PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF CORDIA MACLEODII AND LEUCAS CILIATA

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1.0 INTRODUCTION

Millions of people in the third world rely on the herbal medicines because they believe in them and regard them as their medicine in contrast to the “allopathic” (conventional modern) system of medicine brought in from “outside”. These medicinal herbs are available locally and are prescribed by the practitioners of traditional medicine. Even in Western countries, there is now an increased use of herbal preparation, because of belief that powerful synthetic agents used in western medicine can exert more unwanted side effects and are too often used indiscriminately and irrationally. Many people carry impression that medicines derived from natural plants are harmless. Although natural medicines induce fewer side effects than conventional drugs, there are plants that cause severe side effects.

*Cordia macleodii*

*Cordia macleodii* belongs to the family Boraginaceae. It includes a variety of shrubs, trees, and herbs, totaling about 2,000 species in 100 genera found worldwide. A number of familiar plants belong to this family. In India, the fruits of local species are used as a vegetable, raw, cooked, or pickled, and are known by many names, including *lasora* in Hindi. One such species is *Cordia dichotoma* (fragrant manjack), which is called *gunda* in Hindi and *lasura* in Nepali.

*Cordia macleodii* (Griff) Hook. F. & Thomas, is commonly known as Dahiphals or Dahivan in Hindi and Bhoti in Marathi. It is an 8-10 m high tree with a corky grey bark. The leaves are broad ovate, 5 – 10 cm as long as broad, scabrous, base cordate and crenate-serrate margins. They are arranged alternate to subopposite. The flowers are white in colour and polygamous, in short terminal axillary corymb. The calyx is densely tomentose the corolla lobes are oblong in shape and 0.6 to 0.8cm long. The drupes are 1.2 to 1.9 cm long, ovoid, acuminate at apex, seated at persistent calyx. The flowers and fruits appear in February – August.

*Leucas ciliata:*

*Leucas ciliata* belongs to the family Lamiaceae. Lamiaceae (Labiatae) also known as the mint family, is a family of flowering plants. The Lamiaceae family contains 233 to 263 genera and 6900 to 7200 species (Cantino et al. 1992).

*Leucas ciliata* (Benth) is commonly known as Burumbi. It occurs as herbs or under shrubs 30-100 cm high with stems and branches obtusely four angled and has brownish hairs. The leaves are ovate or lanceolate in shape, about 3 – 9cm long and 2.5 - 4 cm wide. The lamina is membranous, sparsely hairy on both sides with an acute apex, a cuneate base and serrate margin. The flowers are white in colour and have dense globose whorls. They have slender spinulose bracts equaling calyx. The calyx is tubular, hairy outside, with a ring of hairs at mouth and measures 1.2 – 1.8 cm in length. The corolla is long tube annulate inside and measures about 1.8-2.0 cm in length. The nutlets are smooth oblong-obvoid in shape and brown in colour. The flowers and fruits appear in May – August.

**Antioxidant activity:**

Antioxidants are radical scavengers, which protect the human body against free radicals. They may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms and thus prevent disease (Miller et al. 1997). They are used to counteract deleterious effects of free
radicals in oxidative stress. Antioxidants also have hepatoprotective activity. A large number of plants and plant products are used for their antioxidant activity. Many phyto-constituents like phenolics, polyphenolics, flavonoids etc. have been reported to have antioxidant activity.

**Antimicrobial activity:**

Infections diseases are the second leading cause of death world wide. The emergence of multidrug-resistant bacteria has created a situation in which there are few or no treatment options for infections with certain microorganisms (Wenzel et al., 2000). Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents. Many plants have been reported to have antifungal activity.

**Hepatoprotective activity:**

In the modern medicine, plants occupy significant berth as raw materials for some important drug preparations. The traditional Indian medicinal plants act as antiradicals and DNA cleavage protectors. *Moringa oleifera, Eclipt alba, Phyllanthus niruri, Picrorhiza kurroa* etc. possess hepatoprotective property against toxins and drugs induced hepatotoxicity.

**Anti-inflammatory activity:**

Inflammation, though a defense mechanism, owing to its tendency to induce, maintain and even aggravates several diseases, has always been a matter of concern for the physicians. It is a complex pathophysiological response of tissue to injury leading to local accumulation of plasmic fluid and blood cells. Although scores of anti-inflammatory agents are available, the search for better anti-inflammatory agents continues to avert the side effects of the available agents. Many traditionally used plants have been claimed to have anti-inflammatory potential. Several plants containing flavonoids have been studied for anti-inflammatory effect.

**Analgesic activity:**

The process of nociception describes the normal processing of pain and the responses to noxious stimuli that are damaging or potentially damaging to normal tissue. Chronic pain can be a major problem for some people and affect their quality of life. It can be caused by alterations in nociception, injury or disease and may result from current or past damage to the peripheral nervous system (PNS), CNS, or may have no organic cause.
2.0 LITERATURE REVIEW

**Pharmacognostical and phytochemical studies on Cordia macleodii:**
- Dettrakul et al. have isolated a new meroterpene from the root extract of *C. globifera*.
- Medeiros et al. have isolated and characterized two sesquiterpenes viz. α-Humulene and trans caryophyllene from the essential oil of *Cordia verbanacea*.
- Mori et al. have isolated Cordiachromes A, B and C, Cordiaquinol C and Alliodorin, Cordiaquinol I, J and K. from wood extract of *Cordia fragrantissima*.
- Siddiqui et al. have isolated four new aromatic compounds from fruits of *Cordia latifolia*.
- Lim et al. have isolated magnesium lithospermate, calcium rosmarinate and magnesium rosmarinate from water extract of *Cordia spinescens*.
- Ioset et al. have reported two isomeric meroterpenoid naphthoquinones from dichloromethane extract of *Cordia linnaei*.
- Menghini et al. have reported (+)-spathulenol from dichloromethane extract of leaves of *Cordia salicifolia* Cham.
- Ficarra et al. have reported four flavonoid glycosides, robinin, rutin, datiscoside and hesperidin, one flavonoid aglycone, dihydrorobinetin, two phenolic derivatives, chlorogenic and caffeic acid in *C. francisci*, *C. martinicensis*, *C. myxa*, *C. serratifolia* and *C. ulmifolia* leaves.
- Sinha et al. have reported microwave assisted rapid synthesis of methyl 2,4,5-trimethoxyphenylpropionate, a metabolite of *Cordia alliodora*.
- Kuroyanagi et al. have isolated dammarane-type triterpenes from *Cordia multispicata*.

