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HPLC-MS/MS Profile, Analgesic and Anti-inflammatory Activities of *Lawsonia inermis* Seed Fractions

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ABSTRACT

Pain and inflammation are common symptoms present in many medical conditions, typically managed with analgesic and anti-inflammatory medications. However, these medications, whether targeting peripheral or central pathways, are often associated with adverse side effects. This study aimed to identify the constituents of *Lawsonia inermis* seed fractions, and evaluate the analgesic, and anti-inflammatory activities of these fractions. Chemical constituents of the fractions were characterized using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The analgesic properties were evaluated using the acetic acid-induced writhing and tail immersion tests, while anti-inflammatory activity was assessed via the carrageenan-induced paw edema model in mice. A total of 29 compounds were identified, with the ethanol fraction exhibiting the highest concentration of the constituents, amounting to 93.89 mg/g fraction. Major compounds detected in the ethanol fraction included gallic acid, catechin, tannic acid, vanillin, epicatechin gallate, isoquercitrin, and ellagic acid. The study revealed that the ethanol fraction demonstrated a notable peripheral analgesic effect, achieving 34.75% inhibition at a dose of 250 mg/kg in mice, while the aqueous fraction showed substantial central analgesic activity in rats at 500 mg/kg throughout the experimental period. Additionally, at a dose of 400 mg/kg, the ethanol fraction resulted in a 74.05% inhibition of inflammation in mice, outperforming indomethacin, which achieved a 58.09% inhibition at 10 mg/kg after 4 h. These findings present novel evidence that the fractions of *L. inermis* seed exhibit significant analgesic and anti-inflammatory activities, supporting the traditional medicinal use of this plant for the management of conditions involving pain and inflammation.

Keywords: *Lawsonia inermis* seed, HPLC-MS/MS, Pain, Inflammation, Analgesic activity, Anti-inflammatory activity.

Introduction

Pain and inflammation are fundamental biological responses that serve to protect the body from injury and infection.[1-3] However, when these responses become chronic, they can be harmful, impairing quality of life and increasing the risk of developing conditions such as cardiovascular diseases, neurological disorders, obesity, and certain cancers.[4-6]

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While common treatments for pain and inflammation, including non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, are effective, they are often associated with significant side effects. NSAIDs, for instance, can cause serious gastrointestinal complications like ulcers and bleeding, and long-term use may lead to renal and cardiovascular issues.[7,8] Opioids, although potent for managing severe pain, pose a high risk of addiction and abuse, with side effects such as drowsiness, constipation, and potentially life-threatening respiratory depression in cases of overdose.[9] These limitations highlight the urgent need for new therapeutic agents that are both effective and safe, with the aim of reducing risks and improve patients' quality of life.[10,11] This pursuit has fueled growing interest in natural products, which are considered valuable sources of novel therapeutic compounds.[12,13] Medicinal plants, in particular, are rich in diverse bioactive substances and represent a promising reservoir for the development of drugs that are safer and better tolerated while maintaining therapeutic efficacy.[14]*Lawsonia inermis*, commonly known as henna, is a perennial plant native to tropical and subtropical regions of Africa, Asia, and the Middle East.[15] It is primarily recognized for its leaves, which, once dried and ground, are used as a natural dye. For centuries, various cultures have utilized henna not only for colouring hair and nails but also for creating intricate skin decorations, especially in religious rituals and wedding ceremonies. Beyond its cosmetic applications, traditional medicine has employed *L. inermis* for

its therapeutic benefits, such as promoting the healing of cuts and burns and alleviating headaches and joint pain through compresses made from its leaves. In Ayurvedic and Arabic medicinal practices, it is also used to treat skin disorders like eczema and to strengthen hair and prevent hair loss.[16] Recent pharmacological research has substantiated several therapeutic benefits of *L. inermis*, attributing these effects to the plant's bioactive components.[17,18] Also one of the most notable effects of henna is its anti-inflammatory and analgesic properties.[19] Studies have indicated that extracts of *L. inermis* can modulate the production of key inflammatory mediators, such as tumour necrosis factor-alpha (TNF-α), and various interleukins, which are critical in controlling inflammatory processes.[20] By inhibiting the production or action of these cytokines, henna may help to reduce inflammation and pain.[21] Furthermore, its flavonoid and tannin content contributes to membrane stabilization, which helps modulate the body's inflammatory responses. These mechanisms support henna's traditional use for conditions such as joint pain, headaches, and other inflammatory ailments. Additionally, research has highlighted henna's antimicrobial and antioxidant properties, reinforcing its potential as a promising source for developing novel natural therapeutic agents.[22] As a result, henna is currently a plant of great interest for the development of new natural therapeutics.

