

PHARMACOLOGICAL STUDY OF ANTI-OXIDANT AND WOUND HEALING CAPACITY IN ACHYRANTHES ASPERA L STEM

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ABSTRACT

The present study was carried out to evaluate the phytochemical profile, antioxidant potential, and wound healing activity of the ethanolic extract of *Achyranthes aspera* L. stem. Although the plant is widely used in traditional medicine for treating wounds and oxidative stress-related disorders, limited scientific evidence is available regarding the medicinal value of its stem. Therefore, this investigation aimed to validate its pharmacological potential through experimental studies. The plant material was subjected to organoleptic, morphological, and physicochemical evaluation for authentication and quality assessment. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, and steroids. Thin layer chromatography confirmed important constituents such as rutin, gallic acid, and flavone glycosides. Quantitative estimation showed total phenolic content of 108 mg GAE/g

DW and total flavonoid content of 160.2 mg QE/g DW. Antioxidant activity was assessed by DPPH and FRAP assays. The extract exhibited strong free radical scavenging activity with 86.39% inhibition at 100 µg/ml in the DPPH assay and significant ferric reducing power in the FRAP method. Acute toxicity studies as per OECD guideline 423 confirmed safety up to 2000 mg/kg body weight. In the excision wound model using Wistar albino rats, the extract showed dose-dependent wound contraction and reduced epithelialization period, with 400 mg/kg showing activity comparable to Nitrofurazone.

KEYWORDS: *Achyranthes aspera*, antioxidant activity, wound healing, phytochemical screening.

INTRODUCTION

Wound healing is a complex and highly coordinated biological process essential for restoring the integrity and function of damaged skin and underlying tissues after injury. It involves four overlapping phases: haemostasis, inflammation, proliferation, and remodelling.^[1] Immediately after injury, haemostasis occurs through vasoconstriction, platelet aggregation, and fibrin clot formation to prevent blood loss. This is followed by the inflammatory phase, where neutrophils and macrophages remove debris, control infection, and release cytokines and growth factors required for tissue repair.^[5,6] During the proliferative phase, fibroblasts synthesize collagen, granulation tissue forms, angiogenesis develops new blood vessels, and epithelial cells migrate to cover the wound surface. Finally, the remodelling phase strengthens the tissue through collagen reorganization and wound contraction, although scar tissue generally remains weaker than normal skin. Any disturbance in these phases may delay healing and result in chronic wounds. interest.^[4,3]

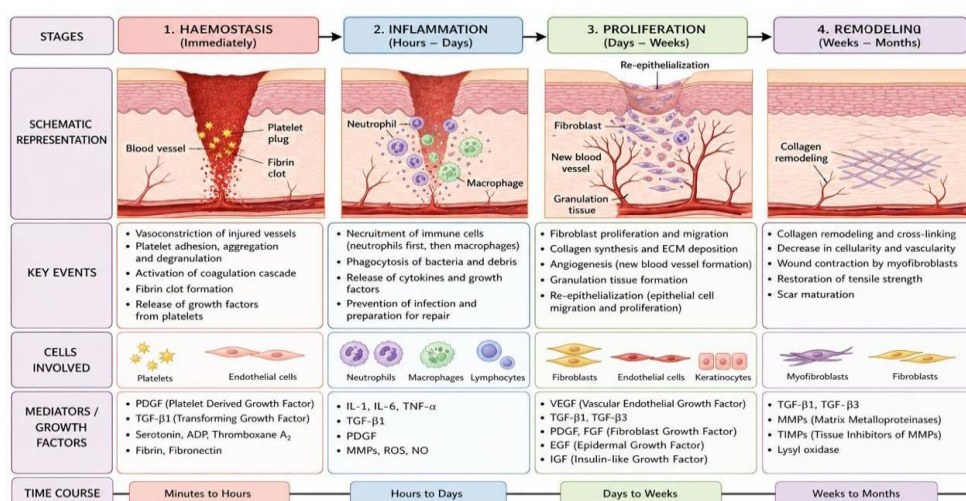


Fig. 1: Cellular and Molecular Mechanisms of Wound Healing.

Medicinal plants have been widely used in traditional healthcare systems for treating wounds, burns, ulcers, and skin infections. Their therapeutic value is attributed to the presence of phytochemicals such as flavonoids, tannins, alkaloids, saponins, glycosides, and terpenoids. These constituents possess antioxidant, anti-inflammatory, antimicrobial, and regenerative properties that support healing. Flavonoids neutralize free radicals and reduce oxidative stress, tannins promote wound contraction, while saponins aid tissue regeneration. Plant-

based remedies also enhance collagen deposition and neovascularization, accelerating repair.^[18,20,21]

