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Evaluation of wound healing potential of the essential oil of *Ocimum sanctum* L. (Thulasi/basil) containing ointment in female Wistar albino rats

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Abstract
A skin ointment containing the essential oil of *Ocimum sanctum* L. and another skin ointment without essential oil were prepared. 5 mm diameter wounds were created on the back of female Wistar Albino rats. Essential oil containing ointment was applied over the ulcer of one group of rats, while the ointment without essential oil was applied to the ulcer of a second group of animals. The third group of animals was not treated with any ointment. The areas of the ulcers were measured on every fourth day until the ulcers completely healed. The ulcers healed completely in 21.33, 36.00, and 34.67 days in the essential oil ointment group, ointment without essential oil group and untreated group, respectively. Thus the essential oil containing ointment healed the ulcers in significantly lesser number of days (13.34 days earlier) when compared with the ulcers of untreated animal group.

Keywords: *Ocimum sanctum*, essential oil, Wistar albino rats, ointment, ulcer, wound healing potential

Introduction
Wounds are considered to be a major human health problem. Wounds affect the normal activities and diminish the quality of their life and cause enormous social and economic implications. Chronic ulcers are caused mostly by diabetes, venous pressure, or pressure sores [1]. Today we need new therapeutic agents to treat wounds.

Incorporation of biological materials such as stem cells or vegetable extracts to solutions or gels to treat wounds are being reported [2]. Therapeutic dressings with plant extracts of wounds are gaining importance because of the multifactorial properties of plant extracts (such as anti-bacterial, anti-oxidant, and anti-inflammatory). There are many experimental evidences to suggest that plant products have beneficial properties on wound healing as well on many skin diseases [3, 4, 5, 6].

Essential oils extracted from plants have been reported to have anti-inflammatory, anti-oxidant, and anti-microbial properties [7, 8, 9]. Essential oils are one of the commonly used natural products, with one of their main applications being their use in dermatology. At least 90 essential oils have been identified for dermatologic use, with at least 1500 combinations [6] for many decades essential oils have been used safely in perfumery, food and sanitation products. However, essential oils or their main components are scarcely used in wound healing.

Therefore the present study is designed to evaluate the wound healing property of an ointment containing *Ocimum sanctum* L essential oil in experimentally induced wound in female Wistar albino rats.

Ocimum sanctum Linn, a commonly available plant, belongs to the class Magnoliopsida, is found to have anti-inflammatory, analgesic, immunostimulatory, free radical scavenging and anti-microbial activity [7-12]. It is widely distributed throughout India and in different parts of the world.

Materials and Methods
Extraction of essential oil: 200 grams of *Ocimum sanctum* L (Thulasi/Basil) leaves, washed in clean water were boiled with 400 ml of distilled water in the Clevenger apparatus and the vapor was cooled to collect the essential oil and water. The essential oil was separated from water and stored at 2º to 8 °C in a tightly closed container and kept in dark.

4% (v/w) *Ocimum sanctum* Essential oil (EO) containing skin ointment was prepared as per the British Pharmacopoeia [13]. A simple skin ointment was prepared by fusion method. 4ml of Essential oil was added to Wool fat 5 gm, Cetostearyl alcohol 5gm, hard paraffin 5gm, and White soft paraffin 85 gm and stirred continuously in a closed condition.

The preparation was stored at 2º–8 °C. A 4% (v/w) ointment base without EO was prepared by mixing the above ingredients without essential oil and was stored at 2º-8 °C.

Earlier we evaluated the skin-irritancy potential of the above essential oil containing ointment in female Wister Albino rats and found that the ointment falls under the category of "negligible irritant" [14].

The test protocol was approved by the Institutional Animal Ethical Committee (approval of the protocol number: 08/IAEC/MG/08/2019-II dated 28-08-2019) and care of the animals was taken according to the guidelines of CPSCEA, Ministry of Forests and Environment, Government of India.

