PHARMACOGNOSTICAL, PHYTOCHEMICAL INCLUDING ISOLATION OF PHENOLIC RICH FRACTIONS, HPTLC ANALYSIS AND PHARMACOLOGICAL STUDIES OF THE LEAVES OF *Ixora pavetta Andrews*

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CHAPTER I

INTRODUCTION

HERBAL DRUGS:

Plants are the only economic source of a number of well established and important drugs. In addition, they are also the source of chemical intermediates need for the production of drugs [1].

Inspite of tremendous advances made in the modern system of medicine, there are still lack of conditions for which suitable drugs are not available in the allopathic system Moreover the modern system of medicine producing lot of side effects to the patients [2]. Nowadays herbs have been the main source of medicine throughout the human history. About 25-30% of todays prescription drugs contain chemicals derived from herbs [3].

The factors responsible for the continued and extensive use of herbal drugs in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners in rural areas.

Natural products are having complex molecular architecture and they show amazing arrangements of functional groups, ring system and other attractive structural attributes. The reasons for interest in natural products are [4]

- Serve as a lead compounds for newer drugs.
- Give information about the bio mechanism.
- Natural products are permanent challenge with respect to total synthesis and stimulate the development of new reagents and reactions.
- Collection of natural products as well as their derivatives and analogues are valuable starting points for drug discovery.[5]

The main limitation of plant is the lack of standardization of raw materials, processing methods, the final products, dosage, formulations and non existence criteria for quality control. [1]

Undoubtfully, the plant kingdoms still hold many species of plants containing substance of medicinal value which have yet to be discovered and large numbers of plants are constantly being screening for their pharmacological value. So that we have to made an attempt to identify the medicinal herbs useful to mankind.

ACUTE RENAL FAILURE [6, 7, 8]

DEFINITON:

Acute renal failure is characterized by a rapid decline in glomerular filteration rate over hours to days.

EPIDEMIOLOGY:

Acute renal failure complicates 5-7% of hospital admissions and upto 30% of admissions in intensive care units.

CAUSES:

(1) Prerenal ARF (Acute Renal Failure)

- ❖ Hypovolemia
- ❖ Altered renal hemodynamics resulting in hypertension.

(2). Intrinsic ARF:

- ❖ Renovascular Obstruction
- ❖ Diseases of the glomeruli (or) vasculature
- ❖ Acute tubular Necrosis

(i) Ischemia

(ii) Infection

(iii) Toxins

It has two types,

1. Exogenous:

- Radio contrast,
- Calcineurin inhibitors,
- Antibiotics,
- Chemotherapy (eg. Cisplatin)
- Antifungals,
- Ethylene glycol.

2. Endogenous:

- Hemolysis,

❖ Interstitial nephritis

❖ Intratubular Obstruction

(3)Postrenal ARF (obstruction)

(a) Ureteric

(b) Bladder neck

(c) Urethra

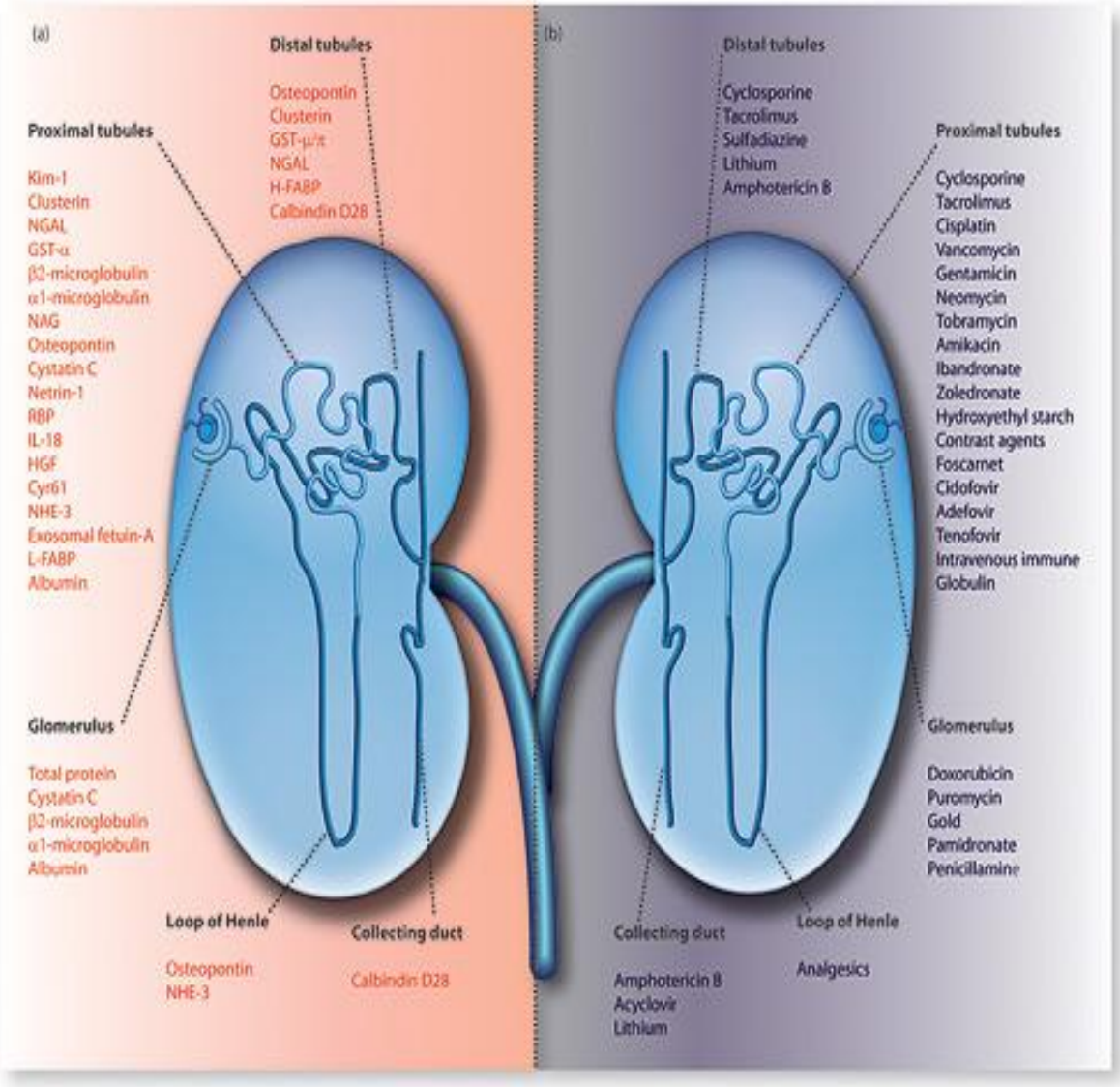
TREATMENT:

Pre-renal ARF	_	Isotonic Saline,Hypotonic Saline
Intrinsic ARF	_	Glucocorticoids, Alkylating agents
Post-renal ARF	_	Placement of bladder catheter, Ureteric Stent Insertion.

DRUG INDUCED RENAL DAMAGE:

Drugs can cause impairment of renal function directly by renal damage (or) changes in the renal functions (or) indirectly via on blood supply.

ADVERSE EFFECTS OF DRUGS ON THE KIDNEY:



Drugs which are predominantly eliminated by the kidneys and have a narrow safety margin are likely to create problems.

TYPES OF DRUG INDUCED RENAL DAMAGE:

(1) Direct Renal Damage

Eg: Lithium cause diabetes insipidus by inhibiting anti diuretic hormone on the collecting ducts.

(2) Indirect damage via effects on blood supply

Eg: Penicillins and sulfonamides cause vasculitis through hypersensitivity.

E-amino caproicacid cause vascular thrombosis through which produce renal insufficiency.

Drugs that causes intravascular hemolysis (quinidine,Dapsone)can cause hemoglobinuria which cause renal insufficiency.

TREATMENT

Elimination of Nephrotoxic agents

HERBAL DRUGS POSSESS NEPHROPROTECTIVE ACTIVITY

BIOLOGICAL SOURCE	**FAMILY**
Pongamia pinnata	Fabaceae
Crataeva nurvala	Capparidaceae
Ocimum sanctum	Labiatae
Eugenia jambolana	Myrtaceae

UROLITHIASIS:

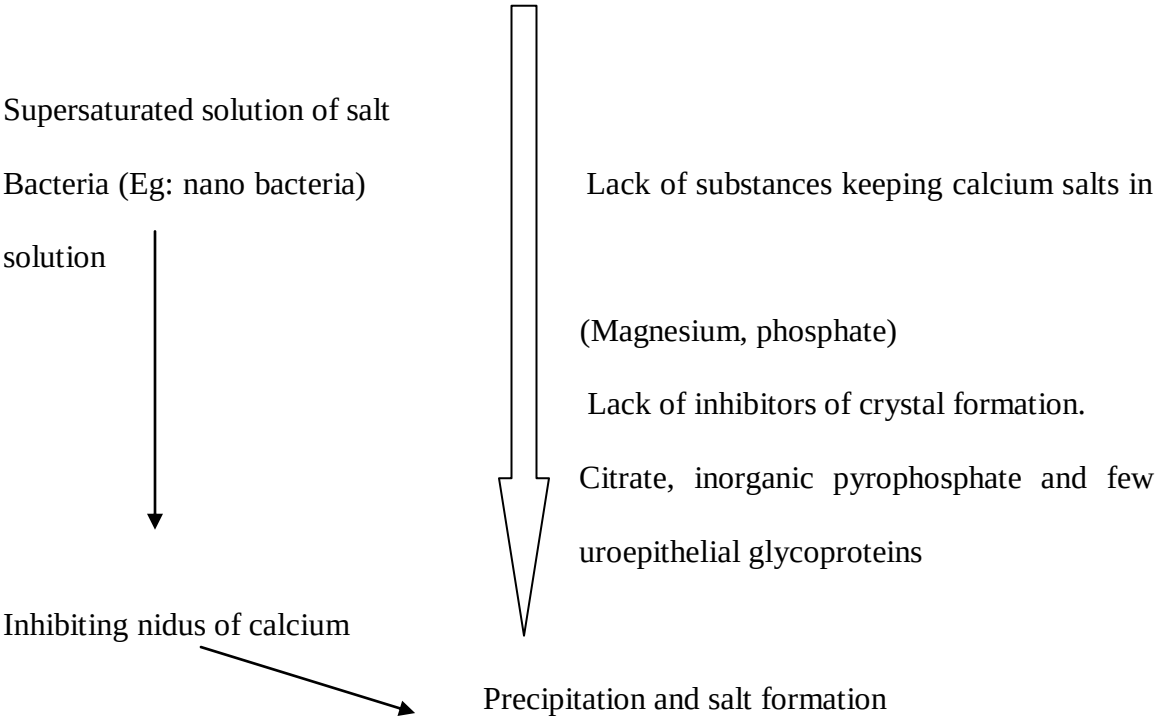
Nephrolithiais term indicates that the disease is characterized by the formation of stone in the kidneys or urinary tract.[9]

Epidemiology:

Nearly 4-15%of the human populations are suffering from urinary stone problem in all over the globe. In the United states 13% of men and 75 of women will develop kidney stone during their life time. The area of high incidence of urinary calculi are British islands, Scandinavian countries, Central Europe, Northen Australia, Northen India, Pakistan, Medditerranean countries. So they are Known as **Stone belts.** [6, 10]

MODE OF FORMATION[]

Excretion of stone substances (Eg:oxalate)+Calcium



PRE DISPOSING FACTORS

Urinary pH

Dehydration- causes increased urinary concentration.

Stasis- obstruction to urine flow encourages salt precipitation

Renal disease

Metabolic factors- Hyper calcuria, hyper phosphaturia, oxaluria, urate excess

EFFECT AND COMPLICATION:

Passage of small size stone ⟹ Colic

↓

Injury to ureter

↓

Stricture

↓

Subsequent impaction of stone

Passage of larger stone ⟹ Impaction at upper and lower ends of ureter or at brim of pelvi

⇩

Stasis

↙ ↘

Hydronephrosis Infection

↓

Staghorn calculus (Suppuration and ulceration of pelvis and calculus leads to squamous metaplasia)

TYPES: [12]

1. Calcium stones:

   Calcium oxalate (50%)

   Calcium phosphate (5%)

   Mixture of calcium oxalate and calcium phosphate (45%)

2. Mixed (struvite) stones

   Magnesium-ammonium-calcium phosphate

3. Uric acid stone (5-10%)

4. Cystine stones (1%)

5. Hereditary xanthinuria, xanthine stones.

TREATMENT: [6]

- Calcium stones are treated with Thiazide diuretics and allopurinol.
- Antimicrobial agents,Judicious surgery are used for the treatment of struvite stones.
- Alkali and allopurinol are useful in uricacid stones.
- Cysine stones are treated with allopurinol and penicillamine.

Herbal drugs used for the treatment of Urolithiasis:

BIOLOGICAL SOURCE	FAMILY
Coleus aromaticus	Labiatae
Cucurbita pepo	Cucurbitaceae
Mallotus philippensis	Euphorbiaceae
Tribulus terrestris	Zygophyllaceae
Bergenia ligulata	Saxifragaceae

URINARY TRACT INFECTIONS[13]

Definition

Urinary tract infection is defined as a disease caused by microbial invasion of the genito urinary tract that extends from the renal cortex of the kidney to the urethral meatus.

PREDISPOSING FACTORS:

- Gender-Females are more frequently affected by UTI
- Pregnancy
- Obstruction to flow of urine(stone,stricture,prostatic hypertrophy and tumour)
- Neurogenic bladder dysfunction(spinal cord injury,Multiple sclerosis)
- Bacterial virulence
- Reflux of urine from bladder up into ureters and sometimes into renal pelvis
- Genetic factors

CAUSATIVE ORGANISMS:

1. Gram negative bacilli:

   Ecoli(70-80%cases), Klebsiella spp, Proteus spp; Pseudomonas aeruginosa, Enterobactor , Serratia.

2. Gram positive bacilli

   Staph.saprophyticus, staph.aures, Staph.epidermidis, Enterococci

3. Micellaneous

   M.Tuberculosis,Str.pyogens,Str.agalactiae,Citobactor,Salmonella,Gardnerella vaginalis

4. Fungus

TYPE:

Lower UTI

It includes Urethrits, cystitis, prostatitis

Upper UTI

It includes Acute pyelitis, Acute Pyelonephritis

TREATMENT

The following drugs are used for the treatment of urinary tract infections

Quinolone, Nitrofurantoin, Amoxycillin, Ceftriaxone and Gentamicin

CHAPTER II

ETHNO MEDICAL INFORMATION

All parts of this shrub are used in various indigenous system of medicine for the treatment of several diseases.

