EVALUATION OF THE WOUND HEALING ACTIVITY OF SELECTED INDIGENOUS MEDICINAL PLANTS

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ABSTRACT

Epipremnum aureum and *Morus alba* leaves were screened for phytochemical constituents. Phytochemical analysis of the extract revealed that the wound healing activity by wound excision and incision methods. The plant material is due to the presence of active constituents like tannins or flavonoids. *Epipremnum aureum* is used in disease related to anti-bacterial , anti-termite , anti-oxidant , anti-malarial , anti-cancerous , anti-tuberculosis , anti-arthritis and wound healing activities and also *Morus alba* is used to treat disorders like dizziness , insomnia , premature aging , atherosclerosis , liver ,kidney disorders and inflammation. In the present study contains *Epipremnum aureum* and *Morus alba* leaves samples were obtained by using Maceration (softening) Process. Phytochemical studies revealed that tannins and flavonoids are present in the sample.

Keywords: *Epipremnum aureum; Morus alba* , wound excision, wound incision.

DOI: 10.48047/ecb/2023.12.Si9.294

INTRODUCTION

Nature reflects the creative power of God, and plants are an integral part of this nature[10]. Plants have been used for medicinal purposes since prehistoric days. With increasing resistance to antimicrobial agents, scientists are encouraged to look for new antimicrobial agents from plants. They have been found very efficient in treating many contagious diseases with a minimal side effect. As per the World health organization (WHO), most of the population uses herbal medicine for most diseases by altering the plant species. Natural products like medicinal plants as primary potential sources of innovative therapeutic agents through investigation and researches[3].

METHODOLOGY

Wound Healing Evaluation

Preparation of ointment

The materials for the ointment base were combined according to the British Pharmacopoeia (1980) in a beaker at 65°C on a water bath. The ingredients were wool fat (5g), hard paraffin (5g), cetostearyl alcohol (5g), and soft paraffin (85g). The mixture was homogenised for 10-15 minutes at 1500 rpm in a homogenizer after chilling. To create a homogenous ointment formulation, the test extract (5 percent w/w) was blended with the ointment base using a mortar and pestle. A new medication formulation was created every fifth day. Wool fat, cetostearyl alcohol, soft paraffin, and hard paraffin are products of Burgoyne Burbidges and Co Company and were acquired from the pharmaceutical school at Vaageswari College of Pharmacy in Karimnagar. Povidine iodine ointment was procured from a local chemist[1].

Preparation of animals for in vivo wound healing study

6 Rabbits each weighing between 180-200g were procured from the animal house of Vaageswari College of Pharmacy, Karimnagar. The animals were housed in regulated climatic settings at a temperature of 25°C, a relative humidity of 45–55% and a natural light cycle. They were also given access to food and water. Before the trial started, they underwent a week of acclimation.

Treatment protocol

Three sets of six rabbits each were created. The institutional animal ethics committee of the School of Pharmaceutical Sciences at Vaageswari College of Pharmacy in Karimnagar gave its approval to the current study project. VCP/1720/12/2021/001 is the registration number.

Group (i) functioned as the control and received topically a straightforward ointment base.

Group (ii) received topical application of a 5 percent Povidone-iodine ointment as the standard of care.

Group (iii) was provided as test treatment with Plant residue ointment topically.

Wound healing study in excision wound model

Excision of wounds[18]. The animals are first given anaesthesia with the anaesthetic ether before being put on a dissection table in their natural position. After using ethanol to disinfect the area, a 1.5 cm wide by 0.2 cm deep square incision was produced in the dorsal thoracic area. A simple ointment base was used to topically treat the animals in Group (i). Povidone iodine ointment was applied topically to the Group (ii) animals. Once daily, until the epithelization was finished, 5 percent test ointment was applied topically to the Group (iii) animals. To protect the wound and avoid infection, all of the rabbits were housed in separate, clean cages right after being injured. Neither an oral nor a systemic antibiotic was given after the procedure. The animals were inspected daily for any indications of an infection. Day 0 stood for the day of the injured. Later on days 0, 4, 8, 12, 17, 20 and 24 the wound contraction, scar

residue, area, and length of complete epithelization were also evaluated. To analyse the wound contraction, the raw wound area was drawn onto graph paper. The length of epithelization as well as the percentage of wound closure were recorded[12] **(Results will be shown in Table.3, 4 & Figure.1, 2)**.

