RESEARCH ARTICLE

Exploring the Therapeutic Potential of *Achyranthes aspera* in Wound Healing: A Comprehensive Investigation into its Anti-Inflammatory and Antioxidant Properties

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ABSTRACT

This study examines the likely activity of *Achyranthes aspera* in the improvement of wound mending. A carrageenan-prompted intense provocative model was utilized to prompt irritation, and an extraction wound model was utilized to make wounds in rodents. Chronic administration of different extracts of *A. aspera*, counting ACAQ (extract aqueous at 200 and 400 mg/kg) and ACME (methyl extract at 200 and 400 mg/kg), initiated for a time of 14 days following injury creation. The application of various extracts of A. aspera was observed to have a moderating impact on inflammation and oxidative stress as well as a considerable ameliorating effect on wound healing. Medicinal herbs used for wound healing and skin illnesses are crucial to opening the doors to countering resistance of infections to medicines and allopathic management. Results of antioxidant, anti-inflammatory, and wound healing tests endorse the use of *A. aspera* for dermatological and wound healing purposes, indicating the necessity for additional studies to identify and isolate its active compounds. As demonstrated in carrageenan-induced acute inflammatory and excision wound models, *A. aspera* may, therefore, contribute to the ongoing injuries by diminishing oxidative pressure and incendiary cytokines.

Keywords: Mitigating, Cell reinforcement, Wound, Mending.

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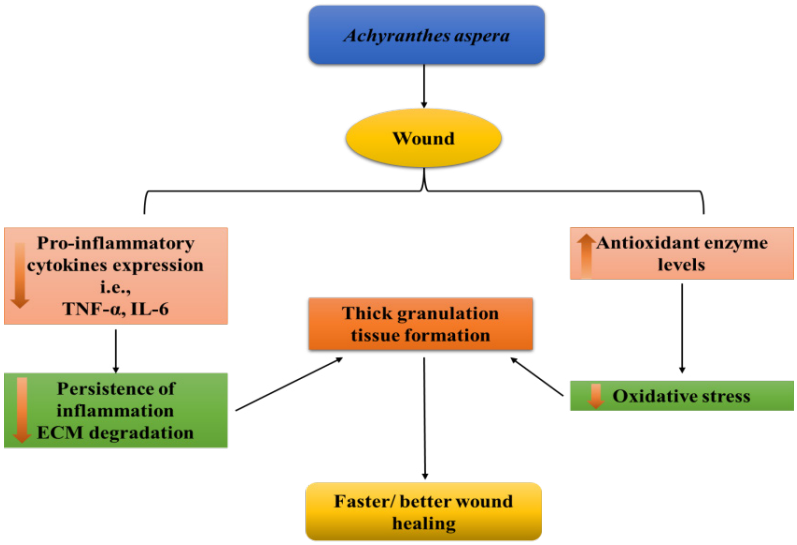

Graphical Abstract

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INTRODUCTION

A dynamic and sophisticated process, including an outpouring of cell and sub-atomic exercises accomplished the restoration of tissue during wound mending. Inadequate wound healing may result in chronic wounds, heightened vulnerability to infections, and prolonged inflammation. Traditional medicinal plants have garnered increasing attention as promising reservoirs of innovative therapeutics for wound care in recent times.[1,2] Among these, *Achyranthes aspera* therapeutic spice, well established in customary medication frameworks, has arisen as a promising competitor because of its rich history of purpose and episodic proof proposing its viability in injury mending.[3,4] *A. aspera*, commonly known as "Prickly chaff flower" or "Aapamarga" in various traditional medicinal systems, has been employed for centuries in different cultures for its diverse therapeutic properties. Recent scientific investigations have shed light on its potential as a natural remedy for wound healing, drawing attention to its anti-inflammatory and antioxidant attributes.[5,6] A wound's ability to heal depends on inflammation, yet too much or too long of an inflammatory response could slow down the healing process.[7,8] The active components found in *A. aspera*, such as triterpenoids, flavonoids, and alkaloids, are thought to be crucial in its ability to reduce inflammation. By obstructing incendiary pathways, these substances decrease the development of favorable to provocative go between such chemokines and cytokines.[5,9,10] By maintaining a stable and regulated inflammatory response, this modulation fosters the best possible conditions for wound healing. Furthermore, the presence of bioactive compounds with antioxidant properties in *A. aspera* has sparked interest in its potential to mitigate oxidative stress, a factor known to hinder the wound healing process.[11-15] Oxidative stress not only exacerbates inflammation but also contributes to cellular damage, impairing the overall regenerative capacity of tissues. The antioxidant dynamics of *A. aspera* add another layer to its therapeutic potential. *A. aspera*'s rich array of antioxidants, including flavonoids, polyphenols, and ascorbic acid, equips it with the capacity to neutralize ROS, mitigating oxidative stress and its detrimental effects on cellular components. This research article aims to comprehensively explore the potential of *A. aspera* in wound healing by delving into its anti-inflammatory and antioxidant mechanisms.[9,16-20] Through a combination of *in-vitro* and *in-vivo* tests, we seek an explication of the molecular pathways involved and evaluate the efficacy of *A. aspera* in promoting optimal wound repair as we unravel the mysteries of this humble herb, the ability to empower wound-mending outcomes and lessen the burden of chronic wounds beckons. This bodes well for a time when *A. aspera* reclaims its rightful place in the pantheon of therapeutic interventions.

METHODS AND MATERIALS

Plant Material and Natural Extract Preparation

A. aspera leaves were collected and authenticated by Dr. S. Shweta and Dr. Ashwini Kumar Dixit of Guru Ghasidas Vishwavidhyalya with authentication no. Bot/GGV/2022/20. The leaves were first rinsed with clean water to remove any debris or dirt that had stuck to them and then dried in an oven set to 35 to 40℃. The powder was made by crushing and grinding the dried leaves, and then the powder was weighed. After that, a solution of ethanol and water was used in a sequential fashion for extraction. After collecting and distilling the menstruum under a vacuum, it was air-dried in an evaporating dish until it reached a consistent weight.

