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EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND WOUND HEALING ACTIVITY OF THE HYDROALCOHOLIC FLOWER EXTRACTS OF *CALENDULA OFFICINALIS*

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ABSTRACT

This study focuses on the extraction, phytochemical investigation, and wound healing activity of the hydroalcoholic flower extracts of *Calendula officinalis*. *Calendula officinalis*, commonly known as marigold, is a medicinal plant with reputed wound healing properties. The hydroalcoholic extracts of its flowers were obtained using a suitable extraction method. The extracts were subjected to phytochemical screening to identify the presence of various secondary metabolites such as flavonoids, terpenoids, saponins, tannins, and alkaloids. The results of the phytochemical screening revealed the presence of flavonoids, terpenoids, saponins, and tannins in the hydroalcoholic flower extracts of *Calendula officinalis*. These compounds have been previously reported to possess wound healing properties. The wound healing activity assessment demonstrated significant improvements in wound closure, increased epithelialization, and enhanced tensile strength in the animals treated with the extracts compared to the control group. Histopathological examination revealed accelerated tissue regeneration and increased collagen deposition in the extract-treated group. Based on these findings, it can be concluded that the hydroalcoholic flower extracts of Calendula officinalis possesses potent wound healing activity. The presence of bioactive compounds, such as flavonoids and terpenoids, may contribute to the observed effects. Further investigation is warranted to isolate and identify the specific active components responsible for the wound healing activity of *Calendula officinalis* extracts.

Keywords: *Calendula officinalis,* Wound healing activity, Hydroalcoholic, Flower Extract

INTRODUCTION

Medicinal plants play an important role in the field of treatment and cure of diseases. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the active constituents, which determine the medicinal properties of plants. It has been worldwide accepted that the plant drugs are safer than synthetic medicines.

Today's healthcare systems rely largely on plant material. Much of the world's population depends on traditional medicine to meet daily health requirements, especially within developing countries. Use of

plant-based remedies is also widespread in many industrialized countries and numerous pharmaceuticals are based on or derived from plant compounds. Similarly, cosmetics and other household products may contain plants of medicinal or therapeutic value. The pharmaceutical industry is both large and highly successful.

Wound healing is a complex regeneration process, which is characterized by intercalating degradation and re-assembly of connective tissue and epidermal layer. The pH value within the wound influences indirectly and directly all biochemical reactions taking place in this process of healing[1]. Wound healing is a complex and dynamic process with the wound environment changing with the changing health status of the individual or it is the body's natural process of regenerating dermal and epidermal tissues. The healing of acute wounds is a well-organized process leading to predictable tissue repair where platelets, keratinocytes, immune surveillance cells, microvascular cells and fibroblasts play key roles in the restoration of tissue integrity.

It is an essential physiological process consisting of the collaboration of many cell strains and their products[2]. A healed wound is one in which the connective tissues have been repaired and the wound completely re-epithelialized by regeneration. In order to complete this regeneration process successfully the wound is metabolically very active. It has returned to its normal anatomical structure and function without the need for continued drainage or dressing. *Calendula officinalis* is an aromatic, erect, annual herb belong to the family asteraceae, it contained a wide range of chemical constituents including saponins, tritrpenes, triterpendiol esters, flavonoids, steroids, tannin, quinines, coumarins, carotenoids, amino acids, polysaccharides, essential and volatile oils and many other chemical groups. *Calendula officinalis* exerted many therapeutic effects including antibacterial, antifungal, anthelmintic, antiviral, cytotoxic, antioxidant, anti-inflammatory, analgesic, hepatoprotective, cardioprotective, gastroprotective, wound healing and many other effects. The aim of the present study was to investigate the wound healing activity of the hydroalcoholic flower extracts of *Calendula officinalis*.

MATERIALS AND METHODS

Collection of plant material

Flowers of *Calendula officinalis* were collected in the month of January 2022, from local area of Bhopal (M.P.). Flowers of *Calendula officinalis* were cleaned by tab water and a portion was dried at room temperature. The dried samples were ground and passed through a sieve (20 meshes). The powdered drugs were kept in sealed containers and protected from light until used. Another portion of sample was used for maceration.

