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Research Article

# Efficacy of Ethanolic Extracts for Two Plants on Wound Healing in Diabetic Albino Rats

Girish Kumar Vyas[1], Hariom Sharma[2], Bhupendra Vyas[3], Anil Sharma[4], Manmohan Sharma[5]

[1]Research Scholar, [2]Professor, [4,5]Asscociate Professor, School of Pharmaceutical Studies, Faculty of Health Sciences, Dr K.N. Modi University, Newai, Rajasthan, India.
[3]Head & Associate Professor, Department of Pharmacognosy, BN Institute of Pharmaceutical Sciences, Bhupal Nobles' University Udaipur, Rajasthan, India.
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## I N F O

**Corresponding Author:**
Girish Kumar Vyas, School of Pharmaceutical Studies, Faculty of Health Sciences, Dr KN Modi University, Newai, Rajasthan, India.
**E-mail Id:**
girishvyas10@gmail.com
**Orcid Id:**
https://orcid.org/0000-0003-3753-0569
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## A B S T R A C T

*Background:* Wounds in diabetic patients are critical to treat. The use of allopathic drugs has various side effects, hence new natural sources for therapies are vital.

*Materials and Methods:* This research aimed to determine the possibility of wound healing by the ethanolic extract of *Eucalyptus globulus* leaves and *Curcuma longa* rhizomes. Albino rats were given oral administration of various plant extracts in order to find out how plant extracts affected diabetic rats' wound healing.

*Results:* These plant extracts greatly accelerated the wound's ability to heal in diabetic or non-diabetic rats ($p < 0.05$). This increases the proliferation and migration of the fibroblast resulting in a faster healing process. These plant extracts do not cause cytotoxicity. After treatment, less amount of cell death was found for both plant extracts. The results were found to be positive.

*Conclusion:* This research indicates that the ethanolic extracts of both plants have the potential to be used as novel therapeutics for diabetic patients' wound healing.

**Keywords:** Diabetes, Healing, Extraction, Contraction, Epithelialization

## Introduction

Natural ingredients have demonstrated the ability to create new opportunities for pharmacological, nutraceutical, and agrochemical products. The most frequent sources of biologically active molecules include plants, bacteria, fungi, and marine natural products.[1] One of the richest plant-based traditions in the world is found in India. Examining medical indications according to the compounds they contain revealed that natural products and very similar medications are utilised to treat 87% of all categorized human disorders, including their use as anticancer, anticoagulant, antiparasitic, and immunosuppressive medicines.[2] Herbs are being used for centuries by people all over the world. They may be the oldest "evidence-based medicine". Herbal medicines have been observed to cure all kinds of human diseases.[3] Traditional healers have a broad knowledge of medicinal plants and their healing properties. Medicinal plant usage formed the backbone of medicine in many rural communities for treating ailments with varying severity.[4] Most people use herbal preparations with

modern medicines. WHO noticed that 80% of people in the world depend on complementary and alternative systems of medicine (CAM); herbal medicines have major contributions to some aspects of primary healthcare needs. According to WHO, twenty-one thousand plant species have been identified to have therapeutic value.[5]

Diabetes is a highly widespread and common disease. Globally, it is thought to impact 381 million adult populations.[6] The number of undiagnosed cases is estimated to be approximately 0.175 billion.[7] Its prevalence is constantly increasing. 0.011 billion people were diagnosed with diabetes in the year 2001, and it is expected to increase to 0.029 billion by 2050.[8] An increase in the number of cases of diabetes in the future has been predicted by International Diabetes Federation (IDF).[9] Only about 10-15% of diabetic people are suffering from type one diabetes whereas, people suffering from type 2 diabetes constitute approximately 85-90% of diabetic patients.[10]

Various drugs from the synthetic class are available for the treatment of Diabetes Mellitus (DM). Despite this, about 80% of diabetic patients die due to vascular complications. If certain herbs are selected to develop polyherbal formulations (PHF) in palatable or oral dosage forms, it will benefit people on multidrug treatment such as antidiabetic, antihyperlipidemic, wound healers etc.

The goal of the current research is to offer a research-based perspective on the efficiency of several herbal plants in the medication and treatment of diabetic wounds. The usage of these herbs in traditional medicine served as the main factor for their selection. Most importantly, the ancestral communities used a vast array of plants to treat wounds, cuts, and skin conditions. Two plant species were chosen based on their ability to repair wounds both in vivo and in vitro. The selected plants have been shown in Figure 1.

