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Research Article

AGERATUM CONYZOIDES LINN., AND WOUND HEALING PROPERTIES

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ABSTRACT

It is unimaginable to even think of the existence of human race on the Earth without vegetation on it. Right from the beginning of the human race, humans depended on plants for their survival. Medicinal plants were the only source of medicine for any ailment. Ayurveda, Unani, and Chinese Ordinary medicine contain statements of medicinal plant description and their use which are the evidences to medicinal plant use. During economic crisis and in less affluent rural areas, the population depends mainly on medicinal plants or traditional medicine and even in urban areas, the population frequently uses this alternative medicine for routine health care and as self-medication against minor and chronic ailments. This traditional/local medicine evolved out from a holistic perspective of human well-being. In the developed countries and more affluent regions, phyto-pharmaceutical therapy is being used as an alternative to biomedicine and also for the treatment of mild and chronic health problems. Wounds are ultimate result of physical disruption of the skin – which is the major hurdle for the infection to establish itself by microbial pathogens in visceral tissues. Infection results when the microbes breach this skin barrier. An intrinsic feature of inflammation is the removal of polluting microbes and fortunately this inflammation also happens to be a routine part of injury recovery process. If for any reason the contaminating microbes are not removed completely, which usually results from ineffective decontamination, there will be a long standing inflammation.5%w/w simple ointments were prepared from the extracts of Ageratum conyzoides and evaluated for wound healing properties and it was found that these ointments significantly reduced the number of days required for wound contraction and for epithelialization when compared to placebo in excisional wound models of rats.

Keywords: plants, ageratum conyzoides, excisional wound model, rate of wound contraction

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INTRODUCTION

It is unimaginable to even think of the existence of human race on the Earth without vegetation on it. Right from the beginning of the human race, humans depended on plants for their survival. Medicinal plants were the only source of medicine for any ailment. Ayurveda, Unani, and Chinese Ordinary medicine contain statements of medicinal plant description and their use which are the evidences to medicinal plant use. The ultimate use of medicinal plants and herbs was to attain a positive interaction or for vitalizing the body. [1] Globally the current market share of plant based pharmaceutical preparations is about 30% which generates a revenue of US $ 60 billion and by the year 2050 it is expected to generate a revenue of US $ 5 trillion. [2] According to a report given by WHO about 80% of world's total population depended on herbal medicines for the management of their primary health-care problems [3]. During economic crisis and in less affluent rural areas, the population depends mainly on medicinal plants or traditional medicine and even in urban areas, the population frequently uses this alternative medicine for routine health care and as self-medication against minor and chronic ailments. This traditional/local medicine evolved out from a holistic perspective of human well being. [4] Business interests have always been the spark plug behind the search for new therapies and medicines. The current observations

suggest that the global economy is tending towards commercialization of indigenous and local medicine. [5] Wounds are ultimate result of physical disruption of the skin – which is the major hurdle for the infection to establish itself by microbial pathogens in visceral tissues. Infection results when the microbes breach this skin barrier. [6,7]

The process of wound healing occurs in four integrated and overlapping phases:

Hemostasis, Inflammation, Proliferation, and The Tissue remodeling/Resolution phases.[8] Dynamism is the characteristic property of the process of wound healing and consists of four phases which are programmed precisely, overlap each other and are continuous. All the events of the four phases must occur in a regulated and precise way so as the process of wound healing occurs without any delay and obstructions. If any prolongation or interruptions or aberrancies occur in the process of wound healing, delayed wound healing and chronic non-healing wounds are the end result.

Multiple factors may lead to impaired wound healing. In particular, it is possible to categorize the factors influencing repair into local and systemic. Local variables are those that directly affect the features of the wound itself, while systemic variables are the individual's general state of health or disease that affects the individual's capacity to cure. Many of these variables are linked, and through the local impacts influencing wound healing, the systemic variables act.

Local Factors:-

- Venous insufficiency
- Infection
- Oxygenation
- Foreign Body

Systemic Factors:-

- Nutrition
- Ischemia
- Stress
- Age and Gender
- **Disease**- uremia, jaundice, diabetes, keloids, hereditary healing disorders, fibrosis
- Sex hormones
- **Immunocompromised conditions**- cancer, radiationtherapy, AIDS.
- Alcoholism and Smoking
- **Drugs** – glucocorticoids, chemotherapy drugs, NSAIDS, Immunomodulatory drugs.
- Obesity [9,10]

There are high risks associated with chronic wounds like- huge expenses, loss of mobility, loss of function, amputations, and in some cases even death. Management of chronic wounds at hospitals is a huge financial burden to individuals and to even governments hence in the recent decades research was diverted to find novel and more potent agents from the environment we live, viz., fungi, microorganisms, plants and marine species etc., that help in efficient management of chronic wounds particularly in those patients with metabolic disorders.[11,12]

