.*, **1**: 173–176.

Hadisi Z, Nourmohammadi J and Nassiri SM (2018). The antibacterial and anti-inflammatory investigation of *Lawsonia inermis*-gelatin-starch nano-fibrous dressing in burn wound. *Int. J. Biol. Macromol.*, **107**: 2008–2019.

Hindi N, Hindi K, Fadhil I, Abdul-Husin I, Jebur M, Al-Mahdi Z, Al-Mahdi A and Almansoori A (2017). Evaluation of anti-bacterial activity of the aquatic henna leaves extract in Hilla City: An *in-vitro* study. *J. Chem. Pharm. Sci.,* **10**(1): 162–165.

Jenkins M and Johnson C (2024). Management of burns. *Surg. (Oxf.)*., **42**: 510–516.

Kalusalingam A and Roshan S (2023). Perspectives on bioactive compounds and pharmacological applications of *Lawsonia inermis* L. *J. Surv. Fish. Sci.*, **10**(1): 4201–4211.

Khan BA, Khan A, Khan MK and Braga VA (2021). Preparation and properties of high sheared poly (vinyl alcohol)/chitosan blended hydrogels films with *Lawsonia inermis* extract as wound dressing. *J. Drug Deliv. Sci. Technol.*, **61**: 102227.

Khantamat O, Dukaew N, Karinchai J, Chewonarin T, Pitchakarn P and Temviriyanukul P (2021). Safety and bioactivity assessment of aqueous extract of Thai henna (*Lawsonia inermis* Linn.) leaf. *J. Toxicol. Environ. Health A.*, **84**: 298–312.

Khare B and Shukla TP (2022). A review on polyherbal formulation used in the treatment of rheumatoid arthritis. *J. Adv. Sci. Res.*, **13**(1): 31–42.

Khatun R, Khanam F, Juhi SA, Khatune NA and Rahman MAA (2022). *In-vitro* evaluation of anti-diabetic and anti-inflammatory activities of ethanolic extract of *Lawsonia inermis* L. leaves (Family: Lythraceae). *J. Pharmacogn. Phytochem.*, **11**:252–255.

Ko WJ, Na YC, Suh BS, Kim HA, Heo WH, Choi GH and Lee SU (2013). The effects of topical agent (Kelo-Cote or Contractubex) massage on the thickness of post-burn

scar tissue formed in rats. *Arch. Plast. Surg.*, **40**(6): 697–704.

Langer A and Rogowski W (2009). Systematic review of economic evaluations of human cell-derived wound care products for the treatment of venous leg and diabetic foot ulcers. *BMC Health Serv. Res.*, **9**(1): 115.

Lavari N, Ostadrahimi N, Rahimi R, Raei M and Abbassian A (2023). The effect of a topical formulation from *Lawsonia inermis* L. (henna) on pain intensity in patients with chronic sciatica: A randomized double-blind clinical trial. *J. Ethnopharmacol.*, **313**: 116519.

Manivannan R, Aeganathan R and Prabakaran K (2015). Anti-microbial and anti-inflammatory flavonoid constituents from the leaves of *Lawsonia inermis*. *J. Phytopharmacol.*, **4**: 212–216.

Mir NT, Saleem U, Anwar F, Ahmad B, Ullah I, Hira S, Ismail T, Ali T and Ayaz M (2019). *Lawsonia inermis* markedly improves cognitive functions in animal models and modulates oxidative stress markers in the brain. *Medicina (Kaunas).*, **55**: 192.

Mouffouk S, Mouffouk C, Bensouici C and Haba H (2020). *In-vitro* cytotoxic effect, hemolytic and antioxidant activities of the Algerian species *Nonea vesicaria* Rchb. *Curr. Bioact. Compd.*, **16**: 1197–1204.

Moutawalli A, Benkhouili FZ, Doukkali A, Benzeid H and Zahidi A (2023). The biological and pharmacologic actions of *Lawsonia inermis* L. *Phytomed. Plus.*, **3**: 100468.

Moutawalli A, Benkhouili FZ, Ouchari L, El Fahime E, Benzeid H, Doukkali A and Zahidi A (2024). Quantitative phytochemical, antioxidant and antimicrobial properties of the seeds of *Lawsonia inermis* L. *Plant Sci. Today.*, **11**(3): 1–16.