Eucalyptus comes under the Myrtaceae family. This genus contains more than or equal to 900 different species and sub-species. Leaves, fruits, buds, and bark of eucalyptus can be used for the extraction of oil and have lethal effects on bacteria. They have oxidative and neoplastic properties and can work on inflammation.[11,12] It is also used in the case of upper respiratory infections like common cold, influenza, and sinus congestion.[13,14]

*Curcuma longa* is an herb which is available throughout the year. It is a member of the genus Curcuma, and Zingiberaceae is the family. The rhizome part of the plant has got most of its pharmacological properties and is widely used as an anti-diabetic,[15-17] hypolipidaemic,[15-18] anti-inflammatory,[17,18] anti-diarrhoea,[16] hepatoprotective,[15,16] and anti-asthmatic.[17] It is also widely used in cosmetic products.[19] The fresh extract of turmeric is believed to have anthelmintic property.[20]

This research was designed to assess the efficacy of ethanolic extracts of Eucalyptus leaves and *Curcuma longa* rhizomes in wound healing in diabetic albino rats. The cytotoxic and lethal effects of these plant extracts on human fibroblast cell lines were also examined in this study.

## Methods

### Identification and Collection of Plant

Leaves of *Eucalyptus globulus* and rhizomes of *Curcuma longa* were collected in July 2021 and October 2021, respectively from nearby locations in Newai. The herbarium department of Rajasthan University authenticated them by receiving voucher specimens (RUBL21218 and RUBL21219).

### Preparation of the Ethanolic Extract

Leaves of *Eucalyptus globulus* and rhizomes of *Curcuma longa* were cleaned, dried, and blended before being submerged in 100% ethanol. For 3 days at 25-28 °C temperature, dried leaves were submerged in ethanol (1:10 weight/volume ratio) with constant shaking.[21] To eliminate the solid contaminants, Whatman filter paper was used and it was dried with the help of a rotary evaporator which was set to 50 °C at low pressure.[22] The unprocessed extracts were kept at -20 °C in sealed containers.

### Wistar Albino Rats

Wistar albino rats were selected for this study. Thirty-six rats were used for this study. No lethality was reported.

### Diabetes Generation and Collection of Samples

Diabetes was brought on by injection which contains streptozotocin (strength 50 mg/kg body weight). It was given to rats by intraperitoneal route. It takes at least 3 days to cause diabetes. After three days, diabetes was determined by using glucometer strips to measure the level of blood glucose from the vein present in the tail (measured by freestyle freedom lite device by Abbott Pharmaceuticals).[23] Diabetic rats were defined as rats having a glucose level of more than 200 micrograms/ml in blood.[24] The blood was collected through various methods of collection. Sampling of blood was done as depicted in Figure 2.[25]

### Animals and Dose Selection for Wound Healing Activity

The anti-inflammatory and healing effects of ethanolic extracts of *Curcuma longa* rhizomes and *Eucalyptus globulus* leaves on wounds were examined in rats (180-200 g). Researchers chose male Wistar albino rats because they thought that female rats responded differently to the same stimuli during the female reproductive cycle due to hormonal changes. This has been proven by several experiments. Institutional Animal Ethical Committee

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(Registration No. 838/PO/Re/S/04/CPCSEA) provided approval to conduct the experiments. Each animal was housed in a typical plastic and stainless-steel cage there.

Room temperature was maintained at 24 °C for rats. Day and night cycle was also maintained from light to dark (12 hours each) according to the biological clock of the animal. The natural environment was maintained for better easy result finding. The Institutional Animal Ethics Committee of Dr K. N. Modi Institute of Pharmaceutical Education and Research granted approval for the study after taking the presentation and viva (KNMIPER/2022/01).

Rats were given a specific time to become used to the new place before studies began. They were kept apart during the study using different cages. Blood samples were obtained using a variety of techniques (Figure 2). The serum was subjected to additional biochemical assays. The doses of plant extracts were given according to the LD50 in rats (Eucalyptus leaves extract can be used at a dose of 5000 mg/kg body weight and *Curcuma longa* rhizomes at a dose of 5000 mg/kg weight).[26,27]

## Wound Creation

Rats were sedated by inhaling diethyl ether. An electrical hair trimmer (Phillips hair trimmer) was used to shave the dorsal portion, and a 10-mm biopsy punch (Disposable Biopsy Punch) was used to remove the skin.[25] The closing of wound was measured until the complete wound was closed or epithelized. Wound creation and closing were measured systematically as shown in Figure 3.


