

UNIVERSITI TEKNOLOGI MARA

***IN VITRO* WOUND HEALING
POTENTIAL AND THE
IDENTIFICATION OF RELATED
BIOACTIVE COMPOUND(S) FROM
RHODOMYRTUS TOMENTOSA
(AITON) HASSK**

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ABSTRACT

Wounds and their treatment significantly strain the healthcare system in terms of cost, time, and energy expended. The productivity and quality of life setbacks are incalculable. Herbal remedies are widely used in complementary and alternative medicine worldwide. *Rhodomyrtus tomentosa* leaves or known as "Kemunting", which are traditionally claimed to treat wounds, have not been proven scientifically. This research aimed to elucidate the mechanism of action of rhodomyrtone, Fraction 5-ethyl acetate (F5-EA) of *R. tomentosa* crude ethanolic extract (SERT) in enhancing the wound healing through *Wnt/β-catenin* pathway. The first part of this study was conducted to profile SERT, identify, and quantify rhodomyrtone in liquid-liquid extraction (LLE) Fractions 1 - 6 of the SERT by conducting liquid chromatography - mass spectrophotometry (LCMS). Free radical scavenging activity (DPPH) was used to assess the antioxidant activity of SERT and LLE Fractions 1-6. The *in vitro* wound healing potential was determined by the cell viability, proliferation of human skin fibroblast (CRL-2522), and human keratinocyte (HaCaT) cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium followed by migration assay, while the cell cycle analysis was conducted using flow cytometry. The molecular mechanism was conducted to investigate gene expression using Fluidigm (RT-PCR) analysis, confirming protein expression *via* western blot analysis and determining protease activity through zymogram analysis. The LCMS results in SERT confirmed rhodomyrtone as one of the major compound and five out of 10 most abundantly identified compounds belongs to flavonoid glycoside family which are myricetin, kaempferol, quercetin, laricitrin and cyanidin 3-O-(acetylglucoside). The highest radical scavenging activity was portrayed by SERT with EC of 14.60 ± 0.284 . Cell viability test showed the non-toxic dose of SERT at 250 $\mu\text{g/mL}$ for CRL-2522, lower concentration of SERT of 62.5 $\mu\text{g/mL}$ for HaCaT, 100 $\mu\text{g/mL}$ for Fractions 1 - 6 and for rhodomyrtone standard (Rhos) was at 2.5 $\mu\text{g/mL}$ for both CRL-2522 and HaCaT. Cell proliferation and migration assay revealed best concentration of SERT at 62.5 $\mu\text{g/mL}$, F5-EA at 100 $\mu\text{g/mL}$ and Rhos was at 0.625 $\mu\text{g/mL}$. CRL-2522 treatment with F5-EA of 62.5 $\mu\text{g/mL}$ showed enhanced *in vitro* wound healing activities of cell viability, proliferation and migration which exceeded the positive control. F5-EA of 62.5 $\mu\text{g/mL}$ portray the percentage of cells in the S phase increased and the upregulation of the *COL1A1*, *CTNNA1*, *FN1*, *MMP-2*, *MMP-3* and *TGFβ1* genes (more than 1.0-fold) were observed with ($p < 0.05$). Wnt3a, CTTNβ, MMP-2 and MMP-9 protein analysis portrayed the effect of F5-EA of 62.5 $\mu\text{g/mL}$ on CRL-2522 via Wnt/β-catenin pathway. Zymogram analysis of culture media treated with F5-EA of 62.5 $\mu\text{g/mL}$ on CRL-2522 were found to only digest the MMP-2. In conclusion, based on F5-EA of *R. tomentosa* may be responsible for accelerating wound healing activities *in vitro*. The wound-healing efficacy is due to the synergistic action of several bioactive components, rather than any single component.

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