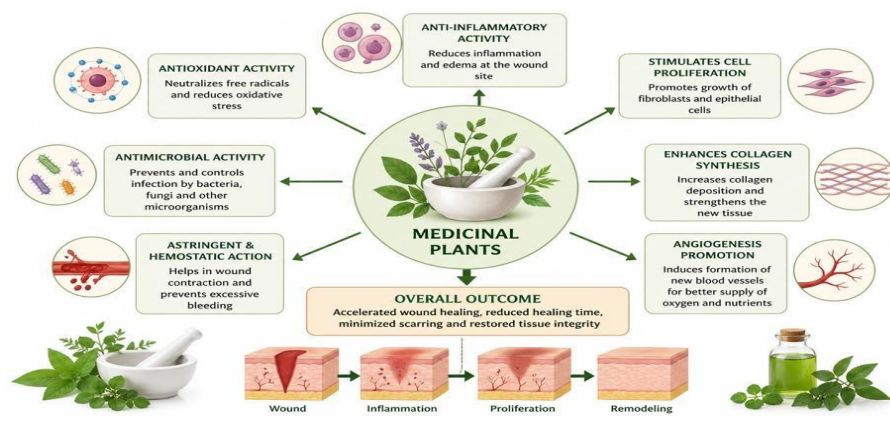


Fig. 2: Role of Medicinal Plants in Wound Healing.

Traditional systems such as Ayurveda, Siddha, Unani, and Chinese medicine rely heavily on natural remedies because of their affordability, accessibility, and fewer side effects.

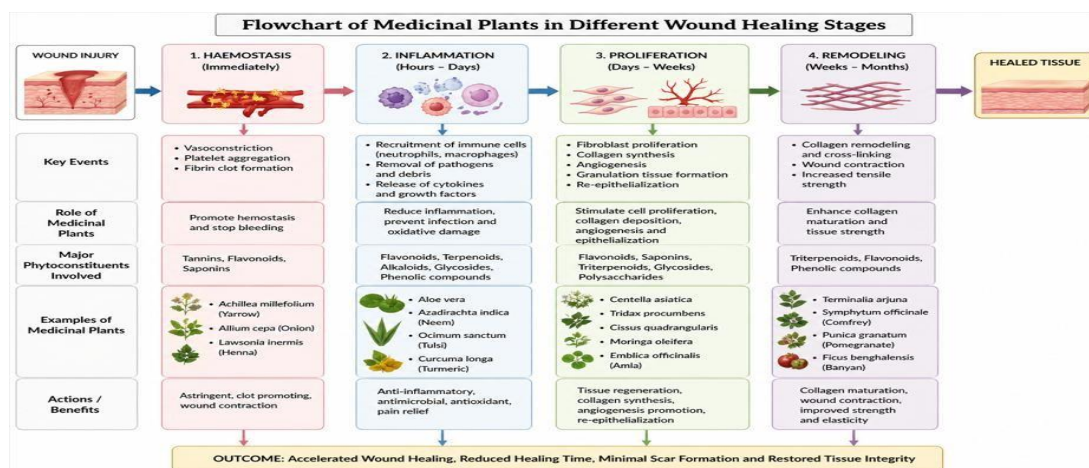


Fig. 3: Flowchart of Medicinal Plants in Different Wound Healing Stages.

Unlike synthetic drugs that often target a single pathway, herbal medicines usually act through multiple mechanisms including infection control, inflammation reduction, oxidative stress management, and tissue regeneration. This makes them valuable alternatives in wound care management.

Table 1: Common Medicinal Plants Used in Wound Healing.

Plant Name	Key Compound	Reported Activity
Aloe vera	Polysaccharides	Anti-inflammatory, cell proliferation
Centella asiatica	Asiaticoside	Collagen synthesis, wound contraction

Curcuma longa	Curcumin	Antioxidant, tissue repair
Azadirachta indica	Nimbidin	Antibacterial, wound healing
Tridax procumbens	Flavonoids	Fibroblast proliferation
Moringa oleifera	Flavonoids	Epithelialization

Plant Profile of *Achyranthes aspera* L

Achyranthes aspera L., commonly known as **Prickly Chaff Flower**, is a perennial herb belonging to the family **Amaranthaceae**. It is widely distributed in tropical and subtropical regions such as India, Nepal, Sri Lanka, Bangladesh, Africa, and Southeast Asia. The plant commonly grows in wastelands, roadsides, and cultivated fields. Morphologically, it has an erect or procumbent angular stem covered with fine hairs and grows up to 1–2 meters in height. Leaves are simple, opposite, ovate-lanceolate, entire, and slightly rough in texture. The flowers are small, greenish or reddish, arranged in long terminal spikes with sharp persistent bracts that give the plant its prickly appearance. Fruits are dry one-seeded capsules (achenes) containing small reddish-brown seeds, while the root system consists of a long woody taproot. Taxonomically, it belongs to Kingdom Plantae, Family Amaranthaceae, Genus *Achyranthes*, and Species *aspera*. It is known by different names such as **Aghada** (Marathi), **Chirchira** (Hindi), **Apamarg** (Sanskrit), and **Chaff flower** (English).