**Figure 1(A, B).Eucalyptus Globulous (Tree and Leaves) (C, D). Curcuma Longa (Plant and Rhizomes)**


**Figure 2.Sites for Collection of Blood. (A).Caudal Vein (B).Femoral Trigone (C).Sinus Retro Orbital Blood Collection**

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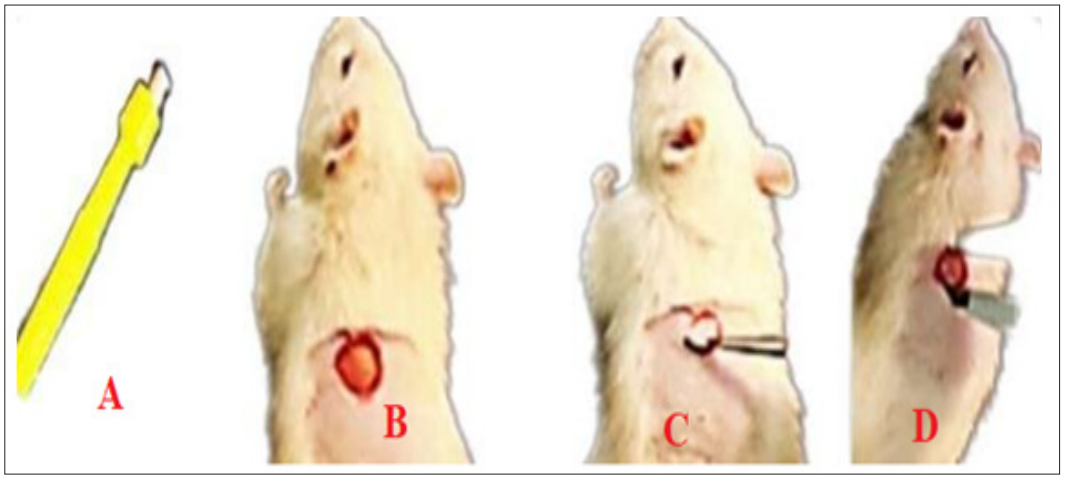


**Figure 3.Creation of Wound. (A).10-mm Punch (B).Wound of Skin (C).Removal of Skin (D). Measurement of Created Wound**

The main factor that was considered was contraction. The contraction % was calculated using the following formula:

$$\text{Contraction \%} = \frac{\text{(Initial wound diameter – day-by-day wound diameter)}}{\text{Initial wound diameter}} \times 100$$

Initial wound diameter is the diameter of the wound which was initiated for the study on day 1.

The duration of complete contraction was determined by counting the days until the scab disappeared, and there was no presence of an open naked wound.[28]

### Experiment Design

Rats were divided into two primary categories: diabetic (group 4-6) and non-diabetic (group 1-3) (Table 1). Three subgroups of five animals each constituted each main group (n = 5) as follows:

- Suspension of CMC in a concentration of 0.05% in normal saline was given to Group 1 which was known as the control group.
- Vitamin E (100 mg/kg body weight) was given to Group 2 (positive control).
- Group 3 received a dose of the extract of the plant (200 mg/kg body weight).

For continuous 15 days, equal doses of both plant extracts (5 ml/kg body weight) were given. The wounds were made as described by Mieczkowski et al.[29] On days 0 through 15, wound areas were gauged with a caliper. Digital photography was used to capture the images of the injuries. The variation in the closing of wounds was observed from day 0 to day 15th.