Animals were kept individually housed in separate cages. The temperature of the experimental animal room was 20 °C (±3 °C). The relative humidity was between 50-60%. Lighting was artificial, the sequence being 12 hours light and 12 hours dark. For feeding, conventional laboratory diet was used with an unrestricted supply of drinking water.

Three groups of female Wistar albino rats, each group consisting of 3 animals were used. The back of the animals were hair clipped one day before the creation of ulcer. The animals were anesthetized with an intramuscular injection of Ketamine (25mg/Kg body weight of animal). The skin was disinfected with a skin antiseptic containing 70% ethyl alcohol and 2.5% chlorhexidine gluconate solution. A sterile, disposable 5 mm diameter skin punch biopsy instrument (Derma India, Perungudi, Chennai, India) was used. The instrument was placed over the skin and a punch was made to create a uniform ulcer in all animals. To the essential oil test group animals (EO group), 0.5 gram of essential oil containing ointment was applied over the ulcer. 0.5 gram of ointment base alone was applied over the ulcer in the ointment base only group of animals (Ointment base only group). The third group animals were not treated with any ointment (No treatment group). The animals were returned to individual cages.

On alternate days, appropriate ointments were applied and the animals were returned to the cages. On every fourth day, a transparent paper was kept over the wound and the margin of the wound was marked on the paper. Later the transparent paper was kept on a graph paper and the area of the wound was counted in square millimeter and recorded. The animals were observed till the complete healing of the ulcers.

The degree of wound healing was calculated as percentage closure in wound area from original wound area. The mean and standard deviation values were calculated. (Table-1 & Figure 1).

$$\text{Percent of wound closure} = \frac{\text{Initial wound area (mm}^2\text{)-Present wound area (mm}^2\text{)}}{\text{Initial wound area (mm}^2\text{)}} \times 100$$

The number of days required for complete healing of the wound in each animal was observed and recorded. After completion of the experiment, the animals were humanely killed in a carbon dioxide chamber.

Results: The Mean ± Standard deviation (SD) of wound closure percentages on different days in the three groups of rats were calculated by Bayesian Repeated Measures ANOVA (Table-1 and Figure-1). The number of days required for complete wound healing in EO group, Ointment base only group, and no treatment group were 21.33± 6.11, 36±6.93 and 34.67 ±2.31, respectively.

Table 1: Mean ± SD of the percentage Wound closure (in mm^2) on different days in Wistar female Albino rats groups.

Groups	Days –(Percentage wound closure - mm^2)										
	Day1	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	Day 32	Day 36	Day 40
No Treatment	0	22.67±4.62	34.67±13.47	44±8.00	66.67±10.07	70.61±9.24	77.33±9.24	84±12.65	90.67±8.33	100±0.00	
Ointment base only	0	14.67±11.55	21.33±16.17	29.33±26.63	60±10.58	64±13.86	77.33±10.07	86.67±11.55	84±0.00	92±0.00	100±0.00
Essential oil ointment	0	36±7.44	46.67±18.48	76±4.00	92±6.93	94±8.49	96±0.00	100±0.00			

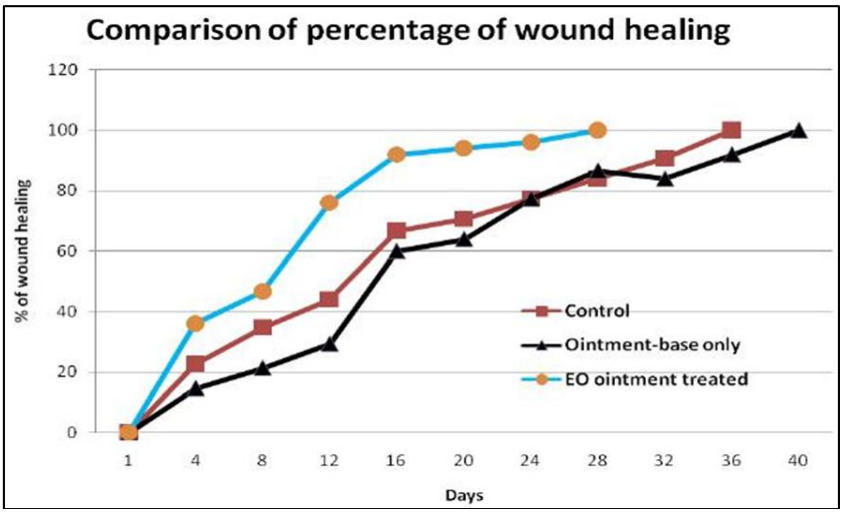


Fig 1: Wound closure percentages on different days in Wistar female albino rat groups.

