A large majority of Indian population is treated by Traditional systems of medicine such as Ayurveda, Unani, Siddha and Homeopathy systems of Medicine. There are about twenty well recognised manufacturers of traditional drugs and around 1200 licensed small manufacturers besides thousands of vaidyas having their own miniature manufacturing facilities. There are about 700 naturally occurring independent drugs (medicinal plants) used in numerous formulations available in the country although literature indicates traditional medicinal uses of 2300 medicinal plants.

With the advent of European scientific methods, many of the reputed medicinal plants came under chemical scrutiny, leading to the isolation of active principles. Beginning with A.D. 1800 there was continuous activity in this area and many of the well known medicinal plants were chemically analyzed and their active principles characterised. Soon after their isolation and characterization, these compounds, either in pure state or in the form of well characterised extracts, became part of pharmacopoeias of several countries.

Before any botanical drug is used it is essential that it be identified correctly as the desired species. Botanical classification depends almost entirely on morphological characteristics, both macroscopical and microscopical. Confirmation of identity, purity and quality are the three parameters used in drug evaluation. Such an evaluation can be done by examining characteristics under the organoleptic evaluation, microscopic evaluation, physical evaluation, chemical evaluation and biological evaluation.
Because of the definite trend throughout the world for people to use many herbal preparations, the need for proper dissemination of information on safety, efficacy and potential hazards involved in their use represent a challenge for the pharmaceutical profession and points out to the need for pharmacology oriented pharmacognosy. The popularity of natural drugs all over the world in recent years is an indication of significant contributions of pharmacognosy in modern medicine.

The problem of urinary stones or calculi is a very ancient one and kidney stones affect up to 5% of the population. A kidney stone is a hard mass developed from crystals that separate from the urine and build up on the inner surface of the kidney. Increased incidence of kidney stones in the industrialised world is associated with improved standards of living and is strongly associated with race or ethnicity and region of residence. Stones form twice as often in men as in women. Once a kidney stone forms, the probability that a second stone will form within five to seven years is approximately 50%.

Since urolithiasis (urinary stone disease) is characterized by a high recurrence rate, it requires a preventive treatment. Even though drug treatment to prevent stones has been effective in many randomized trials, it is not accomplished without side effects. Therefore, it is worthwhile to look for alternative treatments using medicinal plants.

The precipitation and dissolution of sparingly soluble calcium salts such as calcium oxalate and calcium phosphate are of interest due to their
widespread and universal applicability. Oxalates are the main inorganic components in pathological deposits and play a key role in the formation of kidney stones. Precipitation and dissolution studies of calcium oxalate and the influence caused on such process by foreign substances, are of great interest in view of their potential application in the urolithiasis therapy. *In-vitro* methodologies and other non-animal methods are a routine part of basic science studies.

Diuretics are drugs that act primarily by promoting the excretion of Na\(^+\) or Cl\(^-\) or HCO\(_3\)^- ions which constitute the main electrolytes of the extracellular fluid. They also act by decreasing tubular reabsorption, a process which involves the active transport of electrolytes and other solutes from tubular urine to the tubular cells and then to the extracellular fluid. Carbonic anhydrase inhibitors, loop diuretics and thiazide diuretics increase the delivery of Na\(^+\) to the late distal tubule and collecting duct, a situation that often is associated with increased K\(^+\) and H\(^+\) excretion. Diuretics are used in the relief of edema and as adjuvants in the management of hypertension. Untoward effects of diuretics vary according to the class to which they belong. Some effects are minor, whereas others may be very serious, including death. Herbs that stimulate the kidneys were traditionally used to reduce edema. Herbal diuretics do not work the same way that drugs do. Thus it is unclear whether such herbs would be effective for this purpose.

The medicinal value of any plant drug depends on the nature of the chemical constituent(s) present in it and is referred to as active principle. The isolated chemical constituents of plants have various applications in medicine.
In the present investigation the following plants have been subjected to systematic pharmacognostical, inhibitory effect on crystal growth, diuretic and phytochemical studies for the first time:

1. *Dichrostachys cinerea* (Linn.) Wt. And Arn. (Fam. *Mimosaceae*)

2. *Hemidesmus indicus* R.Br. (Fam. *Asclepiadaceae*)

3. *Parmelia perlata* Ach. (Fam. *Parmeliaceae*)


5. *Sida cordata* (Burm.F.) Borssum (Fam. *Malvaceae*)

The main objectives of the present investigations are:

1. To provide information about the distribution and vernacular names of medicinal plants used as diuretics and for urolithiasis, to analyse the macroscopic and microscopic characters of the leaf, stem and root and also to standardize three plant drugs used for the above, performing a systematic pharmacognostical studies.

