

## Research Article

# Evaluation of In Vivo Wound-Healing and Anti-Inflammatory Activities of Solvent Fractions of Fruits of *Argemone mexicana* L. (Papaveraceae)

Teklie Mengie Ayele <sup>1</sup>, Endeshaw Chekol Abebe <sup>2</sup>, Zelalem Tilahun Muche <sup>3</sup>,  
Melaku Mekonnen Agidew,<sup>2</sup> Yohannes Shumet Yimer,<sup>1</sup> Getu Tesfaw Addis,<sup>1</sup>  
Nega Dagnaw Baye,<sup>4</sup> Achenef Bogale Kassie <sup>1</sup>, Muluken Adela Alemu <sup>1</sup>,  
Tesfagegn Gobezie Yiblet,<sup>1</sup> Gebrehiwot Ayalew Tiruneh,<sup>5</sup> and Samuel Berihun Dagneu <sup>1</sup>

<sup>1</sup>Department of Pharmacy, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia

<sup>2</sup>Department of Medical Biochemistry, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia

<sup>3</sup>Department of Medical Physiology, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia

<sup>4</sup>Department of Human Anatomy, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia

<sup>5</sup>Department of Midwifery, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia

Correspondence should be addressed to Teklie Mengie Ayele; [teklielpharm@gmail.com](mailto:teklielpharm@gmail.com)

Received 10 August 2022; Revised 1 September 2022; Accepted 16 November 2022; Published 22 November 2022

Academic Editor: Olufunmiso Olusola Olajuyigbe

Copyright © 2022 Teklie Mengie Ayele et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Introduction.** The solvent fractions of the fruits of *Argemone mexicana* L. (Papaveraceae) have not yet been explored scientifically for in vivo wound healing and anti-inflammatory activities. The objective of this study was, therefore, to evaluate in vivo wound healing and anti-inflammatory activities of the solvent fractions of the fruit of *Argemone mexicana* L. (Papaveraceae) in rats. **Method.** The crude extract of *Argemone mexicana* was fractionated with *n*-hexane, ethyl acetate, and distilled water. Wound healing activity was evaluated using excision and incision wound models while anti-inflammatory activity was evaluated using carrageenan-induced rat paw and cotton pellet-induced granuloma models. The fractions were evaluated at 5 and 10% ointments using moist-exposed burn ointment as the standard drug, and 100, 200, and 400 mg/kg test doses using aspirin, and dexamethasone as standard drugs for wound healing and anti-inflammatory activities, respectively. All treatment administrations were made orally for anti-inflammatory activity and applied topically for wound healing activity. **Result.** The 10% w/w ethyl acetate fraction ointment showed a significant percentage of wound contraction, reduced period of epithelialization, increased amount of fibrosis, neovascularization, and collagen tissue formation ( $p < 0.01$ ). The ethyl acetate fraction also showed a significant increase in tensile strength (55%;  $p < 0.01$ ) and (81.10%;  $p < 0.01$ ) at the tested doses of 5 and 10% w/w ointments, which was comparable to moist-exposed burn ointment. The ethyl acetate fraction also revealed a significant percent edema inhibition (61.41%;  $p < 0.01$ ), suppression of the exudate (38.09%  $p < 0.01$ ), and granuloma mass formations (53.47%  $p < 0.01$ ) at the tested dose of 400 mg/kg. **Conclusion.** The results of this study showed that the Ethyl acetate fraction of *Argemone mexicana* fruit has significant wound healing and anti-inflammatory activities which support the traditional claims of the experimental plant.

## 1. Introduction

A wound is a break in the skin's epithelial integrity caused by physical or chemical traumas or microbial infections [1]. Based on the physiology of wound healing, wounds are

categorized as acute or chronic. Acute wounds are tissue lesions that recover by a predictable set of physiological occurrences, restoring anatomical and functional integrity over the long term. In most cases, they heal in less than eight weeks. *Staphylococcus aureus* is the most common pathogen

in this type of wound [2]. Chronic wounds, on the other hand, are wounds that, even after three months, have failed to progress via an orderly and timely process to create anatomic and functional integrity [3, 4].

Herbal preparations and their products are often regarded as a vital component of modern treatment [5]. Many plants have been shown to have powerful therapeutic effects [6]. Herbal treatments are widely utilized in Ethiopia to treat skin diseases, particularly wounds, and they are widely available [7].

*Argemone mexicana* L. (Figure 1) which belongs to the family of *Papaveraceae*, is naturalized throughout the world's tropical and subtropical regions and grows as a prickly poppy shrub [8–12]. *A. mexicana* has been studied in vitro and in vivo for a variety of biological activities of numerous solvent extracts of the various parts of the plant; for example, it is used in the indigenous system of medicine as an analgesic and anti-inflammatory [13–15], antiulcerogenic [16], anticancer [17, 18], antioxidant [18], thrombolytic [19], antidiabetic [20–22], antiarthritic [23], cytotoxic [24, 25], antipsychotic [26], antiepileptics [27], apoptotic [18, 28], aphrodisiac [29], antiasmatic [30], antihepatotoxic [31], nematocidal [32], antischistosomal [33], and antimalarial [34, 35] wound healing [36, 37] activities.

Moreover, the antibacterial activity of *A. mexicana* solvent crude extracts was evaluated by utilizing a range of solvent systems against the tested microorganisms [38–40]. In order to provide more context, the antibacterial compounds chelerythrine and berberine, which were extracted from *A. mexicana* root and leaf extracts, had substantial action, with the strongest activity being on Gram-positive bacteria [40]. Additionally, phytoconstituents having antibacterial activity were discovered in the ethanol root extract of *A. mexicana*, including alkaloids, triterpenes, free amino acids, phenols, and/or tannins for both Gram-negative and Gram-positive bacteria [39]. Additionally, the ethanol, methanol, and chloroform leaf extract of *A. mexicana* showed a significant suppression of bacterial growth [38].

Despite numerous claims and in vitro experiments demonstrating the wound-healing activity, no animal research on the wound-healing activity of the solvent fractions of *A. mexicana* fruits has been carried out. As a result, this investigation was carried out to look into the fractions' ability to cure wounds in different animal models.

## 2. Materials and Methods

**2.1. Chemicals, Drugs, and Reagents.** In this study, we used sulfuric acid (Loba Chemie, India), *n*-hexane (Loba Chemie, India), ethyl acetate (Loba Chemie, India), methanol (Loba Chemie, India), *n*-hexane (Loba Chemie, India), wool fat, hard paraffin, white soft paraffin, cetostearyl alcohol, indomethacin (Cadila, Ethiopia), Tween 80 (Uni-Chem, India), diethyl ether (NEON Laboratories, India), moist-exposed burn ointment, and ketamine (APF, Ethiopia). Chemicals and reagents were graded analytically.

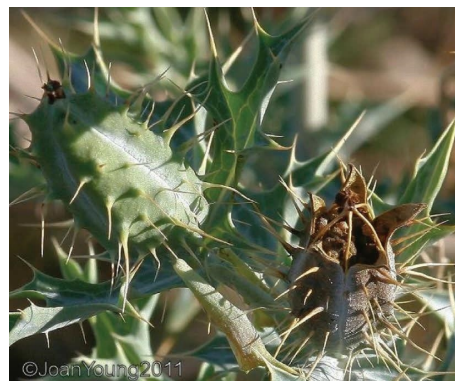


FIGURE 1: *A. mexicana* at the site of collection.

**2.2. Experimental Animals.** Adults in good health, Wistar albino rats (weighing 200–250 grams) of both sexes (age, 6–8 weeks) were procured from the Ethiopian Public Health Institute's animal house in Addis Ababa. They were kept in clean polypropylene cages with connected steel roofs under normal conditions ( $25 \pm 2^\circ\text{C}$ , 55% relative humidity, and 12-hour light/dark cycles), with unrestricted access to clean drinking water and standard laboratory pellets. One week previous to the start of the research, rats were acclimatized to laboratory settings. The National Institute of Health Guidelines for the Care and Use of Laboratory Animals were adhered to for all procedures. At the end of the experiment, the rats were euthanized using a cotton ball soaked with halothanes (inhaled anesthetics) into a bell jar to reduce suffering from pain [41].

**2.3. Collection and Authentication of the Experimental Plant.** The fruits of *A. mexicana*, also known as “Dendero” in Amharic, were gathered from their native habitat in the area of Fogera woreda in Debre Tabor, North West Ethiopia. The IUCN's policy statement on investigations involving species that are endangered or at risk of extinction permits the use of the plant's leaves in the current study. Taxonomists from the Debre Tabor University's Department of Biology, College of Natural Sciences and Computation, recognized and verified the plant, and the specimens were placed under the voucher number TM002/2022 for future use.

**2.4. Extraction and Fractionation of the Experimental Plant.** The extraction and liquid-liquid fractionation of the fruits of *A. mexicana* were carried out using the technique described by Mengie et al. with a few minor modifications [42]. In a flask containing 80% methanol (1 : 6 v/v), 650 grams of the fruits of *A. mexicana* were ground up and macerated for 72 hours. The marc was then twice further macerated with brand-new solvent after the crude extract had been filtered through Whatman filter paper (No. 1) and through. To get rid of the leftover solvent, the filtrates from the three batches were combined and concentrated at  $40^\circ\text{C}$  in a rotary evaporator. The water from the extract was lyophilized at reduced pressure and temperature. A brown powder residue weighing 80 grams with a percent yield of 12.31 of *A.*

*mexicana* fruit extract was found after solvent removal. Following that, the crude extract of *A. mexicana* was fractionated using the procedures previously indicated [43, 44], with diverse polarity index solvents (*n*-hexane, ethyl acetate, and distilled water [45]. Seventy-seven grams of *A. mexicana* 80% methanol extract was suspended in 200 ml of distilled water in a separatory funnel using liquid-liquid fractionation. An equal amount of *n*-hexane was added and thoroughly mixed. After allowing the mixture to separate into discrete layers, the *n*-hexane fraction was isolated by lower-layer elution. Each time, fresh *n*-hexane was used, and this was done three times. The residue was then combined with a similar amount of ethyl acetate and separated in the same manner. In a rotary evaporator set to 40°C, all solvent fractions were concentrated and dried. The percent yields of aqueous, *n*-hexane, and ethyl acetate from the dried fractions were 27.6 g (35.8%), 16.4 g (21.3%), and 28.2 g (36.6%), respectively (Figure 2). The fractions were then kept in airtight bottles at -4°C until the experiment began [46]. For the various tests, all fractions were diluted in simple ointment and 2% tween 80 at an appropriate concentration for wound healing and the anti-inflammatory activity evaluation, respectively.

The British Pharmacopoeia was followed in the preparation of the solvent fractions of simple and medicated ointments [47]. To make 50 g of simple ointment, 2.5 g hard paraffin and 2.5 g cetostearyl alcohol were heated in a beaker. In a separate beaker, 2.5 g wool fat and 42.5 g white soft paraffin were melted (Table 1). After that, the contents of the two containers were blended and swirled till they were cool. The 2.5 g and 5 g of the aqueous, ethyl acetate, and *n*-hexane fractions were combined with 47.5 g and 45 g of the basic ointment base, respectively, to create the 5% w/w and 10% w/w medicated ointments for each fraction.

**2.5. Acute Oral and Dermal Toxicity Tests.** Acute dermal toxicity of the solvent fractions of *A. mexicana* fruit was tested in line with OECD 425 guidelines [48]. For each fraction, three female rats with normal skin texture were chosen at random, contained separately in a cage, and adapted to the laboratory environment for seven days before the test. Rats were anesthetized with intraperitoneal injections of 50 mg/kg ketamine and 5 mg/kg diazepam. The 10 percent of their body's surface area fur was shaved from the dorsal part of the trunk 24 hours before the main study. For 24 hours, a limit test dose of 2000 mg/kg of the 10% w/w solvent fraction ointment preparations was applied consistently over the clean-shaven area. The rats were kept in individual cages during the exposure time. Similarly, the OECD 425 guideline also applied for acute oral toxicity tests but the test agents were administered orally. The left-behind test chemical was detached at the conclusion of the exposure period, and the rats were monitored for 14 days for any adverse skin reactions and behavioral abnormalities using the OECD404 classification system (2002).

