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Evaluation of Wound Healing Activity of Argyreia cuneata Leaf Extract in Wistar Albino Rats

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ABSTRACT:
Argyreia cuneata (Convolvulaceae) plant is an example of a hallucinogenic plant. It has antiseptic, anti-inflammatory, antispasmodic, antibacterial, antiviral, antifungal, anticonvulsant, nootropic, antifertility and aphrodisiac properties. The present work aims to evaluate the wound healing properties in normal and diabetic animals by topical administration of ethanolic leaf extract. Phytochemical investigations revealed the presence of various biochemicals (alkaloids, flavonoids, carbohydrates, triterpenoids, proteins, saponins, steroids, and tannins). A single injection of streptozocin prepared in citrate buffer (0.1 M, pH 4.5) induces diabetes in rats after overnight fasting. To study the wound contraction and epithelization rate, we made incision wounds, excision wounds and dead space wound mechanisms (300 mm2 and 2 mm depth). The means of wound area measurement between groups at different time intervals were compared using a one-way analysis of variance (ANOVA), followed by Dunnet's test. Extracts of A. cuneata showed significant wound healing effects in average (topically treated) and diabetic (topically treated rats). These effects included faster wound closure, reduced inflammation, and improved tissue regeneration. In diabetic rats, the topically treated group with a concentration of 2.5% showed a more significant effect than 0.5% of the ethanolic extract of A. cuneata. The present study demonstrates that A. cuneata leaves extract applied topically promotes the healing of wounds more significantly and can be a promising alternative to synthetic medications.

1. Introduction

Argyreia cuneata (Convolvulaceae), commonly known as the purple morning glory, is a plant whose leaf extract has garnered attention for its potential therapeutic benefits, particularly in wound healing and diabetes management. This plant is often used in traditional medicine, and recent research supports its promising applications in these areas.[1-5] The use of Argyreia cuneata leaf extract in wound healing is rooted in its various bioactive compounds that contribute to tissue repair and regeneration. The leaf extract contains compounds with significant anti-inflammatory effects. [7-9] Reducing inflammation is crucial in the early stages of wound healing, as it minimizes tissue damage and prepares the wound bed for repair. Argyreia cuneata leaf extract is rich in antioxidants, which help neutralize free radicals and oxidative stress at the wound site. Oxidative stress can hinder the healing process, so antioxidants play a vital role in promoting quicker recovery and reducing scarring. The extract has demonstrated antimicrobial activity against a range of pathogens, including bacteria and fungi. [10-14]

This helps prevent infections, which is a common complication in wound healing and can delay recovery. Some studies suggest that the extract may enhance collagen production, a critical component of the extracellular matrix that supports new tissue formation and wound strength. Research indicates that the leaf extract may help lower blood glucose levels. [4,14,15]It appears to influence glucose metabolism, possibly by enhancing insulin sensitivity or promoting the secretion of insulin from pancreatic cells. In diabetes, oxidative stress contributes to complications such as diabetic neuropathy and retinopathy. [16-19] The antioxidant properties of leaf extract can mitigate these effects,

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helping to protect against such complications. A. cuneata leaf extract shows promise in both wound healing and diabetes management, largely due to its anti-inflammatory, antioxidant, and antimicrobial properties. In present study for easy application on wound a hydrogel was prepared and their feasibility was checked by wound healing activity in albino rats as compared to Silverex Heal Gel.

2. Materials and Methods

Collection and identification: The plant leaves of A. cuneata were collected locally and identified in the Botanical Survey of India, Zone Circle, Delhi.
M and HPMC was procured from CDH, New Delhi. Carbopol 934 and PEG was purchased from Agile Chemie, Gujarat. Triethanolamine and Distilled water were procured from CDH, New Delhi. All the chemicals used in this project were of optimum and standard quality.
Selection of animals: Male Wistar rats, weighing 180–250g, were used in the study after obtaining the approval of the Institute's Animal Ethics Committee (Approval code no**. IAEC/ONIO/OPRTCM/M.Pharm/2023-24/007**). Animals were fed on a standard pellet diet and water ad libitum and maintained at 24–28°C temperature and relative humidity (30% - 70%). Animals marked as fasted were deprived of food for 16 hours, but had free access to water. [17-20]