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs:

Defatting of plant material

50.6 gram of powdered flowers of *Calendula officinalis* were coarsely powdered and subjected to extraction with petroleum ether by maceration method. The extraction was continued till the defatting of the material had taken place[3].

Extraction by soxhlet extraction process

Defatted dried powdered of *Calendula officinalis* has been extracted with hydroalcoholic (Methanol: Water: 70:30v/v) as a solvent using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40°C [4].

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} x100$$

Phytochemical analysis

Photochemical examinations were carried out extracts as per the following standard methods [5].

Quantitative studies of phytoconstituents

Total phenol content estimation

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method[6].

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol

Preparation of Extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexes for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminum chloride method[7].

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

***In vivo* wound-healing Activity**

Animals

Adult Wistar rats (180-200gms) of both sexes were procured from College of Veterinary Sciences and Animal Husbandry, Mhow, Indore (M.P). Total 24 animals were divided into four groups (control, standard, Hydroalcoholic extract of *Calendula officinalis* (200mg/kg and 400mg/kg) with 6 animals in each group. Animals were housed under standard environmental conditions of temperature (23^0C) and 12 hours light and dark cycle. All the animals were provided with food and water ad libitum. Study protocol was approved by Institutional Animal Ethical Committee and conducted according to the guidelines of CPCSEA.

Acute dermal toxicity

Swiss albino female mice of 18-22g weight and age of 90 days were used to determine the dermal toxicity of test extracts. The toxicological study was carried out to determine the therapeutic dose of the Hydroalcoholic extract of *Calendula officinalis* as per the OECD guidelines. Testing of the extract was done by applying the extract at two different concentrations on the shaved dorsal sides of the rats. It was observed that the dose was safe and lower dose was considered for further study[8].

Animal testing

For the in vivo wound experiment incision and excision wound models were used. Test extracts were prepared and diluted in double distilled water and applied at a dose of 200 mg/kg and 400mg/kg. Test extract was applied topically on the wounded site immediately after creating circular wounds by a surgical blade. The control group of animals was not treated with any drug and wounds were kept open16. Whereas the standard drug treated group of animals were applied with reference drug cipladine (10 % W/W)[9].

Linear incision wound model

All the animals were anaesthetized with 1:1 ketamine hydrochloride and xylazine and the back hair of the rats were shaved by using a shaving machine and impression was made on dorsal region 1cm away from vertebral column and 5cm away from ear. Linear paravertebral incision of 5cm long was made through the full thickness of the skin. Wounds were closed with interrupted sutures, which were removed on the 10th day after wound creation. Incision wounds were treated with the extracts daily for 14 days. The Wounds in control group of animals were kept open and was allowed to heal naturally. On 14th day

after formation of wound the breaking strength of the wound (in kilograms) and was measured by using Tensiometer[10].

Excision wound model

The animals were anaesthetized by injecting intramuscularly ketamine hydrochloride and xylazine in 1:1 concentration. The dorsal fur of the animals was shaved with shaving machine. Impression was made on dorsal region and area of the wound to be created was marked on the back of the animals by picric acid using circular stainless stencil. Using toothed forceps and pointed scissors circular excision wound of 300 to 400 mm2 were created to full thickness along the markings. Wound areas were measured by tracing the wound on transparency sheet with permanent marker by using millimeter-based graph paper on days 0, 3rd, 6th, 9th, 12th and 15th for all groups [11-12].

Preparation of test samples for bioassay

The extracts, the reference drug and the vehicle were applied topically once a day till the 15th day. At an interval of every three days, changes in wound area were monitored[13] and also the wound area was evaluated by using graph paper. Percentage of the reduction in wounded area was calculated from wound contraction. Histopathological examination[14] and biochemical parameters were carried out by using tissue specimen isolated from the healed skin of each group of rats.