Plant is used in the treatment of dysuria and spider bite poisoning. It is also useful in abortion and easy delivery.[14]

- Leaves are used to treat chest pain, dysuria ,urinary disorders and kidney diseases[15]
- Decoction of the bark is used in anemia, general debility and malnutrition[16].
- Fruits are extensively used as an antidote for high coloured urine and as diuretics [17].
- Flowers are pounded with milk to treat whooping cough and pulmonary troubles.
- Roots have been useful for females in white discharge .It also used for urinary diseases and as diuretics[18, 19].
- Aerial parts of the plant are used as antiviral, hypotensive and spasmolytic.
- Wood is used for torches , fuel and lighting.

Root, leaf and Stem are used to treat scabies, leprosy, pus formation and eczema. It is also used to treat dysuria and spider bite poisoning[20].

Root, stem and bark were crushed with garlic and the filterate was administered 2-3 spoons twice dily for five days for chest pain[21].

REVIEW OF LITERATURE

PHARMACOGNOSY

- Saxena. M.C., and Chawla. A. et al (1985) reported that the *Ixora arborea* possess C4 photosynthetic pathway [22].
- Umapathi. P et al,(1979) study reveals that the herbicidal influence on the leakage of electrolytes from the leaves of *Ixora arborea*[23].
- Rao JVS, et al (1981) screened the physiological studies of *Ixora arborea*[24].
- Bhuyan. D.K. et al (1994) documented fifteen plants used by the tribal people in Lohit district of Arunachal Pradesh for abortion and easy delivery[25].
- Anand, S.P.; and Jeyachandran, R. et al (2008) studied the ethnomedical importance of *Ixora arborea* [26].
- Khan SS, et al (1984) reported the folklore uses of *Ixora parviflora* [27].
- Mehta, P.M. et al (1999) studied the effect of mineral salts and growth regulatory substances on some biochemical changes during senescence in *Ixora* and vinca flowers[28].
- Dhivahara, V. et al (2008) reported that the medicinal plants used as home remedy[29] .

PHARMACOLOGY

- Srinivas, K^1, Celestin Baboo $R.U^2$ et al (2011) reported that the antiulcer activity of flowers of *Ixora pavetta* against aspirin-induced and pylorus ligation induced ulcer in rat model [30].

- Suvarna, V. and Patil, S. et al (2009) studied the antifungal activity of *Ixora parviflora* against Candida albicans and Aspergillus niger by cup plate method [31].
- Serrame, E. et al (1995) documented that the decoction and expressed juice from *Ixora coccinea* having antitumour activity that was 67% in skin tumour, 50% in liver tumour and 33% in colon tumour[32].
- Latha, P.G. ; and Panikkar , K.R. et al (1994) isolated the active principle from *Ixora coccinea* flowers and screened against ascitic tumours in Swiss albino mice at the dose of 100 mg/kg body weight [33].
- Latha, P.G. ; and Panikkar , K.R. et al (1999) isolated Ursoli acid from *Ixora coccinea* flowers and evaluated the chemoprotective effect on cyclophosphamide induced toxicity in mice [34].
- Latha, P.G. ; and Panikkar , K.R. et al (2001) studied the chemoprotective effect of active fraction of *Ixora coccinea* flowers on cisplatin induced toxicity in mice [35].
- Menon, T. V, et al (1980) reported that the pama (scabies) with secondary infection was treated with Aragwatha kwatham and pamari ointment containing *Ixora coccinea*[36]
- Nayak, B.S ; et al (1999) screened the effect of *Ixora coccinea* flowers on dead space wound healing in rats [37].

- Saha, M.R., et al (2008) studied the invitro free radical scavenging activity of *Ixora coccinea* by DPPH free radical scavenging assay, reducing power and total antioxidant capacity using Phosphomolybdenum method [38].
- Panikkar , K.R. et al (1986) screened the antitumour activity of ayurvedic oil preparation containing flowers of *Ixora coccinea* and Costus sativum in preventing the development of Dalton's Lymphoma and also retard the already formed tumour. [39]
- Nair, S. C., et al (1991) studied the potential antitumour activity of *Ixora javanica* leaves extract against Myelogenious leukemia cells [40].
- Pillai,R. R* et al (1990) reported the alcoholic root extract of *Ixora coccinea* possess anti-inflammatory, antibacterial, antifungal, CNS activity[41].
- Sadeghai – Nejad, B., et al (2007) studied the invitro antifungal activity of *Ixora brachiata* leaves and root extracts against microsporum, trichophyton and Epidermophyton by agar dilution method[42].
- Jachariah, R. et al (1994) isolated and evaluated the anti-inflammatory and antimitotic effect of Lupeol from the petroleum ether fraction of ethanolic extract of leaves of *Ixora coccinea* [43].
- Kumar, S. R, et al (2009) studied the anthelmintic activity of methonolic extract of flower of *Ixora coccinea* against Indian earthworm pheretima posthuma in 0.1, 0.5, and 1% w/v concentration [44].

PHYTOCHEMISTRY

- Chauhan J S, et al (1984) reported that the New flavones glycoside isolated from the stem of *Ixora araborea* has been characterized as chrysin 5-o-beta-D-xylo pyranoside [45].
- Daulatabad C D, et al (1982) studied the seed oils of *Ixora parviflora* contain lauric acid (3.1% wt), capric acid (1.3 % wt), myristic (4.7 % wt), palmitic (11.4% wt), stearic (11.9% wt), arachidic (2.9 % wt), behenic (2 % wt), oleic (18.7 % wt) and linoleic acid (44 % wt) [46].
- Ragasa, C . Y. ; et al (2004) reported the *Ixora coccinea* have new cycloartenol esters, lupeol fatty ester, lupeol, ursolic acid, oleaninc acid and sitosterol [47].
- Chauhan, J S . ; et al (1996) isolated and identified the apigenin-4-o-beta-D-glucopyranodise and a new aliphatic keto alcohol (11-hydroxy-d.dec-5-en-2-one) from *Ixora finlarysoniana* [48].

CHAPTER III

AIM AND SCOPE OF THE STUDY

Ixora pavetta is a large ever green shrub belonging to the family Rubiaceae.

The ethno medical information revealed that almost all parts of this shrub is used in various indigenous system of medicine against several diseases.

- Leaves are used to treat chest pain, dysuria , **urinary disorders and kidney diseases**.
- Fruits are extensively used as an antidote for high coloured urine and as diuretics.
- Roots have been useful for a female in white discharge .It is also used for urinary diseases and as diuretics.
- Aerial parts of this plant is used as antiviral, Hypotensive and spasmolytic.
- Root, leaf and Stem are used to treat dysuria and spider bite poisoning.

Phytochemical studies on the stem has been reported for the presence of a flavone glycoside and it has been characterized as **chrysin 5-o-(beta)-D-xylo pyranoside**.

It has also been reported that the flowers of *Ixora pavetta* was used to treat ulcer, in aspirin induced and pylorus ligation induced rat model. This effect was comparable with standard drug omeprazole.

It has been reported for antifungal activity against candida albicans and aspergillus niger.

So the present study has been designed to carry out the following work on the leaves of *Ixora pavetta*.

1) It is planned to carry out the detailed pharmacognostical studies on the leaves of this plant.
2) Preliminary phytochemical studies were carried out for the crude drug as well as extracts inorder to confirm the presence of primary and secondary metabolites like proteins, flavonoids, tannins, sterols.
3) Extraction of ethanolic extract of the leaves of *Ixora pavetta* (EIP) and preparation of phenolic rich fraction of this extract(CIP).
4) To estimate the total phenolic, tannin and flavonoid content in both extracts(EIP&CIP).
5) Phytochemical evaluation of these extracts by means of TLC and HPTLC studies
6) It is planned to evaluate the extracts of this plant (EIP & CIP) for the following pharmacological studies

    (a) Determination of invitro antioxidant potential by

    - DPPH assay
    - Hydrogen peroxide Scavenging activity
    - Nitric oxide scavenging activity

    (b) Evaluation of nephroprotective activity of *Ixora pavetta* on cisplatin induced nephrotoxicity.

    (c) Invitro calcium oxalate crystallization inhibition by *Ixora pavetta*

    (d) Antibacterial activity of *Ixora pavetta* against urinary tract Pathogens.

CHAPTER IV

PHARMACOGNOSTICAL STUDIES

Ixora pavetta is a large evergreen shrub belonging to the family Rubiaceae.

SECTION A

SYSTEMATIC POSITION [49]:

Kingdom	:	Plantae-plants
Subkingdom	:	Tracheobionta-vascular plants
Super division	:	Spermatophyto-seed plants
Division	:	Magnoliophyta-flowering plants
Class	:	Magnoliopsida-dicotylidons
Subclass	:	Asteridae
Order	:	Rubiales
Family	:	Rubiaceae
Genus	:	*Ixora*
Species	:	*pavetta*

SYNONYM:

Ixora parviflora,Ixora arborea,Ixora decipens

COMMEN NAME:

Small flowed Ixora,Torch tree

VERNACULAR NAME [17]:

English: Torch tree, Torch wood ixora

Hindi: Kotagandhal, Nevari

Kanada: Grorabikattige, Kansuragi

Malayalam: Sujimulla

Sanskrit: Iswara, Nevali

Tamil: Shulundu-kora, Korivi

Telugu: Korivipala, puttupala, Kachipadel, Gorivi

GEOGRAPHICAL DISTRIBUTION:

It is found in Assam, Kerala, Nicobar islands, Bangladesh, Ceylon and Pakistan.

HABIT AND HABITAT OF PLANT [17, 50, 51]**:**

A large evergreen shrub with white flowers. Bark is dark brown , wood bears light reddish brown .The green branches are said to be used for torches.

DESCRIPTION OF THE PLANT:

LEAVES

Arrangement: opposite

Type: simple, subsessile.

Margin: Entire

Shape: Ovate-oblong

Colour: Dark green

Size: 3-6 inch long

Surface: coriaceous

Apex: obtuse

Base: Cuneate

FLOWER

Colour: white

Inflorescence: Terminal cyme

Odour: Fragrant

Size: 1/3 inch long

Shape: lobes oblong

Margin: Curted

Flowers are corymbs or panicles at the end of the branches. Sepal tube have a small teeth and style protrudes out of the flower with bifurcated stigma.

FRUIT

Shape: globose

Diameter: 0.25 inch

Colour: black

SEED

It appears in plane convex shape.

WOOD

It is brown, hard and heavy close grained. It appears smooth and good polish.

SECTION- B

MIROSCOPICAL STUDIES OF THE LEAVES [52, 53 54,55]

MATERIALS AND METHODS:

Fresh leaf was used for the microscopical examination. The cut portion of the leaf was fixed using FAA (Formalin 5ml+Acetic Acid 5ml+70%ethanol 90ml).After 24 hrs of fixing the specimens were dehydrated with graded series of tertiary butyl alcohol, and then the filteration was carried out by gradual addition of paraffin wax (58-60˚c)Then the specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary microtome. The sections were stained with toluidine blue.

ANATOMY OF THE LEAF:

The leaf consists of thick midrib which projects more on the abaxial side. The lamina is smooth and thick. The lateral veins do not raise above or below the leaf surface. The midrib is slightly raised to short wide abaxial hump; the abaxial part is broadly semicircular and thick. The midrib is 1.4mm thick, The adaxial part is 20µm in height and 40µm in breath. The abaxial part is 1.2mm wide.

The midrib consists of a thick epidermal layer of semi circular cells and thick cuticle. The ground tissue is parenchymatous, circular thick walled and compact dark tannin content occurs in many of the parenchyma cells, especially in those cells which are adjoining the vascular cylinder. (Fig.2.1)

The vascular system consists of a wide outer cylinder and narrow central cylinder (Fig.2.2). The outer cylinder is plano convex in sectional view with semicircular lower part and flat plate of upper part.

The xylem part of the cylinder consists of outer thin zone of fibres and inner short radial parellal rows of xylem elements. The xylem elements are narrow, thin walled and circular phloem encircles the xylem cylinder forming thick continous cylinder of small cells.

Within the outer cylinder occurs a narrow, horizontally elliptical small hollow cylinder of xylem with central phloem.(fig.). The xylem elements are mostly fibers.

LAMINA (Fig 3.1) The lamina is 170 µm thick and the cells are wide and squarish in outline. It is 25µm thick. The abaxial epidermis is comparatively thin. The cells are rectangular or cylindrical and are 15 µm thick. The mesophyll tissue consists of upper wide zone of three layers of short, compact cylindrical palisade cells. The lower part consists of about 6 layers of loosely arranged spherical or lobed spongy mesophyll.

LEAF – MARGIN (Fig 3.2) : The marginal parts of the leaf is gradually taperring into slightly bent down tip. The structure of the leaf margin is not much different from middle part of the lamina. The epidermal cuticle is more prominent. The palisade-spongy tissues are distinct. Prominent circular vascular strands are seen in the marginal part. They do not project beyond the levels of the surface. The margin is 200 µm thick.

PETIOLE (Fig 4.1,2;5.1,2) : The petiole is squarish cross sectional view with two prominent adaxial lateral wings. It is 1.5 mm in both vertical and horizontal planes. The petiole consists of a prominent epidermal layer, parenchymatous ground tissue and a closed hollow vascular cylinder.

The epidermal layer is thick comprising wide squarish cells and thick cuticle. The ground tissue consists of fairly thick walled compact parenchyma cells. The vascular system includes a main, central circular hollow cylindrical and one ortho small circular,

solid wing bundles. The main cylinder comprises several long, uniseriate parallel radial lines of xylem elements surrounded externally by thin layer of phloem. In the central core occurs tannin containing parenchyma cells. The vascular cylinder is 850 µm in diameter.

The wing part is thick and conical; it iis 300 µm long and 200 µm thick. The epidermal layer of wing is thin with thick cuticle. The ground tissue is parenchymatous and compact embedded in the wing are one or two circular vascular strands. The wing traces are circular with radiating files of xylem elements surrounded external layer of phloem.