Preparation of the extract: The freshly collected leaves were shade-dried and pulverized using a mechanical grinder. The powdered leaves were macerated with 90% ethanol for 3 days, with occasional shaking. The extract was subjected to preliminary phytochemical tests and percentage yield was calculated in the extract after drying.
Phytochemical screening: Phytochemical screening was carried out to identify the presence of alkaloids, carbohydrate, glycoside, flavonoids, triterpenoids, protein, saponins, steroids, tannins, etc. in the ethanolic extract of A. cuneata

Preparation of Hydrogel
The formulation of a gel incorporating Carbopol 934, HPMC, AND Ethanolic extract in low (0.5% EELAC) and high concentration (2.5%) were done. [14,17-19]

Chemical Induction of Diabetes
In this study involving rats, the experimental groups were treated as follows:

- **Group I**: Control group, administered with 0.1 M citrate buffer.
- **Groups II-IV**: Experimental groups, induced with diabetes using a single intraperitoneal dose of 50 mg/kg of streptozotocin (STZ), freshly prepared in 0.1 M citrate buffer with a pH of 4.5.

Wound Creation [19]**:**
Incision wound healing model[20-24]
This in-vivo model using Wistar rats were divided into 4 groups, (n= 6) rats, and treated as follows:
Selection of dose
Reviewing existing studies is critical step in selection of dose. Initially maximum tolerated dose is selected and acute toxicity studies were done. This encouraged us to choose an optimum dose of 2.5 % of leaf extract of A. cuneata in managing wound healing activity for Wistar rats (**Table No 1**).

Table no. 1 Details of animals for incision wound model

Sr.no	Type	Rats
1.	Group A	Control
2.	Group B	Standard
3.	Group C	Treated ȣ
4.	Group D	Treated ȸ

ȣ =0.5% A cuneata Leaves Ethanolic extract hydrogel formulation
ȸ= 2.5% A cuneata Leaves Ethanolic extract hydrogel formulation
The experimental procedure involved the following steps:

1. **Anesthesia**: Rats were anesthetized with diethyl ether before and throughout the experiment.
2. **Incision Wound Creation**: The dorsal fur of the rats was shaved, and two parallel incisions of 6 cm length were made in the paravertebral

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region on both sides of the vertebral column, cutting through the entire of the skin.

3. **Wound Closure**: After creating the incisions, the wounds were sutured using surgical thread and a curved needle, with sutures placed 1 cm apart.
4. **Treatment Application**: Each group received their respective treatments as specified (Petroleum Jelly for Control, Silverex Heal Gel for Standard, 0.5% Treated with 2.5% Ethanolic extract of Leaves of A.cuneata hydrogel)
5. **Suture Removal and Assessment**: Sutures were removed on day 8 post-wounding. Treatment application continued thereafter.
6. **Wound Breaking Strength (WBS) Measurement**: On the 10th post-wounding day, the rats were anesthetized and fixed on a table top. The wound breaking strength (WBS) was measured using the following method:

- Two cut-lines were made on both sides of the incision, approximately 3 mm away from the wound edge.
- These lines were secured with Allis forceps, one fixed and one attached to a measuring jar.
- Water was poured onto the wound until it just began to open (gaping). The amount of water poured before gaping occurred was measured as WBS in grams (g).
- Three readings were taken for each incision wound, and the mean value of these readings was recorded as the WBS for each group.

This method allowed for the evaluation of wound healing efficacy among the different treatment groups based on their ability to withstand external mechanical stress, as indicated by the WBS measurements on the 10th post-wounding day.

Excision Wound Model [20-24]

The experimental setup involved grouping rats into different categories, with 6 animals in each group, and treating them as follows (**Table 2**):

Table No. 2 Details of Animal Groups For Excision Wound Model

Sr.no	Type	Rats
1	Group A	Control
2	Group B	Standard
3	Group C	Treated ɛ
4	Group D	Treated ɸ

ɛ =0.5% A cuneata Leaves Ethanolic extract hydrogel formulation

ɸ= 2.5% A cuneata Leaves Ethanolic extract hydrogel formulation

The procedural steps were as follows:

1. **Anesthesia**: Rats were anesthetized using diethyl ether before and throughout the experiment.
2. **Wound Induction**: The dorsal fur of the rats was shaved, and a circular area of approximately 500 mm², marked with methylene blue, was excised to create a full-thickness skin wound.
3. **Measurement of Wound Area**: On the day of wounding (day 0), the initial wound area was outlined and measured using graph paper. Subsequent measurements were taken every 4 days until day 12, and then alternately until complete wound closure (epithelialization). Epithelialization was defined as the complete removal of the eschar without leaving any open wound.
4. **Calculation of % Wound Closure**: The percentage of wound closure was calculated using the following formula:

Percentage Wound closure =(IA−FA)/IA×100

Where **IA=** initial area of wound **FA=** Final area of wound

This formula allowed for quantification of the healing progress over time, comparing the initial and final areas of the wound. This experimental design enabled the evaluation of wound healing efficacy among the different treatment groups (Control, Standard, and Test groups), based on the rate and extent of wound closure measured over the observation period. **(Das S et al., 2013)**

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Dead Space Wound Screening Model [25-29]

The experimental procedure (**Table no. 3**) involved grouping rats into different categories, with 6 animals in each category, and treating them as follows:

Table no. 3 Details of animals for dead wound model

Sr.no	Type	Rats
1	Group A	Control
2	Group B	Standard
3	Group C	Treated ɛ
4	Group D	Treated ϕ

ɛ =0.5% Ethanolic extract of Leaves of A cuneata hydrogel

ϕ= 2.5% Ethanolic extract of Leaves of A cuneata hydrogel

The procedure included the following steps:

1. **Anesthesia**: Rats were anesthetized using diethyl ether.
2. **Wound Induction**: A 1 cm long incision was made in the dorso-lumbar area of the rats' bodies. Two 5 mg cotton pellets were placed on each side of the incision. The wound was then sutured.
3. **Post-wounding Day 10**: On the tenth day after the wound was induced, the rats were sacrificed.
4. **Tissue Collection and Measurement**: Granulation tissue formed around the pellets was carefully dissected. The wet weight of the granulation tissue was recorded.
5. **Drying of Tissue**: Approximately 250 mg of wet granulation tissue from each rat was dried for 24 hours at 50°C to obtain the dry weight of the tissue (40 mg per sample).
6. **Hydroxyproline Measurement**: The dried tissue (40 mg) was hydrolyzed by adding 1 mL of 6N HCl and heated in a boiling water bath for 24 hours at 110°C (12 hours daily for two days). The hydrolysate was then neutralized with 10N NaOH using phenolphthalein as an indicator. This neutralized hydrolysate was diluted to 20 mg/mL.
7. **Colorimetric Analysis**: To measure hydroxyproline (a collagen tissue parameter), 1 mL of the neutral hydrolysate was added to tubes containing 1 mL each of 2.5N NaOH, 6% H_2O_2, and 0.1M $CuSO_4$. The tubes were incubated at 80°C for 5 minutes, then cooled in an ice bath. To the cooled tubes, 4 mL of 3N H_2SO_4 and 2 mL of 5% p-dimethyl-amino-benz-aldehyde were added. The tubes were heated for 15 minutes at 70°C in a water bath and cooled again in an ice bath. The intensity of the resulting color was measured spectrophotometrically at 540 nm, comparing against a blank.

This detailed experimental protocol ensured precise measureme4nt of wound healing parameters such as granulation tissue weight, dry tissue weight, and hydroxyproline content in the treated groups compared to the control, providing robust data for evaluating the efficacy of the treatments derived from Argyreia cuneata hydrogel. **(Liu et al., 2023)**

5.8 Statistical Evaluation

The results were recorded in the form of mean ± standard error of the mean (SEM). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. This approach allowed for rigorous evaluation of the observations, ensuring robust statistical inference regarding the differences among the experimental groups compared to the control.[30-31]

3. Results

Incision Wound Model

On the 10th post-wounding day (PWD), (**Table No. 4** and **Figure No. 1)** every experimental group showed superior wound breaking strength (WBS) and a more favorable diabetic wound healing pattern compared to the control group. [5,13,19] These differences were statistically significant, highlighting the effectiveness of the treatments in enhancing wound healing in diabetic conditions.