Chemicals

The substances that were utilized included carrageenan, diagnostic kits, and analytical grade for all others. The chemicals that were acquired from HiMedia (Mumbai, India) were sodium carbonate, methanol, chloroform, ethanol, thiazolyl blue (MTT), aluminum chloride, and dimethyl sulfoxide (DMSO) of cell culture grade. Sigma (Bangalore, India) provided MSTFA and DPPH (1,1-diphenyl-2-picrylhydrazyl).

Animals

The Institutional Animal Ethics Committee approved the protocols for this investigation, approving protocol number 1355/PO/Re/S/10/CPCSEA. Following the guidelines set out by the CPCSEA, the animals were cared for appropriately. Wistar rats (male) weighing about 180 to 220 g were housed in a standard habitat. The circumstances included keeping a temperature of 23 ± 2℃ and a light/dull pattern of 12 hours. The rodents were given standard rat feed and unhindered admittance to new water.

In-vitro Antioxidant Activity

Assay of reducing activity

The components for the concoction included 2.5 mL of sample ranging in concentration from 20 to 320 µg/mL, 2.5 mL of 0.1M sodium phosphate buffer has pH of 6.6, and 2.5 mL K_3Fe (CN) 6 containing 1% by weight. After 20 minutes of incubation at 50°C, the diminishing power was measured in the second step. The following stage is to add 2.5 mL of 10% w/v $C_2HCl_3O_2$ trichloroacetic acid corrosive and afterward turn the combination at 5000 rpm for 10 minutes. By mixing 4 mL of the top layer at 0.4 mL of fresh $FeCl_3$ (0.1% w/v), the absorbance was calculated at a wavelength 700 nm. To determine the inhibitory concentration (IC_{50}) value, a reference chemical known as ascorbic acid was used.[21]

Assay of H_2O_2 scavenging activity

In this review, a mix of concentrates at fixations somewhere in the range of 20 and 320 µg/mL was blended with 2.5 mL of 0.1M phosphate cradle (pH 7.4) and 600 µL an answer containing 41 mM of H_2O_2. The mixture was given a good shake and then left to oxidize for ten minutes at room temperature. Next, we took a 230 nm absorbance measurement of the reaction mixture. We utilized virtual reality (VC) to regulate the experiment positively. The procedure for calculating H_2O_2 scavenging activity is as follows:

$$\text{Scavenging outcome\%} = [1- (A_1-A_2)/A_0] \times 100$$

Here, A_0 denotes control absorbance of water, A1 denotes sample absorbance, and A2 stands for the sample absorbance alone (instead of H_2O_2 solution, phosphate buffer is taken). Half of a chemical's H_2O_2 inhibitory impact was at half its susceptibility concentration or its IC_{50} value.[22-24]

Activity of nitric oxide radical scavenging

The action of rummaging nitric oxide was evaluated utilizing the Griess reagent. This included combining 5 mM sodium nitroprusside in phosphate-cushioned saline. Consequently, an answer of the concentration at focuses going from 20 to 320 mg/mL was added to 3.0 mL. Consequently, the blend was kept at a temperature of 25°C for the accompanying 150 minutes. Besides, 5 extra mL Griess reagent containing 1% $C_6H_8N_2O_2S$, 2% H_3PO4 and 0.1% naphthalene-diamine dihydrochloride was introduced. To establish a positive control, we carried out identical measurements at 546 nm using ascorbic acid standard solutions prepared with Griess reagent.

With this formula, we were able to determine the inhibition percentage:

$$\%\text{inhibition} = [(A_0-A_T)/A_0 \times 100]$$

Where when the extract is present, the absorbance is represented by at, and when the control is present, it is represented by A0 (empty, extract-free). For a better picture of the overall picture, we repeated each test three times and analyzed the results graphically.[24,25]

TAOC

A variant of the phosphomolybdate technique was expanded upon.[26] Initially, a plant sample of 0.3 mL was heated with 1-mg per mL of sugar at 95°C for 10 minutes using 3 mL of phosphomolybdate. It was then decided to measure the absorbance at 695 nm. Afterward, the TAC was determined as milligrams of AAE per gram of raw sample weight by referring to the calibration curve of ascorbic acid (10–320 g/mL).

Assessment of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity[27]

The initial stage involved creating an ethanol solution containing 0.1 mM DPPH. Following that, a blend comprising 1.0 mL of the substance and 3.0 mL of a water-based extract solution, having concentrations from the range 20 to 320 µg/mL, was prepared. Ascorbic acid was used as the reference chemical later 30 minutes when measuring the absorbance at 517 nm. A lower absorption value shows an enhanced capacity to counteract free radicals. This is the equation for the sample's activity for free radical scavenging DPPH or the percentage of free radical inhibition.

$$\%\text{inhibition} = [(A_0-A_t)/A_0 \times 100]$$

The presence of absorbance of the extract is represented as At, while the absorbance in the absence of the extract is denoted as A0. We plotted the graph with the mean values after doing all the experiments three times.

Estimation of total phenolic compounds

In this activity, gallic acid was used as a reference phenolic component, and the Folin-Ciocalteau reagent was employed for the estimation process. After following the protocol, the extract solution was measured using a volumetric flask, achieving a concentration of 1-mg/mL. The last response blend comprised 0.5 mL of plant remove arrangement, 2.5 mL of water containing a 5% Folin-Ciocalteu reagent, and 2.5 mL of a fluid arrangement containing 7.5% $NaHCO_3$. Following a 45-minute incubation at 45°C, the samples were withdrawn. The wavelength at which the blue color was detected was 760 nm. Milligrams per gram of dry extract were used to express the concentration of total phenols. It was necessary to make each determination three times. Milligrams of gallic acid equivalents (GAE) were used to evaluate the total amount of phenolic components in plant extract.[28,29]

Estimation Parameters

Acute inflammatory model induced by carrageenan

In order to test the anti-inflammatory activity, the rat paw edema experiment was conducted using carrageenan. A 100 µL subplantar injection of a newly made carrageenan solution in distilled water was administered to each rat in every group (with the exception of Group I) to induce edema. Group II animals were given 20 mg/kg of diclofenac sodium as a single dose 30 minutes before carrageenan injection, while groups III-VII received a single dose of plant extract. The paws were measured at "0 hours," the time immediately prior to the injection of carrageenan, and again at 1, 2, 4, and 8 hours after the injection. The measurement of paw thickness increase was done by subtracting the paw thickness at "0 hour" from the paw thickness at each hour.[29]

Experimental Design

The experimental design for the carrageenan-induced inflammatory model is given in (as shown in Table 1).

