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The Assessment of Indian Henna (*L. Inermis*) Aqueous Extracts of Phenolic Composition and Potential for Wound Healing

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Abstract

Background

Henna (Lawsonia inermis) is a medicinal plant with significant cultural and traditional value. Its leaves have been used for centuries in various cosmetic and medicinal applications. However, the phytochemical composition and wound healing potential of henna from different regions have not been thoroughly investigated.

Material and Methods

Samples of Lawsonia inermis leaves were collected from three locations in Uttar Pradesh: Sharavasti, Bahraich, and Lucknow. Phytochemical analysis was performed on water-soluble extracts to determine the concentrations of phenolic compounds, flavonoids, tannins, and saponin. Swiss albino mice were used to evaluate the efficacy of specific compositions derived from these extracts in injury rehabilitation. Chromatographic analysis on Sephadex-G50 gel was used to determine the lawsone content in the extracts.

Results

Phytochemical analysis revealed significant concentrations of phenolic compounds, flavonoids, and tannins in the water-soluble extracts. Sharavasti henna extract exhibited higher levels of phenolic compounds (13.48%), flavonoids (9.25%), and tannins (2.57%), while Bahraich henna extract had a greater saponin concentration (0.32%). Chromatographic analysis confirmed higher lawsone content in Sharavasti henna. Application of 10% henna extract in petroleum jelly significantly reduced burnt area with contraction percentage (CP) and complete re-epithelialization duration (CRD) similar to 1% Flamazine treatment.

Conclusion

This study suggests the potential of henna-based formulations rich in phenolic chemicals for dermatological, cosmetic, and medicinal applications. The findings highlight the importance of phytochemical analysis and wound healing potential of henna from different regions, which can be used to develop effective henna-based products for various applications.

Keywords: Phytochemical; wound healing activity; Indian Henna; Lawsonia inermis; Phenolic composition; Wound healing; Bioactive compounds.

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Introduction

These natural herbs and shrubs are often utilized for numerous presentations—together within healthcare and traditional practices as well as cultural use, outstanding to their squat price tag and easy in use. Grown in tropical locations, henna is a plant used for cosmetic purposes. Due to its active ingredients' supposed therapeutic qualities, henna fine powder is also widely utilized, potentially helping with wound healing. Around the world, cultures have always been fascinated by plants, and people have long utilized natural formulations for cosmetic and therapeutic commitments to enhance their comfort.[1,2] Consequently, the discovery of cosmetic items with a mineral or vegetable basis at some Indian archaeological sites has included henna. [3-4]

A cosmetic item is any material that is meant to originate into proximity to the anthropological body in order to clean, scent, alter, and maintain it in a healthy state. The growing body of research revealing the therapeutic benefits of natural cosmetics has led to their increasing popularity. Africa is home to more than 60,000 plant kinds, of which 8,000 are employed in herbal medicine, mostly in cosmetic items.[5-7] This highlights the plants' potential economic and cultural significance as well as their sustainable environmental qualities. Primary medical care Plants are essential to daily life and the treatment of many ailments because of increased security buffers and the low cost of conventional medications. The use of these plants in Indian culture, folklore, prophecy, and religion is also intimately associated with them.[8,9] In tropical regions, L. inermis—also known as henna in Arab countries—is the most widely used and well-liked.[10] L. inermis is a member of the Lythraceae family, which is notable for its leaves. Due to their plenty of the vigorous Lawsone constituents also acknowledged as hennotannic acid, which is hand-me-down in the hair, nail, and hand dyeing sections of the cosmetic marketplace for antifungal, antibacterial, analgesic, antioxidant, and anti-inflammatory agents, they are used in an extensive assortment of industries, together with drugs and makeup's. [11–13] Notwithstanding all of these advantages, the cosmetics industry based on L. inermis is still in its infancy, and India is among the richest nations in the world when it comes to the potential benefits of henna. Although plantations are still utilized in many other nations, including Iran, they are still only found in a small number of unstable locations, have little additional value, and have not yet undergone industrialization. Indian henna's chemical and phytochemical properties are well studied, but less is known about its other qualities, particularly its positive effects on human skin, or its cosmetic and biological activity and technical utility qualities. [14]

All things considered, the active ingredients in this plant have curative qualities and may have therapeutic effects on the healing of wounds. The current study aims to examine the effects of silver sulfadiazine on wound healing by means of in vivo examination of our ointment formulations that are founded on a water-based extract of L. inermis, which was gathered in three different districts of India.

Henna may, in fact, have medicinal uses in the treatment of wounds, given that its active ingredients are traditionally recognized for their healing qualities. The unprejudiced of this study was to examine the phenolic substance of Indian Henna, which was collected from three different locations across the nation. Additionally, ointment formulations based on aqueous extracts of Indian Henna leaves were prepared, and by means of mice, the in vivo wound healing potential of these formulations was evaluated.

Material and Methods

Leaves of *Lawsonia inermis*

Garden-fresh leaves of L. inermis were collected in July 2023 from 3 different areas in state of Uttar Pradesh India chiefly, Sharavasti (LI1), Bahraich (LI2) and Lucknow (LI3). Leaves was cleaned with water and air-dried for three weeks at± 25^0C and then grinded into fine powder.

Aqueous Extract (AQ) Obtention

To gain the water soluble extract (AQ), leaf fine residues (10 g) from the three deliberate henna were liquefied in distilled water (100mL).Subsequently, the combination was subjected to magnetic spinning at 700 rpm for duration of 72 hours at room temperature, prior to filtering. At 40^0C, the separated from gathered and focused with a rotary evaporator operating under vacuum. The extracted material was labeled, placed in a sterile, airtight container, and maintained at 4 ^{0}C until needed.