Achyranthes aspera has been widely used in Ayurveda, Unani, Siddha, and folk medicine for centuries. Different plant parts possess important medicinal uses. Leaves are used for wounds, skin infections, cough, fever, and digestive disorders. Seeds show diuretic, laxative, and anti-parasitic properties and are used for kidney stones and urinary disorders. Roots are valuable in treating respiratory problems, rheumatism, menstrual irregularities, and deworming. The stem is used as an astringent, febrifuge, and sometimes as a natural toothbrush. Phytochemically, the plant contains alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and phenolic compounds. Due to these constituents, it exhibits anti-inflammatory, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, hepatoprotective, anti-asthmatic, and wound healing activities. Hence, *Achyranthes aspera* is considered an important medicinal plant with strong potential for herbal drug development.

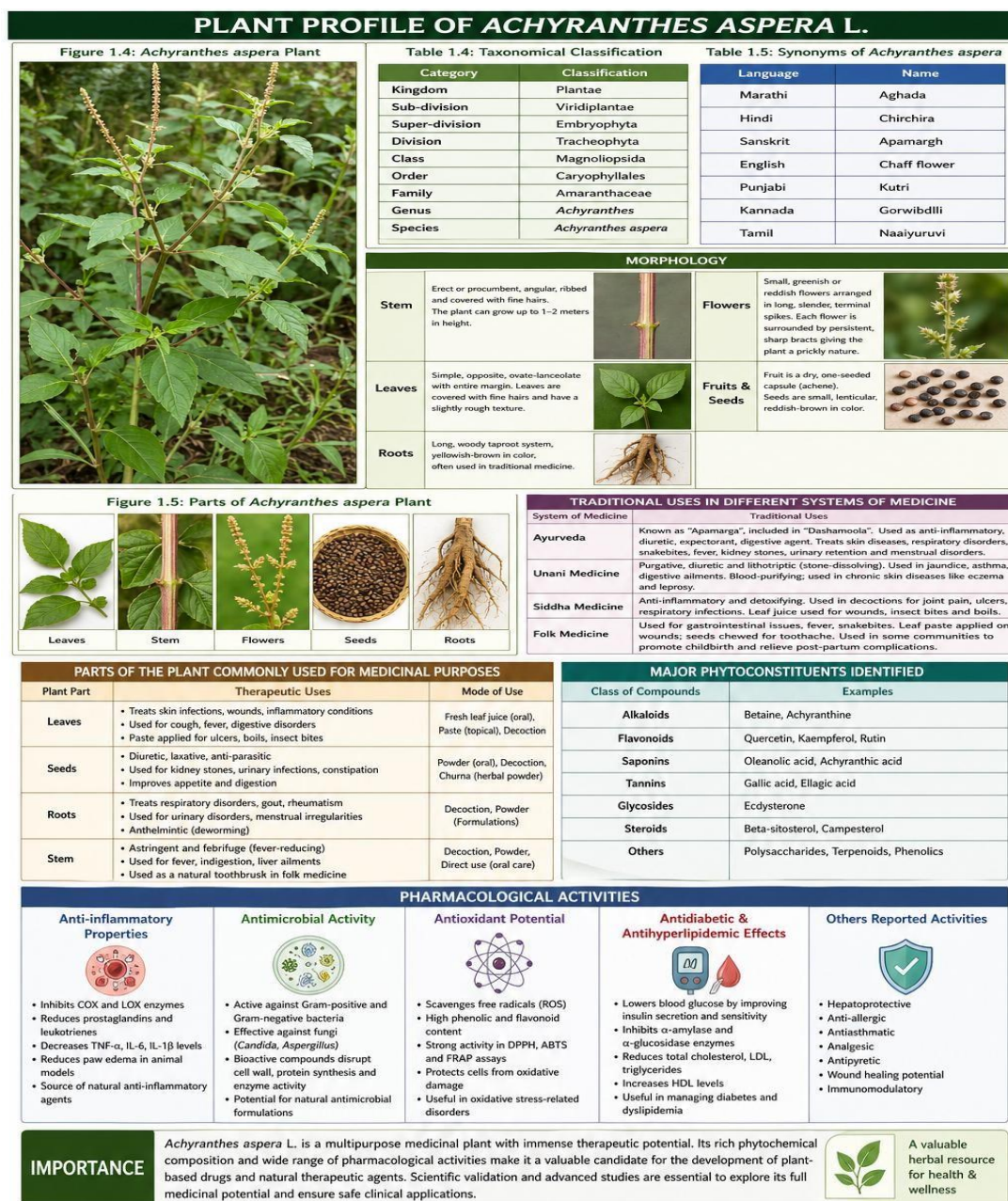


Fig. 4: Plant Profile of *Achyranthes aspera* L.

EXPERIMENTAL WORK AND RESULT

Determination of Ash Values

Ash values are crucial for assessing the inorganic content and purity of herbal drugs. The total ash, acid-insoluble ash, and water-soluble ash content of the stem were determined as per standard procedures.

Table 2: Ash Value Determination of *Achyranthes aspera* Stem Powder.

