Objective and Scope of the study

2.1. Objective of the study
2.2. Hypothesis and rational of the study
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Herbs have been used by all cultures throughout history. India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants. An increasing number of individuals are adopting complementary and alternative medicine, thus it is essential to identify clinically useful and safe products from medicinal plants. Herbs contain a variety of chemical compounds that act upon the body and are used to prevent or treat disease or promote health and well-being. Standardization of the herbal preparations is essential to get the optimal concentrations of known active constituents present, and in preserving their activities.

Skin aging is a complex evitable process of human life involves age-dependent decline of skin cell function. It causes harmful proteolytic degradation of extra-cellular matrix (ECM) that leaves visible signs on the surface of the skin. Skin aging expresses the clinical signs including irregular dryness, dark/light pigmentation, sallowness, deep furrows or severe atrophy, telangiectases, premalignant lesions, laxity, leathery appearance etc. Several scientific evidences on skin wrinkle highlights the degradation of ECM, which is significantly associated with increased dermal enzymatic (hyaluronidase, elastase, MMP-1 etc.) activities and formation of wrinkle. Skin-care ingredients from natural resources have the potential to inhibit these enzymes in aged skin and would also be used as anti-aging agent. Hence an attempt has been taken to search new skin-care ingredients from natural resources which have inhibitory potential on the dermal enzymatic activities and could also be used as ingredient in skin aging.

Salient objectives of the works represented in this thesis are:-

- Three Indian medicinal plants such as *Piper betel*, *Tagetes erecta*, and *Clitoria ternatea* were selected.
- All the herbs were standardized with their respective marker compounds through reverse phase high performances liquid chromatography (RP-HPLC).
- To evaluate the anti-aging activity depending upon previous pharmacological and phytochemical reports of the selected Indian medicinal plants, the experiments were designed.
- The methanol extracts, ethyl acetate, n-butanol and aqueous fractions of each plants were screened for *in-vitro* hyaluronidase, elastase (UV spectrophotometric) and MMP-1 (fluorescence) enzymes inhibition assays. The MMP-1 fluorescence assay is more precise and sensitive compare to spectrophotometric assays, which uses a difference in
the fluorescence intensity of fluorescein conjugated substrate from product to measure the enzymatic reaction.

- Bioactive compounds were obtained through bio-assay guided isolation and the activities were correlated with respect to individual phytoconstituents and their RP-HPLC standardization and validation through ICH guideline.
- The plan of work has been represented in the schematic diagram (Figure 2.1).

![Figure 2.1. Plan of work](image)

### 2.2. Hypothesis and rational of the study

Skin aging involves degradation of ECM in both the epidermal and dermal layers. It leaves visible signs on the surface of skin and the physical properties of the skin are modified. Skin aging is occurred due to some environmental factors on skin produces visible signs such as irregular dryness, dark/light pigmentation, sallowness, severe atrophy, telangiectases, premalignant lesions, laxity, leathery appearance and deep wrinkling. There are several synthetic skincare cosmetics existing in the market to treat premature aging and the most
common adverse reactions of those include allergic contact dermatitis, irritant contact dermatitis, phototoxic and photo allergic reactions. Recent trends in anti aging research projected the use of natural products derived from ancient era after scientific validation. Varieties of phytomolecules such as aloin, ginsenoside, curcumin, epicatechin, asiaticoside, ziyuglycoside I, magnolol, gallic acid, hydroxychavicol, hydroxycinnamic acids, hydroxybenzoic acids etc. scavenges free radicals from skin cells, prevent trans-epidermal water loss, include a SPF of 15 or higher contribute to protect skin from wrinkles, leading to glowing and healthy younger skin. Present era of treating aging skin has become technologically more invasive; but herbal products including botanicals are still relevant and combining them with molecular techniques will help to maximize the results and maintain the desired anti aging benefits. The scientific validity on the use of herbs as anti-wrinkle activity should be explored further based on different models. The plants from traditional and other resources need to be evaluated based on the combined approaches of exploitation and exploration to find effective leads from natural resources useful in the treatment of skin aging. The RP-HPLC methods have been developed for the identification and quantification of lead molecules. The methods are rapid, simple, accurate, specific, precise, and reproducible and have wide scope for separation and quality assessment of botanicals. The methods were successfully validated as per ICH guidelines and statistical analysis proves sensitivity, specificity, and repeatability. Assay methods are cost effective, environment friendly and satisfactory precision and accuracy. These can be conveniently employed for routine screening of new leads and their quality control analysis as bulk drug in marketed formulations. This thesis covered wide aspects for evaluation of three different plants species for their phytochemical and biological potential related with skin aging.

Salient features of the thesis are:-

- Collection and authentication of plant materials: all the plant materials were collected from local market and authenticated from genuine sources. The voucher specimen of each plant was deposited for future references.
- Plant materials were washed with distilled water and grounded for size reduction.
- All the plant materials were initially extracted with methanol by cold maceration and then methanol extract was fractionated using water, ethyl acetate and n-butanol.
➢ To assess the anti-aging activity, the extracts and fractions were screened for *in-vitro* hyaluronidase, elastase (UV spectrophotometric) and MMP-1 (fluorescence) enzymes inhibition assays with reference to oleanolic acid as standard.

➢ These evaluations helped us to select the bioactive extract or fractions from each plant to isolate, identify, quantify and screen the leads as bioactive compounds.

➢ Isolation of lead molecules was performed by silica gel and diaion column chromatography and characterization by Mass and NMR spectroscopic methods.

➢ Lead molecules were screened for *in-vitro* hyaluronidase, elastase (UV spectrophotometric) and MMP-1 (fluorescence) enzymes inhibition assays with reference to oleanolic acid.

➢ RP-HPLC standardization and related method development were made for each plant using isolated biomarker. Since the bioactive moiety always cannot be reliably quantified in a complex mixture, marker analysis and standardization of the medicinal plant is of great importance to maintain quality as well as safety.