The normality of the data was tested by Leven's test and found suitable for parametric test (p=0.19), (Table 2).

Table 2: Comparison of mean time for complete cure among the three groups (ANOVA)

Cases	Sum of squares	DF	Mean square	F	P
Group	394.67	2.00	197.33	6.53	0.031
Residual	181.33	6.00	30.22		

ANOVA was used to find the variations in wound closure time among the three groups. The average number of days required for complete wound healing in EO group, Ointment base only group, and no treatment group were 21.33±6.11, 36±6.93, and 34.67±2.31 days, respectively. The result of ANOVA shows that the differences in wound closure time was significantly difference between at least two groups. (F=6.53, p=0.031), (Table 2).

The results of post-hoc test (Tukey HSD) shows that the number of days required for complete wound healing was significantly lesser in the EO group compared with ointment only group (p<0.05). Likewise, the average number of days for complete cure was significantly less in EO group compared to No treatment group (p<0.05). There was no significant difference between the ointment base only group and No treatment group (p<0.05), (Table 3).

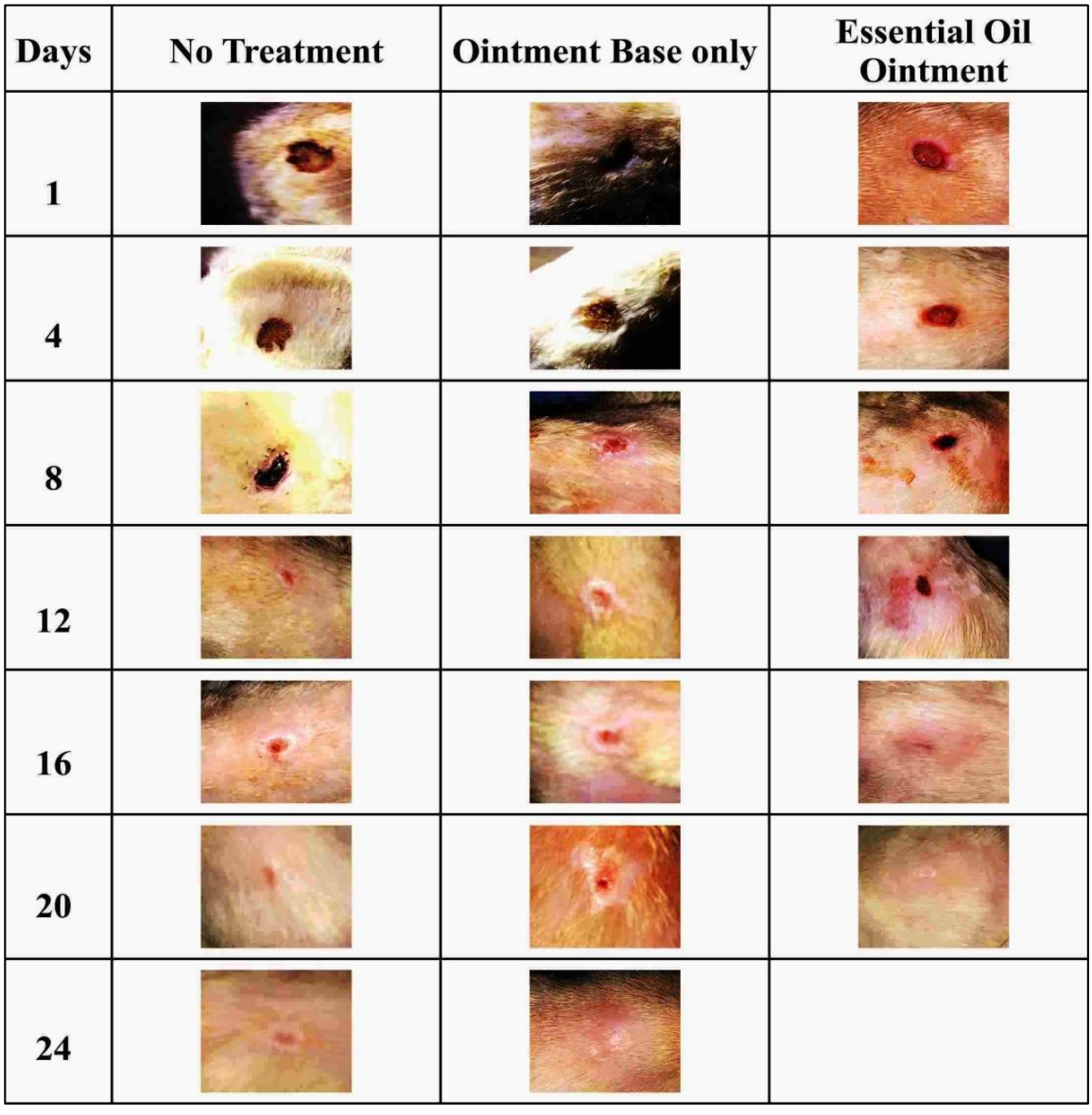
Table 3: Tukey HSD (Honest Significant Difference) test