**Pharmacological studies on Cordia macleodii:**
- Al-Awadi et al. have reported anti-inflammatory effects of *C. myxa* fruits on experimentally induced colitis in rats.
- de Menezes et al. have reported the chemical composition and larvicidal activity of the essential oil from leaves of *Cordia globosa*.
- Dettrakul et al. have reported antimycobacterial, antimalarial antifungal and cytotoxic activity of the isolated constituent and its derivative.
- Frydman et al. have reported the effects of *C. salicifolia* extract on the radiolabeling of blood constituents and on the morphology of red blood cells.
- Sen et al. have reported use of *C. macleodii* as a wonderful wound healing plant.
- Kuroyanagi et al. have reported antiandrogenic activity of six triterpenes isolated from *Cordia multispicata*.
- Makari et al. have reported antioxidant activity of hexane and methanol extracts of leaves of *C. wallichii* by DPPH radical scavenging and reducing power methods.
- Lim et al. have reported inhibitory activity against HIV 1 reverse transcriptase and HIV1 protease by water extract of *Cordia sapinensis* and its isolated constituents.
Afzal et al have reported antioxidant and hepatoprotective effects of *Cordia myxa* extracts by DPPH radical scavenging method and CCl₄ induced liver damage.

Kuppast et al have reported wound healing activity of fruits of *C. dichotomata*.

Roldão et al have reported antiulcer and analgesic activities of ethanolic extract of leaves of *C. verbanaceae*.

Kuppasta et al have reported anthelmintic activity of fruits of *Cordia dichotomata*.

Passos et al have reported the anti-inflammatory and anti-allergic properties of the essential oil and active compounds from *Cordia verbenaceae*.

Singh et al have reported the analgesic activity on different extracts of *Cordia dichotoma* bark.

Hernandez et al have reported the antimicrobial activity of the essential oil and extracts of *Cordia curassavica* (Boraginaceae).

**Pharmacognostical and phytochemical studies on *Leucas ciliata*:**

Chaudhury et al have reported chemical constituents present in *Leucas aspera*.

Manivannana et al have isolated glycoside Baicalin and baicalein from fresh flowers of *Leucas aspera*.

Scheen et al have reported the molecular phylogenetics of the *Leucas* group.

Pradhan et al have reported a triterpenoid lactone from *Leucas aspera*.

Mangathayaru et al have reported the volatile constituents of the *Leucas aspera*.

**Pharmacological studies on *Leucas ciliata*:**

Reddy et al have reported Analgesic activity of *Leucas aspera* leaves.

Saha et al have reported antibacterial activity of "*Leucas lavandulaefolia*.

Saha et al have reported Antiinflammatory activity of *Leucas lavandulaefolia* plant extract against carrageenan, histamine, serotonin, dextran induced rat paw edema.

Mukherjee et al have reported antipyretic activity of methanolic extract of aerial parts of *Leucas lavandulaefolia* against yeast induced pyrexia in rats.

Mangathayaru et al have reprotoed hepatoprotective effect of methanolic extract of aerial parts of *Leucas aspera* in rats against CCl₄ induced liver damage model.

Saha et al have reported hypoglycemic activity of methanolic extract of *Leucas lavandulaefolia* Rees extract on streptozotocin (STZ) induced diabetes in rats.

Ku et al have reported the antiinflammatory activity of the isolated fractions *Leucas Mollissima*.

Sadhu et al have reported inhibitory effect of fraction of *Leucas aspera* on prostaglandin-induced contraction in guinea pig ileum.

Reddy et al have reported effect of *L. aspera* on snake venom poisoning in mice.

Rahman et al have reported the antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root.
3.0 OBJECTIVES AND PLAN OF WORK

The world of plants and indeed all natural sources represents a virtually untapped reservoir of novel drugs. It has been estimated that only 5-15% of the approximately 250,000 species of higher plants have been systematically investigated for presence of bioactive compounds. There is a dire need to expand the exploration of nature as a source of novel active agents that may serve as the leads and scaffolds for elaboration into desperately needed efficacious drugs for a multitude of disease indications.

Nature is the prime source of novel, active chemotypes as leads for effective drug development. Much of nature remains to be explored, a host of novel, bioactive chemotypes await discovery from both terrestrial and marine sources. Plants known for medicinal activities are rich in secondary metabolites, which are potential source of drugs and essential oils.

The work embodied in this thesis is an effort on this line to explore the untapped nature for development of drugs for treatment of a multitude of human diseases.

Objectives:

1. To investigate the organoleptic, morphological, physico-chemical, microscopic and histological characteristics of C. macleodii and L. ciliata leaves to establish the pharmacognostical standards of the plants.

2. To carry out phytochemical studies on the extracts of leaves of C. macleodii and L. ciliata.

3. To evaluate the extracts of C. macleodii and L. ciliata leaves for antibacterial and antifungal activities against common pathogenic gram positive and gram negative bacteria and common pathogenic fungi.

4. To evaluate the extracts of C. macleodii and L. ciliata leaves for antioxidant activity by different radical scavenging methods and methods based on reducing ability of extracts.

5. To evaluate the anti-inflammatory activity of extracts of C. macleodii and L. ciliata leaves against carrageenan induced paw edema in rats.

6. To evaluate the analgesic activity of extracts of C. macleodii and L. ciliata leaves by hot plate method.

7. To evaluate the hepatoprotective activity of extracts of C. macleodii and L. ciliata leaves against carbon tetrachloride induced liver damage in rats.

Plan of Work:

The research work would be carried out according to a well planned and systematic scheme. The steps in systematic work plan are as under.

Literature review and Selection of plants:

The plant kingdom is rich with a vast array of plants having potential therapeutic utility. A large number of plants used traditionally are still unexplored. Similarly a large number of widely distribute plants need to be explored for therapeutic utility. The first step is to review the literature for such potential plants and select few of them for a systematic study.
Collection and authenticication of Plants:
The plants selected and the parts of plant decided would be collected from their natural habitat in healthy flourishing form. Sample of plant and/or its parts would be submitted to the Botanical Survey of India, Pune for authentication. After authentication, the parts of plant would be collected in sufficient quantity so as to suffice the requirement of work.

Pharmacognostic investigations on plants:
The plant parts selected would be investigated for macroscopic morphological and organoleptic features. The plants would then be studied for microscopic features.