Ointment Formulation

A 5% water-soluble extract of L. inermis and white soft paraffin were used to create the formulations on a galenic foundation. Thus, 10 g of modest ointment base were made by combining 9.5 g of paraffin and 0.5 g of quotation. Each ointment combination was moved to a sanitized container on its own. Different ointments were made using three different aqueous extracts of henna from Bahraich, Lucknow, and Sharavasti. We took advantage of the natural carbohydrate polymers present in significant amounts in the extracts as well as the high viscosity of soft paraffin, which is brilliant with a subtle consistency fibrous, to guarantee a good spreading of the water-based extracts in the hydrophobic matrix. On the other arrow, this also improved the dispersion of the particles even hydrophilic. Consequently, in the case of elements with a non-spherical geometric construction (fibrillar, lamelar, dendritic), an entanglement can be generated mechanically and manifest. In light of this, we used an IKA RW 20 Digital Propeller Stirrer, which is made to appropriate into even the maximum intricate inspiring applications for high thickness mixes. The stability of the ointments for an extended duration was monitored to ensure the dispersion was successful.

Total Phenolic Compounds Content

The Folin–Ciocalteu substance was used to portion the overall phenolic contented of extracts, with modifications, in accordance with the Singleton and Rossi (1965) technique.[15] Gallic acid was utilized to create an ordinary calibration arc that was acquired by spectroscopic analysis at a wavelength of 765 nm, and distilled water was employed as a blank. The findings were given as gm of Gallic acid equivalent (GAE) per 100 gm of extract dry matter (g GAE/100 gm DM).

Total Flavonoids Content

The colorimetric scheme of Folin-Ciocalteu, as labeled [16] was secondhand to determine the level of tannin. The blank was distilled water. Tannic acid standard solutions were made in the manner mentioned above. In mg of equivalent tannic acid (TAE) per 100 gm of dry substance (g TAE/100 g DM), the entire tannin gratified (TTC) was reported.

Total Tannin Content

The Folin-Ciocalteu colorimetric technique, [17] was used to determine the tannin concentration. As a blank, distilled water was utilized. Tannic acid solutions according to standard procedure were made. The equivalent tannic acid (TAE) concentration (mg) per 100 g of dry substance (g TAE/100 g DM) was used to represent the total tannin content (TTC).

Total Saponin Content

The Spectro-photometric technique, modified from Hiai et al. (1976), was used to determine the total saponin content. [18] In instead of the sample extract, a blank comprising 30% aqueous methanol was used to test the absorbance at 544 nm. Total Saponin Concentrations (TSC) was determined using a standard calibration curve and represented as diosgenin equivalents (DE µg/100g DM).

Gel Filtration Chromatography

The majority of the molecules in the raw watery extract of L. inermis must be separated and purified because it is a complex matrix that requires additional characterization. Fractionation of crude extract was carried out in an open Sephadex-G50 column. Through the use of size-exclusion chromatography, molecules can be separated according to their molecular sizes using this technique. The molecules with the greatest molecular mass only diffuse outside the pores of the very porous micro beads that make up Sephadex-gel, which is why they depart the column first. Smaller molecules, on the other hand, disperse inside each micro bead, move more slowly, and eventually leave the column.[19] Based, with slight adjustments, on the technique of Siddiqui et al., a column measuring 2.5 cm in diameter and 50 cm in length was utilized, with the rate of flow set at 1 mL/min. [20] A mixture of 150 mL of lithium chloride buffer solution and 20 gm of Sephadex-G50 was added. Each extract was fractionated at a concentration of half milligrams per milliliter on Sephadex-gel. The unglued segments were subsequently taken in test tubes at a

capacity of two milliliters and examined using a spectrophotometer at 380 nm of Phenolic complexes and 490 nm of Lawsone. [21]

Wound Healing Activity Assessment

Swiss albino mice are frequently used for wound curing experiments because of their biological traits somewhat than for research purposes. In comparison to human beings, these animal prototypical exhibits more rapidly epidermis rejuvenation and matrix manufacture. This variety it conceivable to study all stages of wound therapeutic, such as dermal reorganization as well as mutations in genes that mimic human pathologies like diabetes, immunodeficiency, and obesity. [22] This study targets to associate the efficaciousness of a cosmetic product including leaf extracts of L. inermis on wound healing to the standard treatment administered in burn care units, which involves 1% silver sulfadiazine. The method of creating wounds on the backs of mice permits the observation of all stages of wound healing, from re-epithelialization to retrenchment.

Ethics statement

Swiss albino mice (either sex) were delivered by the Hygia Institute of Pharmaceutical Education and Research Lucknow (Reg. No.1098/PO/Re/S/07/CPCSEA: Dated. 30-07-2022), Prior to existence used in biological actions, the animals were housed in normal polystyrene cages in groups of five for a two-week acclimation period. The animals were reserved at a consistent temperature of 23 ± 1^0C and were given unrestricted access to food and drink. For every activity that involved animals, an ethics statement was obtained.

Animal preparation

We employed clusters of mice, weighing between 40 and 41.4 g, as an experimental archetypal to produce warm air burns. Every animal was kept in a separate polystyrene cage and had unlimited access to water and food. Each of the five groups that the animals were placed in contained five mice:

. Negative control group (NC): mice were left unprocessed and preserved with white soft paraffin;

. Positive control group (PC): mice were cured with 1% silver sulfadiazine, also recognized as Flamazine;

. LI1 group: mice were cured with the Sharawasti ointment preparation;

. LI2 group: mice were cured with the Bahraich ointment preparation;

. LI3 group: mice were cured with the Lucknow ointment preparation.

