CHAPTER 2

AIM AND OBJECTIVES
2. **CHAPTER 2: AIM AND OBJECTIVES**

2.1. **Aim**

The aim of the present research study was to provide a scientific rationale for the effectiveness of traditional uses of phytochemicals from selected plants in the management of wounds and to explore the wound healing activity of such phytochemicals from these plants.

2.2. **Objectives**

The objectives proposed for the present research study were:

1. To evaluate and check the *in vitro* and *in vivo* wound healing efficacy of the phytochemicals from selected plants.

2. To evaluate and perform *in vivo* toxicity studies or preclinical safety studies for the phytochemicals of the extracts of the plants that could be potential promoters of wound healing.

3. To carry out

   a. Detailed pharmacognostic studies of the plant *Epipremnum aureum* (Linden & Andre) G.S. Bunting.

      The pharmacognostic studies of the plant *E. aureum* included:

      i. Macroscopic and microscopic studies of different parts of the plant

      ii. An evaluation of the physicochemical parameters

      iii. UV fluorescence analysis of the leaf powder

      iv. UV and FTIR spectral analysis of methanolic extracts of plant *E. aureum*

      v. Qualitative phytochemical analysis of the methanolic extracts of the plant for the detection of various phytoconstituents

      vi. Screening and detection of various phytoconstituents by thin layer chromatography (TLC)
vii. HPTLC fingerprint analysis of the plant methanolic extracts

viii. Determination of microbial load

ix. Estimation of total phenolic content and tannin content in methanolic extracts

b. Screening for antimicrobial activity of the methanolic extracts of the plant *E. aureum*.

**2.3. Research envisaged and plan of experimental work**

An outline of the research envisaged and plan of the experimental research work undertaken for the project is explained through a flow chart in Figure 2.1.

![Flow Chart](image-url)

*Figure 2.1: Research envisaged and experimental research plan*
For the present research project, it was proposed that initially plant material from the four selected plants would be identified and authenticated and methanolic extracts would be prepared. The plant methanolic extracts would be then processed and evaluated for a pilot screening for in vivo wound healing efficacy by excision model using rats as experimental animals to narrow down the number of plants from four to two plants. Eventually these two plants that were potential promoters of wound healing activity would be studied in detail for the actual wound healing potential by in vivo wound healing studies. The aim of the pilot screening study was to eliminate few of the selected plants which were not possessing better wound healing activity. On the basis of the initial pilot screening results, the plants that were potential promoters of wound healing activity were further evaluated for in vivo toxicity studies which included acute dermal toxicity study and acute systemic toxicity study to ascertain their preclinical safety. On the basis of the results of the in vivo toxicity studies undertaken for evaluation of preclinical safety of the phytochemicals, the plant extracts that were safe and showing no signs of in vivo toxicity were further evaluated in detail for preclinical pharmacological efficacy for wound healing properties by various in vitro and in vivo wound healing efficacy testing assays.

During the course of the present study, it was observed that one of the plants, viz. E. aureum, which was selected and proposed to be included in the present study, was not evaluated for in vivo toxicity study so far hence its in vivo toxicity studies were evaluated. Whereas, the preclinical safety of the other plant was well documented and reported earlier.

2.4. Organization of thesis

The thesis is organized into eight chapters and a summary of the research work undertaken is as described below:

Chapter 1 gives a brief introduction on a wound; how a wound is formed; and incidences and present status of occurrence of wounds. Further in the chapter, the process of wound healing is described in brief which also includes a short explanation about the unfolding of systematic events of this highly; well organized and overlapping phases of the wound healing process leading to an acquirement of a
normal or near normal structure and function. Later in this chapter the importance and the need of proper wound healing is emphasized. Also highlighted and included in the chapter is the importance of a better wound healing agent for restoration of lost function and integrity of the injured skin or any tissue. Different commercially available therapies which are commonly and routinely employed for wound healing are also mentioned in brief. The importance and effectiveness of the phytochemical based therapies from natural sources and bioactive plant secondary metabolites are discussed.

Chapter 2 describes the aim and objectives of the present research work. Through a flow chart, an outline of the research envisaged and plan of experimental research work undertaken for the project is explained.

Chapter 3 includes a review of literature on the plant species under investigation and having a potential for wound healing. This review of literature includes a profile of each plant including classification, botanical and morphological description of the selected plants; and traditional uses of these selected plants. This chapter also emphasizes the rationale for use of these selected plants for the present research study.

Chapter 4 comprises of two sections; Section 4.1 and Section 4.2.