Sr. NO.	Parameter	Wt. of Crucible + Ash (Z)	Wt. of Crucible (X)	Wt. of Drug (Y)	% Ash = $\frac{(Z - X)}{Y} \times 100$
1	Total Ash	26.05 g	25.87 g	2.00 g	9.00%
2	Acid-Insoluble Ash	44.12 g	44.07 g	2.00 g	2.50%
3	Water-Soluble Ash	26.46 g	26.35 g	2.00 g	5.50%

The ash values indicate the inorganic content of the crude drug. The total ash value (9.0%) reflects the overall mineral residue, while the low acid-insoluble ash (2.5%) suggests minimal contamination with silica or earthy matter. The water-soluble ash (5.5%) confirms the presence of water-soluble minerals, indicating good purity and quality of the plant material.

Extractive Values in Various Solvents

Extractive values help to estimate the approximate amount of phytoconstituents extracted by different solvents, thereby providing a preliminary idea of the type of compounds present based on polarity.

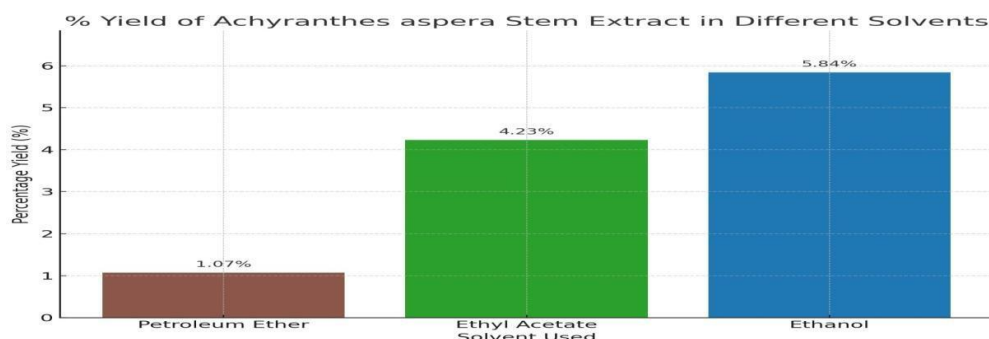
Table 3: Extractive Value Determination of *Achyranthes aspera* Stem Extracts.

Sr. No.	Solvent	Wt. of Petri Dish (g)	Wt. with Extract (g)	Wt. of Extract (g)	% Extractive Value
1	Petroleum Ether	47.40	47.77	0.37	18.5%
2	Acetone	47.80	48.01	0.21	10.5%
3	Ethyl Acetate	44.06	44.80	0.64	32.0%
4	Chloroform	42.40	42.85	0.45	22.5%
5	Methanol	47.99	48.21	0.22	11.0%
6	Ethanol	38.80	39.43	0.63	31.5%
7	Water	41.80	42.58	0.78	39.0%

Extractive values represent the quantity of phytoconstituents extracted by different solvents. The highest extractive value was observed in water (39.0%), followed by ethyl acetate and ethanol, indicating the presence of both polar and moderately polar compounds. This supports the selection of ethanol for further extraction due to its ability to dissolve a wide range of bioactive compounds. The highest yield was observed with ethanol, a highly polar solvent, which gave 15.19 grams, corresponding to a 5.84% yield. This significant increase demonstrates that the stem of *Achyranthes aspera* is rich in polar bioactive compounds such as tannins, saponins, and polyphenols—well-known for their antioxidant and therapeutic properties.

Table 4: Appearance and % yield of AA Extract.

Sr. No.	Drug Taken (gm)	Solvent used (ml)	Consistency	Colour of Extract	Yield ((gm)	(%) Yield
1	270gm	1500 (Pet. ether)	Sticky	Dark Brown	2.80	1.07%
2	270gm	1500 (Ethyl acetate)	Sticky	Dark Brown	11.01	4.23%
3	270gm	1500 (Ethanol)	Sticky	Dark brown	15.19	5.84%

**Fig. 5: Percentage yield of *Achyranthes aspera* stem extract in different solvents.**

The percentage yield reflects the efficiency of extraction. Ethanol showed the highest yield (5.84%), indicating its superior ability to extract phytoconstituents compared to non-polar solvents like petroleum ether. This suggests that the plant contains predominantly polar compounds such as flavonoids and phenolics.

Table 5: Preliminary phytochemical screening of *Achyranthes aspera* stem extracts.

Phytochemical Test	Petroleum Ether	Ethyl Acetate	Ethanol
Carbohydrates			
Molisch's Test	—	+	+
Fehling's Test	—	+	+
Benedict's Test	—	—	—
Proteins			
Biuret Test	—	+	+
Millon's Test	+	—	—
Amino Acids			
Ninhydrin Test	—	—	—
Steroids			
Salkowski Test	—	+	+
Libermann-Burchard Test	+	—	—
Glycosides	Foam Test	—	—
Flavonoids			
Shinoda Test	—	+	+
Lead Acetate Test	+	+	+
Sulphuric Acid Test	—	—	+
Tannins and Phenolic Compounds			
Lead Acetate Test	+	+	+

Dil. Nitric Acid Test	—	—	—
Ferric Chloride Test	—	+	+
Alkaloids			
Mayer's Test	—	+	+
Hager's Test	—	+	+
Wagner's Test	—	—	—