Performance of experimental burns

Weighing the mice in individually cluster at the start of the experimentation allowed us to track their weight variation throughout the course of therapy. The animals were given ether for general anesthesia, and each one's previously shaved back was used for the burns because it was the most accessible area. A circular metal bar with a diameter of 10 mm was animated with boiling water at 100^0C in order to cause burns. [23]The object was rapidly applied to the study region for 20 seconds

without applying any pressure after it reached 100 °C.

Treatment application

Mice in the NC group were not given any therapy, while burned mice received a daily application of a cream that corresponded to their lot. The mice's injuries were not covered, but daily pictures were taken to track the mice's recovery. To prevent underestimating the size of the wound, the photographs were all obtained at the same angle.

Study of scar evolution using digital planimetry

Through the use of digital planimetry, which entails taking pictures of wounds and utilizing Image J-software to analyses their surface area, the progression of burn wound healing has been investigated. [24] During photography, a ruler was utilized to allow the program to determine injuries in quadrangular millimeters.

Percentage of contraction

Using the following Equation (1), the average wound size of five mice from the same group was calculated and compared with the initial burn surface area [25] to determine the wound contraction percentage (WCP):

$$\mathbf{WCP} = \frac{\mathbf{InitialwoundsizeD0 - WoundSizeDn}}{\mathbf{Initialwoundsize}} \times \mathbf{100} \ldots\ldots\ldots (\mathbf{1})$$

Where:

WCP: Wound contraction percentage;

D_0:Day0

D_n: Dayn.

Epithelialization period

The number of times needed for dermal mutilation and full wound closure, with no residual injury, was used to measure the epithelialization phase, which is marked by the proliferation of epithelial stem cells.[26,27]

Statistical Analysis

Three separate regions' intra- and inter-variety variability was ascertained by statistical analysis of the obtained results. Software called Minitab version 20.2 was used to process the data. For the purpose of comparing means, two-factor ANOVA was utilized, and for quantitative variables, Pearson's correlation test was employed.

Results and Discussion

Phenolic Compounds Analysis

The table below (Table 1) shows the results for extraction yield, total phenolic content, flavonoids

contents, tannins, and saponin. Table-1 data demonstrates the high concentration of water-soluble chemicals, tannin, flavonoids, and phenolic complexes discovered in henna from the Sharawasti area. Lucknow region and Bahraich region are the next regions where henna is found to be highly concentrated in these compounds. This study focuses on these families of molecules since they are critical to the healing process.

Table1. The median values and standard deviations for the phytochemical properties of the examined henna extract using water (AQ).

Extracts	**AQ-LI 1**	**AQ-LI 2**	**AQ-LI 3**
Aqueous extract (AQ) Yields (%)	50.88 ± 3.04	23.81 ± 1.73	37.32± 1.81
Total phenolic compounds (g GAE)/100 g DM)	13.38 ± 0.83	6.45± 0.51	8.33± 0.40
Total flavonoids (g QE/100 g DM)	9.22± 0.50	4.39± 0.41	5.44± 0.26
Total tannin (g TAE/100 g DM)	2.55± 0.12	1.40± 0.11	0.36± 0.04
Saponin (mg/100 g DM)	291.50 ± 17.40	83.35± 5.74	321.51±15.10

Gel Filtration Chromatography

Highly sensitive compounds can be quickly and easily separated using gel filtration chromatography, sometimes referred to as exclusion chromatography or gel permeation chromatography. The images below (Figure 1) display the chromatographic results of the spectrophotometric examination of henna from Sharawasti, Bahraich, and Lucknow.

After 20 minutes of elution from the 15th to the 35th sample tube, the graph of L. inermis from Sharawasti (Figure 1a) shows a solitary main peak with a visual density of three for the extract from water and 0.7 for Lawsone. 32.7% is the yield of the two curves. Sixty-seven percent of the other chemical components that make up the total phenolic compounds are distributed in the area between the two curves.

After 24 minutes of elution from the twelve to the twenty-four illustration tube, the chromatogram of Henna from Bahraich (Figure 1b) displays a significant highest with an optical concentration of 1.047 for the water soluble extract and 0.356 for Lawsone. The two curves' combined yield is 33.94%. The remaining chemical components, which make up the phenolic compounds, account for 66.06% of the space between the two curves.

Afterward elution duration of 32 minutes from the 18^{th} to the 34^{th} sample tube, the water-based extract of L. inermis from Lucknow was primarily consisted of a peak at 381nm containing Lawsone, according to the consequences of Figure 1c. The water soluble extract and Lawsone have maximal optical densities of 2.289 and 0.719, respectively, and a yield of 31.41% for the two curves.