Physicochemical evaluation of Plants:
The plant parts would be subjected to physicochemical evaluation. Extractive values, ash values and loss on drying would be determined for the plant parts selected.

Successive Solvent extraction:
The plant parts selected would be extracted with solvents of different polarity to study the phytoconstituents present in them. The plant parts would be extracted with petroleum ether (40-60), chloroform and ethanol successively.

Phytochemical investigations of plant extracts:
The extracts obtained would be subjected to phytochemical investigation for the presence of various classes of compounds present in plants. Tests for alkaloids, sterols, saponins, Flavonoids, glycosides and carbohydrates would be performed on the extracts to detect their presence. Chromatographic studies will also be carried out.

Pharmacological screening of plant extracts:
The plant extracts would be subjected to determination of pharmacological activities. The extracts would be subjected tests for following pharmacological studies;

I. In-vitro activities:
   a. Antioxidant activity
   b. Antibacterial activity
   c. Antifungal activity

II. In-vivo activities:
   a. Acute toxicity study
   b. Anti-inflammatory activity
   c. Analgesic activity
   d. Hepatoprotective activity
4.0 MATERIALS AND METHODS

Collection and authentication of leaves: Fresh leaves of *Cordia macleodii* were collected in the month of June from Nandurbar district of Maharashtra. *Leucas ciliata* leaves were collected in the month of September from Satpuda hills of Amravati district of Maharashtra. The leaves were authenticated by Shri PSN Rao, joint director, Botanical Survey of India, Pune. The herbarium of the plant specimen has been deposited at Govt. college of Pharmacy, Karad (*C. macleodii* – Voucher no. QMNM1 and *L. ciliata* – QMNMQMNM2)

Macroscopy: Fresh leaves of *C. macleodii* and *L. ciliata* were studied for organoleptic and morphological characteristics such as colour, odour, taste, size, shape of lamina, apex, base, margin, surface and texture etc.

Microscopy: The leaves of *C. macleodii* and *L. ciliata* were studied for microscopical characteristics such as microscopical morphology, cellular arrangements in mid rib and laminar regions, types of stomata and trichomes, stomatal index and stomatal frequency etc. Transverse sections of the leaves were prepared using microtome. The sections were stained with Sudan red III and observed under compound microscope at 100X. Surface preparations of leaves were made for the study of stomata and trichomes.

Physicochemical evaluation of Leaf powders:

**Water soluble extractive value:** 5 gm of air dried powdered leaves of *C. macleodii* and *L. ciliata* were separately macerated with 100 ml of chloroform water in a closed flask for twenty four hours shaking frequently during six hours and allowing standing for eighteen hours. It was filtered rapidly. 25 ml of the filtrate was evaporated to dryness in a tared flat bottom shallow dish. The residue was dried at 105 °C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drug (IP 1985).

**Alcohol soluble extractive value:** 5 gm of air dried powdered leaves of *C. macleodii* and *L. ciliata* were separately macerated with 100 ml of ethanol in a closed flask for twenty four hours shaking frequently during six hours and allowing standing for eighteen hours. It was filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in a tared flat bottom shallow dish. The residue was dried at 105 °C and weighted. The percentage of alcohol soluble extractive was calculated with reference to the air dried drug (IP 1985).

**Ash values:** Ash values of *C. macleodii* and *L. ciliata* leaves were determined by method I. 2 gm accurately weighed powdered leaves of *C. macleodii* and *L. ciliata* were separately placed in a dry crucibles. The content was incinerated at a temperature not exceeding 450 °C until free from carbon. The crucible was cooled in a desiccators and weighed. The percent ash was calculated with reference to the air dried drug (IP 1985).

**Acid insoluble ash value:** Total ash of *C. macleodii* and *L. ciliata* leaves were separately boiled with 25ml of 2N hydrochloric acid for 5 min. The insoluble matters were collected in Gooch crucibles and washed with hot water. The residues were
ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug (IP 1985).

Water soluble ash value: The total ash of *C. macleodii* and *L. ciliata* leaves were separately boiled with 25 ml of distilled water for five minutes. The insoluble matters were collected in Gooch crucibles and washed with hot water. The residues were ignited for fifteen minutes at a temperature not exceeding 450 ºC. The insoluble matters were weighed and the weight of the insoluble matter was subtracted from the weight of the total ash. Differences of the weights were taken as water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug (IP 1985).

Sulphated ash value: Silica crucibles were heated to redness for ten minutes and then allowed to cool in a desiccator and weighed. 2 gm accurately weighed powdered leaves of *C. macleodii* and *L. ciliata* were separately placed in the crucibles. They were ignited gently until it got thoroughly charred. The residues were cooled and moistened with 1 ml sulphuric acid and heated gently until white fumes ceased to evolve. The residues were then ignited at 800 ± 25 ºC in a place protected from air currents until all black particles disappeared. The crucibles were cooled in a desiccator. Few drops of sulphuric acid were added and the crucibles were again heated to ignite as before. The crucibles were allowed to cool in a desiccator and then weighed. The operation was repeated until two successive readings did not differ by more than 0.5 mg (IP 1985).

Loss on drying: Glass stoppered, shallow weighing bottles were dried for 30 min and weighed. Powdered leaves of *C. macleodii* and *L. ciliata* were separately kept in the bottles and weighed accurately. Samples were distributed evenly by gentle sidewise shaking. The bottles were placed in oven at 105ºC and weighed intermittantly. The leaves were kept in the oven until dried to a constant weight. After complete drying, the bottles were allowed to come to room temperature in a desiccator before weighing. Difference in the initial and final weight in percent gave loss on drying (IP 1996).

Florescence study: Many herbs fluoresce when cut surface or poser is exposed to UV light and this can help in their identification. For fluorescence study, powdered leaves of *C. macleodii* and *L. ciliata* were separately treated with different reagents and were examined under near UV (254 nm) and far UV (366 nm) lights.

Estimation of volatile oil content: About 50 g Fresh leaves of *C. macleodii* and *L. ciliata* were separately subjected to hydrodistillation for 20 min. in a Clevenger-type apparatus. The oils were separated from the water by decantation and were dried by filtration over anhydrous sodium sulfate. Oil yields were 0.43% for *C. macleodii* and 0.87%, for *L. ciliata*.

Extraction of leaf powders: The dried leaf powder of *C. macleodii* and *L. ciliata* separately were successively extracted with petroleum ether (40- 60ºC), chloroform and ethyl alcohol (95%/v/v).