Plethysmometer/Paw volume

The inflammatory paw of the rat is placed in the column's liquid before and after the inflammation-causing procedure, and the weight is then recorded. The formula $V = m/\rho$ makes calculating the paw volume a breeze (Here, "m" stands for the mass of the displaced fluid, "p" for its density, and "v" for its volume, which is the same as the paw's input volume).[30,31]

Table 1: Grouping of animals for carrageenan-induced inflammatory model

Group No	*Group*
Group I	Normal control (0.5% carboxymethyl cellulose)
Group II	Carrageenan control
Group III	Carrageenan + Standard (Diclofenac Sodium 20mg/kg)
Group IV	Carrageenan + ACAQ (*A. aspera* aqueous extract 200 mg/kg)
Group V	Carrageenan + ACAQ (*A. aspera* aqueous extract 400 mg/kg)
Group VI	Carrageenan + ACME (*A. aspera* methanol extract 200 mg/kg)
Group VII	Carrageenan + ACME (*A. aspera* methanol extract 400 mg/kg)

Paw Diameter

The paw diameter was measured using the vernier caliper both before and after the injection of carrageenan and medications at 0 hours (1, 2, 4, and 8 hours). By contrasting the control group's average swelling with that of the test and standard groups, we were able to ascertain the paw diameter inhibition%. The formula used for this calculation is:

$$\text{Rate Restraint} = [(V1 - V2)/(V1)] \times 100,$$

where V1 is the paw diameter prior to injection with carrageenan and V2 is the paw diameter following therapy.[31]

Wound Healing Measurement

Excision wounds

After anesthesia, the hairs were removed from the middle region of the dorsal thorax of the rats. To create a wound that was approximately 300 mm^2 in size, the entire designated area was surgically removed. Using an alcohol-soaked cotton swab, the wound was cleansed. Applying the going with condition yielded the percent of wound end:

$$\text{Percent of closure wound} = \frac{100 * (\text{Initial area of wound}) - (\text{Nth day area of wound})}{(\text{Initial area of wound})}$$

Using GraphPad Prism v8.0, regression analysis was conducted, and a graph depicting the relationship between the percentage of closure of wound and time of closure (measured in days) were generated. When the X value is equal to Y = 50%, the CT50 is the amount of time it takes for the wound to close by 50%.[32]

Experimental Design

The experimental design for the carrageenan-induced inflammatory model is given in (Table 2).

Homogenizing Tissue

Collection, rinsing with saline, and homogenization of tissues from various groups took place at 4°C in a 0.1 M Tris-HCl buffer with a pH of 7.4. Following that, homogenates were centrifuged (3000 rpm) for the time period of 10 minutes at 4°C. After that, aliquots were obtained for further analysis. The BSA technique was used to measure the amount of protein in the tissue homogenate.[33]

Catalase

After incorporating a detergent, like 1% Triton X-100, into the pH 7 mixture (1:100), the 10% tissue homogenate was reduced using pH-7 phosphate buffer. The method for estimating catalase was reported.[34]

GSH estimation

Mixing 2.5 mL of 5% TCA with 0.5 grams of skin tissue yielded a tissue homogenate. For 10 minutes, the protein that had precipitated was spun in a centrifuge at 1000 rpm. The GSH concentration was estimated using the supernatant (0.1 mL).[35,36]

Lipid Peroxidation Assay

The TCAe-TBA HCl solution was combined with 1-mL of tissue homogenate in each tube, followed by brief vortexing of the tubes in a water bath set to boiling temperature. When the tubes reached room temperature, centrifugation was applied for a duration of 15 minutes. Preceding evaluating the optical thickness (OD) at 535 nm wandered from an undeniable, the supernatants were moved into a cuvette utilizing a pipette.[36]

Biochemical Analysis

Estimation of hydroxyproline

The recuperated tissue was extracted and moved to vials for drying in a broiler at 110°C for a term of 48 hours. To assess the hydroxyproline content, 5 mg of freeze-dried material was hydrolyzed with 5 mL of 6N HCl in a tube, which was sealed at 110°C for a duration of 18 to 20 hours, adhering to the specified procedure.[37]

Estimation of collagen

Hydroxyproline concentration can be multiplied by 7.46 to obtain collagen with the same molecular weight.[37]

Estimation of hexosamine

About 5 mg of lyophilized tissue were heated for six to seven hours at 110°C in 5 mL of 2 N HCl. Following vanishing, the buildup was broken up in a foreordained volume of water. The solution was heated to boiling for 15 minutes after adding 1-mL of freshly manufactured 2% acetyl acetone in 1/2 mM of sodium carbonate. Once cooled, 1-mL of Ehrlich's reagent was mixed with 5 mL of 95% ethanol. The evolution of the purple-red hue was revealed by a spectrophotometric measurement performed at 530 nm for 30 minutes later.[38-47.]

Statistical Analysis

The mean ± SEM of the edema volume is displayed, and an ANOVA is performed before Tukey's post hoc test is utilized to look at the bundle.

(a*): Normal control versus carrageenan control versus wound control

(b*): Wound control vs different doses of plant extract

(***): $p < 0.001$, **(**)**: $p < 0.01$, **(*)**: $p < 0.005$

RESULTS

***In-vitro* Antioxidants**

DPPH free radical scavenging process

The IC_{50} values for ascorbic acid, ACAQ and ACME were 121.73, 194.3 and 175.05 µg/mL, respectively. The findings

Table 2: Grouping of animals for excision-induced model

Group no	*Group*
Group I	Wound control
Group II	Wound + Standard
Group III	Wound + ACAQ (*A. aspera* aqueous extract 200 mg/kg)
Group IV	Wound + ACAQ (*A. aspera* aqueous extract 400 mg/kg)
Group V	Wound + ACME (*A. aspera* methanol extract 200 mg/kg)
Group VI	Wound + ACME (*A. aspera* methanol extract 400 mg/kg)

demonstrate that the DPPH scavenging activity of both plant extracts is on par with ascorbic acid, the industry standard as shown in Figure 1.

