



## Effects of *Tagetes erecta* gel on experimentally induced oral ulcer model in rats

Lakshana S<sup>\*1</sup>, Vijayalakshmi S<sup>2</sup>, Dinakar J<sup>3</sup>, Asok Kumar K<sup>4</sup>

<sup>1</sup>Department of Research & Development, Saveetha Institute of Medical And Technical Sciences (Deemed University), Thandalam, Chennai-602105, Tamil Nadu, India

<sup>2</sup>Department of Anatomy, Saveetha Institute of Medical And Technical Sciences (Deemed University), Thandalam, Chennai-602105, Tamil Nadu, India

<sup>3</sup>Department of Oral Pathology, Sri Ramakrishna Dental College, Coimbatore-641006, Tamil Nadu, India

<sup>4</sup>Department of Pharmacology, Sri Ramakrishna Institute of Para Medical Sciences, Coimbatore-641044, Tamil Nadu, India



### Article History:

Received on: 01 Jan 2020  
Revised on: 01 Feb 2020  
Accepted on: 18 Feb 2020

### Keywords:

Antiulcer,  
Histopathology,  
Oral ulcers,  
*Tagetes erecta*

### ABSTRACT

*Tagetes erecta* (African marigold) has various medicinal values. The present study has been undertaken to evaluate the effects of extracts of the fresh leaves and flowers of *Tagetes erecta* on oral ulcer models in wistar albino rats. The anti-ulcer activity of the extracts of *Tagetes erecta* (2.5% and 5%) was compared among leaf and flower extract and with the standard drug, Triamcinolone. Phytochemical screening of plant extract, extract action on oral ulcer and histopathology analysis were carried out. *Tagetes erecta* leaf and flower extracts have showed the presence of alkaloids, flavonoids, and carotenoids. Results has that *Tagetes erecta* leaf extract showed significant oral ulcer protective activity (83.6%) compared with the standard drug Triamcinolone. In the present work it can be concluded that the hydro alcoholic leaf and flower extracts of *Tagetes erecta* gel have better potential against oral ulcer which supports the traditional claims in folklore medicine.

### \*Corresponding Author

Name: Lakshana S  
Phone:  
Email: [lakshana.dr@gmail.com](mailto:lakshana.dr@gmail.com)

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i2.2090>

Production and Hosted by

IJRPS | [www.ijrps.com](http://www.ijrps.com)

© 2020 | All rights reserved.

### INTRODUCTION

An oral ulcer is a rupture in the mucous membrane, which positions inside of the mouth. It generally seems to be a depression in the mucous membrane and typically has white or yellow colour and is usually very painful (Knecht and Mishriki, 1999; Scully,

2000). Since the pharmacological drugs encompass numerous adverse effects, the present paper is a step to open insight for therapeutic efficacy of *Tagetes erecta* against oral ulcer. The effectiveness of *Tagetes erecta* has not been tested in oral ulcers or ulcers of the mucous membrane. With the rising requirement to discover better antiulcer agents in therapeutics the study has been conducted.

*Tagetes* species fit in to family Asteraceae as well as it is used in diverse areas like medicines, cosmetic preparation and is most extensively used as ornamentals (Piccaglia et al., 1998; Gutierrez et al., 2006). Lutein is one among the major components and the main colorant of this plant (Patel et al., 2017; Nguyet et al., 2019). The leaves are described to be operative against piles, kidney troubles, and wounds. The flower is beneficial in fevers, carminative, astringent, scabies, stomachic and liver complaints and is also hired in diseases

of the eyes (Nikkon *et al.*, 2011; Dasgupta *et al.*, 2012). It displays diverse pharmacological effects like antibacterial activity, hepato-protective activity, anti-microbial activity, insecticidal activity, nematocidal activity, mosquitocidal activity, wound healing activity, larvicidal activity, analgesic and antioxidant activity (Wang *et al.*, 2016; Bhattacharyya, 2017).

However, the effectiveness of *Tagetes erecta* extract gel has not been tested in ulcers of the mucous membrane. Thus, the current study was carried out to examine the effects of the fresh leaves and flowers of *Tagetes erecta* extract gel on oral ulcer models in wistar albino rats. The research intended at comparing the healing potential of the leaf and the flower extract gel. The study also compared the healing potential of the plant extract versus the standard drug, triamcinolone and the duration taken to heal and potency of the extract against the ulcer model.

