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Healing potentials of Marigold flower *(Tagetes erecta)* on full thickness dermal wound in caprine model

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ABSTRACT

Objectives: In the modern days multiple drug resistance has been developed against many microbes due to the random use of existing antimicrobial drugs in the treatment of infectious diseases. This paves the way for reconsidering traditional medicine; hence we have carried out to evaluate the wound healing potentials of Marigold flower *(Tagetes erecta)* in the surgical wound model in black Bengal goats.

Methods: A total of sixteen surgical wounds were made in eight goats under proper restraint and analgesia. Wounds were topically treated with Marigold flower paste (Group A) and normal saline (Group B, control). Post-treatment information was recorded from day 1 to day 21. Planimetric features such as swelling of the wound area, elevation of suture line, and length of the wound were monitored. Histopathological and *in vivo* anti-microbial studies were also investigated.

Results: Results revealed that aqueous paste of *Tagetes erecta* flower modulated inflammation and promoted wound contraction leading to earlier healing than those with saline. Histological findings highlighted the normal cutaneous architecture of the marigold treated wound more than that appeared in the saline-treated wound. The antibacterial study revealed that the aqueous paste of marigold flower was highly effective against *Staphylococcus aureus* which is the ubiquitous bacterial pathogen both in humans and animals.

Conclusions: These results, thus, demonstrate that the aqueous paste of *Tagetes erecta* flower possesses wound healing activities and it could be a potential candidate for the treatment of dermal wounds by topical application.

Keywords: *Tagetes erecta*, wound healing, histopathology, antibacterial efficacy, goats

Wound healing or repairing is a process of replacing devitalized and missing cellular structures and tissue layers. This process can be categorized into four phases i.e. *hemostasis, inflammatory,* proliferative, and remodeling. The process involves growth factors, cytokines, extracellular matrix, and relevant enzymes along with the differentiated cells that modify molecular components of the matrix [1]. The initial events of the wound healing mechanism occurred smoothly under favorable condition but the healing rate may differ among species, tissues, nature of infection or infestation, even between sites of the same tissue as well as pathological features of tissues [2, 3].

The trend of using herbal medicines are not new, many herbal preparations are extensively utilized in rural areas. The uses of herbal products are not only because of the easier availability in local areas but also herbal products have no side effects on the body as

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well as this is economic. The beneficial effect of herbal medicine typically results from the combination of secondary metabolites produced within the herbs like flavonoids, tannins, glycosides, alkaloids, gums, etc. The resistance of organisms against antibiotics is a matter of global concern and using herbal products may help to cope up with this problem. Plant-derived antimicrobials have received tons of attention recently [4]. The current threat of antimicrobial resistance and easy availability of Marigold flower (*Tagetes erecta* Linn) has drawn attention to use for therapeutic purposes.

Marigold has about 56 species but two basic types are *Tagetes erecta* Linn and *Tagetes patula* Linn. It is a good remedy for inflamed skin, open fresh wounds, and a range of microbial and parasitic infections [5]. Phytochemical constituents of Marigold indicate the presence of carotenes, flavonoids, resin, saponin, sterols, triterpenes, bitter glycosides, volatile oils, calcium, and alkaloids [5]. It shows different pharmacological activities like anti-bacterial, anti-microbial activity, hepatoprotective, insecticidal, mosquitocidal, larvicidal, nematocidal, wound healing, antioxidant, and analgesic [6]. In a combination of some substances with the high resin, content provide Marigold a powerful anti-inflammatory action.

Among different species of Marigold, *Tagetes erecta* is the most common and widely available in Bangladesh. Different study at a different place on Marigold was reported but with this species, there is still limited study. Based on having active constituents and therapeutic potentials mentioned above and easy availability of the plants in the country, we have conducted the study to investigate the healing potentiality of Marigold *(Tagetes erecta)* on full-thickness dermal wound in goat.

