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ORIGINAL ARTICLE

Evaluation of Wound healing by Topical Administration of *Achyranthus aspera* Cream on Circular Excision Wound in Wistar Albino rats

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ABSTRACT

Skin is a complex tissue, and thus a 'full-thickness' wound results in damage to many structures, cell layers and lineages, including the epidermal keratinocyte layer (the body's barrier to the outside world), together with associated epidermal appendages such as hair follicles and sweat glands; the basement membrane (BM) that underlies the epidermis; and the dermis, which is an intricate structure that consists of fibroblasts, extracellular matrix (ECM), nerves and blood and lymphatic vessels. A wound also causes damage at the level of individual cells. Wound healing is an essential physiological process that is important for tissue homeostasis, but it can be impaired in disease and contributes to numerous pathologies. Wound healing process is particularly in skin, has been well characterized by measurement of wound area. On this basis A.aspera herbal cream was prepared by the traditional formulation and subjected for the evaluation of wound healing activity. Excision *wound* healing *model was performed using albino Wister rats. Cream was applied on the wound surface and the rate of wound healing was analysed by taking photographs of wound surface on regular intervals. Wound surface images were measured and calculated with the help of UTHSCSA image tool software. Qualitative and quantitative phytochemical analysis is also done by standard protocols. Result showed that the wound healing activity of A.aspera herbal cream prepared by the formulation method was significant (P<0.05) than the control.*

Key words: *Wound healing, Excision wound, Herbal medicine, A.aspera cream and UTHSCSA image tool*

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INTRODUCTION

Over one-third of the populations in developing countries need access to essential medicines. The major form of healthcare in the rural parts of developing countries is traditional medicine, which is mostly plant-based. WHO shows more than 80 % of the world's population depends on traditional herbal medicine for their primary health care[24]. The leaves of *Achyranthes aspera L.* (Amarenthaceae) has been utilized traditionally for the treatment of wound in various parts of India [7].*A. aspera*has diverse therapeutic uses in the folk medicinal system [16]. The proved *Achyranthes aspera L.* (Amaranthaceae) (*A. aspera*) locally termed as nayuruvi(in Tamil) is one of the plant used for medicinal purposes. It is an erect, annual herb which is a typical plant found throughout Bangladesh, India, tropical Asia and other parts of the world as weed [24] (Table 1).

Table 1. Taxonomic classification

Kingdom	Plantae
Subkingdom	Angiosperms
Super division	Eudicots
Division	Core eudicots
Order	Caryophyllales
Family	Amaranthaceae
Genus	*Achyranthus*
Species	*A.Aspera*

The review reveals that wide variety of phytochemical constituents have been isolated from the plant which possesses activities like diuretic, laxative, antiasthmatic, anti-allergic and various other important medicinal properties[24].Wound healing involves diverse phases including inflammation, granulation, fibrogenesis, neovascularization, wound contraction, and epithelialization[6]. In wound healing angiogenesis, a complex physiological process required for healing wounds and for restoring blood flow to tissues followinginjury,[27] and[26] has become a major focus of study for wound biologists. The depth of the ulcer can be checked with a tweezer, swab or thin urethral probe, which should be inserted into deepest point of the ulcer; the quantity of material required to fill the cavity can also recorded [1]. The measurement of foot ulcers or wounds is necessary for podiatrists to evaluate or assess the effectiveness of dressings, debridement, pressure-relieving techniques and footwear modifications. Measurement techniques vary greatly and may range from rulers to newly developed sophisticated methods involving computer vision technology [4].

MATERIAL AND METHODS

Preparation of plant extract

Achyranthes aspera plant was collected from campus of PRIST University and authenticated from Tamil university, Thanjavur. A known portion of whole plant sample was washed with running tab water, and dried under shadow. This sample was then macerated and packed in a soxhlet apparatus for aqueous extraction for about 24hrs. This extract was subjected to the following preliminary phytochemical analysis.