**2.6. Grouping and Dosing of Animals.** The previous study by Mengie et al. with minor modifications was used for grouping and dosing of experimental animals [42]. The

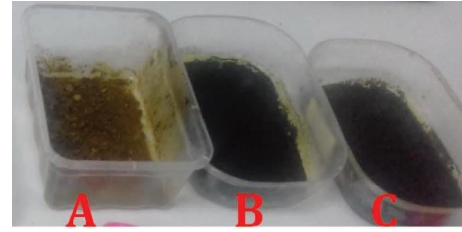


FIGURE 2: Solvent fractions of *A. mexicana* fruit: (a) aqueous fraction; (b) ethyl acetate fraction; (c) *n*-hexane fraction.

TABLE 1: Simple ointment preparation utilizing the master and reduced formulas.

Ingredients	Master formula	Reduced formula
Wool fat	50 grams	2.5 grams
Hard paraffin	50 grams	2.5 grams
Cetostearyl alcohol	50 grams	2.5 grams
White soft paraffin	850 grams	42.5 grams
	1000 grams	50 grams

animals were separated into eight groups of six rats each for the excision wound model, as follows: the 1<sup>st</sup> group was treated with simple ointment. The 2<sup>nd</sup> group was given a moist-exposed burn ointment (MEBO). The 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> groups were given 5 and 10% aqueous (AF) and ethyl acetate fraction (EAF) ointments, respectively. The 7<sup>th</sup> and 8<sup>th</sup> groups received 5 and 10% ointments of *n*-hexane fraction (*n*-HF) of *A. mexicana*, respectively. The rats were divided into 9 groups each with 6 rats for the incision wound model, with the identical grouping and dosage as the excision wound model except for the addition of the left-untreated group. The rats were randomly divided into 11 groups each with 6 rats to assess anti-inflammatory activity in two models. The 2% tween 80, aspirin, and dexamethasone were used as control at the test doses of 10 ml/kg, 200 mg/kg, and 0.5 mg/kg, respectively for both models. The test groups were given 3 different doses for each fraction i.e., at the tested doses of 100, 200, and 400 mg/kg of aqueous, *n*-hexane, and ethyl acetate fractions. For prepare of suspensions, the fractions and aspirin powder were diluted in 2% tween 80. The tested doses were identified based on the toxicity test results.

## 2.7. Wound-Healing Models

**2.7.1. Excision Wound Model.** Rats were sedated with intraperitoneal injections of 50 mg/kg ketamine and 5 mg/kg diazepam [49]. The hair from the dorso-thoracic area was detached. A circular mark of 250 mm<sup>2</sup> was created with a marker and a graft consisting of the epidermis and the entire depth of the dermis was detached using sterilized scissors on day 0, as stated by Mengie et al. and Nagar et al. [42, 50]. After 24 hours of establishing the wound area, the rats were treated daily with ointments until the wound healed fully. The wound area was measured every two days with a transparent paper and marker to check for wound

closure. The traced area for each rat was then estimated by using a millimeter-scaled ruler to measure the diameter. As

mentioned in the prior study, the percentage of wound contraction was assessed [51].

$$\% \text{Wound contraction} = \frac{\text{wound area on da y zero} - \text{wound area on da y n}}{\text{wound area on da y zero}} \times 100, \quad (1)$$

where  $n$  = the number of days i.e. 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, and 18<sup>th</sup>. The eighteenth day is the day the wound in the fraction and standard treated groups completely healed.

The number of days required for the dead tissue remnants to fall off from the wound surface exclusive of leaving a raw wound behind was taken as the endpoint of complete epithelialization and the days required for this were considered to be a period of epithelialization [52, 53].

**2.7.2. Incision Wound Model.** For the excision wound model, rats were sedated by intraperitoneal 5 mg/kg diazepam and 50 mg/kg ketamine, and their fur was detached

[42]. One centimeter from the midline, a three-centimeter elongated linear paravertebral incision was created through the whole thickness of the skin on either side of the spinal column. The excised skin was stitched at 1 cm 47 intervals using chromic catgut (2/0 metric-1/2 Circle) with a curve needle on day 0. The ointments were applied daily as indicated in the grouping and dosing section starting from the first day for a total of 9 days. On the 8<sup>th</sup> day after wounding, the sutures were detached [54]. The tensile strength was measured [55] on the 10<sup>th</sup> day to estimate the extent of healing using a continuous constant water flow technique [54, 56].

$$\begin{aligned} \% \text{TS of fractions} &= \frac{\text{Tensile strength (fractions)} - \text{Tensile strength (SO)}}{\text{Tensile strength (SO)}} \times 100, \\ \% \text{TS of reference} &= \frac{\text{Tensile strength (reference)} - \text{Tensile strength (SO)}}{\text{Tensile strength (SO)}} \times 100, \end{aligned} \quad (2)$$

$$\% \text{TS of simple ointment} = \frac{\text{Tensile strength (SO)} - \text{Tensile strength (LU)}}{\text{Tensile strength (LU)}} \times 100,$$

where TS = tensile strength, SO = simple ointment and LU = left untreated [57].

**2.7.3. Histopathology.** On the 18<sup>th</sup> day of the experiment, to check for histological alterations, deep granulation tissues and cross-sectional full-thickness skin specimens from the implanted tube were collected. After that, the samples were sectioned into 5-micrometer sections, fixed with 10% formalin, parain-blocked, and stained with hematoxylin and eosin.

## 2.8. Evaluation of the Anti-Inflammatory Activity of Solvent Fractions of *A. mexicana* Fruits

**2.8.1. Carrageenan-Induced Rat Paw Edema Model.** The method mentioned by Mengie et al. and Belay [42, 58] was used with a minor change to evaluate the effect of fractions of fruits of *A. mexicana* on acute inflammation. Rats fasted for 12 hours with unrestricted access to water until the main research was instigated. Before administering the controls and solvent fractions as stated in the dosing and grouping section, each rat's basal volume, i.e., the amount of water displaced by the left hind paw, was measured using a calibrated plethysmometer. Rats were randomly assigned to their respective groups. After that, the rats were given test agents (controls and fractions) via oral

gavage. An hour after the test agents were given, freshly prepared 1% carrageenan (w/v) was injected into the subplantar-surface left hind paws of the rats for induction inflammation. Parameters such as a change in the paw volume was recorded after a different point of time i.e., at 1, 2, 3, and 4 hours of induction through 1% w/v carrageenan using a plethysmometer [57].

$$\text{Percentage inhibition of edema} = \frac{\text{PEC} - \text{PET}}{\text{PEC}} \times 100, \quad (3)$$

PEC = paw edema of negative control and PET = paw edema of solvent fractions (test groups and the standard).

**2.8.2. Cotton Pellet-Induced Granuloma.** The chronic inflammatory process and transudative and proliferative elements were assessed using the method mentioned by Mengie et al. and Afsar et al. [42, 59]. Male rats weighting 20–30 g were fasted for 12 hours with access to the water add liptum. The rats were then treated with test substances as described in the grouping and dosing section.

An autoclave-disinfected cotton pellet weighing  $10 \pm 1$  mg was used to induce granuloma in rats. The rats received their respective treatments as described in the grouping and dosing section. After twenty minutes, rats

were anesthetized through intra-peritoneal 5 mg/kg diazepam and 50 mg/kg ketamine hydrochloride. After both groin regions of the rat were shaved, a subcutaneous tunnel was prepared aseptically using blunted forceps. Disinfected cotton pellets weighing  $10 \pm 1$  mg were then inserted into the subcutaneous tunnel in each groin and sutured with chromic catgut (2/0 metric-1/2 Circle). The rats were then orally treated with a daily dose of 2% tween 80, dexamethasone, and solvent fractions for a total of seven consecutive days in their respective groups as stated in the grouping and dosing section. The pellets that were enclosed by granuloma tissue were carefully removed and isolated from extraneous tissue after the rats were scarified by cervical dislocation on the 8<sup>th</sup>

day of the experiment. As soon as the cotton was removed and constantly dried up at 60°C for 24 hours, its wet weight was measured. Its net dry weight i.e., the weight remaining after subtracting the weight of the cotton pellets was then calculated.

The measure of exudate formation = immediate wet weight of pellet – constant dry weight of the pellet.

The measure of granuloma tissue formation = constant dry weight – initial weight of the cotton pellet.

The following formula was used to calculate the amount of exudate, granulation tissue formation in mg, % inhibition of exudate, and granuloma tissue formation [60]:

$$\text{Exudate inhibition (\%)} = \left( 1 - \frac{\text{exudate in the treated group}}{\text{exudate in controls}} \times 100 \right). \quad (4)$$

**2.9. Phytochemical Screening of Solvent Fractions.** Standard tests were used to conduct preliminary phytochemical screening of secondary metabolites in the fraction of fruits of *A. mexicana* [42, 61, 62].

**2.9.1. Test for Saponins.** 5 ml distilled water was added to 0.25 g of each fraction of *A. mexicana*. The solution was then violently shaken and stable continuous foam was seen. Saponins were detected by the formation of a steady froth that lasted about half an hour.

**2.9.2. Test for Terpenoids.** 2 ml chloroform was added to 0.25 g of each fraction of *A. mexicana*. Then, to build a coating, 3 ml of concentrated sulfuric acid was carefully applied. The presence of terpenoids was indicated by a reddish-brown coloration of the interface.

**2.9.3. Test for Tannins.** In a test tube, 0.25 g of each fraction of *A. mexicana* was cooked in 10 ml water and then filtered using filter paper (Whatman No. 1). To the filtrate, a few drops of 0.1 percent ferric chloride were added. The presence of tannins was indicated by a brownish-green or blue-black precipitate.

**2.9.4. Test for Flavonoids.** A water bath was used to boil 10 ml of ethyl acetate to 0.2 g of each fraction of *A. mexicana* for 3 minutes. The mixture was filtered and chilled. After that, 4 ml of the filtrate was mixed with 1 ml of weak ammonia solution and shaken. The layers were allowed to separate, and the presence of flavonoids was revealed by the yellow color in the ammonia layer.

**2.9.5. Test for Cardiac Glycosides.** 2 ml glacial acetic acid containing one drop of ferric chloride solution was added to 0.25 g of each fraction of *A. mexicana* that had been diluted with 5 ml of water. 1 ml of concentrated sulfuric acid was used as a base. The presence of a deoxysugar, which is

characteristic of cardenolides, was identified by a brown ring at the interface.

**2.9.6. Test for Steroids.** 0.25 g of each fraction of *A. mexicana* was mixed with 2 ml sulfuric acid and 2 ml acetic anhydride. Some samples changed color from violet to blue or green, indicating the presence of steroids.

**2.9.7. Test for Alkaloids.** A few drops of freshly made Mayer's reagent were added to 0.5 g of each fraction of *A. mexicana*. The presence of alkaloids was determined by the production of the cream.

**2.10. Data Analysis.** SPSS version 20.0 for Windows was used to analyze the data. The statistical significance test was conducted using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test for multiple comparisons to compare results between groups, with *p* values <0.05 regarded as statistically significant. Tables and figures were used to present the examined data.

### 3. Results

**3.1. Phytochemical Screening.** Phytochemical determination of solvent fractions of *A. mexicana* fruits revealed the presence of a variety of phytochemicals such as Alkaloids, saponins, tannins, glycosides, terpenoids, steroids, and flavonoids. According to the present research findings, the EAF tested positive for almost all phytochemicals (except saponins). Among the solvent fractions, the AF relatively lacks phytochemicals such as alkaloids, terpenoids, and steroids. On the other hand, *n*-HF is devoid of terpenoids and saponins. However, both the EAF and *n*-HFs lacks saponins as described in Table 2.

**3.2. Acute Oral and Dermal Toxicity Test.** There was no evidence of dermal and systemic toxicity within 24 hours or over the next 2 weeks for both the acute oral and cutaneous

TABLE 2: Preliminary phytochemical screening of *A. mexicana* fruit solvent fractions.

Secondary metabolite	AF	<i>n</i> -HF	EAF
Saponin	+	–	–
Terpenoids	+	+	+
Tannins	–	–	+
Flavonoid	+	+	+
Glycosides	+	+	+
Steroids	–	+	+
Alkaloids	–	+	+

(+, present); (–, absent); AF = aqueous fraction; *n*-HF = *n*-hexane fraction; EAF = ethyl acetate fraction.

toxicity investigation of *A. mexicana* fruits solvent fractions [48], The oral LD50 of each fraction was larger than 2000 mg/kg in rats, confirming the “limit test” of OECD 425 guideline [46]. Following that, the ointment doses of *A. mexicana* solvent fractions of 5% and 10% were chosen for the evaluation of wound healing, whereas 100, 200, and 400 mg/kg were selected for the evaluation of anti-inflammatory activity in the main experiment.