Wound contraction rate

Every two days, the rate of wound contraction was assessed. It is a percentage decrease in the size of the wound. It may also be thought of as a portion of wound protection. Transparent paper and an appropriate marker were used to track the shrinkage of wounds at predetermined intervals. The percentage of wound closure obtained as a result shows the development of new epithelial tissue to heal the lesion. The proportion of the initial wound size that was reduced to represent wound contraction.

% of wound = (initial area of wound day 0-area of wound on N^{th} day) / (wound area on day 0) x100.

Wound healing study in the incision model

Ether was used to make the animals unconscious. The animals were kept in the standard posture on the operation table. Using a scalpel blade, a six-centimeter-long paravertebral straight incision was created on either side of the vertebral segment. Cotton balls dipped in 70% alcohol were used to disinfect the wound. The animals were housed in separate cages. Animals in Group (i) received topically applied treatments with a basic ointment base, those in Group (ii) received povidone iodine ointment, and those in Group (iii) had 5 percent test ointment administered topically daily for ten days. Sutures were taken out nine days after the wound. Tensile strength was assessed on the tenth day following injury[2] **(Results will be shown in Table.5 & Figure.3)**.

Determination of tensile strength

The process of repair results in wound healing and tissue strength recovery. In the procedure described above, the ultimate tensile strength, or breaking strength, is the most important step. The elastic fibre networks and collagen in the dermis are in charge of giving skin its mechanical qualities. The minimal amount of effort needed to separate the incision, which indicates the degree of healing, the resilience of the wound tissue, and the effectiveness of the healing process.

The skin sutures are taken out nine days after surgery. On the tenth day, one side of the incision received application with progressively more weight while the other was fixed. The breaking strength, also known as tensile strength, is the weight at which the wound completely detaches from the incision line. The average breaking strength at the two paravertebral incisions on the animals opposite sides was used to calculate the breaking strength of each individual animal.

Statistical analysis

The values were expressed as mean ± standard deviation. For each parameter, the One-Way ANOVA was used to detect significant differences between the groups. When significant differences existed, the Waller–Duncan test ($p < 0.05$) was used to compare the means.

RESULTS

Table 1. Preliminary phytochemical screening of the methanolic extract of *Epipremnum aureum* leaves.

Phytoconstituents	**Methanolic extract**
Alkaloids	+
Flavonoids	+
Glycosides	+
Steroids	-
Terpenoids	+
Tannins	+
Carbohydrates	+
Anthraquinone	-
Reducing sugars	+
Saponins	+

Table 2. Preliminary phytochemical screening of the methanolic extract of *Morus alba* leaves.

Phytoconstituents	**Methanolic extract**
Alkaloids	-
Flavonoids	+
Steroids	-
Triterpenes	+
Tannins	+
Anthraquinones	-
Coumarins	+

Table 3. Effect of methanolic leaf fraction of *Epipremnum aureum* on excision wound model.

	Initial day	4th day	8th day	12th day	Final Day
Test drug length	2.41±0.1.6	2.16±0.14	1.8±0.16	1.41±0.20	0.76±0.19 (19 th day)
width	2.4±0.17	2.080.19±	1.7±0.16	1.3±0.25	0.9±0.42 (19 th day)
Standard drug length	2.26 ±0.17	1.96 ±0.14	1.6±0.08	1.23±0.11	0.7± 0.12 (16 th day)
width	2.23 ±0.14	2.1±0.15	1.78± 0.14	1.33 ±0.12	0.78±0.13 (16 th day)
Control drug length	2.3±0.13	2.55± 0.22	1.65 ±0.09	1.25±0.09	0.73±0.14 (23 th day)

width	2.66±0.11	2.3±0.1	1.81±0.15	1.38 ±0.22	0.88±0.21 (23 th day)

The values are expressed as Mean ± SEM, n=6 in each group.

Table 4. Effect of methanolic leaf fraction of *Morus alba* on excision wound model.