Any agent either from plant or other natural sources can be classified as a wound healing agent if it possesses two or more of the following properties:

- Capable of stimulating the process of fibroblast proliferation
- Capable of inducing keratinocyte differentiation and proliferation
- Capable of increasing collagen formation
- Capable of exhibiting anti-oxidant properties
- Capable of exhibiting anti-inflammatory properties
- Capable of exhibiting anti-microbial property. [13,14]

It is evident from literature that Ageratum conyzoides possesses antioxidant, anti-inflammatory, and anti-microbial properties and hence is of interest for wound healing evaluation. Also others have reported its uses.

AIMS & OBJECTIVES:

The aim of the study is to evaluate the healing properties of A.conyzoides L., in excisional wound models of rats

The objective of the study is to assess the rate of wound contraction in extract treated groups and also to assess the epithelialization period.

MATERIALS & METHODS:

Plant extraction- the plants were picked from around the University campus in Alwar in the early hours of October 2015. The plants were confirmed by The Department Of Botany, Sunrise University. The plants were hacked into pieces and washed with copious amounts of water and rinsed off and allowed to dry at room temperature for two weeks. The dried plant material was coarsely pounded utilizing a mallet process at the department of Pharmacognosy, Sunrise University.

Hydro extract preparation- 250 g of the coarsely powdered plant material was mixed with 1.5 L of distilled water. This mixture was decocted for 4 hours at 65^0C. After this, the solution was allowed to cool for 2 hours at room temperature and then filtered. The filtrate was concentrated with a rotary evaporator water bath set at 50^0C and the yield obtained was 32g. This yielded extract was stored in a sterile wide mouthed container and stored at 8^0C in a refrigerator.

Methanol extract preparation- 250 g of the coarsely powderd plant material was mixed with 1 L of methanol and allowed to macerate on an orbital shaker for 48 hours. The solution was filtered after 48 hours of maceration and the filtrate obtained was concentrated with a rotary evaporator water bath set at 40^0C and the yield obtained was 24g. This yielded extract was stored

in a sterile wide mouthed container and stored at 8^0C in a refrigerator.

Hydro/ethanolic (50/50) extract preparation- 250 g of the coarsely powdered plant material was mixed with 1.5 L of hydroethanolic solution and allowed to macerate on an orbital shaker for 72 hours. The solution was filtered after 72 hours of maceration and the filtrate obtained was concentrated with a rotary evaporator water bath set at 50^0C and the yield obtained was 27g. This yielded extract was stored in a sterile wide mouthed container and stored at 8^0C in a refrigerator.

Ethyl acetate extract preparation- 250 g of of the coarsely powdered plant material was mixed with 1 L of ethyl acetate and allowed to macerate on an orbital shaker for 72 hours. The solution was filtered after 72 hours of maceration and the filtrate obtained was concentrated with a rotary evaporator water bath set at 70^0C and the yield obtained was 21g. This yielded extract was stored in a sterile wide mouthed container and stored at 8^0C in a refrigerator.

Induction of excisional wounds in animals- the research topic was approved by CPCSEA; Registration no- 769/2011/CPCSEA, Proposal no-287. All the animals (35 numbers) were procured from SICRA LABS PVT.LTD, KUKATPALLY, HYDERABAD. Wistar strain adult albino rats of age ranging from 2-4 months of both the sex and weighing between 250-300 g were collected and housed individually in cages. These animals were acclimatized to in-house conditions for a period of 10 days and were later used in the study. Standard in-housing conditions were nurtured like- 12 hours light and 12 hours dark cycle, 27 ± 5^0C, and Humidity was maintained at 50±10%. The animals were fed with std. Pellet diet for rats. The animals were provided with safe drinking water daily.

Preparation of simple ointments (5% w/w)- the method of preparation of simple ointments was adopted from **Cooper & Gun's, 1987**. 5 g of each of the extracts were weighed and kept aside. 95 g X 4 of White soft paraffin base was weighed and and placed into 4 different ceramic mortars that are labeled as hydro, hydroethanolic, methanolic, and ethyl acetate. Now each of the 5 g of the extracts were added into their respective mortars and triturated into ointments of concentration 5% w/w. The resultant end products were collected into labeled, sterile, wide mouthed containers.

Placebo ointment preparation- 100 g of white soft paraffin is placed in a ceramic mortar and triturated with a pestle. The soft paste obtained now is collected and labeled as placebo.

The animals were divided into 5 groups and each group consists of 6 animals. Group I- placebo group, Group II- Hydro group, Group III- Hydroethanol group, Group IV- Methanol group, Group V- Ethyl acetate group.