CRYSTAL DISTRIBUTION: Calcium oxalate crystals are abundant in leaf and petiole. In the petiole, the crystals are prismatic type and are located in the phloem parenchyma (Fig 6.1).

In the leaf, crystals druses (sphaerocrystals). They occur in the palisade and spongy mesophyll tissues. The druses are located in dilated circular specialized cells called Idioblasts (Fig 7.1,2). The crystal bearing idioblasts are random in distribution.

STOMATA (Fig 8.1,2) : The leaf is hypostomatic. The stomata are predominantly paracytic type. The two subsidiary cells are equal in size. The guard cells are broadly elliptical measuring 50 X 100 µm. The epidermal cells are polygonal with fairly thick straight anticlinal walls.

The epidermal cells along the costal region are vertically elongated and are parallel to each other. Their walls are comparatively thick. No stomata are seen on the costal region (Fig 9.1,2).

VENATION : The venation is reticulate with dense thick veins and veinlets (Fig 10.1,2). The veins of different orders are different in thickness. The vein islets are fairly distinct with boundaries of thick veins. The islets are variable in size and shape. The vein terminations are thick and are straight or curved. (Fig 10.2).

EPIDERMAL CELLS: small pieces of epidermis are often seen in the powder of the leaf. The adaxial epidermis is apostomatic. The cells are 4-6 sided, the walls are thick, prominent parallel plates of cuticular striations are seen on the periclinal walls of the cells (Fig 11.1,2) . Peeling of the abaxial epidermis are also common in the powder (Fig 12.1,2). The abaxial epidermis is densely stomatiferous. The stomata are paracytic type with more or less equal subsidiary cells lying parallel on either side of Guard cells. The periclinal walls exhibit thick cuticular plates in dense parallel lines(Fig 12.2).

SECTION C

POWDER MICROSCOPY

ORGANOLEPTIC CHARACTERS:

Nature : Coarse

Colour : Green

Odour : Characteristic

Taste : Bitter followed by astringent taste.

Powder microscopy of the leaves shows the following characters,

- Epidermal cells with paracytic stomata.
- Fragment of leaf showing prismatic and druses of calcium oxalate crystals in abundance.
- Parenchyma cells containing tannin are present.
- Presence of polygonal epidermal cells with thick anticlinal walls.
- Fragment of leaf showing lignified annular xylem vessel

SECTION-D

QUANTITATIVE MICROSCOPY

DETERMINATION OF LEAF CONSTANTS: [56, 57]

The vein islet number, vein termination number, stomatal number and stomatal index were determined on fresh leaves using standard procedure.

VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER:

The term vein islet is used to denote the minute area of photo synthetic tissue encircled by the ultimate division of the conducting strands. The number of vein islets per square mm area is called vein- islet number.

Vein termination number may be defined as the number of vein terminations present in one square mm area of the photosynthetic tissue. [67]

Determination of vein islet number and vein termination number:

Pieces of leaves were cut from various regions of the leaves between midrib and the margin, cleared in chloral hydrate and mounted on a slide.

Camera Lucida and drawing board were arranged. With the help of a stage micrometer, camera Lucida and microscope, 1mm square was drawn on the paper. Then the stage micrometer was replaced by the preparation and the veins were traced in that square. Then the vein islets and vein terminations were counted in the square. Ten such readings were taken and the average was calculated and the results were presented in [67,68]

Table-1.

STOMATAL NUMBER:

The average number of stomata per square mm area of epidermis of the leaf is called stomatal number.

Determination of Stomatal Number:

Pieces of upper and lower epidermal peelings were mounted on a slide with the help of camera Lucida and stage micrometer 1 mm square was drawn on a paper. The stage micrometer was replaced by the preparation. Then the preparation was observed and the stomata marked in that unit area. Number of stomata present in those unit area was calculated. Ten such readings were taken and the average of stomatal number was calculated.[67]

STOMATAL INDEX:

Definition:

It is the percentage which the numbers of stomata form to the total number of epidermal cells, each stoma beings counted as one cell.

Stomatal index $\mathbf{S.I = \frac{S}{E+S} \times 100}$

Where, S=number of stomata per unit area

E= number of epidermal cells in the same unit area

Determination of Stomatal Index:

The procedure adopted in the determination of stomatal number was followed and then the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula [68].

SECTION E

The physical parameters such as total ash, Acid insoluble ash, Water soluble ash, Water insoluble ash, Extractive values and Loss on drying were determined separately for air dried powdered leaves of this plant as per standard method.

PHYSICAL PARAMETERS

POWDER ANALYSIS [58, 59]

The behaviour of the powder with different chemical reagents was carried out as mentioned by Kay (1938) and Johansen (1940) [40, 43]. The observations are presented in T**able 2**

FLUORESCENCE ANALYSIS [60]

The fluorescent analysis of the drug powder as well as the plant extracts of *Ixora pavetta* were carried out by using the method of Chase and Pratt (1949) [44]. The observations are tabulated in **Tables 3 and 4.**

Determination of Volatile Oil [61]

Volatile oils are characterized by their odor, oil like appearance and also it has ability to volatilize at room temperature. Chemically they are mixtures of monoterpenes, sesquiterpenes and their oxygenated derivatives. Volatile oils can be estimated by hydro distillation method by standard procedure.

The result is presented in **Table 5.**

Determination of foreign organic matter[62]

The part of organ or organs other than those specified in the definition or description of the crude drugs is defined as foreign organic matter. An accurately weighed 100g of air dried coarse drug and spread out in a thin layer.

The sample drug was inspected with the unaided eye or with the use of 6X lens and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the drug taken. The result is presented in **Table 5.**

Determination of Moisture Content (Loss on Drying) [61, 62]

An accurately weighed 10g of coarsely powdered drug was placed in a tared evaporating dish. Then the dish was dried at 105^{o}C for 5h and weighed. The drying and weighing was continued at one hour intervals until the difference between the two successive weighing is not more than 0.25%. The loss on drying was calculated with reference to the amount of powder taken. The readings are tabulated in **Table 5**

ASH VALUES: [63, 64]

The ash values were determined by using air dried powdered leaves as per the official method.

(A) Total ash:

2 grams of the crude leaf powder was accurately weighed in a tarred nickel crucible. The ground drug was scattered in a fine even layer on the bottom of the crucible and incinerated by gradually increasing the heat not exceeding 450˚ c [dull red heat] until free form carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried drug was calculated.

(B) Acid insoluble ash:

The ash obtained in (A) was boiled for 5 minutes with 25ml of 2M hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, ignited to constant weight, cooled in a desiccator and weighed. The percentage of acid insoluble ash with reference to the air – dried drug was calculated.

(C) Water soluble ash:

For water soluble ash, ash obtained in (A) was boiled with 25ml of distilled water. The insoluble matter was collected in a Gooch crucible, washed with hot water ignited to a constant weight. Cooled in a desiccator and weighed. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference gave the weight of the water soluble ash. It was calculated with reference to the air-dried powder. The results were represented in **Table 5**.

Determination of Extractive values: [62,63]

I) Petroleum ether soluble extractive value

5 gm of the air –dried drug, coarsely powdered, was macerated in 100ml of petroleum ether in a closed flask for 24 hours, shaking frequently during 6 hrs and allowed stand for 18 hrs, filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚ c, to constant weight. The percentage of the petroleum ether soluble extractive with references to the air - dried drug was calculated as presented in **Table 5**

II) Alcohol (Ethanol) and 75% Ethanol soluble extractive:

10 gm of the air –dried drug, coarsely powdered, was macerated in 100ml of ethanol and 100ml 75% ethanol 5gm for each solvent, in two separate closed flask for 24 hours, shaking frequently during 6 hrs and allowed stand for 18 hrs, filtered rapidly, taking precautions against loss of solvent. 25ml of the each filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚ c, to constant weight. The percentage of alcohol soluble extractive with references to the air - dried drug was calculated as presented in **Table 5.**

III) Water-Soluble Extractive:

5 gm of the air dried drug coarsely powdered, was macerated in 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during 6 hours, and allowed to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred-flat-bottomed shallow dish and dried at 105˚ C to constant weight. The percentage of water – soluble extractive with reference to the air-dried drug was calculated and presented in **Table -5**.

IV) Ether soluble Extractive:

The above mentioned procedure for the determination of petroleum ether soluble extractive was followed for the determination of ether soluble extractive also.

V) Determination of Chloroform, Acetone, Methanol and Hexane soluble extractives:

The procedure followed for the determination of alcohol soluble extractive value was adopted for the determination of Chloroform soluble extractive, acetone soluble

extractive, methanol soluble extractive and hexane soluble extractive. Instead of alcohol, respective solvents were used for the determination of their extractive values.

The percentage of chloroform, acetone, methanol and hexane soluble extractives were calculated and presented in Table 5.

Table 1: Quantitative analytical microscopical parameters of the leaf of *Ixora pavetta*

S. No.	Parameters*	Values obtained
1	Stomatal number in lower epidermis	31 ± 1.32
2	Stomatal index in lower epidermis	16 ± 0.53
3	Vein islet number	3±0.44
4	Vein termination number	12 ± 1.27

*** mean of 6 readings ± SEM**

Table 2: Behavior of the *Ixora pavetta* powder with various chemical reagents

Powder + Reagents	Colour / Precipitate	Presence of active principle
Picric acid	Yellow precipitate	Protein present
Conc. sulfuric acid	Reddish brown color	Phyto sterols present
Lieberman Burchard reagent	Reddish brown color	Phyto sterols present
Aqueous ferric chloride	Greenish black color	Tannins present
Iodine solution	Blue color	Starch present
Mayer's reagent	No cream color	Absence of alkaloids
Spot test	No stain	Fixed oils absent
Sulfosalicylic acid	White precipitate	Protein present
Aq. Sodium hydroxide	Yellow color	Flavanoids present
Mg – HCl	Magenta color	Flavanoids present
Aq. Lead acetate	White precipitate	Presence of tannins

Note :- Colour reactions are viewed under natural light by naked eye

Table 3: Fluorescence Analysis of powder of *Ixora pavetta*

Powder +reagent	Day light	UV light (254 nm)	UV light (366 nm)
Drug powder	Green	Green	Brown
Drug powder +aqueous 1M sodium hydroxide	Green	Green	Brown
Drug powder + alcoholic 1M sodium hydroxide	Green	Green	Brown
Drug powder + iodine	Red	Brown	Brown
Drug powder + 10% potassium hydroxide	Yellow	Greenish yellow	Brown
Drug powder + 1M hydrochloric acid	Green	Green	Brown
Drug powder + glacial acetic acid	Yellow	Greenishyellow	Brown
Drug powder + 50% sulphuric acid	Green	Green	Brown
Drug powder + 50% nitric acid	Green	Green	Brown
Drug powder + 50% hydrochloric acid	Green	Green	Brown

Table 4: Fluorescence Analysis of extracts of *Ixora pavetta*

Extracts	Consistency	Colour in Day Light	Colour under UV Lamp	
			360 nm	254nm
Petroleum extract	Semisolid	Yellow	Orange	Yellow
Ether extract	Semisolid	Greenish brown	Green	Greenish brown
Chloroform extract	Semisolid	Yellowish brown	Orange	Yellowish brown
Ethanol extract	Semisolid	Yellowish green	Orange	Green
Methanol extract	Semisolid	Yellowish green	Orange	Green
Aqueous extract	Semisolid	Brown	Green	Dark green
Hexane extract	Semisolid	Yellow	Reddish orange	Green
Acetone Extract	Semisolid	Yellowish green	Orange	Green

Table 5: Standardization parameters of *Ixora pavetta*

S. No	Parameters*	Values* expressed as %
1	**Volatile oil**	Nil
2	**Foreign organic matter**	0.01 ± 0.12
3	**Moisture content**	5.83 ± 0.01
4	**Ash values**	
	Total ash	7.39 ± 0.42
	Acid insoluble ash	1.09 ± 0.17
	Water soluble ash	5.09 ± 0.90
	Water insoluble ash	2.29 ± 0.90
5	**Extractive Values**	
	Petroleum extract	0.58 ± 0.03
	Ether extract	4.92± 0.02
	Chloroform extract	1.22± 0.04
	Ethanol extract	2.12 ±0.03
	75 % Ethanol extract	6.81 ±0.03
	Methanol extract	5.25 ± 0.01
	Aqueous extract	4.8± 0.03
	Hexane extract	2.26 ± 0.01
	Acetone Extract	2.18 ± 0.01

*** mean of three readings**

CHAPTER-V

PHYTO CHEMICAL STUDIES

The leaves of *Ixora pavetta* was collected in an around Madurai and it was authenticated by taxonomist.

The Shadow dried leaves were powered and then subjected to the following preliminary phytochemical studies

SECTION-A

ORGANOLEPTIC EVALUATION

Nature - Coarse powder

Colour - Dark Yellowish brown

Odour - Characteristic odour

Taste - Bitter followed by astringent taste

The powdered plant material and extracts were subjected to the following chemical tests and the results were tabulated.

SECTION-B

PRELIMINARY PHYTOCHEMICAL SCREENING [65, 66, 67, 68]

1. TEST FOR STEROLS

The powdered leaf was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

A) SALKOWSKI'S TEST

A few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in color indicating the presence of sterols.

B) LIEBERMANN – BURCHARD'S TEST

To the chloroform solution a few drops of acetic anhydride and 1 ml of concentrated Sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

2. TEST FOR CARBOHYDRATES

A) MOLISCH'S TEST

The aqueous extract of the powdered leaf when treated with alcoholic solution of α-naphthol in the presence of sulphuric acid gave purple color indicating the presence of carbohydrates.

B) FEHLING'S TEST

The aqueous extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

3. TEST FOR PROTEINS

A) MILLON'S TEST

A small quantity of aciduous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating Indicated the presence of proteins.

B) BIURET TEST

To one portion of aciduous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution was added, followed by this one drop of dilute copper sulphate solution was added. Violet color was obtained indicating the presence of proteins.

4. TEST FOR ALKALOIDS

A) About 2gm of the powdered material was mixed with 1gm of calcium hydroxide and 5ml of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200ml of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was

filtered and the chloroform was evaporated. To this 5ml of dilute hydrochloric acid was added followed by 2ml of each of the following reagents.

a) Mayer's Reagent _ No cream precipitate

b) Dragendorff's Reagent _ No orange brown precipitate

c) Hager's Reagent _ No Yellow precipitate

d) Wagner's Reagent _ No Reddish brown precipitate

B) TEST FOR PURINE GROUP (MUREXIDE TEST)

The residue obtained after the evaporation of chloroform as described in (a) was treated with 1ml of hydrochloric acid in a porcelain dish and 0.1gm of Potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple Color was obtained indicating the absence of purine group of alkaloids.