	Initial day	4th day	8th day	12th day	Final Day
Test drug length	2.41±0.1.6	2.3±0.09	2.1±0.08	1.73±0.17	0.68±0.24 (20 th day)
width	2.4±0.17	2.3±0.14	2.03±0.16	1.6±0.2	0.8±0.4 (20 th day)
Standard drug length	2.26 ±0.17	1.9 ±0.14	1.65±0.11	1.11±0.06	0.61±0.13 (17 th day)
width	2.23 ±0.14	2.1±0.11	1.8± 0.18	1.25 ±0.12	0.06±0.09 (17 th day)
Control drug length	2.3±0.13	2.3± 0.16	1.9 ±0.08	1.2±0.10	0.61±0.19 (24 th day)

wi dth	2.66±0.11	2.6±0.13	2.3±0.11	1.35 ±0.16	0.68±0.34 (24 th day)

The values are expressed as Mean ± SEM, n=6 in each group.

Table 5. Effect of the Methanolic extract of *Epipremnum aureum* & *Morus alba* on the incision wound model (n=6, Mean ± SEM).

Group	Drugs	Dose and route	Breaking strength (g)
Group 1	Control	2 mL of distilled water	275.94±2.36
Group 2	*E. aureum*	400 mg/kg, oral	321.15±1.83
Group 3	*M. alba*	400 mg/kg, oral	322.18±1.86
Group 4	Dexamethasone	0.17 mg/kg, im	151.15±0.84

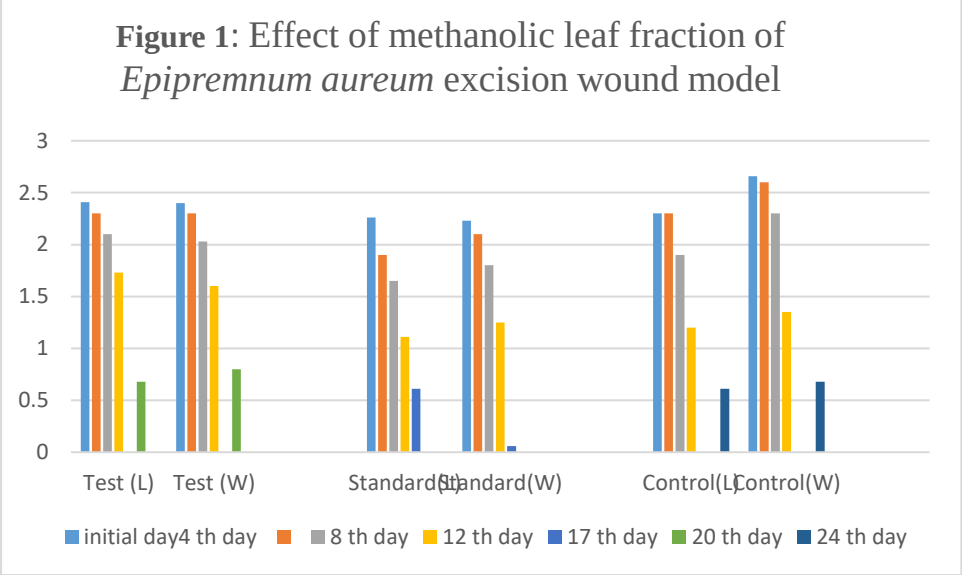


Figure 1: Effect of methanolic leaf fraction of *Epipremnum aureum* excision wound model

Figure 2: Effect of methanolic leaf fraction of *Morus alba* on excision wound model