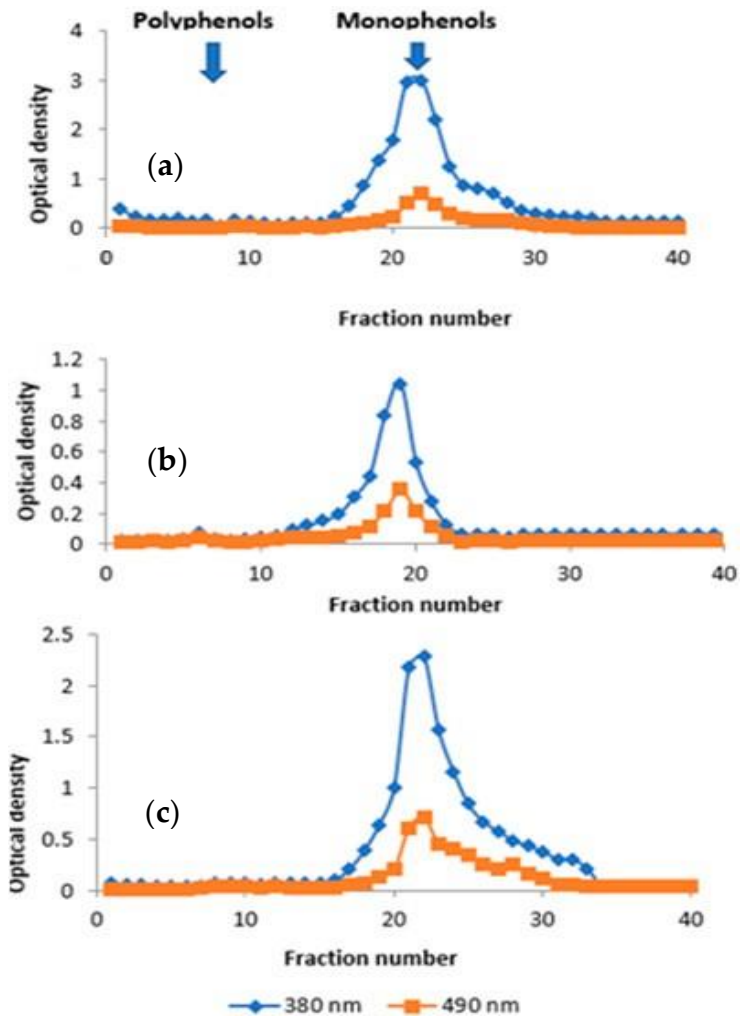


Figure1. Molecular weight distribution of phenolic compounds and Lawsone in *Sharawasti* (**a**), *Bahraich* (**b**) and *Lucknow* (**c**) extracts.

The distribution of the other chemical components, which make up 68.59% of the phenolic complexes, is characterized by the range among the two curvatures. The segment Lawsone was faultlessly isolated by distilled water, and this segment consisted of an individual monophenol peak that was discovered to have an absorbance of 490 nm, according to the data shown in Figure 1a–c. It container be shown from Figure 1a–c that the Lucknowian henna water soluble extract characterizes the ridiculous extract in Lawsone had owed by Bahraich and then Sharawasti. *Henna* from Lucknow could be valorized within the agenda of prospective industrialization.

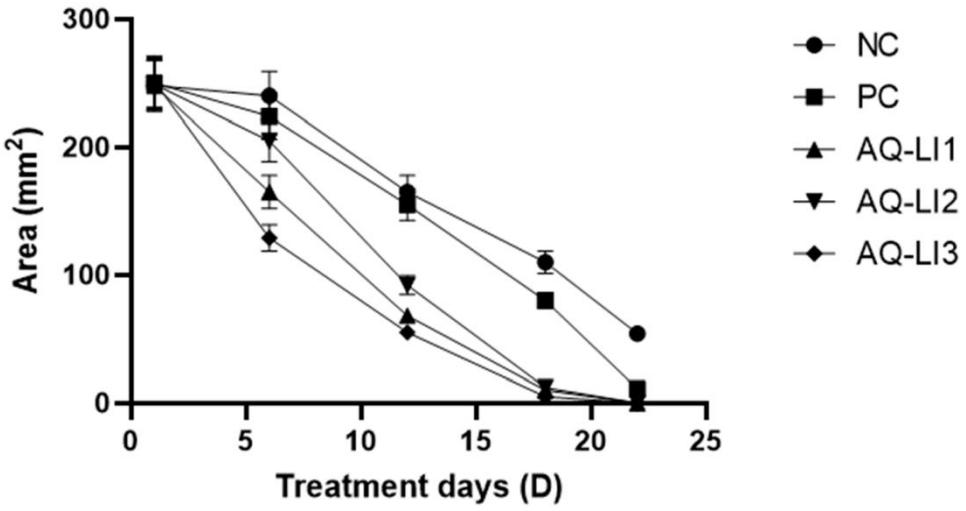
Evaluation of in Vivo Wound Healing Activity

Epithelialization period mean values and standard errors.

The middling complete healing periods of burns from the LI1, LI2, and LI3 groups wereAQ-LI1(21.31± 4.55),AQ-LI2 (20.66± 5.2), AQ-LI3 (19.35± 0.04) to differ significantly from those of the NC(41.32± 2.42) and PC(31.62± 3.67) groups, with the managements involving L. inermis cream preparations winning out.

Note: L. inermis from Sharawasti is AQ-LI1; from Bahraich is AQ-LI2; and from Lucknow is AQ-LI3; NC is negative control; PC is positive control. For every extract, values are given in the form of mean ±SE, where SE stands for standard error and n=5.

The PC cohort recorded an average duration of 31.62 3.67 days, whereas the latter recorded average durations of full epithelialization of 19.35 ± 0.04, 21.31 ± 4.55, and 20.66 ± 5.2 days for Lucknow LI, Sharawasti LI, and Bahraich LI, respectively. Eventually, it required 41.32 2.42 days for the burns in the mice without treatment group (NC) to fully re-epithelialize. Between the LI1, LI2, LI3, PC, and NC groups, there was a very statistically significant difference (ANOVA: $F = 185.53$; $df = 4$; $p < 0.001$). Average surface and mean percentage of burn contraction



Variations in burning superficial and retrenchment percentage are obtainable in Figure2; Figure3 below.

Figure 2. Evolution of the AQ-LI1, AQ-LI2, AQ-LI3, PC, and NC groups' burn surface mean values and standard errors. Note: PC stands for positive control, NC for negative control, and D for days. AQ-LI1 is L. inermis from Sharawasti; AQ-LI2 is L. inermis from Bahraich; and AQ-LI3 is L. inermis from Lucknow. Values are given as mean ± standard error of the mean (SE = Standard error); $p < 0.05$ indicates significance when compared to the control group (n = 5).