The animals were well accoustomed to human touch due to frequent encounter with humans. The dorsolateral flank area was decided for inducing excisional wounds in animals. A day before induction of wounds, the desired area for inducing wounds was marked with ink and the hair was removed by shaved off with a twin blade razor. The next day, the animals were anaesthesized with the help of chloroform and the shaved area was prepared with surgical spirit and povidone iodine solution. In the shaved area, a 2cm circle was marked with a marker and using a pointed foreceps, the circled area is lifted and was cut off with a scissor. Any irregular edges of the wounds were trimmed off to a final wound area corresponding to 400 mm^2. By applying pressure on the wound area, hemostasis was achieved. The final area of the wound was measured with the help of a graph sheet.

The wounds were occluded with their respective group ointments and dressed with sterile gauze. The groups were dressed with their respective ointments and the animals were left to recover. The wound dressing was changed every alternate day.

Measurement of wound healing parameters- the wound healing capabilities were estimated by measuring **rate of contraction of wound and epithelialization period**. The rate of wound contraction was measured by the following :

$$\text{Percentage wound contraction} = \frac{(\text{initial wound size} - \text{wound size on specific day})}{\text{Initial wound size}} \times 100.$$

The **epithelialization period** was assessed by noting the day on which the Eschar falls off from the wound.

RESULTS & DISCUSSION:

Rate of wound contraction: the animals of all groups were dressed up with their ointments on every alternate day and the rate of wound contraction was calculated on every 5^{th} day i.e day 5, day 10, day 15, day 20,…..

The day on which wound was induced was taken as day 0. Now the rate of wound contraction can be calculated by the formula

$$\% \text{ wound contraction} = \frac{\text{wound size on day 0} - \text{wound size on specific day}}{\text{wound size on day 0}} \times 100$$

Table 1 % wound contraction observed on day 5 in all groups

	Group-I	Group-II	Group-III	Group-IV	Group-V
Animal 1	**16.75%**	**27.75%**	**27.75%**	**31.93%**	**27.75%**
Animal 2	**14.4375%**	**23.375%**	**32%**	**27.75%**	**29.875%**
Animal 3	**14.5%**	**27.75%**	**29.875%**	**34%**	**36%**
Animal 4	**19%**	**23.5%**	**25.625%**	**27.75%**	**27.75%**
Animal 5	**16.75%**	**23.375%**	**27.75%**	**31.93%**	**36%**
Animal 6	**14.4375%**	**23.375%**	**27.75%**	**31.93%**	**34%**
Mean	**15.9792±1.8577**	**24.8542±2.2436**	**28.4583±2.1947**	**30.8817±2.5548**	**31.9375±3.9135**

It can be observed from the tables that the rate of wound contraction varies considerably between control group and ethyl acetate group i.e. there is almost double the rate of contraction in ethyl acetate treated group when compared to control group.

Table 2 % wound contraction observed on day 10 in all groups

	Group-I	Group-II	Group-III	Group-IV	Group-V
Animal 1	**43.74%**	**60.94%**	**59.375%**	**64%**	**64%**
Animal 2	**43.74%**	**56.125%**	**64%**	**56.125%**	**54.43%**
Animal 3	**39.94%**	**61%**	**57.75%**	**62.5%**	**64%**
Animal 4	**52.75%**	**57.83%**	**57.75%**	**64%**	**64%**
Animal 5	**49.25%**	**57.75%**	**62.5%**	**64%**	**57.75%**
Animal 6	**43.74%**	**56.125%**	**59.375%**	**60.93%**	**57.75%**
Mean	**45.5267±4.6223**	**58.2950±2.2020**	**60.1250±2.5715**	**61.9258±3.0944**	**60. 3217±4.2078**

Among all the 5 groups, methanol extract treated group of animals exhibited a faster wound healing process with a mean of 61.9258±3.0944%, followed by ethyl acetate extract treated group with a mean of 60.3217±4.2078%. it was also observed that the hydroethanolic extract treated group had a mean wound contraction rate of 60.1250±2.5715%, the hydro extract treated group had a mean wound contraction rate of 58.2950±2.2020%, and the placebo treated group had a mean wound contraction rate of 45.5267±4.6223%.

