ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

A Pharmacological Study on the Effect of *Calendula officinalis* Leaf Extract on Excision Wound Healing in Swiss Albino Mice

Priyanka Pandey[1]*, Bhagyashree Despande[1], Ashish Kumar Sarkar[2]

Abstract

Calendula officinalis is one of those plants which have medicinal importance due to its leaf and flowers have pharmacological activity, the important pharmacological parameters have been less investigated properly and can be scientifically proved by reverse pharmacology. It was found of interest to evaluate these properties of the extract of the leaf of *C. officinalis*. The present study is to screen wound healing activity of gels prepared from methanolic extracts of *C. officinalis* Linn. using excision wound models in albino mice. Formulations of the extracts were prepared in the gels of Carbopol individually and also in combination in equal ratio. In excision wound models, the so treated animals showed significant reduction in a period of epithelization and wound healing activity. The enhanced wound healing activity of hydroalcoholic extracts may be due to free radical scavenging action and the phytoconstituents present in it which either due to their individual or additive effect fastens the process of wound healing. It was found that *Calendula* extract enhanced the wound healing activity as seen by increased synthesis of connective tissue, especially collagen. There was a significant increase in the granuloma tissue. The wound contraction and increased tensile strength were found to be statistically significant.

Keywords: *Calendula officinalis*, Epithelization and methanolic extract, Wound healing activity

Asian Pac. J. Health Sci., (2022); DOI: 10.21276/apjhs.2022.9.2.22

Introduction

A wound is a break in the tissue's integrity that causes harm and usually leads in loss of function. Wound healing is a biological process that is started by trauma and usually ends with scar development. Coagulation, epithelization, granulation, college nation, and tissue remodeling are all aspects of the wound healing process. Wound healing frequently occurs in a direction that is different from its normal path, and underhealing, overhealing, or no healing of the wound are common.[1] Management of wounds that are not healing properly is a time consuming and costly process, and research into medications that speed up wound healing is a growing field in modern biomedical science. Many Ayurveda medicinal herbs have a vital part in the treatment of wounds. Plants are more effective healers because they support natural mending processes.[2]

Calendula officinalis, sometimes known as pot marigold, is a flowering plant in the Compositae family. *Calendula* is a flowering plant native to South Europe that grows up to 60 cm tall and has enormous yellow or orange blooms.[3-5]

C. officinalis Linn. (Compositae), a traditional medicinal plant, has been shown to have a variety of pharmacological properties. *C. officinalis* extract was discovered to have strong anti-inflammatory effect when applied topically.[6] It also has wound healing properties.[7] *C. officinalis* extract was proven to have preventative efficacy against acute dermatitis after irradiation in clinical studies[8] *C. officinalis* flower extract has been shown to have anti-genotoxic properties.[9] These pharmacological effects might be linked to *Calendula* extract's antioxidant activity, albeit this has not been shown conclusively. *Calendula's* antioxidant action has been shown in only a few studies extract,[10,11] and we have conducted a thorough examination into the antioxidant activity of this extract *in vitro* and *in vivo* in this work.

The herb's leaves and petals are used medicinally,[11] either as infusions, tinctures, liquid extracts, lotions or ointments, or in one of a multitude of over-the-counter skin and hair treatments available across the world. The goal of this study was to determine the combination wound healing efficacy of Carbopol gels containing *C. officinalis* leaves extract on excision wounds in Swiss albino mice.

[1]School of Sciences, MATS University, Raipur, Chhattisgarh, India.

[2]Department of Sciences, Shri Rawatpura Institute of Science and Technology, Raipur, Chhattisgarh, India.

Corresponding Author: Priyanka Pandey, School of Sciences, MATS University, Raipur, Chhattisgarh, India. E-mail: priyankapandey2907@gmail.com

How to cite this article: Pandey P, Despande B, Sarkar AK. A Pharmacological Study on the Effect of *Calendula officinalis* leaf extract on Excision Wound Healing in Swiss Albino Mice. Asian Pac. J. Health Sci., 2022;9(2):109-113.

Source of support: Nil

Conflicts of interest: None.

Received: 12/11/2021 **Revised:** 31/12/2021 **Accepted:** 28/01/2022

Materials and Methods

Plant Material

C. officinalis leaf (Fig.1) was taken from Raipur (Chhattisgarh), India's Local Herbal Garden.

Reagents and Authentic Samples

The reagents used were bought from Sigma Chemical Co. and were of the highest purity (>99.5%) (Germany). Varian's Lambda 532 nm ultraviolet spectrometer was used to measure sample absorbances.

