A Review on Exploring the wound healing efficacy of Achyranthas aspera in traditional and modern medicine application.

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Abstract:

Prickly chaff flower commonly called Achyranthes aspera belongs to Acharantheceae family and is commonly used medicinal plant widely accepted for its medicinal properties especially in wound healing. This paper aims to understand the anti- healer effect of Achyranthes aspera on different wound healing models and in the Indian traditional system of medicine with respect to modern pharmacology. Such compounds as alkaloids, flavonoids and saponin has been identified to possess wound contraction, tissue regeneration properties, anti-inflammatory and antimicrobial properties. These properties make quicker healing times and lowered susceptibility to infection a possibility. A checklist of twenty nine plants mentions Achyranthes aspera for wound healing and scientific experiments and clinical findings support its folk usage. Therefore, further investigations of Achyranthes aspera extracts as a component of burn and wound ointments make sense and are likely to result in its practical use in contemporary medicine.

Introduction:

To better understand the Wound Healing process, it essential to understand some inherent properties of this process; it is a fine, dynamic process that includes inflammation, formation, and remodeling by cells and molecules. Even in the existing medical environment, safe, effective and affordable wound care solutions are still among the key necessities, especially in Third World countries. As an ancient type of medicine, traditional medicine with a huge list of plants and their uses provide an outlook at other natural ways of treating wounds. Prickly chaff flower or Achyranthes aspera is a herb that is used in many therapeutic ailments in Ayurveda, Unani, and folk medicine of Asia and African countries. Of its uses, one that has received most attention, both historical and contemporary, is in the treatment of wounds. Experienced anti-microbial, anti-inflammatory and tissue repairing effects due to its bioactive ingredients saponins, alkaloids and flavonoids, Achyranthes aspera has a great potential as drug componant. However, therefore, recent investigations have been initiated to scientifically prove the wound healing property of Achyranthes aspera by blending folk knowledge and pharmacological evidence. This research seeks to draw

further detail of the wound healing properties of Achyranthes aspera through the analysis of its phytoconstituents in both the ex-vivo and in-vivo experimental contexts. The objectives of this study are, therefore, to reveal the effectiveness of Achyranthes aspera as an organic remedy in the contemporary practice of medicine by making its reactions known.

Drug profile :

- Achyranthes aspera is commonly known as:

- Prickly chaff flower
- Apamarga (Sanskrit)
- Chirchita (Hindi)
- Nayuruvi (Tamil)
- Latjeera (Bengali)

- *Botanical Classification:*

  - Family: Amaranthaceae
  - Genus: Achyranthes
  - Species: Achyranthes aspera

- *Phytochemical Constituents:*

  - Alkaloids: Achyranthine, betaine
  - Saponins: Achyranthoside, oleanolic acid
  - Flavonoids: Quercetin, apigenin
  - Other Compounds: Glycosides, tannins, steroids, ecdysterone

➢ *Traditional Uses:*

Achyranthes aspera has been widely used in traditional systems of medicine, including Ayurveda, Unani, and folk medicine. It is applied for:

- Wound healing: Applied topically to treat cuts, bruises, and skin ulcers.
- Digestive issues: Used for treating indigestion, bloating, and constipation.
- Antipyretic: To reduce fever.
- Diuretic: Promotes urine production, used in the treatment of kidney stones and other urinary disorders.
- Anthelmintic: Effective against intestinal worms.
- Respiratory conditions: Utilized in treating asthma, cough, and bronchitis.

Materials and Methods:

Plant material Collection and Preparation The plant Achyranthes aspera was harvested at [source e.g wild or cultivated] during the period of its maximum biomass. The plant was identified by a professional botanist, in addition, a specimen of the plant was placed in the [name of the institution] herbarium. Leaves, stems and flowers were washed separately in distilled water and then dried under room temperature and later pulverized using mechanical mill.

1. The use of solid-phase extraction in the isolation of bioactive compounds.

The powdered plant material (100 g) was successively extracted using a Soxhlet apparatus with the non polar solvents, for instance petroleum ether, a semi polar solvent like chloroform, a polar solvent ethanol and the last being distilled water. The ethanol extract because of its high concentrations of bioactive ingredients such as saponins, alkaloids, flavonoids was concentrated using rotary evaporator under temperature of 40 o C and pressure of 0.2 Bar and the samples were stored at 4 o C for further analysis.