## MATERIALS AND METHODS

### Collection and preparation of the sample

The fresh flower plant of *Tagetes erecta* was collected from the Tamil Nadu Agricultural University botanical garden, Coimbatore. The plant was recognized and authenticated by BSI, the Botanical Survey of India, and a voucher specimen was dropped with a voucher specimen sample No. BSI/WRC/Tech./2012/431 The shade dried clean leaves and petals were powdered with the help of an electric mixer separately.

### Extraction Procedure

The fresh flower and leaf part of plant was dried in shade for 20 days. About a significant amount of dry flower petals were extracted with hydro alcohol at temp. 40-60°C using cold maceration. The hydro-alcoholic extract was filtered and intense to a dry mass with the help of a rotary evaporator. A deep reddish brown viscous residue obtained having characteristic odour. The weight of the dried mass was calculated. The yield was expected to be around 10%. Further the solvents were evaporated to dryness by using vacuum oven. Extract was kept in sterile glass bottles.

### Preliminary phytochemical screening

The flower and leaf extracts were exposed to phytochemical screening to find the existence of several phytoconstituents like flavonoids, saponins, alkaloids and carotenoids.

### Formulation of the therapeutic gel

A water soluble base like carbopol containing methyl paraben and propyl paraben was selected

as base for both the extracts individually. 2.5 g of the extract was taken and mixed vigorously with the gel base containing Carbopol: 2.5g, propyl paraben: 0.10gms, methyl paraben: 0.01g, and distilled water: 97.50ml.

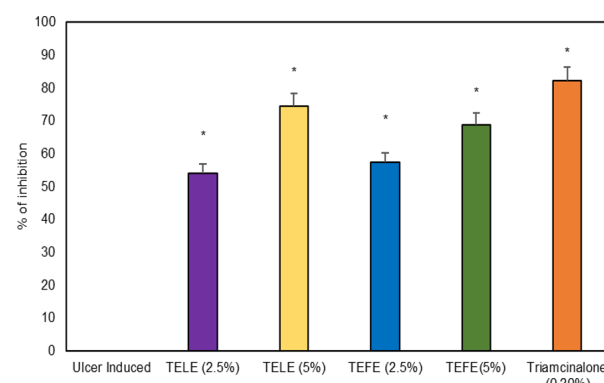
### Experimental animals

Healthy adult albino wistar rats weighing between 200-250 g were procured and boarded in ordinary cages at room temperature and were given food and water *ad libitum*. The experimental procedure was accepted by institutional animal ethics committee and animals were sustained under ordinary conditions in an animal house permitted by CPCSEA.(1559/PO/E/S/11/CPCSEA).

### Experimental induction of ulcer

Paraffin wax heated to above 100° C and poured on the dorsal surface of the tongue as a drop to induced oral ulcer. The animals were divided into 7 groups with 6 rats in each group. The ulcer was induced to Group II, III, IV, V, VI and VII rats.

Group I served as normal control. Group II served as ulcer induced ones. Group III and Group IV animals are given 2.5% and 5% of leaf extract respectively to the ulcer induced ones. Correspondingly, Group V and Group VI were given 2.5% and 5% of flower extract to the ulcer induced ones. Group VII rats were given 0.2% standard drug (Triamcinolone) to the ulcer induced ones.



**Figure 1: Percentage of ulcer inhibition by *T. erecta* gel treatment on ulcer induced rats**

Results were expressed as Mean  $\pm$  SEM (n=6). \*\*\*p<0.001 statistically significant as compared with ulcer induced control group.

### Administration of drug & duration

The animals in all subgroups were treated at the same time, twice daily. A cotton swab dunked in appropriate drug was applied topically to the ulcer from the second day for a period of 7 days.

### Observation of wound healing

**Table 1: Phytochemical screening of *T. erecta* flowers (TEFE) and leaves extract (TELE)**

Test	Compounds	TELE	TEFE
Flavonoid test	Flavonoid	Positive	Positive
Alkaloid test	Alkaloid	Positive	Positive
Saponin test	Saponins	Negative	Negative
Carotenoid test	Carotenoids	Positive	Positive

**Table 2: Ulcer size of control and experimental rats**

Variable	Ulcer induced	TEFE (2.5%)	TEFE (5.0%)	TELE (2.5%)	TELE (5.0%)	STD
Ulcer size	14.75 ± 1.1	9.23 ± 0.8**	6.01 ± 0.5***	6.7 ± 0.4***	4.0 ± 0.1***	3.83 ± 0.2***
Inhibition (%)	-	37.4 ± 2.5	59.2 ± 2.9	54.57 ± 3.2	72.8 ± 5.6	74 ± 4.9

Results were expressed as Mean ± SEM (n=6). \*\*, \*\*\*p<0.05, 0.01, 0.001 statistically significant as compared with ulcer induced control group. TELE= *Tagetes erecta* leaf extract, TEFE= *Tagetes erecta* flower extract

Apart from observing the behavioral changes, the animals were also being examined for the size and appearance of the ulcer, pus formation, exudate release, weight gain or loss and behavioral changes from the day of infliction of the wound. At the end of 7 days, all the animals were sacrificed.

