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WOUND HEALING EFFECT OF VARIOUS EXTRACTS OF *ADHATODA VASICA*

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ABSTRACT

In the Present study, methanolic, chloroform and diethyl ether extracts of *Adhatoda vasica* Plants were evaluated for its wound healing activity in the form of Ointment dosage form in excision wound model in albino rats. The methanolic extract ointment of *Adhatoda vasica* showed a significant effect in excision wound model as comparable to standard drug and other two extracts of ointment, by calculating the parameters, percentage closure of excision wound model. The Isolated compounds were characterized by instrumental analysis and IR.

KEY WORDS

Adhatoda vasica, Wound healing, excision model

INTRODUCTION

Herbal medicine has become an integral part of standard healthcare, based on a combination of time honoured traditional usage and ongoing scientific research. Burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections (Atal et al., 1986).

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world due to their potent pharmacological activities and low toxicity.

Medicinal plants are coming into prominence because of the conventional medicine such as antibiotics which have developed resistance to many of the infection organisms which no longer responsive to conventional medicines. Herbal preparation can be more effective and safer than conventional medicines. Non-toxic could be administered for a long period.

The treatment of diseases in early days has begun by using various medicinal plants. They served as a good tool in altering different clinical conditions. Our land is having a vast heritage of knowledge and expertise in herbal medicine from different cultures and civilization. The purpose of the plant work is to identify the active ingredient through scientific methods and to study the pharmacological activities of the plant in shoot and root extracts of *Adhatoda vasica.*

Adhatoda vasica (acanthaceae) known as chue Mue, is a stout stragling prostrate shrubby plant with the compound leaves which gets sensitive on touching, spinous stipules and globose pinkish flower heads, grows as

weed in almost all parts of the country. Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root contains tannins. *Adhatoda vasica* is used for its anti – hyperglycemic (Uma maheswari,2007), anti – diarrhoeal, anti – convulsant and cytotoxic properties. The plant also contains turgorins, leaves and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. Plant is also used in treatment of sore gum and is used as a blood purifier. In ayurvedic and Unani system of medicine, this plant has been used in diseases arising from corrupted blood and bile, bilious fever, piles, jaundice, leprosy, ulcers, small pox. The objective of present investigation was to study the Wound healing activity of the Methanolic, Chloform and Diethyl Ether extracts of the leaves of *Adhatoda vasica* in animal models.

Plant Material and Extract Preparation

Plant material: The plant material was collected in Arulmigu kalasalingam college of pharmacy Medicinal Garden, Krishnankoil,Tamilnadu.It was authenticated by Dr.Stephan, Dept of Botany, The American college, Madurai.

Extract Preparation

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with methanol, chloroform and Diethyl ether and the residual marc was collected. The extract was filtered through a cotton plug, followed by Whatman filter paper (no.1). The extract was evaporated under reused pressure using a rotovac evaporator at a low temperature (40-60oC) until all the solvent had been removed to give an extract sample with a yield of 16% w/w, 14 %w/w and 12% w/w in relation to the dried starting material. Preliminary phytochemical analysis was carried out to identify presence of phytoconstituents in the crude extract.

The percentage yields of Adhatoda vasica Extract

WEIGHT OF DRUG	EXTRACTION PATTERN	SOLVENT USED	WEIGHT OBTAINING)	PERCENTAGE YIELD
500gms *Adhatoda vasica* powder	Soxhlet apparatus	Methanol	16gm	7%
		Chloroform	14gm	6.2%
		Diethyl Ether	12gm	5.2%

Experimental Animals

Adult male wistar rats150-200gm of either sex were used for this study. They were provided with a standard diet (Pranav Agro, India) and water ad libitum in animal house facility and maintained under standard laboratory conditions. The experimental protocol has been approved by institutional animal ethics committee, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. (Regd No.509/02/C/CPCSEA/2002.)

Qualitative Chemical Evaluation

All the extracts obtained were subjected to qualitative tests for various plant constituents and observed that presence of Flavonoids, Essential oil, Tannins, glycosides, Phytosterol and alkaloids as major active constituents are confirmed by suitable chemical tests.

***Formulation of Ointment**:*

Table 1
Bases used in the formulation of Adhatoda vasica

S.No.	Ingredients	Official Formula	Working Formula
1.	Emulsifying wax	30gm	3gm
2.	White soft paraffin	50gm	5gm
3.	Liquid paraffin	20gm	2gm

Type: Water miscible base.
***Method of Preparation*:** Mixed the ingredients, heated gently with stirring until homogenous mixture forms. Stirred to cool (Table 1).