Mssillou I, Bakour M, Slighoua M, Laaroussi H, Saghrouchni H, Ez-Zahra Amrati F, Lyoussi B and Derwich E (2022). Investigation on wound healing effect of Mediterranean medicinal plants and some related phenolic compounds: A review. *J. Ethnopharmacol.*, **298**: 115663.

Nallajerla SK and Ganta S (2022). Evaluation of anti-inflammatory mediated anti-hemorrhoidal activity of *Lawsonia inermis* on croton oil induced hemorrhoidal rats. *Antiinflamm. Antiallergy Agents Med. Chem.*, **21**: 62–73.

Nayak BS, Isitor G, Davis EM and Pillai GK (2007). The evidence-based wound healing activity of *Lawsonia inermis* Linn. *Phytother. Res.*, **21**: 827–831.

Philip JP, Madhumitha G and Mary SA (2011). Free radical scavenging and reducing power of *Lawsonia inermis* L. seeds. *Asian Pac. J. Trop. Med.*, **4**: 457–461.

Rahmany E, Cakici A and Cakir E (2015). Antioxidant activity and phenolic compounds of lawson molecule extracted from *Lawsonia inermis* (Henna). *Int. J. Food Eng. Res.*, **7**: 1–17.

Rekik DM, Ben Khedir S, Daoud A, Ksouda Moalla K, Rebai T and Sahnoun Z (2019). Wound healing effect of *Lawsonia inermis*. *Skin Pharmacol. Physiol.*, **32**: 295–306.

Rowan MP, Cancio LC, Elster EA, Burmeister DM, Rose LF, Natesan S, Chan RK, Christy RJ and Chung KK (2015). Burn wound healing and treatment: Review and advancements. *Crit. Care.*, **19**:243.

Salma R, Vijay V, Sivakumar M, Vijayakumar AR and Deepa N (2024). A review on phytochemical, pharmacological and toxicological properties of *Lawsonia inermis* L. *Zenodo*, **2024**.

Singer AJ and Boyce ST (2017). Burn wound healing and tissue engineering. *J. Burn Care Res.*, **38**: e605–e613.

Skowroaska W and Bazylko A (2023). The potential of medicinal plants and natural products in the treatment of burns and sunburn-A review. *Pharmaceutics.*, **15**:633.

Taha M, Parveen B, Osman B, Abdoon IH, Mohamed MS, Osman WJA and Ahmad S (2019). *In-vitro* profiling of plants used in Sudanese traditional medicine for antioxidant and anti-breast cancer activities. *Ann. Phytomed. Int. J.*, **8**: 119-126.

Towfik A, Hamza AS and Munahi A (2015). The effect of henna (*Lawsonia inermis*) on the wound healing of local Arabian horses. *Univ. J. Appl. Sci.*, **13**: 1–5.

United States Pharmacopeial Convention. *United States Pharmacopeia and National Formulary (USP–NF).* Rockville (MD): USP Convention; 2024.

Vinchurkar A, Valsange A, Dama L, Sonawane S, Gaikwad N, Mane P and Dama SB (2014). Evaluation of *in-vitro* anti-inflammatory activity of crude *Lawsonia inermis* leaf extract using egg albumin denaturation assay. *Trends Biotechnol. Res.*, **8**: 44.

Wandira A, Astriani D and Wahyudin M (2022). Formulation and effectivity of henna leaves (*Lawsonia inermis* L.) extract ointment against burn healing of rabbit (*Orctolagus cuniculus*). *Ad-Dawaa J. Pharm. Sci.*, **5**: 103–113.

WHO (2023). Burns fact sheet. *World Health Organization*, https://www.who.int/en/news-room/fact-sheets/detail/burns (accessed 15 janvier 2025).

Youl O, Konate S, Sombie EN, Boly R, Kabore B, Koala M, Zoungrana A, Savadogo S, Tahita CM, Valea I, Tinto H, Hilou A and Traore-Coulibaly M (2024). Phytochemical analysis and antimicrobial activity of *Lawsonia inermis* leaf extracts from Burkina Faso. *Am. J. Plant Sci.*, **15**: 552–576.

Yusuf M (2016). Phytochemical analysis and antibacterial studies of *Lawsonia inermis* leaves extract. *J. Chem. Pharm. Res.*, **2016**: 571–575.