METHODS

Animals

This study was reviewed and approved by the Animal Experimental Ethics Committee (AEEC) of the Department of Surgery and Obstetrics, Bangladesh Agricultural University (Permission number: AEEC/DSO-BAU/01/2017) and strictly obeyed the rules of animal experiment ethics to reduced number as well as the suffering of animals. The research was conducted on eight healthy goats with body weight ranged from 8-10 kg with irrespective of sexes. The animals were kept in the Veterinary Teaching Hospital (VTH), BAU under standard housing system and veterinary monitoring with no restrictions on water and food. They were acclimatized to the experimental conditions for 14 days. Deworming and vaccination against Peste des petits ruminants (PPR) virus were done. The overall health of the animals was monitored before and throughout the study.

Preparation of Marigold Petal Paste

Fresh flower petals were collected from the premises of BAU. The petals were properly cleansed with distilled water and blended with sterile double distilled water to make homogeneous paste. The procedure was followed during each application on the wound.

Surgical Wound Model

All the wounds were created aseptically considering animal welfare to provide minimal discomfort to the experimental goats. Local analgesia was done before wounding to minimize the pain sensation. A total of sixteen full-thickness cutaneous wounds of 2.0 cm length and 0.5 cm depth were made on either side of the vertebral column of eight goats with 2 wounds in each animal following the technique described by Tamanna *et al*. [7].

Experimental Protocol

Following treatment, all the animals were maintained carefully to avoid contamination of wounds. Other than experimental herbal therapy, no other drugs were applied to the wound in order to avoid their influential effects on the healing process. Goats were divided into two groups:

Group A - Fresh aqueous Marigold flower paste was applied locally once daily to eight surgical wounds made in four animals.

Group B - This group received normal saline (NS) and served as control.

Plenimetry

Follow-up information was obtained since day of surgical operation (day 1) up to the end of experiment (day 21). Gross morphology such as swelling of the wound area, elevation of suture line from the skin surface, length of wound area was recorded to evaluate

the healing potentials of Marigold flower. Elevation of suture line was recorded up to 7th days of wounding, length of suture line was measured on the day 1 (D1), day 3 (D3), day 7 (D7), day 14 (D14) and day 21 (D21) post-operation, for bacteriological study, wound swabs were collected from both the groups on D3 post-wounding. To examine histological changes of wounds following treatment, full-thickness lesion biopsies were collected at D3, D7, and D21 from both the groups.

Monitoring and Data Collection

Swelling area (mm), elevation of suture line (mm), and length of suture line (mm) of the wound were measured using digital slide calipers (FstDgte, China). Healing progress of animals in both groups was monitored daily. Healing was scored as follows:

a) *Excellent* - No inflammation, no exudation, no infection, no dehiscence, gradual decrease of a width of the wound area.

b) *Good* - Minimum inflammation with minimum exudation, no dehiscence, gradual decrease of a width of the wound area.

c) *Poor* - Marked inflammation, presence of infection, and exudation.

Antibacterial Evaluation

Antimicrobial activity was determined through the dilution method. All of the samples were collected aseptically from the wounds by using sterile cotton buds circling onto the wound and immediately transferred into screwed capped test tubes containing nutrient broth. The tubes were taken to the laboratory to culture and counting of viable bacteria. For this purpose, the spread method was used Swab collection, culture, and staining of collected bacteriological samples were done by following the method described by Jaman *et al*. [8].

Histopathological Study

The full-thickness cutaneous biopsies (1.5 cm x 1.5 cm) were collected from the wound of each experimental animal on 3rd, 7th, and 21st days post-wound using the surgical procedure described by Tamanna *et al*. (2020). The samples were fixed in 10% formalin for 48 hours for proper fixation and slides were prepared and stained in the histopathology laboratory of the Department of Surgery and Obstetrics, BAU following the protocol described by Ashraf *et al*. [9]. The stained slides were assessed under a photographic microscope (Micros®, Austria) for epidermal thickness, inflammatory infiltration, fibroblasts generation, vascularization, and other essential histological changes and then images were captured accordingly.

Statistical Analysis

All the data were expressed as Mean ± SE (Standard Error). To compare data among groups, one-way ANOVA (Analysis of variance) was performed using Statistical Package for the Social Sciences (SPSS) version 22.0.