Reducing power activity

ACAQ and ACME extracts were tested for their reducing power at various concentrations ranging from 10 to 320 µg/mL. Results showed that ACAQ and ACME extracts had a concentration-dependent increase in their reducing power. At 320 µg/mL, the extracts exhibited the maximum reducing power. With ACAQ, the EC50 was determined to be 23.77 µg/mL. With ACME, it was 26.41 µg/mL, and with ascorbic acid, had 14.72 µg/mL (Figure 2).

Hydrogen peroxide-scavenging activity

When tested at a concentration of 320 µg/mL, which was the maximum concentration, the ACAQ extract inhibited hydroxyl radicals by 62.37% and the ACME extract by 64.0%. Ascorbic acid, on the other hand, had a 77.7% inhibition. The extracts demonstrated a concentration-dependent capacity to scavenge these radicals. It was discovered that the IC_{50} values of the extracts of ACAQ and ACME were 221.10 and 207.4 µg/mL, respectively. The IC_{50} value of ascorbic acid was determined to be 148.71 µg/mL as shown in Figure 3.

Total antioxidant capacity (TAC)

When measured against an ascorbic acid reference standard, ACAQ, ACME displayed potential antioxidant ability, that is 102.43 and 143.9 mg/AAE (Ascorbic acid equivalent), respectively (Figure 4).

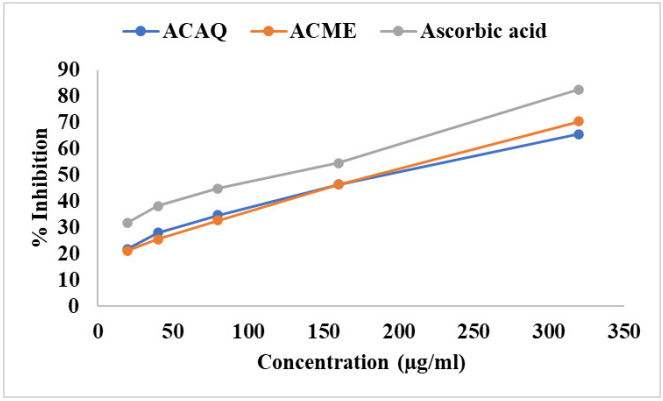

Figure 1: DPPH free radical scavenging activity

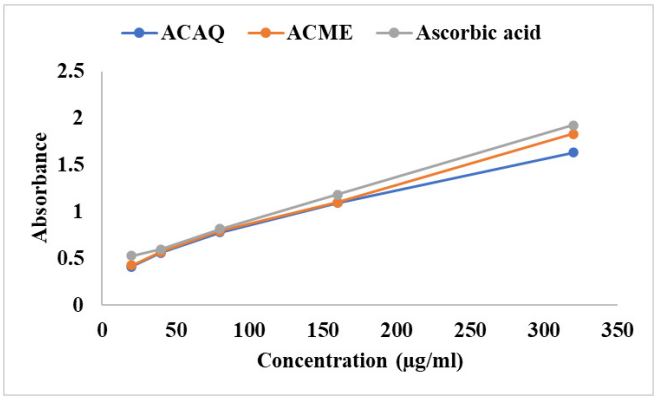

Figure 2: Reducing power activity

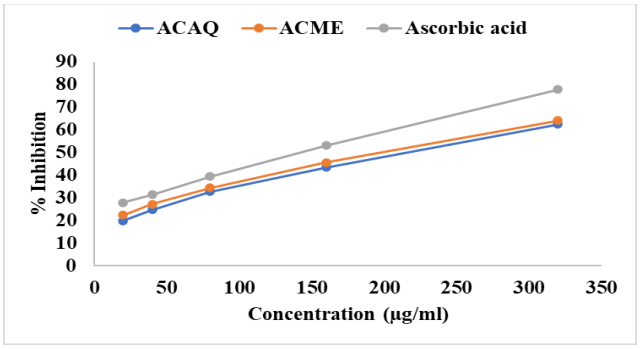

Figure 3: Hydrogen peroxide-scavenging activity

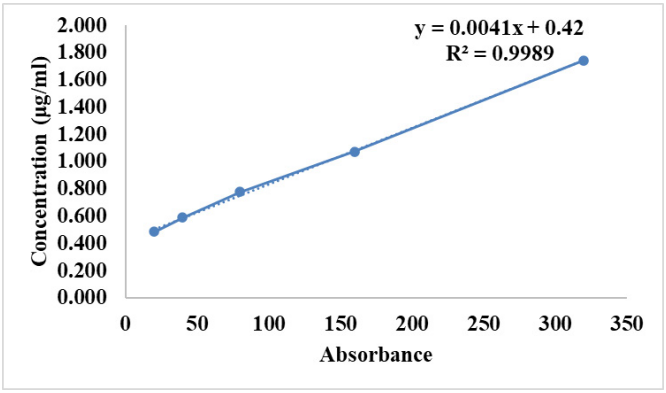

Figure 4: Total antioxidant capacity

Nitric oxide radical scavenging activity

The activity to scavenge nitric oxide was measured using a Griess reagent. The initial steps involved dissolving 5 mM sodium nitroprusside in PBS. Next, 3.0 mL of extract varying with concentrations ranging from 20 to 320 µg/mL were subsequently added. A further 150 minutes were spent incubating the mixture at 25°C. There was no evidence of scavenging activity; the ascorbic acid with IC_{50} value was found to be 152.97 µg/mL, and the ACAQ IC_{50} value was 203.43 ACME 208.82 µg/mL as shown in Figure 5.