1 kg of powdered leaves of *C. macleodii* and *L. ciliata* were filled separately in soxhlet apparatus. The powders were first extracted with petroleum ether (40-60) until the marc got exhausted. The mother liquor was then concentrated by distillation to
recover the petroleum ether. The concentrated petroleum ether extract was kept in desiccators for drying. The dried petroleum ether extracts of *C. macleodii* (CM-pet) and *L. ciliata* (LC-pet) were preserved at 2 to 4ºC in sealed container until used.

The marc after petroleum ether extraction was dried and then extracted with chloroform in Soxhlet apparatus. The extraction was continued until the marc got exhausted with chloroform. The mother liquor obtained was then concentrated by distillation to recover the chloroform. The concentrated chloroform extract was then kept in desiccators for drying. The dried chloroform extracts of *C. macleodii* (CM-chl) and *L. ciliata* (LC-chl) were preserved at 2 to 4ºC in sealed container until used.

The marc after chloroform extraction was dried and then extracted with ethanol (95%v/v) in Soxhlet apparatus. The extraction was continued until the marc got exhausted with ethanol. The mother liquor obtained was then concentrated by distillation to recover the ethanol. The concentrated ethanol extract was then kept in desiccators for drying. The dried ethanol extracts of *C. macleodii* (CM-eth) and *L. ciliata* (LC-eth) were preserved at 2 to 4ºC in sealed container until used.

**Preliminary Phytochemical investigations:** The extracts of *C. macleodii* and *L. ciliata* were separately subjected to chemical characterization. Different test for various classes of phytoconstituents i.e. alkaloids, carbohydrates, glycosides, sterols, proteins, flavonoids, saponins and phenolics were performed on the extracts of *C. macleodii* and *L. ciliata*. The tests performed are given below. (Harborne 1984)

**Tests for alkaloids:** The extracts were shaken separately with 5 ml. of 1.5 v/v hydrochloric acid and filtered. The filtrate was then tested by the Dragendorff’s test and Mayer’s test for detection of alkaloids.

**Tests for carbohydrates:** Few mgs of the residue was tested with Molisch’s test for presence of carbohydrates.

**Tests for glycosides:** Legal’s test: The residue was tested for presence of glycosides with Legal’s test.

**Tests for sterols:** The presence of sterols was tested by Salkowski Reaction, Liebermann Burchardt Reaction and Liebermann Reaction.

**Tests for proteins:** Biuret test, Millon’s test and Xanthoproteic test were performed to detect the presence of proteins.

**Tests for flavonoids:** For detection of flavonoids, Shinoda Test, Lead acetate test and Alkali test were performed.

**Tests for saponins:** Foam Test and Haemolysis Test were performed to detect the presence of saponins.

**Tests for phenolic compounds:** For detection of phenolic compounds Ferric chloride test and Lead acetate test were performed.

**Isolation of phytoconstituents:**

Portions of the extracts (5g) were subjected to column chromatography on silica gel of 60-120 mesh size. The column was first eluted with petroleum ether and then petroleum ether with increasing portions of ethyl acetate for Pet ether extract, chloroform and then chloroform with increasing portions of ethyl acetate for chloroform extract and with chloroform and then chloroform with increasing portions of ethanol for ethanolic extract. The fractions obtained were concentrated and those
yielding workable quantity of extract were further subjected to preparative TLC. The bands on preparative TLC were scrapped and recovered in warm ethanol. These subfractions were chromatographed on TLC plates and the constituents were characterized by co-TLC or spectrophotometric methods.

**Pharmacological screening of C. macleodii and L. ciliata extracts:**

**Preparation of extract:** Fresh Leaves of C. macleodii and L. ciliata were shade dried and crushed to make coarse powder. The powder (250g) was extracted with three liters of ethanol (95%v/v) by continuous extraction method for 48 hours for C. macleodii and 36 hours for L. ciliata. Solvent was distilled off and the extracts were concentrated and dried under reduced pressure. Extracts were preserved at 2 to 4°C.

**In vitro activity studies on the extracts:**

**Evaluation of Antioxidant activity:** The antioxidant activity was determined by four established methods. Diphenyl Picryl Hydrazyl (DPPH) radical scavenging method, Nitric Oxide (NO) radical scavenging method, Iron chelation method and reducing power method.

DPPH radical scavenging activity was measured by spectrophotometric method (Blois et al. 1958). 1ml each of ethanolic solutions of various concentrations (25–800 μg/ml) of the extract were mixed with 1ml of ethanolic solution of DPPH (200 μM). Similarly 1ml ethanolic solution of Ascorbic acid of various concentrations (25–400 μg/ml) were mixed with 1ml of DPPH solution. A mixture of 1ml of ethanol and 1ml of ethanolic solution of DPPH (200 μM) served as control. After mixing, all the solutions were incubated in dark for 20 minutes and then absorbance was measured at 517 nm. The experiments were performed in triplicate and % scavenging activity was calculated by using the formula:

\[
\text{% Scavenging} = \frac{\text{Absorbance of control – absorbance of test}}{\text{Absorbance of control}} \times 100
\]

Nitric oxide radical scavenging activity was measured by using Griess’ reagent (Green et al. 1982). Different concentrations of the ethanolic extract (25–800 μg/ml) in standard phosphate buffer solution (pH 7.4) were incubated with equal volume of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4) at 25°C for 5Hrs. In an identical manner solutions of different concentrations of Ascorbic acid (25–400 μg/ml) in standard phosphate buffer (pH 7.4) were also incubated with equal volume of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4). Control experiments without the test compound but with equivalent amount of buffer were also conducted. After incubation, 0.5ml of the incubation mixture was mixed with 0.5 ml of Griess’ reagent (Sulphanilamide 1%, O-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%) and the absorbance was measured at 546nm. From the absorbance the % scavenging activity was calculated using the same formula. The experiments were performed in triplicate.

Iron chelating activity is also a measure of antioxidant activity (Benzie et al. 1996). Solutions of extract (2ml in 5%v/v methanol) of different concentrations (25–800 μg/ml) and solutions of Ascorbic acid (2ml in 5% v/v methanol) of different concentrations (25–400 μg/ml) were incubated with methanolic O-phenanthroline
solution (1ml, 0.05%) and ferric chloride solution (2ml, 200 μM) at ambient temperature for 10 minutes. After incubation, the absorbance of solutions was measured at 510nm. The experiments were performed in triplicate.