**3.3. Evaluation of Wound Healing Activity.** In an excision wound model, rats treated with EAF and *n*-HF ointments showed wound healing. In most postwounding days, the % of wound contraction in the rats treated with the 10% w/w EAF ointment was significant ( $p < 0.01$ ) compared to the negative control (Table 3; Figure 3), and the period of epithelialization was decreased (Table 4). Additionally, the % of wound contraction in rats treated with 10% w/w EAF ointment was comparable with the positive control (MEBO 15.3 g). For 10% w/w EAF and MEBO ointments, the % of wound contraction values were 59.16 and 60.12% on day 9, 70.31 and 75.25 percent on day 12, 89.17 and 92.16 percent on day 15, and 98.14 and 99.07 percent on day 18. Conversely rats given 5% w/w and 10% w/w AF ointments showed insignificant wound healing activity compared to simple ointment until the third day after wounding. In addition, 5% w/w AF ointments showed a statistically insignificant % of wound contraction until the 12<sup>th</sup> day of wounding. Among the rats treated with the EAF, *n*-HF, and AF ointments, there was a significant difference in the % of wound contraction. However, 5% w/w EAF ointment initiated significant wound healing on the 6<sup>th</sup> day and showed a significant difference in wound contractions when compared to 5% w/w AF and simple ointment on the 9<sup>th</sup>, 15<sup>th</sup>, and 18<sup>th</sup> days (Figure 3;  $p < 0.01$ ). On the 15<sup>th</sup> day, the wound contraction of the group treated with 10% w/w EAF ointment indicated a significant wound healing activity when compared to all 5% w/w *A. mexicana* solvent fraction ointments ( $p < 0.01$ ). Furthermore, when compared to 5% w/w EAF, *n*-HF, and AF ointments, the maximal dose of EAF demonstrated significant wound contraction on most wound contraction measurement days ( $p < 0.01$ ). Consequently, EAF is the most active fraction, as indicated by a larger % of wound contraction and shorter epithelialization duration (Tables 3 and 4).

When compared to the control groups (simple ointment-treated and left untreated), rats treated with 5 and 10% w/w ointments of all fractions showed a significant ( $p < 0.01$ ) increase in tensile strength in the incision wound model. MEBO & 10% w/w EAF ointments considerably increased tensile strength when compared to all *A. mexicana* solvent fractions of 5% w/w ointments ( $p < 0.01$ ). The tensile strength recorded in the group treated with 10% *n*-HF was significantly increased compared with the rats treated with 5% AF ointment (Table 5) ( $p < 0.01$ ).

Histology of the granulation tissue of the inner structure of the control rat displayed the presence of inflammatory cells (IC), disseminated fibroblasts (*F*), and limited blood vessels (BV) in the granulation tissues, whereas the granulation tissue of rats treated with 400 mg/kg dose of EAF and *n*-HF revealed the abundance of collagen tissue (*C*) and formation of blood vessels (BV) with negligible inflammatory cells indicative of healing by fibrosis (Figure 4).

**3.4. Evaluation of the Anti-Inflammatory Effect of Solvent Fractions of *A. mexicana* Fruits.** A progressive increase in paw thickness was seen after a subplantar injection of 0.05 ml of 1% carrageenan into the left hind paw of the rat. This increment peaked two hours after induction with the negative control (Table 6). Compared to the negative control, all tested doses of the EAF significantly inhibited paw edema beginning at 1 hour and continuing for 4 hours postinduction ( $p < 0.01$ ). At 4 hours after induction of inflammation, the maximum % of inhibition from the 100, 200, and 400 mg/kg dosages of EAF was observed dose-dependently with the values of 34.85%, 54.36%, and 61.41%, respectively ( $R^2 = 0.963$ ). There was a significant difference between the dosages of the EAF with the AF. For instance, the effects produced by the EAF at the doses of 200 and 400 mg were significantly different from those of AF at tested doses of 100 and 200 mg/kg at 1, 2, and 4 hours of follow-up ( $p < 0.01$ ). Moreover, the EAF produced a significant reduction ( $p < 0.01$ ) of the paw edema at all-time points of measurement as compared with the AF (at tested doses of 100 and 200 mg/kg), *n*-HF and EAF (at a tested dose of 100 mg/kg). At the tested dose of 100 mg/kg, the AF revealed statistically insignificant inhibition of paw edema at all-time points of the measurement after the induction of inflammation as compared with the negative control ( $p < 0.01$ ). Furthermore, both 100 and 200 mg/kg doses of *n*-HF showed only a significant reduction in paw volume as compared with negative control ( $p < 0.01$ ) at 1, 2, and 3 hours after the induction of inflammation. Likewise, the maximum percent of inhibition of the paw edema for the *n*-hexane fraction was recorded at the 4<sup>th</sup> hour of induction of the inflammation and the values were 31.53, 35.27, and 44.81% for the doses of 100, 200, and 400 mg/kg, respectively ( $R^2 = 0.896$ ).

Following induction of inflammation, 200 mg/kg aspirin, the standard drug, significantly reduced the paw edema as compared with AF (at the doses of 100 and 200 mg/kg), *n*-HF (at the dose of 100), and negative control ( $p < 0.01$ ). The maximum % of inhibition by the positive control was found

TABLE 3: Effect of *A. mexicana* fruit solvent fractions on percentage wound contraction in rats.

Test Group	Days of wound area measurement in mm <sup>2</sup> (mean ± SEM) and % of contraction					
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
SO	258.27 ± 26.87 (0.97)	244.32 ± 26.26 (6.36)	189.99 ± 36.00 (47.76)	147.40 ± 34.58 (55.98)	105.08 ± 7.52 (58.81)	79.63 ± 11.25 (66.84)
MEBO	217.42 ± 8.45 <sup>a,cd,fig</sup> (13.03)	168.30 ± 6.79 <sup>a,af</sup> (32.68)	103.28 ± 3.68 <sup>a,af</sup> (60.12)	61.88 ± 11.88 <sup>a,af</sup> (75.25)	19.61 ± 15.11 <sup>a,af</sup> (92.16)	2.33 ± 1.25 <sup>a,af</sup> (99.07)
5% EAF	242.31 ± 2.64 (3.08)	182.17 ± 3.61 <sup>a,a</sup> (27.13)	131.34 ± 1.53 <sup>a,af</sup> (35.32)	98.73 ± 1.10 <sup>a,a</sup> (60.51)	49.36 ± 1.90 <sup>a,af</sup> (80.25)	13.58 ± 3.15 <sup>a,af</sup> (94.57)
10% EAF	229.22 ± 2.58 <sup>a,ad</sup> (8.31)	171.50 ± 3.11 <sup>a,adf</sup> (31.40)	109.21 ± 1.72 <sup>a,af</sup> (59.16)	74.23 ± 6.92 <sup>a,af</sup> (70.31)	27.07 <sup>a,acdf</sup> (89.17)	4.65 ± 4.49 <sup>a,af</sup> (98.14)
5% <i>n</i> -HF	255.06 ± 21.48 (2.02)	204.49 ± 40.15 (18.21)	123.20 ± 29.46 <sup>a,a</sup> (32.53)	90.06 ± 34.09 <sup>a,af</sup> (63.98)	46.13 ± 14.01 <sup>a,af</sup> (81.55)	13.57 ± 1.02 <sup>a,af</sup> (94.57)
10% <i>n</i> -HF	232.52 ± 2.51 <sup>a,a</sup> (7.1)	173.96 ± 3.8 <sup>a,a</sup> (30.41)	112.74 ± 1.66 <sup>a,af</sup> (36.77)	80.44 ± 8.28 <sup>a,af</sup> (67.82)	24.86 ± 11.53 <sup>a,af</sup> (90.06)	8.77 ± 2.21 <sup>a,af</sup> (96.49)
5% AF	246.93 ± 2.95 (1.23)	210.16 ± 5.62 (15.94)	167.12 ± 2.08 (48.81)	127.97 ± 6.93 (49.61)	89.77 ± 13.56 <sup>a,af</sup> (64.09)	34.04 ± 18.48 <sup>a,a</sup> (86.39)
10% AF	241.82 ± 3.04 (3.28)	193.89 ± 5.18 <sup>a,af</sup> (22.45)	117.23 ± 3.15 <sup>a,af</sup> (52.39)	89.17 ± 1.65 <sup>a,a</sup> (64.33)	29.27 ± 13.68 <sup>a,af</sup> (88.29)	11.83 ± 0.66 <sup>a,af</sup> (95.27)

Values are expressed as mean ± SEM (*n* = 6 rats in each group) and analyzed by one-way ANOVA followed by post hoc Tukey's test; <sup>a</sup>compared with simple ointment; <sup>b</sup>compared with MEBO; <sup>c</sup>compared with 5% EAF; <sup>d</sup>compared with 5% *n*-HF; <sup>e</sup>compared with 10% *n*-HF; <sup>f</sup>compared with 10% AF; <sup>g</sup>compared with 10% AF; <sup>h</sup>w/w = weight by weight; SO = simple ointment; \* = *p* < 0.05; # = *p* < 0.01; MEBO = moist-exposed burn ointment; EAF, *n*-HF & AF are ethyl acetate, *n*-hexane, and aqueous fractions of *A. mexicana*, respectively. The initial wound area was 250 mm<sup>2</sup>. Values in the bracket are % of contractions.



FIGURE 3: Effect of *A. mexicana* fruit solvent fractions on excision wound model in rats.

to be 56 which is comparable with the maximum dose of EAF. Additionally, all the tested doses of the EAF, 200, 400 mg/kg of the *n*-HF, and 400 mg/kg of AF exhibited a significant suppression of paw edema from the first hour up to the fourth-hour postinduction ( $p < 0.01$ ). The onset or duration of effect among the dosages of EAF was not observed. However, there was a delayed in the onset and short duration of action of the AF at the tested doses of 100 and 200 mg/kg. Furthermore, EAF in both 200 and 400 mg/kg doses (41.01%, 47.622%, and 48.29%, 53.96% at the 2<sup>nd</sup> and 4<sup>th</sup> hour, respectively) inhibited paw edema to a comparable extent as 200 mg/kg aspirin (41.44% and 50% at the 2<sup>nd</sup> and 4<sup>th</sup> hour, respectively). The EAF was the most active fraction, which is evidenced by the higher percent edema inhibition values of all tested doses throughout the observation period compared to the same doses of the *n*-HF and AF as indicated in Table 6.

The result from the cotton pellet-induced granuloma model revealed that the EAF, at all tested doses, significantly inhibited exudate and granuloma mass formation ( $p < 0.01$ )

as compared with the negative control. The production of exudate and granuloma mass was also significantly reduced at all the tested doses of *n*-HF. In contrast to EAF, the percent of reduction was minimal with *n*-HF. When ethyl acetate dosages were compared to those of other groups, it was found that the 400 mg dose had a significantly different effect on exudate and granuloma inhibition as compared with the 100 and 200 mg/kg doses of the aqueous fraction, and the 100 mg/kg dose of *n*-HF and EAFs ( $p < 0.01$ ). Moreover, the EAF showed a statistically significant exudate and granuloma inhibition at the tested dose of 200 mg/kg in comparison to the 100 mg/kg dose of AF and *n*-HF ( $p < 0.01$ ). Additionally, a dose-dependent increase in the anti-inflammatory impact of EAF was seen ( $R^2 = 0.891$  for exudate inhibition;  $R^2 = 0.924$  for granuloma inhibition). Comparing with 400 mg/kg of EAF with AF (at the doses of 100 and 200 mg/kg), EAF, and *n*-HF (at the dose of 100 mg/kg), the maximum percentage suppression of exudate and granuloma mass formations were found to be 38.09 and 53.47%, respectively.



TABLE 4: Effect of *A. mexicana* fruit solvent fractions on *n* period of epithelialization (no. of days) post wound creation in rats.

Test group	Period of epithelialization in days (mean $\pm$ S.E.M)
SO	17.83 $\pm$ 0.41
MEBO	12.50 $\pm$ 0.55 <sup>#acdefg</sup>
5% EAF	14.33 $\pm$ 0.52 <sup>#afg</sup>
10% EAF	12.67 $\pm$ 0.52 <sup>#acdefg</sup>
5% <i>n</i> -HF	15.33 $\pm$ 0.52 <sup>#af</sup>
10% <i>n</i> -HF	14.17 $\pm$ 0.42 <sup>#adf</sup>
5% <i>n</i> -AF	16.50 $\pm$ 0.55 <sup>*a</sup>
10% <i>n</i> -AF	15.83 $\pm$ 0.41 <sup>*af</sup>

Values are expressed as mean  $\pm$  SEM ( $n = 6$  rats in each group) and analyzed by one-way ANOVA followed by post hoc Tuckey's test; <sup>a</sup>compared with simple ointment; <sup>b</sup>compared with MEBO; <sup>c</sup>compared with 5% EAF; <sup>d</sup>compared with 5% *n*-HF; <sup>e</sup>compared with 10% *n*-HF; <sup>f</sup>compared with 5% AF; <sup>g</sup>compared with 10% AF; w/w = weight by weight; SO = Simple ointment; \* =  $p < 0.05$ ; # =  $p < 0.01$ ; MEBO = moist-exposed burn ointment; EAF, *n*-HF, and AF are ethyl acetate, *n*-hexane and aqueous fractions of *A. mexicana* fruit, respectively. The initial wound area was 250 mm<sup>2</sup>.