***Types of Ointment prepared**:* There are three types of ointments are prepared. There are,
1. Base + *Adhatoda vasica* Methanolic Extract
2. Base + *Adhatoda vasica* Chloroform Extract
3. Base + *Adhatoda vasica* Diethyl Ether Extract

10% concentration of ointment was prepared.

Method of preparation: In this preparation 1gm of suitable extract is mixed with 10gms of ointment base (10%). Then it is stirred well until homogenous base is obtained.

Pharmacological screening

Excision Wound Model:

Animal:

Wister Albino rats (150-200gms).

Procedure:

Wister Albino rats (150-200gms) were selected and made into five groups of 6 animals each for the experiment. The animals were housed in the experimental room which was maintained as per IAEC guide lines. The experimental animals were anaesthetized using lignocaine 2% injections, over the local selected region. The rats were depilated over the region excision wound was infected by cutting a way of 500mm square thickness of skin from the predetermine area, the wound was left and rest to the open environment then the drugs reference standard (0.2 % w/w Nitrofurazone ointment) control (simple ointment base B.P) and *Adhatoda vasica* diethyl ether, chloroform, alcohol leaves extracts were applied till the wound was healed (Fig. 1). This model was used to monitor the wound contraction and wound closer time. Wound contraction was calculated as % reduction in wound area (Table 2).

Table 2

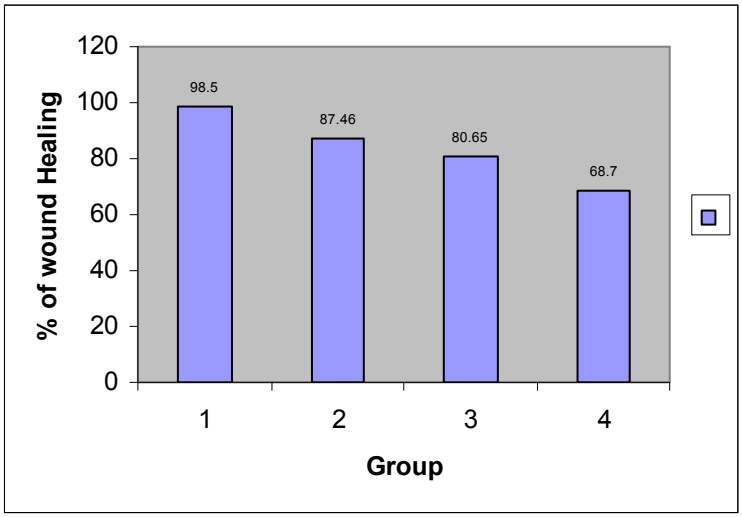
Effect of Methanolic, Chloroform and Diethyl ether extract ointments of Adhatoda vasica on excision wound model.

Group	Avg. wt of animal	Drug / extract	Size of wound surface area (mm^2)						Percentage of wound healing
			Day 0	Day 1	Day 4	Day 8	Day 12	Day 16	
I	150-200 gm.	Control	50.24	50.24	48.24	44.20	40.46	30.42	39.46
II		Nitrofurazone ointment (0.2% w/w)	50.36	50.36	28.26	12.56	3.14	0.758	98.50
III		Alcoholic extract (10%w/w)	51.16	51.16	38.14	27.84	16.12	6.42	87.46
IV		Chloroform extract(10% w/w)	50.62	50.62	36.90	27.52	18.42	9.80	80.65
V		Diethyl ether extract (10%w/w)	49.84	49.84	37.10	29.34	21.64	15.60	68.70

Values are mean ± SEM of 6 animals in each group. Numbers in Parenthesis indicate percentage of wound contraction. P<0.001Vs respective control by students Control"t" test.

Fig. 1

Effect of Methanolic, Chloroform and Diethyl ether extract ointments of Adhatoda vasica on excision wound model.