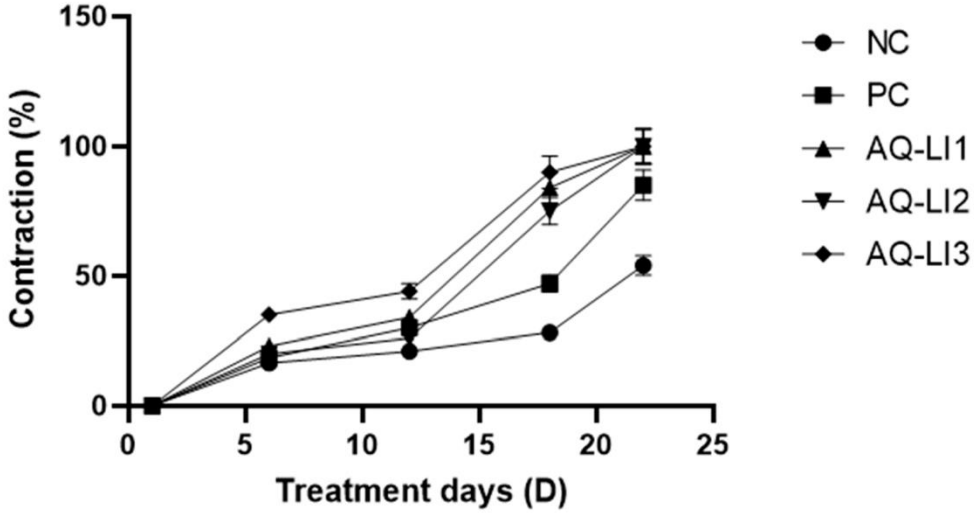


Figure3. Evolution of each group's burn contraction means values and standard errors. Note: PC stands for positive control, NC for negative control, and D for days. AQ-LI1 is L. inermis from Sharawasti; AQ-LI2 is L. inermis from Bahraich; and AQ-LI3 is L. inermis from Lucknow. Values are given as mean ± standard error of the mean (SE = Standard error); $p < 0.05$ indicates significance when compared to the control group (n = 5).

Based on the acquired data, we observed that the burning superficial diminished throughout the managements, and the evolution of this superficial differed throughout the groups. The Lucknow AQ-LI group's burns had the largest diminutions in superficial area from 241.76± 2.12 to 127.92± 2.05 mm2, with a contraction percentage of 33.13%, during the first six days after the burn, known as the therapeutic inflammatory phase. Sharawasti AQ-LI's burns decreased from 242.63 ± 2.34 to 164.70 ± 2.15 mm2, with a contraction percentage of 22.77%. Following that, results for the variety Bahraich AQ-LI ranged from 241 ± 2.51 to 201.53 ± 2.27 mm2.

The NC group did, however, show the lowest reductions in burn surface, with a low contraction percentage of 14.83% and a range of 241.33 ± 2.86 to 239.73 ± 3.07 mm2. At the level of the AQ-LI1, AQ-LI2, AQ-LI3, PC, and NC groups, statistical analysis indicated a highly significant effect based on the contraction percentage.

On the one hand, the size disparities between the groups' burns were more noticeable starting on day 12, and the groups that were treated with our formulations continued to differ significantly from the PC and NC clusters—that is, from variety cluster (AQ-LI3). As a result, after 22 days, every burn in the AQ-LI1, AQ-LI2, and AQ-LI3 groups had fully healed. However, mice given PC (Flamazine) only needed 24 days to recuperate.

With a contraction percentage of 21.88% and the greatest average superficial expanse of 162.42 2.72 mm2, the NC group showed the first instances of full burn therapeutic on the thirty-five day. The

outcomes (Figure 4) support the customary use of the leaves of the L. inermis species that our investigation revealed. These findings also support previous research that has demonstrated the efficacy of this species in the treatment of burns [30] and wounds.[28, 29]

Lawsone, which was extracted from henna leaves (L. inermis), was assessed by a research team for its analgesic and anti-inflammatory properties. The study's findings demonstrated that the bioactive fragment Lawsone produced a significant analgesic effect in comparison to the aspirin-containing positive control. They also determined that Lawsone possesses both analgesic and anti-inflammatory properties, indicating the L. inermis species' practical medical significance. The latter has historically been employed extensively due to its safety and profitability; nevertheless, more research is required to as certain Lawsone's systemic safety. However, it should be highlighted that this is the first study to compare the healing effects of a galenic formulation of L. inermis leaf extracts with the standard burn treatment, silver sulfadiazine (SSD). Because of its antibacterial properties, silver sulfadiazine, also known as Flamazine, is used to treat underlying infections; nonetheless, prevention is necessary for improved wound healing. Its simplicity of use is linked to median cutaneous infiltration and little discomfort.