TABLE 5: Effect of *A. mexicana* fruit solvent fractions on tensile strength in rats.

Test Group	Tensile strength in grams (mean $\pm$ S.E.M)	Percent of the tensile strength (%)
SO	461.66 $\pm$ 65.81	16.12
LU	397.55 $\pm$ 15.65	—
MEBO	852.34 $\pm$ 36.06 <sup>#abdfhi</sup>	84.60
5% EAF	715.60 $\pm$ 25.92 <sup>#ab</sup>	55
10% EAF	835.48 $\pm$ 59.96 <sup>#abdfh</sup>	81.10
5% <i>n</i> -HF	704.183 $\pm$ 56.41 <sup>#ab</sup>	52.7
10% <i>n</i> -HF	810.06 $\pm$ 13.09 <sup>#abh</sup>	75.5
5% <i>n</i> -AF	656.90 $\pm$ 71.63 <sup>#ab</sup>	42.3
10% <i>n</i> -AF	745.10 $\pm$ 30.92 <sup>#ab</sup>	61.4

Values are expressed as mean  $\pm$  SEM ( $n = 6$  rats in each group) and analyzed by one-way ANOVA followed by post hoc Tuckey's test; <sup>a</sup>compared with simple ointment; <sup>b</sup>compared with LU; <sup>c</sup>compared with MEBO; <sup>d</sup>compared with 5% EAF; <sup>e</sup>compared with 10% EAF; <sup>f</sup>compared with 5% *n*-HF; <sup>g</sup>compared with 10% *n*-HF; <sup>h</sup>compared with 5% AF; <sup>i</sup>compared with 10% AF; w/w = weight by weight; SO = simple ointment; \* =  $p < 0.05$ ; # =  $p < 0.01$ ; LU = left untreated; MEBO = moist-exposed burn ointment; EAF, *n*-HF, and AF are ethyl acetate, *n*-hexane, and aqueous fractions of *A. mexicana* fruit, respectively.

Inhibition of the production of exudate and granuloma mass caused by cotton pellets was another impact that the *n*-HF demonstrated at all tested doses. Comparisons among the doses of the EAF revealed a significant inhibition against exudate and granuloma formation in 400 mg versus 100 mg/kg AF, *n*-HF, and EAF ( $p < 0.01$ ). Besides, the anti-inflammatory effect of the EAF was ascertained to increase in a dose-dependent manner ( $R^2 = 0.988$  for exudate inhibition;  $R^2 = 0.981$  for granuloma inhibition).

On the contrary, the AF at the doses of 100 and 200 mg/kg was devoid of a statistically significant inhibition of both exudate and granuloma mass formation as compared with the negative control, *n*-HF, and EAF at the tested doses of 100 and 200 mg/kg. However, significant inhibitory effects were observed at the dose of 400 mg/kg, being 31.59% ( $p < 0.01$ ) and 47.15% ( $p < 0.01$ ) for percent inhibition of

exudate and granuloma mass formation, respectively, as shown in Table 7. The dose-dependent activity was also observed in AF ( $R^2 = 0.884$  for exudates inhibition;  $R^2 = 0.892$  for granuloma inhibition) but failed to show significant inhibition of exudate formation at the dose of 100 mg/kg.

Dexamethasone (the standard drug), both the formation of both exudates and granuloma mass (39.74;  $p < 0.01$ ) and (53.42%;  $p < 0.01$ ), respectively, compared with 2% tween 80. Comparing all doses of the three fractions to the negative control in terms of granuloma inhibition revealed a statistically significant difference. The 400 mg/kg of EAF revealed a comparable % inhibition of both the exudates and granuloma mass formation to the standard drug. However, a larger % of inhibition showed that the EAF was most effective at preventing the development of exudate and granuloma mass as described in Table 7.

#### 4. Discussion

Herbal medications have been demonstrated to help with wound healing [61]. Medicinal herbs help wounds heal faster and with less pain, suffering, and scarring for the patient [63]. Wound healing could be achieved with medicinal plant ointment compositions [64]. This enhanced wound contraction by the crude extract ointments could be linked to plant extracts' ability to increase epithelial cell proliferation [65].

In an excision wound model, ointments made from solvent fractions of *A. mexicana* had different wound-healing activities. In rats given EAF ointment, there was a faster rate of contraction and a shortened period of re-epithelialization. The wound healing activity of *A. mexicana* EAF showed a significantly higher wound contraction in the majority of post wound days ( $p < 0.01$ ,  $p < 0.05$ ), along with a quicker epithelialization time ( $p < 0.01$ ). This could be because of the presence of secondary active metabolites (Table 2) [66, 67]. Secondary active metabolites may aid wound healing either individually or in combination [68, 69]. By encouraging tissue regeneration and organization, tannins, due to their astringent and antioxidant properties, could contribute to the wound healing process [70, 71]. By preventing or delaying the onset of cell necrosis and enhancing vascularity, flavonoids decrease lipid peroxidation [72]. Moreover, the histopathological finding from the current study supports the potential wound healing activity from the aforementioned secondary active metabolites (Figure 4).

An infection largely brought on by *S. aureus* and anaerobic bacteria could extend the inflammatory phase of the wound during the healing process, leading to wound failure [73, 74]. Solvent extracts from different plant parts of *A. mexicana* demonstrated substantial effectiveness against tested bacterial species in in vitro research. On *E. coli*, *P. mirabilis*, and *B. subtilis* test strains, methanol extracts of *A. mexicana* leave demonstrated considerable inhibition of bacterial growth [40, 75]. Reports from the previous study revealed that the chloroform and methanol seed extracts of *A. mexicana* significantly lowered the mortality rate of

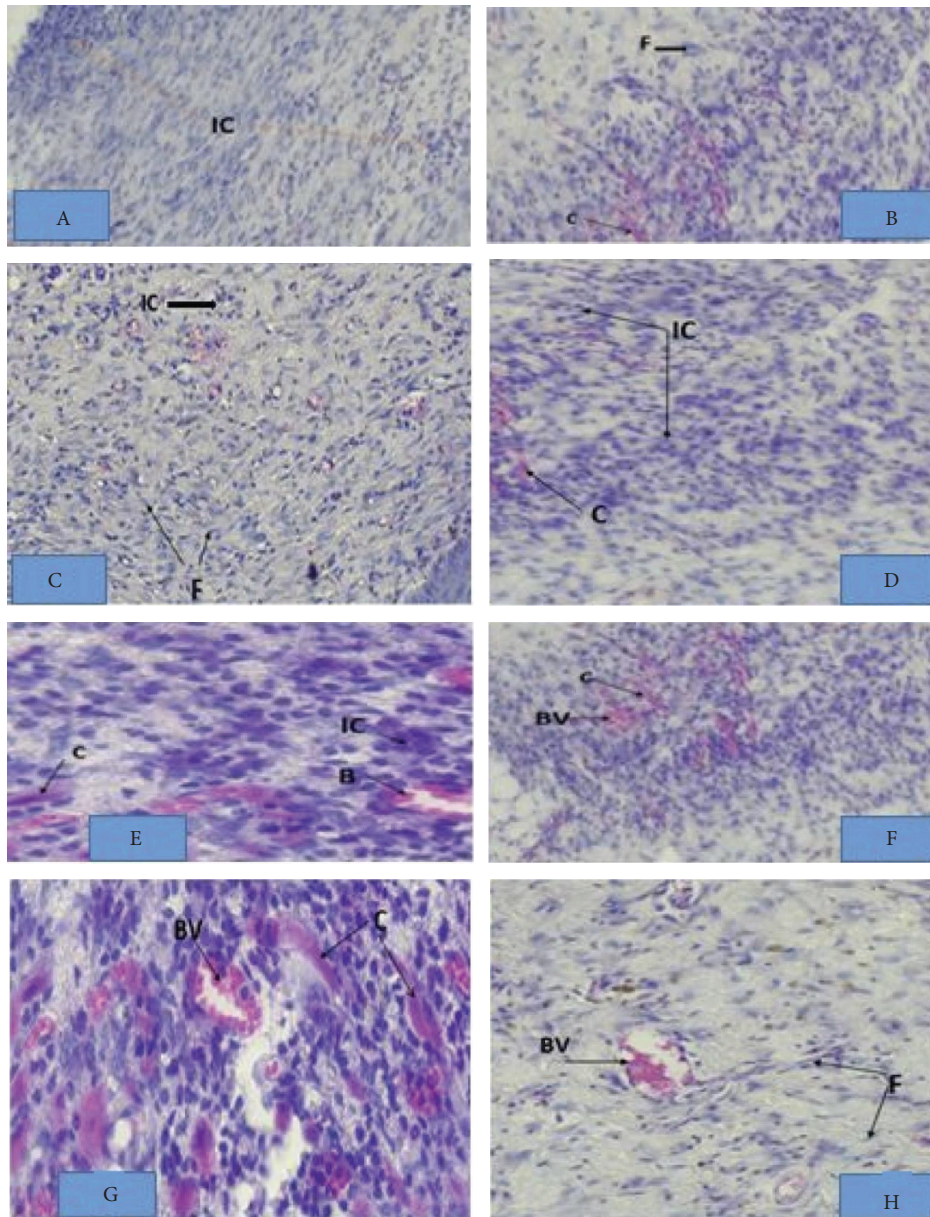


FIGURE 4: Hematoxylin and eosin are used to stain the histopathology of the skin at day 18 (100 XS). (a-b) The black arrow indicates granulation tissue with many inflammatory cells (IC) and lacks collagen tissue and blood vessels in rats treated with simple ointment and 5% aqueous fraction. (c-d) The black arrow indicates granulation tissue in 5% ethyl acetate and n-hexane fraction treated rats with few inflammatory cells (IC), fibroblasts (F), collagen tissues (C), and less vascularized (BV). (e-g) The 10% ethyl acetate fraction and MEBO-treated rats' skin healed a well-formed, nearly normal epidermis, repaired adnexa, and a significant amount of fibrosis and collagen tissue in the dermis. (f-h) The 10% n-hexane and aqueous fraction treated rats also showed a significant fibrosis, accumulation of collagen tissues, and blood vessel formation or vascularization.

aquatic animals infected with *Bacillus cereus* [76]. Moreover, essential oils and alkaloids isolated from *A. mexicana*'s aerial and root portions exhibited broad-spectrum antibacterial activity against tested bacterial species [77, 78]. Methanol extracts of *A. mexicana* leaves and seeds were found to be effective against Gram-positive and Gram-negative multidrug-resistant pathogenic bacteria [79]. Tannins isolated from ethyl extract of *A. mexicana* aerial parts showed a significant antibacterial activity against the wound-causing bacterial strains [80]. Besides, other studies showed that

phytoconstituents from the crude extract of *A. mexicana* were found to have antioxidant, antibacterial, and cytotoxic properties [81]. These could support the results of the present study [82].

Saponins, especially, cause significant damage to the bacteria strains tested by dissolving the cell wall, breaking the cytoplasmic membrane proteins, and causing the contents of the cell to leak out [83]. The previous research also found that triterpenoid saponins derived from medicinal plants had cytotoxic and antibacterial activity, probably due

TABLE 6: Anti-inflammatory effect of the solvent fractions of *A. mexicana* fruits on cotton pellet-induced granuloma in rats.