RESULTS

In this study, the efficacy of aqueous paste of Marigold flower was evaluated on the basis of morphological changes such as an area of swelling of wounds, elevation of suture line, length of wounds, mean time to get healed, histopathological changes and *in vivo* antimicrobial performance.

Morphological variables of wounds of group-A and B are shown in Table 1. Swelling of the wound edges was observed in both group of animals. Treatment with Marigold flower paste resulted in significant ($p < 0.05$) decreased swelling of the wound (4.02 ± 0.11 mm) than wound treated with NS (5.36 ± 0.12

Table 1. Morphological characteristics of wounds after treatment with Marigold petal paste and NS

Groups	Area of swelling of wounds (mm)	Elevation of suture line (mm)	Length of wounds (mm)
Group A	4.02 ± 0.11^{a}	2.73 ± 0.19^{a}	16.04 ± 0.06^{a}
Group B	5.36 ± 0.12^{b}	3.59 ± 0.12^{b}	17.11 ± 0.08^{b}

Data are shown as Mean± SE. Values with different superscript letter in the same column indicate significance ($p < 0.05$). NS = normal saline

mm), Elevation of suture line was remarkably lower in Marigold treated wound (2.73 ± 0.19 mm) than those of control (3.59 ± 0.12 mm). Decreased length of wounds after treatment with this herbal paste was recorded (16.04 ± 0.06 mm) whereas in NS treated wound, it was as high as 17.11 ± 0.08 mm.

The morphological changes of wounds of group A and group B at indicated days are shown in Fig. 1, Fig. 2 and Fig. 3.

In this study, we have found a significant difference ($p < 0.05$) in the average healing time between the groups. The mean days to complete healing for the treatment group was 14.17 ± 0.31 days where it was much higher in control wounds (18.67 ± 0.33 days) (Fig. 4) indicates that Marigold has potent accelerating wound healing effect.

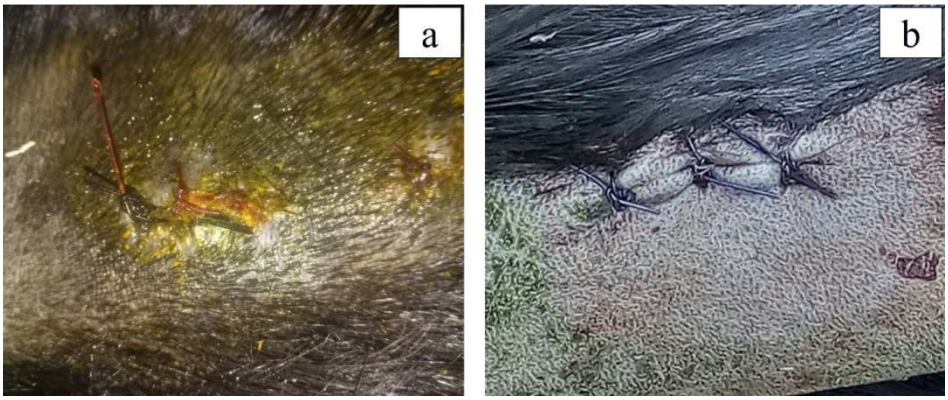

Fig. 1. Gross observation of wounds on day 1 treated with (a) Marigold, (b) Normal saline (control).