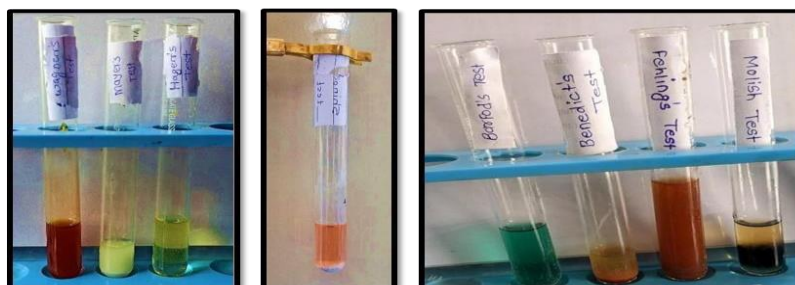


Fig. 6: preliminary phytochemical evaluation.

Preliminary phytochemical screening confirmed the presence of important secondary metabolites in the ethanol extract. Compounds such as flavonoids and phenolics are well known for antioxidant and wound healing properties, supporting the pharmacological potential of the plant extract.

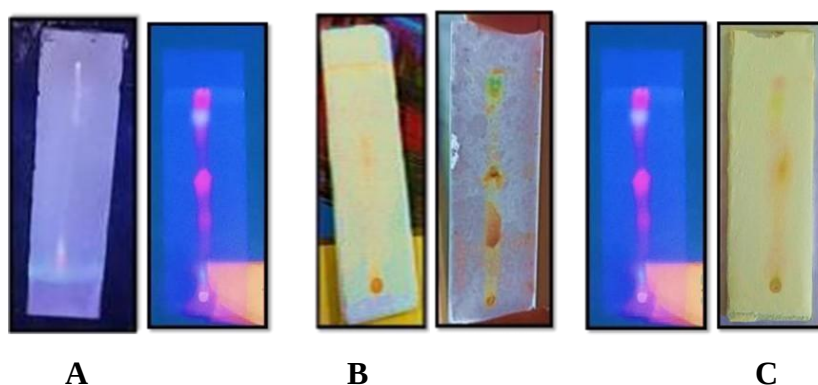


Fig. 7: A) TLC of AA Petroleum ether B) TLC of AA Ethyl Acetate C) TLC of AA Ethanol.

Table 5: Thin Layer Chromatography Results.

Sr. No.	Extract	Solvent System (v/v)	Spraying Agent	Rf Value	Colour	Identified Compound
1	Pet. AEther	Toluene:Acetone:Methanol (8:1:1)	H ₂ SO ₄	0.87	Brown	Flavone Glycosides
				0.52	Yellow	Rutin
2	Ethyl Acetate	Ethyl Acetate:n-Hexane:Ammonia (5:5:0.2)	H ₂ SO ₄	0.85	Blue	Chlorogenic Acid
				0.55	Green	Flavonoid

				0.45	Yellow	Rutin
				0.20	Brown	Flavone
						Glycosides
				0.18	Pink	Gallic Acid
3	Ethanol	Toluene:Acetone:Methanol (6:3:1)	H ₂ SO ₄	0.66	Brown	Flavone Glycosides
				0.49	Green	Flavonoid
				0.38	Pink	Gallic Acid

The TLC analysis revealed distinct R_f values corresponding to specific phytoconstituents such as flavonoids and gallic acid. These compounds are associated with antioxidant activity, confirming the chemical richness of the ethanol extract and validating its selection for further studies.

Table 6: Total Phenolic & Flavonoid Content.

Parameter	Value
TPC	108 mg GAE/g
TFC	160.2 mg QE/g

The high total phenolic and flavonoid content indicates a significant presence of bioactive compounds responsible for antioxidant activity. These compounds act as free radical scavengers and contribute to the therapeutic potential of the extract. Flavonoid content of the ethanol extract was estimated and expressed as milligrams of quercetin equivalents per gram of dry weight.

Table 7: Absorbance of Gallic acid, Rutin, Quercetin.

Sr.No.	Conc. (µg/ml)	Absorbance		
		Gallic acid	Rutin	Quercetin
1	20	0.423 ± 0.002	0.251 ± 0.002	0.484 ± 0.02
2	40	0.380 ± 0.001	0.218 ± 0.001	0.375 ± 0.001
3	60	0.316 ± 0.002	0.193 ± 0.001	0.282 ± 0.001
4	80	0.287 ± 0.001	0.163 ± 0.002	0.194 ± 0.001
5	100	0.195 ± 0.002	0.150 ± 0.001	0.155 ± 0.002

These findings indicate a concentration-dependent increase in antioxidant activity. Among the three standards tested, Quercetin exhibited the highest free radical scavenging activity at 100 µg/ml (88.92%), followed by Rutin (81.68%) and Gallic acid (76.14%). This confirms the potent antioxidant capability of Quercetin.