2.2Phytochemical Screening

The extracts was analyzed for alkaloids, flavanoids, pholabatannins, glycosides, saponins, lipids and fat, tannins, quinines, cardiac glycosides, coumarines acids, steroids, phytosterols, proteins and carbohydrates etc.

Chemical signature by GC-MS profiles

Gas chromatography ("GC") and mass spectrometry ("MS") make an effective combination for chemical analysis. GC analysis is a widely used confirmation test. It separates all components available in a sample and provides unique spectral output for each compound which can be qualitatively and quantitatively distinguishable (Frederic Douglas, 2010). Aqueous extract in powder form was dissolved in methanol and centrifuged for clear supernatant before analysis. About 100 mg each extract powder was dissolved in methanol and centrifuged at 5000 rpm for 10 min. Supernatant collected was given to Gas chromatography - mass spectrophotometer analysis. GC-MS analysis was performed in Periyar maniammai university, Thanjavur, Tamilnadu.

Acute toxicity studies of aqueous extracts

It is not necessary to conduct acute, single-dose toxicity studies in animals for herbal medicines being developed under IND, because they should have sufficient previous human experience(Kuei-Meng et al., 2008). These plants are in human use from the time immemorial. Recent studies show that these plants toxicity data are well established. established data for *Achyranthes aspera* (5g/Kg) [5].

Preparation of herbal cream

A 100 ml beaker was placed on a weighing balance and its weight was noted. Weigh the quantities of petroleum jelly and coconut oil as mentioned in the (Table 2). Heat the beaker in a water bath until all the ingredients have melted completely. In the next step plant extract and tween 20 is added to the mixture. This mixture was heated at 80° to 90°C. Continue stirring until you have a smooth, uniform paste. Tween 20 is added to the mixture to form a stable cream. The amount of tween 20 consumed during the experiment is calculated. (Table 2)

Table 2. Composition and percentage of cream ingredients

S.no	Ingredients	Wt in gms (%)
1	Coconut oil	**23.8%**
2	Petroleum jelly	**47.6%**
3	Tween 20	**23.8%**
4	Plant extract	**4.76%**

Animals

Two weeks old healthy, Swiss albino Wister rats of either sex were maintained under standard laboratory conditions. The experiment was conducted in CPCSEA (Committee for the purpose of control and supervision of experiments on animals) approved animal house after obtaining the prior approval from the Institutional Animal Ethics Committee (IAEC). Approval Number: PRIST / IAEC / Ph.D. BT – 02 - 2012-2013.Acclimation Period: 7 days. Housing condition: Bodyweight 200g ± 10%, Temperature 22 ± 3° C, Humidity 30 – 60 %, Diurnalcycle: Light / dark cycle 12 / 12 h, *Ad libitum* food (Normal feed – commercially available) and (RO) water (Table 3).