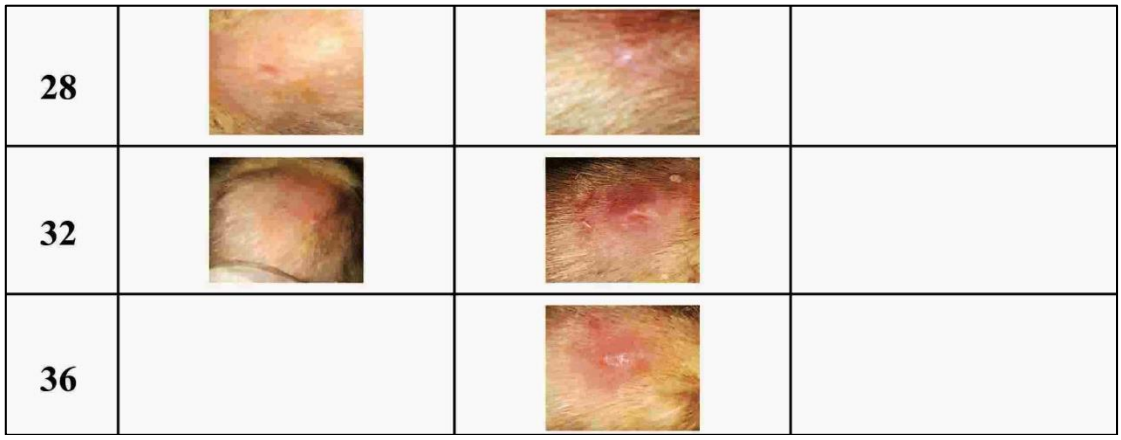
Group	Comparison Group	Mean Difference	DF 1	DF 2	P-Value
No treatment	Ointment only	-1.33	4.49	-0.30	0.95
No treatment	EO	13.33	4.49	2.97	0.056
Ointment only	EO	14.67	4.49	3.27	0.039

The trend in percentage of wound healing over a period of 40 days for the three groups was presented in the graph, which shows that the trend was very high in EO group at 8-12 days where as it was 12 to 16 days for no treatment and ointment base only groups. The lines for no treatment and ointment base only groups were close to each other, suggesting that they were equally responding to the process of wound healing while the line for EO group was above these two lines inferring that the healing process was faster when compared to the other two groups.