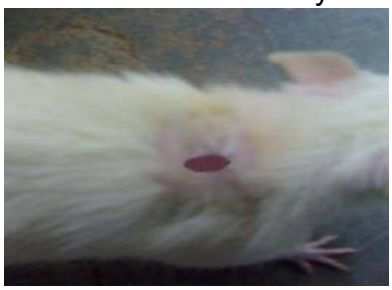


GROUP – 1 Nitrofurazone ointment (0.2% w/w) GROUP-2: Alcoholic extract (10%w/w)

GROUP-3 Chloroform extract(10%w/w) GROUP-4 Diethyl ether extract (10%w/w)

Control 0 day

Standard 0 day



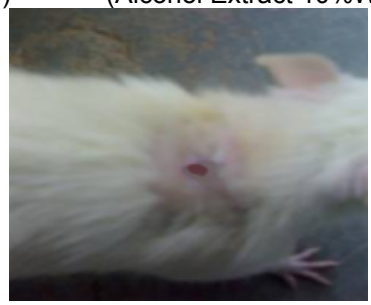
Control 16th day

Standard 16th day

Adhatoda vasica
(Diethyl ether Extract10%W/W)

Adhatoda vasica
(chloroform Extract10 %W/W)

Adhatoda vasica
(Alcohol Extract 10%W/W)



RESULT & DISCUSSION

The present work is the pharmacological studies on the extracts of *Adhatoda vasica*. The Soxhlet extraction procedure carried out using *Adhatoda vasica* powder with by successive solvent Methanol, Chloroform and Diethyl Ether. The preliminary chemical analysis indicates the presents of Alkaloids-Quinazoline,Flavonoids,Tannins, Vasicinone,Essential oil.

The wound healing activity was studied by using five groups; Group I negative control simple ointment, In Group II positive control Nitrofurazone ointment (0.2% w/w), Group III AVME, Group IV AVCE and Group V AVDEE. The size of the wound in surface area, On the Day 1 (50.24) (50.36) (51.16) (50.62) (49.84). On the Day 4 (48.24) (28.26) (38.14) (36.90) (37.10). On the Day 8 (44.20) (12.56) (27.84) (27.52) (29.34). On the Day 12 (40.46) (3.14) (16.12) (18.42) (21.64). On the Day 16 (30.42)

(0.758) (6.42) (9.80) (15.60). The mean percentage closure of excision wound model on Day 16 (39.46) (98.50) (87.46) (80.65) (68.70).

Contraction of the excision wound was promoted from Day 1 of the treatment till Day 16. In excision wounds, wound contraction was 98.50, 87.46, 80.65 and 68.70% respectively on 16th day for Nitrofurazone, Methanolic, Chloroform and Diethyl ether extract treated groups(Table no 2).Significant wound contraction was also observed on 16th day for all treated groups($p<0.001$ for standard and methanolic extracts; $p<0.01$for chloroform and diethyl ether extract), in comparison with the control group.Time for complete epithelization was significantly short in drug and standard treated groups.

The epithelization of wound in case of rat treated with extracts was found to be quite earlier than control. It is also comparable with the marketed preparation. It suggests that the leaves extracts of *Adhatoda vasica* promoted wound healing activity. The excision wound model showed excellent wound healing property in alcoholic leaf extract which was well compared with standard drug. The results are shown in Table no 2.

CONCLUSION

Wound healing comprises of different phases such as contraction, epithelization, granulation and collagenation. It normally involves an initial inflammatory phase followed by fibroblast proliferation, formation of collagen fibres and shrinking, occurring concurrently but independent of one another. Several plants are having wound healing potential. Phytoconstituents such as flavonoids, glycosides and tannins are reported to be responsible for wound healing property in Leucas hirta, Ocimum sanctum, and Gratissimum. Wound healing effect is also attributed to free radical scavenging activity of flavonoids. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing onset of cell necrosis, but also by improving vascularity. Lipid peroxidation is an important process in several types of injuries like burns, infected wounds and skin ulcers. Hence any drug that inhibits lipid peroxidation is believed to increase strength of collogen fibres, by increasing circulation or by preventing cell damage or by promoting DNA synthesis. Flavonoids, glycosides and tannins are known to promote wound healing process mainly by their astringent and antimicrobial property. Preliminary phytochemical analysis of the leaves of *Adhatoda vasica* revealed presence of flavonoids, tannins, proteins, phenolic compounds, glycosides and organic acids. Presence of flavonoids and tannins in extracts of leaves of *Adhatoda vasica* may be responsible for its wound healing activity.

On the basis of the results obtained in the present investigation it is possible to conclude that the methanolic, chloroform and Diethyl ether extract ointment (10%w/w) of *Adhatoda vasica* has significant wound healing activity. In both extract ointment, the methanolic extract ointment (10%w/w) showed significant effect when compare to standard drug and other two extract in excision wound model.

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