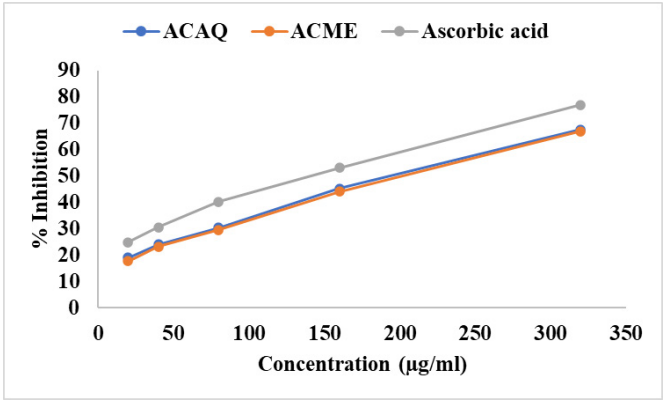

Figure 5: Nitric oxide radical scavenging activity

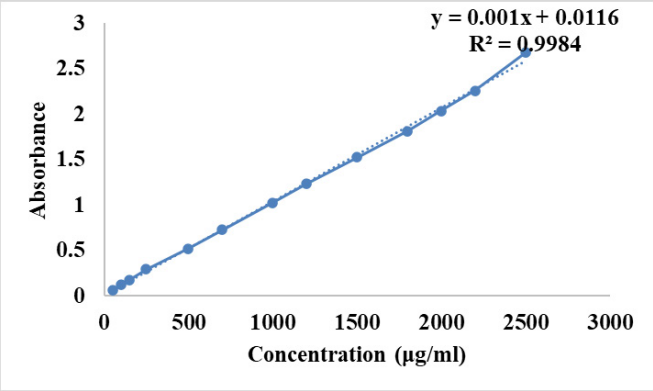

Figure 6: Total phenol content

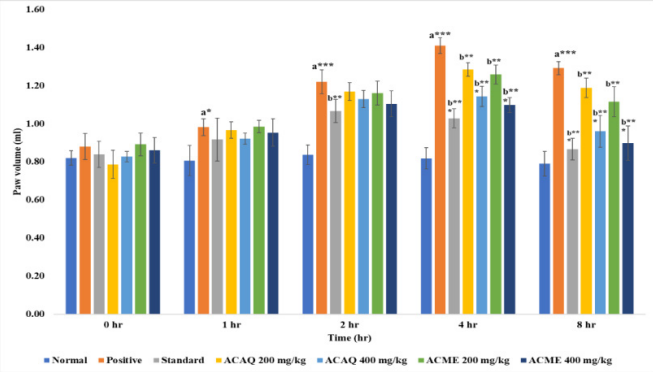

Figure 7: Effect of *A. aspera* on paw volume

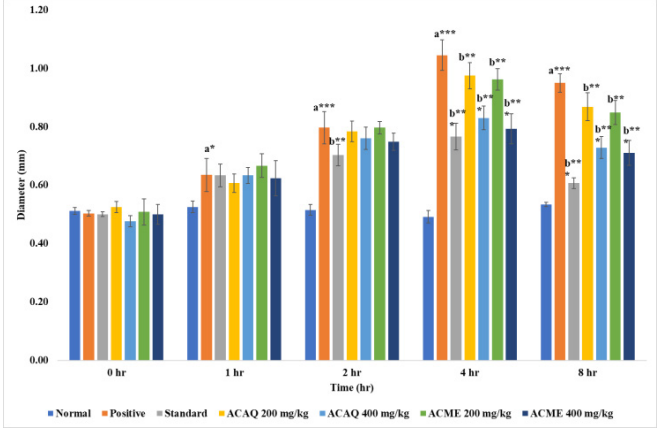

Figure 8: Effect of *A. aspera* on paw diameter

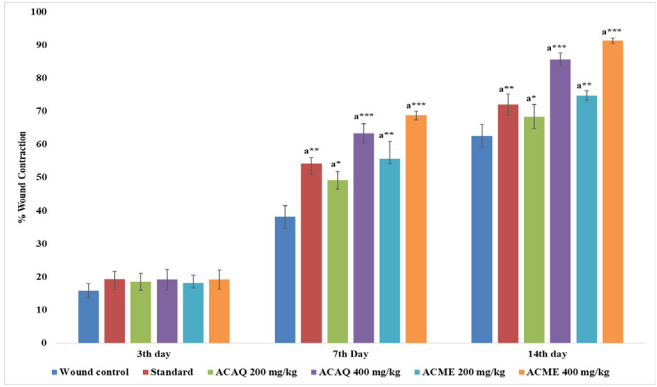

Figure 9: Wound contraction

Total phenol content

The ACAQ and ACME extracts had total phenol contents of 500.4, 508.4, and 530.5 mg per gallic acid equivalent (GAE), correspondingly (Figure 6).

Carrageenan-Induced Acute Inflammatory Model

Effect of A. aspera extracts on paw volume

In contrast with the typical rats, the control rats showed an imperative increment ($p < 0.001$) in the volume of their paws following a one-hour treatment of subplantar carrageenan. Individual rats that were given *A. aspera* **(**ACAQ, ACME 200 mg/kg and 400 mg per kg) demonstrated a substantial decrease in paw volume starting at 2 hours and increasing with increasing dose ($p < 0.001$, respectively) as it was tally to control rats (Figure 7).

*Effect of **A. aspera** extracts on Paw diameter*

After one hour of subplantar carrageenan injection, the paw volume of control rats exhibited a notable elevation ($p < 0.001$) compared to that of normal rats. A substantial reduction in paw diameter ($p < 0.001$ respectively) was observed in rats administered with *A. aspera* (ACAQ, ACME 200 and 400 mg per kg) tally to control rats starting at 2 hours and continuing thereafter (Figure 8).

Wound Contraction

In this study, wounds treated with extracts healed at a considerably faster rate than wounds treated with control or standard. The groups who received extracts showed a statistically significant difference from day seven onwards, and this difference remained until day 14, after the wound had formed (Figures 9 and 10).