5. TEST FOR GLYCOSIDES

A) BORNTRAGER'S TEST

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate, benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly.

No color reaction observed in ammoniacal layer showing the absence of Anthraquinone glycosides.

B) MODIFIED BORNTRAGER'S TEST

About 0.1g of the powdered drug was boiled for 2minutes with dilute hydrochloric acid and few drops of ferric chloride solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the Benzene extract.

No pink color was observed in ammoniacal layer showing the absence of anthraquinone glycosides.

6. TEST FOR CARDIAC GLYCOSIDES (for deoxysugar)

A) KELLER KILIANI TEST

About 1g of the powdered leaf was boiled with 10ml of 70% alcohol for 2 minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of leadsubacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid. To this 2 drops of ferric chloride solution was added. Then 3 ml of concentrated sulphuric acid was added to the sides of the test tube carefully and observed.

No reddish brown layer was observed indicating the absence of deoxysugars of cardiac glycoside

7. TEST FOR CYANOGENETIC GLYCOSIDES

Small quantity of the powder was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the

stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place.

No brick red color was produced on the paper indicating the absence of Cyanogenetic glycosides.

8. TEST FOR SAPONINS

About 0.5g of the powdered drug was boiled gently for 2 minutes with 20ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously. Frothing was produced indicating the presence of saponins.

9. TEST FOR TANNINS

A small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added.

Bluish black color was produced indicating the presence of tannins.

10. TEST FOR FLAVONOIDS

A) MAGNESIUM TURNING- CON HCl TEST

A little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour was obtained indicating the presence of flavonoids.

B) ALKALI TEST

To the small quantity of test solution 10% aqueous sodium hydroxide Solution was added. Yellow orange color was produced indicating the presence of flavonoids.

C) ACID TEST

To the small quantity of test solution, few drops of concentrated Sulphuric acid was added. Yellow orange color obtained indicates the presence of flavonoids.

11. TEST FOR TERPENOIDS

A little of the powdered leaf was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. Pink color solution appeared which indicated the presence of terpenoids.

12. TEST FOR THE PRESENCE OF VOLATILE OIL

Weighted quantity (250 gm) of fresh leaves were extracted the subjected to hydrodistillation using volatile oil estimation apparatus (BP 1980).

No volatile oil was obtained indicating the absence of volatile oil.

13. TEST FOR MUCILAGE

Few ml of aqueous extract prepared from the powdered crude drug was treated with ruthenium red. Red color reaction indicating the presence of Mucilage.

The results were shown in TABLE- 6 and 7

TABLE –6

PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE LEAF POWDER OF *Ixora pavetta*

S.NO	TEST	RESULTS
1.	**TEST FOR STEROLS**	
	a. Salkowski's test	+
	b. Libermann- burchard's test	+
2.	**TEST FOR CARBOHYDRATES**	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
3.	**TEST FOR PROTEINS**	
	a. Millon's test	+
	b. Biuret test	+
4.	**TEST FOR ALKALOIDS**	
	a. Mayer's reagent	-

	b. Dragendroff's reagent	-
	c. Hager's reagent	-
	d. Wagner's reagent	-
	e. Test for Purine group (Murexide test)	-
5.	**TEST FOR GLYCOSIDES**	
	a. Anthraquinone glycosides	
	i) Borntrager's test	-
	ii) Modified Borntrager's test	+
	b. Cardiac glycosides	
	i) Keller Killiani test	-
	c. Cyanogenetic glycosides	-
	d. Coumarin glycosides	-
6.	**TEST FOR SAPONINS**	-
7.	**TEST FOR TANNINS**	
	$Fecl_3$ test	+

8.	**TEST FOR FLAVONOIDS**	
	a. Shinoda test	+
	b. Alkali test	+
	c. Acid test	+
9.	**TEST FOR TERPENOIDS**	+
10.	**TEST FOR VOLATILE OILS**	-
11.	**TEST FOR MUCILAGE**	+

(+) indicates positive reaction **(-) indicates negative reaction**

PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE VARIOUS EXTRACTS OF LEAF POWDER OF *Ixora pavetta*

Tests	Pet.ether Extract	Chloroform Extract	Ether extract	Acetone Extract	Methanol Extract	Ethanol Extract	Aqueous extract	Hexane extract
I. Test For Sterols								
Salkowski's test	+	+	+	+	+	+	-	-
Libermann-burchard's test	+	+	+	+	+	+	-	-
II. Test for Carbohydrates								
Molisch's test	-	-	-	-	+	+	+	-
Fehling's test	-	-	-	-	+	+	+	-
Benedict's test	-	-	-	-	+	+	+	-
III.Test for Proteins								

Millon's test	-	-	-	-	+	+	+	-
Biuret test	-	-	-	-	+	+	+	-
IV.Test for Alkaloids								
Mayer's reagent	-	-	-	-	-	-	-	-
Dragendroff's reagent	-	-	-	-	-	-	-	-
Hager's reagent	-	-	-	-	-	-	-	-
Wagner's reagent	-	-	-	-	-	-	-	-
Test for Purine group (Murexide test)	-	-	-	-	-	-	-	-
V. Test for Glycosides								

A) Anthraquinone glycosides								
Borntrager's test	-	-	-	-	-	-	-	-
Modified Borntrager's test	-	-	-	-	+	+	+	-
B)Cardiac glycosides								
Keller Killiani test	-	-	-	-	-	-	-	-
Cyanogenetic glycosides	-	-	-	-	-	-	-	-
Coumarin glycosides	-	-	-	-	-	-	-	-
VI.Test for Saponins	-	-	-	-	+	+	+	-
VII.Test for Tannins								

$Fecl_3$ test	-	-	-	+	+	+	+	+
VIII.Test for Flavonoids								
Shinoda test	-	-	-	+	+	+	+	-
Alkali test	-	-	-	+	+	+	+	-
Acid test	-	-	-	+	+	+	+	-
IX.Test for Terpenoids	+	+	+	+	+	+	-	+
X.Test for Volatile Oils	-	-	-	-	-	-	-	-
XI.Test for Mucilage	-	-	-	-	+	+	+	-

(+) indicates positive reaction **(-) indicates negative reaction**

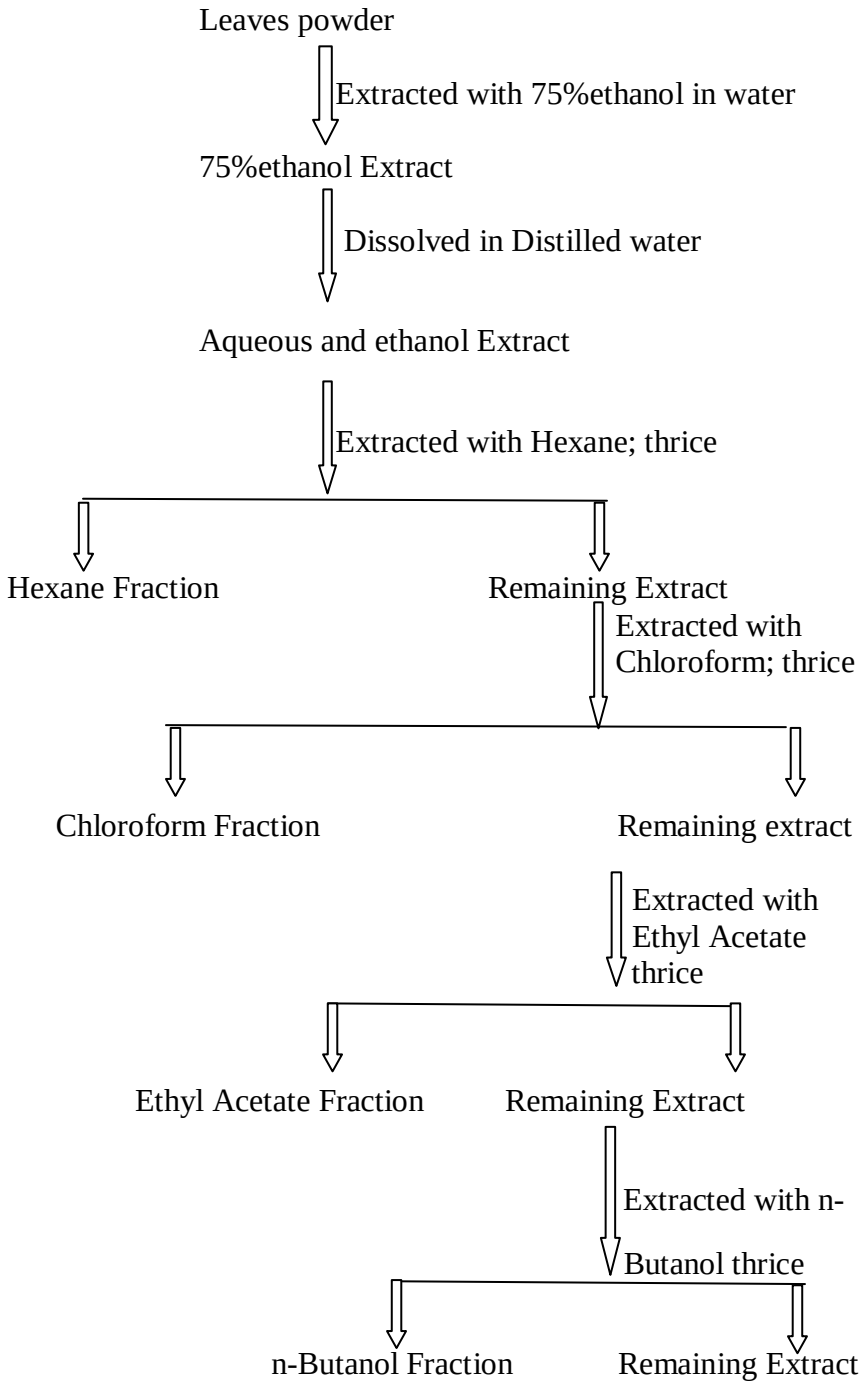
PREPARATION OF EXTRACTS

The shade dried and coarsely powdered leaves of *Ixora pavetta* was defatted with petroleum ether (60-80˚c)for three days by cold maceration. The defatted marc was extracted with 75% ethanol by triple maceration and filtered. The filterate was concentrated under reduced pressure to obtain a solid residue which has dark yellowish brown in colour.

ISOLATION OF PHENOLIC RICH FRACTIONS[69]

The ethanolic extract(75%) of *Ixora pavetta* was fractionated using solvent such as hexane, chloroform, ethylacetate, n-butanol. Each fractions were concentrated under reduced pressure to get the dry residue.

FLOW CHART OF PREPARATION OF PHENLIC RICH FRACTION



SECTION-C

TOTAL PHENOL DETERMINATION [70, 71,72]

PRINCIPLE

Total phenolic content of the various extracts of *Ixora pavetta* by Folin Ciocalteu reagent method.

All the phenolic compounds are oxidised by the Folin-Ciocalteu Reagent. This reagent, which is reduced during oxidation of the phenolic substances, into a mixture of blue molybdenum and tungsten oxides.

The blue colour produced has a maximum absorption at about 750-760nm. The absorption is proportional to the quantity of oxidised phenolic compounds.

REAGENTS REQUIRED

a) **Folin Ciocalteu Reagent (1N)**

Commercially available Folin Ciocalteu Reagent (2N) was diluted with an equal volume of distilled water. The resultant solution was kept in a brown color bottle and stored in refrigerator at 4 ^{o}c.

b) **Sodium carbonate solution (10%)**

c) **Standard gallic acid solution**.

PROCEDURE.

To the 1ml (1mg/ml and o.5mg/ml) of various leaf extracts of *Ixora pavetta* (ehanol and phenolic compound rich fraction of ethanol) 0.5ml of Folin Ciocalteu reagent (1N) was added and allowed to stand for 15 mins. Then 1ml of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made upto 10ml with distilled water and allowed to stand for 30 minutes at room temperature and total phenols were determined by spectrophotometrically at 760nm.

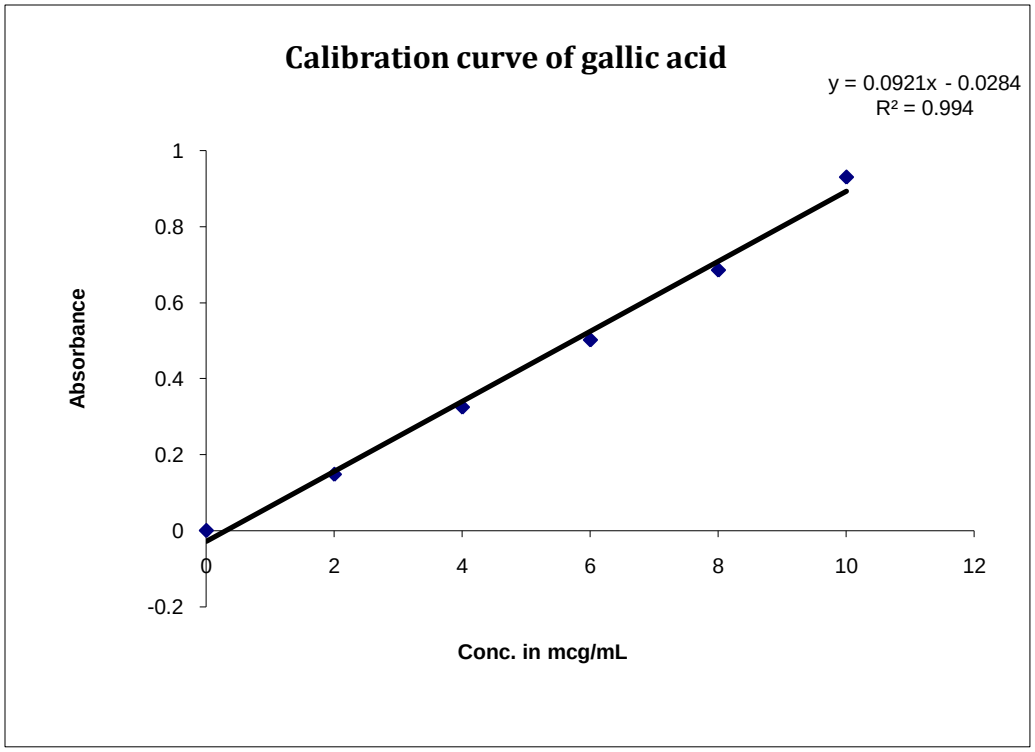
The calibration curve was generated by preparing Gallic acid at different concentration (2, 4, 6, 8, 10µg/ml). The reaction mixture without sample was used as blank. Total phenol content of various leaf extracts are expressed in terms mg of Gallic acid equivalent per gm of extract (mg GAE/g)

Table-8

ESTIMATION OF TOTAL PHENOL CONTENT OF *Ixora pavetta*

STANDARD		**ABSORBANCE**	**TEST**		**ABSORBANCE**	**TOTAL PHENOL CONTENT**
sample	**Conc.in µg/ml**		**Sample**	**Conc in µg/ml**		**Mg of GAE/gm of extract**
Gallic acid	2 4 6 8 10	0.148 0.325 0.502 0.813 0.823	Ethanol PHENOLIC RICH FRACTIONS	50 100	1.60±0.02 3.01 ±0.005	277.75±1.60 260.41±0.81
			Hexane	100	1.79 ±0.040	216.2± 0.25
			Ethyl acetate	100	2.88 ±0.42	321.7± 0.58
			chloroform	100	2.92 ±0.73	329.6 ±0.45
			n-Butanol	100	1.98 ±0.61	237.3 ±0.32

Fig-13: Calibration curve of Gallic acid



SECTION-D

TOTAL TANNIN DETERMINATION [71, 72]

PRINCIPLE

Total tannin content of extract was determined by Folin Denis reagent method.

Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution. The intensity of which is proportional to the amount of tannins and it was estimated by spectrophotometer at 700nm.

REAGENTS REQUIRED

a) Folin Denis Reagent (sodium tugstate 100g and phospho molybdic acid 20gm were dissolved in distilled water 750ml along with phosphoric acid 50ml. The mixture was refluxed for 2 hours and volume was made upto 1 litre with distilled water)

b) Sodium carbonate solution (10%)

c) Standard tannic acid solution.

PROCEDURE

0.2ml of (1mg/ml) leaf extract of *Ixora pavetta* was made upto1ml with distilled water . Then add 0.5ml of Folin Denis reagent and allowed to stand for 15 mins, then 1ml of sodium carbonate solution was added to the mixture and it was made upto 10ml

with distilled water. The mixture was allowed to stand for 30mins at room temperature and the tannin content was determined spectrophotometrically at 700nm.

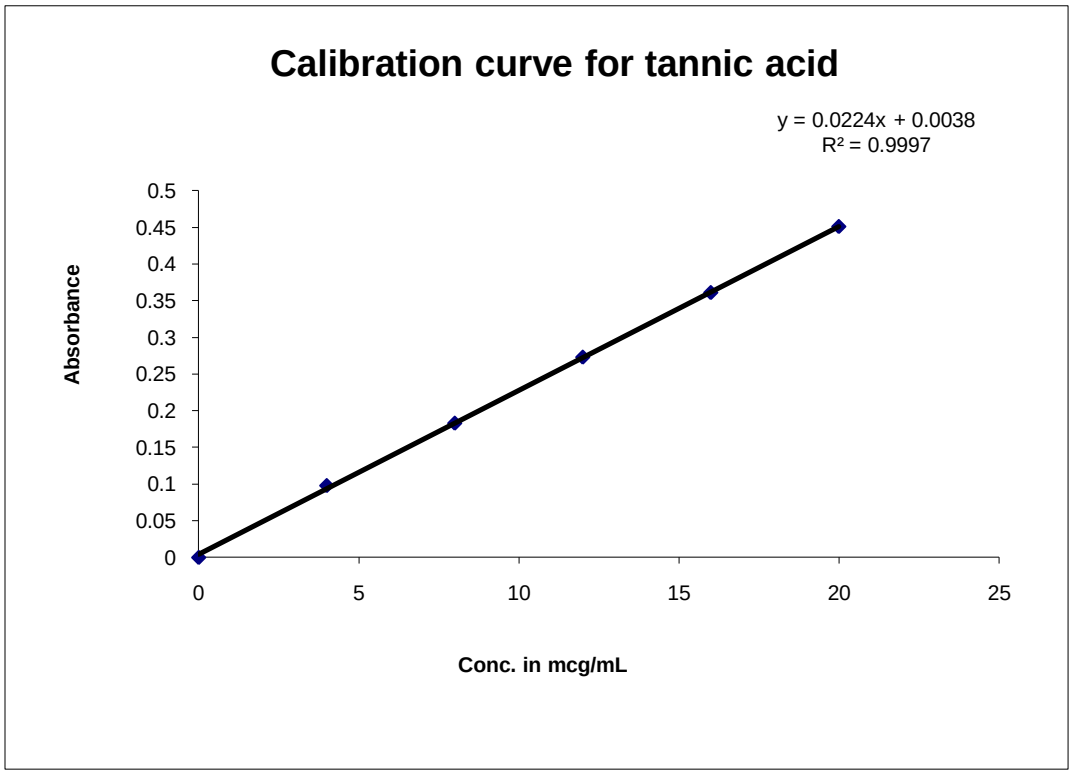
The calibration curve was generated by preparing tannic acid at different concentration (4, 8, 12, 16, 20µg/ml). The reaction mixture without sample was used as blank. The total tannin content in the leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract.

TABLE-9
ESTIMATION OF TANNIN CONTENT

STANDARD		**ABSORBANCE**	**TEST**		**ABSORBANCE**	**TOTAL TANNIN CONTENT**
sample	**Conc.in µg/ml**		**Sample**	**Conc in µg/ml**		**Mg of GAE/gm of extract**
Gallic acid	2	0.148		50	0.105± 0.006	467.21±0.071
	4	0.325	Ethanol			
	6	0.502		100	0.232± 0.003	486.36±0.052
	8	0.813				
	10	0.823			**Average:**	**476.785**

***** mean of three readings ± SEM

Fig-14: Calibration curve for Tannic acid



SECTION-E

TOTAL FLAVONOID DETERMINATION [73, 74, 75]

PRINCIPLE

The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation. Aluminum ions form stable complexes with C4 keto group and either to C_3 or C_5 hydroxyl groups of flavones and flavonols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavanoids . These complexes showed a strong absorption at 415nm which is used for the estimation of flavanoids.

REAGENTS REQUIRED

10% aluminum chloride

1M potassium acetate

PROCEDURE

1mL of Ethanolic extract at concentrations 50µg/mL and 100µg/mL were taken and0.1mL of aluminum chloride solution, 0.1mL of potassium acetate solution and 2.8mL of ethanol were added and the final volume was then made up to 5mL with distilled water. After 20min the absorbance was measured at 415nm. A calibration curve was constructed by plotting absorbance reading of quercetin at different concentrations. The sample without aluminium chloride was used as a blank. The total flavonoid content

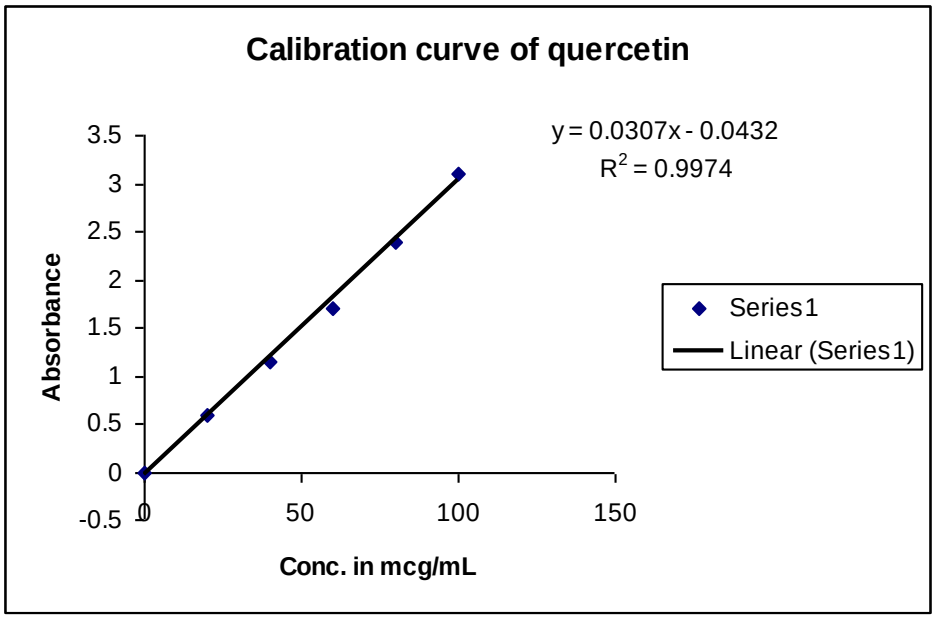
in the extract was expressed as milligrams of quercetin equivalent per gram of extract and the results were tabulated.(**Fig.13)**

Table-10 ESTIMATION OF TOTAL FLAVONOID CONTENT OF *Ixora pavetta*

S.No.	**Conc. of quercetin in µg/mL**	**Absorbance at 415nm**	**Conc. of ethanolic extract in µg/mL**	**Absorbance at 415nm**	**Amt of total flavonoid content in terms mg quercetin equivalent/ g of extract**
1	20	0.589 ± 0.01	50	0.0383± 0.001	467.21±0.351
2	40	1.151 ± 0.04	100	0.0896±0.006	486.36 ±0.06
3	60	1.710 ± 0.09			
4	80	2.390 ± 0.03			
5	100	3.112 ± 0.03			
				Average	**24.08**

***mean of three readings** ± SEM

Fig. 15: Calibration curve of Quercetin



CHROMATOGRAPHY

Thin layer chromatography [76, 77, 78]

Thin layer chromatography is the technique used for the separation of the natural components .Here the principle is adsorption. Silica gel is used as stationary phase. The special advantage of TLC is versality, speed, sensitive. Preliminary phytochemical screening of the extract revealed that the presence of flavonoids, phenols, tannins. Hence an attempt has been made to evaluate these extracts by TLC and HPTLC studies.

Table 11:TLC finger profile of the ethanolic extract and phenolic rich fraction of *Ixora pavetta*

Sample	Solvent system	Detecting agent	No of spots	R_f values
Ethanolic extract			**3**	0.447
				0.533
				0.761
Phenolic rich Fraction				
Hexane	Toluene: ethyl acetate : formic acid (7 : 3 : 0.2)	UV light	2	0.83
				0.48
Ethyl Acetate			2	0.83
				0.53
Chloroform			5	0.40
				0.48
				0.63
				0.75
				0.80
n-Butanol			1	0.67

High Performance Thin Layer Chromatography [79]

High performance thin layer chromatography, also known under the synonym Planar Chromatography, is a modern, powerful, analytical technique with separating power, performance and reproducibility superior to classic TLC. Based on the use of high performance TLC plates with small particle sizes (3-5 µ) and precise instruments for each step of the chromatographic process.

HPTLC provides the means not only for flexible screening procedures and qualitative analyses but also for demanding quantitative determinations. While traditional TLC often relies on visual inspection of the chromatographic plate and its documentation by either tracing or photography, HPTLC features have highly sensitive scanning densitometry and video technology for rapid chromatogram evaluation and documentation.

Today most HPTLC instruments are computer controlled and can therefore, often dramatically improved reproducibility of the analytical result. At the same time HPTLC is still as flexible and user friendly as classic TLC, but provides even greater advantages.

MATERIALS AND METHODS

EQUIPMENT

A Cammag make HPTLC system equipped with a sample applicator Linomat5, Twin trough glass chamber (20x10 cm^2) with SS lid, TLC scanner III, Rota vapour.

CHEMICALS AND REAGENTS

Toluene, ethylacetate and formic acid analytical grade were used. TLC aluminium pre coated plate with silica gel 60 GF 254. (20x10 cm^2 ; 0.2mm thick)was used as stationary phase.

CHROMATOGRAPHY

TLC aluminium pre coated plate with silica gel 60 GF 254 (20x10 cm^2 ; 0.2mm thick) was used with

Toluene : ethyl acetate : formic acid (7:3:0.2)

as mobile phase. Ethanolic extract (EIP)and chloroform fraction of ethanolic extract (CIP)of sample solution were applied on plate by using Linomat 5 applicator. Cammag Twin trough glass chamber (20x10 cm) with SS lid was used for development of TLC plate. The twin trough glass chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8cm distance above the position of the sample application. The plates were removed from the chamber and air dried at room temperature. Plates were scanned immediately using Cammag TLC Scanner III at wavelength 366 nm and 254nm

HPTLC PROFILE:

Instrument used : CAMAG make HPTLC.

Software : winCATS 1.4.3

Sample Applicator : Linomat 5.

Detection : @254nm & @366nm in Densitometry TLC Scanner 3

Sample preparation : The sample was prepared in corresponding solvents

Stationary Phase : HPTLC plates silica gel 60 F 254.