Biochemical parameters

Circular wound area was excised and evaluated for various biochemical parameters at the end of the study. Especially Collagen content, Hydroxyproline[15] and Hexosamine[16] was estimated for evaluating the healing properties of Hydroalcoholic extract of *Calendula officinalis.*

Statistical analysis

Results obtained from the two wound healing models have been expressed as Mean ± SD and were compared with the corresponding control group by one way ANOVA test for assessing statistical significance[18].

RESULTS AND DISCUSSION

In the present study, the *Calendula officinalis* an indigenous medicinal plant of Asia was evaluated for the wound healing activity. This plant is widely distributed and it's each and every part having noble pharmacological activity. Percentage yield of extracts of hydroalcoholic of *Calendula officinalis* were found to be 5.28%. Hydroalcoholic extract of *Calendula officinalis* revealed the presence of carbohydrates, flavonoids, diterpenes, saponins, proteins and phenols. The phytochemical compounds detected are known to have medicinal importance. This secondary metabolite also was reported as one of the medicinal plants which have a lot of biological and therapeutic trait. Hence this species was expected to have many medicinal purposes.

The total phenolic content of the crude extract calculated from the calibration curve, where we obtained linear regression y = 0.029x - 0.004, with R^2 value = 0.999. The TPC of crude extracts was expressed as milligrams of gallic acid equivalents (GAE) per 100 milligrams crude extracts. The TPC was obtained from hydroalcoholic extract 0.234mg/ 100mg. Total flavonoids content was calculated from Quercetin calibration curve, where we obtained regression equation y = 0.018x + 0.016 with R^2 value = 0.998. Total flavonoids content of plant was stated in QE (Quercetin equivalent) or defined as an equivalent amount of mg quercetin per 100 milligrams sample. Result of flavonoids content indicated that hydroalcoholic extract has the flavonoids content (0.758mg QE/100mg extract).

Hydroalcoholic extract at 200 mg/kg was effective in increasing wound contraction but to a lesser degree than 400 mg/kg. In the excision wound model we observed that the (200 and 400 mg/kg) showed 95.79% and 98.86% wound contraction whereas, the standard drug, cipladine treated group and untreated control group showed 98.45% and 88.33% wound contraction on the 15 days study period. In the linear incision wound model, we measured the tensile strength of the incision wound. In this study we found that the topical application of (400 mg/kg) showed significantly higher tensile strength than the aqueous extracts, whereas standard and disease control groups showed much lesser tensile strength needed to break the wound than the extracts treated groups.

Table 7.1: Extractive values obtained from *Calendula officinalis*

S. No.	Solvent	Time of extraction	% Yield
1.	Hydroalcoholic extract	48 hours	5.28%

Table 7.2: Preliminary phytochemical screening of *Calendula officinalis*

S. No.	Phytoconstituents	Test Name	Hydroalcoholic extract
1	Alkaloids	Hager's Test	Absent
2	Glycosides	Legal's Test	Absent
3	Carbohydrates	Fehling's Test	Present
4	Flavonoids	Lead acetate	Present
5	Diterpenes	Copper acetate Test	Present
6	Saponins	Froth Test	Present
7	Proteins	Xanthoproteic Test	Present
8	Phenols	Ferric Chloride Test	Present

Table 7.5: Estimation of total phenolic and flavonoids content of *Calendula officinalis*

S. No	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.234	0.758

Table 7.6: Effect of Hydroalcoholic extract of *Calendula officinalis* (HACO) in excision wound contraction

Group	0 DAY	3rd DAY	6th DAY	9th DAY	12th DAY	15th DAY
Control	388.5±7.06	325.0±5.8	292.0±5.5	141.0±8.0	60.5±6.1	45.3±7.9
Standard Cipladine	405.3±7.9	236.7±25.4***	149.8±9.8***	61.8±6.4***	23.3±3.6***	6.3±3.8***
HACO (200mg/kg)	404.8±6.5	259.7±45.2***	187.3±5.9***	75.2±7.5***	53.5±6.2***	17.0±7.9***
HACO (400 mg/kg)	398.0±8.0	202.3±14.7***	90.3±5.7***	38.2±2.9***	18.7±2.5***	4.5±2.4***

Data: Mean± SD *** P<0.05 when compared with control group.

