For this study, the selected medicinal plants belonging to the family Caesalpiniaceae viz., roots of *Peltophorum pterocarpum*, stem of *Colvillea racemosa* and stem of *Bauhinia purpurea* were collected from area of Andhra University, Visakhapatnam, Andhra Pradesh, India and were identified following Gamble and Fischer (1928) and Matthew (1983). As pointed out by Jain (1980) and Martin (1995), the
folk knowledge on medicinal value of plants was intensively enquired from local people of the survey area during the collection of plants.

The plant samples were air-dried and ground into uniform powder using a milling machine. The aqueous extract of each sample was prepared by soaking 100g of dried powdered samples in 200ml of distilled water for 12h. The extracts were filtered using Whatman filter paper No.42 (125mm).

Chemical tests were carried out on the aqueous extract and also on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

**Test for Tannins**

About 0.5 g of the dried powdered samples were boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or blue-black colouration.
Test of Phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence of phlobatannins presence.

Test for Saponin

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath, and then it was filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. Few drops of olive oil was added on the froth and shaken vigorously, and then observed for the formation of emulsion.

Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the selected plant sample (Sofowara, 1993; Harborne, 1973). 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated sulphuric acid. Development of yellow coloration was observed in each extract which indicated the presence of flavonoids,
and then yellow colouration was disappeared on standing. Few drops of 1% aluminium solution were added to a portion of filtrate, presences of flavonoids are noticed by the development of yellow colouration.

A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The yellow colour developed on the solution indicates a positive test for presence of flavonoids.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml of sulphuric acid. The colour was changed from violet to blue or green indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

Five ml of plant extract was mixed with 2 ml of chloroform, and then 3 ml of concentrated sulphuric acid was added carefully in
order to form a layer. A reddish brown colouration was formed in the inter face indicates positive results for the presence of terpenoids.

Test for Cardiac Glycosides (Keller/ Killani test)

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride. This was underplayed with 1ml of concentrated sulfuric acid. Appearance of brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Quantitative determination of the Chemical constituent

Preparation of fat free sample

2 g of the sample was defatted with 100ml of diethyl ether using a Soxhlet apparatus for 2 hr.

Determination of Total Phenols by Spectrophotometric method

The fat free sample was boiled with 50ml of ether for extraction of the phenolic component for 15 min. From this 5 ml of the extract was pipetted in to a 50 ml flask, then 10ml of distilled water was
added. Then 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

Alkaloid determination using Harborne (1973) method

Around 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 h. Then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract until the precipitation was completely dissolved. The whole solution was allowed to settle and the collected precipitated was washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin determination by Van-Burden and Robinson (1981) method

Around 500 mg of the sample was weighed into a 50 ml plastic bottle. To this 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This solution was filtered in to a 50 ml
volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M. FeCl₃ in 0.1 N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Saponin determination

The method used was that Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethylether was added and shaken vigorously. The aqueous layer was recovered while the ether was discarded. The purification process was repeated, and then 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.
Flavonoid determination by the method of Boham and Kocipai - Abyazan (1974)

About 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm) and the filtrate was transferred to a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

ANTIMICROBIAL ACTIVITY

Plant material and preparation of extracts

The plant materials used in this study consisted of *P. pterocarpum* (root), *C. racemosa* (stem) and *B. purpurea* (stem) species of the family Caesalpiniaceae, which were collected from area of Andhra University, Visakhapatnam, Andhra Pradesh, India. The air-dried plant materials were ground into powder in Willey Mill. The crude dried powdered materials are separately extracted with ethanol and water concentrated to a small bulk under reduced pressure at 50°C was suspended in water and pH of the water is adjusted to neutral.
Test for Microorganisms

Eight clinical strains were used in the study: methicillin-resistant *Staphylococcus aureus*, multi-drug resistant *Pseudomonas aeruginosa* (i.e resistant to ampicillin, cefuroxine, nalidixic acid, norfloxacin, ciprofloxacin and amoxicillin - clavulanic acid), *Staphylococcus epidermidis*, Klebsilla pneumonia, Bacillus subtilis, *Staphylococcus marcescens*, Escherchia coli and *Pseudomonas fluorescens*. A standard ciprofloxacin solution was also tested.