Treatment group	BV (ml)	Change in paw volume (mean $\pm$ S.E.M) (ml) (percent inhibition (%))			
		1 hour	2 hours	3 hours	4 hours
2% tween	0.22 $\pm$ 0.1	0.43 $\pm$ 0.02	0.44 $\pm$ 0.03	0.42 $\pm$ 0.03	0.40 $\pm$ 0.03
Aspirin, 200 mg/kg	0.21 $\pm$ 0.1	0.28 $\pm$ 0.04 <sup>*abcd</sup> (30.89)	0.25 $\pm$ 0.04 <sup>*abcde</sup> (41.44)	0.21 $\pm$ 0.03 <sup>*abcde</sup> (50)	0.17 $\pm$ 0.02 <sup>*abcd</sup> (56)
100 mg/kg AF	0.20 $\pm$ 0.01	0.39 $\pm$ 0.04 (3.85)	0.38 $\pm$ 0.04 (14.67)	0.36 $\pm$ 0.03 (14.92)	0.35 $\pm$ 0.03 (22.50)
200 mg/kg AF	0.20 $\pm$ 0.01	0.37 $\pm$ 0.01 (8.55)	0.34 $\pm$ 0.01 <sup>*a</sup> (27.41)	0.31 $\pm$ 0.1 <sup>*a</sup> (37.09)	0.27 $\pm$ 0.03 <sup>*a</sup> (45)
400 mg/kg AF	0.21 $\pm$ 0.01	0.32 $\pm$ 0.01 <sup>*ab</sup> (20.94)	0.30 $\pm$ 0.03 <sup>*ab</sup> (29.34)	0.28 $\pm$ 0.03 <sup>*ab</sup> (42.34)	0.23 $\pm$ 0.01 <sup>*ab</sup> (45.83)
100 mg/kg n-HF	0.22 $\pm$ 0.01	0.36 $\pm$ 0.05 <sup>*a</sup> (13.01)	0.36 $\pm$ 0.06 <sup>*a</sup> (17.87)	0.33 $\pm$ 0.06 <sup>*a</sup> (26.63)	0.28 $\pm$ 0.02 <sup>*ab</sup> (31.54)
200 mg/kg n-HF	0.22 $\pm$ 0.01	0.34 $\pm$ 0.05 <sup>*a</sup> (16.26)	0.35 $\pm$ 0.03 <sup>*a</sup> (21.29)	0.30 $\pm$ 0.01 <sup>*a</sup> (28.57)	0.26 $\pm$ 0.01 <sup>*ab</sup> (35.27)
400 mg/kg n-HF	0.21 $\pm$ 0.02	0.29 $\pm$ 0.02 <sup>*abc</sup> (28.05)	0.28 $\pm$ 0.02 <sup>*abc</sup> (37.26)	0.29 $\pm$ 0.10 <sup>*abc</sup> (43)	0.22 $\pm$ 0.01 <sup>*ab</sup> (44.81)
100 mg/kg EAF	0.22 $\pm$ 0.01	0.34 $\pm$ 0.04 <sup>*a</sup> (18.29)	0.32 $\pm$ 0.03 <sup>*a</sup> (27.37)	0.28 $\pm$ 0.03 <sup>*a</sup> (33.33)	0.26 $\pm$ 0.02 <sup>*ab</sup> (34.85)
200 mg/kg EAF	0.21 $\pm$ 0.01	0.29 $\pm$ 0.04 <sup>*abc</sup> (27.64)	0.26 $\pm$ 0.05 <sup>*abcde</sup> (41.01)	0.22 $\pm$ 0.05 <sup>*abcd</sup> (47.62)	0.18 $\pm$ 0.06 <sup>*abc</sup> (54.36)
400 mg/kg EAF	0.21 $\pm$ 0.01	0.26 $\pm$ 0.02 <sup>*abcde</sup> (35.77)	0.22 $\pm$ 0.01 <sup>*abcde</sup> (48.29)	0.19 $\pm$ 0.01 <sup>*abcde</sup> (53.96)	0.15 $\pm$ 0.01 <sup>*abcd</sup> (61.41)

Values are expressed as mean  $\pm$  SEM ( $n=6$  rats in each group) and analyzed by one-way ANOVA followed by post hoc Tukey's test; <sup>a</sup>compared with 2% tween 80, <sup>b</sup>compared with 100 mg/kg AF, <sup>c</sup>compared with 200 mg/kg AF; <sup>d</sup>compared with 100 mg/kg n-HF; <sup>e</sup>100 mg/kg EAF; \* =  $p < 0.01$ ; BV = basal volume; Dexa = dexamethasone; 2% tween 80 = negative control; AF, n-HF, and EAF are aqueous, n-hexane, and ethyl acetate fractions of *A. mexicana*, respectively.

to cellular component changes [84]. Therefore, the solvent fruit extract of *A. mexicana* could also increase the percentage of wound contraction by this mechanism.

Flavonoids are the primary active phytoconstituents that promote the wound-healing process [85]. These phytoconstituents may also promote wound healing by shortening the inflammatory phase, facilitating re-epithelialization, and possessing antimicrobial properties [72, 86]. Furthermore, the wound healing and antimicrobial properties of several *A. mexicana* extracts (stem, leaf, flower, and root) are investigated [36, 37]. Phytochemicals such as flavonoids were attributed to these previously reported wound-healing activity investigations.

Alkaloids also play an enormous role in the process of wound healing. They can enhance macrophage chemotactic and neutrophil mobilization towards the wounded area [87]. Alkaloids also have wound-healing activity, which may be attributed to their anti-inflammatory properties [88].

The EAF- and n-HF-treated groups showed a significantly increased wound contraction and shortened re-epithelialization period than the AF and simple ointment-treated groups. On the other hand, longer period re-epithelialization and wound closure was observed in the groups treated with simple ointment treated. However, rats treated with solvent fractions of *A. mexicana* fruit and MEBO were clean and healthy. This could be attributed to the presence of bacteria and enterotoxin in the simple ointment-treated group, which limits wound contraction and slows wound healing.

Collagen synthesis, maturation, angiogenesis, and fiber stabilization may all be contributing factors to improved tensile strength [89]. Consequently, the fractions could promote collagen production, maturation, and stabilization. The antioxidant and antibacterial capabilities of phytochemicals may attribute to the wound-healing activity of the fractions. For instance, flavonoids are powerful antioxidants and free radical scavengers that protect cells from oxidative damage [90, 91]. Additionally, the hydroxylation and alkoxylation patterns of flavonoids are crucial in influencing their antioxidant activity [42].

In the previous study, the EAF of the flower *A. mexicana* was found to have good antioxidant and anti-inflammatory properties. It is been hypothesized that it is because of high phytochemical contents such as flavonoids [13, 81, 92]. The present study is in line with the previous reports as it shows a significant increase in wound contraction, shortened re-epithelialization period, and a high percentage of tensile strength.

Moreover, extracts from numerous portions of *A. mexicana* have been used in a number of pharmacological researches [93], wound healing activity was found in all three fractions, with the ethyl acetate fraction being the most active. There were additional biologically active components found, which could be responsible for the wound healing actions.

Both acute and chronic models of inflammation were employed to evaluate the in vivo anti-inflammatory properties of *A. mexicana*. Since the relative potency estimates obtained from the majority of medications tend to mirror clinical experience, the carrageenan-induced hind paw edema model has been utilized extensively for the discovery and evaluation of anti-inflammatory agents [94]. Overproduction of the inflammatory prostaglandins is the major mechanism responsible for carrageenan-induced paw edema formation [95]. For this reason, the present study employed for investigation of the acute anti-inflammatory effect of the solvent fractions of the fruits of *A. mexicana*.

In the carrageenan-induced rat paw edema model, the AF, EAF, and n-HFs produced significant anti-inflammatory effects at their maximum dose, with the EAF being the most active fraction ( $p < 0.01$ ). However, the levels of significance among the solvent fractions were different in terms of the magnitude of reduction of carrageenan-induced rat paw edema, and it was further entertained as described below.

The AF at a tested dose of 100 mg/kg was devoid of any significant anti-inflammatory effect throughout the observations. However, the percentage of inhibition associated with this dose was greatest in the 4<sup>th</sup> hour after inflammation induction. A secondary metabolite's inability to accumulate to a sufficient concentration could account for the AF's

TABLE 7: Effects of solvent fractions of *A. mexicana* fruits on cotton pellet-induced granuloma in rats.

Treatment group	Mean weight of exudates in mg (mean $\pm$ S.E.M) (% inhibition)	Mean weight of granuloma in (mg) (mean $\pm$ S.E.M) (% inhibition)
2% Tween 80, 10 ml/kg	124.80 $\pm$ 0.64	144.41 $\pm$ 1.02
Dexa, 0.5 mg/kg	75.83 $\pm$ 1.9 <sup>abcde</sup> (39.24)	67.27 $\pm$ 0.94 <sup>abcdef</sup> (53.42)
100 mg/kg AF	123.15 $\pm$ 0.87 (1.32)	132.27 $\pm$ 2.06 <sup>aa</sup> (8.41)
200 mg/kg AF	98.06 $\pm$ 2.31 <sup>aa</sup> (21.42)	100.24 $\pm$ 2.74 <sup>ab</sup> (30.58)
400 mg/kg AF	85.36 $\pm$ 0.60 <sup>abd</sup> (31.59)	76.32 $\pm$ 0.67 <sup>ab</sup> (47.15)
100 mg/kg n-HF	101.70 $\pm$ 0.58 <sup>ab</sup> (18.51)	81.46 $\pm$ 1.30 <sup>ab</sup> (43.09)
200 mg/kg n-HF	92.53 $\pm$ 1.46 <sup>ab</sup> (25.86)	78.29 $\pm$ 1.11 <sup>abc</sup> (45.78)
400 mg/kg n-HF	85.79 $\pm$ 2.10 <sup>abcd</sup> (31.25)	70.25 $\pm$ 0.95 <sup>abcd</sup> (51.35)
100 mg/kg EAF	98.92 $\pm$ 1.07 <sup>ab</sup> (20.73)	80.78 $\pm$ 1.67 <sup>abc</sup> (44.06)
200 mg/kg EAF	89.04 $\pm$ 2.08 <sup>abd</sup> (28.65)	75.79 $\pm$ 0.76 <sup>abc</sup> (47.52)
400 mg/kg EAF	77.26 $\pm$ 2.73 <sup>abcde</sup> (38.09)	67.19 $\pm$ 0.88 <sup>abcde</sup> (53.47)

Values are expressed as mean  $\pm$  SEM ( $n=6$  rats in each group) and analyzed by one-way ANOVA followed by post hoc Tuckey's test; <sup>a</sup>compared with 2% tween 80, <sup>b</sup>compared with 100 mg/kg AF, <sup>c</sup>compared with 200 mg/kg AF; <sup>d</sup>compared with 100 mg/kg *n*-HF; <sup>e</sup>100 mg/kg EAF; <sup>f</sup>compared with 200 mg/kg *n*-HF; \* =  $p < 0.01$ ; Dexa = dexamethasone; 2% tween 80 = negative control); AF, *n*-HF, and EAF are aqueous, *n*-hexane, and ethyl acetate fractions of *A. mexicana*, respectively.

insignificant rat paw edema inhibition activity at the low dose. The activity would be detectable with an increasing dose, which supports this viewpoint. This may also indicate that the active components are differentially concentrated in the EAF and *n*-HFs. Additionally, it is conceivable to infer that nonpolar secondary metabolites prevent the carrageenan from inducing rat paw edema which is supported by studies [96, 97].

As compared with the negative control, both the EAF (at the tested dose of 100 mg/kg) and *n*-HFs (at the tested doses of 100 and 200 mg/kg) significantly reduced inflammation ( $p < 0.01$ ). However, the EAF of *A. mexicana* fruits demonstrated a significant anti-inflammatory effect by all the tested doses at all-time points of observation with different % of inhibition ( $p < 0.01$ ), the dose 400 mg/kg being the highest percentage of edema inhibition (61.41%) at 4<sup>th</sup> hour after induction of inflammation ( $p < 0.01$ ). The EAF and *n*-HFs of several plants were shown to diminish the carrageenan-induced paw edema in previous research, and this investigation was consistent with those findings [98, 99].

In all the solvent fractions at the tested doses, % of inhibition of inflammation was observed at the latter phase of inflammation (Table 6). This was comparable to the effects of the standard drug (aspirin), showing that the anti-inflammatory activity might be mediated through cyclooxygenase enzyme inhibition.

