3.1 Scope of the study

Three medicinal plants *C. asiatica* L., *O. basilicum* L., *O. vulgare* L. subsp *hirtum* were selected for the present investigation. As described above, these three plants have good antioxidant, anti-inflammatory and antifibrotic properties attributed to the secondary metabolites viz. triterpenoid, volatile oils and terpenes. These biological activities have been reported in wound healing, inflammation and in liver and pulmonary fibrosis. According to Widegrow *et al.* in 2011 therapies should be aimed at targeting:

(i) Inhibition of collagen synthesis  
(ii) Inhibition of excessive matrix deposition  
(iii) Modulation of fibroblast activation  
(iv) Stimulation of matrix degradation

3.2. Aim:
To demonstrate antioxidant, anti-inflammatory and antifibrotic activities in crude ethanolic extracts (*Centella asiatica* Linn., *Ocimum basilicum* Linn. and *Origanum vulgare* L. subsp *hirtum*) and pure compounds (asiatic acid, linalool, thymol) of the three selected species in an arecoline induced fibrosis model.

3.3. Objectives:

i. Assessment of antioxidant activity of ethanolic crude extracts of *Centella asiatica* L., *Ocimum basilicum* L. and *Origanum vulgare* L. subsp *hirtum*.

ii. Quantification and standardization of asiatic acid in *Centella asiatica* L., linalool in *Ocimum basilicum* L. and thymol in *Origanum vulgare* L. subsp *hirtum*. 
iii. Developing a human buccal fibroblast (HBF) cell line model and characterizing the cell type using vimentin, s100a4, phalloidin and transforming growth factor beta receptor 1.

iv. Assessment of inflammatory (IL-1β, IL-6, TNFα), anti-inflammatory (IL-10) and fibrotic markers (TGFβ1, COL1A2, COL3A1) post treatment with arecoline in arecoline inducible fibrosis model.

v. Assessment of anti-inflammatory and antifibrotic potential of three plant extracts (Centella asiatica L., Ocimum basilicum L., Origanum vulgare L. subsp hirtum) and their secondary metabolites (asiatic acid, linalool, thymol) on pretreatment in an arecoline induced human buccal fibroblast (HBF) cell line model.

vi. Morphological assessment of human buccal fibroblasts (HBFs) and qualitative assessment of collagen, post arecoline treatment and on pretreatment with pure compounds (secondary metabolites).

3.4 Hypothesis

3.4.1 Null Hypothesis: Ethanolic plant extracts (Centella asiatica L., Ocimum basilicum L., Origanum vulgare L. subsp hirtum) and pure compounds (asiatic acid, linalool, thymol) do not exhibit potent in-vitro antifibrotic activity.

3.4.2 Alternative Hypothesis: Ethanolic plant extracts (Centella asiatica L., Ocimum basilicum L., Origanum vulgare L. subsp hirtum) and pure compounds (asiatic acid, linalool, thymol) exhibit potent in-vitro antifibrotic activity.
Figure 3.1: WORK PLAN

**ETHANOLIC LEAF EXTRACTS**
- *C. asiatica* L.
- *O. basilicum* L.
- *O. vulgare* L.

**PURE COMPOUNDS**
- Asiatic acid
- Linalool
- Thymol

Quantification and Standardization by HPTLC & GC/MS

*In vitro* human buccal fibroblast cell line model for screening antifibrotic activity of plant compounds in Oral Submucous Fibrosis

**Primary cell culture**
- Cell viability
- Cell morphology
- Karyotyping

Establishment & characterization of Human oral buccal fibroblast cell line (HBF)

**ORAL SUBMUCOUS FIBROSIS**

**MTT Cytotoxicity Assay**
- Assessment of IC₅₀ concentration

**Molecular characterization of cell line (RT-PCR)**
- Inflammatory markers (IL-1β, IL-6, TNFα and IL-10)
- Fibrotic markers (TGFβ1, COL1A2 and COL3A1)

**Immunocytochemistry for fibroblast type**
- Vimentin,
- s100a4,
- TGFβR1
- Phalloidin

**Microscopy**
- Masson Trichrome Staining
- Fibroblast morphology
- Collagen deposition

**Anti-inflammatory and antifibrotic activity in OSMF cell line model**

**In – vitro OSMF cell line model induced by arecoline**

**MTT Cytotoxicity Assay**
- Assessment of IC₅₀ concentration of extracts & compounds

**Ethanolic leaf extracts of plants**

**Pure compounds**

**Molecular characterization of cell line (RT-PCR)**
- Inflammatory markers (IL-1β, IL-6, TNFα and IL-10)
- Fibrotic markers (TGFβ1, COL1A2 and COL3A1)

**Microscopy**
- Masson Trichrome Staining
- Fibroblast morphology
- Collagen deposition