Preparation of Extract

The extract was made by continuously mixing dried powdered (Fig.2) *C. officinalis* leaf (10 g) in 100 ml 50% methanol for 24 h at room temperature (Fig.3). After filtering, methanol was evaporated on a water bath at 60–70°C until only water remained. The dry powder was stored in an airtight container (Fig.4).

Figure 1: *Calendula officinalis* leaf

Figure 2: Dry leaf of *Calendula officinalis*

Figure 3: Extraction of *Calendula officinalis*

Wound Healing Activity

Requirements

- Scale
- Surgical blades (No. 18)
- Xylocaine (local anesthetics)
- Annie French (hair removal cream)
- Forceps
- Cotton.

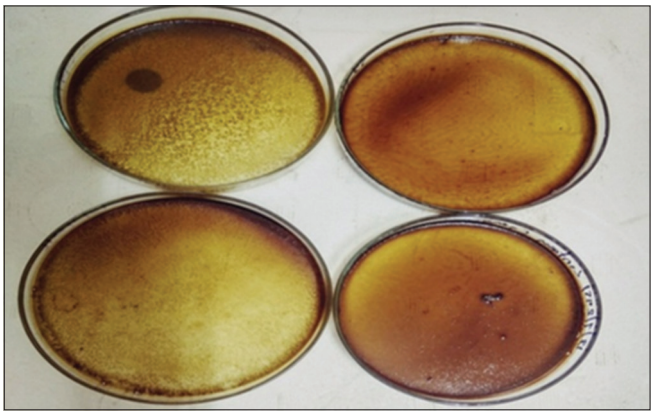


Figure 4: Powder of *Calendula officinalis*

Figure 5: Photographs showing the wound healing activity of *Calendula officinalis* leaf extract in the 0th day and 21st day

Figure 6: Photographs showing the wound healing activity of *Calendula officinalis* leaf extract in the 0th day and 21st day

Animals

The experiment was carried out on Swiss albino mice that were 3–4 months old and were placed into three groups based on the therapy they received.

Procedure

It is carried out according to Morton and Malone's (1972) and Manjunatha *et al*. methods (2005).[12] Excision was used to generate the wound. Hairs were removed from the posterior sides of mice using hair removal lotion for this experiment. A sterile scale was used to measure a 314 mm^2 area, which was then marked with a marker pen. The specified region was treated with xylocaine, a local anesthetic. The specified region of skin was removed with surgical blade no. 18 and forceps after 2 min of xylocaine application. After generating a 314 mm^2 wound, the skin was removed. The animals were separated into four groups of four. The animals in Group I were left untreated and were used as the control group. The animals in Group II were treated with a 5% w/w ointment Betadine as a reference standard, whereas those in Group III were given a 1% w/v extract. Starting on the day of the procedure and continuing until full epithelialization, the extract and Betadine were administered topically once a day. Wound closure and epithelialization time were the variables evaluated. The area of the wound was determined using transparent paper, a marker, and a scale. The wound closure % was determined. The number of days necessary for the dead tissue remains of the wound to fall without any remaining raw wound was used to compute the epithelialization duration.

Groups of *C. officinalis* Leaf Extract

- Group 1: This group consisted of four animals no treatment was given. The size of the wound was measured by Vernier calipers
- Group 2: This group consisted of four animals; standard drug Betadine was given by topically. During the treatment, the size of the wound was measured by Vernier calipers
- Group 3: This group consisted of four animals; 1% *C. officinalis leaf* extract was given by topically. During the treatment, the size of the wound was measured by Vernier calipers.

Experimental Design

The animals were divided into three different groups for each extract as follows:

- Total no. of animals for each group: 4 mice
- *C. officinalis* leaf extract – 1% w/v (topical application)
- Betadine ointment (5%).

Study Parameters

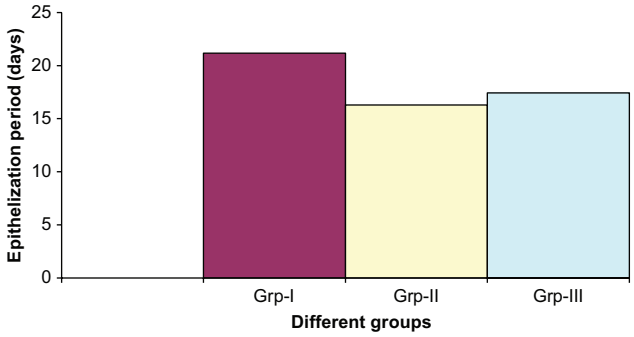
- Wound closure
- Epithelialization time.