#### Gross lesion and oral ulcer size evaluation

The length (mm) and width (mm) of the ulcer was dignified by a sliding caliper and ruler. The inhibition percentage (1%) was measured by the following formula,

$$1\% = \frac{UA_{control} - UA_{treated}}{UA_{control}}$$

UA: The sum of the areas of all lesions

#### Tissue sampling and histopathological analysis

The tissue samples of the tongue tissue from the ulcer area along with the margins were excised and processed for Hematoxylin Eosin (H&E) staining. Then the tissues were hydrated and dehydrated in sorted alcohol series. It was then cleared using xylene and chloroform, and then it was fixed in paraffin wax. Using rotary microtome, tissues sections were taken (10µm) out and kept overnight at room temperature. It was then de-paraffinised and moistened with descending alcohol concentrations followed by dist.H<sub>2</sub>O. Using haematoxylin and eosin stain, the sections were stained and then passed through the ascending alcohol series. The permanent slide was prepared using a DPX mount. The slides were scrutinized under a light microscope (20x) (Olympus microscope) and photomicrographs were captured using a Sony digital camera.

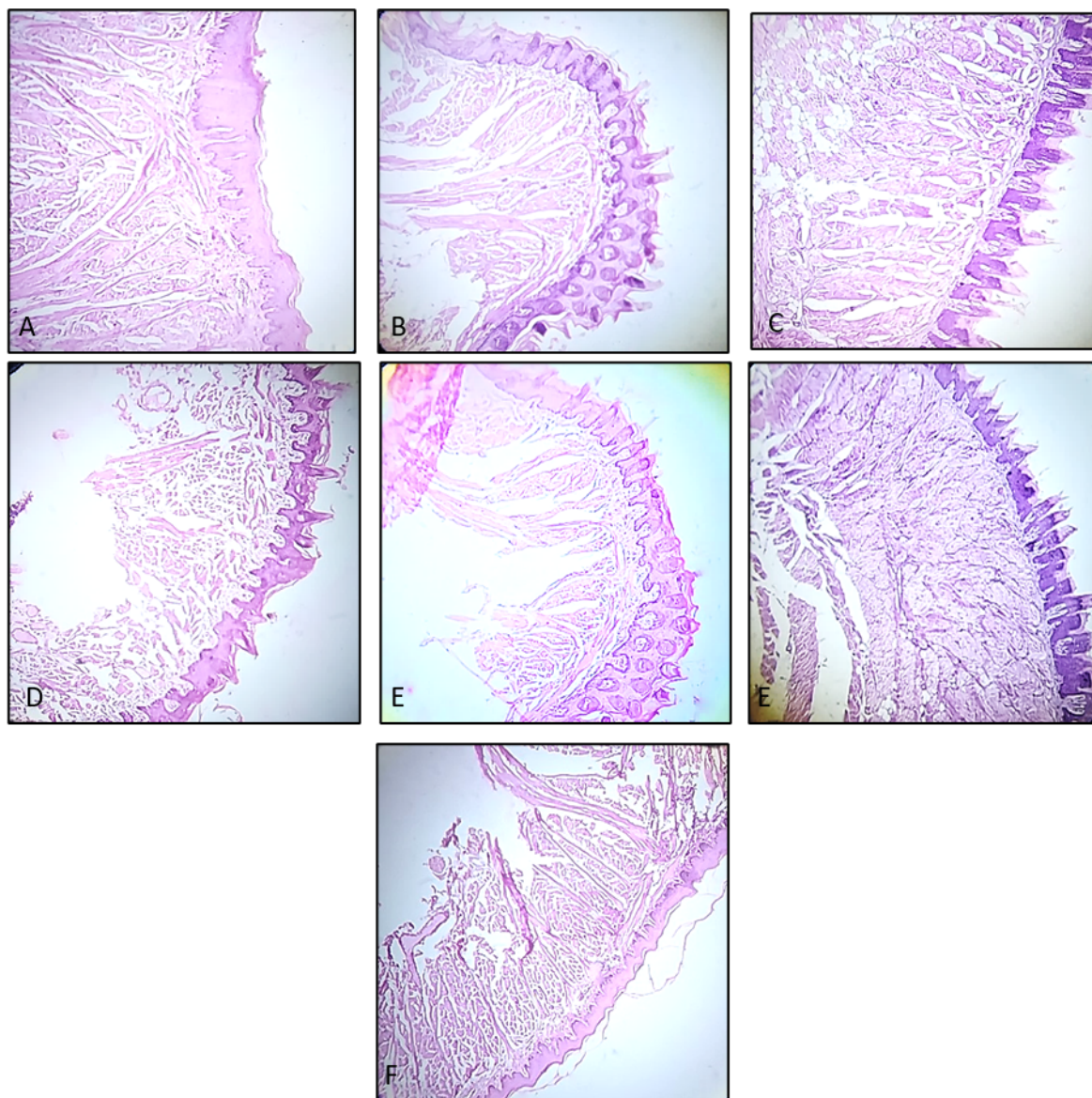
#### Statistical Analysis

The results were measured as the Mean ± SEM for each group. Statistical differences were evaluated using a one-way analysis of variance (ANOVA). Results were measured to be statistically significant at p<0.05.

#### RESULTS AND DISCUSSION

Phytochemical screening of *Tagetes erecta* leaf and flower extracts have indicated the presence of alkaloids, flavonoids and carotenoids (Table 1). Many of these compounds have been reported to have various therapeutic properties such as antiulcer, wound healing and mucosal protective activity (Alapaka *et al.*, 2019). Earlier investigation on bioactive property of *T. erecta* has identified the flavonoid patulitrin isolated from the flowers of plant as the active ingredient (Kiranmai *et al.*, 2011).

The pharmacological activities of marigold are related to the content of several classes of secondary metabolites such as flavonoids, sterols, carotenoids, tannins, saponins, triterpene alcohols, polysaccharides, a bitter principle, mucilage and resin (Verma *et al.*, 2018). The present study found the presence of carotenoids which agree with the result of (Verma *et al.