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Table No. 4 Results for A cuneata wound healing activity for incision wound model

Sr.no	Treatment	Wound healing strength (g) (mean ± SEM)
1	A*	189.2 ± 3.2
2	B*	484.5 ± 2.6
3	C*	424.3 ± 6.5
4	D*	401.1 ± 3.5

***A= Standard, B = Control, C = 0.5% EELAC and D= 2.5 % EELAC**

In comparison to control animals, which exhibited a wound breaking strength (WBS) of 189.2 ± 3.2g on the 10th post-wound day (PWD), animals treated with a standard gel showed a significantly higher WBS of 484.5 ± 2.6g. [2,4,20,] Treatment with 0.5% and 2.5 % EELBV and EELAC resulted in WBS values of 424.3 ± 6.5g and 401.1 ± 3.5g, respectively. These findings indicate that the 2.5% EELAC hydrogel formulation exhibits substantial effects in promoting diabetic wound healing. [32]

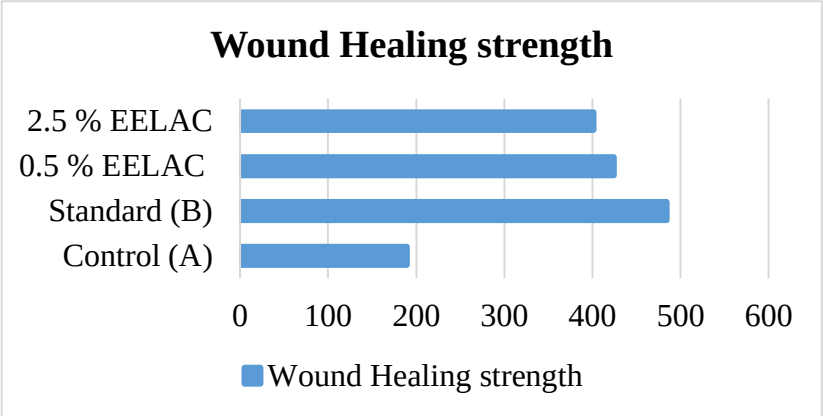

Fig. 1 Representation of A. cuneata wound healing in Incision healing model

Excision Wound Model

It is evident that superficial application of hydrogels containing EELAC (Ethanolic Extract of A. cuneata Leaf) exhibited significant wound healing potential (**Table No 5** and **Figure No.2)**. This was demonstrated by a shortened epithelialization period and increased wound contraction area compared to a standard group. The results underscore the effectiveness of A. cuneata in wound healing compared to control treatments, validating its traditional and medicinal use. [24-26,33]

The standard group animals showed wound contraction from 385 ± 2.3 mm² at day 0 to 23.3 ± 0.5 mm² by day 22, with complete epithelialization observed by day 28, totaling an epithelialization period of 25 days. [31]

- Animals in the control group displayed wound contraction from 423 ± 5.2 mm² at day 0 to 0 ± 0 mm² by day 22, achieving epithelialization in 21 days.
- Rats treated with 0.5% EELAC and 2.5% EELAC both demonstrated wound epithelialization within 22 days, with wound closure percentages of approximately 99.02% and 98.7%, respectively.

These outcomes collectively support the conclusion that A. cuneata, particularly formulations containing EELAC effectively promote wound healing compared to standard treatments, highlighting its therapeutic potential in traditional and modern medicinal contexts**.** [34]

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Table no. 5 results for wound healing activity for excision wound model[#]

Sr.no	Days*	A*	B*	C*	D*
1	0	385 ± 2.3	423±5.2	412±2.3	402±2.8
2	4	245±3.1	345±4.2	385±4.1	379±5.3
3	8	210 ±1.3	212±5.9	296±3.2	256±3.5
4	12	185±2.5	157±5.7	203±2.3	205±4.6
5	14	154±2.1	85±6.8	155±5.6	193±1.5
6	16	99±1.2	32±4.6	96±7.6	101±8.5
7	18	65±2.6	12±1.3	36±8.5	55±3.9
8	20	45±1.9	2.3±0.2	12±5.9	18±6.3
9	22	23±4.3	0.00	3.5±2.1	4.6±3.2
% Wound Closure		94.02	99.99	99.02	98.75