Reducing power of the extract was also evaluated by Oyaizu method (Oyaizu et al. 1986). 2.5ml of Solutions of different concentrations of the extract (25–800 μg/ml) in standard phosphate buffer solution (pH 6.6) were incubated with 2.5ml of potassium ferricyanide solution (1% w/v) at 50°C for 20 min. In an identical manner solutions of different concentrations of Ascorbic acid (25–400 μg/ml) were also incubated. After incubation, 2.5ml of 10% trichloro acetic acid solution was added to each of the tubes and the mixture was centrifuged at 650 rpm for 10 min. 5ml of the upper layer solution was mixed with 5ml of deionized water and 1ml of ferric chloride solution (1%w/v) and the absorbance was measured at 700nm.

**Evaluation of Antibacterial activity:** The antibacterial activity was evaluated on five common pathogenic bacteria viz. *Escherichia coli* 2109 (NCIM, Pune), *Klebsiella pneumoniae* 2719 (NCIM, Pune), *Pseudomonas aruginosa* 2036 (NCIM, Pune), *Bacillus Subtilis* 6633 (ATCC, Chandigarh), *Staphylococcus aureus* 2079 (NCIM, Pune).

For evaluation of antibacterial activity of alcohol extract of *C. macleodii* and *L. ciliata* leaves, Agar diffusion assay method was used. Sterile Muller Hinton agar media (Hi-media) were prepared in Petri dishes. The bacteria (1x10^8 bacteria/ ml) were inoculated separately in the media. In each Petri dish four wells (diameter 6mm) were prepared under aseptic conditions. In these wells, DMSO (1ml/well, as control), alcohol extract of *C. macleodii* (1ml of 500μg/ml solution in DMSO), alcohol extract of L. ciliata (1ml of 500μg/ml solution in DMSO) and Chloramphenicol (1ml of 10μg/ml solution in DMSO) were added. All the dishes were incubated at 35°C for 24 Hrs. At the end of the incubation period, the media were observed for zone of inhibition. The zones of inhibition were measured in millimeter using Vernier Calipers.

**Evaluation of Antifungal activity:** The antifungal activity of the extracts was evaluated on two common pathogenic fungi viz. *Aspergillus niger* 545 (NCIM, Pune) and *Candida albicans* 3471 (NCIM, Pune).

For evaluation of antifungal activity of ethanolic extract of *C. macleodii* and *L. ciliata* leaves, Agar diffusion assay method was used. Sterile Potato dextrose agar media (Hi-media) were prepared in Petri dishes. The fungal spores (1x10^6 spores/ ml) were inoculated separately in the media. In each Petri dish four wells (diameter 6mm) were prepared under aseptic conditions. In these wells, DMSO (1ml/well, as control), alcohol extract of *C. macleodii* (1ml of 500μg/ml solution in DMSO), alcohol extract of L. ciliata (1ml of 500μg/ml solution in DMSO) and Nystatin (1ml of 100U/ml solution in DMSO) were added. All the dishes were incubated at 35°C for seven days. At the end of the incubation period, the media were observed for zone of inhibition. The zones of inhibition were measured in millimeter using Vernier Calipers.

**In vivo activity studies on the extracts:**

Animals: Healthy albino mice weighing 20 to 22g and albino rats weighing 120-170g of either sex were procured from National Toxicology Center, Pune and were kept in standard plastic animal cages in groups of 5 animals with 12 hrs. of light and dark
cycle. They were fed on standard feed and provided water *ad libitum*. Prior to initialization of experimentation the animals were acclimatized to laboratory conditions for a week. The experiments were carried out according to guidelines of ‘Committee for Prevention and Control of Scientific Experimentation on Animals’ (CPCSEA) New Delhi, and the procedures were approved by Institutional Animal Ethics Committee (IAEC), Govt. College of Pharmacy, Karad.

**Acute toxicity study:** The acute toxicity study was carried out by staircase method (Dixon et al 1965). Initially the dose of 500mg/kg was administered individually in 2 mice and mortality was observed for next 24 hr. The next dose 1000mg/kg was administered individually in 2 mice and mortality were observed for next 24 hr. Finally the dose was increased by 200mg/kg up to the dose 2000mg/kg and animal were observed for the mortality and toxicity.

**Evaluation of Anti-inflammatory activity:** The anti-inflammatory activity was evaluated using Carrageenan induced rat hind paw edema method (Winter et al 1962). The ethanolic extracts were incorporated in carbopol based Hydrogels (Salunkhe et al, 2005).

For preparation of hydrogels, one gram carbopol was dissolved in 100 ml of 10%v/v aqueous acetic acid solution. To the solution a 10%w/v sodium hydroxide solution was added gradually till a white cloudy precipitate appeared and then the mixture was incubated overnight at 37°C. The gel formed was washed extensively with distilled water and then 50%w/w propylene glycol was added and mixed. The extract was added to this gel base so as to contain 5%w/w of the extract. Finally, 2%w/w ethanol was added as penetration enhancer.

Twenty rats were divided in four groups comprising of five rats in each group. Group I served as control group and it was applied plain hydro gel. The Group II served as reference standard and it was applied Enacgel (Marketed preparation). To the rats in Group III Hydrogel containing *C. macleodii* extract was applied similarly Hydrogel containing *L. ciliata* extract was applied to the rats in Group IV.

One hour after the application of 50 mg of respective hydrogels to the paw, 0.1ml of 1%w/v solution of carrageenan was injected subcutaneously. At different time intervals the paw volume was noted and accordingly activity of each formulation was evaluated in terms of percent inhibition of edema using following formula;

\[
\% \text{ Inhibition of edema} = \left( \frac{V_t}{V_c} \right) \times 100
\]

Where \(V_t\) means paw volume of treatment group at time \(t\) and \(V_c\) means paw volume of control group.

**Evaluation of Analgesic activity:** Several in vivo models are available for evaluation of analgesic activity. Tail clip method, tail flick or radian heat method, tail immersion method, hot plate method, grid shock method in mice or rats, formalin test in rats and monkey shock titration are some of the commonly used methods for evaluation of analgesic activity.

The evaluation of analgesic activity of the extract was carried out on mice using hotplate analgesio-meter (Woolfe etal 1944).
The mice were divided in eight groups comprising of five animals each. One group received Pentazocin (10mg/kg i.p.) and one group received vehicle. Three groups received three different doses (100, 200 and 400mg/kg) of C. macleodii extract while the remaining three groups received three different doses (100, 200 and 400mg/kg) of L. ciliata.