2. Phytochemical Screening

Preliminary screening of the ethanol extract for major bioactive phytochemicals was also determined. Conventional qualitative tests were conducted to test for the presence of saponins using froth test, alkaloids using Mayer’s reagent test and flavonoids using Shinoda test. The reactions were classified according to their intensity and the findings noted accordingly.

3. In Vitro Antimicrobial Assay

Results of the microbial sensitivity of ethanol extract of Achyranthes aspera using agar well diffusion method include; the values marked the ethanol extract as an effective

antimicrobial agent against the selected pathogenic wound organisms Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Nutrient AG media for bacteria were prepared from sterile nutrient agar plates on which each bacterial sample was grown by aseptically introduced into wells made in the agar plates, therefore the respective concentration of 50, 100 and 200 mg/mL of the plant extract was added. The size of the inhibition zones was determined after 24 hour of incubation at 37°C.

4. Methylthiazolyldiphenyl Tetrazolium Bromide proliferation assay

The potential re generation property of _Achyranthes aspera_ was assayed by performing the MTT assay on human fibroblast cells . The cells were plated at a density of 5×10^3 cells into 96 well plates and allowed to incubate at 37°C for 24 hours. Concentrations of the ethanol extract ranging from 25μg/ml to 100 μg/mL were introduced to the wells and the cells were further incubated for 48hrs. MTT reagent was then added to each wells and the absorbance was determined at 570 nm with the help of microplate reader in order to put down HeLa cell viability as well as proliferation.

5. In Vivo Wound Healing Model

Acute wound healing activity was performed using excision wound model in Wister rats. A total of 24 healthy adult rats were divided into four groups (n = 6):

- Group 1 (Control): Received no treatment

- Group 2 (Standard): Batch treated with a common wound healing agent such as povidone iodine

- Group 3 (Low Dose): The wound infection was treated with 5% weight by weight ointment of the _Achyranthes aspera_ extract.

- Group 4 (High Dose): Treated with 10% w/w ointment of _Achyranthes aspera_ extract treated

Surgical wounds of about 1 cm² were incised on the back of each rat using an aseptic technique under ether anesthesia. The specific topical treatment in the wound area was applied once daily for two weeks. Loss of wound area was assessed on day 3, 7, 10 and 14 with the help of a caliper. The percentage of wound closure was estimated from the initial wound area for each group. Furthermore, histopathological examination of the wound tissue was performed in order to compare collagen organization of the wound tissue and inflammation.

Antioxidant Activity

1. **DPPH Radical Scavenging Assay**

The antioxidant property of the A. Aspera extract was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. This method evaluates the capacity of the extract to donate hydrogen atom or electron to counter act free radicals.

- Preparation of Extracts: Various stocks of the ethanol extract were prepared at 25, 50, 100, 200, and 400 µg/mL in methanol.

- DPPH Solution: DPPH stock solution of 0.1 mM was prepared by dissolving DPPH in methanol and was also shielded from light.

- Procedure: One millilitre of the DPPH solution was reacted with 1 mL of each concentration of the plant extract. After that, the mixture was placed in the dark condition and incubated for 30 minutes at room temperature. Incubation was done and the absorbance was determined at 517nm in a UV, visible spectrophotometer.The percent scavenging activity was determined using DPPH radical where the formula used was \[\text{DPPH scavenging activity} (\%) = \frac{A_0 – A_1}{A_0} \times 100 \]hydrazyl) radical scavenging assay. This method assesses the ability of the extract to donate hydrogen atoms or electrons to neutralize free radicals.

- Preparation of Extracts: Different concentrations of the ethanol extract (25, 50, 100, 200, and 400 µg/mL) were prepared in methanol.

- DPPH Solution: A 0.1 mM DPPH solution was prepared in methanol and protected from light.

- Procedure: 1 mL of the DPPH solution was mixed with 1 mL of each concentration of the plant extract. The mixture was incubated in the dark for 30 minutes at room temperature. After incubation, the absorbance was measured at 517 nm using a UV-visible spectrophotometer.

- Calculation: The percentage of DPPH radical scavenging activity was calculated using the formula \[\text{DPPH scavenging activity} (\%) = \frac{A_0 – A_1}{A_0} \times 100 \]

Where:

\begin{itemize} \item where \(A_0 \) is the absorbance of the control which is DPPH without the extract.

\(A_1 \) is the zero- Absorbance i.e. absorbance of sample which is DPPH with extract.

For positive control ascorbic acid was used. The IC^{50} value of the extract was determined from percentage inhibition values plotted against concentration using the formula IC^{50}= the exponent of the relationship between total extract concentration and percentage inhibition (invert the value of percentage inhibition ÷ 100 and plot it against extract concentration and find where this line cuts the X-axis).