Table 3. Grouping and animal Distribution

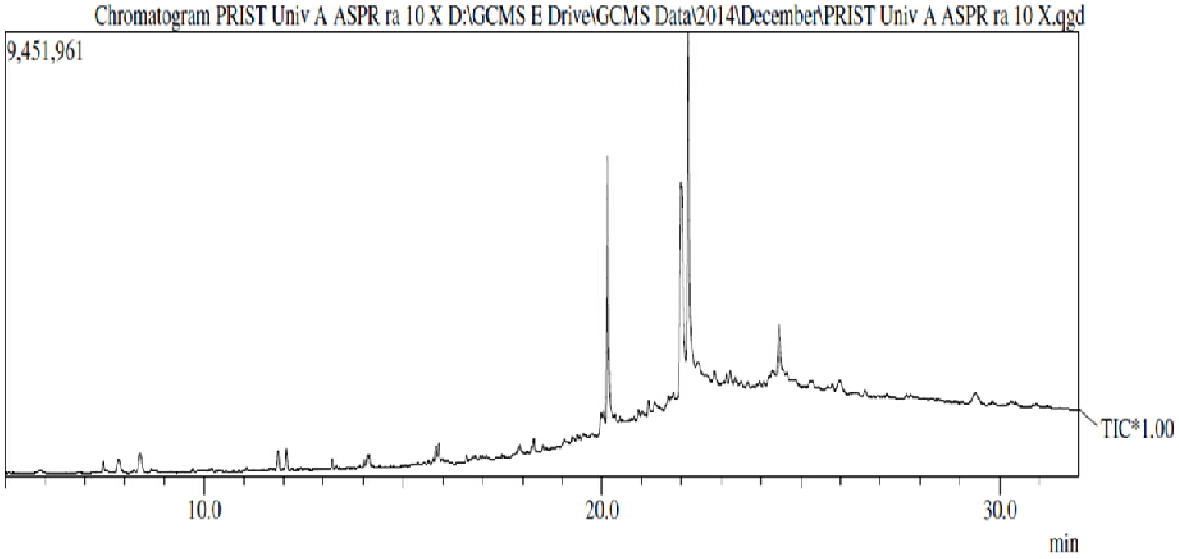
Group	Description	No.of.animals	Treatment
I	Diseased control	4	Nil
II	STD drug	4	Soframycin
III	Test drug	4	*Achyranthus aspera* cream

Experiment

Excision Wound Model

This animal model is used to observe wound contraction and wound closure time. The required animals were grouped into three. All the animals were anaesthetized with 100 mg/Kg Body weight with Phenobarbitone and the hair on the back of the rats were removed using a shaving machine. A circular wound was created on the dorsal intercapsular region of each animal by excising the skin for 5mm. The wounds were kept open. The extracts and the reference drug were applied topically twice a day on the wounds till they are entirely healed. Framycetin (SOFRAMYCIN FROM AVENTIS PHARMA) skin cream was used as standard drug. Progressive changes in the wounded areas were monitored every other day using a camera followed by photography (Fig -1). The wounded areas were later evaluated using computer aided program UTHSCSA image tool. Wound healing rates was calculated based on the reduction in wounded area [25].

Figure 1. Gas Chromatography and Mass Spectrophotometer Profile of *Achranthus aspera* Extract



Immuno-modulatory activity

After euthanasia with sodium pentobarbital (100 mg/kg), the wounds of animals were excised with a margin of normal skin around the edges of the wound on the 3rd, 6th,9th and12th days after wound was created. Then, the tissues were preserved in 10% buffered formalin. About 4 µm thickness sections were stained with Hematoxylin-Eosin and with Masson Trichrome. The semears were analyzed microscopically by pathologist and took photograph.

UTHSCSA Image Tool

UTHSCSA (University of Texas Health Science Centre in San Antonio) image tool is software used to measure the digital images upon calibration of measurements and units. It was developed by team of Don Wilcox, Brent Dove, Doss Mc David and David Greer.

Statistical Analysis

One-way ANOVA (Friedman's test) with posttest Dunnett's Multiple Comparison was performed using Graph Pad Prism version 5.00 for Windows, Graph Pad Software, and San Diego California USA**[8].**

RESULTS

Preparation of aqueous extract from experimental plants

Preparation of extract

About 15.14 grams of dried leaf powder of *A. aspera* was extracted in water using a soxhlet extractor. The yield of aqueous extract was 3.30%.

Acute toxicity studies

The crude extract did not show any toxicity in mice even at the highest dose tested. There were no changes in behavioural pattern and mortality was not observed.

Phytochemical analysis and identification of chemical signature

Qualitative phytochemical analysis

The aqueous extract of *Achyranthus aspera*revealed the presence of alkaloids, flavanoids, pholabatannins, glycosides, saponins, lipids and fat, tannins, anthraquinones, cardiac glycosides, coumarines acids, steroids, proteins and carbohydrates etc., and the result were reported .