2. To grow calcium oxalate crystals *in-vitro* in silica gel media in Hane's tubes by single diffusion method and to study the inhibitory effects of methanolic and aqueous extracts of the said five medicinal plants on calcium oxalate crystal growth *in-vitro*.

3. To characterize the grown crystals by optical microscopy, FT – IR, SEM, and by measuring the lengths of crystal columns.

4. To evaluate the diuretic action of methanolic extract of selected three
plants on male albino rats using Lipschitz et al method.

5. To determine the effect of the drugs on $\text{Na}^+$, $\text{K}^+$ and $\text{Cl}^-$ content of the urine of albino rats.

6. To perform the preliminary phytochemical analysis, thin layer and paper chromatographic studies and also to estimate quantitatively the amount of flavonoids, total free amino acids, L-proline and sugars in the medicinally important parts of the said five medicinal plants.

The thesis consists of five chapters.

Chapter 1 deals with the general introduction, the scope of the present study and it highlights the main objectives of the present investigation.

In chapter 2, the review of pharmacognostical studies on medicinal plants has been highlighted. The past work related to the medicinal plants of present investigation has been presented. Pharmacognostic studies on three plants viz. Parmelia perlata, Sida acuta and Sida cordata have been investigated since the pharmacognostic studies on Dichrostachys cinerea and Hemidesmus indicus have already been published.

Parmelia perlata was collected from Thanthrikudi, Dindigul District of Tamil Nadu and Dichrostachys cinerea, Hemidesmus indicus, Sida acuta and Sida cordata were collected respectively from Thirukurunkudi, Thenmalai, Tirunelveli and Shenkotta of Tirunelveli District of Tamil Nadu, India in the month of September. The plants were identified by Dr. V. Chelladurai, Research Officer (Botany) Survey of Medicinal and Aromatic
plants Unit-Siddha, CCRAS, Palayamkottai, Tirunelveli District, Tamil Nadu, India and Voucher specimens have been deposited at the Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli District, Tamil Nadu, India. [Dichrostachys cinerea (MSU 051), Hemidesmus indicus (MSU 052). Parmelia perlata (MSU 053), Sida acuta (MSU 054) and Sida cordata (MSU 055)].

*Dichrostachys cinerea* known as “Vidattalai” in Tamil is a thorny shrub or a small tree. The root is astringent and is used in rheumatism, urinary calculi, renal troubles and in the diseases of vagina and uterus. Tender shoots of the plant are bruised and applied to the eyes in the case of ophthalmia.

*Hemidesmus indicus* popularly known as "Indian Sarsaparilla" is a twining wiry shrub with polymorphous leaves and occurring over the greater part of India. It is commonly known as "Nannari" in Tamil and finds extensive application in the Indian system of medicine. The root is used as tonic, alterative, diaphoretic, diuretic and blood purifier. It is employed in chronic rheumatism, gravel and other urinary diseases and skin affections.

*Parmelia perlata* is a lichen known as "Kalpasi" in Tamil which spreads itself upon trees like oak and pine. In Ayurvedic system of medicine it is used as fragrant, vulnerant and antipyretic. In Unani system of medicine it is used as an astringent, laxative, tonic, alternative, carminative and aphrodisiac. It is also useful in inflammations, stomach disorders and vesicular calculus. When burnt, the smoke relieves headache.
**Sida acuta** known as "Vattatiruppi" in Tamil is an erect, perennial shrub distributed throughout the hotter parts of India. The root is bitter and said to possess astringent, cooling, tonic, stomachic, diaphoretic and antipyretic properties. It is useful in nervous and urinary diseases, disorders of blood and bile and in chronic bowel complaints.

**Sida cordata** known as "Palampasi" in Tamil is a more or less hairy herb, often procumbent and sometimes rooting at the nodes is distributed throughout the hotter parts of India and Nepal. The herb is considered to possess astringent and tonic properties and is used in fever and urinary complaints. Oral administration of a decoction of the herb is reported to prevent the swelling of joints due to arthritis in experimental animals.

The macroscopic and microscopic characters of the leaf, stem and root of the three plants viz. *Parmelia perlata, Sida acuta* and *Sida cordata* have been discussed with photographic illustrations. The results of fluorescence analyses of the plant body of *Parmelia perlata*, root of *Sida acuta* and aerial parts of *Sida cordata* and their extracts in various solvents such as petroleum ether (40° – 60°C), benzene, chloroform, methanol and water have been discussed. The total ash, acid-insoluble ash, water-soluble ash, residue on ignition and extractive values in different solvents have been determined. The fluorescing spots are located by using UV-fluorescence viewing cabinet (365 nm). These macroscopic and microscopic characters, physico-chemical characters such as acid-insoluble ash, water-soluble ash, residue on ignition, extractive values and fluorescence characters can be used as a
diagnostic tool for the correct identification of these plant drugs and also to test adulteration (if any).