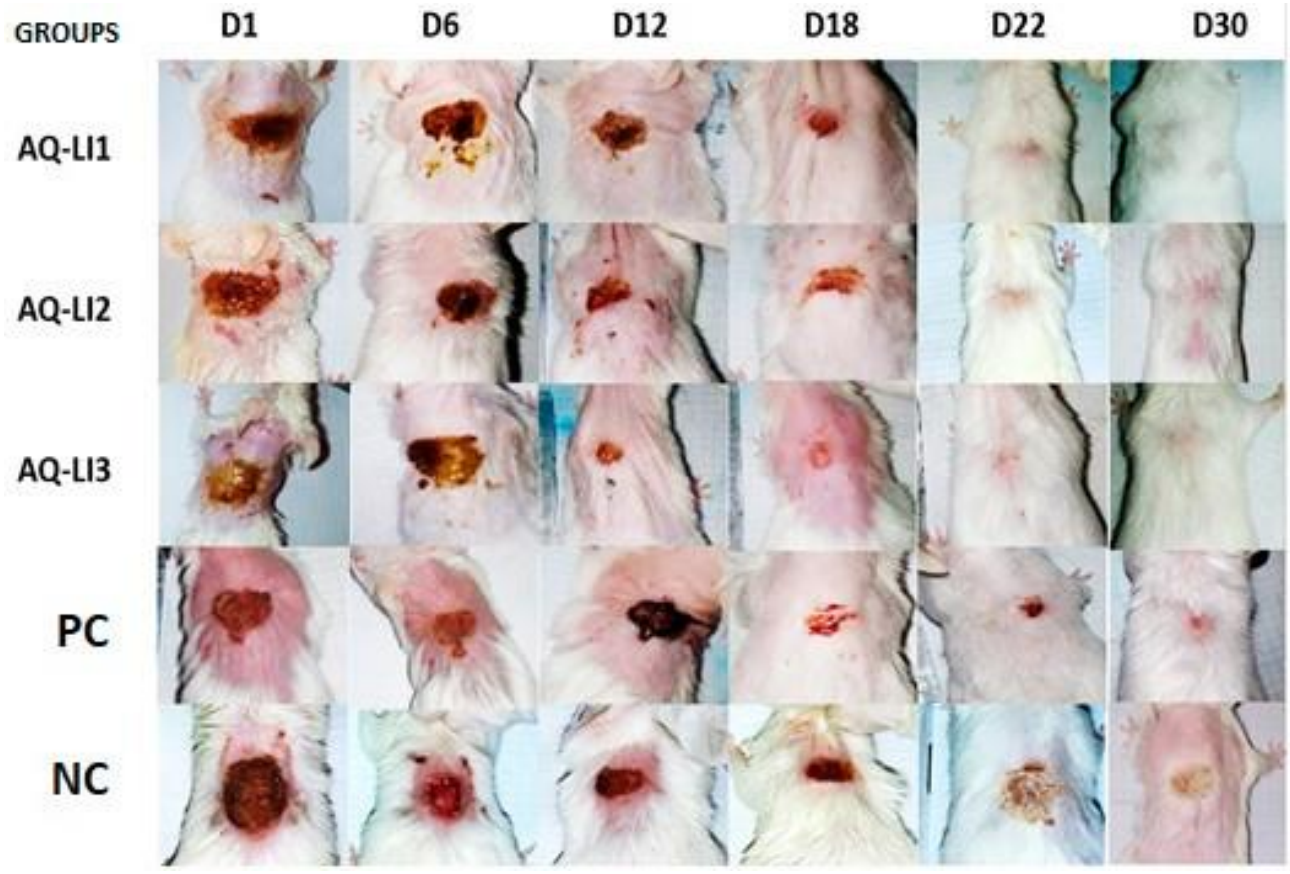


Figure 4. Observations made over the course of 30 days of the burns preserved with AQ-LI1, AQ-LI2, and AQ-LI3, as well as controls (TP and TN, where TP stands for positive control and TN for negative control). Note: PC stands for positive control, NC for negative control, and D for days. AQ-LI1 is L. inermis from Sharawasti; AQ-LI2 is L. inermis from Bahraich; and AQ-LI3 is L. inermis from Lucknow. Values are given as mean ± standard error of the mean (SE = Standard error); $p < 0.05$ indicates significance when compared to the control group ($n = 5$).

Notwithstanding these benefits, a number of SSD adverse effects, including renal toxicity, have been documented, indicating that it should not be used for extended periods of time or on big wounds. Flamazine usage in some patients is limited due to reported latter instances of healing and allergic responses which are noted by. In order to do this, researchers have concentrated in the past few centuries on trainings assessing the anti-inflammatory and restorative properties of plant extracts. Flamazine is not nearly as effective at healing as Crocus sativus, also known as saffron. In a similar vein, the research team evaluated the healing properties of aloe Vera and Flamazine, showing that aloe Vera promotes better burn re-epithelialization than Flamazine.

When associating injuries from consignments AQ-LI1, AQ-LI2, and AQ-LI3 to PC and NC batches, the wound healing activity of our formulated L. inermis was more noticeable throughout the inflammatory phase of healing. Because of its anti-inflammatory properties, this may be explained. Similarly, the many chemical components in natural product formulations contribute to their therapeutic properties. According to a study, these natural mixes' healing properties stem from mechanisms such cell proliferation, collagen synthesis activation, antioxidant capacity, and antibacterial and anti-inflammatory activities. Regarding L. inermis, there has been evidence of its abundance in bioactive compounds, including but not limited to fatty acids, triglycerides, tannins, saponin, flavonoids, and polyphenols. These substances have strong antioxidant properties due to the hydroxyl activity that enables them to snare free radicals. This could account for why groups treated with L. inermis formulations healed completely faster than groups treated with NC and PC. Furthermore, the higher rate of burn healing in comparison to controls may be explained by the high concentration of saponin found in the L. inermis leaf extracts (see biochemical studies). These findings are in line with those of Ankita et al. who assessed the fruit of Ficusreligiosa and created a healing ointment. Based on their research, they concluded that saponin, when combined with the powdered leaves of Ficusbenghalens is, play a significant role in promoting the healing process and minimizing wounds over an 18-day period. Furthermore, compared to mice handled without the use of antioxidants, the inclusion of antioxidants to the slant of therapies for burned mice has positive effects such a reduction in the severity of wound impairment and a shorter overall healing period.

Indeed, the effects of the burn cause damage to the construction of the epidermis, or higher coatings of skin, as well as the loss of the skin's protective hydrolipidic film. As a result, rather than serving as a barrier, the skin serves as a point of entrance for infection and eventually dehydrates. Our tailored remedies' ability to shield burned skin is strongly correlated with their lipid content. El Massoudi et al.'s research has demonstrated the high total lipid content of extracts made from L. inermis leaves, revealing this.

Variation in weight of mice during treatment

The weight change of the mice in each of the groups (AQ-LI1, AQ-LI2, AQ-LI3, NC, and PC) throughout therapy is depicted in Figure 5.