Statistical Analysis

The means of wound area measurement at various time intervals and epithelization period across groups were compared using a one-way analysis of variance followed by a Student's "*t*" test, with $P = 0.05$ assigned for all analyses.

Results

The wound healing efficacy of *C. officinalis* leaf extract was demonstrated in a Swiss albino mouse excision wound model. Animals given *C. officinalis* leaf extract, Betadine, or a control treatment group showed considerable wound healing activity. *C. officinalis* leaf extract treated mice demonstrated a substantial decrease in wound area and epithelization duration in an excision wound model. As indicated in the table, the mean percentage of wound closure was determined on the 3rd, 5th, 8th, 11th, 14th, and 17th wounded days. The animals treated with *C. officinalis* leaf extract had a quicker wound epithelization (Fig.5) (17.42 ± 0.29 days) than the control group (Fig.6). In the case of the conventional medicine 5% Betadine ointment, the epithelization took 16.30 ± 0.24 days. The information is summarized in Table 1.



Effect of *Calendula officinalis* leaf extract on the wound healing

Discussion

The word medicine plant refers to a variety of plants used in herbal medicine, some of which have medicinal properties. These medicinal plants are thought to be a rich source of components for medication discovery and synthesis. *C. officinalis*, a member of the *Asteraceae* family, is a well-known medicinal herb. English marigold and pot marigold are two frequent names for it. *C. officinalis* processes carotenoids, flavonoids, saponins, sterols, phenolic acids, lipids, and other biological active components chemically. In herbal, folk, and traditional medicine in India, around 6000 plants are employed. Approximately 500 of the 1500 medicinal plants found are routinely used.[13] *C. officinalis* (pot marigold) is a widespread medicinal plant in India, China, Europe, and the United States. In old English, *Calendula* was called as "golds" and was linked with Virgin Mary and Queen Mary. Various types of substances, including triterpenoids, flavonoids, coumarins, quinones, volatile oil, carotenoids, and amino acids, have been discovered through chemical investigations. Many herbal preparations are utilized for many elements, including those of the skin, and herbal therapy is becoming increasingly popular among patients and physicians. The historic practice of completely treating dermatologic conditions using plant-derived medications predates Egyptian civilization and is still practiced in developed nations today. Herbal remedies, which have been utilized for generations, are now being researched scientifically.

The wound healing efficacy of *C. officinalis* leaf extract was demonstrated in a Swiss albino mouse excision wound model. Animals given *C. officinalis* leaf extract, Betadine, or a control treatment group showed considerable wound healing activity. *C. officinalis* leaf extract treated mice demonstrated a substantial

Table 1: The effect of *Calendula officinalis* leaf extract on the wound healing

S. No.	Groups	Wound closure $(mm)^2$ (percentage wound closure in parenthesis)						Epithelization period (days)
		0 days	3rd day	5th day	8th day	11th day	14th day	17th day	
I	Control (untreated)	313.66±0.23 (0.00)	286.24±0.25 (8.74)	244.67±0.23 (21.99)	207.78±0.21 (33.75)	172.45±0.26 (45.02)	111.30±0.12 (64.86)	43.01±0.25 (87.10)	21.17±0.37
II	Standard (5%Betadine)	312.50±0.26 (0.00)	250.48±0.28* (19.84)	189.45±0.25* (39.37)	148.14±0.19* (52.59)	92.23±0.26* (70.48)	29.12±0.22* (90.62)	0* (100)	16.30±0.24
III	*Calendula officinalis* extract	313.48±0.24 (0.00)	260.29±0.21* (16.96)	212.20±0.18* (32.30)	159.12±0.23* (49.10)	97.02±0.21* (69.19)	31.49±0.75* (89.94)	0* (100)	17.42±0.29

Calculated on the original wound size of 314 mm^2; *$P<0.05$, $n=4$, *level significance among different groups at $P<0.05$

decrease in wound area and epithelization duration in an excision wound model. As indicated in the table, the mean percentage of wound closure was determined on the 3rd, 5th, 8th, 11th, 14th, and 17th wounded days. The animals treated with *C. officinalis* leaf extract had a quicker wound epithelization (17.42 ± 0.29 days) than the control group. In the case of the conventional medicine 5% Betadine ointment, the epithelization took 16.30 ± 0.24 days. The information is summarized in Table 1.

The therapeutic benefits of *C. officinalis* extract wound in animals can now be plainly detected. Furthermore, this research will assist veterinarians in prescribing this herbal medication for wound healing with just little side effects. The cellular processes induced by *C. officinalis* extract can be researched in the future to determine the herbal plant's true therapeutic potential. Future research might include the extraction of particular biomaterials from *C. officinalis* extract, their molecular characterization, and direct application in wounds. Given the positive results of *C. officinalis* extract in wound healing and infection control, future research should focus on the active component responsible for its effects on infection control and wound healing.