The cotton pellet-induced granuloma model is one of the most often used animal research models for analyzing the long-lasting anti-inflammatory effects of herbal treatments [100]. The proliferative and transudative components of chronic inflammation are studied using this animal [101]. This kind of model is therefore employed to further confirm the anti-inflammatory effects of the solvent fractions of fruits of *A. mexicana* on the proliferative and transudative features of chronic inflammation. In this model, the steroidal anti-inflammatory medication dexamethasone was found to have higher activity. With the exception of the 100 mg/kg AF, all of the tested doses of the solvent fractions of *A. mexicana* fruit demonstrated a significant suppression of both the

exudate and granuloma formation in comparison to the negative control ( $p < 0.01$ ). Moreover, the experimental data revealed that the EAF significantly reduces the weights of both exudate and granuloma mass at all tested doses ( $p < 0.01$ ). This significant inhibitory effect of the EAF further substantiates its anti-inflammatory effect in the acute inflammatory model (Table 7). The significant inhibition of granuloma formation rationalizes the effectiveness of EAF in inhibiting the proliferative phase of inflammation ( $p < 0.01$ ). Secondary metabolites such as alkaloids, flavonoids, tannins, steroids, glycosides, and terpenoids were found in the EAF after the phytochemical screening. This result is in line with other reports which used a series of solvents to conduct phytochemical analyses of the various plant parts of *A. mexicana* [102, 103]. The anti-inflammatory properties are attributed to the presence of such types of secondary metabolites. For instance, terpenoids [104], alkaloids [105, 106], and flavonoids [107–109] contribute to the anti-inflammatory effect of herbal medicines. The anti-inflammatory activity of the solvent fractions of *A. mexicana* compared with the standard (drug dexamethasone) may be through reduction of the production, release, and/or action of various inflammatory mediators such as cytokines, chemokines, and mediators, including histamine, serotonin, prostaglandins, and leukotrienes.

Several active secondary metabolites, including alkaloids, phenols, terpenoids, amino acids, steroids, flavonoids, and fatty acids, have been isolated from the various plant sections of *A. mexicana* and were tested for their biological activities [86, 93, 110–112]. According to reports from earlier investigations, *A. mexicana's* diverse plant parts have a range of in vitro and in vivo pharmacological actions [12]. Among these, the cytotoxic activity of the alkaloids such as chelerythrine, berberine, and sanguinarine isolated from the chloroform fraction of the aerial part of *A. mexicana* [25, 113], the antioxidant and wound healing activities of chitosan flavonoids isolated from the *n*-butanol fraction of the leaves of *A. mexicana* [114], the antioxidant and anti-inflammatory activities of phenols and flavonoids extracted

from the EAF of the flowers of *A. mexicana* [14, 102], and the anti-inflammatory activity of isorhamnetin-3-O $\beta$ -D-glucopyranoside, cysteine, and phenylalanine  $\beta$ -amyryn derived triterpenoids from the leaves, seeds, and roots extract of *A. mexicana* [115] were investigated. Additionally, earlier study findings showed that the *A. mexicana* aqueous leaves extract significantly reduced edema (76.75%) at the highest tested dose of the extract which compares favorably to the 400 mg/kg dose of ethyl acetate fraction the results of the current test in the same anti-inflammatory model [116]. Results from the current study collectively revealed that the reduction of the rat paw edema and percentage inhibition of cotton pellet-induced exudate and granuloma mass formation was in the order of efficacy: ethyl acetate > *n*-hexane fraction > aqueous fraction for both anti-inflammatory models.

## 5. Conclusion

In all the models of wound healing and inflammation employed for the present study, it was revealed that the order of efficacy was ethyl acetate > *n*-hexane fraction > aqueous fraction for both wound healing and anti-inflammatory activities of the *A. mexicana* fruits, with the ethyl acetate fraction being the most active. The difference in activity across fractions could be attributed to the amount and concentration of phytochemicals in the ethyl acetate fraction, which could be the most potent and effective.

## Abbreviations

ANOVA:	One-way analysis of variance
<i>B. subtilis</i> :	<i>Bacillus subtilis</i>
<i>n</i> -AF:	Aqueous fraction
<i>n</i> -HF:	<i>n</i> -hexane fraction
EAF:	Ethyl acetate fraction
OECD:	Organization for Economic Co-Operation and Development
LD:	Lethal dose
MEBO:	Moist-exposed burn ointment
SO:	Simple ointment
<i>E. coli</i> :	<i>Escherichia coli</i>
<i>P. mirabilis</i> :	<i>Proteus mirabilis</i>
<i>S. aureus</i> :	<i>Staphylococcus aureus</i> .

## Data Availability

All the data sets generated and analyzed during the study are included in the text.

## Ethical Approval

Ethical clearance and permission were obtained from the Debre Tabor University Research and Ethical Review Committee and the approval was obtained by protocol number CHS/134/2022.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

The authors are responsible for the content and writing of the paper. TM developed the draft proposal under the supervision of AB, EC, YS, GT, TG, GA, ND, MA, and SB. MM and ZT critically conceptualized, collected the data, analyzed the study, and wrote the paper. TM, AB, EC, YS, GT, TG, GA, ND, MA, SB, MM, and ZT also prepared the manuscript, and they read and approved the final manuscript.

## Acknowledgments

The authors were very grateful to Debre Tabor University for funding the study. Debre Tabor University provided all the required resources for conducting this research project.

## References

- [1] M. R. Farahpour and M. Habibi, "Evaluation of the wound healing activity of an ethanolic extract of Ceylon cinnamon in mice," *Veterinarni Medicina*, vol. 57, no. No. 1, pp. 53–57, 2012.
- [2] P. G. Bowler and B. J. Davies, "The microbiology of infected and noninfected leg ulcers," *International Journal of Dermatology*, vol. 38, no. 8, pp. 573–578, 1999.
- [3] P. G. Bowler, "Wound pathophysiology, infection and therapeutic options," *Annals of Medicine*, vol. 34, no. 6, pp. 419–427, 2002.
- [4] G. S. Lazarus, D. M. Cooper, D. R. Knighton et al., *Archives of Dermatology*, vol. 130, no. 4, pp. 489–493, 1994.
- [5] P. Garg and S. Sardana, "Pharmacological and therapeutic effects of ocimum sanctum," *Eur J Pharm Med Res*, vol. 3, no. 8, pp. 637–640, 2016.
- [6] S. Mittal and P. K. Dixit, "International journal of comprehensive pharmacy natural remedies for wound healing: a literary review," *Int Journal Compr Pharm*, vol. 04, no. 03, pp. 1–6, 2013.
- [7] T. Teklehaymanot, M. Giday, G. Medhin, and Y. Mekonnen, "Knowledge and use of medicinal plants by people around Debre Libanos monastery in Ethiopia," *Journal of Ethnopharmacology*, vol. 4, p. 13, 2006.
- [8] A. T. Heard and R. Segura, *Argemone mexicana L. And Argemone ochroleuca Sweet--Mexican poppy*, pp. 65–72, Biol Control Weeds Aust CSIRO Publ Victoria, Australian Forestry, 2012.
- [9] J. Rubio-Piña and F. Vázquez-Flota, "Pharmaceutical applications of the benzylisoquinoline alkaloids from *Argemone mexicana* L.," *Current Topics in Medicinal Chemistry*, vol. 13, no. 17, pp. 2200–2207, 2013.
- [10] R. Otto and F. Verloove, "A new natural hybrid in *Argemone* (Papaveraceae)," *Phytotaxa*, vol. 255, no. 1, pp. 57–65, 2016.
- [11] I. Vandebroek and D. Picking, "*Argemone mexicana* L.(Papaveraceae)," in *Popular Medicinal Plants in Portland and Kingston, Jamaica*, pp. 45–54, Springer, Berlin Heidelberg, Germany, 2020.
- [12] A. Alam and A. A. Khan, "*Argemone mexicana* L.: a weed with versatile medicinal and pharmacological applications," *Annals of Phytomedicine: International Journal*, vol. 9, no. 1, pp. 218–223, 2020.
- [13] H. A. Ibrahim, M. A. Umar, B. A. Bello, A. Aliyu, and A. Ahmad, "Analgesic and anti-inflammatory studies on the roots *argemone mexicana* linn (family: papaveraceae) a,"

- Journal Pharmacy Biological Science IOSR-JPBS*, vol. 11, no. 3, pp. 92–95, 2016, <https://www.iosrjournals.org>.
- [14] M. Prabhakaran and D. Rajeshkanna, “Antioxidant and Anti-inflammatory activities of the flower extracts of *Argemone mexicana* L,” *International Journal of Research in Pharmacy and Science*, vol. 11, no. 1, pp. 323–330, 2020.
- [15] M. H. Jang, X. L. Piao, J. M. Kim, S. W. Kwon, and J. H. Park, “Inhibition of cholinesterase and amyloid- $\beta$  aggregation by resveratrol oligomers from *Vitis amurensis*,” *Phytotherapy Research*, vol. 22, no. 4, pp. 544–549, 2008, <http://www3.interscience.wiley.com/journal/117934759/abstract>.
- [16] C. Ravi Kiran, C. Rajkuberan, A. Y. Sangilimuthu, F. L. Hakkim, H. Bakshi, and S. P. Manoharan, “Intrinsic evaluation of antiuro lithiatic capacity of argemone mexicana L. In wistar albino rats,” *Journal of Herbs, Spices, & Medicinal Plants*, vol. 27, no. 3, pp. 289–304, 2021.
- [17] N. V. More and A. S. Kharat, “Antifungal and anticancer potential of argemone mexicana L.” *Medicine (Baltimore)*, vol. 3, no. 4, p. 28, 2016.
- [18] A. Alam, A. M. A. A. Khan, L. der Westhuizen, P. Mpedi, N. K. Sangwan, and M. S. Malik, “Induction of apoptosis in A431 cells via ROS generation and p53-mediated pathway by chloroform fraction of *Argemone mexicana* (Pepavraceae),” *Nat Prod Res [Internet]*, vol. 3, no. 1, pp. 61–68, 2021, <http://www.iosrjournals.org>.
- [19] A. H. Al-Baizyd, *Phytochemical Screening & in Vitro Antioxidant and Thrombolytic Activities of Argemone Mexicana Extracts*, East West University, Dhaka, Bangladesh, 2012.
- [20] P. Nayak, D. Kar, and S. Nayak, “Antidiabetic activity and modulation of antioxidant status by fractions of *Argemone mexicana* in alloxan induced diabetic rats,” *International Journal of Green Pharmacy*, vol. 6, no. 4, pp. 321–329, 2012.
- [21] P. Nayak, D. M. Kar, and L. Maharana, “Antidiabetic activity of aerial parts of *Argemone mexicana* Linn. in alloxan induced hyperglycaemic rats,” *Pharmacologyonline*, vol. 1, pp. 889–903, 2011.
- [22] S. P. Rout, D. M. Kar, and P. K. Mandal, “Hypoglycaemic activity of aerial parts of *Argemone mexicana* L. in experimental rat models,” *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, pp. 533–540, 2011.
- [23] A. Tilak, R. Sharma, R. Thakur, S. Gangwar, and R. Sutar, “Screening of leaf extracts of *Argemone Mexicana* for its antiarthritic activity in experimental animals,” *World Journal of Pharmaceutical Research*, vol. 7, no. 3, pp. 1054–1063, 2017.
- [24] S. Singh, M. Verma, M. Malhotra, S. Prakash, and T. D. Singh, “Cytotoxicity of alkaloids isolated from *Argemone mexicana* on SW480 human colon cancer cell line,” *Pharmaceutical Biology*, vol. 54, no. 4, pp. 740–745, 2016.
- [25] Y. C. Chang, F. R. Chang, A. T. Khalil, P. W. Hsieh, and Y. C. Wu, “Cytotoxic benzophenanthridine and benzylisoquinoline alkaloids from *Argemone mexicana*,” *Zeitschrift für Naturforschung C*, vol. 58, no. 7–8, pp. 521–526, 2003.
- [26] R. D. Bhalke, Y. P. Mandole, and N. B. Mali, “Phytochemical investigation and effect of various extracts of *Argemone mexicana* (Papaveraceae) leaves on clonidine and haloperidol-induced catalepsy in mice,” *Journal of Pharmacy Research*, vol. 2, no. 4, pp. 765–767, 2009.
- [27] G. Asuntha, Y. P. Raju, C. R. Sundaresan, A. Rasheed, V. H. Chowdary, and K. R. Vandana, “Effect of argemone mexicana (L.) against lithium-pilocarpine induced status epilepticus and oxidative stress in wistar rats,” *Indian Journal of Experimental Biology*, vol. 53, 2015.
- [28] S. Attri, P. Kaur, D. Singh et al., “Induction of apoptosis in A431 cells via ROS generation and p53-mediated pathway by chloroform fraction of *Argemone mexicana* (Pepavraceae),” *Environmental Science & Pollution Research*, vol. 29, no. 12, pp. 17189–17208, 2022.
- [29] G. Asuntha, R. Y. Prasanna, C. V. Harini, K. R. Vandana, R. Arun, and K. Prasad, “Pharmacological profiling of *Argemone mexicana* for its aphrodisiac potentials in male Wistar rats,” *Asian Pacific Journal of Reproduction*, vol. 3, no. 2, pp. 110–115, 2014.
- [30] R. Tilak, S. S. Gangwar, A. Tilak, R. N. Thakur, and R. C. Sutar, *Screening of Leaf Extract of Argemone Mexicana for It's Antiasthmatic Activity in Isolated Goat Tracheal Chain Preparation*, 2017.
- [31] T. S. Sourabié, H. M. Kone, J. B. Nikiéma, O. G. Nacoulma, and I. P. Guissou, “Evaluation of the antihepatotoxic effect of *Argemone mexicana* leaf extracts against CCl<sub>4</sub>-induced hepatic injury in rats,” *International Journal of Biological and Chemical Sciences*, vol. 3, no. 6, 2009.
- [32] J. H. Elizondo-Luévano, R. Castro-Ríos, J. López-Abán et al., “Berberine: a nematocidal alkaloid from *Argemone mexicana* against *Strongyloides venezuelensis*,” *Experimental Parasitology*, vol. 220, Article ID 108043, 2021.
- [33] J. H. Elizondo-Luévano, R. Castro-Ríos, B. Vicente et al., “In vitro antischistosomal activity of the argemone mexicana methanolic extract and its main component berberine,” *Iranian Journal of Parasitology*, vol. 16, no. 1, pp. 91–100, 2021.
- [34] C. A. Simões-Pires, E. A. Diop, J. R. Ioset et al., “Bioguided fractionation of the antimalarial plant *Argemone mexicana*: isolation and quantification of active compounds from effective clinical batches,” *Planta Medica*, vol. 75, no. 09, p. PD22, 2009.
- [35] S. Bapna, P. K. Choudhary, M. Ramaiya, and A. Chowdhary, “Antiplasmodial activity of *Argemone mexicana*: an in vivo and in vitro study,” *World Journal of Pharmaceutical Research*, vol. 4, pp. 1653–1663, 2015.
- [36] R. Saravanan, K. Senthilkumar, D. Dhachinamoorthi, P. U. Mahesh, G. V. Krishna, and K. V. Pavani, “Evaluation of wound healing and anti microbial activity of the *Argemone mexicana* linn (Papavraceae),” *Research Journal of Pharmacy and Technology*, vol. 10, no. 6, pp. 852–857, 2017.
- [37] G. K. Dash and P. N. Murthy, “Evaluation of *Argemone mexicana* Linn. Leaves for wound healing activity,” *Journal of Natural Product and Plant Resources*, vol. 1, no. 1, pp. 46–56, 2011.
- [38] S. Andleeb, A. Alsalmé, N. Al-Zaqri, I. Warad, J. Alkahtani, and S. M. Bukhari, “In-vitro antibacterial and antifungal properties of the organic solvent extract of *Argemone mexicana* L,” *Journal of King Saud University Science*, vol. 32, no. 3, pp. 2053–2058, 2020.
- [39] D. Más, Y. Martínez, M. Bullain, C. Betancur, and C. Ruiz, “Secondary metabolites and in vitro antimicrobial activity of roots of Cuban *Argemone mexicana* Linn,” *World Journal of Pharmaceutical and Medical Research*, vol. 4, no. 6, pp. 46–51, 2018.
- [40] D. A. Orozco-Nunnally, J. Pruet, C. P. Rios-Ibarra, E. L. Bocangel Gamarra, T. Lefeber, and T. Najdeska, “Characterizing the cytotoxic effects and several antimicrobial phytochemicals of *Argemone mexicana*,” *PLoS One*, vol. 16, no. 4, Article ID e0249704, 2021.
- [41] National acadamy council, *Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals*, p. 248, 8th ed