Chemical signature by GC-MS profiles

The finger print analysis was obtained from GC-MS analysis (Fig 1).Many comopunds were obtained from the GC-MS analysis of *Achyranthus aspera* . Stearic acid, oleic acid and ascorbic acid compounds were identified from the crude extract of the sample (Fig 1.1).Stearic acid compound was obtained from Peak 1,molecular formula and molecular weight of the compound is $C_{18}H_{36}O_2$ and 284 (Fig 1.2).Oleic acid compound was obtained from Peak 2,molecular formula and molecular weight of the compound is $C_{18}H_{34}O_2$ and 282(Fig 1.3). Ascorbic acid compound was obtained from Peak 3, molecular formula and molecular weight the compound is $C_{38}H_{68}O_8$ and 652(Table 4).

Figure 1.1 Gas Chromatography and Mass Spectrophotometer Profile of *Achranthus aspera* Extract (Compound Name: Stearic Acid)

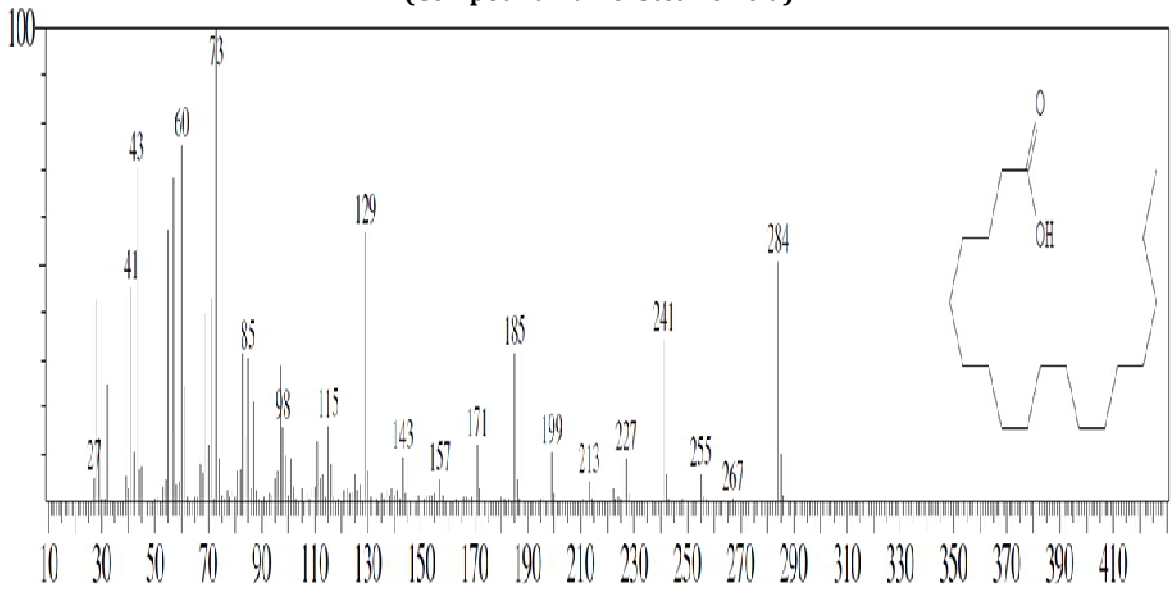


Figure 1.2. Gas Chromatography and Mass Spectrophotometer Profile of *Achranthus aspera* Extract (Compound Name: Oleic Acid)

