WOUND HEALING ACTIVITY OF SOME LUPEOL DERIVATIVES USING SCRATCH WOUND ASSAY

Nisansala S. Bopage[1], K. Hector Jayawardena[2], S. Chandrani Wijeyaratne[3], Ajita M. Abeysekera[4] and G. M. Kamal B. Gunaherath[1]*

[1]Department of Chemistry, The Open University of Sri Lanka, Nawala, Nugegoda
[2]Department of Zoology, The Open University of Sri Lanka, Nawala, Nugegoda
[3]Department of Botany, Sri Jayewardenepura University, Nugegoda
[4]Department of Chemistry, Sri Jayewardenepura University, Nugegoda

INTRODUCTION

Lupeol is a penta-cyclic lupane-type triterpenoid which shows an array of biological activities such as antiprotozoal, anti-inflammatory, antitumor, chemopreventive, antibacterial, and wound healing (Gallo and Sarachine, 2009, Harish *et al.*, 2008). It was also reported that lupeol is one of the constituents responsible for many biological activities among several plant species (Gallo and Sarachine, 2009). The wound healing activity of lupeol has been established using animal cell culture model using Madin-Darby Canine Kidney (MDCK) cells and Baby Hamster Kidney (BHK-21) cells (Bopage, *et al.,* 2014). The present study was designed to compare the wound healing potential of lupeol (**1**) and few common lupane triterpenoids, lupeol acetate (**2**), betulinic acid (**3**), betulin (**4**), and lupenone (**5**) with a view to understanding structure activity relationships (SAR) if any. The investigation was carried out using Scratch Wound Assay (SWA).

METHODOLOGY

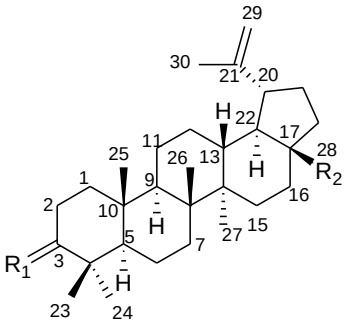
Scratch Wound Assay (SWA)

A monolayer of MDCK and BHK cells were distributed on 12-well tissue culture plates with growth medium (GM) [GM, 10% fetal bovine serum (FBS) in Dulbecco's modified Eagles medium (DMEM) supplemented with L-glutamine (Sigma Aldrich), antibiotics, penicillin 50 μg/mL and streptomycin 50 μg/mL (Sigma Aldrich) and 7.5% sodium bicarbonate]. Cultures were incubated in a humidified incubator maintained at 37 °C with 5% CO_2 (Liang, *et al.,* 2007). After the formation of a monolayer of cells, a scratch (wound) was performed on monolayer of cells along the vertical axis of each well under the microscope and washed with 750 μL of phosphate buffer saline (PBS). Each test well was then filled with DMEM (990 μL) and added 10μL of DMSO containing appropriate amount of test sample, such that the final concentration of test sample is 25 μM. Plates were incubated for 24 hours at 37 °C with 5% CO_2. Initial width of the scratch and the width of the scratch after treatment, at different time intervals (12 h, 18 h, and 24 h) were measured by using a stage micrometer. The percentage healing of the wound at 24 h was taken as an indication of the relative activity of an extract. Two negative controls, 1% DMSO in growth medium and 100% DMEM were used in this experiment while asiaticoside (25μM), a potent wound healing active compound (Shukla *et al.*, 1999) was served as the positive control.

Chemical Constituents

Lupeol (**1**) and lupeol acetate (**2**) were isolated from stem bark of *F. racemosa* (Bopage *et al.,* 2014). Other lupeol derivatives, betulinic acid (**3**), betulin (**4**), and lupenone (**5**) were obtained from the Chemistry Laboratory chemical collection (Jayasinha, 1999) and purified by preparative TLC.

Corresponding author: Email - **kbgun@ou.ac.lk*



	R_1	R_2
Lupeol (**1**)	α-H, β-OH	Me
Lupeol acetate (**2**)	α-H, β-OAc	Me
Lupenone(**3**)	O	Me
Betulin (**4**)	α-H, β-OH	CH_2OH
Betulinic acid (**5**)	α-H, β-OH	CO_2H

RESULTS AND DISCUSSION

The percentage wound closure at 24 h representing the wound healing activity is given in **Table 1** and **Figure 1** for both BHK and MDCK cells.

Table 1: Wound healing ~~Activity~~ activity (WHA) of lupeol (**1**), lupeol acetate (**2**), lupenone (**3**), betulin (**4**), and betulinic acid (**5**).

Sample[a]	**% Closure of the wound**[b] **at *t* = 24 h**[b]	
	BHK	**MDCK**
Lupeol (**1**)	83.1 (0.1)	78.6 (0.3)
Lupeol acetate (**2**)	33.7 (0.2)	33.2 (0.4)
Lupenone (**3**)	45.5 (0.1)	42.4 (0.4)
Betulin (**4**)	76.4 (0.2)	72.6 (0.3)
Betulinic acid (**5**)	42.4 (0.4)	42.4 (0.3)
Asiaticoside (**A**)	84.6 (0.7)	83.1 (0.1)
1% DMSO (Con1)	10.1 (0.7)	10.1 (0.1)
100% DMEM (Con 2)	11.2 (0.8)	11.3 (1.4)

[a]Sample concentration at a 25 μM
[b]The mean value follows the standard error of mean within the parentheses
Three triplicates for each sample and three measurements for each wound were taken.