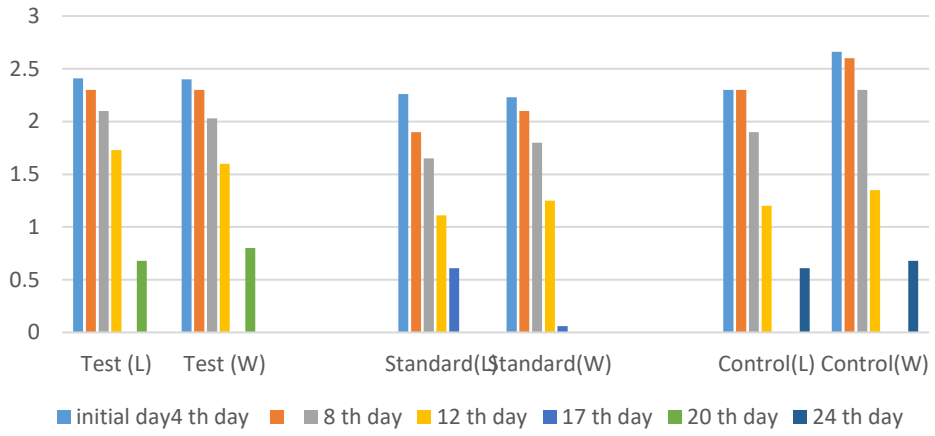
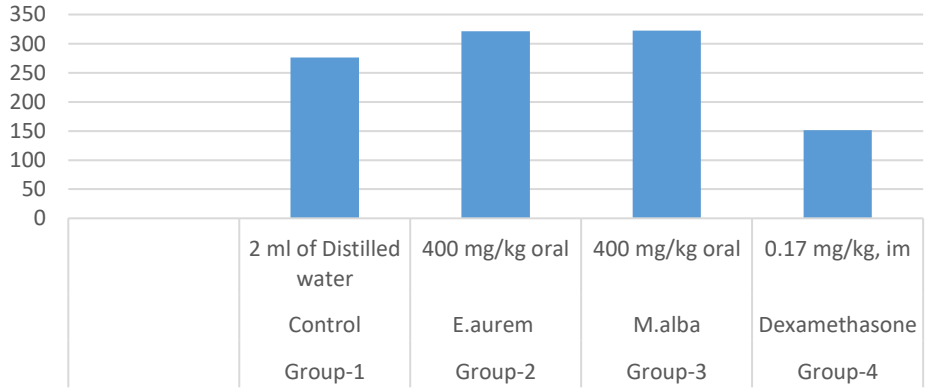


Figure 3: Effect of the methanolic extract of *E. aureum & M. alba* on the incision wound model (n=6, Mean±SEM)



DISCUSSION

The Ethnobotanical studies and folklore claiming reviewed that the leaves of the plants *Epipremnum aureum & Morus alba* are used for wound healing, anti-inflammatory and antibacterial activities. The young leaves are used as tonic in the diseases of the digestive function and is said to be remedy for toothache. Tannin has a broad scale of biological activities among which anti- inflammatory and wound healing effects stands out. *Epipremnum aureum & Morus alba* is a wide spread plants in India and commonly used as for antiseptic, anthelmintic, wound healing and in inflammatory conditions. It has a high content of tannins and flavonoids substances

reviewed from literature. The methanolic leaf fraction of leaf plants was formulated in the ointment form and studied for wound healing activity.

Phytochemical study

Phytochemical screening was carried out to identify the phyto-constituents present in the methanolic extracts and its fraction.

Wound healing activity

Wound healing, a complex sequence of events, is initiated by the stimulus of injury to the tissues. A positive stimulus may result from the release of some factors by wounding of tissues. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue. The results of present study indicates that methanolic extract of leaf ointment of title plants at both strengths (5% leaf *Epipremnum aureum* and 5% leaf *Morus Alba*) exhibited significant wound healing promoting activity. However, this effect was found to be concentration related fashion where 5% ointment promotes significant wound-healing activity by increasing cellular proliferation , formation of granulation tissue, synthesis of collagen and by increase in the rate of wound contraction as compared to the control animals. This was evident by faster rate of wound closure and epithelization period in excision wound model and also incision wound model. Further phytochemical studies are needed to isolated and identified the compounds .which is responsible for wound healing activity.

CONCLUSION

From this study, it is concluded that *Epipremnum aureum & Morus alba* leaves of methanolic extract fractions have significant wound healing models. That methanolic rich fraction of leaves of the plants was formulated in the ointment form and it was screened for In vivo wound healing. It showed significant percentage wound protection at the tested concentration.

The wound healing activity is probably due to the presence of tannin (gallic acid) & flavonoids (quercetin). Further studies need to be isolate individual tannin & flavonoids explore its biological potency by various preclinical and clinical trials of the isolated compounds.

Acknowledgements

We thank Vaageswari College of Pharmacy, Telangana, India, for offering their support and research facility to conduct this research work.

Conflicts of Interest

No conflicts of interest is declared.

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