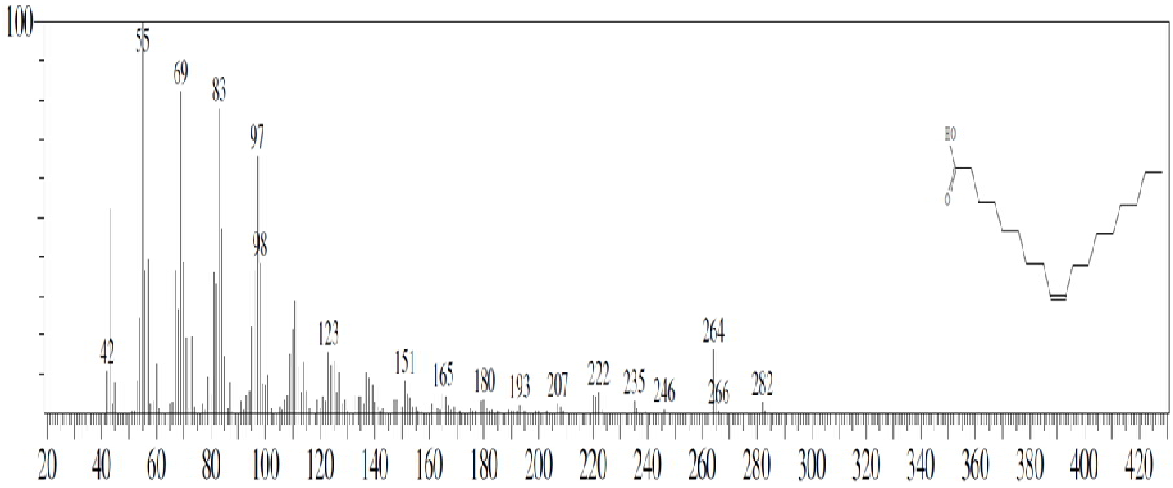


Figure 1.2. Gas Chromatography and Mass Spectrophotometer Profile of *Achranthus aspera* Extract (Compound Name: Ascorbic Acid)

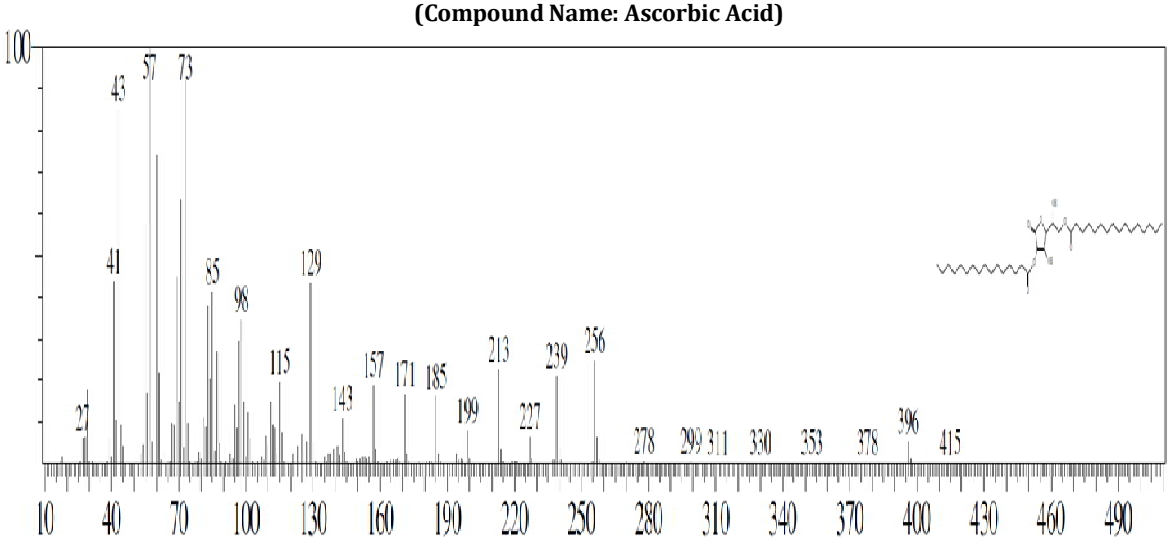
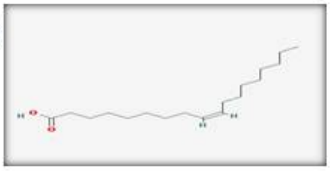
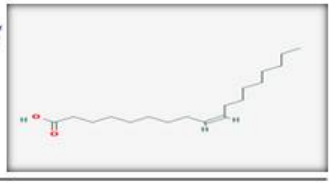