Section 4.1 gives an account of the pharmacognostic studies of the plant *E. aureum* while Section 4.2 focuses on the antimicrobial activity of the methanolic extracts of the leaves of the plant *E. aureum*.

The data from our preliminary studies indicated that the plant *E. aureum* showed very good wound healing activity. Further on the basis of the literature reviewed, it was observed that no systematic pharmacognostic study has been reported on this plant species; hence this aspect of the plant was studied. This includes: (i) A macroscopic and microscopic study; (ii) Evaluation of physicochemical parameters (iii) UV fluorescence analysis of the leaf powder; (iv) Qualitative phytochemical analysis of the plant methanolic extracts (v) Screening and detection of various phytoconstituents by TLC and HPTLC fingerprint analysis. (vi) UV and FTIR spectral analysis of the methanolic extracts of the plant *E. aureum* was also undertaken. Further this chapter includes results and discussion about the estimation of total phenolic content and
tannins in the methanolic extracts. This is followed by a detailed discussion on the antimicrobial activity screening study for the methanolic extracts of the plant *E. aureum* against a battery of different Gram +ve and Gram –ve bacterial cultures and one of the fungal strains by the agar cup diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the hot methanolic extract were determined against susceptible bacteria.

**Chapter 5** describes *in vivo* toxicity studies undertaken to evaluate preclinical safety of the methanolic extract of the plant *E. aureum*. The *in vivo* toxicity studies were conducted to identify and evaluate possible adverse effects resulting from exposure to a new pharmacological agent. For this purposes, an *in vivo* toxicity study as per the standard OECD guidelines is undertaken with modifications according to CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) in order to reduce the number of animals eventually being used in the experimental studies. Hence, a limit test using a single highest dose of the drug was administered to mice and the animals were observed for a period of 2 weeks for any signs of deviant behavior and rate of mortality, if any, recorded. For the acute systemic toxicity studies, a single highest dose of 2000 mg/kg body weight was administered (OECD, 2001, Test no.423) and for the acute dermal toxicity, topical application of different doses of the plant methanolic extracts was evaluated (OECD, 1987, Test No.402). The histopathological evaluations of the skin tissues from *in vivo* dermal toxicity study and different organs of the treated animals from the *in vivo* acute systemic toxicity study are reported in this chapter. These data confirmed the *in vivo* preclinical safety of the methanolic extract of the plant *E. aureum*.

**Chapter 6** includes a description and a discussion regarding the screening of selected plants for *in vitro* wound healing efficacy. Many cell based *in vitro* assays can be employed to check and screen plant extracts that may indicate at which stage of a wound healing process the drug/plant extract is effective. Since wound healing is a complex process that involves interactions of many different types of cells, various cytokines, growth factors and many extracellular matrix proteins; there are possibilities that the plant phytochemicals could be effective in any of these phases.
One of these cell based assays routinely used is the *in vitro* scratch assay using 3T3 mouse fibroblast cells. This commonly used cell based assay checks the migration and proliferation of fibroblast cells in a gap/scratch created in the cell monolayer that mimics a wound. Thus a pilot screening of methanolic extracts of the selected four plants were undertaken by the *in vitro* scratch assay using 3T3 fibroblast cells.

**Chapter 7** includes a detailed description of the two *in vivo* experimental models employed for the present study for evaluation of wound healing potential.

*In vivo* wound healing efficacy is evaluated by different experimental animal models and each experimental model evaluates different parameters that are characteristics of one or the other phases of the wound healing process. The commonly employed *in vivo* experimental models for wound healing are the excision model, incision model and dead space model. Initially the ethical approvals were confirmed for all the *in vivo* studies involving animals prior to start of these studies and all these studies were performed as per CPCSEA guidelines and rules for usage and safety of animals.

The methanolic extracts of leaves of two of the short listed plants, *viz.* *E. aureum* and *H. rosa-sinensis* were evaluated for wound healing efficacy by the excision model using rats as an experimental model. Through the excision model, the percent wound contraction and total number of days required for complete closure of the wound was evaluated for the methanolic extracts. The *in vivo* dead space model was studied by using mice as experimental animals. This model evaluated the formation of granuloma tissues in plant extract treated animals and later these granuloma tissues were subjected to a histopahological evaluation. An estimation of hydroxyproline content in the newly formed granuloma tissue was also undertaken to check collagen formation and deposition in treated animals. The chapter concludes with the histopathological reports and evaluation of the results of the skin tissue from the excision model study and the granuloma tissues from the dead space model study.

**Chapter 8** describes the conclusions drawn on the basis of the present research project results.

The thesis ends with a list of references followed by the appendices and information on the publications and presentations.