Antioxidant Activity in Wound Healing

The levels of glutathione and catalase showed a colossal extension in the social occasion controlled the arrangement stood out from the benchmark bunch. The control group recorded catalase levels at 1.80 ± 0.04 µ/gm, while the group receiving standard drugs showed 3.87 ± 0.11 µ /gm, and animals treated with ACME at 400 mg per kg showed 3.75 ± 0.12 µ/gm catalase in dry tissue. Glutathione levels measured with the control group, standard drug group, ACAQ at 200 mg/kg, ACAQ at 400 mg/kg, ACME at 200 mg/kg, and ACME at 400 mg/kg groups were observed to be 178.8 ± 6.34, 333.16 ± 5.74, 253.5 ± 5.99, 325.66 ± 6.11, 262.33 ± 4.63, and 325.5 ± 11.34 mol/gm, respectively. Conversely, the control group exhibited higher tissue MDA values at 254.16 ± 6.14 U/gm. In contrast, the standard drug-treated group recorded 108.66 ± 4.54 U/gm. The ACAQ with 200 mg/kg, ACAQ with 400 mg/kg, ACME at 200 mg/kg, and ACME with 400 mg/kg groups showed 66.16 ± 2.52, 97.5 ± 2.75, 66.33 ± 6.20, 100.5 ± 2.87 U/gm, respectively, indicating a considerable decrease in MDA levels upon using the formulation ($p < 0.001$) (Figure 11).

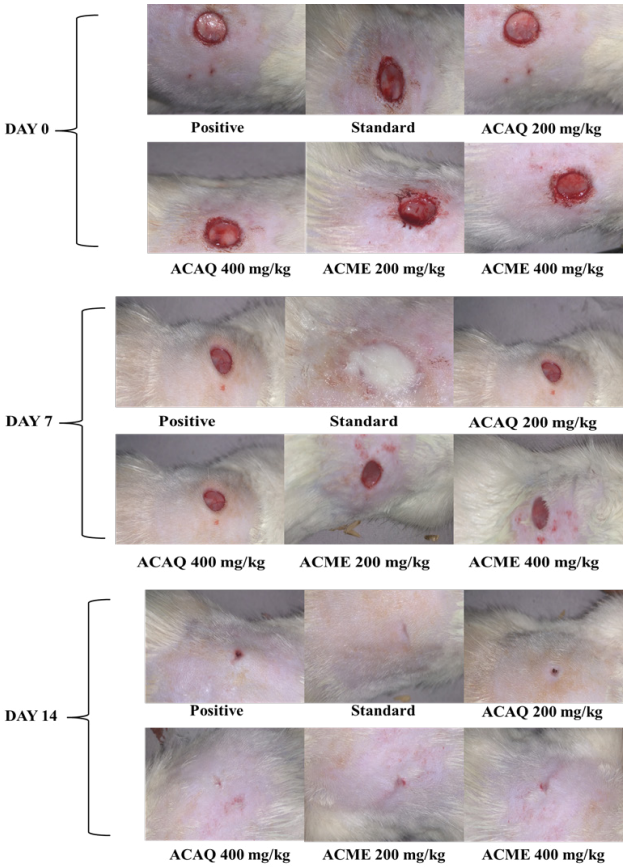

Figure 10: Visual depiction of the rate of contraction illustrating the percentage of wound area under contraction on various days following excision

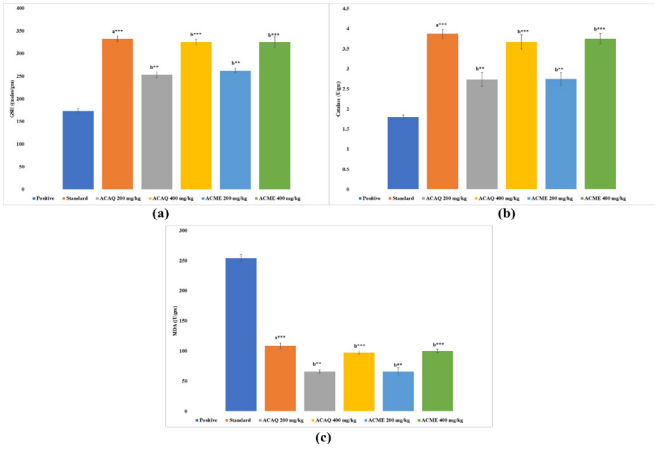

Figure 11: Antioxidant activity in wound healing

Biochemistry of Wound Healing

A bunch of methyl extract showed more elevated levels of hydroxyproline, collagen, and hexosamine per gram of dried recovered tissue contrasted with the benchmark group, demonstrating prevalent injury-mending capacities. In the control animals, hydroxyproline levels measured 32 ± 1.42 mg/g, while animals treated with the standard drug exhibited 54.33 ± 3.7 mg/g. Animals treated with 200 mg/kg of ACAQ,

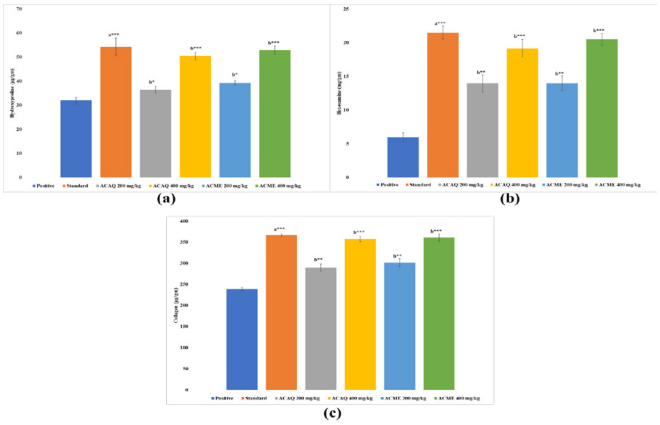

Figure 12: Biochemistry of wound healing: Hydroxyproline, collagen, and hexosamine concentrations

ACAQ at 400 mg per kg, ACME at 200 mg/kg, and ACME at 400 mg/kg showed hydroxyproline levels of 36.5 ± 1.46, 50.5 ± 1.68, 39.33 ± 0.99, and 52.83 ± 1.81 mg/g of tissue, respectively.