Antibacterial activity

Antibacterial activity was determined by the Agar cup plate (Kavanagh, 1963). Petriplates containing 20ml of nutrient agar medium (pH 7.2 - 7.4) were seeded with a 24hr culture of the bacterial strains. Wells (8mm diameter) were cut into the agar and 50µl of the plant extracts were tested in a concentration of 100mg /ml. The inoculum size was adjusted so as to deliver a final inoculum of approximately 10⁸ colony - forming units (CFU) ml. Incubation was performed at 37°C for 24hrs. The assessment of anti-bacterial activity was based on measurement of diameter of the inhibition zone formed around the well. A standard ciprofloxacin solution was also tested.
ANTI-INFLAMMATORY ACTIVITY

Plant Material

The plant materials used in this study consisted of *P. pterocarpum* (root), *C. racemosa* (stem) and *B. purpurea* (stem) species of the family Caesalpiniaceae, which were collected from area of Andhra University, Visakhapatnam, Andhra Pradesh, India. The selected parts were shade dried at room temperature for 10 days and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No.60.

Preparation of Extract

Air-dried and powdered leaves were extracted successively with H$_2$O, MeOH and ChCl$_3$ at 80°C, 40°C and room temperature respectively (Kokate, 1994; Owoyele et al., 2001). The dried extract was stored at 4°C until use. The extract yields of the plants were 1.2g, 3.0 g and 2.0g from 20.0g, 30.0g and 20.0g of powdered leaves in 150 ml water, 300 ml methanol and 250ml chloroform respectively. The aqueous extract was dissolved in 0.9% saline while the methanol extracts and chloroform extracts were dissolved in 2.5% Tween 80 and subsequently in normal saline.
Animals

Wister rats of either sex and of approximately the same age, weighing about 150-175g were used for the study. They were housed in polypropylene cages are fed with standard clow diet and water ad libitum. The animals were exposed to alternate cycle of 12h of darkness and light. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethical Committee and cleared by the same.

Acute Toxicity Studies

The animals were divided into control and test groups containing six animals each. The Control group received the vehicle (1% acacia) while the test groups received different extracts orally and were observed for mortality till 48h and the LD50 were calculated (Ghosh, 1994).

Carrageenan Induced Rat Paw Edema

Anti-inflammatory activity was assessed by the method described by Winter et al. (1968).
The rats were divided into eleven groups of six animals each. First group (negative control) received 1ml of normal saline, second group (positive control) received 10 mg/kg p.o. Diclofenac sodium, third, fourth and fifth groups were received aqueous, methanolic and chloroform extracts (100mg /kg p.o.) of *P. pterocarpum* respectively. Groups sixth, seventh and eighth were received aqueous, methanolic and chloroform extracts (100 mg/kg, p.o) of *C. racemosa* respectively. Groups ninth, tenth and eleventh were received aqueous, methanolic and chloroform extracts (100 mg/ kg p.o) of *B. purpurea* respectively. After 1h, the rats were challenged with subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan (Sigma, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury upto the mark. The plethysmometer apparatus was used for the measurement of rat paw volume Singh and Ghosh (1968). The paw volume was measured immediately after injection (0h) and then first, second and third hour after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume.
Percent inhibition of inflammation was calculated using the formula, Percent Inhibition = 100 (1 - Vt/ Vc), where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with various plant extracts.

DIURETIC ACTIVITY

Plant Materials

The plant materials used in this study consisted of *P. pterocarpum* (root), *C. racemosa* (stem) and *B. purpurea* (stem) are belonging to the family Caesalpiniaceae, which were collected from the area of Andhra University, Visakhapatnam, Andhra Pradesh, India. After collection, the chosen plant parts were sun-dried for nine days and made into fine powder in Willey Mill. The powder of 320g of each plant extracted with 900 ml of methanol (40-50°C) in soxhlet apparatus, the extraction was carried out until the process was completed. The methanolic extracts were concentrated to one third of its initial volume and a dark greenish colored mass was obtained the concentrated mass was kept in room temperature to remove by evaporation and finally heated it at 40-50°C until it became methanol free and highly dense.
Experimental Animal

Male rats weighing about 200g were used for the study. They were kept in clean and neat environment with adequate food and water. The animals were maintained under standard conditions of temperature and humidity. They were deprived of any food and water for preceding 12 h and were divided into six groups containing 5 animals in each group. They were weighed before the experiment.