- edition, The national acadampy press, Washinton DC, 2011.
- [42] T. Mengie, S. Mequanente, D. Nigussie, B. Legesse, and E. Makonnen, "Investigation of wound healing and anti-inflammatory activities of solvent fractions of 80% methanol leaf extract of *Achyranthes aspera* L. (Amaranthaceae) in rats," *Journal of Inflammation Research*, vol. 14, pp. 1775–1787, 2021.
- [43] A. H. Al-Saeedi, M. T. H. Al-Ghafri, M. A. Hossain, S. Goswami, R. Das, and P. Ghosh, "Brine shrimp toxicity of various polarities leaves and fruits crude fractions of *Ziziphus jujuba* native to Oman and their antimicrobial potency," *Sustainable Chemistry and Pharmacy*, vol. 5, pp. 122–126, 2017.
- [44] S. Goswami, R. Das, P. Ghosh, T. Chakraborty, A. Barman, and S. Ray, "Comparative antioxidant and antimicrobial potentials of leaf successive extract fractions of poison bulb, *Crinum asiaticum* L.," *Industrial Crops and Products*, vol. 154, Article ID 112667, 2020.
- [45] A. R. Abubakar and M. Haque, "Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes," *Journal of Pharmacy and BioAllied Sciences*, vol. 12, no. 1, p. 1, 2020.
- [46] S. Fentahun, E. Makonnen, T. Awas, and M. Giday, "In vivo antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei* infected mice," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, p. 13, 2017.
- [47] Pharmacopoeia British, "British pharmacopoeia, department of health and social security scottish home and health department," *Office of the British Pharmacopoeia commission*, vol. 2, p. 713, 1988.
- [48] oecd.org, "OECD 404 Guideline for the Testing of Chemicals: Acute Dermal Irritation/Corrosion.2002. Adopted: 2017. OECD 402 Guideline for the Testing of chemicals.Acute Dermal Toxicity: Fixed Dose Procedure," 2017, <http://www.oecd.org/termsandconditions/>.
- [49] R. Thakur, N. Jain, R. Pathak, and S. S. Sandhu, "Practices in wound healing studies of plants," *Evidence-based complementary and alternative medicine*, vol. 2011, Article ID 438056, 17 pages, 2011.
- [50] H. K. Nagar, A. K. Srivastava, R. Srivastava, M. L. Kurmi, H. S. Chandel, and M. S. Ranawat, "Pharmacological investigation of the wound healing activity of *cestrum nocturnum* (L.) ointment in wistar albino rats," *Journal of Pharmaceutics*, vol. 2016, pp. 1–8, 2016.
- [51] Y. Shivhare, P. K. Singour, U. K. Patil, and R. S. Pawar, "Wound healing potential of methanolic extract of *Trichosanthes dioica* Roxb (fruits) in rats," *Journal of Ethnopharmacology*, vol. 127, no. 3, pp. 614–619, 2010.
- [52] T. Liu, H. Liu, Y. Li, X. Zheng, Y. Gu, and S. Guo, *Asian Journal of Surgery*, vol. 37, no. 1, pp. 30–34, 2014.
- [53] R. S. Pawar, P. K. Chaurasiya, H. Rajak, P. K. Singour, F. Toppo, and A. Jain, "Wound healing activity of *Sida cordifolia* Linn . in rats," *Indian Journal of Pharmacology*, vol. 45, no. 5, pp. 474–479, 2013.
- [54] J. P Wang, J. L Ruan, Y. L Cai, Q. Luo, H. X Xu, and Y. X Wu, "In vitro and in vivo evaluation of the wound healing properties of *Siegesbeckia pubescens*," *Journal of Ethnopharmacology*, vol. 134, no. 3, pp. 1033–1038, 2011.
- [55] K. Ilango and V. Chitra, "Wound healing and anti-oxidant activities of the fruit pulp of *limonia acidissima* linn (rutaceae) in rats," *Tropical Journal of Pharmaceutical Research*, vol. 9, no. 3, pp. 223–230, 2010.
- [56] A. Fikru, E. Makonnen, T. Eguale, A. Debella, and G. Abie Mekonnen, "Evaluation of in vivo wound healing activity of methanol extract of *Achyranthes aspera* L.," *Journal of Ethnopharmacology*, vol. 143, no. 2, pp. 469–474, 2012.
- [57] E. Mulisa, K. Asres, and E. Engidawork, "Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus* J. (Polygonaceae) in mice," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, p. 341, 2015.
- [58] R. Belay, "Evaluation of the anti-inflammatory activities of 70 % ethanol leaves extract and solvent fractions of *Zehneria scabra* (L. f.) Sond (Cucurbitaceae) in Rodents Rediet Belay (B pharm)," A Thesis Submitted to the Department of Pharmacology, Addis Ababa University, Ethiopia, 2016.
- [59] A. Afsar, K. Rajesh Kumar, and J. Venu Gopal, "Assessment of Anti-inflammatory activity of *Artemisia vulgaris* leaves by Cotton Pellet Granuloma method in wistar albino rats," *Journal of Pharmacy Research*, 2014.
- [60] T. Ahmed Aziz, B. Hasan Marouf, Z. Aorahman Ahmed, and S. Abdulrahman Hussain, "Anti-inflammatory activity of silibinin in animal models of chronic inflammation," *American Journal of Pharmacological Sciences*, vol. 2, no. 1, pp. 7–11, 2014;2(January).
- [61] A. Sharma, S. Khanna, G. Kaur, and I. Singh, "Medicinal plants and their components for wound healing applications," *Future Journal of Pharmaceutical Sciences*, vol. 7, no. 1, pp. 53–13, 2021.
- [62] S. Adesegun, G. Ayoola, H. Coker et al., "Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria," *Tropical Journal of Pharmaceutical Research*, vol. 7, no. 3, pp. 1019–1024, 2008.
- [63] O. Yazarlu, M. Iranshahi, H. R. K. Kashani et al., "Perspective on the application of medicinal plants and natural products in wound healing: a mechanistic review," *Pharmacological Research*, vol. 174, Article ID 105841, 2021.
- [64] I. D. Gwarzo, S. P. Mohd Bohari, R. Abdul Wahab, and A. Zia, "Recent advances and future prospects in topical creams from medicinal plants to expedite wound healing: a review," *Biotechnology & Biotechnological Equipment*, vol. 36, no. 1, pp. 82–94, 2022.
- [65] T. F. Belachew, S. Asrade, M. Geta, and E. Fentahun, "In vivo evaluation of wound healing and anti-inflammatory activity of 80% methanol crude flower extract of *Hagenia abyssinica* (Bruce) JF Gmel in mice," *Evidence-Based Complement Altern Med*.vol. 2020, Article ID 9645792, 12 pages, 2020.
- [66] U. Saleem, S. Khalid, S. Zaib et al., "Phytochemical analysis and wound healing studies on ethnomedicinally important plant *Malva neglecta* Wallr.," *Journal of Ethnopharmacology*, vol. 249, Article ID 112401, 2020.
- [67] M. Prasathkumar, K. Raja, K. Vasanth et al., "Phytochemical screening and in vitro antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of *Senna auriculata* (L.) Roxb. leaves," *Arabian Journal of Chemistry*, vol. 14, no. 9, Article ID 103345, 2021.
- [68] M. Wink, "Modes of action of herbal medicines and plant secondary metabolites," *Medicine (Baltimore)*, vol. 2, no. 3, pp. 251–286, 2015.
- [69] M. Fumakia and E. A. Ho, "Nanoparticles encapsulated with LL37 and serpin A1 promotes wound healing and synergistically enhances antibacterial activity," *Molecular Pharmaceutics*, vol. 13, no. 7, pp. 2318–2331, 2016.
- [70] K. Li, Y. Diao, H. Zhang et al., "Tannin extracts from immature fruits of *Terminalia chebula* Fructus Retz. promote

