

## THE WOUND HEALING PROPERTY OF THYME OLEORESIN FROM *THYMUS VULGARIS* L. ON HACAT KERATINOCYTES

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### ABSTRACT

**Objective:** The objective of the present study was to evaluate the wound healing property of thyme oleoresin using HaCaT keratinocytes.

**Methods:** The effect of thyme oleoresin on cell migratory activity of HaCaT keratinocytes was investigated and analyzed using scratch assay. The HaCaT cell line was obtained from NCCS Pune and maintained in Dulbecco's Modified Eagle's Media. The keratinocyte cells (HaCaT) were trypsinized for 30 s and passaged to T25 flasks in complete aseptic environment. The effect of thyme oleoresin on wound closure was determined using a 12-well plate. Dulbecco's Modified Eagle's medium with dimethyl sulfoxide was used as control. The effect of thyme oleoresin on wound closure was determined microscopically at 20× magnification using Nikon microscope. The experiment was performed in triplicate. The wound area was photographed and analyzed.

**Results:** Thyme oleoresin at 25 µg/ml and 50 µg/ml has significantly promoted the migration of HaCaT cells, thereby leading to wound closure.

**Conclusion:** The study has proved the wound healing property of thyme oleoresin, and hence, it may be used for wound healing purpose in a natural way.

**Keywords:** Thyme oleoresin, HaCaT cell line, Keratinocytes, Wound healing, Scratch assay.

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### INTRODUCTION

*Thymus vulgaris* L. is an aromatic medicinal plant. It is used in folk medicine, phytopharmaceutical preparations, and also for food preservation [1]. In folk medicine, some *Thymus* spp. are used for their antihelminthic, expectorant, antiseptic, antispasmodic, antimicrobial, antifungal, antioxidative, antiviral, carminative, sedative, and diaphoretic effects [2,3]. In this study, scratch assay was used to explore the wound healing property of thyme oleoresin. Oleoresins are rich in antioxidants and are normally composed of essential oils and resins from herbs [4]. The essential oil of thyme contains  $\alpha$ -pinene, 3-carene, p-cymene, 1-8 cineole, limonene, borneol,  $\alpha$ -terpineol, thymol, carvacrol,  $\beta$  caryophyllene, geraniol, linalool, gamma-terpineol, carvacrol, thymol, and trans-thujan-4-ol/terpinen-4-ol [5-7]. Thymol and carvacrol are known for its antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial effects [8-12]. The constituents reported in thyme oleoresins are thymol, p-cymene, borneol, terpinol, carvacrol, and linalool [13,14].

The conventional method to screen the wound healing property of plant products is invasive procedures in animal models. Many *in vitro* assays can be used to screen many plant products having antioxidant, cell mobilization, and angiogenic properties essential for wound healing [15]. Moreover, plants are explored for their various pharmacological activities such as antihyperglycemic activity, anticancer, and antimicrobial activities [16-18]. Here, in this study, the effect of thyme oleoresin on cell migratory activity of HaCaT keratinocytes was investigated and analyzed using scratch assay.

### METHODS

#### Cell culture

HaCaT cell line was obtained from NCCS Pune and maintained in Dulbecco's Modified Eagle's Media (DMEM) supplemented with 10% fetal bovine serum (FBS) and grown to confluency at 37°C in 5% CO<sub>2</sub>

in a CO<sub>2</sub> incubator. The keratinocyte cells (HaCaT) were trypsinized for 30 s and passaged to T25 flasks in the complete aseptic environment.