 # Post wounding Day (Days)(epithelialization period)

***A= Standard, B = Control, C = 0.5% EELAC and D= 2.5 % EELAC**

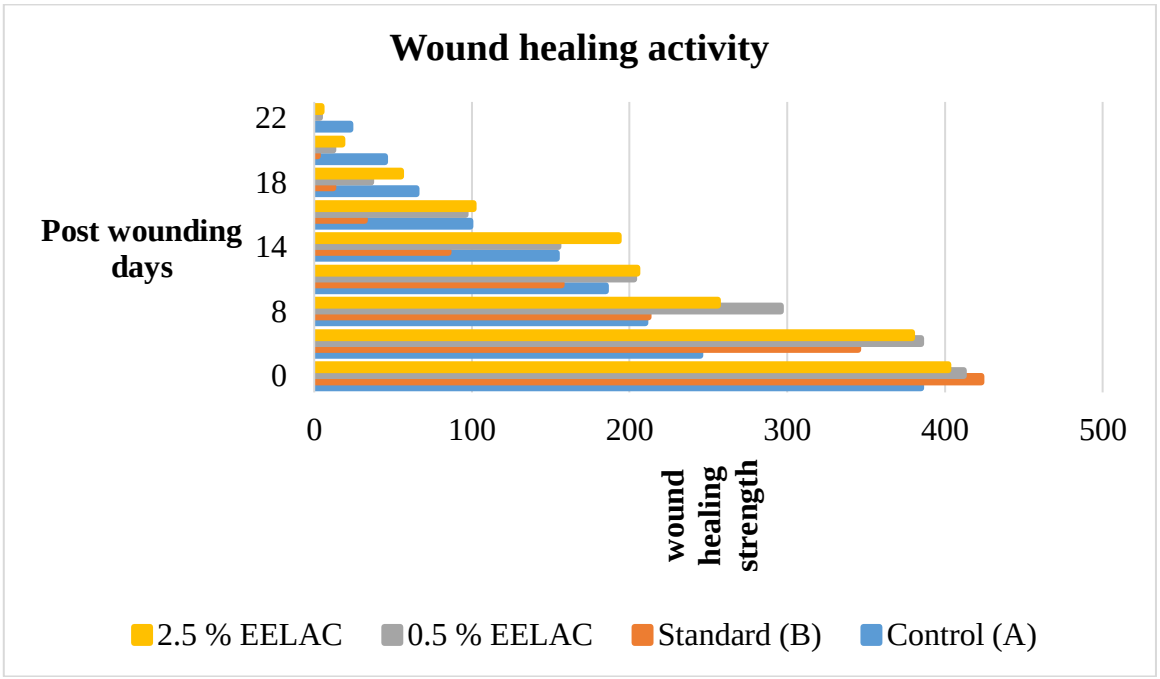

Fig. 2 Representation of A cuneata extract healing strength in Excision model

Dead Space Wound Model

It can be inferred that the hydrogel containing 0.5% EELAC and 2.5 % EELAC (Extract of A. cuneata Leaf) demonstrated the highest levels of hydroxyproline, along with increased weights of wet and dry granulation tissue **(Table 6** and **Figure**

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No.3). These findings suggest that A. cuneata may possess superior diabetic wound healing properties. Both graded doses of EELAC and EELAC

showed significant increases in the weights of dry and wet granulation tissue compared to a control group. [26,29,35,]Additionally, both low and high dose of EELAC and EELAC formulations reported a notable increase in hydroxyproline levels, indicating enhanced wound healing capabilities. Therefore, based on these results, it can be concluded that extracts from A. cuneata have promising potential for diabetic wound management.

Table no.6 Results for wound healing activity of A cuneata extract for dead wound model

Sr.no	Treatment	Weight of Granulation Tissue (mg/100g body weight)		Hydroxyproline
1	A*	123.2 ± 5.3	25.3 ± 5.3	12.23 ± 0.2
2	B*	312.5 ± 2.3	65.2 ± 2.5	25.30 ± 5.6
3	C*	325.2 ± 4.1	70.13 ± 2.5	39.56 ± 5.3
4	D*	354.6 ± 5.3	64.21 ± 2.6	36.52 ± 5.9