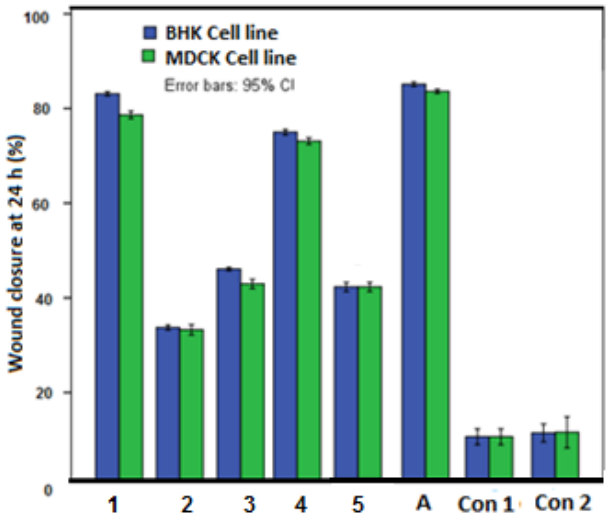


Fig. 1: Percentage wound closure of the compounds in the SWA ~~SAR assay~~. Bars represent the mean ±S.E.M. of nine measurements in the three experiments.

It is evident that among these compounds, only lupeol (**1**) and betulin (**4**) showed significantly high wound healing activity. When wound healing activity of lupeol (**1**) compared with those

of lupeol acetate (**2**) and lupenone (**3**)**,** it is evident that the presence of 3-OH group is an essential structural feature for the WHA of lupane skeleton.

Despite the presence of 3-OH in betulin (**4)** and betulinic acid **(5**), only **4** showed a significant activity which is slightly less than that of **1** while **5** was found to be significantly less active. This may be attributed to the increase of hydrophilic nature at C-28. In **4** the C-28 Me group of lupeol has replaced by CH_2OH group which is an H-bond donor while in **5** it has been replaced by CO_2H group which is an H-bond donor as well as an H-bond acceptor, thereby immensely increasing the hydrophilicity at C-28 (**Fig 2**).

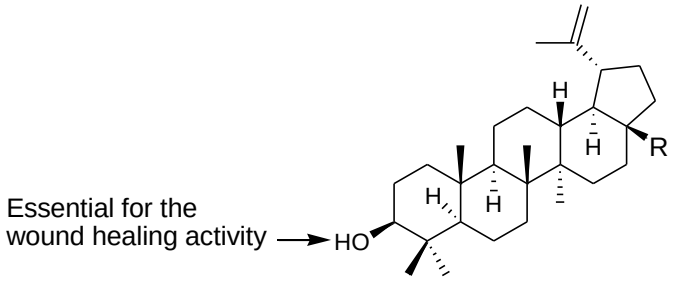


Fig. 2: Lupane Skeleton: Representation of wound healing active sites.

CONCLUSIONS

Lupeol and betulin showed the wound healing activity among the tested compounds of lupane skeleton. Further it was found that 3-OH is an essential feature in the lupane skeleton for its wound healing activity, Further these results indicate that the substituent at C-28 can influence the activity although the nature of the interaction involving the C-28 group cannot be elucidated based on the limited results of this study.

REFERENCES

Bopage, N. S., Jayawardena, K. H., Wijeyaratne, S. C., Abeysekera, A. M. and Gunaherath, G. M. K. B. (2014). Wound healing active constituents from *Ficus racemosa* Linn. Bark. *Proceedings of YSF symposium*, Colombo, Sri Lanka, 29–32.

Gallo, C. B. M., Sarachine, M. J., (2009). Biological activities of lupeol. In: Chattopadhyay D (Ed) Ethnomedicinal Phytophores in Disease Management. *International Journal of Biomedical and Pharmaceutical Sciences*, **3** (Special issue 1), 46–65.

Harish, B. G., Krishna, V., Kumar, H. S., Ahamed, B. M. K., Sharath, R., & Swamy, H. M. K. (2008). Wound healing activity and docking of glycogen-synthase-kinase-3-β-protein with isolated triterpenoid lupeol in rats. *Phytomedicine*, **15**, 763–767.

Jayasinha, P. M. (1999). *Chemical investigation of some Sri Lankan Diospyros species*. (Unpublished M. Phil Thesis), The Open University of Sri Lanka.

Liang, C. C., Park, Y. A., Guan, J. L., (2007). *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*. *Nature Protocols*, **2**, 329–333.

Shukla, A., Rasik, A. M., Jain, G.K., Shankar, R., Kulshrestha, D. K., Dhawan, B. N. (1999). *In vitro* and *in vivo* wound healing activity of Asiaticoside isolated from *Centella asiatica*. *Journal of Ethnopharmacology*, **65**, 1–11.

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