Although the leaves of *L. inermis* have been the subject of numerous studies, the seed of this plant are still relatively unexplored despite their therapeutic potential. The seed may contain bioactive compounds with analgesic and anti-inflammatory properties. High-performance liquid chromatography is particularly well suited to the separation and precise analysis of complex components in plant extracts. The identification of compounds often requires the use of specific detectors or a combination with other analytical techniques, such as mass spectrometry. The aim of this study was to evaluate the analgesic and anti-inflammatory properties of *L. inermis* seed fractions, and to characterize the compounds present using HPLC-MS/MS. The results could pave the way for the development of new natural treatments for pain and inflammation.

Materials and Methods

Collection and identification of plant material

The seed of *Lawsonia inermis* were collected from Draa-Tafilalet region, Zagora province, Tinzouline municipality, Morocco, in October 2019. The plant material was identified, and authenticated at the Herbarium unit of the Department of Botany, Scientific Institute of Rabat, Morocco. A specimen with voucher number: RAB114594 was deposited in the Institute's Herbarium. The dried seed were ground manually using a mortar, and the resulting powder was stored in green vials at room temperature. These vials were kept in a dry environment, protected from moisture and light, until they were used.

Animals

Wistar rats, weighing between 150 and 270 g, and Swiss mice, with weights ranging from 20 to 30 g, were sourced from the animal facility at the Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco. The animals were kept in a controlled environment with a temperature of 22 ± 2°C, 14-h light cycle, and 10-h dark cycle, and were provided with unrestricted access to food and water.

All experimental procedures were conducted in compliance with the "Principles of Laboratory Animal Care" and followed the guidelines outlined in the National Academy of Sciences' "Guide for the Care and Use of Laboratory Animals," approved by the National Institutes of Health. Each animal received an accurate dose calculated based on its body weight and the method of administration used.

Preparation of fractions

A total of 100 g of *L. inermis* seed was extracted in sequential steps using a Soxhlet apparatus. Initially, the powder was extracted with 400 mL of n-hexane for 8 h. The remaining solid residue (pomace) was then dried in an oven at 30°C for 24 h before undergoing a second 8-h Soxhlet extraction with dichloromethane. Following this, the process was repeated using ethanol as the solvent.

To ensure thorough extraction, the ethanol-treated pomace was subsequently macerated in 400 mL of water at room temperature for 48 h. The extracts obtained were categorized into four fractions: hexane (F_1), dichloromethane (F_2), ethanol (F_3), and aqueous (F_4) fractions. These fractions were then concentrated using a rotary evaporator and stored in opaque glass containers for further use.[22]

HPLC-MS/MS analysis of L. inermis seed fractions

The quantification of phytochemicals was conducted by a previously developed and validated method.[23] The four fractions of *L. inermis* seed were solubilized in a suitable solvent and filtered through a 0.2 µm filter prior to HPLC-MS/MS analysis. A Shimadzu-Nexera ultra-high performance liquid chromatography (UHPLC) coupled to a tandem mass spectrometer was used for the quantification of the phytocompounds. The reverse phase UHPLC was equipped with an automatic sampling probe (model SIL-30AC), binary pumps (model LC-30AD), a column oven (model CTO10ASvp), and a degasser (model DGU- 20A3R). Chromatographic conditions were adapted to achieve optimal separation of the compounds and to overcome suppression effects. The chromatographic separation was performed on an Agilent Poroshell 120 EC-C18 model reversed-phase chromatographic column (150 mm×2.1 mm, 2.7 µm). The column temperature was set at 40°C. The elution gradient consisted of eluent A (5 mM ammonium formate + 0.1% formic acid + water) and eluent B (5 mM ammonium formate + formic acid + 0.1% methanol). The following gradient elution profile was used: 20%-100% B (0-25 min), 100% B (25-35 min), and 20% B (35-45 min). Additionally, the solvent flow rate was set to 0.5 mL/min and the volume injected was set to 5 µL. Detection was performed in negative and positive modes using a Shimadzu LCMS-8040 tandem mass spectrometer equipped with an electrospray ionization (ESI) source. LabSolutions software (Shimadzu) was used to acquire and process the LC-ESI-MS/MS results. Multiple Reaction Monitoring (MRM) modes were used to quantify the phytocompounds. The detection and selective quantification of the phytochemicals was carried out by the MRM method based on the screening of specific phytochemical precursor ionic transitions to fragments. Collision energies were optimized to achieve optimal fragmentation of the phytochemicals and maximum transfer of the desired product ions. The MS operating parameters were as follows: drying gas flow rate (N_2), 15 L/min; nebulizing gas flow rate (N_2), 3 L/min; DL temperature, 250°C; thermal block temperature, 400°C and interface temperature, 350°C. [23]