- cutaneous wound healing in rats," *BMC Complementary and Alternative Medicine*, vol. 11, no. 1, pp. 86–89, 2011.
- [71] X. Su, X. Liu, S. Wang et al., "Wound-healing promoting effect of total tannins from *Entada phaseoloides* (L.) Merr. in rats," *Burns*, vol. 43, no. 4, pp. 830–838, 2017.
- [72] M. T. B. Carvalho, H. G. Araújo-Filho, A. S. Barreto, L. J. Quintans-Júnior, J. S. S. Quintans, and R. S. S. Barreto, "Wound healing properties of flavonoids: a systematic review highlighting the mechanisms of action," *Phytomedicine*, vol. 90, Article ID 153636, 2021.
- [73] W. Xu, E. Dielubanza, A. Maisel et al., "*Staphylococcus aureus* impairs cutaneous wound healing by activating the expression of a gap junction protein, connexin-43 in keratinocytes," *Cellular and Molecular Life Sciences*, vol. 78, no. 3, pp. 935–947, 2021.
- [74] F. Alminderej, S. Bakari, T. I. Almundarij, M. Snoussi, K. Aouadi, and A. Kadri, "Antimicrobial and wound healing potential of a new chemotype from piper cubeba L. Essential oil and in silico study on *S. aureus* tyrosyl-tRNA synthetase protein," *Plants*, vol. 10, no. 2, p. 205, 2021.
- [75] S. Arokiyaraj, M. Saravanan, N. Udaya Prakash, M. Valan Arasu, B. Vijayakumar, and S. Vincent, "Enhanced antibacterial activity of iron oxide magnetic nanoparticles treated with *Argemone mexicana* L. leaf extract: an in vitro study," *Materials Research Bulletin*, vol. 48, no. 9, pp. 3323–3327, 2013.
- [76] G. Chandra, I. Bhattacharjee, and S. Chatterjee, "Bacillus cereus infection in stingray catfish, *Heteropneustes fossilis* (Siluriformes: heteropneustidae) and their recovery by *Argemone mexicana* seed extract," *Iranian Journal of Fisheries Sciences*, vol. 14, no. 3, pp. 741–753, 2015.
- [77] K. Vagionas, K. Graikou, I. B. Chinou, D. Runyoro, and O. Ngassapa, "Chemical analysis and antimicrobial activity of essential oils from the aromatic plants *artemisia afra* jacq. And *leonotis ocyimifolia* (Burm. F.) iwarsson var. *Raineriana* (vision1) iwarsson growing in Tanzania," *Journal of Essential Oil Research*, vol. 19, no. 4, pp. 396–400, 2007.
- [78] L. Opletal, M. Ločárek, A. Fraňková et al., "Antimicrobial activity of extracts and isoquinoline alkaloids of selected papaveraceae plants," *Natural Product Communications*, vol. 9, no. 12, Article ID 1934578X1400901, 2014.
- [79] I. Bhattacharjee, S. K. Chatterjee, S. Chatterjee, and G. Chandra, "Antibacterial potentiality of *Argemone mexicana* solvent extracts against some pathogenic bacteria," *Memorias do Instituto Oswaldo Cruz*, vol. 101, no. 6, pp. 645–648, 2006.
- [80] R. S. Kushtwar, S. Tripathy, C. Dilip, S. Pharmacy, P. Science, and M. Pratap, "Study on antibacterial activity of aerial Part Of *argemone mexicana* linn case report," *Innovat International Journal Of Medical & Pharmaceutical Sciences*, vol. 2, no. 3, pp. 3–5, 2017.
- [81] K. D. Datkhile, S. R. Patil, M. N. Patil, and P. Pratik, "Studies on phytoconstituents, in vitro antioxidant, antibacterial, and cytotoxicity potential of *argemone mexicana* linn. (family: papaveraceae)," *Journal of Natural Science, Biology and Medicine*, vol. 11, pp. 198–205, 2020.
- [82] M. Kaur, Y. Thakur, and R. C. Rana, "Antimicrobial properties of *Achyranthes aspera*," *Ancient Science of Life*, vol. 24, no. 4, pp. 168–173, 2005.
- [83] S. Dong, X. Yang, L. Zhao, F. Zhang, Z. Hou, and P. Xue, "Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria," *Industrial Crops and Products*, vol. 149, Article ID 112350, 2020.
- [84] J. Yu, Z. Yu, Y. Wang, J. Bao, K. Zhu, and T. Yuan, "Phytochemistry triterpenoids and triterpenoid saponins from *dipsacus asper* and their cytotoxic and antibacterial activities," *Phytochemistry [Internet]*, vol. 162, 2019.
- [85] Y. Pang, Y. Zhang, L. Huang et al., "Effects and mechanisms of total flavonoids from *blumea balsamifera* (L.) DC. on skin wound in rats," *International Journal of Molecular Sciences*, vol. 18, no. 12, pp. 2766–2812, 2017.
- [86] S. Singh, V. B. Pandey, and T. D. Singh, "Alkaloids and flavonoids of *Argemone mexicana*," *Natural Product Research*, vol. 26, no. 1, pp. 16–21, 2012.
- [87] M. B. Elsaid, D. M. Elnaggar, A. I. Owis, S. F. Abouzid, and S. Eldahmy, "Production of isoquinoline alkaloids from the in vitro conserved *Fumaria parviflora* and their in vitro wound healing activity," *Natural Product Research*, vol. 36, no. 10, pp. 2575–2579, 2021.
- [88] H. Xia, Y. Liu, G. Xia, Y. Liu, S. Lin, and L. Guo, "Novel isoquinoline alkaloid litcubanine A - a potential anti-inflammatory candidate," *Frontiers in Immunology*, vol. 12, Article ID 685556, 2021.
- [89] C. Onursal, E. Dick, I. Angelidis, and H. B. Schiller, "Collagen biosynthesis, processing, and maturation in lung ageing," *Frontiers in Medicine*, vol. 8, pp. 1–23, 2021.
- [90] M.-Y. Shon, S.-D. Choi, G.-G. Kahng, S.-H. Nam, and N.-J. Sung, "Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions," *Food and Chemical Toxicology*, vol. 42, no. 4, pp. 659–666, 2004.
- [91] F. Nessa, Z. Ismail, N. Mohamed, M. R. H. M. Haris, and H. Mas, "Free radical-scavenging activity of organic extracts and of pure flavonoids of *Blumea balsamifera* DC leaves," *Food Chemistry*, vol. 88, no. 2, pp. 243–252, 2004.
- [92] D. Prabhakaran, A. Rajeshkanna, and M. M. Senthamselvi, "International Journal of Research In Antioxidant and Anti-inflammatory activities of the lower extracts of. 2020," vol. 11, no. 1, pp. 323–330, 2020.
- [93] G. Brahmachari, R. Roy, L. C. Mandal, P. P. Ghosh, and D. Gorai, "A new long-chain secondary alkanediol from the flowers of *Argemone mexicana*," *Journal of Chemical Research*, vol. 34, no. 11, pp. 656–657, 2010.
- [94] S. S. Sakat, K. Mani, Y. O. Demidchenko et al., "Retraction note: release-active dilutions of diclofenac enhance anti-inflammatory effect of diclofenac in carrageenan-induced rat paw edema model," *Inflammation*, vol. 44, no. 2, pp. 810–819, 2021.
- [95] D. B. Duarte, M. R. Vasko, and J. C. Fehrenbacher, "Models of inflammation: carrageenan air pouch," *Current Protocols in Pharmacology*, no. 56, pp. 1–8, 2012.
- [96] I. Rivero-Cruz, G. Anaya-Eugenio, A. Pérez-Vásquez, A. L. Martínez, and R. Mata, "Quantitative analysis and pharmacological effects of *artemisia ludoviciana* aqueous extract and compounds," *Natural Product Communications*, vol. 12, no. 10, Article ID 1934578X1701201, 2017.
- [97] R. Sahukari, J. Punabaka, S. Bhasha et al., "Phytochemical profile, free radical scavenging and anti-inflammatory properties of *Acalypha Indica* root extract: evidence from in vitro and in vivo studies," *Molecules*, vol. 26, no. 20, pp. 6251–6321, 2021.
- [98] M. Shah, Z. Parveen, and M. R. Khan, "Evaluation of antioxidant, anti-inflammatory, analgesic and antipyretic activities of the stem bark of *Sapindus mukorossi*," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, pp. 526–616, 2017.



- [99] S. Makni, S. Tounsi, F. Rezgui, M. Trigui, and K. Z. Bouassida, "Emex spinosa (L.) Campd. ethyl acetate fractions effects on inflammation and oxidative stress markers in carrageenan induced paw oedema in mice," *Journal of Ethnopharmacology*, vol. 234, pp. 216–224, 2019.
- [100] A. A. Kareem, T. A. Aziz, Z. A. Ahmed, H. H. Othman, and S. A. Hussain, "Anti-inflammatory activity of ginkgo biloba extract in cotton pellet- induced granuloma in rats: a comparative study with prednisolone and dexamethasone," *Iraqi Journal of Pharmaceutical Sciences*, vol. 31, no. 1, pp. 184–193, 2022.
- [101] K. R. Patil and C. R. Patil, "Anti-inflammatory activity of bartogenic acid containing fraction of fruits of *Barringtonia racemosa* Roxb. in acute and chronic animal models of inflammation," *Journal of Traditional and Complementary Medicine*, vol. 7, no. 1, pp. 86–93, 2017.
- [102] A. Nancy and A. Praveena, "Argemone mexicana: a boon to medicinal and pharmacological approaches in current scenario," *Cardiovascular and Hematological Agents in Medicinal Chemistry*, vol. 15, no. 2, pp. 78–90, 2018.
- [103] A. M. Khan and S. Bhadauria, "Analysis of medicinally important phytochemicals from *Argemone mexicana*," *Journal of King Saud University Science*, vol. 31, no. 4, pp. 1020–1026, 2019.
- [104] T. Kim, B. Song, K. S. Cho, and I. S. Lee, "Therapeutic potential of volatile terpenes and terpenoids from forests for inflammatory diseases," *International Journal of Molecular Sciences*, vol. 21, no. 6, p. 2187, 2020.
- [105] R. Bai, C. Yao, Z. Zhong et al., "Discovery of natural anti-inflammatory alkaloids: Potential leads for the drug discovery for the treatment of inflammation," *European Journal of Medicinal Chemistry*, vol. 213, Article ID 113165, 2021.
- [106] H. Pei, L. Xue, M. Tang et al., "Alkaloids from black pepper (*piper nigrum* L.) exhibit anti-inflammatory activity in murine macrophages by inhibiting activation of NF- $\kappa$ B pathway," *Journal of Agricultural and Food Chemistry*, vol. 68, no. 8, pp. 2406–2417, 2020.
- [107] S. J. Maleki, J. F. Crespo, and B. Cabanillas, "Anti-inflammatory effects of flavonoids," *Food Chemistry*, vol. 299, Article ID 125124, 2019.
- [108] M. Serafini, I. Peluso, and A. Raguzzini, "Flavonoids as anti-inflammatory agents," *Proceedings of the Nutrition Society*, vol. 69, no. 3, pp. 273–278, 2010.
- [109] R. Shukla, V. Pandey, G. P. Vadhane, and S. Lodhi, "Role of flavonoids in management of inflammatory disorders," in *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*, pp. 293–322, Elsevier, Amsterdam, Netherlands, 2nd ed edition, 2019.
- [110] L. J. A. Loza-Muller, J. I. Laines-Hidalgo, M. Monforte-González, and F. Vázquez-Flota, "Alkaloid distribution in seeds of *Argemone mexicana* L.(Papaveraceae)," *J Mex Chem Soc*, vol. 65, no. 4, pp. 501–506, 2021.
- [111] I. Bhattacharjee, A. Ghosh, N. Chowdhury, S. K. Chatterjee, G. Chandra, and S. Laskar, "n-Alkane profile of *Argemone mexicana* leaves," *Zeitschrift für Naturforschung C*, vol. 65, no. 9–10, pp. 533–536, 2010.
- [112] M. B. Magaji, Y. Haruna, and A. Muhammad, "Phytochemical screening and antioxidant activity of *Argemone mexicana* leaf methanol extract," *Ann Pharmacol Pharma*, vol. 4, pp. 1167–1172, 2019.
- [113] M. L. Díaz Chávez, M. Rolf, A. Gesell, and T. M. Kutchan, "Characterization of two methylenedioxy bridge-forming cytochrome P450-dependent enzymes of alkaloid formation in the Mexican prickly poppy *Argemone mexicana*," *Archives of Biochemistry and Biophysics*, vol. 507, no. 1, pp. 186–193, 2011.
- [114] R. D. F. Soares, M. G. N. Campos, G. P. Ribeiro et al., "Development of a chitosan hydrogel containing flavonoids extracted from *Passiflora edulis* leaves and the evaluation of its antioxidant and wound healing properties for the treatment of skin lesions in diabetic mice," *Journal of Biomedical Materials Research Part A*, vol. 108, no. 3, pp. 654–662, 2020.
- [115] D. Sukumar, R. Arivudai Nambi, and N. Sulochana, "Studies on the leaves of *argemone mexicana*," *Fitoterapia*, 1984.
- [116] T. S. Sourabie, N. Ouedraogo, W. R. Sawadogo, J. B. Nikiema, and I. P. Guissou, "Biological evaluation of anti-inflammatory and analgesic activities of *Argemone mexicana* Linn. (Papaveraceae) aqueous leaf extract," *Int J Pharma Sci Res [Internet]*, vol. 3, no. 9, pp. 451–458, 2012, <https://www.ijpsr.info/docs/IJPSR12-03-09-006.pdf>.