Photographs of wound contracture of one of the representative animals from each group on different days

Photographs of one (out of three) representative animal from each of the three groups on different days show increasing wound contracture over the period of the experiment. The wound of the Essential oil treated animal heals earlier than the wounds of the ointment base only group and no treatment group.





Discussion

For many decades essential oils have been used safely in perfumery, food and sanitation products. Essential oils are used mostly for the treatment of human skin infections (such as bacterial, fungal and viral), inflammatory skin conditions (such as dermatitis, eczema, and lupus), and general skin manifestations (such as wrinkles, scars, and scabs). In dermatology, essential oils are one of the commonly used natural products. 90 essential oils have been identified for use in dermatology and about 1500 essential oil combinations have been reported [6]. Essential oils also have anti-inflammatory and wound healing applications [6]. However, essential oils or their main components are scarcely used to treat wounds.

A summary of the experimental evidences on the remarkable wound healing effects of essential oils by *in vitro* and *in vivo* tests have been reported by Mercedes Perez- Recalde, *et al*. Furthermore, they have reported on the synergic effects of a combination of essential oil with resorbable polymers for wound dressings [1].

The wound healing property of *Ocimum sanctum* plant extracts have been studied in animals by investigators using aqueous suspension or alcoholic extract. Combined orally administered aqueous solution of *Ocimum sanctum* and locally applied silver sulfadiazine ointment have been reported to be effective in wound healing in rabbits by Vaibhav K Gupta, *et al*. [15] Somashekar, *et al*. have reported that alcoholic and aqueous extracts of *Ocimum sanctum* Linn favor faster wound healing in Wistar albino rats [16]. The oral aqueous extract administration and the topically applied aqueous extract of *Ocimum sanctum* L in glycerin was reported by Asha, *et al*. to promote better granulation tissue, early and complete epithelialization and better tensile strength compared to the controls in albino rats [17]. Asha, *et al*. have reported that topical aqueous *Ocimum sanctum* L extract promoted better granulation tissue, early and complete epithelisation and better tensile strength compared to control in albino rats [18]. All these studies reveal that *Ocimum sanctum* has great potential as a wound healing plant.

The efficacy of essential oils on human skin ulcers have been evaluated in certain clinical studies. PH Warnke, *et al*. Have reported a decrease in inflammation, reduction of the odor, and improved wound healing of malodorous necrotic ulcers of cancer patients by the use of the essential oil of *Eucalyptus globulus* [19]. Chin and Cordell have reported that *Melaleuca alternifolia* essential oil gel showed an accelerated healing rate of abscessed wounds and cellulitis [20]. Blackwood, *et al*. *et al* have opined that there was a concluding evidence to show that there was definitely a potential for the use of *Melaleuca alternifolia* (tea tree) oil as an additional /alternative treatment to standard wound treatments [21]. Hartman, *et al*. have reported that *L. Angustifolia* and *Matricaria recutita* essential oil mixture lead to complete wound healing in four of five patients with chronic leg ulcers [22]. A successful essential oil combination of *L Angustifolia*, *Artemisia vulgaris* (mugwort), and *Salvia officinalis* (sage) in treating chronic wounds such as venous ulcers, pressure sores, skin tears, and abrasions had been reported by R Guba, *et al*. and they have speculated that the essential oils increased the circulation and vascular permeability resulting in accelerated angiogenesis [23].

Conclusion

The present study reveals that the *Ocimum sanctum* L essential oil containing ointment heals the wounds in Wistar Albino female rats in significantly lesser number of days (21.33) when compared with the number of days required for wound healing without any treatment (34.67 days). Thus the EO ointment completely healed the wounds in 13.34 days earlier to the group without any treatment. To our knowledge, this is the first report of the wound healing beneficial effect of *Ocimum sanctum* L essential oil containing ointment in experimental animals. The beneficial effect of wound healing property of *Ocimum sanctum* L. essential oil in human skin ulcers needs to be evaluated in clinical trials in future.

Conflict of interest

The authors have no conflict of interest regarding this investigation.

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