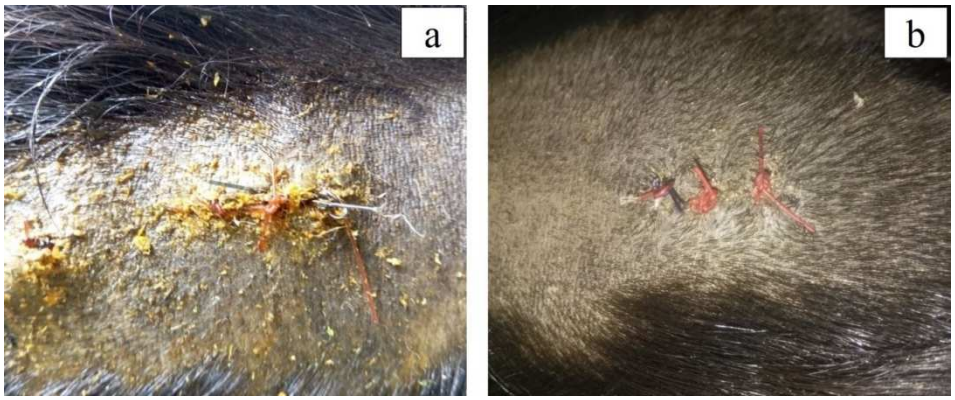

Fig. 2. Gross morphology of wounds on day 7 treated with (a) Marigold, (b) Normal saline (control).

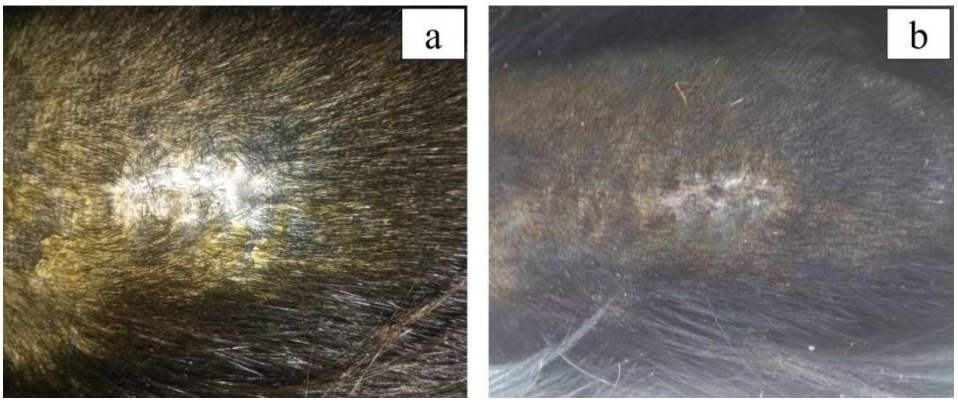

Fig. 3. Gross observation of wounds on day 21 treated with (a) Marigold, (b) Normal saline (control).

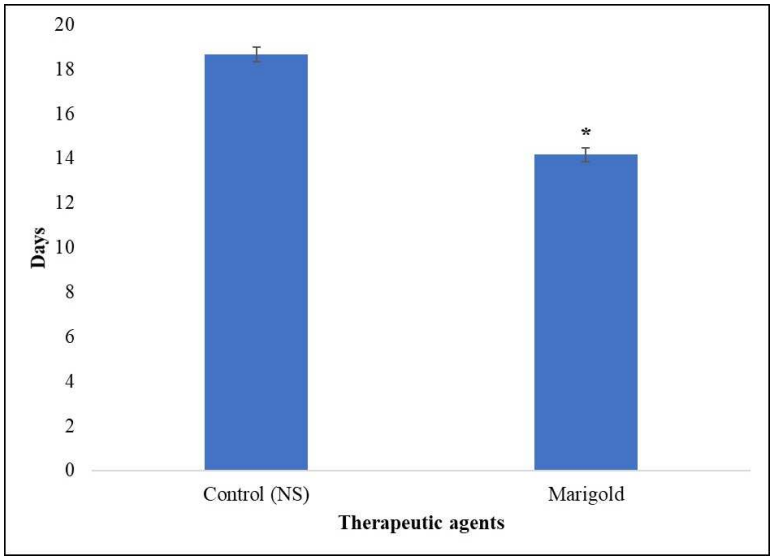

Fig. 4. Mean days to wound healing. * denotes healing time of Marigold is significantly lower than control group ($p < 0.05$).

