CHAPTER 8

SUMMARY

AND

CONCLUSIONS
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8.1. Summary of results

The purpose of this chapter is to summarize the findings of the present study, discuss its implications, and suggest avenues for future research.

The primary aim of the present research was to evaluate wound healing activity of the selected plants employing the *in vivo* and *in vitro* wound healing assays. The present research work endeavors to identify and develop new a therapeutic agent for wound care management.

Keeping in view the aim and objectives of the study, initially an extensive review of literature was undertaken and from which it was observed that the leaves of none of the under mentioned plants selected for the present study had been studied earlier for their wound healing properties:

1. *Epipremnum aureum* (Linden & Andre) G. S. Bunting
2. *Hibiscus rosa-sinensis* L.
3. *Tabernaemontana divaricata* L.
4. *Polyalthia longifolia* var. pendula

After collection and identification of the plant material, their identification was by an expert botanist. Additionally, the plant material of *E. aureum* was authenticated at the Blatter Herbarium, Mumbai. Thereafter, the methanolic extracts of the leaves of all the four plants were prepared using soxhlet apparatus. At an initial stage of the study, the methanolic extracts of the leaves of these four plants were subjected to a pilot screening for their wound healing efficacy by the *in vivo* wound healing by excision model. The findings of this pilot screening study revealed that amongst the four plants, two of them, *viz.* (1) *E. aureum* and (2) *Hibiscus rosa-sinensis* showed good wound healing properties. Hence a detailed investigation on the wound healing efficacy was undertaken for these two plants employing various *in vitro* and *in vivo* wound healing models.

The outcome and findings of these studies are discussed and summarised as below:
**In vivo toxicity studies**

An extensive review of literature had revealed that the preclinical efficacy and safety profile of the plant *E. aureum* had not been reported earlier. Subsequently, the plant methanolic extract was subjected to the *in vivo* acute toxicity studies. The findings of these studies established the preclinical safety of the plant and hence it was concluded that the methanolic extract of the leaves of this plant species was safe to be used as a pharmacological agent for wound care management. Incidentally, the preclinical safety of the other plant species, *viz. H. rosa-sinensis* had already been reported by earlier researchers.

On establishing the preclinical safety of the plant methanolic extracts, they were further subjected to various *in vivo* and *in vitro* wound healing efficacy tests to evaluate their wound healing capacity.

**In vitro wound healing study**

*In vitro* wound healing by scratch assay was employed for evaluation of the wound healing efficacy of the four selected plants. During this experimental study, promotion of migration and proliferation of fibroblasts were evaluated in response to the treatment with the plant methanolic extracts and compared with that of the controls. It was observed that out of the four plants evaluated, the extract of the plant *P. longifolia* showed the highest percent migration of the fibroblasts while the migration observed in response to the treatment with the plant extracts of *H. rosa-sinensis* and *T. divaricata* were of moderate whereas the plant extract of *E. aureum*, in fact, suppressed the migration of the fibroblasts. The findings of this study revealed that migration of the fibroblasts with plant methanolic extract treatment was not statistically significant. Keeping in view all these inconclusive findings of the *in vitro* scratch assay, it was decided to employ *in vivo* wound healing assays for evaluation of the wound healing potential of the two plants.

In contrast to the above observations, the results of the pilot screening for the same four plant species employing the *in vivo* wound healing efficacy by excision model, indicated that only two of these plants, *viz. E. aureum* and *H. rosa-sinensis* showed good wound healing properties. The predominant inconclusive and contradictory findings of the *in vitro* wound healing efficacy study by scratch assay prompted to
initiate and undertake further studies. Accordingly, the in vivo wound healing assays aimed at testing and establishing the wound healing efficacy of these plant species were initiated for the present study.

The primary limitations of the in vitro scratch assay undertaken for the present study were that the only one cell type, fibroblasts, was evaluated in the study, whereas it is an established fact that the wound healing is a complex process involving interactions of many cell types, growth factors and cytokines.

**In vivo wound healing study**

For evaluation of in vivo wound healing, the excision wound model and the dead space wound model were employed for the present study.

**A.** The methanolic extract of *E. aureum* (in two doses) was evaluated for its wound healing effect in vivo by the excision model using rats as experimental animals. The methanolic extract of *E. aureum* was found to be a potential promoter of wound healing activity. Out of the two doses evaluated in the study, the plant extract at a concentration of 5% (w/v) gave a positive response to the treatment such that the values obtained were statistically significant in terms of percent wound contraction as well as the lesser number of days required for complete healing of the wound as compared to those obtained in response to the treatment with 1% (w/v) plant extract in the test group animals. This observation was further reconfirmed by a histopathological evaluation of the healed skin tissues of all the experimental animals of the study.