Table 4. Result of GC-MS analysis of *Achyranthus aspera*

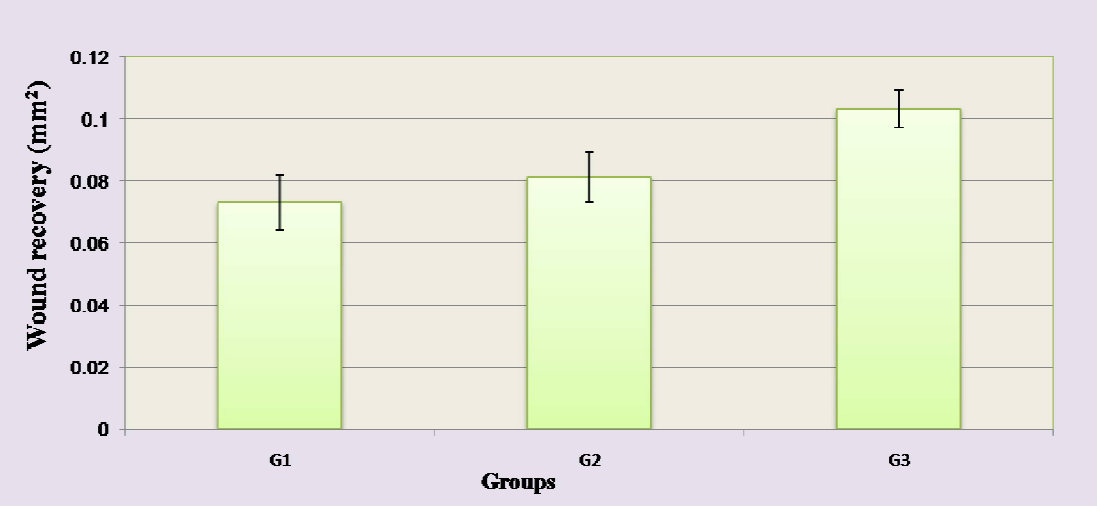
S.No	Name of the compound	Mol. formula	Mol. mass	Name of the species	Chemical structure
1	Stearic acid	$C_{18}H_{36}O_2$	284.47	*Achyranthus aspera*	
2	Oleic acid	$C_{18}H_{34}O_2$	282.46	*Achyranthus aspera*	

Wound recovery and wound contraction

Cream prepared by this experiment applied topically to analyze the efficacy profiles of wound healing activity. Results observed during the study were represented. It shows that rate of recovery in wound healing activity of the Test Drug (Group III) and STD Drug (Group II) and Control (Group I). On

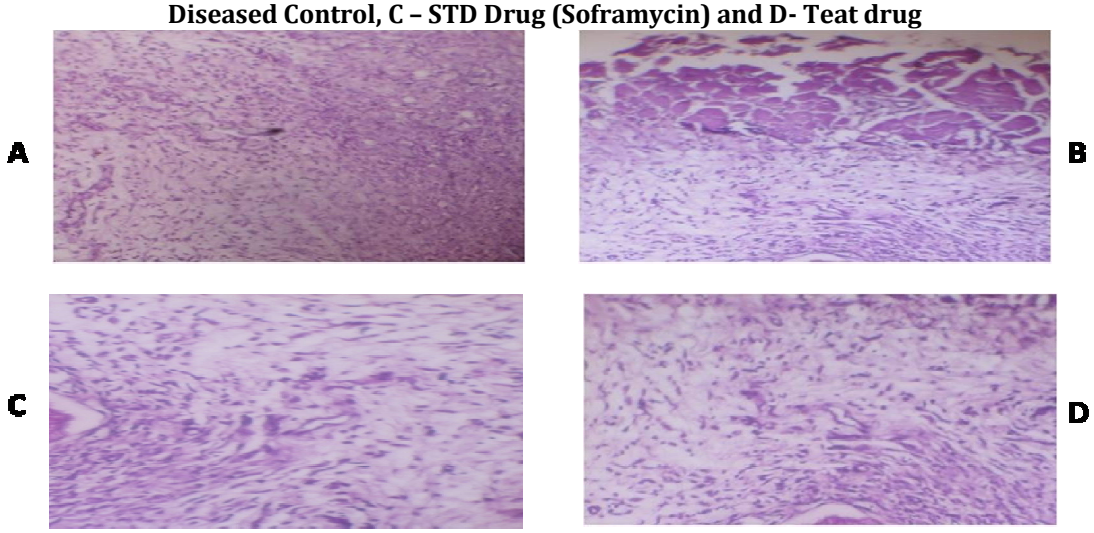
comparison of Test Drug and STD Drug there was a significant (P<0.05) improvement in Test Drug than the STD Drug and Control animals. (Figure: 2)