Mobile Phase : Toluene: Ethyl acetate: Formic Acid (7:3:0.2)

Sample : Track 1: IP 1, Track 2: IP 2

Fingerprinting: **3D DISPLAY**

@ 254nm **@366nm**

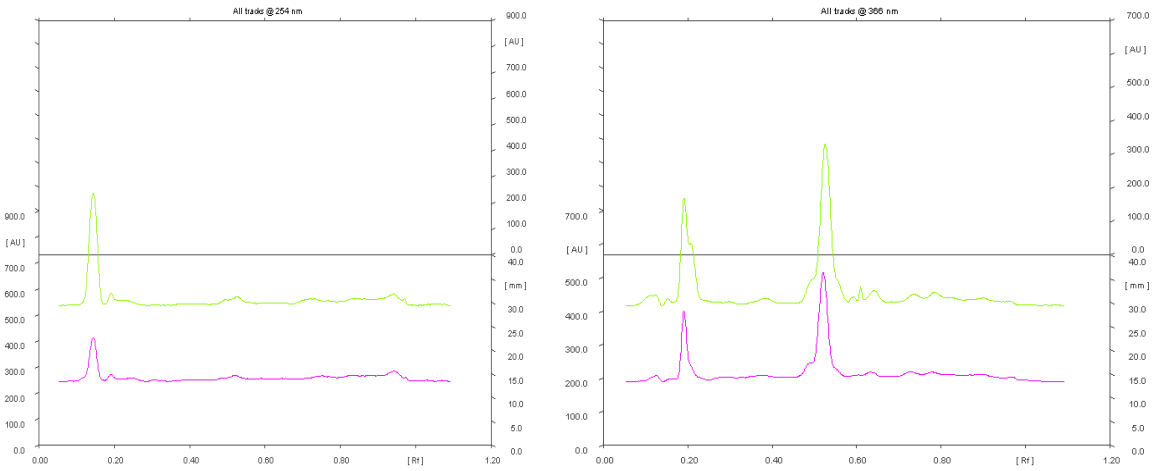


Fig : 16 Visualization

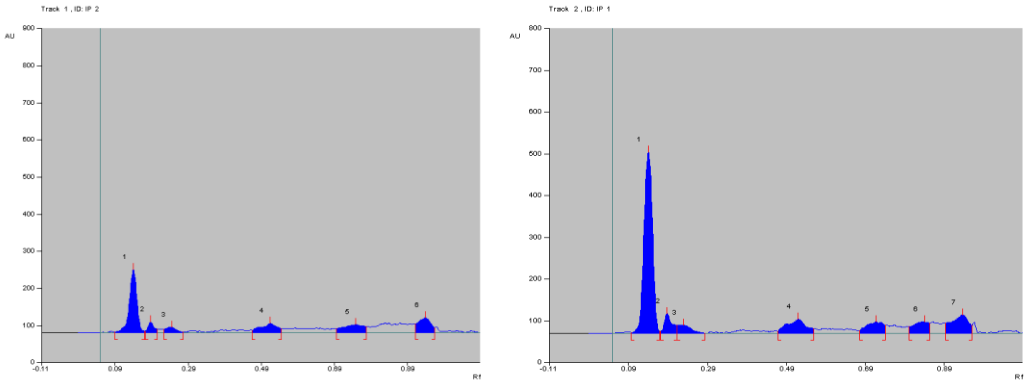
@ 254nm

@366nm

PEAK DISPLAY

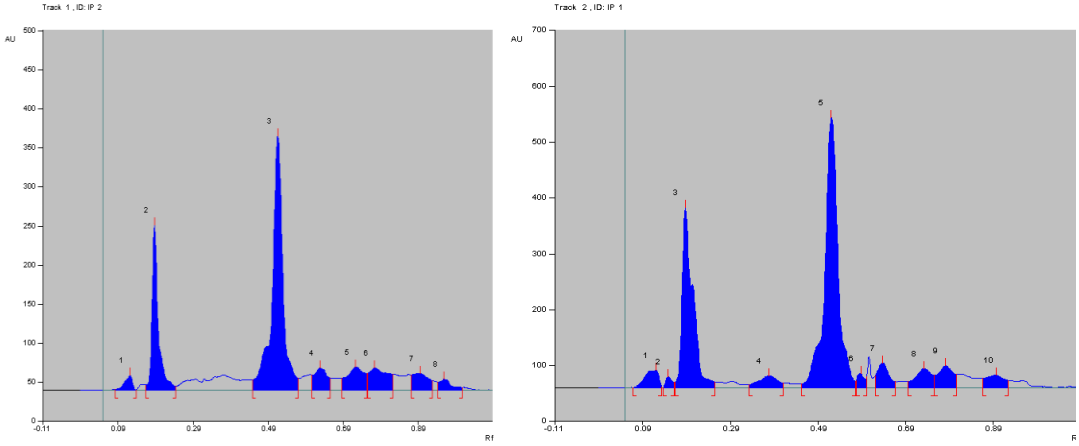
@ 254nm

Track 1 **Track 2**



@ 366nm

Track 1 **Track 2**



TABLES: 12

Peak	@ 254nm			
	Track 1		Track 2	
	Rf value	Area (AU)	Rf value	Area (AU)
1	0.14	3056.9	0.14	8148.9
2	0.19	415.3	0.19	811.0
3	0.25	379.1	0.23	585.4
4	0.52	972.9	0.52	1414.8
5	0.75	1077.2	0.72	1025.5
6	0.94	1220.8	0.84	951.5
7			0.94	1620.0

Peak	@366nm			
	Track 1		Track 1	
	Rf value	Rf value	Rf value	Rf value
1	0.13	339.3	0.12	874.1
2	0.19	3069.3	0.15	243.7
3	0.52	8146.3	0.19	6884.2
4	0.63	851.7	0.38	819.1
5	0.73	1174.3	0.52	12784.1
6	0.78	1233.1	0.59	379.9
7	0.90	809.5	0.64	1014.8
8	0.96	399.7	0.73	1091.5
9			0.79	1185.5
10			0.90	820.7

CHAPTER-VI

PHARMACOLOGICAL SCREENING

SECTION-A

INVITRO ANTIOXIDANT ACTIVITY OF LEAF EXTRACT OF *Ixora pavetta.*

Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects. Antioxidants are capable of stabilizing or deactivating free radicals or reactive oxygen species before they attack the cells. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from the plant materials. That's why plants with antioxidant properties are becoming more and more popular all over the world.

The causes for many degenerative diseases such as atherosclerosis, cancer, cirrhosis and diabetes are the presence of reactive oxygen species [80-83].

Synthetic antioxidants like butylated hydroxyl anisole, BHT, tertiary butylated hydroxyl quinine and gallic acid esters are available, they are suspected to be carcinogenic and have low solubility [84-86]. Hence strong limitations have been placed on their use and there is a necessity to replace them with naturally occurring antioxidants[87].

Plants derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechin, anthocyanins and

proanthocyanins could delay or prevent the onset of degenerative diseases because of their redox properties which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals scavengers and nitric oxide scavengers[88-89].

Many in vitro models for evaluating antioxidant activity are available. Some of them are enumerated below

1. DPPH method
2. Superoxide radical scavenging activity
3. Hydroxyl radical scavenging activity
4. Nitric oxide radical inhibition assay
5. Reducing power method
6. Phosphomolybdenum method
7. carotene linoleate model
8. Xanthine oxidase method
9. Ferric reducing ability of Plasma
10. Thiobarbituric acid assay etc.

METHOD 1: FREE RADICAL SCAVENGING ACTIVITY USING DIPHENYL PICRYL HYDRAZYL (DPPH) FREE RADICAL [90]

PRINCIPLE:

Total antioxidant assays are used to compare the antioxidant activities of different molecules. DPPH is a stable free radical with a distinctive ESR signal. It is widely accepted that DPPH can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the ethanol solution of DPPH, which is purple in colour shows a strong absorption at 517nm[91]. DPPH radicals react with

suitable reducing agents and then electrons become paired off and the solution loses colour stochiometrically with the number of electrons taken up [92]

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$$

REAGENTS

0.1mM diphenyl picryl hydrazyl in ethanol

Ascorbic acid

PROCEDURE[93,94]

DPPH stock solution was prepared by 4mg of DPPH in 100mL of ethanol. To the 1mL of the extract (EIP and CIP) of different concentrations, 4mL of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance of test mixtures was measured at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Vitamin C was used as standard. The percentage scavenging was calculated using the formula

% inhibition = [(Control-Test)/Control] X 100

The concentration of the sample required for 50% reduction in absorbance (IC_{50}) was calculated using linear regression analysis. The results obtained are presented in **Table 13** and F**ig 17.**

Table 13: Percentage inhibition of Ethanolic extract of

***Ixora pavetta* and standard ascorbic acid against DPPH at 517nm**

S. No.	Conc. in µg/mL	Percentage inhibition by standard ascorbic acid	Conc. in µg/mL	Percentage inhibition by Ethanolic extract	Conc. in µg/mL	Percentage inhibition by Choloroform fraction of Ethanolic extract
1	20	25.86 ± 5.63	4	13.52± 3.07	2	26.54 ±0.025
2	40	53.32 ± 4.84	8	33.98± 1.94	4	39.58 ±0.084
3	60	62.2 ± 7.35	12	56.95 ±1.52	6	76.64± 0.035
4	80	81.21 ± 5.87	16	64.69± 2.15	8	89.36± 0.024
	IC_{50}	**45.51 µg/mL**	IC_{50}	**43.14 µg/mL**	IC_{50}	**58.71 µg/mL**

***mean of three readings ± SEM**

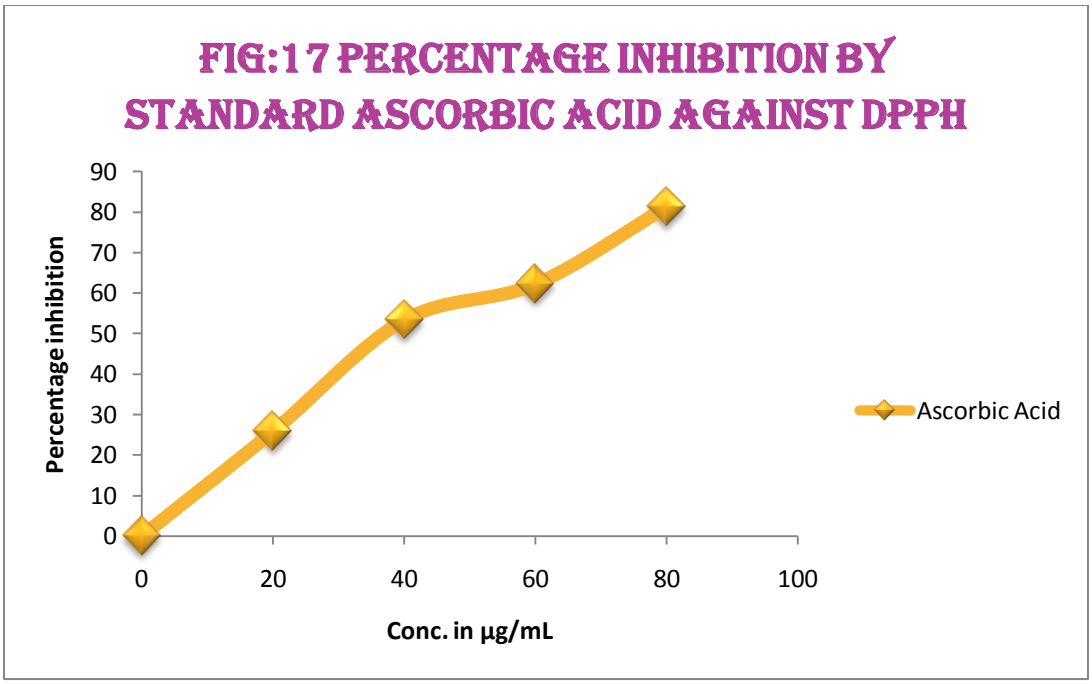


FIG: 18 PERCENTAGE INHIBITON OF EIP

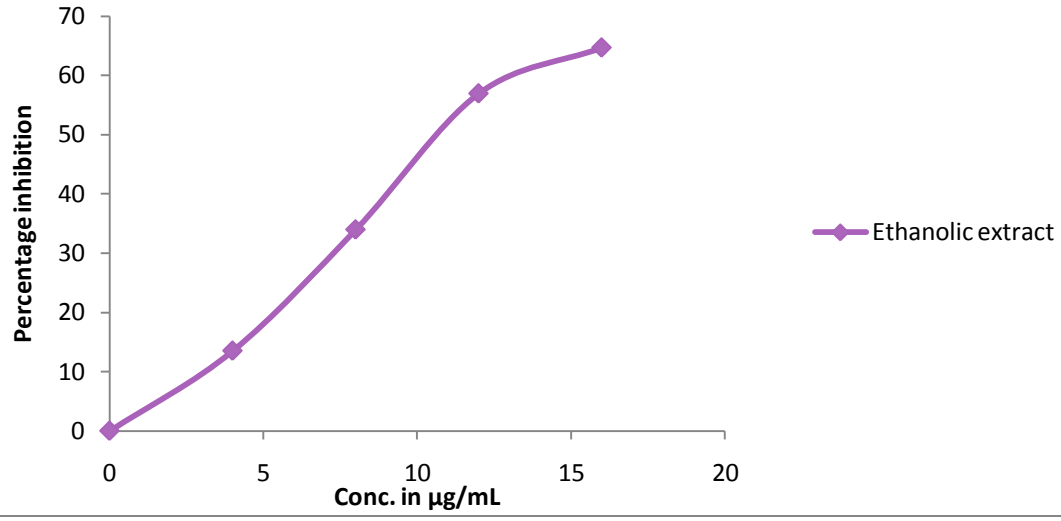
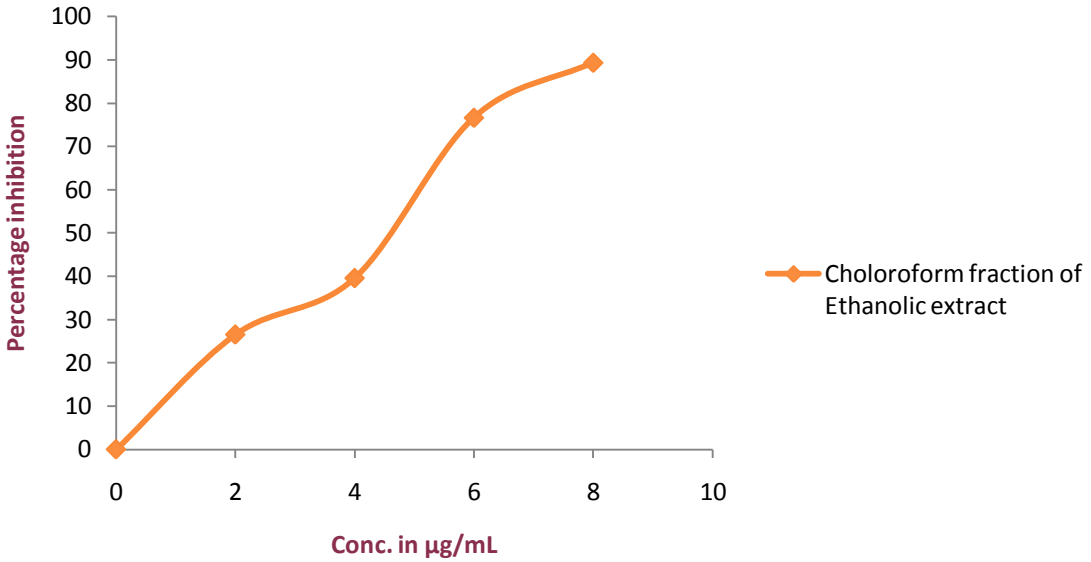


FIG:19 PERCENTAGE INHIBITION OF CIP



METHOD 2: NITRIC OXIDE SCAVENGING ACTIVITY ASSAY [95, 96]

PRINCIPLE

Nitric oxide scavenging activity was determined according to the method reported by Green *et al.,* 1982 [78]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. The nitrite ions produced diazotizes sulphanilamide and then the diazonium salt reacts with N,N-naphthyl ethylene diamine dihydrochloride to give a pink colour chromophore which has a maximum absorption at 546nm.

