

## ABSTRACT

The present study was conducted to evaluate the comparative wound healing activity associated with Liposome-encapsulated *Hygrophila auriculata* (K. Schum.) Heine Root and Betulin. The research was done in four phases. Initially, studies on phytochemicals and free radical scavenging activities were carried out on various extracts from *H. auriculata* leaves and roots. Subsequently, *in silico* studies were conducted to identify lead compounds against wound targets from secondary metabolites of *H. auriculata* roots. Phase III involved the synthesis and characterization of liposomes using EEHA (Ethanol Extract of *H. auriculata*) and betulin. In the final phase, the *in vitro* and *in vivo* wound healing potential of the synthesized liposomes was assessed using the human keratinocyte cell line, HACAT, and swiss albino rats.

The qualitative phytochemical analysis revealed the presence of eight major phytoconstituents: alkaloids, phenols, tannins, flavonoids, proteins, carbohydrates, cardiac glycosides, and terpenoids. The ethanol extract of *H. auriculata* demonstrated significant scavenging effects on DPPH, FRAP, and ABTS, compared to other solvent extracts. Due to its superior phytochemical content and free radical scavenging capability, the ethanol extract was selected for further investigation. Enzymic antioxidants (catalase, superoxide dismutase, peroxidase, polyphenol oxidase, and glutathione S transferase) and non-enzymic antioxidants ( $\alpha$ -tocopherol, polyphenols, and ascorbic acid) were evaluated in the ethanol extract. Notably, the roots exhibited the highest concentration of antioxidants, prompting the selection of roots for subsequent studies. LC-MS analysis of *H. auriculata* root identified 15 significant compounds, and their physicochemical properties were examined. Ten ligands were analyzed for docking characteristics with proteins crucial for wound healing prognosis. Among the top-scoring complexes, since betulin exhibited the highest affinity, LB (Liposome encapsulated betulin) was synthesized and further evaluated for *in vitro* and *in vivo* studies along with the LHA (Liposome encapsulated *H. auriculata*). Liposome ointment formulations (LHA and LB) were developed using both betulin and *H. auriculata* root extract for a comparative analysis of their wound healing properties.

The synthesized liposomes demonstrated favourable characteristics in FTIR, XRD, TEM, and ZETA potential measurements, indicating enhanced stability. In antibacterial activity, liposomes of *H. auriculata* root exhibited superior inhibition against all bacterial species, with zone of inhibition values ranging from  $12.3 \pm 0.57$  mm to  $22.2 \pm 2.3$  mm.

Cytotoxicity assessments on HACAT cells revealed an IC<sub>50</sub> of 125.77 µg/ml and 97.65 µg/ml For LHA and LB respectively revealing less cytotoxicity.

The Wound Healing assay results show both *H. auriculata* root and betulin liposomes effectively promote wound healing in Human Skin Keratinocytes (HACAT), reducing wound area in an incubation-dependent manner. Flow cytometry after 48 hours of *H. auriculata* liposome treatment revealed the expression of Collagen 1 (78.35%) in HACAT cells, when compared to the standard control human Epidermal Growth Factor (hEGF- 84.89%). In *in vivo* studies, 10% LHA liposome ointment demonstrated the highest wound contraction on day 16 and subsequent post-wounding days ( $p < 0.01$ ) compared to the standard ointment (Povidone). LB ointment also showed significant wound contraction on all post-wounding days ( $p < 0.01$ ). Histological examination indicated normal healing, and reduced IL-6, CRP, and procalcitonin levels were observed in treated rats compared to untreated rats. Haematological parameters showed a more favourable decline in macrophages, T lymphocytes, platelets, and ESR in the LHA treated rats than in LB treated rats. This study underscores the importance of synergistic effects (LHA) in enhancing efficiency, especially when compared to the impact of a single bioactive compound (LB).