### Fibroblast Migration and Proliferation in an In Vitro Study of the Effects of Ethanolic Plant Extract on Wound Healing

1. **In Vitro Cytotoxicity Test:** Various extracts obtained from plants were tested in a biochemistry lab for cytotoxicity against a fibroblast cell line at Dr K. N. Modi University. The cells were grown in a medium known as Dulbecco's MEM. They were grown at 37 °C in 5% $CO_2$. Cells were fractionated after three to four days by withdrawing the liquid culture, separating the cells with 2 mL of trypsin, and then adding fresh media.[30] Different quantities of the plant extract (10-100 g/mL) were examined. Triplicates of each experiment were run. The IC50 was determined by using the dose-response curve. A 24-well plate was used to test the cytotoxicity of various plant extract concentrations (10, 25, 50, and 100 g/mL). Changes in cell morphology and shape were treated as cytotoxic markers. Cell lines lacking extract were utilised as a negative control.[31]

   **Dulbecco's Modified Eagle Medium:** It is also known as DMEM. It contains high glucose without L-GLT with HEPES. It also contains sodium bicarbonate and sodium pyruvate. The medium was sterilised and filtered carefully with a 0.2-micron filter. It was stored at 2-8 °C.

2. **Antiproliferative Assay:** After the investigated substances were applied to cell lines, the hindrance to cell proliferation was measured using the G-banding stain method. The wounds were cleaned with 0.5 mL SPB (saline phosphate buffer) after aspiration of the media and fixed by ethanol at 36 °C for nine minutes. Before washing and fixing, the plates were dried for 2 minutes. Giemsa stain (1:10 in SPB) was used to stain each well for 9 minutes. The dye was aspirated followed by a deionized water rinse. The antitumor activity was assessed using an ELISA reader at 630 nm by microplate after the bound dye was removed with 0.1 N HCl (Instrument ELX 800). The proportion of live cells in the tested sample as compared to the control was a measure of cell mortality.[32] The formula listed below was used to compute mortality.[33]

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$$\text{Mortality (\%)} = 100 - \frac{\text{Controlled absorbance - Treated cells Absorbance}}{\text{Controlled absorbance}} \times 100$$

The wound-healing effects of *Eucalyptus globulus* leaves ethanolic extract on different groups (diabetic and non-diabetic albino rats) are shown in Table 2 (diameter of wound and percentage contraction). Table 3 shows the wound contractions in non-diabetic and diabetic albino rats.

The wound-healing effects of *Curcuma longa* rhizomes ethanolic extract on different groups (diabetic and non-diabetic albino rats) are presented in Table 4 (as diameter of wound and percentage contraction). Table 5 shows the wound contractions in non-diabetic and diabetic albino rats.

3. **Migration Assay:** The wound was showing a density of 35000 cells per wound; fibroblast was developed in the wound until it got closed. Juszczak et al. mentioned in an article that a scratch was made in each well with a 200 L pipette tip.[34] The media was removed, and cells were washed with SPB, prior to the addition of medium to which suitable doses of each plant extract were added in concentrations of 10 and 20 g/ml (saline phosphate buffer). An optical microscope was used to analyse various regions along each well's scratches at 0, 12, 24, and 48 hours after the damage was induced. Optical microscopy was used to investigate several places along the scratches of each well. The distance between the scratch's edges was measured in comparison to untreated control cells and was reported as a percentage of area closure.[35]

## Statistical Analysis

The information was displayed as means and standard deviation (SD). The statistical significance of differences between groups was calculated using Graph Pad Prism version 7 with a significance level of p = 0.05.

**Table 1.Oral Extract Treatment given to the Rats as per their Groups**

| Group No. | Category | Treatment Given |
|---|---|---|
| 1. | Non-diabetic rats | Normal saline |
| 2. | Non-diabetic rats | Vitamin E (Tocopherol) (100 mg/kg) |
| 3. | Non-diabetic rats | Plant extract (200 mg/kg) |
| 4. | Diabetic rats | Normal saline |
| 5. | Diabetic rats | Vitamin E (Tocopherol) (100 mg/kg) |
| 6. | Diabetic rats | Plant extract (200 mg/kg) |