Chapter 3 deals with the inhibitory effect of all the five medicinal plants on calcium oxalate crystal growth *in-vitro*. The epidemiological factors in the formation of urinary stones, types of stones and causes of urinary stone formation have been presented. Past work on inhibitory effect of some medicinal plants on calcium oxalate crystal growth and agglomeration has been highlighted. The inhibitory effects of methanolic and aqueous extracts of the medicinally important parts of *Dichrostachys cinerea*, *Hemidesmus indicus*, *Parmelia perlata*, *Sida acuta* and *Sida cordata* on calcium oxalate crystal growth *in-vitro* have been studied at 20 mg /5 ml and 10 mg /5 ml doses by single diffusion method. The thickness of crystal columns were measured on different dates and the results are tabulated. The test of significance was statistically analysed using Student’s ‘t’-test. The sizes of the crystals formed were also noted in microns on different dates and the values thus obtained were compared with that of the controls. After the crystal growth, the crystals were subjected to IR and SEM studies. The IR spectra of the extract treated calcium oxalate crystals rule out any possibility of binding between the drug and oxalate crystals. SEM analysis clearly supported the view that all the five plants are found to be beneficial in controlling calcium oxalate crystal growth *in-vitro*.

Chapter 4 highlights the classification of diuretics and the past work on the diuretic activity of medicinal plants. It deals with the diuretic activity of the methanolic extracts of the roots of *Dichrostachys cinerea*, *Hemidesmus*
indicus and the plant body of Parmelia perlata. The diuretic activity was evaluated on male albino rats using Lipschitz's method. The extracts were suspended in normal saline at three different doses namely 100, 250 and 500 mg/kg body weight and were administered orally to three different groups. A group of rats receiving normal saline (25 ml /kg bw) alone was taken as the control and another group of rats received urea (750 mg in 25 ml of normal saline) as a reference drug. The parameters measured for diuretic activity were total urine volume, Na⁺, K⁺ and Cl⁻ contents and the results were analysed using Student's 't'-test for statistical significance. The methanolic extract of Dichrostachys cinerea acted as a better diuretic at a dose of 250 mg/kg of body weight. At this dose potassium and chloride excretion levels were significantly increased as nearly 1.5 fold when compared to urea, the reference diuretic. The data also revealed that the methanolic extract of H. indicus is a good aquaretic rather than a kaliuretic or saluretic. Promising results were not obtained for Parmelia perlata with regard to diuretic activity.

In chapter 5, the past work on the phytochemical studies related to the family, genus and species of the present investigation has been presented. This chapter deals with the phytochemical analyses of the various extracts of all the five medicinal plants under investigation. Preliminary phytochemical screening of the various extracts of the five plants has been performed to find out the presence of steroids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, xanthoproteins, saponins, tannins, flavonoids and aromatic acids and the results are presented. Thin layer chromatographic behaviour of the various extracts of the medicinally important parts of all the
five medicinal plants have been studied in different solvent systems. The fluorescing spots are located by using UV light (365 nm) and the Rf values of the fluorescing spots have been determined. The plates were then developed in an iodine chamber and the Rf values of the spots have been measured and compared. The amino acids present in the methanolic and aqueous extracts were identified by paper chromatographic technique by comparison with the standard amino acids, based on their Rf values. The chromatogram was developed in a solvent mixture of n–butanol : acetic acid : water (12:3:5) by volume and 0.2 % ninhydrin in acetone was used as a spraying reagent.

Quantitative estimation of amino acids, flavonoids and sugars have been performed in all the five plants. *Sida cordata* contains the maximum amount of flavonoids (4.93 A/g) followed by *P. perlata* (4.22 A/g), *H. indicus* (4.19 A/g) and *D. cinerea* (2.96 A/g). *Sida acuta* has the lowest amount of flavonoids (2.62 A/g). Total free amino acid content is the maximum in *H. indicus* (0.702 mg/g) and minimum in *S. acuta* (0.095 mg/g) and all the five plants have considerable amount of sugars.

In the present study the diuretic activity and inhibitory effect on calcium oxalate crystal growth *in-vitro* may be due to the presence of these phytochemicals. The diuretic effect is thought to be due to the inhibition of the renal absorption of Na⁺, K⁺ and Cl⁻. Among the five plants tested *Dichrostachys cinerea* possesses the highest inhibitory effect on calcium oxalate crystal growth *in-vitro* and diuretic activity. The present study confirmed the use of these medicinal plants traditionally for urinary disorders.