Sengottuvelu S *et al.* / *International Journal of Phytopharmacology.* 7(4), 2016, 233-236.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

WOUND HEALING ACTIVITY OF MONOTERPENE RICH *ORIGANUM MAJORANA* ON EXPERIMENTALLY INDUCED WOUND IN RATS

Anjana Yogesh[1], Latha M[2], Saravana Balaji MD[3], Premlal KR[4], Vinod Mony[5] and Sengottuvelu S[6*]

[1]Department of Periodontics, [2]Department of Oral Pathology, KGF College of Dental Sciences & Hospitals, BEML Nagar, Kolar Gold Fields, Kolar, Karnataka, India.
[3]Department of Periodontics RVS Dental College & Hospital, Coimbatore, Tamilnadu, India.
[4]Division of Oral Pathology & Microbiology, Indira Gandhi Institute of Dental Science, Sri Balaji Vidyapeeth University, Puducherry, India.
[5]Division of Oral Pathology PMS College of Dental Science & Research, Trivandrum, Kerala, India.
[6]Department of Pharmacology, Nandha College of Pharmacy, Erode, Tamilnadu, India.

ABSTRACT

Wound may be defined as a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection and is produced due to any accident or cut with sharp edged things. It may be produced due to physical, chemical, thermal, microbial or immunological exploitation to the tissues. Wound healers are one of the most critical requirements in the essential medicaments. India has a rich tradition of plant based knowledge on healthcare. A large number of plants/plant extracts/decoctions or pastes are equally used by tribals and folklore traditions in India for treatment of cuts, wounds, and burns. *Origanum majorana* is a frost perennial undershrub used traditionally for various ailments which is rich source in monoterpenes. Monoterpenes are the secondary metabolite of various plants, which were used extensively for wound healing. The study was conducted to evaluate the wound healing potential of ethanolic flower extract of *Origanum majorana* on excision and incision wound models in rats. Two concentrations, 5% and 10% of *Origanum majorana* flower extract and nitrofurazone ointment was used to compare in the both models. The test drugs were topically applied for 14 days on the wounded area and the wound contraction, tensile strength were observed on the excision and incision wound models respectively. *Origanum majorana* flower extract promotes the healing of wound on the both models in effective manner. From the results it was concluded that, ethanolic flower extract of *Origanum majorana* exhibits wound healing property.

Key words: *Origanum majorana*, Monoterpenes, Wound healing, Tensile strength.

INTRODUCTION

The monoterpenes are secondary metabolites of plants. Monoterpenes, belonging to a large and diverse group of chemical compounds named ‘terpenes’ or ‘terpenoids’.

Corresponding Author

S. Sengottuvelu
Email: sengt@rediffmail.com

The former represent a group of naturally occurring organic compounds whose basic structure consists of two linked isoprene units, which are formed by a 5-carbon-base (C5) each and the later which may include some oxygen functionality.

Monoterpenes are the most representative molecules constituting 90% of the essential oils and have a great variety of structures (Bakkali *et al.*, 2008), with several functions such as antimicrobial, wound healing, hypotensive, anti-inflammatory and antipruritic (Kordali *et al.*, 2005; Bastos *et al.*, 2010; Menezes *et al.*, 2010).

These compounds are inexpensive and have been widely used in flavorings and fragrances since the beginning of the 19th century. More recently, the therapeutic potentials of monoterpenes (Ghasemi *et al.,* 2009) were investigated for various disorders. The *Origanum majorana* L. is an aromatic, perennial, herbaceous plant belonging to the family Lamiaceae. O majorana (L.) is an endemic medicinal plant of Cyprus and is commonly known as 'Sampsishia'. Previous studies show the presence of monoterpenes, sabinene linalyl acetate and Cis-sabinene hydrate from the essential oil of this plant species (Johannes *et al.,* 2002). It is used against common cold, as spasmolytic and as an antirheumatic. Dried leaves and flowering tips of this species are used in formulation of vermouths and bitters. The essential oil is used for flavouring sauces, condiments and other products (de Vincenzi *et al.,* 1997).

