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**IDENTIFICATION OF POTENTIAL WOUND
HEALING COMPOUNDS FROM SELECTED
PLANTS USED IN AYURVEDA BY
EMPLOYING CELL MIGRATION AND
ANGIOGENIC ASSAYS**

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ABSTRACT

The plant secondary metabolites belonging to various chemical classes have been identified as active principles responsible for facilitating of wound healing. Even though many plants are being used for the treatment of wounds, most of them are scientifically unexplored for their efficacy. This thesis describes the identification of potential wound healing compounds from the three plant species used in Ayurveda by employing cell migration assay (scratch wound assay) and angiogenic assay.

This thesis consists of 4 chapters. Introduction to the thesis which includes the literature review on plants and plant preparations used for wound management and their chemistry is presented in the Chapter 1. It also describes the general experimental procedures for cell migration assay (scratch wound assay), chorioallantoic membrane assay and antibacterial assay which were employed to investigate the wound healing potential of selected plant species.

Chapter 2 describes the scratch wound assay (SWA) directed chemical investigation of hexanes extract of *Jeffreyia zeylanica*, which led to the isolation and identification of lupeol (17), friedelin (33), β -amyrin acetate (36), β -amyrin (46), ethuliacoumarin (48), stigmasterol (49), and oleana-9(11),12-dien-3 β -ol (50) and a non-resolved triterpene alcohol mixture (JHF_{5D1}) containing glut-5-en-3 β -ol (47) and friedelin-3 β -ol (34). All of these compounds except friedelin (33) and β -amyrin acetate (36) showed enhanced cell migration at 12.5 μ M and considerable proangiogenic activity at 10 μ g/ disc. The mixture (JHF_{5D1}) also showed enhanced cell migration at a concentration of 12.5 μ M equivalent to glut-5-en-3 β -ol and considerable proangiogenic activity at 10 μ g/ disc. The structure of ethuliacoumarin (48) was established by

the unambiguous assignment of all NMR data to the structure, supported by HSQC and HMBC data for the first time. It was noticed that the reported ^1H NMR assignments of oleana-9(11),12-dien-3 β -ol (**50**) were incomplete, while some ^{13}C NMR assignments were inconsistent with the observed data. Hence reassignment of ^1H and ^{13}C NMR data of **50** was carried out with the aid of 1D and 2D NMR data. The combined effect of ethuliacoumarin (**48**) and stigmasterol (**49**) in *in-vitro* cell migration was studied using several pharmacological models, and it is plausible that the effective cell migration activity observed for the tested combinations of compounds **48** and **49** is due to the synergistic effect of them.

Chapter 3 describes the SWA directed chemical investigation of dichloromethane extract of *Leea indica*, which led to the identification of lupeol (**17**), β -sitosterol (**28**), betulin (**76**) and a rare phytosterol, stigmast-5-en-3 β ,7 α ,22 α -triol (**77**). All of these compounds showed enhanced cell migration at 12.5 μM and considerable proangiogenic activity at 10 μg /disc.

Chapter 4 describes the SWA directed chemical investigation of hexanes extract of leaf of *Ziziphus oenoplia*, which led to the isolation of two fractions ZHF_{2F1} and ZHF_{3C1}. The fraction ZHF_{2F1} consist of α -amyrinone (**98**), β -amyrinone (**99**) and lupenone (**100**) while the fraction ZHF_{3C1} consist of lupeol (**17**), α -amyrin (**101**), β -amyrin (**46**) and two alkanols, hexacosanol (**102**) and octacosanol (**103**). The fraction ZHF_{3C1} only showed enhanced cell migration at 5 mg/L and considerable proangiogenic activity at 10 μg /disc. SWA directed chemical investigation of methanol extract of leaf of *Z. oenoplia* led to the isolation of quercetin-3-*O*- β -D-galactoside (**104**) which showed enhanced cell migration activity in SWA at 5 mg/L (10.8 μM) and proangiogenic activity in CAM assay



at 10 μg / disc. The identified active constituents may be responsible for the efficacy of these three plant species used in Ayurveda for wound healing.