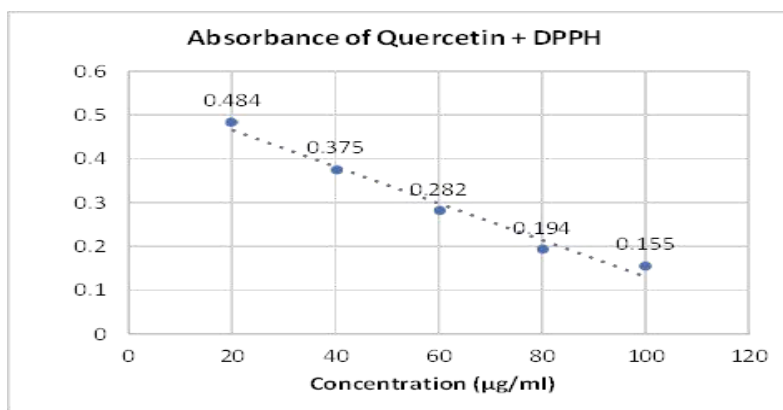


Fig. 8: Absorbance of Quercetin + DPPH (Standard).

Table 8: % Inhibition of Standard Antioxidants in DPPH Assay.

Sr. No.	Concentration (µg/ml)	% Inhibition (Gallic acid)	% Inhibition (Rutin)	% Inhibition (Quercetin)
1	20	48.30 ± 0.36	69.26 ± 0.28	44.40 ± 0.10
2	40	53.59 ± 0.21	73.33 ± 0.17	54.20 ± 0.18
3	60	61.37 ± 0.28	76.43 ± 0.18	65.56 ± 0.18
4	80	64.95 ± 0.18	80.09 ± 0.25	76.22 ± 0.17
5	100	76.14 ± 0.24	81.68 ± 0.13	88.92 ± 0.10

Table A9: % Inhibition of Quercetin.

Sr. No	Concentration (µg/ml)	Absorbance (Quercetin)	Absorbance (Ethanol Extract)
1	20	0.484 ± 0.02	0.188 ± 0.011
2	40	0.375 ± 0.001	0.154 ± 0.015
3	60	0.282 ± 0.001	0.131 ± 0.005
4	80	0.194 ± 0.001	0.094 ± 0.007
5	100	0.155 ± 0.002	0.060 ± 0.018

Table 10: % Inhibition of DPPH Radical by Ethanolic Extract.

Sr. No	Concentration (µg/ml)	% Inhibition (Quercetin)	% Inhibition (Ethanol Extract)
1	20	44.4 ± 0.10	57.36 ± 0.17
2	40	54.20 ± 0.18	65.07 ± 0.12
3	60	65.56 ± 0.18	70.29 ± 0.14
4	80	76.22 ± 0.17	78.68 ± 0.19
5	100	88.92 ± 0.10	86.39 ± 0.22

The ethanolic extract of *Achyranthes asperastem* displayed potent DPPH radical scavenging activity, with over 57% inhibition at the lowest tested concentration of 20 µg/ml. Since 50% inhibition was achieved at a lower concentration, the IC₅₀ is estimated to be approximately 15 µg/ml, suggesting a stronger antioxidant potential than quercetin, whose IC₅₀ is estimated around 38 µg/ml. This highlights the extract's remarkable efficacy as a free radical scavenger, likely due to the synergistic action of phenolic and flavonoid compounds in the extract. These

findings validate its traditional use and justify further investigation into its potential therapeutic applications.

Table 11: IC₅₀ value of Standard and Extract.

Sample	IC ₅₀ (µg/ml)
Quercetin (Standard)	~38 µg/ml
Ethanollic Extract of AA Stem	~15 µg/ml

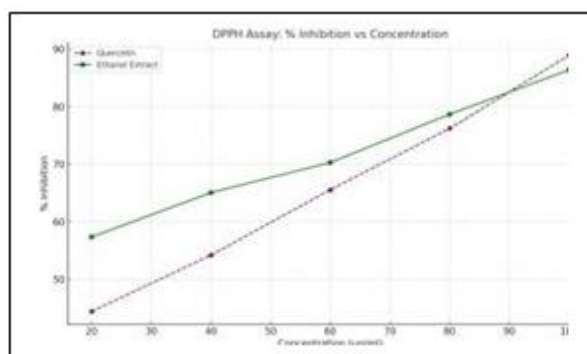


Fig. 8: DPPH Assay for ethanolic extract of AA stem for *in-vitro* antioxidant activity.

Table 12: % Antioxidant Activity (% Inhibition) and IC₅₀ of Ethanolic Extract vs. Standard.

Sr. No	Conc. (µg/ml)	% Inhibition (Ascorbic Acid)	% Inhibition (Ethanol Extract)
1	20	32.39 ± 0.45	22.67 ± 0.48
2	40	53.33 ± 0.38	35.45 ± 0.56
3	60	70.58 ± 0.34	50.34 ± 0.42
4	80	85.74 ± 0.26	63.85 ± 0.39
5	100	100.00 ± 0.00	76.12 ± 0.40

Table 13: IC₅₀ value of Standard and Extract.