The group receiving conventional treatment had tissue hexosamine levels of 21.5 ± 0.96 mg/gm, whereas the control group had values of 6 ± 0.5 mg/gm. Animals treated with ACAQ at 200 mg/kg, ACAQ at 400 mg/kg, ACME at 200 mg/kg, and ACME at 400 mg/kg exhibited hexosamine levels of 14 ± 1.24, 19 ± 1.31, 14 ± 1.13, and 20 ± 0.85 mg/gm, respectively. There was an existence of a direct correlation between hexosamine and collagen synthesis. In the control group, tissue collagen content was measured at 239.33 ± 3.89 mg/gm, while animals treated with the standard drug showed 366.83 ± 3.37 mg/gm. ACAQ at 200 mg/kg, ACAQ at 400 mg/kg, ACME at 200 mg/kg, and ACME at 400 mg/kg treated groups exhibited collagen levels of 289.83 ± 8.60, 357.33 ± 6.15, 302 ± 9.54, and 361.33 ± 8.37 mg/gm, respectively (as shown in Figure 12).

DISCUSSION/CONCLUSION

There are three stages to the healing process of a wound: Inflammation, angiogenesis, and collagen deposition. When diverged from the benchmark bunch that sought no treatment, the *A. aspera* isolates showed a quantifiably immense lessening in injury size following 15 days. Collagen plays a critical part in the extracellular lattice, which supports wound recovery. There is a one-to-one relationship between hydroxyproline and collagen production. Contrasted with the benchmark group, animals treated with a polyherbal formulation had considerably higher levels of hydroxyproline in their newly created tissue. Traditional methods for evaluating plant-based wound healing agents rely on invasive and distressing procedures carried out on animal models. The *in-vitro* tests outlined here allow us to screen a plethora of plant compounds for the angiogenic, antioxidant, and cell mobilization characteristics that are crucial to wound healing. This article outlines *in-vitro* assays that can be used to screen herbal preparations for wound healing qualities. According to the results of our research, several extracts (ACAQ 200 mg/kg, ACAQ 400 mg/kg, ACME 200 mg/kg and ACME 400 mg/kg) of *A. aspera* speed up the

mending system of wounds by empowering angiogenesis and fibroblast and keratinocyte assembly.

REFERENCES

1. Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S, Saparov A. Immunology of acute and chronic wound healing. Biomolecules. 2021;11(5):700.
2. Bowers S, Franco E. Chronic wounds: evaluation and management. *American family physician*. 2020;101(3):159-166.
3. Thorat V, More HN, Tamboli F, Jadhav A, Gaikwad R, Rangari D. Pharmacognostic account and medicinal uses of *Achyranthes aspera* L. linn. International Journal of Pharmacognosy. 2020;8(8):100-108.
4. Mengie T, Mequanente S, Nigussie D, Legesse B, Makonnen E. 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*. 2021; 14: 1775.
5. Ha H-A, Al-Humaid LA, Aldawsari M, Bharathi D, Lee J. Evaluation of phytochemical, antibacterial, thrombolytic, anti-inflammatory, and cytotoxicity profile of *Achyranthes aspera* aerial part extracts. *Environmental Research*. 2024; 243: 117802.
6. Viswanatha GL, Venkataranganna MV, Prasad NBL, Shylaja H. *Achyranthes aspera* Linn. alleviates cerebral ischemia-reperfusion-induced neurocognitive, biochemical, morphological and histological alterations in wistar rats. *Journal of ethnopharmacology*. 2019; 228: 58-69.
7. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: A cellular perspective. Physiological reviews. 2019; 99 (1):665-706.
8. Visha M, Karunagaran M. A review on wound healing. *In*ternational Journal of Clinicopathological Correlation. 2019;3(2):50-59.
9. Ganesh SS, Rao P, Nandal D, Kunkulol R. A review on pharmacological and phytochemical constituent of *Achyranthes aspera* L. linn. International Journal of Pharmacognosy. 2021;8(8):58-64.
10. Chauhan S, Gupta S, Yasmin S, Saini M. Antihyperglycemic and Antioxidant Potential of Plant Extract of Litchi chinensis and Glycine max. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2021;11(3):225-233.
11. Ahmad W, Singh V, Ahmed S, Nur-e-Alam M. A comprehensive study on antibacterial antioxidant and photocatalytic activity of *Achyranthes aspera* mediated biosynthesized Fe2O3 nanoparticles. *Results in Engineering*. 2022; 14: 100450.
12. Chauhan S, Chalotra R, Rathi A, et al. Current Approaches in healing of wounds in Diabetes and Diabetic foot ulcers. Current Bioactive Compounds. 2023;19(3):104-121.
13. Dhankhar S, Chauhan S, Mehta DK, et al. Novel targets for potential therapeutic use in Diabetes mellitus. *Diabetology & Metabolic Syndrome*. 2023;15(1):17.
14. Lalit K, Navpreet K, Samrat C, Randhir S. Phyto-pharmacological review of Coccinia indica. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;3(2):1734-1745.
15. Rohilla M, Bansal S, Garg A, et al. Discussing pathologic mechanisms of Diabetic retinopathy & therapeutic potentials of curcumin and β-glucogallin in the management of Diabetic retinopathy. Biomedicine & Pharmacotherapy. 2023;169: 115881.
16. Tamta G, Malik N, Gauri V, Nand V. Phytochemical Analysis and *in-vitro* Antioxidant and Antibacterial Activities of Ethanolic Leaf Extract of *Achyranthes aspera* L. 2022;
17. Dhankar S, Mujwar S, Garg N, et al. Artificial Intelligence in The Management of Neurodegenerative Disorders. CNS & Neurological Disorders - Drug Targets. 2024; 23: 1-10.
18. Mittal P, Dhankhar S, Chauhan S, et al. A Review on Natural Antioxidants for Their Role in the Treatment of PParkinson'sDisease. Pharmaceuticals. 2023;16(7):908.
19. Panchal M, Rana P, Garg N, Dhankhar S, Chauhan S, Sharma H. A Comprehensive Review of Alternative Therapeutic Approaches for Nausea and Vomiting Relief in Pregnancy. New Emirates Medical Journal. 2024; 5:1-8.
20. Saharan R, Kaur J, Dhankhar S, et al. Hydrogel-based Drug Delivery System in Diabetes Management. Pharmaceutical Nanotechnology. 2024;12: 1-11.
21. Yen GC, Hsieh PP. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. Journal of the Science of Food and Agriculture. 1995;67(3):415-420.
22. Ruch RJ, Cheng S-j, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-1008.
23. Chawla A, Devhare LD, Dharmamoorthy G, Ritika, Tyagi S. Synthesis and In-vivo Anticancer Evaluation of N-(4-oxo-2- (4-((5-aryl-1,3,4 thiadiazole-2yl) amino) Phenyl thiazolidine-3-yl) Benzamide derivative. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):470-474.
24. Ahmed MS, Khan IJ, Aman S, et al. Phytochemical investigations, in-vitro antioxidant, antimicrobial potential, and in-silico computational docking analysis of *Euphorbia milii* Des Moul. Journal of Experimental Biology and Agricultural Sciences. 04/30 2023;11(2):380-393.
25. Shukla S, Mehta A, Bajpai VK, Shukla S. *In-vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. Food and Chemical Toxicology. 2009;47(9):2338-2343.
26. Marcocci L, Maguire JJ, Droylefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochemical and biophysical research communications. 1994;201(2):748-755.
27. Wijewardhana U, Gunathilaka U, Navaratne S. Determination of total phenolic content, radical scavenging activity and total antioxidant capacity of cinnamon bark, black cumin seeds and garlic. 2019.
28. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-1200.
29. Prasad C, Khemchandani N, Bhagat R, Pimpale A. Ethnomedicinal plant -A review. Journal of Pharmaceutical Research International. 2021;33 (29B):17-30.
30. Dirar A, Alsaadi D, Wada M, Mohamed M, Watanabe T, Devkota H. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. South African Journal of Botany. 2019;120: 261-267.
31. El-Shitany NA, Eid BG. Icariin modulates carrageenan-induced acute inflammation through HO-1/Nrf2 and NF-kB signaling pathways. *Biomedicine & Pharmacotherapy*. 2019;120: 109567.
32. Kumar KK, Kiran V, Choudhary RK, Devhare LD, Gunjal SD. Design Development and Characterization of Nicardipine Solid Lipid Nano-Particulars. International Journal of Drug Delivery Technology. 2024;14(1):71-78.