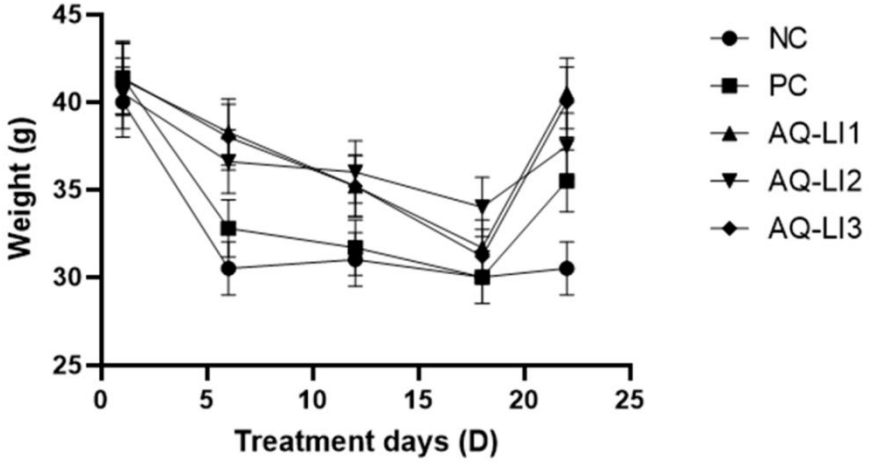


Figure 5. Variation in the treatment-related batch mouse weightiness of the groups under study (mean values and standard errors). Note: L. inermis from Sharawasti is identified as AQ-LI1, L. inermis from Bahraich as AQ-LI2, and L. inermis from Lucknow as AQ-LI3. Days; PC stands for positive control; NC for negative control. Values are given as mean ± standard error of the mean (SE = Standard error); $p < 0.05$ indicates significance when compared to the control group ($n = 5$).

Weights dropped for all groups during the healing process, according to these results (Figure 5). The first 12 days saw the biggest drops in recorded values. The NC and PC groups dropped 9 g, while the groups administered with our preparations lost 6 g. This discrepancy, which was seen in the group who had Flamazine treatment, could be attributed to the harmful effects of the chemical compounds in this medication.

These findings further demonstrate that mice in groups AQ-LI1, AQ-LI2, and AQ-LI3 were able to regain their starting weight shortly after the burns absolutely healed at 22 days.

The mice's weight analysis of variance revealed an important distinction between the three groups under investigation (ANOVA: df = 4; F = 2.62; $p < 0.001$). One of the variables that affect how quickly wounds heal is weight. Neuro-hormonal factors are produced after burn, including glucocorticoids, catechol amines, and humoral mediators like TNF, IL1, and IL6. The patient's body experiences hyper catabolism as a result, which is primarily characterized by the breakdown of nutritional stores of protein and carbohydrates by decreasing the production of insulin and lipid stores necessitating greater energy disbursement. These metabolic aftereffects are crucial for supplying the hyper activated cells with the nutrients (amino acids, fatty acids, and glucose) needed for tissue repair. The degree of the harm is correlated with these processes' intensity. The findings suggest that L. inermisis an intriguing plant whose greenery extracts may find application in the management of burns. To identify the active ingredients that are responsible for the healing effect and to determine the machinery of action of the produced preparations, pharmacodynamic and phytochemical studies must be conducted.

Conclusions

In comparison to the control groups (PC and NC), the study's findings indicate that the L. inermis formulations had a statistically significant impact on the overall healing durations and contraction percentages of burn patients. The formulation-treated burns displayed a reduction in burn surface area as well as a variance in the percentage of contraction; the Lucknow variety (AQ-LI3) had the greatest reduction in burn surface area. The current study's results validated the traditional applications of L. inermis leaves, and they are in line with previous research demonstrating the species' utility in burn and wound healing.

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Author Contributions

Conceptualization & methodology, GM, MIA & KN.; validation & investigation, SV; writing—original draft preparation, GM, AKY. Writing review TA & AKY; editing, KN. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The animal study protocol was approved by the Institutional Animal Ethics Committee of the Hygia

Institute of Pharmaceutical Education and Research Lucknow with the protocol (Reg. No.1098/PO/Re/S/07/CPCSEA: Dated. 30-07-2022) for studies involving animals.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All required data will be available with the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Lydia, B.Y. Petite Histoire Du Maquillage; Stock: Paris, France, 1999.
2. 2. Hughes, K.; Ho, R.; Butaud, J.-F.; Filaire, E.; Ranouille, E.; Berthon, J.-Y.; Raharivelomanana, P. A selection of eleven plants used as traditional Polynesian cosmetics and their development potential as anti-aging ingredients, hair growth promoters and whitening products. J. Ethnopharmacol. 2019, 245, 112159.
3. 3. Walter, P.; Martinetto, P.; Tsoucaris, G.; Brniaux, R.; Lefebvre, M.A.; Richard, G.; Talabot, J.; Dooryhee, E. Making make-up in Ancient Egypt. Nature 1999, 397, 483–484.
4. González-Minero, F.J.; Bravo-Díaz, L. The Use of Plants in Skin-Care Products, Cosmetics and Fragrances: Past and Present.Cosmetics 2018, 5, 50.
5. Bouhlal, T.; Benkhnigue, O.; Zidane, L.; Sobh, M.; Fadli, M. Vasculat Plants Used in Tradional Cosmetic by the Human Population in the Plain of the Gharb (Morocco) Full Paper. Nat. Prod. Indian J. 2013, 9, 326–330.
6. Zrira, S. Some Important Aromatic and Medicinal Plants of Morocco. Med. Aromat. Plants World 2017, 3, 91–125.
7. Saive, M.; Frederich, M.; Fauconnier, M.-L. Plants Used in Traditional Medicine and Cosmetics in Mayotte Island (France): An Ethnobotanical Study. Indian J. Tradit. Knowl. 2018, 17, 645–653.
8. Semwal, R.B.; Semwal, D.K.; Combrinck, S.; Cartwright-Jones, C.; Viljoen, A. Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects. J. Ethnopharmacol. 2014, 155, 80–103.
9. El Ghanjaoui Mohammed Cervera, M.L.; El Rhazi, M.; De La Guardia, M. Assessment of Trace Elements in Traditional Moroccan Cosmetics by Inductively Coupled Plasma Atomic Emission Spectroscopy. Int. J. Sci. Technol. Res. 2014, 3, 104–112.
10. Bafghi, M.F.; Salary, S.; Mirzaei, F.; Mahmoodian, H.; Meftahizade, H.; Zareshahi, R. Antibacterial and anti-trichomunas characteristics of local landraces of Lawsonia inermis L. BMC Complement. Med. Ther. 2022, 22, 203.
11. Al-Snafi, A.E. A review on Lawsonia inermis: A potential medicinal PLANT. Int. J. Curr. Pharm. Res. 2019, 11, 1–13.