*, 2018). Saponins were found negative in the analysis which is similar to the result of (Koilkil and Devika, 2012; Rajvanshi and Dwivedi, 2017). The main carotenoid in *Tagetes erecta* petals is lutein, which is found either free or esterified to one or two fatty acids. Marigold flower petals are an excellent and important source of carotenoids, particularly the yellow carotenoids such as α- and β-carotenes and the xanthophylls, lutein and zeaxanthin (Siriamornpun *et al.*, 2012).



**Figure 2: Histopathological changes in the tongue tissue of control and experimental rats**

In the present study, leaf and flower extracts of *Tagetes erecta gel* were evaluated for its oral anti-ulcer activity. The results of study are tabulated in (Table 2). Among the ulcer induced rat model, *Tagetes erecta* leaf extract (2.5%) showed lesser inhibition when compared to the flower extract and higher concentration of leaf extract (5%) showed more oral ulcer protective activity when compared with the standard drug (Figure 1). This research proved that the flower extract (5%) of *T. erecta gel* possess also highly significant beneficial action on oral ulcer. The present research agrees with the study done by (Aslani *et al.*, 2016), who found that flower extract also exhibits its anti-ulcer activity. Oral ulcer size measurement and percentage of inhibition in the oral cavity were observed and tabulated under (Table 2).

Histology image of normal control group has been depicted in (Figure 2A). The histopathology findings of ulcer induced group showed an infiltration of the connective tissue with inflammatory cells mainly neutrophils and histiocytes, with increased vascularity and perivascular inflammatory infiltrate (big arrow) (Figure 2B). Healing process with reepithelization, formation of epithelial ridges (small arrow) and granulation tissues can be observed in the herbal and standard treatment groups (Figure 2C-G). It can be concluded from the study that ulcer induced rats with 2.5% and 5% of flower extract showed the healing of ulcer with few inflammatory cells and leaf extract (5%) showed the healed ulcer, normal mucosa with no inflammatory cells (Figure 2). Figure 2 Shows that ,Histopathological changes in the tongue tissue of control and experimental rats. A: normal control rats; B: ulcer induced

rats; C: Ulcer rats administered with 2.5% of leaf extract gel; D: Ulcer rats administered with 2.5% of flower extract gel; E: Ulcer rats administered with 5% of leaf extract gel; F: Ulcer rats administered with 5.0% of flower extract gel; G: Ulcer rats treated with 0.2% Triamcinolone.