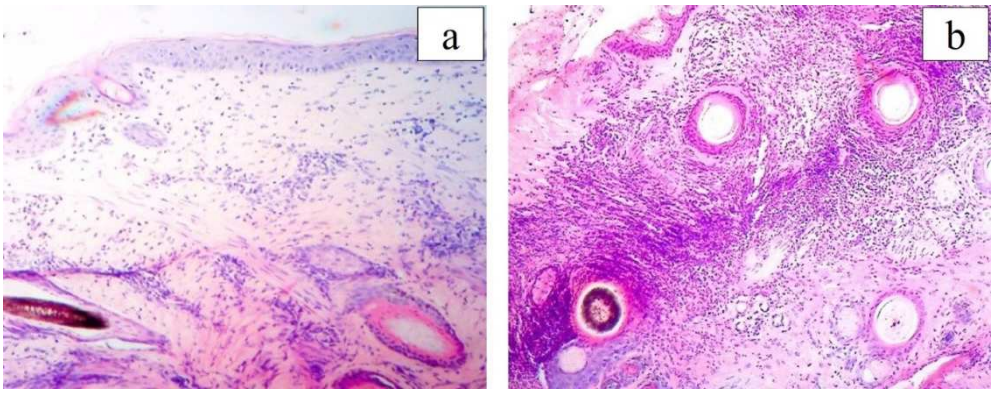

Fig. 5. Histological features on day 3. (a) Moderate infiltration of inflammatory cells with fibroplasia in group A, (b) Huge inflammation, and less fibroplasia in group B. (H & E stain, original magnification × 200).

The inflammatory lesions in the regenerating tissues were evaluated based on the infiltration of different reactive cells. Reactive cells decreased gradually in wounds treated with aqueous Marigold flower paste. Moderate infiltration of inflammatory cells with fibroplasia was seen on day 3 (Fig. 5a) and gradually decreased on day 7 (Fig. 6a) and on day 21, there was the least degree of inflammation present with marked thickening of the keratinized layer of the epidermis (Fig. 7a).

On the other hand, in the control group, huge inflammation and fibroplasia were encountered along with widespread hemorrhages and congestion on day 3 (Fig. 5b) which continued up to day 7 where fibroblastic proliferation with minimal infiltration inflammatory cells seen (Fig. 6b). On day 21, there was the least degree of inflammation present with a thin keratinized layer of the epidermis (Fig. 7b). Based on the histological scoring as described in materials and methods, Marigold flower provided excellent score whereas NS has given poor score in this experiment.

We have performed in vivo antibacterial efficacy study with the swab samples collected on day 3 post-operation. After culture and colony counting, we found that bacterial colonies were markedly reduced in Marigold treated wound (Fig. 8a) but there was a huge colony in saline-treated wound (Fig. 8b). To check the type of bacteria involved in the wound infection, we performed Gram's staining and it revealed Staphylococcus spp. characterized spherical shaped clustered Gram-positive bacteria (Fig. 9).

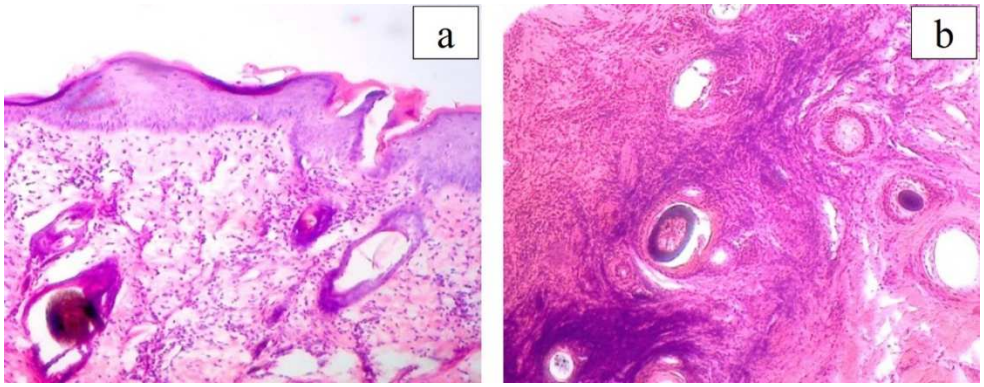

Fig. 6. Histological features on day 7. (a) Less inflammatory cells than those of fibroblastic cells in group A, (b) Fibroblastic proliferation with minimal infiltration inflammatory cells in group B. (H & E stain, original magnification × 200).