Table 7.7: Effect of Hydroalcoholic extract of *Calendula officinalis* on biochemical parameters of wound healing

Group	Hydroxyproline (µg/gm)	Collagen (µg/gm)	Hexosamine (mg/gm)
Control	42.88±3.82	313.45±7.33	9.77±0.83
Standard	68.90±4.84	507.53±8.20	21.73±1.67
HACO (200mg/kg)	81.85±4.10	604.14±30.61	25.75±1.48
HACO(400 mg/kg)	87.03±4.19	642.81±31.27	28.28±2.32

Data: Mean± SD *** P<0.05 when compared with control group.

CONCLUSION

In the present experiment, the plant increases not only granulation and Hexosamine formation but also, showed significant increase in hydroxyproline content of the granulation tissue of the excision wound which indicated rapid collagen formation. Both the dose (200 and 400mg/kg) also showed an increase in Hexosamine content which leads to rapid healing of wounds. Considering the obtained results, we can assume that the plant of *Calendula officinalis* might become a useful component for healing the wounds. Thus, further efforts will be put forth towards emphasizing its active components responsible for its wound healing potential.

REFERENCES

1. Schneider Lars Alexander, Korber Andreas, Grabbe Stephan, Dissemond Joachim. Influence of pH on wound-healing: a new perspective for wound-therapy. Archives of Dermatological Research. 2006; 7-9.
2. Gosain Ankush, Luisa MD A Di Pietro, DDS. Aging and wound healing. World Journal Surgical. 2004; 28, 321–326.
3. Silva GO, Abeysundara AT, Aponso MM. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. American Journal of Essential Oils and Natural Products. 2017; 5(2):29-32.
4. Evans WC. Trease and Evans Pharmacognosy. 16th Edn. Saunders Elsevier, 2009, 135-415.
5. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. International Pharmaceutica Sciencia. 2011; 1:98-6.
6. Singleton VL, Orthofer R, Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999;299:152-78.
7. Madaan R, Bansal G, Kumar S, Sharma A. Estimation of total phenols and flavonoids in extracts of Actaea spicata roots and antioxidant activity studies. Indian J Pharm Sci 2011;73(6):666-9.
8. Shenoy C, Patil MB, Kumar R, Patil S. Preliminary phytochemical investigation and wound healing activity of Allium cepa linn (liliaceae). Int J Pharm Pharmaceut Sci 2009; 2(2):167-175.
9. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK. Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var. paradisiaca) in rats. Indian J Exp Biol 2009; 47:32-40.
10. Suguna L, Singh S, Sivakumar P, Sampath P, Chandrakasan G. Influence Of Terminalia Chebula on dermal wound healing in rats. Phytotherapy Research 2002; 16: 227-231.

11. Diwan PV, Tilloo LD, Kulkarni DR. Influence of Tridax procumbens on wound healing. Ind J Med Res 1982; 75: 460-466.
12. Gupta N, Jain U K. Investigation of wound healing activity of methanolic extract of stem bark of Mimusops elengi Linn. Afr J Trad Comp Alt Med 2011; 8(2): 98-103.
13. Sadaf F, Saleem R, Ahmed M, Ahmad SI, Navaid-Ul Z. Healing potential of cream containing extract of Sphaeranthus indicius on dermal wounds in guinea pigs. J Ethnopharmacol 2006; 107:161– 163.
14. Oommen ST, Rao CM, Raju CVN. Effect of oil of Hydrocarpus on wound healing. Int J Lepr Other Mycobact Dis 1999; 69:154 –158.
15. Mukherjee PK, Verpoorte R, Suresh B. Evaluation of in vivo wound healing activity of Hypericum patulum (Family: Hypericaceae) leaf extract on different wound model in rats. J Ethnopharmacol 2000; 70: 315-321.
16. Mundada S, Shivhare R. Pharmacology of Tridax procumbens a Weed: Review. Int J Pharm Tech Res 2010; 2 (2): 1391-1394.