Evaluation of analgesic activity

Peripheral analgesic activity screening using acetic acid-induced writhing test

Following the method described by Ameggouz *et al* (2024),[24] mice were weighed and randomly assigned to ten groups, with five animals in each group. Group 1 served as the negative control and did not receive any treatment. The remaining groups were given oral administration of fractions F_1, F_2, F_3, and F_4 at doses of 500 mg/kg and 250 mg/kg, respectively, as well as acetylsalicylic acid at 125 mg/kg as a reference treatment. Thirty minutes after administering the fractions, each mouse was injected intraperitoneally with a 3% (v/v) acetic acid solution at a dose of 3.75 mL/kg body weight. Ten minutes following the acetic acid injection, the number of abdominal contractions displayed by each mouse was counted over a ten-minute observation period. Percentage inhibition of abdominal contractions was calculated using the following formula:

$$PI\%\ Inhibition = \left(1 - \frac{\text{Number of contortions of mice in the treated group}}{\text{Number of contortions of the negative control group}}\right) \text{x } 100$$

Central analgesic activity screening using tail immersion test

The tail immersion test was conducted according to the procedure described by Benkhouili *et al*. (2024).[25] Female rats weighing 150–270 g were used, following the same group distribution as in the abdominal writhing test. The rats received treatments with the four fractions of *L. inermis* seed at doses of 500 mg/kg and 250 mg/kg, while morphine at 0.1 mg/kg served as the reference drug. During the test, the distal 6 cm of each rat's tail was immersed in a water bath maintained at 55.0 ± 0.5°C. The latency time between tail immersion and tail withdrawal was

recorded using a digital stopwatch at 0, 30, 60, 90, and 120 min post-administration of the treatments.

Evaluation of anti-inflammatory activity

The anti-inflammatory activity was assessed using the carrageenan-induced paw edema test in mice.[26] Carrageenan solution at 1% (m/v), prepared in 0.9% sodium chloride, was used to induce edema in the mice's paws. Thirty mice were allocated into six groups of five mice per group, and injected with the carrageenan solution. Groups 1 to 4 were orally administered aqueous and ethanol fractions of *L. inermis* seed at doses of 300 mg/kg and 400 mg/kg, 30 minutes before the carrageenan injection. Group 5 received 0.9% sodium chloride as a negative control, while group 6 was treated with indomethacin at 10 mg/kg serving as a positive control. Paw volumes were measured prior to the injection and then at 1, 2, 3, and 4 h post-injection using an LE 7500 plethysmometer (Panlab, Spain).

Statistical analysis

Data were reported as mean ± standard error of mean (SEM). Statistical analysis was conducted by comparing group means using one-way analysis of variance (ANOVA) with GraphPad Prism version 8 software. $P < 0.05$ was considered statistically significant.

Results and Discussion

Compounds identified from L. inermis seed fractions

The results of the HPLC-MS/MS analysis of *L. inermis* seed fractions are presented in Table 1. A total of 29 compounds were identified, with the ethanol fraction having the highest content of total compounds analyzed (93.89 mg/g EX), while the dichloromethane fraction had the lowest content (8.585 mg/g EX).