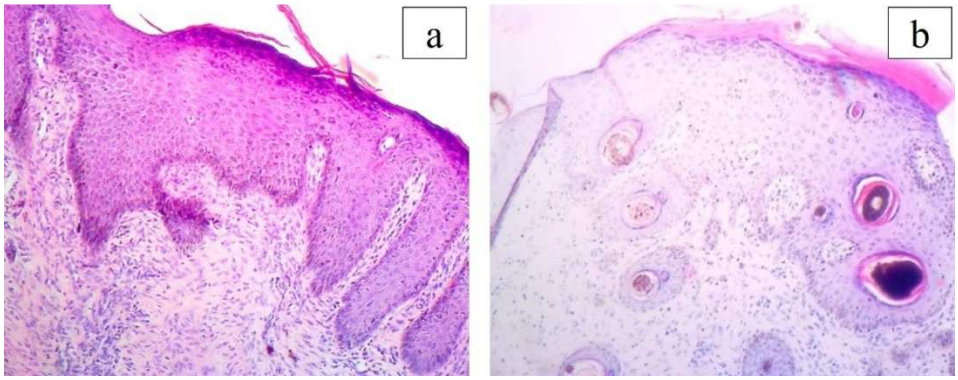

Fig. 7. Histological features on day 21. (a) Marked thickening of the keratinized layer of the epidermis in group A, (b) Thin keratinized layer of epidermis present in group B. (H & E stain, original magnification × 200).

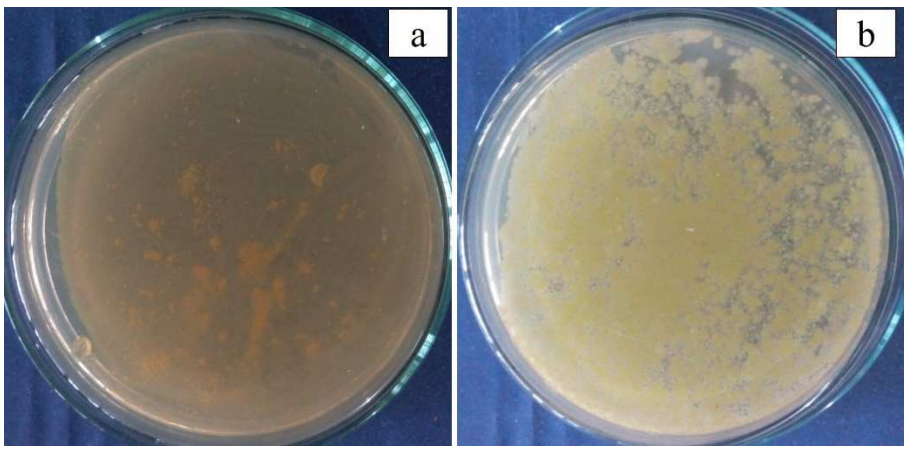

Fig. 8. Presence of bacterial colony in primary culture of Mannitol salt agar observed in samples collected from wound treated with (a) Marigold petal paste and (b) Normal saline (Control).

DISCUSSION

Medicinal plants, their derivatives, and metabolites are widely used for medicinal purposes, either phamacopoeial, non harmacopoeial or synthetic drugs. They are becoming popular all over the world as a natural alternative to synthetically produced medicine. In developing countries like Bangladesh, most of the people lived in rural areas do not get access to sophisticated and expensive medical support. A greater portion of them rely on traditional or herbal therapy for both humans and animals [7, 10, 11]. The plant