12. Tang, Y.; He, W.; Yang, S.; Liu, L. Stabilisation and detoxification of henna (Lawsonia inermis L.) extract for hair dye cosmetics by spray-drying encapsulation. Color. Technol. 2019, 135, 439–450.
13. Obat, R.; Bosire, C. Formulation and evaluation of herbal lipstick using beta vulgaris and Lawsonia inermis as natural colorants. J. Phys. Appl. Sci. (JPAS) 2022, 1, 28–37.
14. Benzeid, H.; Gouaz, F.; Touré, A.H.; Bouatia, M.; Idrissi, M.O.B.; Draoui, M. Inventory of Toxic Plants in Morocco: An Overview of the Botanical, Biogeography, and Phytochemistry Studies. J. Toxicol. 2018, 2018, 4563735.
15. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol. Vitic. 1965, 16, 144–158.
16. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999, 64, 555–559.
17. Ba, K.; Tine, E.; Destain, J.; Cissé, N.; Thonart, P. Étude Comparative Des Composés Phénoliques, Du Pouvoir Antioxydant De Différentes Variétés De Sorgho Sénégalais Et Des Enzymes Amylolytiques De Leur Malt. Biotechnol. Agron. Soc. Environ. 2010, 14, 131–139.
18. Hiai, S.; Oura, H.; Nakajima, T. Color reaction of some sapogenins and saponins with vanillin and sulfur1c acid. Planta Medica 1976, 29, 116–122.
19. Mottaghipisheh, J.; Iriti, M. Sephadex® LH-20, Isolation, and Purification of Flavonoids from Plant Species: A Comprehensive Review. Molecules 2020, 25, 4146.
20. Uddin, N.; Siddiqui, B.S.; Begum, S.; Ali, M.I.; Marasini, B.P.; Khan, A.; Choudhary, M.I. Bioassay guided isolation of urease and α-chymotrypsin inhibitory constituents from the stems of Lawsonia alba Lam. (Henna). Fitoterapia 2013, 84, 202–207.
21. Ettayebi, K.; Errachidi, F.; Jamai, L.; Tahri-Jouti, M.A.; Sendide, K.; Ettayebi, M. Biodegradation of polyphenols with immobilized Candida tropicalis under metabolic induction. FEMS Microbiol. Lett. 2003, 223, 215–219.
22. Davidson, J.M. Animal Models for Wound Repair. Arch. Dermatol. Res. 1998, 290, S1–S11.
23. Cai, E.Z.; Ang, C.H.; Raju, A.; Tan, K.B.; Hing, E.C.H.; Loo, Y.; Wong, Y.C.; Lee, H.; Lim, J.; Moochhala, S.M.; et al. Creation of Consistent Burn Wounds: A Rat Model. Arch. Plast. Surg. 2014, 41, 317–324.
24. Nunes, J.P.S.; Dias, A.A.M. ImageJ macros for the user-friendly analysis of soft-agar and wound healing assays. Biotechniques 2017, 62, 175–179.
25. Abeje, B.A.; Bekele, T.; Getahun, K.A.; Asrie, A.B. Evaluation of Wound Healing Activity of 80% Hydromethanolic Crude Extract and Solvent Fractions of the Leaves of Urtica simensis in Mice. J. Exp. Pharmacol. 2022, 14, 221–241.
26. Shivananda Nayak, B.; Isitor, G.; Davis, E.M.; Pillai, G.K. Wound healing activity of Lawsonia inermis linn. 827 The Evidence Based Wound Healing Activity of Lawsonia Inermis Linn. Phytother. Res 2007, 21, 827–

831.

27. Dev, S.K.; Choudhury, P.; Srivastava, R.; Sharma, M. Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. Biomed. Pharmacother. 2019, 111, 555–567.
28. Muheyuddeen G, Rav DS, Verma S, Gautam SK, Gupta SK. Lawsonia inermis linnaeus: Pharmacological peculiarity and modern progression. Research Journal of Pharmacognosy and Phytochemistry. 2023;15(1):63 76.
29. Muheyuddeen G, Divy SR, Gautam SK, Gupta SK. Lawsonia inermis L. phytopharmacological characteristics and recent advancement. Research Journal of Pharmacognosy and Phytochemistry. 2023;15(1):11-23.
30. Muheyuddeen G, Rayini SH, Yadav P, Gupta SK. In vivo Analgesics and in vitro Antioxidants Activity of Newly Synthesized Mannich Bases of Lawsone. Asian Journal of Pharmaceutical Research. 2023;13(1):11-7