Preparation of sample solution

Urea (52.6mg) was accurately weighed and taken into graduated test tube. Then it was dissolved slowly in saline by gentle shaking, finally the volume was adjusted upto 2.5ml with saline solution. To prepare suspension of the methanolic extract of *P. pterocarpum* (18.4 mg), *C. racemosa* (20.6 mg) and *B. purpurea* (22.0 mg) of the extracts were measured accurately and suspended in saline solution using 0.1% tween.80 as the suspending agent. The final volume was adjusted to 2.5ml so that each animal of group IV; V and VI received 0.5ml of *P. pterocarpum*, *C. racemosa* and *B. purpurea* extract respectively which was equivalent to a dose of 150mg/ kg body weight.
Screening of diuretic activity

The test animal was divided into six groups, containing five rats in each group. Group I was provided only with saline solution and 0.1% tween-80 i.e. control group. Group III was provided with standard diuretic drug frusemide at a dose of 150 mg / kg body weight. Group IV, V and VI were received extracts of *P. pterocarpum*, *C. racemosa* and *B. purpurea* respectively. These preparations were given by oral route Twenty four hours prior to the experiment the test animals were placed into metabolic cages with total withdrawals of food and water. After oral administration of test samples, the urinary output of each group was recorded at different time intervals from the graduated urine chamber of metabolic cage. Urine samples, which are collected from metabolic cage, were analyzed for Na$^+$ and K$^+$ concentration by Flame photometric method (Mukherjee *et al.*, 1997) while Cl$^-$ concentration was determined by the Argentometric titration method (Ramesh and Anbu, 1996).

The volume of the urine excreted in 5 h of study by each group was expressed as percent of the liquid administered giving rise to a measure of urinary excretion (UE) independent of group weight.
Total urinary output

\[
\text{Urinary Excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100
\]

The ratio of urinary excretion (UE) in test group and control group was denoted Diuretic Action which was used as the measure of degree of diuresis.

\[
\text{Diuretic Action} = \frac{\text{UE in test group}}{\text{UE in control group}}
\]

\[
\text{Diuretic Activity} = \frac{\text{Diuretic action of drug}}{\text{Diuretic action of urea}}
\]

**TOXICOLOGICAL STUDIES**

**Plant materials and extracts**

The plant material used in this study is *Peltophorum pterocarpum* (root), *Colvillea racemosa* (stem) and *Bauhinia purpurea* (stem) of the family Caesalpiniaceae, which were collected from the area of Andhra University, Visakhapatnam, Andhra Pradesh, India. The medicinal plants were dried in shade and pulverized. The powder was packed in shoxhlet apparatus and subjected to hot
continuous percolation using ethanol (90%, v/v) as solvent. The extract was concentrated under vacuum, dried and then suspended in 5% aqueous solution of gum acacia for the toxicological studies.

**Toxicity studies**

The sub acute toxicity studies (LD$_{50}$) by intra peritoneal route of the extracts were determined as suggested by Turner (1965). For each plant extract the rats were grouped into four groups. Ethanolic extracts of plant were administered intra peritoneally to rats. The individuals were screened for two hours for mortality. LD$_{50}$ was calculated and rats were observed for period of delayed toxicity.

LD$_{50}$ of the ethanol extract was determined by following standard method using probit scale (Miller and Tainter 1944; Ghosh, 1984) and based on the value, graded doses (75, 150 and 225 mg/kg body weight) were used to study sub acute toxicity.

For each plant sample, sixty adult male albino rats (150-175g) were used in the study and, these individuals were kept in polypropylene cages under identical animal house condition, provided with standard feed pellet and water *ad libitum* and were
divided into 4 groups. In the first three groups, extracts were given orally at the doses level of 75, 150 and 225 mg/kg body weight respectively for 28 days. The fourth group which served as control received equivalent quantity of 5% aqueous solution of gum acacia orally. During the experimental period rats were observed for signs and symptoms, behavior alterations, feed and water intake and body weight changes.

Haemato-biochemical Indices

The blood sample was collected after 24 hour of the last dose of plant extract. The relative blood indices i.e. total RBC, WBC count, Hb, ESR and PCV were determined using standard methods. In serum, analysis of blood glucose (Hugget and Nixon, 1957), Cholesterol (Wybenga and Pileggi, 1970), Serum glutamate pyruvate (GPT) and glutamate oxaloacetate transaminase (GOT) (Reitmen Frankel, 1957), alkaline phosphatase (King and King, 1954), total bilirubin (Mallay and Evelyn, 1937), total protein (Lowry et al., 1951) and creatinine (Henry, 1974) were estimated in control treated groups.
Statistical Analysis

The experimental results were expressed as a mean ±SEM. The data were statistically analyzed through student’s t-test.
Peltophorum pterocarpum
Colvillea racemosa
Bauhinia purpurea