**Table 2.Effects of Eucalyptus globulus Ethanolic Extracts on Wounds of Rats**

| Day | Case | Control Negative | | Tocopherol (100 mg/kg) | | Eucalyptus Extract (200 mg/kg body weight) | |
|---|---|---|---|---|---|---|---|
| | | Diameter of wound (mm) | Wound contraction (%) | Diameter of wound (mm) | Wound contraction (%) | Diameter of wound (mm) | Wound contraction (%) |
| 0 | Non-diabetic | 10 | 0 | 10 | 0 | 10 | 0 |
| | Diabetic | 10 | 0 | 10 | 0 | 10 | 0 |
| 3 | Non-diabetic | 9.2 | 8 | 8.25 | 17.5 | 8.1 | 19 |
| | Diabetic | 9.3 | 7 | 8.3 | 17 | 8.15 | 18.5 |
| 6 | Non-diabetic | 8.25 | 17.5 | 7.7 | 23 | 7.1 | 29 |
| | Diabetic | 8.4 | 16 | 7.9 | 21 | 7.1 | 29 |
| 9 | Non-diabetic | 7.65 | 23.5 | 6.25 | 37.5 | 6.4 | 36 |
| | Diabetic | 7.9 | 21 | 6.3 | 37 | 6.1 | 39 |
| 12 | Non-diabetic | 6.8 | 32 | 3.5 | 65 | 4 | 60 |
| | Diabetic | 6.8 | 32 | 3 | 70 | 4.1 | 59 |
| 15 | Non-diabetic | 6 | 40 | 1.5 | 85 | 2.1 | 79 |
| | Diabetic | 6 | 40 | 2 | 80 | 2.5 | 75 |

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**Table 3.Wound Contractions by Ethanolic Extracts of Eucalyptus Globulus Leaves in Non-diabetic and Diabetic Albino Rats**

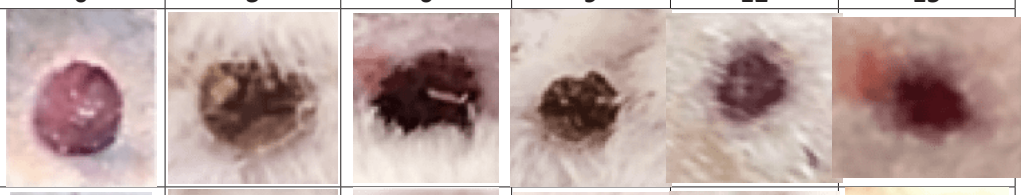
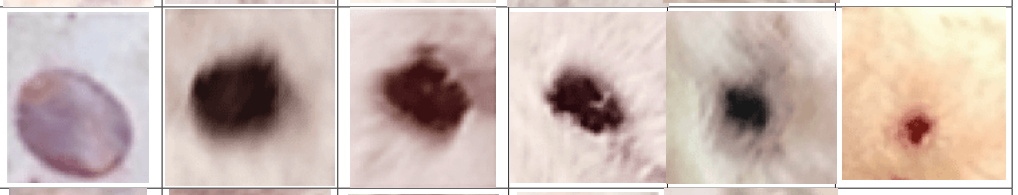
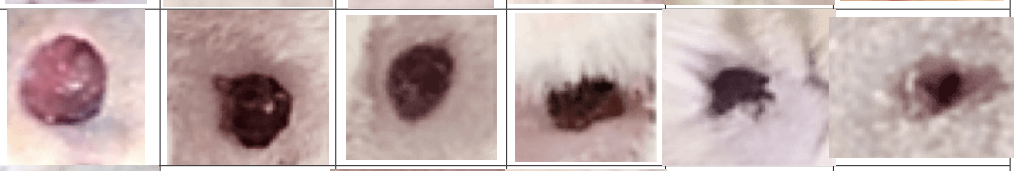
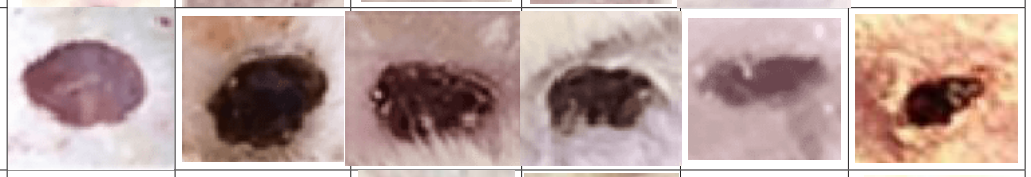
| Groups | Treatment | Days | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 | 15 |
| Non-diabetic A | Normal saline |  | | | | | |
| | Vit. E |  | | | | | |
| | Eucalyptus extract (200 mg/kg) |  | | | | | |
| Diabetic B | Normal saline |  | | | | | |
| | Vit. E |  | | | | | |
| | Eucalyptus extract (200 mg/kg) |  | | | | | |