Conclusion

Yet it comes to wounds, practitioners of "modern" (western) medicine sometimes use dubious phrases such as "alternative," "non-conventional," "indigenous," and "complementary," when many of "modern" medicine's approaches and practices are little different from traditional ones. Coagulation, inflammation, collagenation, wound contraction, and epithelialization are all part of the wound healing process.[14] While the phases of coagulation and collagenation are tightly related, wound contraction and epithelialization are independent of one another and occur simultaneously.[15] Excision wound model on Swiss albino mice revealed wound healing efficacy of *C. officinalis* leaf extract. Animals given *C. officinalis* leaf extract, Betadine, or a control treatment group showed considerable wound healing activity. *C. officinalis* leaf extract treated mice demonstrated a substantial decrease in wound area and epithelization duration in an excision wound model. Thus, the wound healing properties of *C. officinalis* can be linked to the phytoconstituents found in the plant, which may have an additive or individual influence on wound healing. According to the findings of this investigation, all of the 70% methanolic extracts exhibit wound healing ability. The extensive investigation on *C. officinalis* extract based on wound contraction measurement, shorter epithelialization duration, higher tensile strength, enhanced collagenation, and antioxidant analysis supports the concept that successive alcoholic extract has outstanding benefits. To fully comprehend the overall process of wound healing capacity and the elements responsible for the same action, more research using purified constituents is required.

References

1. The Wealth of India. A Dictionary of Indian Raw Material and Industrial Products. Vol. 3. New Delhi: Council of Scientific and Industrial Research; 2004. p. 78.
2. Turner RA. Screening Methods of Pharmacology. New York: Academic Press; 1965. p. 22-41.
3. Udupa SL, Shetty S, Udup AL, Somayaji SN. Effect of *Ocimum sanctum* Linn on normal and dexamethasone suppressed wound healing. Indian J Expt Biol 2006;44:49-54.
4. Sabale P, Bhimani B, Prajapati C, Sabale V. An overview of medicinal

plants as wound healers. J Appl Pharm Sci 2012;2:143-50.
5. AshokKumar P, Rajkumar, Animozhi M. Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. Mid East J Sci Res 2010;5:157-62.
6. Rao SG, Udupa AL, Rao G, Rao PG, Kulkarni DR. Wound healing activity of *Calendula officinalis*. Fitoterapia 1991;62:508.
7. Pommier P, Gomez F, Sunyach MP, D'Hombres A, Carrie C, Montbarbon X. Phase III randomized trial of *Calendula officinalis*. Compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. Clin Oncol 2004;22:1447-53.
8. Perez-Carreon JI, Cruz-Jimenez G, Licea-Vega JA, Popoca EA, Fazenda SF, Villa-Trevino S. Genotoxic and anti genotoxic properties of *Calendula officinalis*. Extracts in rat liver cell cultures treated with diethylnitrosamine. Toxicol *In Vitro* 2002;16:253-8.
9. Cordova CA, Siqueira IR, Netto CA, Yunes RA, Volpato AM, Filho VC, *et al*. Protective properties of butanolic extract of the *Calendula officinalis*. L. (marigold) against lipid peroxidation of the rat liver microsomes and as free radical scavengers. Redox Rep 2002;7:95-102.
10. Herold A, Cremer L, Calugaru A, Tamas V, Ionescu F, Manea S, *et al*. Antioxidant properties of some hydroalcoholic plant extracts with anti-inflammatory activity. Roum Arch Microbiol Immunol 2003;62:217-27.
11. Kiran K, Asad M. Wound healing activity of *Sesamum indicum* L seed and oil in rats. Indian J Exp Biol 2008;46:777-82.
12. Manjunatha BK, Vidya SM, Rashmi KV, Mankani KL, Shilpa HJ, Singh J. Evaluation of wound-healing potency of *Vernonia arborea* HK. Indian J Pharmacol 2005;4:223-6.
13. Chidambaram J, Saritha K, Maheswari R, Muzammil MS. Efficacy of green synthesis of silver nanoparticles using flowers of *Calendula officinalis*. Chem Sci Transc 2014;773-7.
14. Murti K, Kumar U. Enhancement of wound healing with roots of *Ficus racemosa* L. in albino rats. Asian Pac J Trop Biomed 2012;2:276-80.
15. Bairy KL, Rao CM. Wound healing profiles of *Ginkgo biloba*. J Nat Remedies 2001;1:25-7.