33. Junior AJ, Leitão MM, Bernal L, et al. Analgesic and anti-inflammatory effects of Caryocar brasiliense. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents). 2020;19(3):313-322.
34. Lai W, Wang C, Lai R, Peng X, Luo J. Lycium barbarum polysaccharide modulates gut microbiota to alleviate rheumatoid arthritis in a rat model. NPJ Science of Food. 2022;6(1):34.
35. Ahmad SU, Binti Aladdin N-A, Jamal JA, Shuid AN, Mohamed IN. Evaluation of wound-healing and antioxidant effects of Marantodes pumilum (Blume) Kuntze in an excision wound model. Molecules. 2021;26(1):228.
36. Chawla A, Devhare LD, Dharmamoorthy G, Ritika, Tyagi S. Synthesis and In-vivo Anticancer Evaluation of N-(4-oxo-2- (4-((5-aryl-1,3,4 thiadiazole-2yl) amino) Phenyl thiazolidine-3-yl) Benzamide derivative. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):470-474.
37. Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. European journal of pharmacology. 2007;556(1-3):75-83.
38. Raheja S, Girdhar A, Kamboj A, Lather V, Pandita D. *Aegle marmelos* leaf extract ameliorates the cognitive impairment and oxidative stress induced by intracerebroventricular streptozotocin in male rats. Life sciences. 2019; 221: 196-203.
39. Priya MGR, Prasanth LML, Devhare LD, Yazdan SK, Gunjal S. Synthesis, DNA Binding, Molecular Docking and Anticancer Studies of Copper (II), Nickel (II), and Zinc (II) Complexes of Primaquine-based Ligand. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):69-75.
40. Pravalika K, Sarmah D, Kaur H, et al. Trigonelline therapy confers neuroprotection by reduced glutathione mediated myeloperoxidase expression in animal model of ischemic stroke. Life sciences. 2019; 216:49-58.
41. Adimulapu A, Devhare L, Patil A, Chachd O, Dharmamoorthy G. Design and Development of Novel Mini Tablet Cap Technology for the Treatment of Cardiovascular Diseases. International Journal of Drug Delivery.Technology. 2023;13(3):801-806/.
42. Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. Antioxidants. 2019;8(3):72.
43. Devhare L and Gokhale N. In silico anti-ulcerative activity evaluation of some bioactive compound from cassia tora and butea monosperma through moleculer docking approach. International journal of pharmaceutical sciences and research. 2023, 14(2), 1000-08.
44. Sona P, Maya S, Lucy K, et al. Estimation of hydroxy proline and collagen content in skin of turkey (Meleagris gallopavo). Journal of Veterinary and Animal Sciences. 2020;79-81.
45. Gnana RPM, Devhare LD, Dharmamoorthy G, Khairnar MV, Prasidha R. Synthesis, Characterisation, Molecular Docking Studies and Biological Evaluation of Novel Benzothiazole Derivatives as EGFR Inhibitors for Anti-breast Cancer Agents. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):475-480.
46. Ali S, Alam S, Ahmad S, Ali M, Ahsan W, Siddiqui MR. Wound healing activity of alcoholic extract of *Tamarix aphylla* L. on animal models. Biomedical and Pharmacology Journal. 2019;12(1):41-48.
47. Devhare L and Gokhale N. Antioxidant and antiulcer property of different solvent extracts of cassia tora linn. Research journal of pharmacy and technology. 2022, 15(3), 1109-1113.