A hotplate was maintained at 55±1°C. The extracts were administered intraperitoneally. Each mouse was placed on hot plate until the first lick of paw or jump and the latency to lick paw or jump was recorded. The test was terminated at 20 sec. The following formula was used to compute percentage analgesia.

\[
\% \text{ Analgesia} = \frac{t_t - t_c}{20 - t_c} \times 100
\]

Where:
- \(t_t\) – time required to lick paw in treated animals
- \(t_c\) – time required to lick paw in control animals

**Evaluation of Hepatoprotective activity:** The hepatoprotective activity of C. macleodii and L. ciliata leaves extracts was determined by using carbon tetrachloride induced hepatotoxic rat model (Singh et al 1999). Rats were divided in nine groups each comprising of five rats. Before treatment, the rats were fasted overnight with free access to water. Group I served as vehicle control and received normal saline (5ml/kg po) for seven days. The second group served as toxic control and was administered carbon tetrachloride in olive oil (1:1 v/v, 0.7ml/kg, ip) on alternate days for seven days. Group three served as standard control and was administered Silymarin (100mg/kg, po, daily) along with carbon tetrachloride in olive oil (1:1 v/v, 0.7ml/kg, ip) on alternate days for seven days. Groups four, five and six were respectively administered ethanolic extract of C. macleodii (100, 200 and 400mg/kg, po, daily) along with carbon tetrachloride in olive oil (1:1 v/v, 0.7ml/kg, ip) on alternate days for seven days. Similarly Groups seven, eight and nine were respectively administered ethanolic extract of L. ciliata (100, 200 and 400mg/kg, po, daily) along with carbon tetrachloride in olive oil (1:1 v/v, 0.7ml/kg, ip) on alternate days for seven days.

At the end of treatment, blood was withdrawn under light ether anesthesia by retro orbital cannulation and the rats were dissected to isolate liver. The blood samples after coagulation were centrifuged and the sera isolated were used for estimation of the biochemical markers of liver damage viz. SGOT, SGPT, ALP and Bilirubin levels. The livers were excised out, were washed with normal saline and weighed. The materials were fixed in 10% buffered neutral formalin for 48 hrs. Liver slices were then embedded in paraffin and sections of 5µ were taken using Microtome. The sections were stained with Hematoxylin and Eosine (H/E) and were observed under microscope for architectural changes, inflammation, congestion, steatosis and necrosis (Galigher et al 1971).
5.0 RESULTS AND DISCUSSION

The leaves of *C. macleodii* and *L. ciliata* were authenticated by Shri PSN Rao, joint director, Botanical Survey of India, Pune.

Microscopic studies:

*Cordia macleodii*: *C. macleodii* leaves are dorsiventral and hypostomatic and the midrib is prominent adaxially. The upper and lower epidermal cells are barrel shaped, medium sized and compactly arranged in one row. Both the epidermis are covered by a thick cuticle. The outer walls are thicker than the inner walls. The mesophyll consists of palisade and spongy parenchyma. The palisade consists of elongated cells compactly arranged in on layer and contains abundant chloroplast. Parenchyma consists of irregular cells having less chloroplast and intercellular spaces between them. The internal structure of midrib is different from laminar part. The cells of upper epidermis are similar to epidermis of laminar part. It is followed by 3 or 4 layered collenchyma. The cells of lower epidermis are similar to those observed in the laminar part. They are smaller than the upper epidermis, with thick cuticle. The vascular tissue is resolved into four vascular bundles embedded in ground tissues. The trichomes observed are foliar trichomes present on both surfaces i.e. adaxial and abaxial. In *Cordia macleodii* there is presence of unicellular conical glandular trichomes. The leaves are found to contain paracytic stomata significantly in the laminar region. The calculated stomatal index is 17.8 (stomatal frequency – 1.9).

*Leucas ciliata*: *Leucas ciliata* leaves are dorsiventral and hypostomaticand the midrib is flattened adaxially. The upper epidermis is single layered containing large barrel shaped or rectangular compactly arranged cells. The epidermis is covered by a thick cuticle. The cells of lower epidermis are smaller than the upper epidermis. The mesophyll consists of palisade and spongy parenchyma, which is single layered cells containing chloroplast. Parenchyma has loosely arranged rounded or irregular enclosing spaces in between. The internal structure of midrib is different from that of laminar part. The cells of upper epidermis are similar to those in the epidermis in the laminar part. It is followed from within by 3-4 layered collenchyma. The cells of lower epidermis in this region are similar to those observed in the laminar part. However they are slightly smaller than the cells of upper epidermis of midrib. They have also thick cuticle. The vascular tissue is restored into an arc. The cells of ground tissue in between the vascular bundle are fairly larger thin walled and parenchymatous. The trichomes are foliar trichomes, present on both adaxial and abaxial surfaces. In *Leucas ciliata* unicellular conical trichomes as well as multicellular segmented trichomes are present. The leaves are found to contain anomocytic stomata significantly in the laminar region. The calculated stomatal index is 12.4 (Stomatal frequency – 2.7).

Physical constants determination: The physical constants of *C. macleodii* and *L. ciliata* leaves including Water soluble extractive value, Alcohol soluble extractive value, Ether soluble extractive value, Chloroform soluble extractive value, Ash value, Acid insoluble ash value, Water soluble ash value, Sulphated ash value and Loss on drying were determined.
Florescence study: The fluorescence study was carried out on the leaves of *C. macleodii* and *L. ciliata*, on powdered drug as such and by treatment with various reagents like NaOH in methanol, NaOH in water, Benzene, Acetone, Ethyl acetate, Chloroform, Dilute H$_2$SO$_4$, Conc. HCl, Distilled water, 50% HCl, Dilute HNO$_3$, Conc. H$_2$SO$_4$ and Iodine solution (5%w/v).

Estimation of volatile oil content: Volatile oil contents of *C. macleodii* and *L. ciliata* leaves were determined by hydrodistillation. The Oil yields (dried over anhydrous sodium sulfate) were 0.43% for *C. macleodii* and 0.87%, for *L. ciliata*.

Preliminary Phytochemical Analysis: Petroleum ether extract of *C. macleodii* leaves showed presence of alkaloids, sterols and phenolic compounds. The chloroform extract of *C. macleodii* indicated the presence of alkaloids, sterols and phenolics compounds while the alcohol extract was found to exhibit presence of alkaloids, glycosides, flavonoids and saponins.