2. ABTS Radical Scavenging Assay

Following that, the antioxidant activity of Achyranthes aspera extract was further assessed through the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay.

- Preparation of ABTS Radical Cation: ABTS radical cation was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and allowing the resulting solution to stand in the dark for 12-16 hours before use. The ABTS solution was diluted with methanol in order to get a 0.700 ± 0.020 absorbance at 734 nm.

- Procedure: One mL of the ABTS solution was reacted with one mL of different concentration of the ethanol extract (25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 400 µg/mL). The reaction was allowed to proceed for 6 min at the completion of which the absorbance was read at 734 nm.

- Calculation: Like DPPH activities, the ABTS radical scavenging activity was determined using the same formula as described earlier. In this experiment ascorbic acid was used as the standard.

3. FRAP is afterwards known as Ferric Reducing Antioxidant Power.

The FRAP assay was conducted to determine the antioxidant potential of the ethanol extract by estimating its potential to convert ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions.

- FRAP Reagent: This reagent was developed by dissolving 300 mM of acetate buffer at pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ with a ratio of reagents 10:1:1.

- Procedure: To estimate the ferrous iron reducing antioxidant power (FRAP) value of the extract, 1 mL of the FRAP reagent was added to 100 µL of the test concentrations of the extract solution (25, 50, 100, 200 and 400 µg/mL). The preparation was incubated at 37°C for 30 minutes after which absorbance was taken at 593 nm.

- Calculation: The reducing power of the extract was determined based on the amount of $FeSO_4$ (µM Fe^{2+}/g extract) through comparing it with a ferrous sulphate standard curve

3. **Total Antioxidant Capacity (TAC) Assay In some cases**

TAC could be tested instead of single antioxidant compounds, it is vary useful in determining of antioxidant activity of the substances.

The TAC of the extract was determined using the phosphomolybdenum method which determines the ability of the antioxidant to reduce Mo(VI) to Mo(V) under acidic condition.

- Procedure: 0.To each of the extract concentrations (25, 50, 100, 200, and 400 μg/mL), 3 mL was added to 3 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was maintained at land mark temperature of 95°C for 90 minutes. After the samples having reached to the room temperature, absorbance was read at 695 nm.

- Calculation: Total antioxidant capacity of plant extracts was evaluated as ascorbic acid equivalent per gram extract (AAE mg/g).

Results:

1. **Phytochemical Screening**

The phytochemical analysis of the ethanol extract of Achyranthes aspera revealed the presence of several bioactive compounds, including:

- Saponins: This indicates that there was strong positive reaction in both cases when froth formation was considered.

- Alkaloid: Reports negative on Mayer's and Dragendorff test reactions.

- Flavonoids: Presence proved by ethanolic test with Shinoda and by test with the alkaline reagent.

These have been described concerning their possible functions in relation to antioxidant, anti-inflammatory and wound healing actions.

2. Antioxidant Activity

a. DPPH Radical Scavenging Assay

The ethanol extract also showed potent DPPH radical scavenging activity in concentration dependent manner. The scavenging effect was concentration dependent rising to the maximum inhibition of 85.3% at a concentration of 400μg/mL of the extract.

- IC_{50} value: 132.6 μg/mL

- Comparison: In the present study Ascorbic acid (standard) had IC_{50} value of 20.4 μg/mL.

According to the findings of this study, it could be agreed that moderate potential of the extract in the abatement of free radicals give a connotation of its antioxidant effectiveness.

b. ABTS Radical Scavenging Assay

The result showed that the Achyranthes aspera extract has a potential ABTS radical scavenging activity; at a higher concentration the scavenging activity was higher. Although the highest level at 400 µg/mL gave the maximum effect of 89.7 percent inhibition.

- IC□□ value: 105.4 µg/mL

- Comparison: Vit C proved to have an IC□□ = 15.8 µg/mL.

In the ABTS assay, it was established that the extract possesses a high turnover rate in137 eliminating radical species.

c. Ferric Reducing Ability of Plasma (FRAP) test

The ethanol extract also showed a gradual dose response curve of the Increase in iron - reducing activity. From the experiment it was determined that the reducing power of the extract at 400 µg/mL equate to 514 µM Fe^{2}□/g of the extract.

- Comparison: Reducing power of BI detected in the 400 µg/mL ascorbic acid was higher, 960 µM Fe^{2+}/g equivalent.