The ethanol fraction showed the highest content of quinic acid (4.508 mg/g EX), followed by the aqueous fraction with quinic acid content of 1.262 mg/g EX. In fact, protocatechuic aldehyde was only detected in the hexane fraction at a concentration of 0.402 mg/g EX, while catechin, vanillic acid, caffeic acid, quercetin and vanillin were detected in the polar fractions. Gallic acid was abundant in the ethanol fraction with a concentration of 41.795 mg/g EX. On the other hand, vanillic acid was only detected in the aqueous fraction at concentration of 2.992 mg/g EX. Syringic aldehyde, ferulic acid, salicylic acid and hesperetin were detected in the hexane fraction with concentrations of 0.061, 0.376, 0.024 and 0.034 mg/g EX, respectively. Tannic acid was detected in the ethanol and aqueous fractions with concentrations of 0.465 and 0.411 mg/g EX, respectively. Epicatechin gallate and piceid were detected in the ethanol fraction at concentrations of 1.541, and 0.039 mg/g EX, respectively. p-Coumaric acid, naringenin, kaempferol, and apigenin were found in the hexane and ethanol fractions. Cynaroside at concentration of 0.659 mg/g EX was detected in the dichloromethane fraction. Ellagic acid was abundant in the ethanol and aqueous fractions at concentrations of 34.4 mg/g EX, and 12.339 mg/g EX, respectively. Acacetin was detected in the four fractions: ethanol, aqueous, dichloromethane and hexane fractions.

Table 1: Compounds identified from *L. inermis* seed fractions by HPLC-MS/MS

Analytes	**RT**	**M.I. (m/z)**	**F.I. (m/z)**	***F₁*** **(mg/g EX)**	***F₂*** **(mg/g EX)**	***F₃*** **(mg/g EX)**	***F₄*** **(mg/g EX)**
Quinic acid	3.0	190.8	93.0	ND	ND	4.508	1.262
Gallic acid	4.4	168.8	79.0	0.242	ND	41.795	1.802
Protocatechuic acid	6.8	152.8	108.0	0.106	ND	0.844	ND
Catechin	7.4	288.8	203.1	ND	ND	4.876	ND
Protocatechuic aldehyde	8.5	137.2	92.0	0.402	ND	ND	ND
Tannic acid	9.2	182.8	78.0	0.102	ND	0.465	0.411
Epicatechin	11.6	289.0	203.0	6.628	6.391	ND	ND
Vanillic acid	11.8	166.8	108.0	ND	ND	ND	2.992
Caffeic acid	12.1	179.0	134.0	ND	ND	0.043	ND
Vanillin	13.9	153.1	125.0	ND	ND	0.164	0.780
Syringic aldehyde	14.6	181.0	151.1	0.061	ND	ND	ND
Epicatechin gallate	15.5	441.0	289.0	ND	ND	1.541	ND
Piceid	17.2	391.0	135/106.9	ND	ND	0.039	ND

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p-Coumaric acid	17.8	163.0	93.0	1.535	ND	0.318	ND
Ferulic acid	18.8	192.8	149.0	0.376	ND	ND	ND
Salicylic acid	21.8	137.2	65.0	0.024	ND	ND	ND
Cynaroside	23.7	447.0	284.0	ND	0.659	ND	ND
isoquercitrin	25.6	463.0	271.0	ND	ND	3.167	ND
Ellagic acid	27.6	301.0	284.0	2.017	ND	34.400	12.339
Cosmosiin	28.2	431.0	269.0	ND	ND	0.071	ND
Quercitrin	29.8	447.0	301.0	ND	ND	0.023	ND
Astragalin	30.4	447.0	255.0	ND	ND	0.164	ND
Quercetin	35.7	301.0	272.9	ND	ND	1.116	0.023
Naringenin	35.9	270.9	119.0	0.489	ND	0.081	ND
Hesperetin	36.7	301.0	136.0/286.0	0.034	ND	ND	ND
Luteolin	36.7	284.8	151.0/175.0	0.003	1.507	ND	0.085
Kaempferol	37.9	285.0	239.0	0.043	ND	0.030	ND
Apigenin	38.2	268.8	151.0/149.0	0.430	ND	0.149	ND
Acacetin	40.7	283.0	239.0	1.651	0.028	0.096	0.028
Total	-	-	-	14.143	8.585	93.890	19.722

R.T.: Retention time; MI (*m/z):* Molecular ions of the standard analytes (m/z ratio), FI (*m/z):* Fragment ions, ND: Not Detected, F_1: hexane fraction, F_2: dichloromethane fraction, F_3: ethanolic fraction, F_4: aqueous fraction, mg/g EX: mg Analyte/g fraction.