In India, it is used as diuretic, antiasthmatic and an antiparlytic drug (Yadava and Khare, 1995). Stefanakis *et al.,* 2014, have assayed the essential oil extracted from Origanum species as potential antibacterial agents for disinfection of rotifers (Brachionusplicatilis). Abdel Massih *et al.,* 2010 suggested that Marjoram extracts exhibit anti-proliferative effect and have high antioxidant activity as well. Traditionally the plant was used to promote the healing of wound. An attempt was made to evaluate the wound healing effect of *Origanum majorana* flower extract on excision wound model in rats.

MATERIALS & METHODS

Plant Material

Collection and Authentication

The flowers of *Origanum majorana* was purchased from a nursery in Hosur. It was identified and authenticated as *Origanum majorana* by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, Coimbatore. The voucher specimen (BSI/SRC/12/42/2015-16/Sci/1432) has been deposited in department for further references.

Preparation of Extract

The flowers of *Origanum majorana* were, shade dried and then ground into coarse powder. The powder was then subjected to exhaustive extraction by a maceration process using 70% ethanol as a solvent at room temperature for 7 days. The ethanolic extract was concentrated by vacuum distillation to dry. The collected extract was stored in desiccators and used for further pharmacological study.

Animals: Male Wistar albino rats (180 – 220 gm) were used in the study. The animals were obtained from animal house, Nandha College of Pharmacy, Erode**.** The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light:day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed and approved by tthe Institutional Animal Ethics Committee of Nandha College of Pharmacy (Reg No: 688/PO/Re/S/02 /CPCSEA) and were in accordance with the guidelines of the CPCSEA.

WOUND HEALING ACTIVITY

Treatment Schedule

The animals were divided into 4 groups of 6 animals each. The group I served as the control, treated with ointment base, group II served as reference control treated with nitrofurazone ointment (0.2% w/w), group III and IV were treated with OMFE (5% and 10% w/w) respectively. All the test drugs were topically applied once daily for 14 days.

Excision Wound Model

Circular wounds of 50 mm^2 were inflicted on the depilated dorsal thoracic region under mild ether anesthesia. The areas (mm^2) of the wounds were measured by placing a transparent polythene graph paper over the wound. This was taken as the initial wound area reading. The wound was left undressed, open to environment. The parameters studied were percentage closure of excision wound and time of epithelization. The wound area was measured on 2nd, 4th, 8th and 15th day until healing was complete. The percentage of wound closure was calculated with respect to the initial wound area. The period of epithelization was calculated as the number of days required for complete closure of wound (Morton and Malone, 1972).

Incision Wound Model

In the Incision wound model two para vertebral straight incisions of 6 cm each were made on the depilated skin on either side of vertebral column of the rat as described by Ehrlich and Hunt, 1968. Care was taken to see that the incisions were at least 1cm lateral to the vertebral column.

After complete haemostasis, the wounds were closed by means of interrupted sutures of 1 cm apart. The grouping and treatment of animals is similar to that of excision wound model. Sutures were removed on 8th post wounding day and tensile strength was determined on 10th post wounding day according to the method of Lee and Tong, 1968.

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's '*t*' test using GraphPad version 3. P values < 0.05 were considered as significant.

RESULTS

Excision Wound Model

The wound healing effect of ethanolic flower extract of *Origanum majorana* on excision wound model was shown in table 1. In this model, both 5% and 10% of ethanolic flower extract of *Origanum majorana* have shown considerable wound contraction from fourth day onwards. Almost 50% of wound contraction was observed on 8th day with the topical application of ethanolic flower extract of *Origanum majorana*. On 15th day of observation 5% of the extract shown 84% of wound contraction and 10% of the extract shown 92.24% of wound contraction which was comparable to that of reference control nitrofurazone (97.73%).

Incision Wound Model

The wound healing effect of ethanolic flower extract of *Origanum majorana* on incision wound model was shown in table 2. 10% of ethanolic flower extract of *Origanum majorana* has shown maximum tensile strength (602.34 gm) on the 10th day which is comparable to that of the standard drug nitrofurazone (694.89 gm). 5% of the ethanolic flower extract of *Origanum majorana* has shown tensile strength (504.32 gm) on the 10th day which is less as comparable to that of reference control nitrofurazone.