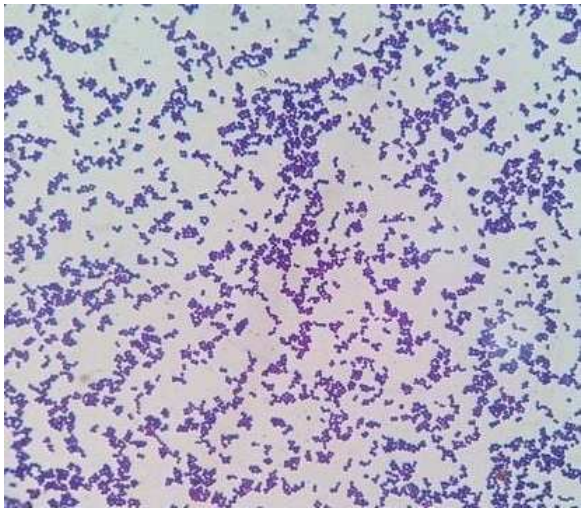

Fig. 9. The bacteria were arranged in a grape-like structure with spherical shaped indicating *Staphylococcus* spp.

Tagetes erecta has been shown to contain quercetagetin, phenolic, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercitin, thienyl, and ethyl gallate [12]. It has been reported that the *Tagetes erecta* flower acts as anti-hemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, and is useful in aromatherapy for its powerful skin healings properties [13]. In the current study, area of swelling, elevation of suture line, length of wounds was observed as gross indicators of wound healing potentials of *Tagetes erecta* petal paste. Results from our study showed that the mean value of swelling area, elevation of suture line and length of wound were remarkably lower in Marigold treated wound than those of control. The mean healing time significantly shorter in Group-A where wounds were treated with Marigold flower paste indicating that Marigold has outstanding healing accelerating properties.

Activation of fibroblasts, endothelial cells, and macrophages are keys of wound healing in which body cells respond to injury. The restoration of structure and function in the wound site is determined by fibroblast proliferation [14]. Therefore, therapeutic bioactive agents that are able to stimulate fibroblast growth and proliferation may be able to improve or promote wound healing as in the case of the present study, petal paste of *T. erecta* flower prepared was demonstrated to enhance the proliferation of fibroblast and collagen deposition in Black Bengal goats.

The mechanisms through which the *T. erecta* flower paste accelerates wound healing is unclear but further research could be done by examining whether this extract increased fibroblast migration and collagen deposition in wound tissue. It can be speculated that quick and maximum deposition collagen fibers in wounds treated with *T. erecta* flower paste may be the hidden mechanism of quick reformation of the wounded area by connective tissues.

The plant used as a wound healer may inhibit bacterial growth by different mechanisms than the presently used antibiotics. It has been shown that the essential oil of the *Tagetes erecta* flower showed noticeable antimicrobial activity against four gram-positive and fifteen gram-negative pathogenic bacteria *(Staphylococcus aureus, Bacillus mycoides, Bacillus pumilus, Bacillus subtilis, Salmonella paratyphi A, Salmonella paratyphi B, Salmonella paratyphi C, Salmonella typhi H, Salmonella enteritides, Salmonella flexneri Salmonella typhimurium, Shigella sonnei, Shigella schimizii, Shigella shigae, Vibrio cholerae Inawa, Vibrio cholerae Ogawa, Vibrio cholera Eltor and Xanthomonas campestris)* strains with minimum inhibitory concentration (MIC) for the extract ranging between 12.5-100 µg/mL [15]. This would explain the wounds given *Tagetes erecta* flower paste in our study were protected from microbial or fungal infection that may compromise wound healing.

CONCLUSION

In this study, the topical application of the *Tagetes erecta* flower paste on the surgical wound in goats caused a significantly higher rate of wound healing and reduced the epithelialization period. The study reveals that flower petals possess good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like alkaloids, and terpenoids present in the extract. Further investigations are necessary to determine the mode of action of constituents present in the paste to prove its potential in clinical studies.

Authors' Contribution

Study Conception: MMA; Study Design: MMA; Supervision: MMA, MR; Funding: MMA, MR; Materials: MMA, MR; Data Collection and/or Processing: AS; Statistical Analysis and/or Data Interpretation: AS, MH; Literature Review: AS, MH;

Manuscript Preparation: MMA, MH and Critical Review: MMA, MR.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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