Analgesic activity of L. inermis seed fractions

The acetic acid-induced writhing test is widely used to assess peripheral analgesic activity in mice models, as it stimulates nociceptors by releasing endogenous substances like bradykinin and prostaglandins, which cause pain and writhing behaviors. The results of this study, summarized in Table 2, demonstrated the analgesic potential of different fractions of *L. inermis* seed in comparison to acetylsalicylic acid. The findings showed that administration of acetic acid significantly increased the number of writhings in mice, indicating pain induction ($p < 0.05$). The ethanol (F_3) and aqueous (F_4) fractions exhibited notable analgesic effects, with dose-dependent reductions in writhing behavior. At a dose of 250 mg/kg, the ethanol fraction reduced the number of writhings by 34.75%, while at 500 mg/kg, the aqueous fraction achieved a 31.91% reduction. In contrast, the hexane (F_1) and dichloromethane (F_2) fractions showed minimal to no significant inhibitory effects on the writhing response.

The tail immersion test results, presented in Table 3, revealed that the latency to thermal nociceptive response significantly increased following the oral administration of 500 mg/kg of various fractions (hexane, dichloromethane, ethanol, and aqueous) of *L. inermis* seed, with a marked antinociceptive effect appearing at 30 minutes and persisting up to 120 minutes ($p < 0.05$). The ethanol (5.0 ± 0.3 s) and aqueous (4.7 ± 0.5 s) fractions exhibited the maximum effect at 120 minutes, which was comparable to the effect of morphine (0.1 mg/kg). These findings align with previous reports demonstrating the analgesic potential of polar fractions from medicinal plants, possibly linked to their phytochemical composition, such as polyphenols, known for their pain-relieving properties. Additionally, the statistical analysis confirmed the significance of these effects, supporting the reliability of the observed antinociceptive response.

Pain is often perceived as an intense, unpleasant sensation that can restrict movement.[27] In contrast, inflammation is the body's protective response to injury, but it can also cause redness, heat, and swelling.[28] Medicinal plants are known to contain a variety of bioactive compounds that have been shown to possess analgesic and anti-inflammatory properties, helping to modulate the pathophysiological responses associated with pain and inflammation.[29] The acetic acid-induced writhing test was used to evaluate the peripheral analgesic activity of *L. inermis* seed fractions due to its high sensitivity and ability to identify antinociceptive effects.

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Table 2: Analgesic effect of fractions of *L. inermis* seed, and Acetylsalicylic acid on acid-induced writhing in mice.

Sample	Dose (mg/kg)	Number of writhings	Percentage Inhibition (%)
F_1	250	$46,5\pm 2.5^{an}$	1.06
	500	45.0 ± 2.6^{is}	4.25
F_2	250	43.6 ± 1.7^{bp}	7.02
	500	40.5 ± 9.5^{jt}	13.82
F_3	250	30.6 ± 2.2^{bq}	34.75
	500	35.3 ± 1.7^{ku}	24.82
F_4	250	45.2 ± 1.5^{br}	3.72
	500	32.0 ± 1.3^{lv}	31.91
Acetylsalicylic acid	125	21.6 ± 1.9^{bm}	41.71
Negative Control		47.5 ± 1.5^{nz}	-

F_1: hexane fraction, F_2: dichloromethane fraction, F_3: ethanolic fraction, F_4: aqueous fraction.
Values in the same column with different superscript letters indicate significant differences ($p < 0.05$)

The injection of acetic acid into the peritoneal cavity causes local irritation, leading to the release of various endogenous inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins. These substances induce chemically induced visceral pain, characterized by abdominal contractions accompanied by the extension of the forelimbs and a prone body position.[30] The results revealed varying degrees of analgesic activity across the fractions, with notable reductions in the number of writhes observed. The inhibitory effect of these fractions ranged from minimal to moderate, depending on both the type of fraction and the administered dose. Interestingly, the aqueous and ethanol fractions showed significant peripheral analgesic activity in mice, at doses of 250 mg/kg and 500 mg/kg, respectively, suggesting their potential therapeutic role. The results suggest that *L. inermis* seed contain bioactive compounds with potential therapeutic value in managing pain. These results could be attributed to the phytoconstituents that possess analgesic activity. The possible mechanism by which the fractions produced peripheral analgesia in this model could be related to inhibition of prostaglandin synthesis, particularly PGE_2, by blocking the cyclooxygenase (COX) enzyme, which would reduce sensitization of nociceptive receptors and therefore pain.[31] These fractions have antioxidant properties and could also reduce oxidative stress, thereby limiting tissue damage associated with inflammatory pain.[22]