Table 1. Wound Healing Activity of Ethanolic Flower Extract of *Origanum majorana* on Excision Wound in Rats

Post Wound Days	Wound Area (mm^2)			
	Control (Ointment Base)	Reference Control (Nitrofurazone)	OMFE (5%)	OMFE (10%)
0	51.38±2.33	52.86±1.72	49.26±2.09	51.77±2.65
2	49.82±2.96 (3.03)	47.03±3.88 (11.11)	46.33±3.78 (5.95)	47.67±3.37 (7.99)
4	39.02±1.66 (23.91)	39.87±2.47 (24.65)	41.72±1.99 (15.31)	40.33±3.31 (22.16)
8	34.58±2.00 (32.57)	18.09±0.82** (65.81)	25.54±1.26* (48.15)	21.97±1.76** (57.59)
15	26.31±1.54 (48.69)	1.20±0.03*** (97.73)	7.88±0.62***(84.00)	4.02±0.11*** (92.24)

Values are in mean ± SEM (n=6); *P<0.05 , **P<0.01, ***P<0.001 Vs Control
Figures in parenthesis indicate the percentage of wound contraction.
OMEF - *Origanum majorana* Flower Extract

Table 2. Wound Healing Activity of Ethanolic Flower Extract of *Origanum majorana* on Incision Wound in Rats

S.No	Groups	Tensile Strength (gm)
1	Control (Ointment Base)	342.66±9.55
2	Reference Control (Nitrofurazone)	694.89±7.41***
3	OMFE (5%)	504.32±8.66*
4	OMFE (10%)	602.34±8.44***

Values are in mean ± SEM (n=6), *P<0.05 , **P<0.01, ***P<0.001 Vs Control OMEF - *Origanum majorana* Flower Extract.

CONCLUSION

The wound healing effect of ethanolic flower extract of *Origanum majorana* on excision and incision wound model in rats were performed. From the result it was concluded that *Origanum majorana* exhibited wound healing property and it may be due to the presence of monoterpenes.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

Abdel-Massih RM, Rida Fares, Samer Bazzi, Nisrine El-Chami, Elias Baydoun. The apoptotic and anti-proliferative activity of *Origanum majorana* extracts on human leukemic cell line. *Leukemia Research*, 34, 2010, 1052–1056.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46, 2008, 446–475.

Bastos JF, Moreira IJ, Ribeiro TP, Medeiros IA, Antoniolli AR, De Sousa DP, Santos MR. Hypotensive and vasorelaxant effects of citronellol, a monoterpene alcohol. *Basic & Clinical Pharmacology & Toxicology*, 106, 2010, 331–337.

de Vincenzi M, Mancini E . Monographs on botanical flavouring substances used in foods. Part VI. *Fitoterapia*, 68(1), 1997, 49-61.

Ehrlich HP, Hunt TK. Effect of cortisone and vitamin A on wound healing. *Annals of Surgery*, 167, 1968, 324-328.

Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Sciences*, 22(3), 2009, 277–281.

Johannes N, Christina B, Freidrisch P, Jan L, Chlodwig FM. Distribution of cis sabinene hydrate acetate chemotype in accessions of marjoram (*Origanum marjorana* L.). *Euphytica*, 127, 2002, 69 –74.

Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *Journal of Agricultural and Food Chemistry*, 53, 2005, 9452–9458.

Lee KH, Tong TG. Study on the mechanism of action of salicylates, retardation of wound healing by aspirin. *Journal of Pharmaceutical Sciences*, 57, 1968, 1042-1046.

Menezes IA, Barreto CM, Antoniolli AR, Santos MR, De Sousa DP. Hypotensive activity of terpenes found in essential oils. *Z Naturforsch C*, 65, 2010, 562–566.

Michalis K Stefanakis, Elias Anastasopoulos, Haralambos E Katerinopoulos, Pavlos Makridis. Use of essential oils extracted from three *Origanum* species for disinfection of cultured rotifers (*Brachionus plicatilis*). *Aquaculture Research*, 45(11), 2014, 1861–1866.

Morton JJ, Malone MH. Evaluation of vulnerary activity by an open wound procedure in Rats. *Archives internationales de pharmacodynamie et de therapie,*196, 1972, 117-126.

Yadava RN, Khare MK. A triterpenoid from *Majorana hortensis*. *Fitoterapia,* 66(2), 1995, 185- 189.