**B.** Similarly, the methanolic extract of leaves of *H. rosa- sinensis* (also in two doses) was evaluated for its wound healing effect in vivo by the excision model. This extract was also found to be a potential promoter of wound healing activity. However, out of the two doses evaluated in the study, the extract at a lower concentration of 1 % (w/v) showed statistically significant values for the same parameters evaluated, viz. percent wound contraction and the lesser number of days required for complete healing of the wound as compared to the methanolic extract at a higher concentration of 5 % (w/v) in the test group animals. In addition, evidences from the histopathological evaluation of the wounded skin from all the experimental animals also indicated that the topical application of the
plant methanolic extracts proved to be highly efficacious in promoting and enhancing the wound healing in response to the treatment.

C. Besides, the hot methanolic extract of *E. aureum* further exhibited its wound healing effect in the experiments employing the *in vivo* dead space model by virtue of facilitating migration of fluid and cells in granuloma tissues. The granuloma tissues were formed in the dead spaces created by implantation of cotton pellets. A measurement of the dry weight of the granuloma tissue formed in the treated group of mice and that of the control group of mice indicated that the dry weight of the granuloma tissue of animals of the treated groups when compared to that of the control group animals was non significant. The migration of different types and total number of cells was almost similar in both the group of animals. From the estimation of hydroxyproline present in the granuloma tissues in the dead space model, it was evident that the amount of hydroxyproline content present in the control group animals was higher and its values were statistically significant as compared to that present in the treated animals. This suggests that the methanolic extract of *E. aureum* may possibly be effective by virtue of facilitating migration of different fluid components in the granuloma tissues or it might be effective at an early stage of the wound healing process.

**Pharmacognostic studies of *E. aureum***

An extensive literature review done so far, indicated that till date, no pharmacognostic studies had been initiated, nor any scientifically validated systematic data was available for this plant. In addition to these facts, this plant species also exhibited very good wound healing activity *in vivo*. Hence all these prompted us to undertake pharmacognostic studies of the plant. This included macroscopic and microscopic studies; physicochemical parameter evaluation, preliminary phytochemical analysis and HPTLC fingerprint analysis. The preliminary phytochemical studies of the hot and cold methanolic extract showed presence of different phytochemical constituents such as alkaloids, tannins, flavonoids, terpenoids, and saponins. Their presence was further confirmed by HPTLC fingerprint analysis.

Through this study, for the first time we hereby report systematic pharmacognostic analysis of the plant *E. aureum*. 
The findings of this study will, no doubt, prove of immense use in the correct identification of the plant amongst the different varieties. Also it will be helpful in preparation of the plant monograph and will serve as reference information.

**Antimicrobial activity of E. aureum**

The hot and cold methanolic extracts of *E. aureum* were evaluated for antimicrobial effect using different Gram +ve; Gram –ve bacterial cultures and a fungal strain as test cultures for evaluation of antimicrobial activity by the agar cup diffusion method. Further, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the hot methanolic extract were also determined. The hot methanolic extract showed better antibacterial activity as compared to the cold methanolic extract, however, both the hot and cold methanolic extracts did not show antifungal activity when tested against *Candida albicans* MTCC 227.

**8.2. Conclusions**

This research study had thrown light on the remarkable potential of commonly available plant *E. aureum* in terms of its pharmacological benefits it offers. The strength of this research work lies in establishing and reporting for the first time the wound healing efficacy of this plant. Additionally, results of the pharamcognostic study of the plant *E. aureum* in future will serve as a referential standard. The other important finding of the study is antibacterial efficacy of the plant *E. aureum*.

1. In brief, the present study has established the wound healing potential of the methanolic extracts leaves of two of the plants species, (1) *E. aureum* and (2) *H. rosa-sinensis*

   Incidentally, through the present study, the wound healing capabilities of the plant *E. aureum* are being reported for the first time.

   Thus these can be explored further as a source of an economical therapeutic agent for wound management as a pro-healer, as well as to facilitate faster wound healing processes without formation of residual scar tissues.

2. The methanolic extract of *E. aureum* is safe and showing no signs of systemic and dermal toxicity *in vivo.*
3. The pharmacognostic studies on the plant *E. aureum*, undertaken for the first time and which include macroscopic and microscopic examination of different parts of the plant, preliminary phytochemical analysis, and HPTLC fingerprint analysis will be of immense use to future researchers in correct identification and standardization of plant material. These results will also aid in preparation of plant monograph.

4. The plant *E. aureum* holds promise of serving as a good source of an antibacterial agent.

8.3. Future scope of the study

As in the case of any research, the present study has opened up quite a few potentially fruitful avenues for future investigations.

1. In more detailed investigations, the plant extracts could be subjected to bio-assay guided isolation and purification procedures for the isolation and identification of specific phytoconstituents responsible for promotion of wound healing.

2. The tissue exudates from the experimental animals of the *in vivo* wound healing study by the excision wound model could be subjected to various genetic and proteomic analytic procedures for evaluating and checking the expression pattern of various genes and proteins associated with the wound healing process.

3. Another important possible direction for the future research could be to explore the plant *E. aureum* for development of a new antimicrobial agent as well as an agent for dental carries and in the treatment of paratyphoid fever.