The second model for the detection of spinal nociception and possible involvement of central antinociceptive mechanisms was the tail immersion test. Based on the results shown in Table 3, oral treatment of rats with the fractions significantly (p <0.05) increased the reaction time to the nociceptive thermal stimulus. This antinociceptive effect begins at 30 min and persists throughout the experiment, with a maximum effect at 120 min for the ethanol and aqueous fractions at 500 mg/kg of the order of 4.7±0.5 and 5.0±0.3 min, respectively. Antinociceptive activity against temperature-induced pain can be attributed to several mechanisms. It can inhibit pain receptors, such as TRPV1 receptors, or activate inhibitory pathways in the central nervous system, thereby reducing pain perception. It can also release analgesic neurotransmitters such as endorphins.[32] To the best of our knowledge, no previous studies have investigated the peripheral and central analgesic activity of *L. inermis* seed fractions. This lack of prior research underscores the novelty and significance of the present findings.

Anti-inflammatory activity of L. inermis seed fractions

The anti-inflammatory activity, as indicated by the percentage of inflammation inhibition induced by carrageenan for *L. inermis* seed fractions and indomethacin, is detailed in Table 4. Compared to the control group, the ethanol fraction of *L. inermis* seed demonstrated a significant reduction in paw edema from one to four hours post-carrageenan injection. The ethanol fraction exhibited the most potent anti-inflammatory effect, comparable to that of indomethacin (positive control), with a marked effect observed two hours post-injection for both doses (300 and 400 mg/kg). Specifically, at a dose of 400 mg/kg, the ethanol fraction achieved a 74.05% inhibition of edema, while indomethacin at 10 mg/kg resulted in a 58.09% inhibition after four hours of treatment. These findings indicate that four hours following carrageenan administration, the ethanol fraction of *L. inermis* seed exhibits significant anti-inflammatory properties, effectively reducing paw edema progression compared to the response observed at one hour post-injection.

To assess the anti-inflammatory activity, *L. inermis* seed fractions were studied using the carrageenan-induced acute inflammation model. This model is particularly relevant because it simulates the different phases of the inflammatory response: an initial transient increase in vascular permeability followed by a prolonged period of cellular infiltration and proliferation.[33] To obtain a complete assessment of the efficacy of the fractions, it is essential to use a test capable of measuring these different stages of inflammation. This allows us not only to observe the immediate reduction in swelling, but also to determine the effect of the fractions on the subsequent inflammatory phases. In this study, acute inflammation was induced by subplantar injection of carrageenan into the left hind paw of mice. This injection induced acute local inflammation resulting in the release of several endogenous inflammatory mediators: histamine, serotonin, bradykinin, pro-inflammatory prostaglandins (PGs), oxygen-derived free radicals (superoxide anion, hydroxyl radicals) and nitric oxide (NO). These mediators are essential for increasing vascular permeability and promoting polymorphonuclear leukocyte (neutrophil) infiltration, which contributes to the development and progression of acute inflammation.[34,35] Both fractions at all doses used (300 and 400 mg/kg), significantly ($p < 0.05$) reduced edema formation from 1h after carrageenan induction and the effects persisted until the fourth hour of observation. The effect of the ethanol fraction began as early as the first hour, while the aqueous fraction began as early as the second hour and continued until the fourth hour of inflammation. This observation suggests that the bioactive constituents of the fractions can suppress acute inflammation by interfering with the release and/or activity of chemical mediators. The maximum percentage of edema inhibition by all doses of the fractions was observed at the 4th hour observation time with values of 66.33 ± 5.94, 74.05 ± 3.68, 52.78 ± 1.25, and 59.61 ± 3.76%, respectively. These effects confirmed that the anti-inflammatory effect of the fraction was dose-dependent. The potential for edema inhibition shown by the highest dose of the ethanol fraction (400 mg/kg) was comparable to that of the standard drug (Indomethacin 10 mg/kg) with respective values of 74.05 ± 3.68 and 58.09 ± 5.80% at the 4th hour observation time. The results show that *L. inermis* seed fractions have significant anti-inflammatory effects.

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Table 3: Analgesic activity of *L. inermis* seed fractions, and morphine on nociceptive responses in the tail immersion test in rats.