***A= Standard, B = Control, C = 0.5% EELAC and D= 2.5 % EELAC**

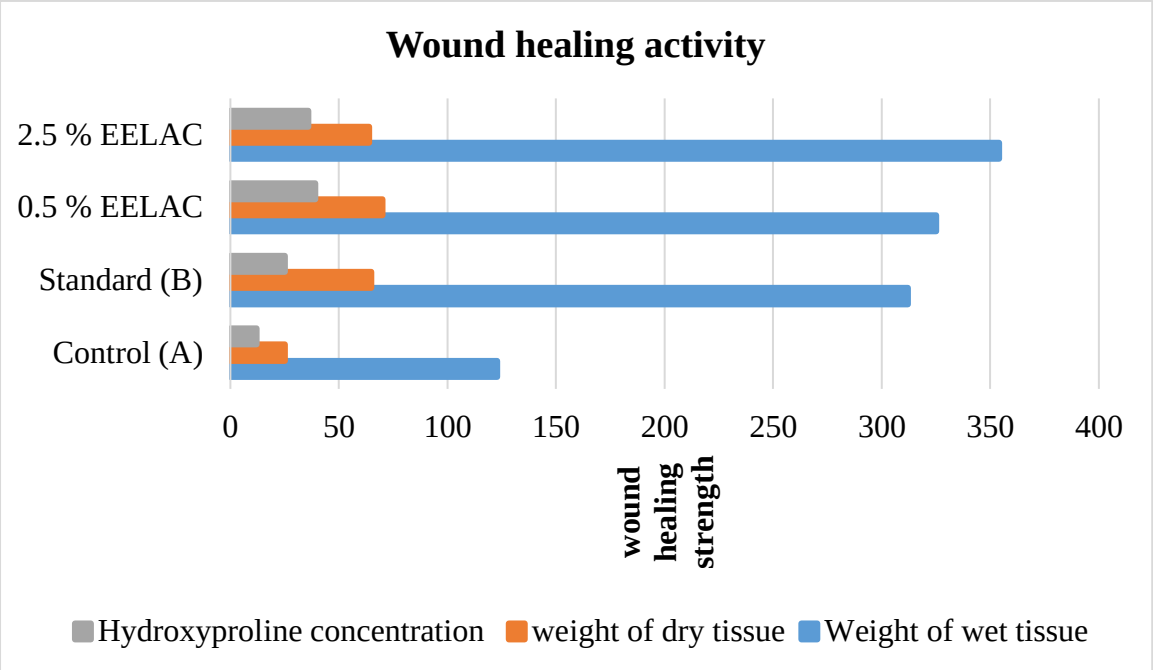

Fig. 3 Representation of A cuneata extract activity in dead space wound healing model

4. Discussions:

Wound healing is a complex process involving several phases: hemostasis, inflammation, proliferation, and remodeling. The application of Argyreia cuneata leaf extract can potentially enhance this process through multiple mechanisms. Inflammation is a critical early response in wound healing but must be carefully regulated. Prolonged or excessive inflammation can impair healing and lead to chronic wounds. [2-6,20,26] A cuneata leaf extract exhibits notable anti-inflammatory properties, attributed to its bioactive compounds, such as flavonoids and tannins. These compounds inhibit pro-inflammatory cytokines and enzymes, thus reducing inflammation at the wound site and facilitating faster progression through the healing phases. [9,15,35]

Oxidative stress results from an imbalance between free radicals and antioxidants and can impede

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wound healing by damaging cellular components and tissues. The leaf extract is rich in antioxidants, which neutralize free radicals and protect the wound site from oxidative damage. This antioxidant activity is vital in promoting cellular repair and minimizing oxidative stress, thus enhancing the overall healing process. Collagen is essential for the structural integrity of newly formed tissue during wound healing. Some studies suggest that A cuneata leaf extract may promote collagen synthesis, which is critical for the formation of a strong extracellular matrix. This enhancement in collagen production supports the formation of new tissue and strengthens the wound, contributing to better healing outcomes and reduced scarring. [31]

Diabetes, characterized by chronic hyperglycemia and associated metabolic disturbances, poses significant health challenges. [36-39] The use of A cuneata leaf extract in diabetes management is supported by several promising mechanisms:

Regulation of blood glucose levels is crucial for diabetes management. Research indicates that Argyreia cuneata leaf extract may help lower blood glucose levels through several mechanisms. It may enhance insulin sensitivity, which facilitates better uptake of glucose by cells and reduces blood sugar levels. Additionally, it might stimulate insulin secretion from pancreatic beta cells, further contributing to improved glycemic control. [8,23,37]

5. Conclusion

Argyreia cuneata leaf extract offers significant potential in enhancing wound healing and managing diabetes due to its anti-inflammatory, antioxidant, antimicrobial, and metabolic effects. While traditional uses provide a foundation, scientific research increasingly supports these applications. [36] However, further studies are necessary to fully understand the mechanisms, establish optimal dosages, and confirm the efficacy of the extract in clinical settings. As with any therapeutic agent, it is essential for patients to consult healthcare professionals before incorporating herbal extract into their treatment regimens to ensure safety and appropriate use.

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