Sample	IC ₅₀ (µg/ml)
Ascorbic Acid (Std)	36.24
Ethanollic Extract of AA Stem	62.85

These findings indicate that *Achyranthes aspera* ethanolic stem extract possesses substantial antioxidant activity, likely attributed to its phytochemical constituents such as flavonoids and phenolics, which act as electron donors in redox reactions. The FRAP assay shows increasing reducing power with concentration. Although the extract exhibited lower activity than ascorbic acid, it demonstrated considerable antioxidant capacity, confirming its ability to donate electrons and reduce oxidative stress.

Table 14: Acute Toxicity Symptoms of Ethanolic Extract of *Achyranthes aspera* Stem.

Dose (mg/kg)	Signs of Toxicity	Mortality (n/N)	Observation Period
5	No signs of toxicity observed	0/3	14 days
50	No behavioral abnormalities	0/3	14 days
300	Mild sedation and slight reduction in locomotor activity(temporary)	0/3	14 days
2000	Mild sedation and transient decrease in motor activity	0/3	14 days

Table 15: Effect of Ethanolic Extract of *Achyranthes aspera* Stem on Body Weight of Rats.

Dose (mg/kg)	Body Weight Day 0 (g)	Body Weight Day 7 (g)	Body Weight Day 14 (g)
5	182.4 ± 3.2	186.1 ± 3.5	190.3 ± 3.8
50	181.8 ± 3.4	185.6 ± 3.6	189.9 ± 3.7
300	183.1 ± 3.6	186.4 ± 3.9	191.2 ± 4.1
2000	182.7 ± 3.5	185.8 ± 3.7	190.5 ± 3.9

Values are expressed as Mean ± SEM (n = 3).

Experimental Design

To evaluate the wound healing potential of the ethanolic extract of *Achyranthes aspera* L. stem, an in vivo excision wound model was employed using healthy Wistar albino rats. The animals were randomly divided into four experimental groups, each containing an equal number of animals. This experimental grouping enabled a comparative assessment of the wound healing effect of the plant extract against both untreated and standard treated groups.

- a. **Group I (Control Group):** Animals in this group received only the vehicle treatment (normal saline or no treatment). This group served as the baseline to observe the natural wound healing process without pharmacological intervention.
- b. **Group II (Standard Group):** Animals in this group were treated with Nitrofurazone ointment, a well-known topical antimicrobial agent widely used as a standard reference drug for wound healing studies.
- c. **Group III (Test Group – Low Dose):** Animals in this group were treated with the ethanolic extract of *Achyranthes aspera* stem at a dose of 200 mg/kg body weight, administered to evaluate the wound healing activity of the extract at a lower dose level.
- d. **Group IV (Test Group – High Dose):** Animals in this group were treated with the ethanolic extract of *Achyranthes aspera* stem at a dose of 400 mg/kg body weight to assess the dose- dependent wound healing effect of the extract.

Observation Parameters Wound Contraction (%)

The primary parameter used to assess wound healing activity in the excision wound model was the percentage of wound contraction, which represents the reduction in wound area over time. Wound contraction is an important indicator of tissue regeneration and reflects the ability of the treatment to accelerate the healing process. The wound area was measured at regular intervals (Day 0, Day 3, Day 7, Day 14, and Day 21) and the percentage of wound contraction was calculated using the following formula:

$$\text{Wound Contraction (\%)} = \frac{\{\text{Initial Wound Area} - \text{Final Wound Area}\}}{\{\text{Initial Wound Area}\}} \times 100$$

A higher percentage of wound contraction indicates faster and more efficient wound healing. This parameter helps evaluate the therapeutic potential of the test extract in promoting tissue repair. The results of the excision wound model demonstrated that the ethanolic extract of *Achyranthes asperastem* significantly enhanced the wound healing process when compared with the untreated control group. The control group (Group I) showed the slowest wound healing response throughout the 21- day observation period, with only partial wound contraction observed by the end of the study. This group represented the natural wound healing process without pharmacological treatment. The standard drug group (Group II) treated with Nitrofurazone ointment exhibited rapid wound healing with almost complete wound closure by Day 14, confirming the effectiveness of the standard treatment. The groups treated with ethanolic extract of *Achyranthes asperastem* demonstrated a dose-dependent improvement in wound healing activity. The group treated with 200 mg/kg showed moderate wound contraction and progressive healing during the study period. In contrast, the group treated with the higher dose (400 mg/kg) exhibited a faster rate of wound contraction and significantly improved wound closure, approaching the healing effect observed in the standard treatment group. The enhanced wound healing activity of the extract may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, tannins, and saponins, which are known to possess antioxidant, anti-inflammatory, and antimicrobial properties. These compounds likely contribute to increased collagen synthesis, fibroblast proliferation, and improved tissue regeneration at the wound site.

Table 16: Effect of Ethanolic Extract of *Achyranthes aspera* Stem on Wound Contraction (%) in Excision Wound Model.