#### Cell migration assay

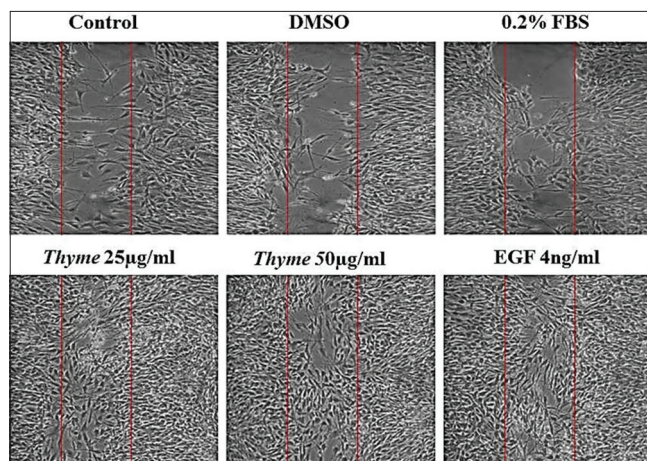
The effect of thyme oleoresin on wound closure was determined using a 12-well plate. Exponentially growing HaCaT cells in DMEM (1% FBS) were trypsinized and then seeded at a density of 200,000 cells into a 12-well plate and cultured overnight to allow adhering and reaching a 90% confluence. The scratch wound was made by a sterile 1 mL pipette tip through a pre-marked line. The cell monolayer was subsequently rinsed 3 times with PBS followed by incubation with indicated concentrations of thyme extract (25 µg/mL and 50 µg/mL) and epidermal growth factor (EGF) (4 ng/mL) for 30 min and incubated at 37°C for 24 h in a CO<sub>2</sub> incubator. DMEM medium with dimethyl sulfoxide (DMSO) was used as control. The effect of thyme extract on wound closure was determined microscopically (20× magnification, Nikon microscope). The experiment was performed in triplicate. The wound area was photographed and analyzed [19].

### RESULTS

The effect of thyme extract on cell migratory activity of HaCaT keratinocytes was investigated and analyzed using scratch assay. Thyme extract at 25 µg/ml and 50 µg/ml has significantly promoted the migration of HaCaT cells, thereby leading to wound closure (Fig. 1) compared to control, DMSO, and EGF.

### DISCUSSION

In the present study, the effect of thyme oleoresin on the migration of HaCaT cells was studied and was observed that the HaCaT cells mobilized toward the artificially created cell injury under the influence of thyme.



**Fig. 1: Photographs showing the cell migratory activity of HaCaT keratinocytes of thyme oleoresin**

It was reported that the wound healing and antioxidant properties exist together and the wound healing process can be aided by the presence of antioxidants [20]. Thyme oleoresin is known for its antioxidant activity. Thyme oleoresin is distilled from the aerial parts. The aerial part of the plants is reported to have major components such as p-cymene,  $\gamma$ -terpinene, and thymol [21]. Tissue injury initiates a cascade of events such as inflammation, new tissue formation, and remodeling. The healing process is modulated by the associated molecular signals through cytokines and growth factors [22]. Rapid migration and proliferation of keratinocytes are required for the wound healing process [23]. Re-epithelialization of the epithelium occurs by proliferation and migration of epithelial cells atop of the wound bed providing cover for the new tissue [24]. Hence, the effect of thyme oleoresin on cell migratory activity of HaCaT keratinocytes was investigated and analyzed using scratch assay. The scratch wound healing assay enables a large number of substances to be tested and analyzed which is relatively simple to carry out [25]. Animal models are also used extensively for the evaluation of wound healing property of plant extracts but which always have invasive procedures [26].

Tribals and folklore in many countries use a number of plants for the treatment of wounds and burns as they have real potential to heal wounds. Multiple mechanisms are involved behind this healing and regeneration of the lost tissue [27]. Several plant extracts and natural compounds have been reported to have anti-inflammatory effects in activated keratinocytes and in animal models [26,28-30]. *Aloe vera* is one such plant reported to have wound healing property. The possible mechanisms in wound healing may be its ability to keep the wound moist, enhancing epithelial cell migration, rapid maturation of collagen and reducing the inflammation, increasing the collagen synthesis, increase in synthesis of hyaluronic acid and dermatan sulfate in the granulation tissue of the healing wound, and improving the oxygenation by increased blood supply [31-34]. Thyme extract at different concentrations used in the study has significantly promoted the migration of HaCaT cells, thereby showing its ability in wound healing.

## CONCLUSION

Thyme oleoresin has significantly promoted the migration of HaCaT cells, thereby leading to wound closure. Hence, it may be used in formulations for the management of wound closure in a natural way.

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## AUTHOR'S CONTRIBUTION

All the authors have equally contributed towards the compilation of this research article.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest in publishing this paper.

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