3 SCOPE AND PLAN OF THE WORK

3.1 SCOPE OF THE WORK

In the course of the centuries, important contributions have been made in the use of plants for therapeutics as traditional medicine. Herbal preparations were the original therapeutic interventions used by man for years to treat diseased conditions in humans and livestock. Development of herbal products depended on local botanical flora. As a result, remedies for different ailments developed in different parts of the world.

Peptic ulcer is a condition associated with a number of factors involving autonomic nervous system, which occurs due to excessive acid secretion in the stomach. This condition is difficult to control with single drug therapy and complete cure cannot be achieved. Even after discontinuation of the drug the relapse rate is high, and the long-term use of synthetic drugs produces adverse effects on the human body.

*Rhodomyrtus tomentosa* (Ceylon hill gooseberry) of family Myrtaceae is a flowering plant, native to Southern and Southeastern Asia, from India, east to southern China, Taiwan and the Philippines, and south to Malaysia. The buds and tender leaves have been used traditionally in abscesses, diarrhoea, colic, dysentery, furunculosis, haemorrhage and the concentrated decoction of the leaves have been used as an antiseptic for abscess and wounds.
*Mallotus philippensis* (Lam.) Mull Arg (Kamala tree) is a small to medium-sized monoeccious tree, up to 25 m tall of the family Euphorbiaceae. Kamala tree is widespread, from the western Himalaya, through India, Sri Lanka, to southern China, Taiwan and the Ryukyu Islands, Burma (Myanmar), Thailand, and throughout Malaysia to Australia. Kamala is used as anthelmintic and an extract of kamala in hexachlorethane may be useful in treating liver fluke in cattle. The leaves are bitter, cooling, stimulates appetite, causes flatulence and constipation. The bark and fruits have been used traditionally to treat ulcers and tapeworms infestations. The pound leaves or seeds are used for fungal infections. Seeds of the plant are used for wound healing.

Studies on the role of free radicals in gastrointestinal ulceration have been reported in various literatures. The ethnomedical uses of plants selected in the present study are related to their antioxidant activities. Flavonoids, terpenoids and phenolic compounds present in these plants are known to have protective effects due to their antioxidant properties.

Therefore, an attempt was made to investigate the antioxidant activities of the various extractives of the leaves of *Rhodomyrtus tomentosa* and *Mallotus philippensis* using various *in vitro* methods and to study the anti ulcer activity using acute and chronic gastric ulcer models including *in vivo* antioxidant activities.
3.2 PLAN OF WORK

3.2.1 Preliminary Phytochemical Investigations

I. Collection and authentication of the selected plants, *Mallotus philippensis* and *Rhodomyrtus tomentosa*

II. Successive extraction of active constituents with solvents in the increasing order of polarity

III. Determination of phytoconstituents present in the extractives

IV. Estimation of phenolic content

3.2.2 Pharmacological Studies

I) Evaluation of toxicity profile by 24 h and 14 days single dose acute toxicity study and assessment of gross behavioural changes.

   a) Mortality    b) Clinical signs and symptoms

II) Assesment of *in vitro* antioxidant activity by different methods

   a] DPPH radical scavenging method

   b] H$_2$O$_2$ radical scavenging method

   c] Nitric oxide radical scavenging method

   d] ABTS radical decolourising method

   e] Reducing ability

III) Exploring the Anti ulcer activities of the extractives by four different models and to carry out the biochemical estimations and short term toxicity profile to support the study.
A. Ethanol induced ulcers in rat model

Parameters observed

i) Ulcer index,

ii) % Ulcer inhibition

iii) Histopathological studies

B. Aspirin plus pylorus ligation induced ulcers in rat model

Parameters Observed and Biochemical Evaluation

i) Ulcer index

ii) % Ulcer inhibition

iii) Gastric volume

iv) pH

v) Free and total acidity

vi) Total proteins

vii) Total Carbohydrate content

viii) Fucose

ix) Hexosamine

x) Total hexoses

C. Acetic acid induced chronic ulcers in rats (Ulcer healing model)

Parameters observed and biochemical evaluation

i) Ulcer index

ii) % Ulcer inhibition
iii) Ulcer area

iv) Histopathological studies

**Gastric mucosal defensive factors**

Estimation of mucous barrier

**In vivo enzymatic antioxidant parameters in rat stomach tissue**

i) Superoxide dismutase (SOD)

ii) Catalase activity (CAT)

iii) Lipid peroxidation products (LPO)

**Short term toxicity profile**

i) RBC and WBC count

ii) Food and water intake

iii) Body weight

iv) Clinical signs & symptoms

**D. Forced Swimming stress induced gastric ulceration in rats**

**Parameters Observed and Biochemical Evaluation**

i) Ulcer index

ii) % Ulcer inhibition

iii) Histopathology of stomach

iv) Alkaline phosphatase activity

**Biochemical estimations**

i) Cholesterol

ii) ALT (SGPT)

iii) AST (SGOT)

iv) Triglycerides
In vivo enzymatic antioxidant parameters in rat stomach tissue and blood

i) Superoxide dismutase (SOD)

ii) Catalase activity (CAT)

iii) Lipid peroxidation products (LPO)

3.2.3 Statistical Analysis

Graph Pad Prism, Version 4.03, One Way Analysis of Variance (ANOVA) followed by Dunnett’s test or with Tukey’s multiple comparison test.

3.2.4 Isolation And Characterization Of Active Constituents From The Selected Extractives

i) Column chromatography and Preparative TLC

ii) Characterization by spectral studies

3.2.5 Estimation Of Betulin In Selected Extractives By HPTLC.

3.2.6 GC-MS Analysis Of Selected Extractives.