Day	Group I (Control)	Group II (Standard Drug)	Group III (200 mg/kg)	Group IV (400 mg/kg)
0	0%	0%	0%	0%
3	8%	28%	18%	32%
7	18%	55%	40%	65%
14	32%	88%	68%	92%
21	52%	100%	86%	100%

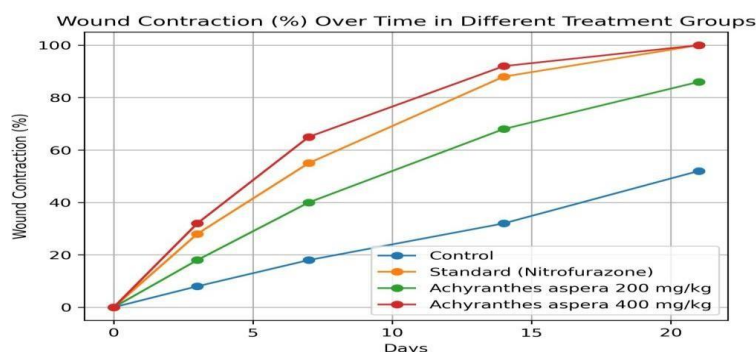


Fig. 9: Wound Contraction (%) Over Time.

Epithelialization Period

The epithelialization period represents the number of days required for complete closure of the wound and formation of new epithelial tissue over the wound surface. It is an important parameter used to evaluate the efficiency of wound healing treatments. In the present study, the epithelialization period was evaluated in Wistar albino rats using an excision wound model to determine the wound healing potential of the ethanolic extract of *Achyranthes asperastem*. The control group exhibited the longest epithelialization period, requiring 21 days for complete wound closure. This delayed healing reflects the normal physiological wound repair process in the absence of any therapeutic intervention. The standard drug group treated with Nitrofurazone ointment showed significantly accelerated wound healing, with complete epithelialization occurring within 14 days. This confirms the effectiveness of the standard drug in promoting wound repair. Animals treated with the ethanolic extract of *Achyranthes asperastem* demonstrated improved wound healing compared with the control group. The group receiving the lower dose (200 mg/kg) achieved complete epithelialization within 18 days, indicating moderate improvement in the wound healing process. The higher dose group (400 mg/kg) showed a more pronounced healing effect, with complete epithelialization occurring within 14 days, which was comparable to the standard drug treatment. The enhanced wound healing activity of *Achyranthes asperastem* extract may be

attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, saponins, and phenolic compounds.

Table 17: Epithelialization Period (Days).

Group	Treatment	Epithelialization Period (Days)
Group I	Control (Vehicle)	21
Group II	Standard Drug (Nitrofurazone)	14
Group III	<i>Achyranthes aspera</i> Stem Extract (200 mg/kg)	18
Group IV	<i>Achyranthes aspera</i> Stem Extract (400 mg/kg)	14

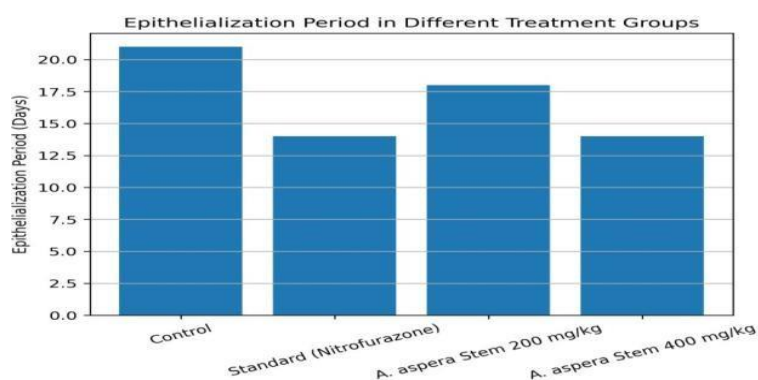


Fig. 9: epithelialization period in different treatment groups.



Fig. 10: Experimental wound area before and after treatment on day 21.



Fig. 11: Control wound area before and after day 21.

CONCLUSION AND SUMMARY

The present study systematically evaluated the pharmacognostic, phytochemical, antioxidant, and wound healing potential of *Achyranthes aspera* stem extract. The plant material was authenticated and standardized through morphological, microscopic, and physicochemical parameters, confirming its purity and quality. Phytochemical screening and TLC analysis revealed the presence of important bioactive compounds such as flavonoids, phenolics, tannins, and alkaloids, with the ethanolic extract showing the richest composition. High total phenolic and flavonoid content further supported its strong therapeutic potential. The in-vitro antioxidant studies demonstrated significant free radical scavenging activity of the ethanolic extract. In the DPPH assay, the extract showed a lower IC₅₀ value than the standard quercetin, indicating superior antioxidant activity, while the FRAP assay confirmed its good reducing power. The acute toxicity study established the safety of the extract, with no mortality observed up to 2000 mg/kg, suggesting a wide safety margin for pharmacological use.

In the in-vivo wound healing study, the ethanolic extract exhibited a dose-dependent enhancement in wound contraction and epithelialization. The higher dose (400 mg/kg) showed healing effects comparable to the standard drug, indicating its strong therapeutic efficacy. Overall, the findings suggest that *Achyranthes aspera* stem extract possesses significant antioxidant and wound healing properties, supporting its traditional use and highlighting its potential as a natural agent for developing effective herbal formula.

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