Figure 2. Wound healing Activity of *Achyranthus aspera* G1 –Diseased Control G2 – STD Drug (Soframycin) and G3 –Test Drug



Immunomodulatory activity – Histopathological studies

Fig 3. Histopathological Studies – Development of cells in wounded skin A- Non-wounded skin, B – Diseased Control, C – STD Drug (Soframycin) and D- Teat drug



Histopathological evaluation of dermal section revealed that when compared to vehicle control group, topical application of *Achyranthus aspera* cream on dermal wounds increased the infiltration of inflammatory cells, fibroblasts and re-epithelization with moderate vascularity Treatment with standard drug (soframycin) led to marked increase in vascularity, fibroblast number and re-epithelization.

DISCUSSION

In the last two decades scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicine[16].Physiological and environmental factors influence the healing process and affect the duration and quality of wound healing. Many isolated natural products and medicinal plants with potential anti-inflammatory effects could be used to promote wound healing.

Achyranthus aspera influences wound healing,anti-inflammatory and anti-microbial properties. A combination of these properties is also possible in some of the medicinal plants used in wound care. Conversely, other reports have shown that linoleic acid and oleic acid could promote wound healing in a dose-dependent manner, increasing secretion of vascular endothelial growth factor (VEGF- α) and interleukin-1 beta (IL-1β), and decreasing TNF-α, consistent with proposed proangiogenic and anti-inflammatory effects of linoleic acid and oleic acid [20].

There are many methodologies available for wound healing activity in the world. Applying cream on an open wound is simple and convenient method for wound treatment. Usage of this cream is not difficult and it does not involve complicated dressing procedures [21][29]reported that topical application of essential fatty acids in pressure ulcers, surgical wounds and skin burns could promote wound healing.
The untreated group showed a constant recovery on all days during the experiment and it took 12 days for complete wound healing. The test cream and standard cream showed significantly minimum duration for complete recovery against the untreated group. In histopathology, development of cells from nucleus were analysed [11].The importance of cross-linking between collagen molecules and physical weave of collagen fibers in contributing to the tensile strength of wound is well acknowledged. Increase in tensile strength may be due to increase in collagen concentration per unit area and stabilization of fibers. *A. aspera* treated animals showed collagen deposition, fibroblast proliferation and formation of epidermis [2]. The use of fatty acids to treat skin problems is studied since 1929, several studies have reported on faster wound healing observed a healing effect on skin wounds after local application of sunflower oil. In this study we have identified fatty acid compounds (stearic acid, oleic acid and linoleic acid) form *Achyranthus aspera* extract which improved wound healing in animal models.

CONCLUSION

The present study discovered that the tested *Achyranthus aspera* cream has the significant wound healing activity. The wound contraction and wound recovery capability of this formulation was found to be higher than the standard drug available in the current commercial market. The mechanism of this accelerated wound healing might be by the presence of one or more phyto-constituents in this formulation. Hence, the research related to these molecules and their role in the healing process to be subjected for further study.

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