## CONCLUSIONS

This study established that the *Tagetes erecta* leaf and flower extract would be a valuable lead, which has the prospective to be explored for its mouth antiulcer activity in the approaching years by the scientific fraternity.

## REFERENCES

- Alapaka, M. R., Arava, S., Popuri, Y., Thummala, L., Manda, M., Penubolu, V. K. R., Shaik, A. 2019. A Glimpse on Herbal Anti-ulcer Agents. *Research Journal of Pharmacognosy and Phytochemistry*, 11(2):97-97.
- Aslani, A., Zolfaghari, B., Davoodvandi, F. 2016. Design, Formulation and Evaluation of an Oral Gel from Punica Granatum Flower Extract for the Treatment of Recurrent Aphthous Stomatitis. *Advanced Pharmaceutical Bulletin*, 6(3):391-398.
- Bhattacharyya, M. 2017. Use of marigold (*Tagetes* sp.) for the successful control of nematodes in agriculture. *The Pharma Innovation*, 6(11).
- Dasgupta, N., Ranjan, S., Saha, P., Jain, R., Malhotra, S., Saleh, M. A. A. M. 2012. Antibacterial activity of leaf extract of Mexican marigold (*Tagetes erecta*) against different gram positive and gram negative bacterial strains. *J Pharm Res*, 5(8):4201-4203.
- Gutierrez, R. M. P., Luna, H. H., Garrido, S. H. 2006. Antioxidant Activity of *Tagetes Erecta* Essential Oil. *Journal of the Chilean Chemical Society*, 51(2):883-886.
- Kiranmai, M., Kazim, S. M., Ibrahim, M. 2011. Combined wound healing activity of *Gymnema sylvestre* and *Tagetes erecta* linn. *International Journal of Pharmaceutical Applications*, 2(2):135-140.
- Knecht, K., Mishriki, Y. Y. 1999. More than a mouth ulcer. *Postgraduate Medicine*, 105(5):200-203.
- Koilpillai, R., Devika, J. 2012. Phytochemical screening studies of bioactive compounds of *Tagetes erecta*. *International Journal of Pharma and Bio Sciences*, 3(4):596-602.
- Nguyet, N. T. M., Ha, V. T. T., Binh, N. T., Dang, N. M., Bay, N. T. 2019. Quantification of lutein from Marigold flower (*Tagetes erecta* L.) petals by liquid chromatography - tandem mass spectrometry method. *Vietnam Journal of Chemistry*, 57(2):240-244.
- Nikkon, F., Habib, M. R., Saud, Z. A., Karim, M. R. 2011. *Tagetes erecta* Linn. and its mosquitocidal potency against *Culex quinquefasciatus*. *Asian Pacific Journal of Tropical Biomedicine*, 1(3):186-188.
- Patel, V., Patel, G. D., Desai, K. D., Desai, M., P, U. 2017. Integrated nutrient management in African marigold (*Tagetes erecta* L.). *International Journal of Chemical Studies*, 5(4):1352-1354.
- Piccaglia, R., Marotti, M., Grandi, S. 1998. Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. *Industrial Crops and Products*, 8(1):45-51.
- Rajvanshi, S. K., Dwivedi 2017. Phytochemical screening studies of bioactive compounds of African marigold (*Tagetes erecta* L.). *Journal of Pharmacognosy and Phytochemistry*, 6(4):524-527.
- Scully, C. 2000. ABC of oral health: Mouth ulcers and other causes of orofacial soreness and pain. *BMJ*, 321(7254):162-165.
- Siriamornpun, S., Kaisoon, O., Meeso, N. 2012. Changes in colour, antioxidant activities and carotenoids (lycopene,  $\beta$ -carotene, lutein) of marigold flower (*Tagetes erecta* L.) resulting from different drying processes. *Journal of Functional Foods*, 4(4):757-766.
- Verma, P. K., Raina, R., Agarwal, S., Kour, H. 2018. Phytochemical ingredients and pharmacological potential of *Calendula officinalis* Linn. *Pharmaceutical and Biomedical Research*, 4(2):1-17.
- Wang, W., Xu, H., Chen, H., Tai, K., Liu, F., Gao, Y. 2016. In vitro antioxidant, anti-diabetic and antilipemic potentials of quercetagen extracted from marigold (*Tagetes erecta* L.) inflorescence residues. *Journal of Food Science and Technology*, 53(6):2614-2624.