Sample	Dose (mg/Kg)	Reaction time in seconds				
		0 min	30 min	60 min	90 min	120 min
F_1	500	2.9±0.2[a]	3.8±0.3[a]	4.1±0.6[a]	3.9±0.4[a]	4.3±0.4[a]
F_2	500	3.2±0.8[a]	3.5±0.5[b]	3.6±0.5[b]	3.1±0.7[b]	3.0±0.3[b]
F_3	500	2.9±0.1[a]	3.5±0.1[c]	3.4±0.1[c]	3.5±0.4[c]	4.7±0.5[c]
F_4	500	3.1±0.3[a]	4.0±0.6[d]	4.4± 0.9[d]	4.9±0.3[d]	5.0±0.3[d]
Morphine	0.1	2.53±0.43[a]	6.46±0.13[e]	6.75±0.12[e]	7.22±0.15[e]	7.70±0.18[e]
Negative control	-	2.35±0.32[a]	2.62±0.39[f]	2.63±0.47[c]	2.33±0.39[c]	2.03±0.31[c]

F_1: hexane fraction, F_2: dichloromethane fraction, F_3: ethanolic fraction, F_4: aqueous fraction.
Values in the same column with different superscript letters indicate significant differences ($p < 0.05$)

Table 4: Anti-inflammatory activity of *L. inermis* seed fractions on carrageenan-induced mouse paw edema

	Dose (mg/kg)	Percentage inhibition %			
		1 h	2 h	3 h	4 h
F_1	-	ND	ND	ND	ND
F_2	-	ND	ND	ND	ND
F_3	300	21.60±5.53[a]	42.92±3.96[a]	54.33±1.76[a]	66.33±5.94[a]
	400	25.32±3.81[b]	52.42±2.78[b]	58.02±1.50[b]	74.05±3.68[b]
F_4	300	4.91±1.21[c]	39.35±2.60[c]	47.71±0.85[c]	52.78±1.25[c]
	400	11.27±2.67[d]	45.61±4.85[d]	53.74±2.73[d]	59.61±3.76[d]
Indomethacin	10	14.60±1.54[e]	30.93±5.16[e]	51.92±2.25[e]	58.09±5.80[e]

F_1: hexane fraction, F_2: dichloromethane fraction, F_3: ethanolic fraction, F_4: aqueous fraction, h: hour, ND: Not Detected. Values in the same column with different superscript letters indicate significant differences ($p < 0.05$)

They inhibit endogenous inflammatory mediators such as serotonin and histamine. In addition, edema inhibition peaks at the fourth hour time interval, suggesting that the fractions and indomethacin act effectively against various inflammatory mediators, such as COX, prostaglandins, bradykinins, and leukotrienes. They may also have free radical scavenging properties. To the best of our knowledge, no studies on the *in vivo* analgesic and anti-inflammatory activities have been reported for *L. inermis* seed. Nevertheless, Wiem *et al* reported an *in vitro* study, which showed that the aqueous fraction of henna seed exhibited a low anti-inflammatory activity by scavenging nitric oxide (NO) with an IC_{50} of 1.514 ± 0.050 mg/mL compared to the reference drug rutin (IC_{50} = 0.055 ± 0.050 mg/mL),[36] while Chaibi *et al* reported that the methanol extract had *in vitro* anti-inflammatory activity superior to that of all extracts tested with IC_{50} value of 51.00 ± 0.23 mg/L.[37] The ethanol and aqueous fractions had highly active anti-inflammatory and analgesic effects. These activities could be attributed to the presence of polyphenol compounds in the fraction. The results of the HPLC-MS/MS analysis showed that the ethanol and aqueous fractions were rich in quercetin, tannic acid, apigenin and luteolin, which are anti-inflammatory and analgesic agents.[38,39] Polyphenols regulate the production of cytokines and the expression of genes associated with inflammation. They also inhibit certain enzymes involved in inflammation by inhibiting cyclooxygenase and lipoxygenase.[40]

Conclusion

In conclusion, the fractions of *L. inermis* seed exhibited notable analgesic and anti-inflammatory effects, underscoring their potential as therapeutic agents for managing pain and inflammation. The dual activity in modulating nociceptive pathways and reducing inflammation suggests a multifaceted mechanism of action, making them promising candidates for both central and peripheral pain relief. Additionally, the impact on inflammatory processes further supports their application in treating inflammatory conditions. These encouraging results call for more in-depth studies to confirm their clinical safety and effectiveness, as well as to clarify the precise molecular mechanisms driving these pharmacological actions.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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