REAGENTS

10mM sodium nitroprusside

Phosphate buffered saline pH 7.4

2% sulphanilamide in ortho phosphoric acid

0.1% naphthyl ethylene diamine dihydrochloride

PROCEDURE

To 1mL of extracts at various concentrations was added to 1ml of sodium nitroprusside, 2.5mL phosphate buffered saline pH 7.4 and mixed then the mixture was incubated at 25^{o}C for 30min. From the incubated mixture 1.5mL was taken. To it, 1mL of sulphanilamide in phosphoric acid and 0.5mbL of naphthyl ethylene diamine dihydrochloride were added and the absorbance was measured at 546nm. Sample blank was also prepared without reagents. The reaction mixture without sample was used as blank. Ascorbic acid was used as a standard.

The percentage inhibition of nitric oxide radical generated was calculated using the following formula:

% inhibition = [(Control-Test)/Control] X 100

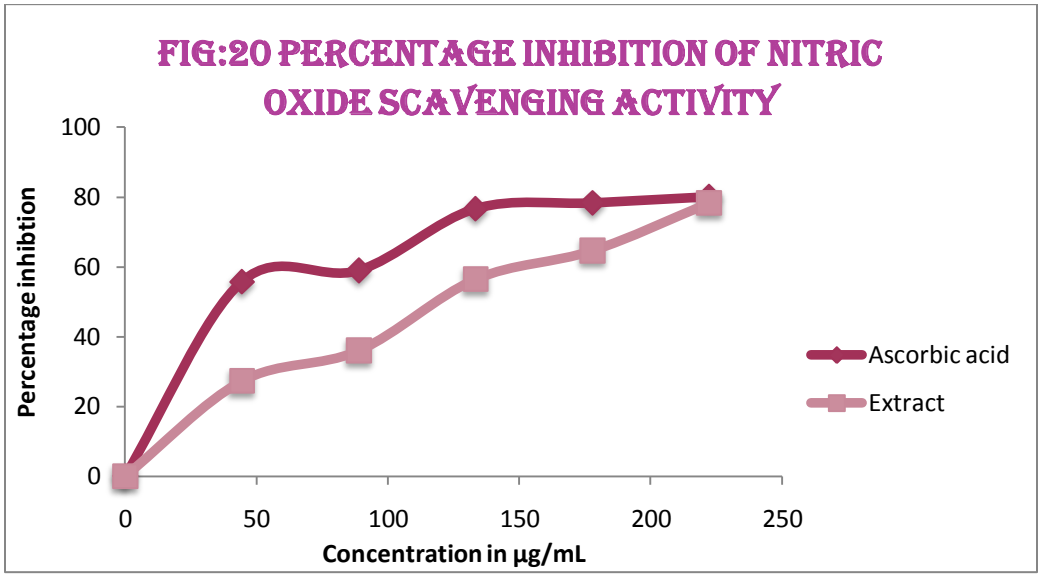
The IC_{50} was calculated using linear regression analysis. The results obtained for the nitric oxide scavenging activity assay are presented in **Table 14** and **Fig. 20**.

Table 14: Percentage inhibition of Ethanolic extract of *Ixora pavetta* against nitric oxide at 546nm

S. No.	Conc. in µg/mL	Percentage inhibition by standard ascorbic acid	Percentage inhibition by extract
1	44.44	55.71 ± 0.45	27.27± 0.151
2	88.89	58.94 ± 0.38	35.98 ±0.065
3	133.33	76.61 ± 3.13	56.43 ±0.016
4	177.78	78.27 ± 2.56	64.62 ±0.003
5	222.22	80.03 ± 1.97	78.25 ±0.017
	IC_{50}	**84.65µg/mL**	**129.65 µg/mL**

***mean of three readings ± SEM**

Fig. 20: Nitric oxide radical scavenging by Ethanolic extract of *Ixora pavetta*



METHOD 3: DETERMINATION OF SCAVENGING ACTIVITY AGAINST HYDROGEN PEROXIDE [97]

PRINCIPLE:

The radical scavenging activity against hydrogen peroxide of plant extract was determined by using the method of Ruch *et al* [83]. The principle is based the capacity of the extracts to decompose the hydrogen peroxide to water.

REAGENTS

6% hydrogen peroxide diluted with water in the ratio of 1:10

0.1M, pH 7.4 phosphate buffer

PROCEDURE

To the 1ml of extract at different concentrations were added to 3.8mL of 0.1M phosphate buffer solution (pH 7.4) and then mixed with 0.2mL of hydrogen peroxide solution. The absorbance of the reaction mixture was measured at 230nm after 10min. The reaction mixture without sample was used as blank. Sample blank was also prepared without reagents .Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100$$

The results are presented in the table

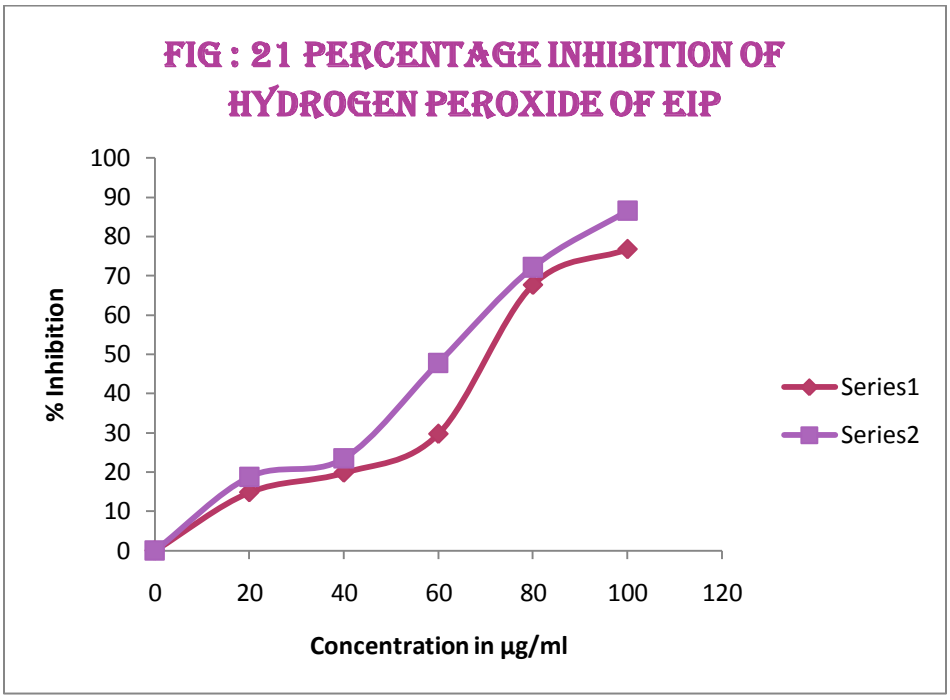
Table 15: Percentage inhibition of hydrogen peroxide by Ethanolic extract of *Ixora pavetta*

S.No.	Conc. in µg/mL	Percentage inhibition	
		Ethanolic extract of *Ixora pavetta*	Ascorbic acid
1	20	14.845 ±2.0854	18.81
2	40	19.84 ±2.336	23.52
3	60	29.72 ±3.967	47.85
4	80	67.69 ±3.075	72.18
5	100	76.8 ±1.659	86.56
	IC_{50}	**59.65 µg/mL**	**69.24 µg/mL**

***mean of three readings ± SEM**

Fig. 21: Percentage inhibition of hydrogen peroxide

by Ethanolic extract of *Ixora pavetta* and ascorbic acid



SECTION-B

NEPHROPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF *Ixora pavetta* ON CISPLATIN INDUCED NEPHROTOXICITY

BACKGROUND:

In traditional medicine *Ixora pavetta* leaves, root and fruits are used for treating kidney diseases.

MATERIALS & METHODS:

ANIMALS:

Male wistar albino rats (100-200 gms) were used for the study. The rats were kept in polyethylene cages and maintained under standard laboratory conditions at 25±2°C relative humidity of 50±15% and under a 12 hrs light dark cycle throughout the entire period of study. Commercial pellet diet (M/S.S.K.M animal feeds) and water were provided ad libitum. The animals were acclimatized to these conditions for one week before commencing the experiments. Experiments were carried out according to the guidelines of the Institutional animal ethics committee.

CHEMICALS:

All the chemicals used for the estimation of serum parameters and tissue homogenate were analytical grade and obtained from commercial sources. Cisplatin (Cisteen 10mg/10ml) was procured from local pharmacy shop.

PLANT EXTRACT:

Ethanolic extract of *Ixora pavetta* (EIP) and phenolic rich fraction of EIP was made into a suspension with 1% Carboxy Methyl Cellulose.

EXPERIMENTAL DESIGN [98]:

Animals were divided into 5 groups of six animals each . Group II, III, IV &V animals were received single dose of 4 mg/kg of cisplatin by intraperitonially on the first day alone to induce nephrotoxicity.

GROUP I: This group of animals were served as control which received 1% CMC for five consecutive days.

GROUP II: Rats were subjected to the adminintration of single dose of cisplatin at 4 mg/kg by i.p route on the first day alone.

GROUP III: Rats were treated with EIP (50mg/kg/p.o), 6 hrs prior to the administration of cisplatin (4 mg/kg/ip) injection on the first day alone and remaining four days EIP (50 mg/kg /p.o)alone has been administered to the animals.

GROUP IV: Rats were treated with EIP(100 mg/kg/p.o), 6 hrs prior to the administration of cisplatin (4 mg/kg/ip) injection on the first day alone and remaining four days EIP(100 mg/kg/p.o) alone has been administered to the animals.

GROUP V: Rats were treated with phenolic rich fraction of EIP (10mg/kg/p.o CIP), 6 hrs prior to the administration of cisplatin(4mg/kg/i.p) injection on the first day alone and remaining four days phenolic rich fraction of EIP (10mg/kg/p.o) alone has been administered to the animals.

On the sixth day of the experiment , blood and urine samples were collected from rats of all groups for the assessment of renal function by urinary and serum biochemicals parameters[99]. Finally all the groups of animals were sacrificed as per CPCSEA guidelines.

ASSESSMENT OF RENAL FUNCTIONS [100-106]:

(a) Serum parameters:

(i) Urea level was estimated by Urease enzymatic method.

(ii) Creatinine level was measured by alkaline picrate method

(iii) Uric acid level and Blood Urea Nitrogen were estimated by diacetyl monoxime method .

(iv) Total protein was estimated by Biuret method .

(v)Serum sodium and potassium levels were estimated by flame photometry.

(b) Urine Parameters:

(i) Creatinine clearance was estimated by alkaline picrate method.

And

(ii) urinary protein was measured by Sulpho salicylic acid method .

(ii) Urine sodium and potassium levels were estimated by flame photometry.

(c)Anti-oxidant enzymes:

The tissue samples were weighed and 10% homogenate was prepared by 0.15MTris-HCl buffer (pH 7.4) and used for the estimation of tissue anti-oxidant parameters like TBARS ,GSH& Proteins .

Thiobarbituric acid Reactive Substances (TBARS):

TBARS was determined by the method described by Rajkumar DV and Rao MNA ,et.al., (1993)

To 1ml homogenate 2.5ml of 20% TCA was added and centrifuged .The supernatant liquid was dissolved in 2.5ml of 0.05M Sulphuric acid and 3.5ml of

Thiobarbituric acid and incubated at 37°C for 30 mins. Contents were extracted into n-butanol and the absorbance was measured at 530nm.

Reduced glutathione:

Reduced Glutathione was determined by the method Morn et al., (1979) 1 ml of tissue homogenate was precipitated with 1 ml of 10 % TCA and the precipitate was removed by centrifugation. To 0.5 ml of the supernatant added 2 ml of 0.6 mM 5,5'Dithiobis-2-nitrobenzoic acid(DTNB) in 0.2 M Sodium phosphate and the total volume was made upto 3 ml with 0.2 M Phosphate buffer (pH 8).

The absorbance was read out at 412nm.

Table 16.

Effect of *E*thanolic leaf extract of *Ixora pavetta* on tissue biochemical parameters

Groups	TBARS (nmol/mg of protein)	GSH (µg/mg of protein)
Control	13.55 ± 0.69	43.68± 0.71
Cisplatin	35.23± 1.03	21.98 ±0.77
EIP(50mg/kg/p.o)	24.47± 0.51*	30.81 ±0.78*
EIP(100mg/kg/p.o)	19.72± 0.48*	35.46 ±0.52*
CIP(10mg/kg/p.o)	17.85± 0.52*	39.15 ±0.45*

Values are expressed as mean±SEM; *P<0.001

When compared with Cisplatin.

TBARS-Thiobarbituric acid reactive substances; GSH-Reduced glutathione

Table-17

Effect of *E*thanolic leaf extract of *Ixora pavetta* on Urine biochemical parameters

GROUPS	CREATININE CLEARANCE (ml/min)	TOTAL PROTEIN (mg/dl)	SODIUM(mEq/L)	POTASSIUM (mEq/L)
Control	16.96± 0.65	8.03± 0.70	0.4± 0.03	1.84 ±0.28
Cisplatin	7.12 ±0.75	15.74 ±0.57	0.88± 0.03	0.86 ±0.05
EIP(50mg/kg/p.o)	10.16± 0.55*	12.35± 0.52*	0.66 ±0.03*	1.08 ±0.07**
EIP(100mg/kg/p.o)	12.29± 0.77**	11.59± 0.38**	0.63 ±0.03**	1.34 ±1.77**
CIP(10mg/kg/p.o)	13.69±0 .84**	10.98± 0.46**	0.55 ±0.02**	1.4 ±0.17**

Values are expressed as mean ± SEM; *P<0.01 and **P<0.001

When compared with Cisplatin.