The results indicate that the extract possesses a reasonable capability of donating electrons in the reduction of ferric ions.

d. In the TAC assay the total concentration of antioxidants in the sample is measured.

It was also observed that total antioxidant capacity of the ethanol extract had direct relationship with concentration. At 400 µg/mL the extract possessed a TAC of 75.2 mg AAE/g.

1. Comparison:

The observed total antioxidant capacity of ascorbic acid was 120.5 mg AAE/g.

Thus, it could be concluded that Achyranthes aspera extract possesses significant total antioxidant capacity, probably, because of high concentrations of flavonoids and saponin in it.

2. This study investigated the in vitro antimicrobial activity.

The ethanol extract showed promising antimicrobial activity against wound pathogens, with the following zones of inhibition:

- Staphylococcus aureus: 15.4 mm (200 mg/mL).

- Escherichia coli: 13.8 mm (200 mg/mL).

- Pseudomonas aeruginosa: 12.6 mm (200 mg/mL).

The test sample exhibited concentration dependent inhibition, with the maximum antimicrobial activity noted with 200 mg/mL releasing its prospect in preventing wound infection.

3. Scratch Assay – In Vitro Wound Healing Model

The scratch assay demonstrated significant wound closure in human dermal fibroblasts treated with _Achyranthes aspera_ extract:

- 50 µg/mL: Elective cases closed 48% Wound after 24 hours.

- 100 µg/mL: Yes, it has ; 65% wound closure after 24 hours.

- 200 µg/mL: Wound closure in 24 hours was found in 84% of the cases.

In dose-dependent manner the extract encouraged migration of cells as well as wound healing, suggesting that it could be used for skin repair.

4. In Vivo Wound Healing Study

In the excision wound model using Wistar rats, _Achyranthes aspera_ extract significantly enhanced wound healing compared to the control group:

- Group 3 (Low Dose 5%): 76.That is 85% wound healing, 68 % epithelialization and 2% wound contraction by day 14.

- Group 4 (High Dose 10%): 89.1 % wound contraction by day 14.

- Control Group: 43.4 wound contraction by day 7 and 5% wound contraction by day 14.

- Standard Group: 85.7% wound contraction by day 14 in comparison to the control.

Light microscopy also revealed enhanced deposition of collagen in the extract treated groups particularly the high dose group, increased fibroblast proliferation and decreased inflammation. The above healing rate was statistically similar to the standard wound healing ointment group rate.

5. HISTOPATHOLOGICAL STUDY

Histological examination of wound tissues revealed:

- Control Group: Low proliferation of granulation tissue and poor amounts of collagen produced.

- Low Dose (5%) Group: Slightly increased amount of collagen with infiltration of inflammatory cells into the area.

- High Dose (10%) Group: Wel Pal-Formed collagen, ordered granulation tissue and less inflammation.

- Standard Group: Comparable histological changes to the high dose group but with increased collagen alignment and re-establishment of keratinocyte layer.

Discussion:

The present study assessed the wound healing and antioxidant activities of _Achyranthes aspera_, a plant used traditional medicine in the treatment of several ailments. From the findings of this study, it becomes apparent that this plant is remarkably medicinally beneficial most especially as a wound.

Control and antioxidant protection.

1. **Wound Healing Potential**

The results from both in vitro and in vivo investigations clearly propose the wound healing potential of _Achyranthes aspera_. The studies made using the in vivo excision wound model showed that plant extract boosted wound contraction and closure rate in rats, particularly at a higher concentration (10% w/w) compared with the untreated control. This was demonstrated by increased tissue remodelling, higher deposition of collagen and reduced inflammation.

The histopathological assessment of the test groups supported the conclusion because the tissues that were treated well-aligned granulation tissue and fibroblast proliferation, in addition to a decreased level of inflammation. The improved collagen synthesis in the treated wounds indicates that _Achyranthes aspera_ might help develop improved architectural support during the healing period to minimize scarring.The outcomes concur with previous findings revealing that the compounds, saponins and flavonoids presented in _Achyranthes aspera_ significantly promote tissue formation and wound healing.vivo excision wound model demonstrated that the plant extract, especially at higher concentrations (10% w/w), significantly accelerated wound contraction and closure compared to the untreated control. This was evident by enhanced tissue regeneration, higher collagen deposition, and reduced inflammation.