**Table 4.Effects of Curcuma Longa Ethanolic Extracts on Wounds of Rats**

| Day | Case | Control Negative | | Tocopherol (100 mg/kg) | | Eucalyptus Extract (200 mg/kg body weight) | |
|---|---|---|---|---|---|---|---|
| | | Diameter of wound (mm) | Wound contraction (%) | Diameter of wound (mm) | Wound contraction (%) | Diameter of wound (mm) | Wound contraction (%) |
| 0 | Non-diabetic | 10 | 0 | 10 | 0 | 10 | 0 |
| | Diabetic | 10 | 0 | 10 | 0 | 10 | 0 |
| 3 | Non-diabetic | 9.1 | 9 | 8.2 | 18 | 8.1 | 19 |
| | Diabetic | 9.2 | 8 | 8.4 | 16 | 8.35 | 16.5 |
| 6 | Non-diabetic | 8 | 20 | 7.3 | 27 | 7.42 | 25.8 |
| | Diabetic | 8.3 | 17 | 7.4 | 26 | 8.42 | 15.8 |
| 9 | Non-diabetic | 7.5 | 25 | 5 | 50 | 6.1 | 39 |
| | Diabetic | 7.8 | 22 | 5.2 | 48 | 6.23 | 37.7 |

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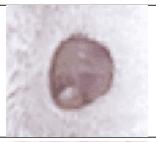
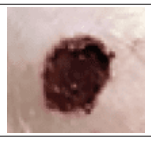
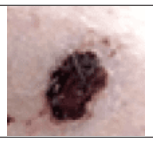
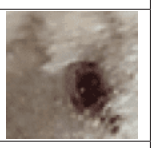
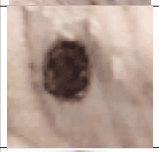
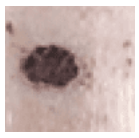
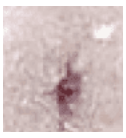
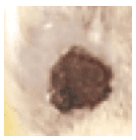
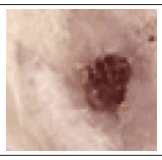
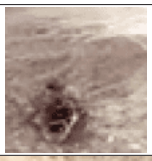
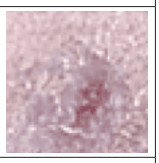
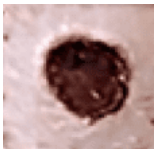
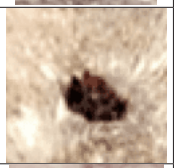
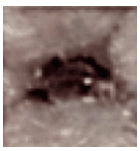
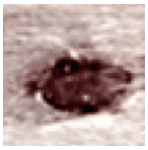
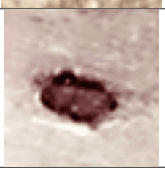
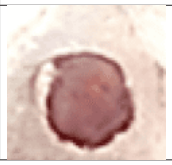
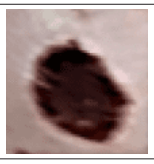
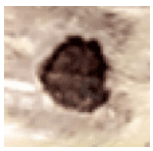
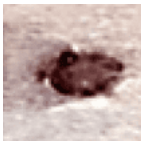
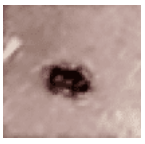
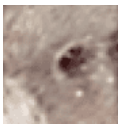
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | Non-diabetic | 7 | 30 | 4.1 | 59 | 4.25 | 57.5 |
| | Diabetic | 7.5 | 25 | 4.2 | 58 | 4.35 | 56.5 |
| 15 | Non-diabetic | 5.8 | 42 | 2.5 | 75 | 2.4 | 76 |
| | Diabetic | 6 | 40 | 2.6 | 74 | 2.65 | 73.5 |

**Table 5.Wound Contractions by Curcuma longa Ethanolic Extract in Non-diabetic and Diabetic Albino Rats**

| Groups | Treatment | Days | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 | 15 |
| Non-diabetic | Normal saline |  |  |  |  |  |  |
| | Vit. E |  |  |  |  |  |  |
| | Curcuma extract (200 mg/kg) |  |  |  |  |  |  |
| Diabetic | Normal saline |  |  |  |  |  |  |
| | Vit. E |  |  |  |  |  |  |
| | Curcuma extract (200 mg/kg) |  |  |  |  |  |  |

## Results and Discussion

### Toxicity Study

Various research articles were used to confirm the lethal concentrations of multiple extracts of *Eucalyptus globulus* and *Curcuma longa*. A dosage of 5000 mg/kg body weight was found to be safe for both extracts.[26,27]

### Effect of Orally Administrated Plants Extracts on Cutaneous Wound in Diabetic Rats

The *Eucalyptus globulus* leaves ethanolic solution (200 mg/kg body weight) had significant ($p < 0.05$) effects on the healing of wounds. This healing was compared with the Vitamin E-treated group. The ethanolic solution of *Curcuma longa* rhizomes (200 mg/kg body weight) showed much better healing effects as compared to *Eucalyptus globulus*.