Preliminary Phytochemical Analysis: Petroleum ether extract of *L. ciliata* showed presence of sterols while the chloroform extract showed glycosides and phenolics along with sterols. The alcohol extract of *L. ciliata* was found to exhibit the presence of alkaloids, carbohydrates, glycosides, flavonoids and phenolics as well.

Isolation and characterization of phytoconstituents of various extracts of leaves of *C. macleodii* and *L. ciliata*:

Three compounds F10Pm2, F14Pb and F19Pm2 were isolated in workable quantities from the petroleum ether extract of *C. macleodii* leaves. Similarly from the chloroform extract of *C. macleodii*, one compound F8Ca and two compounds; F6Em1 and F9Eb from the ethanol extract of *C. macleodii* were isolated in workable quantities.

From the petroleum ether extract of *L. ciliata* leaves the compound F6PLm1 was isolated in workable quantity. Similarly one compound, F11CLm2 from chloroform extract of *L. ciliata* leaves and two compounds F9EL2 and F12E2 from ethanolic extract of *L. ciliata* leaves were obtained in workable quantities.

Structure elucidation:

F10Pm2 (Melting point: 214 °C) on recrystallization from ethanol yielded solid white needles shaped crystals, soluble in chloroform, ethyl acetate, and petroleum ether. It was derivatized by Anisaldehyde H$_2$SO$_4$ reagent, to give violet coloured spot which suggested that it might be a terpenoid. Its R$_f$ value was 0.6 in 2% methanol in DCM and was identified as lupeol with the help of $^1$H NMR, $^{13}$C NMR, MASS and IR spectra.

F14Pb (Melting point: 140 °C) was obtained as white crystals, soluble in chloroform. It was derivatized by Anisaldehyde H$_2$SO$_4$ reagent, to give blue coloured spot which suggested that it might be a terpenoid. Its R$_f$ value was 0.6 in 2% methanol in DCM and was identified as β-sitosterol with the help of $^1$H NMR, $^{13}$C NMR, MASS and IR spectra.

F19Pm2 was obtained as colourless solid, soluble in methanol. It did not melt but decomposed above 215 °C (softens at 195 °C). It was identified as trans caffeic acid based on coupling constants in $^1$H NMR with the help of $^{13}$C NMR, MASS and IR spectra.
F8Ca (Melting point: 207 °C) was solid crystals, soluble in methanol, slightly soluble in ethyl acetate. After acidic hydrolysis, it gave caffeic acid. It was identified as chlorogenic acid with the help of $^1$H NMR, MASS and IR spectra.

F6Em1 (Melting point: 277°C) was obtained as yellow needles, soluble in methanol. It was identified as Kaempferol with the help of $^1$H NMR, MASS and IR spectra.

F9Eb was obtained as yellow compound, slightly soluble in methanol. It decomposed above 315 °C. It was identified as Quercetin with the help of $^1$H NMR, MASS and IR spectra.

F6PLm1 (Melting point: 168-171 °C) was obtained as white crystals, soluble in chloroform, ethyl acetate. It was derivatized by Anisaldehyde H$_2$SO$_4$ reagent, to give blue coloured spot which suggested that it might be a terpenoid. Its R$_f$ value was 0.4 in 2% methanol in DCM and was identified as Stigmasterol with the help of $^1$H NMR, $^{13}$C NMR, MASS and IR spectra.

F11CLm2 (Melting point: 173 °C) was obtained as white needle shaped crystals, soluble in ethyl acetate, methanol. It was identified as Ferulic acid with the help of $^1$H NMR, $^{13}$C NMR, MASS and IR spectra.

Pharmacological screening of C. macleodii and L. ciliata extracts:

Evaluation of Antioxidant activity: The antioxidant activity of C. macleodii and L. ciliata leaf extracts was carried out by using four established methods viz; DPPH radical scavenging method, Nitric oxide radical scavenging method, Iron chelation method and reducing power method.

The C. macleodii extract (800 µg/ml) showed 81.20±1.31 and 72.70±0.44 % inhibition of DPPH and Nitric acid radicals. While ascorbic acid (400 µg/ml) showed 90.91±0.53 % inhibition in DPPH and 94.97±1.90 % inhibition in Nitric acid (200 µg/ml). The extract (800 µg/ml) showed 0.433±0.017 and 1.53±0.02 absorbance in iron chelation and reducing power method. While ascorbic acid (400 µg/ml) showed the 0.330±0.026 absorbance in iron chelation and 1.51±0.032 absorbance in reducing power method (200 µg/ml).

The L. ciliata extract (800 µg/ml) showed 69.09±1.39 and 79.90±1.54 % inhibition of DPPH and Nitric acid radicals. While ascorbic acid (400 µg/ml) showed 90.91±0.53 % inhibition in DPPH and 94.97±1.90 % inhibition in Nitric acid (200 µg/ml). The extract (800 µg/ml) showed 0.357±0.003 and 0.823±0.015 absorbance in iron chelation and reducing power method. While ascorbic acid (400 µg/ml) showed the 0.330±0.026 absorbance in iron chelation and 1.51±0.032 absorbance in reducing power method (200 µg/ml).

C. macleodii and L. ciliata demonstrated dose dependent antioxidant activity comparable with Ascorbic acid. In all the methods, maximum antioxidant activity was found at the dose of 800 µg/ml of C. macleodii extract.

Evaluation of Antibacterial and antifungal activities: Ethanolic extract of C. macleodii and L. ciliata shows antibacterial activity against the E. coli, K. pneumoniae, P. aeruginosa, B. subtilis and S. aureus. C. macleodii extract shows maximum antibacterial effect on E. coli and S. aureus. Ethanolic extract of C. macleodii and L. ciliata shows antifungal activity against the Candida albicans. Both the antibacterial and antifungal effects were less as the standard drugs.
Acute Toxicity Study: The acute toxicity study of ethanolic extracts of *C. macleodii* and *L. ciliata* were performed in mice. The acute toxicity of *C. macleodii* and *L. ciliata* leave extracts were studied in the different doses, the last dose was 2000 mg/kg. None of the doses of the extracts showed any mortality, but the animals were depressed.