Table 3 % wound contraction observed on day 15 in all groups

	Group-I	Group-II	Group-III	Group-IV	Group-V
Animal 1	**68.375%**	**79.75%**	**79.75%**	**81.9375%**	**81.9375%**
Animal 2	**66.9375%**	**77.4375%**	**82%**	**79.75%**	**79.75%**
Animal 3	**66.9375%**	**79.75%**	**79.75%**	**81.9375%**	**79.75%**
Animal 4	**66.9375%**	**77.4375%**	**79.75%**	**83%**	**81.9375%**
Animal 5	**66.9375%**	**78.625%**	**82%**	**83%**	**81.9375%**
Animal 6	**66.9375%**	**77.4375%**	**80.875%**	**80.875%**	**81.9375%**
Mean	**67.1771±0.5869**	**78.4062±1.1379**	**80.6875±1.1061**	**81.75±1.2618**	**81.2083±1.1296**

On day 15 it was observed that the rate of wound contraction was high in methanolic extract treated group with a mean contraction rate of 81.75±1.2618%, which is closely followed by ethyl acetate extract treated group with a mean rate of wound contraction of 81.2083±1.1296%, which was followed by hydroethanolic extract treated group with a mean rate of wound contraction of 80.6875±1.1061%, which in turn was followed by hydro extract treated group with a mean rate of wound contraction of 78.4062±1.1379%, and finally the placebo treated group exhibited a mean rate of wound contraction of 67.1771±0.5869%.

Table 4 % wound contraction observed on day 20 in all groups

	Group-I	Group-II	Group-III	Group-IV	Group-V
Animal 1	**89.4375%**	**94.9375%**	**95.5%**	**96%**	**95.5%**
Animal 2	**88.625%**	**94.9375%**	**95.5%**	**95%**	**94.375%**
Animal 3	**88.625%**	**94.375%**	**96%**	**96%**	**94.9375%**
Animal 4	**89.4375%**	**93.75%**	**96%**	**96.5%**	**95.5%**
Animal 5	**89.4375%**	**94.375%**	**95.5%**	**96.5%**	**96%**
Animal 6	**88.625%**	**94.9375%**	**95.5%**	**96%**	**95.5%**
Mean	**89.0312±0.4450**	**94.5521±0.4799**	**95.6667±0.2582**	**96±0.5477**	**95.3021±0.5652**

On day 20 the methonolic extract treated group exhibited a highest percentage of wound contraction rate with a mean of 96±0.5652%, which is closely followed by hydroethanolic extract treated group with a mean percent rate of wound contraction of 95.6667±0.2582%, followed by ethyl acetate extract treated group with a mean percent of rate of wound contraction of 95.3021±0.5652%,. Next in line was hydro extract treated group with a mean percent of rate of wound contaction of 94.5521±0.4799%, last of the series is placebo treated group with a mean rate of wound contraction of 89.0312±0.4450%.

On day 24 complete wound contraction was achieved in all the animals of groups II to V. But group I could achieve complete wound closure only on day 31.

These observations lead to to the conclusion that all the extracts of the plant A.C possess the wound healing properties and significantly reduced the number of days required for complete wound healing in experimental animals. No mortality occurred among the animals of any study group. Also it was observed that the animals treated with the extracts of A.C were active from day 4 and proceeded with their normal activities without any hindrance from induction of experimental wounds. But as far as placebo group animals are concerned, there was a lethargic attitude observed and the feeding habits have also changed in this group of animals as they were consuming less food when compared to extracts treated groups.

Epithelialization period: the number of days required for the Eschar to fall off from the surface of the wound is considered to be the epithelialization period. The epithelialization period was observed as follows:

Table 5 Epithelializtion period for all groups of animals

	Group-I	Group-II	Group-III	Group-IV	Group-V
Animal 1	**29**	**23**	**23**	**22**	**23**
Animal 2	**30**	**24**	**23**	**23**	**22**
Animal 3	**29**	**22**	**23**	**23**	**23**
Animal 4	**29**	**23**	**22**	**22**	**23**
Animal 5	**30**	**23**	**23**	**22**	**23**
Animal 6	**30**	**23**	**23**	**23**	**23**
Mean	**29.5±0.5477**	**23±0.6325**	**22.8333±0.4082**	**22.5±0.5477**	**22.8333±0.4082**

The epithelialization was almost same for animals in groups II to V, it was 23±0.6325 for group-II animals, for group III animals it was 22.8333±0.4082, for group IV animals it was 22.5±0.5477, for group V animals it was 22.8333±0.4082. The period of epithelialization was almost same for groups II to V, but it was significantly lagging in case of group I which was 29.5±0.5477. These findings clearly validate the claims of traditional medicine that A.C can be used for enhancing wound healing activities.

CONCLUSION:

All the extracts of A.C viz., hydro extract, hydroethanolic extract, methanolic, and ethyl acetate extracts have exhibited significant wound healing properties in excisional model of wounds in rats, when the wound healing parameters were compared with those of placebo treated group. Hence it is formally considered that A.conyzoides possesses significant wound healing properties.

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