In therapeutic treatment, wounds are typically viewed as a severe concern since they represent a clinical problem. Standard wounds heal in a few days, but wounds that do not heal easily are far more problematic due to societal and financial considerations, hence finding novel natural products that perform better and are cheaper is crucial.[36,37] Diabetes can also delay recovery or raise the risk of infection in the area that was injured, necessitating a lengthy hospital stay.[38] In contrast to the majority of past wound-care medications, which are applied externally as ointments, in this study, extracts of plants were taken orally. Improvement of new therapeutic agents employing extracts from medicinal plants was inspired by the current investigation. According to the findings, the untreated group heals wounds to some extent, which may be the result of auto-immunity.[39] However, ethanolic extracts of

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plants used on selected groups in this study revealed a better and faster healing process. In this process, during the treatment, no scratching, discomfort, or irritation was observed at the site of the wound. An activity for better healing of wounds was reported by Sen et al. in 2019. They stated that extracts are also responsible for the contraction and collagen formation.[39,40] The current findings showed that plant extracts promote quick healing of wounds and accelerate mitigation, which supports this interpretation. *Curcuma longa* rhizomes ethanolic extract showed very good results and thus can be preferred as an alternative treatment for diabetic wounds. Some studies reported that the fruit aqueous extract of *Curcuma longa* showed similar results for wound management.

It was reported that Globularia alypum L extract in a hydroalcoholic solvent was rich in flavonoids and phenylethanoids and had antioxidative property.[41] Such antioxidative constituents help in the healing of wounds. An ethanolic mixture of constituents of Eucalyptus leaves showed less healing capability as compared to Curcuma rhizomes.

### In vitro Fibroblast Proliferation and Resettling in Healing of Wound

The results reported the IC50 analysis and the cytotoxicity of extracts of plants at a strength of 100 µgm/ml. *Eucalyptus globulus* had the lowest IC50, while *Curcuma longa* had the lowest cytotoxicity. The score method which demonstrated the resettling of the cell line of fibroblast after the score assay method and at the time of culturing at 10 micro-gram/mL and 20 micro-gram/ml of extract of plants, was used to study the capacity of extract of plants to promote proliferation of fibroblast in vitro and resettling. After 48 hours of treatment, 20 microgram/mL of *Curcuma longa* extract produced the strongest stimulatory effect, followed by *Eucalyptus globulus*.

The growth and migration of cells are crucial to the healing process. The scratch method is generally used for in vitro investigations on the healing of wounds.[35] As protein is necessary to restore the structure, power, and integrity required to restore the architecture and role of damaged tissues, the fibroblasts are crucial for protein settling which is required in the reformation of skin after getting injured or damaged.[42] Cellular replication and migration are the fibroblasts' main activities in the early proliferative phase. However, a large number of fibroblasts start to make and increase the amount of collagen in the wounded tissues on the third day following the injury, which characterises the wound healing activity.[43] The findings of this study demonstrated that the ethanolic extracts of *Curcuma longa* and *Eucalyptus globulus* significantly induced fibroblast migration. These findings corroborate and reinforce the current in vivo investigations which demonstrated that ethanolic extracts of *Eucalyptus globulus* and *Curcuma longa* strongly stimulated fibroblast migration. The medicinal efficacy of these herbal plants in healing injured cells has been revealed in the current study.

## Conclusion

The medicinal advantages of using phytoconstituents of *Eucalyptus globulus* leaves and *Curcuma longa* rhizomes were the focus of this investigation. This study is significant since it established and reported, for the first time, the advantages of plants for wound healing in diabetic and non-diabetic rats. Ethanolic extract of Curcuma and Eucalyptus both displayed strong wound-healing abilities. Furthermore, the extracts were safe, and the rats showed no signs of systemic toxicity.

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