Evaluation of Anti-Inflammatory Activity: Both *C. macleodii* and *L. ciliata* extracts inhibited the carageenan induced edema when compared with the control group. Both the extracts demonstrated significant anti-inflammatory activity. The ethanolic extract of *L. ciliata* leaves showed better anti-inflammatory activity than the extract of *C. macleodii* leaves. Five hours after carageenan challenge, the ethanolic extract of *L. ciliata* inhibited edema to the extent of 61.5% while the *C. macleodii* extract inhibited edema to the extent of 44% in comparison with standard Diclofenac diethylamine which inhibited edema to the extent of 92%.

Evaluation of Analgesic Activity: The extracts of *C. macleodii* and *L. ciliata* were observed for anti-nociceptive activity using hot Plate test. Both the extracts demonstrated good analgesic activity. The analgesic effects of the extracts at doses of 400 mg/kg were comparable with the analgesic effect of Pentazocin at 10 mg/kg. Analgesic effects of the extracts were maximum at 60 and 90 minutes after the administration of the doses. The analgesic effects of *C. macleodii* and *L. ciliata* extracts were comparable with centrally acting analgesic agent pentazocin.

Evaluation of hepatoprotective Activity: In this study significant increase in the total bilirubin content and in the GOT, GPT and ALP activities in the *CCl₄* treated group was taken as an index of liver damage. Treatment with *C. macleodii* extract inhibited *CCl₄* induced increase in total bilirubin and GOT, GPT and ALP activities as compared with *CCl₄* treated group. *C. macleodii* and *L. ciliata* showed significantly decrease in serum biochemical parameters viz. GPT, GOT, ALP and Bilirubin. At a dose of 400mg/kg, both the extract decreased GPT, GOT, ALP and Bilirubin comparable with silymarin 100 mg/kg. in the histopathological study, the extracts demonstrated good inhibition of cellular damage to the hepatic tissue.
6.0 CONCLUSION

The work presented in this thesis embodies pharmacognostic, phytochemical and pharmacological study of leaves of *C. macleodii* and *L. ciliata*.

A detailed pharmacognostical evaluation of the leaves of *Cordia macleodii* and *Leucas ciliata* was carried out. Morphoanatomy of the leaves has been studied to aid pharmacognostic and taxonomic species identification using microscopy, physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological and histological parameters presented may be proposed as parameters to establish the authenticity of the plants.

For phytochemical analysis, the leaves of *C. macleodii* and *L. ciliata* were subjected to successive solvent extraction with petroleum ether, chloroform and ethanol. On preliminary phytochemical analysis, the extracts of *C. macleodii* leaves were found to contain sterols, phenolics, flavonoids and glycosides. The extracts were subjected to column chromatography and other separation techniques for isolation of phytoconstituents and then characterization of isolated phytoconstituents was carried out by various spectrophotometric and physicochemical methods. Two sterols i.e. Lupeol and β-sitosterol were isolated and identified from the extracts of *C. macleodii*. Trans caffeic acid and chlorogenic acid represent the phenolic compounds isolated from *C. macleodii* leaves. Two flavonoids Kaempferol and Quercetin were also isolated and characterized from *C. macleodii* leaves.

On preliminary phytochemical analysis the extracts of *L. ciliata* leaves were found to contain sterols, phenolics, flavonoids and glycosides. Stigmasterol and β-sitosterol constitute the sterols isolated from the extracts of *L. ciliata* leaves. The phenolics isolated from *L. ciliata* leaves include trans caffeic acid and Ferulic acid while Luteolin and Apigenin were the flavonoids isolated from extracts of *L. ciliata* leaves.

For screening of pharmacological activities, the powdered dried leaves were extracted with 95% v/v alcohol to prepare whole alcohol extract. The whole alcohol extracts of leaves of *C. macleodii* and *L. ciliata* were screened for various pharmacological activities like antioxidant activity, antibacterial activity, antifungal activity, acute toxicity potential, analgesic activity, anti-inflammatory activity and hepatoprotective activity.

The whole alcohol extracts of *C. macleodii* and *L. ciliata* were evaluated for antioxidant activity in comparison with ascorbic acid by four in-vitro methods viz. DPPH radical scavenging method, Nitric oxide radical scavenging method, Reducing power method and Iron chelation method. Both *C. macleodii* and *L. ciliata* extracts were found to have good radical scavenging activity against DPPH and Nitric oxide radicals. The extracts also had good reducing ability as evaluated by the Reducing power method and Iron chelation method.

The whole alcohol extracts of *C. macleodii* and *L. ciliata* leaves were screened for antibacterial and antifungal activities. The antibacterial activity was evaluated against five different bacterial species viz. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. *C. macleodii* extract shows maximum antibacterial effect against *E. coli* and *S. aureus*. The *L. ciliata* shows maximum antibacterial effect against *B. subtilis* and *S. aureus*. The evaluation of antifungal activity was carried out against two fungi viz. *Candida albicans* and *Aspergillus niger*. Both *C. macleodii* and *L. ciliata* were effective against *C. albicans*. 

In acute toxicity study the whole alcohol extracts of both *C. macleodii* and *L. ciliata* leaves were found to produce no mortality or any significant toxicity even at a dose of 2000 mg/kg. None of the doses of the extracts showed any mortality, but the animals were depressed.

The extracts of *C. macleodii* and *L. ciliata* were evaluated for anti-inflammatory activity by carrageenan induced rat paw edema method. *C. macleodii* and *L. ciliata* extracts inhibited carrageenan induced edema when compared with the control group. Both the extracts demonstrated significant anti-inflammatory activity. The ethanolic extract of *L. ciliata* leaves showed better anti-inflammatory activity than the extract of *C. macleodii* leaves.

The extracts of *C. macleodii* and *L. ciliata* were evaluated for analgesic activity using hot Plate test in mice. Both the extracts were found to have significant analgesic activity as compared to the reference standard Pentazocin.

The extracts were also evaluated for hepatoprotective activity by carbon tetrachloride (CCl₄) induced liver damage model in rats in comparison with reference standard Silymarin. Extracts of *C. macleodii* and *L. ciliata* leaves inhibited CCl₄ induced increase in total bilirubin and GOT, GPT and ALP levels in serum as compared with CCl₄ treated group as compared to the reference standard Silymarin. The liver histology also suggested the protective effects of the extracts against CCl₄ induced liver damage.

The studies indicate the therapeutic potential of *C. macleodii* and *L. ciliata* leaves and justify the traditional use of these plants. The plants may prove to be promising in management and alleviation of liver diseases, painful inflammatory conditions and aging related disorders.
REFERENCES


M. N. Qureshi
Signature of Research student

Dr. B. S. Kuchekar
Signature of Research guide