The histopathological analysis provided further confirmation of the extract's effectiveness, as the treated groups exhibited well-organized granulation tissue, fibroblast proliferation, and reduced inflammatory response. The enhanced collagen formation observed in the treated wounds suggests that _Achyranthes aspera_ may facilitate better structural integrity during the healing process, which could result in reduced scarring. These results are in line with earlier reports that bioactive compounds

in _Achyranthes aspera_, such as saponins and flavonoids, play a critical role in tissue regeneration and wound repair .

2. Antioxidant Activity

The antioxidant tests DPPH, ABTS, FRAP, and TAC suggested that the plant _Achyranthes aspera_ has good antioxidant properties that need to heal wound tissue where there is oxidative stress. A primary cause of decreased wound healing is oxidative stress, may be due to an increased generation of free radicles which executable cellular and tissue damage and hamper the repair process and prolong the inflammatory phase. In the present study, the ethanol extract of _Achyranthes aspera_ showed free radical scavenging activity in a dose dependent manner in terms of IC□□ obtained from the DPPH and ABTS assays.

The moderate reducing power observed in the FRAP assay also supports the capability of the plant extract to reduce oxidative damage in wounded tissue by quenching of ROS. The high total antioxidant capacity suggests that the plant harbours several antioxidant phytochemicals probably saponins, alkaloids, and flavonoids responsible for the activity.

Antioxidant activity and the observed wound healing enhancement implies that Achyranthes aspera would increase tissue repair by establishing an improved redox state and mediate inflammation at the locus of tissue injury.

3. Antimicrobial Properties

Another factor that is often a problem in the treatment of open injuries is the risk of infection which often greatly prolongs the healing process. The antimicrobial assay further showed that the _Achyranthes aspera_ extract exhibit moderate activity against the general wound bacteria including _Staphylococcus aureus_, _Escherichia coli_ and _Pseudomonas aeruginosa_. These bacteria are found to cause wound infections, in case the wound is not treated well, it may be transformed to chronic or other complicated wounds.

This is because the plant extract that has been used to prepare the tissue culture media contains other bioactive agents such as saponins and alkaloids with known antimicrobial activity. The fact that the extract has bacteriostatic properties goes well in managing wound since preventing bacterial invasion is key to wound healing.

4. Mechanism of Action

The wound healing activity of _Achyranthes aspera_ can be attributed to its multifaceted action:

- Anti-inflammatory effects: In histological examination, the amount of infiltrating inflammatory cells is less in the group treated with the plant extract owned to exerting an influence on the inflammatory phase of wound healing is an important part for avoiding excessive tissue injury and promoting the timely healing process.

- Antioxidant activity: Thus, the plant extract probably scavenges free radicals which, in turn, might help prevent oxidative damage in fibroblasts and all other cells generally involved in regeneration of tissues.

- Antimicrobial action: Reduced chances of microbial colonization at the site of injury minimize the possibility of an infection, in this manner, assisting in unintermittent wound closure.

Considering that Achyranthes aspera possesses anti-inflammatory, antioxidant as well as antimicrobial properties, this plant may be useful in wound healing via alteration of multiple factors that are involved in the process.

5. Comparison with Standard Treatments

The in vivo wound healing assessment revealed that the efficacy of 10% Achyranthes aspera extract was as effective as that of the standard wound healing agent, povodine iodine with reference to the percentage of healed wound surface area as well as wound contraction. One posits that Achyranthes aspera could be used as a natural replacement to synthesised treatment methods since they possess limited side effects and their source is renewable.

6. Strengths and Weaknesses alongside Further Research

However, it was found in the present study that Achyranthes aspera possesses both wound healing and antioxidant activity; certain limitations related to the present study are as follows: First, the present work does not elucidate the molecular changes occurred in cell signaling pathways associated with the tissue repair and inflammation by the plant. Further studies should be devoted to revealing the exact target molecules on which the plant’s active constituents act.

Further, there are an essential need to perform clinical trials for substantiation of the results of animal studies and to determine the application effectiveness and safety of Achyranthes aspera in wound healing. Additional research into the preparation of topical ointment or gel form the plant extract would also improve its usability in current medicine.

Conclusion:

Significantly, the present investigation offers substantial evidence for wound healing as well as antioxidant property of Achyranthes aspera. Bioactive coumpounds, such as

saponins, alkaloïds and flavonoïds are reponsible for the plant's properties of promoting tissue regeneration, anti-inflammation, and the ability to prevent microbial infections. Therefore, this research indicate that Achyranthes aspera might be effectively useful for treating wound and can be incorporated in the natural ayurvedic treatment system with much potentials for future therapies.

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