Table-18

Effect of *E*thanolic extract of *Ixora pavetta* on Serum biochemical parameters

GROUPS	BUN(mg/dl)	UREA(mg/dl)	CREATININE (mg/dl)	TOTAL PROTEIN (mg/dl)	URIC ACID (mg/dl)	SODIUM (mEq/L)	POTASSIUM (mEq/L)
Control	21.47±0.88	50.61± 0.76	0.55±0.29	8.875±0.42	2.12± 0.17	0.46±0.48	5.68±0.48
Cisplatin	77.02±1.05	82.25± 0.61	1.18±.01	4.52±0.32	3.67±0.27	0.88±0.55	8.77±0.27
EIP(50mg/kg/p.o)	36.39 ±1.12***	68.63±0.42**	0.70±0.04**	7.15±0.83**	2.75±0.04**	0.66±0.07**	7.12±1.04*
EIP(100mg/kg/p.o)	30.78±0.76***	61.41± 0 .60***	0.64±0.04***	7.57±0.29***	2.50±0.04***	0.63±0.20***	6.78±0.83***
CIP(10mg/kg/p.o)	25.73± 0.56***	58.61±1.35***	0.61±0.02***	8.54±0.24***	2.31±0.025***	0.55±0.12***	6.18±1.18***

Values are expressed as mean±SEM; *p<0.05, **p<0.01 and ***p<0.001

When compared with cisplatin

HISTOPATHOLOGICAL STUDIES:

Kidney was removed from the sacrificed animals of all groups which were sectioned longitudinally and kept in 10 % neutral formalin solution. These tissues were embedded in paraffin wax and stained with hematoxylin and eosin which was observed under light microscope.

Statistical Analysis

All the results were expressed as standard error of mean (SEM). Data of biochemical parameters were analyzed by using one way ANOVA. Tukey-Kramer Multiple comparisons test was applied for post-hoc analysis. A value of $P<0.001$, $P<0.01$ and $P<0.05$ were considered to be statistically significant.

SECTION-C

INVITRO-CALCIUM OXALATE CRYSTALLIZATION INHIBITION BY ETHANOLIC EXTRACT OF *Ixora pavetta*

MATERIALS AND METHODS:

CHEMICALS:

10mM Calcium chloride dihydrate($Cacl_2.2H_2O$)

4mM Sodium oxalate ($Na_2C_2O_4$)

0.15M Sodium Chloride (NaCl)

PLANT EXTRACT:

Ethanolic extract of leaves of Ixora pavetta

Phenolic rich fraction of EIP

EXPERIMENTAL DESIGN [9,10]:

The effect of extract on calcium oxalate crystallization was determined by the time course measurement of turibidity changes due to the crystal nucleation and aggregation in the calcium chloride dihydrate solution on the addition of 0.15M sodium oxalate .The precipitation of calcium oxalate at 37°c and pH6.5 has been studied by the measurement of turbidity at620nm.A spectrophotometer UV/Visible (Shimadzu 1800) was employed to measure the turbidity of the formation of calcium oxalate.

PREPARATION OF CALCIUM CHLORIDE AND SODIUM OXALATE SOLUTION:

0.15M Sodium chloride solution was prepared by standard procedure.10mM of calcium chloride dihydrate and 4mM of sodium oxalate was prepared by 0.15M sodium chloride solution as solvent.

STUDY WITHOUT INHIBITOR:

About 1.5ml of calcium chloride dihydrate was transferred into the cell and the blank reading was recorded. Then 1.5ml of sodium oxalate was added to the above solution and the absorbance was recorded at 620nm followed by within 10 minutes of the addition of this solution.

STUDY WITH INHIBITOR:

Different concentration (i.e 1mg/ml, 2mg/ml, 3mg/ml) of EIP and(100µg/ml, 200µg/ml, 300µg/ml)of CIP was prepared by using 0.15M sodium chloride solution

A mixture of 1ml of calcium chloride dehydrate and 1ml of plant extract (inhibitor solution) were taken in the cell and the blank reading was recorded. About 1ml of 4mM sodium oxalate was added to the above solution and the absorbance was recorded at 620nm followed by within 10 minutes of the addition of this solution.

The percentage of the inhibition was calculatd by using the following formula.

Percentage of inhibition = [1-(Ti/Tc)] X 100

Ti – turbidimetric slope with inhibitor

Tc - turbidimetric slope without inhibitor

Table-19

Effect of Ethanolic extract of *Ixora pavetta* on calcium oxalate crystallization inhibition

CONCENTRATION(mg/ml) OF INHIBITOR	SLOPE	%INHIBITION
0	0.000614	0
1	0.000266	56.72
2	0.000103	83.22
3	0.0000616	89.96

Table-20 Effect of phenolic rich chloroform fraction of ethanolic extract of *Ixora pavetta*

CONCENTRATION(µg/ml) OF INHIBITOR	SLOPE	%INHIBITION
0	0.000614	0
100	0.00090	73.81
200	0.000614	85.24
300	0.0000616	89.98

MICROSCOPIC STUDY:

A drop of the crystallizable solution as well as a drop of mixture of inhibiting solution with crystallizable solution were placed on the microscopic slide and the crystals were observed by research microscope .Growth of crystals in different stages(t1 and t2) with and without plant extracts were observed using research microscope. Effect of plant extracts on crystallization were noted and the photograph of crystallization were given in figure.

Table-21

TIME CORRESPONDING TO THE STAGE OF CRYSTALLISATION WITH AND WITHOUT INHIBITOR

NUMBER OF PHOTOGRAPHS	STAGES OF CRYSTALLISATION	CONCENTRATION OF INHIBITOR	TIME(seconds)
1	Growth	00	20
2	Aggregation	00	60
3	Growth	3mg/ml(EIP)	50
4	Aggregation	3mg/ml(EIP)	120
5	Growth	300µg/ml(EIP)	50
6	Aggregation	300µg/ml(EIP)	120

IDENTIFICATION TEST FOR CALCIUM OXLATE CRYSTALS:

(1) A drop of crystallizable solution was mixed with a drop of Hydrochloric acid and viewed under microscope. Solubilisation of crystals were noted.

(2) A drop of crystallizable solution was taken with drop of acetic acid and viewed under microscope. No change was observed in the crystallizable solution.

(3) Sulphuric acid 50% was added to the crystallizable solution , a gradual separation of needle like crystal of calcium oxalate at the site of orginal crystal.

SECTION-D

ANTIBACTERIAL ACTIVITY [107-110]

The Ethanolic extract of *Ixora pavetta*(EIP)and its phenoic rich fraction of the extract were screened for antibacterial activity.

Bacteria

The various organisms used in the present study include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis.*

Preparation of media

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/litre) Beef - 2 g, caesin acid hydrolysate 17.5g, starch 1.5g and agar 17 g; pH 7.4 ± 0.2.

MH agar (38g) was weighed and dissolved in 1000ml of distilled water and adjusted to pH 7.3 ± 0.2, sterilized by autoclaving at 121^{o}C for 15 minutes at 15psi pressure and was used for sensitivity tests.

Preparation of bacterial cultures

Few colonies of the bacterial strains selected for study were picked from the agar slants and inoculated into 4ml peptone water in a test tube. These tubes were incubated for 2-4 hours to produce suspensions. The suspensions were then diluted, if necessary with saline to a density visually equivalent to that of standard prepared by adding 0.5ml of 1% barium chloride to 99.5ml of 1% sulphuric acid. These suspensions were used for seeding the bacteria in the agar media.

Preparation of the extracts

The plant extract was dissolved in DMSO to get a concentration of 10mg/mL.

Preparation of agar plates

The media (20mL) was introduced aseptically into sterilized petridishes [89] and the petridishes were swirled until the agar begins to set.

Disc Diffusion technique

The pathogenic strains were then seeded on the MH agar media in a petridish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry.

Test Procedure

The plain sterile discs of 6mm diameter were obtained from Hi Media. The discs were then impregnated with different concentrations of the EIP and CIP of *Ixora pavetta* and solvent DMSO. Amiakacin discs were used as a standard. Each disc contained 30µg of standard and different concentration of extracts and DMSO discs were then placed on the seeded medium plates. The plates were then incubated at 37^{o}C for 24h. The results were read by the presence or absence of zone of inhibition. The zone of inhibition was then measured. The results are tabulated in

Table 22: Antibacterial activity of Ethanolic extract of *Ixora pave*tta against various microorganisms

S.No	Name of the organism	Zone of inhibition (mm)							
		Standard Amikacin (30µg/disc)	EIP				CIP		
			1 mg	2 mg	3 mg	Standard Amikacin	100 µg	200 µg	300 µg
1.	Escherichia coli	24	8	12	20	26	6	16	21
2.	Klebsiella pneumoniae	26	-	8	12	25	6	14	18
3.	Proteus mirabilis	24	4	10	18	24	8	15	20
4.	Pseudomonas aeurginosa	26	-	8	14	26	8	17	22

CHAPTER - VII

RESULTS AND DISCUSSION

Chapter1:

In this chapter a brief introduction was given on acute renal failure, drug induced renal damage , Herbal drug possesses nephroprotective activity, nephrolithiasis, types of renal stones , Herbal drug used for the treatment of urolithiasis, urinary tract infections, causative organisms and treatment were also discussed in this chapter.

Chapter-II:

This part of the study has dealt about the survey of literature. Ethnomedical uses pertaining to *Ixora pavetta* which indicate that the plant is used to treat kidney diseases, urinary disorder and chest pain.

Chapter-III:

Aim and scope of the present study has been discussed in this part of the study, though the plant has been claimed to treat kidney diseases and urinary disorder so far no work has been reported for the above mentioned activities. Already this plant has been reported for the presence of flavone glycosides. Hence it is planned to evaluate the extract of this plant for in vitro antioxidant, nephroprotective activity, invitro calcium oxalate crystallization inhibition and also antibacterial activity against urinary tract bacterial pathogens.

Chapter-IV:

PHARMACOGNOSTICAL STUDIES

In this chapter an attempt has been made to fix certain pharmacognostical standards for the leaves of *Ixora pavetta* which includes the macroscopical, microscopical, quantitative microscopy and physical standards.

Section A

Systematic position, synonym, common name, vernacular name, geographical distribution , Habit and habitat of the plant and morphological characters of leaves, flowers, fruits, seed, wood of this plant were described in detail in this chapter. Salient features of the macroscopy of leaves were observed such as leaves bears ovate oblong shape, coriaceous surface, obtuse apex and cuneate base.

Section B

The following salient anatomical features were observed in the transverse section of the leaves;

- Paracytic stomata are present in abaxial epidermis whereas the abaxial epidermis is apostomatic.
- Ground tissue consists of parenchyma cells and many of them containing Tannin in these cells.
- The vascular system consist of wide outer cylinder and narrow inner cylinder.The xylem part of the cylinder consist of outer thin zone of fibres and inner short radial parallel rows of xylem elements which is encircled by phloem.
- Calcium oxalate crystals are present as druses in the palisade and spongy mesophyll tissues and also in idioblasts.

- T.S of petiole exihibited prominent epidermal layer ,parenchymatous ground tissueand a closed hollow vascular cylinder.
- Tannin containing parenchyma cells are present in the central core .
- Crystals present in the petiole is prismatic type which are located in the phloem parenchyma.

Section c

Powder microscopy of the leaves showed the following anatomical characters.

- Small pieces of epidermis with paracytic stomata.
- Prismatic and druses of calcium oxalate crystals.
- Lignified spiral xylem vessels and fibres
- Parenchyma cells consist of tannins.

Section D

This part of the study has dealt about the quantitative microcopy such as stomatal number, stomatal index, vein islet number and vein termination number were determined and the results were tabulated.

Parameters	Values
Stomatal number in lower epidermis	31 ±1.32
Stomatal index in lower epidermis	16± 0.57
Vein islet number	3± 0.44
Vein termination number	12 ±1.27

Section E

Physical parameters like profile of ash values, loss on drying and various extractive values have been determined for this plant and the results are as follows.

Physical parameters of *I.Pavetta*

S. No	Parameters*	Values* expressed as %
1	**Volatile oil**	Nil
2	**Foreign organic matter**	0.01 ± 0.12
3	**Moisture content**	5.83 ± 0.01
4	**Ash values**	
	Total ash	7.39 ± 0.42
	Acid insoluble ash	1.09 ± 0.17
	Water soluble ash	5.09 ± 0.90
	Water insoluble ash	2.29 ± 0.90
5	**Extractive Values**	
	Petroleum extract	0.58 ± 0.03
	Ether extract	4.92± 0.02
	Chloroform extract	1.22± 0.04
	Ethanol extract	2.12 ±0.03
	75 % Ethanol extract	6.81 ±0.03
	Methanol extract	5.25 ± 0.01
	Aqueous extract	4.8± 0.03
	Hexane extract	2.26 ± 0.01
	Acetone Extract	2.18 ± 0.01

*** mean of three readings**

Highest extractive value was noted in 75%ethanolic extract (6.81%)and the lowest extractive value was observed in chloroform extract(1.22%).

Chapter V:

SectionA

This part of the study has dealt about the organoleptic evaluation of *Ixora pavetta* which indicates that the powder material is dark yellowish brown in colour, chacteristic odour and bitter followed by astringent taste.

Section B

Collection , authentication of the plant material and preliminary phytochemical study of the crude drug and various extracts were discussed in this chapter. This study indicates the presence of flavanoids, proteins, anthroquinone glycosides, tannins ,sterols and terpenoids. Ethanolic extract (75%) has shown the presence of most of the secondary and primary metabolites which are reported to possess various pharmacological activities . preparation of ethanolic extract (EIA) by triple maceration was discussed in this chapter.The EIA also shown highest extractive values when compare to other extracts.Hence this extract has been choosen for phytochemical and pharmacological studies.

Preparation of phenolicrich extract by fractionation of EIP by hexane, chloroform, ethyl acetate and butanol were discussed in this chapter.

Section C

EIP has been evaluated for total flavonoid content(24.08mg/g),total tannin content(269.05mg/g) and phenolic content (269.08mg/g).Above mentioned phenolic rich fractions were quantified for total phenolic content which revealed that chloroform

fraction showed highest phenolic content (329.6mg/g)when compared with hexane(216.2mg/g),ethyl acetate(321.7) and butanol (237.3).

This study indicate that both EIP and chloroform fractions were enriched with phenolic constituents. Hence these extracts were evaluated for phytochemical and pharmacological studies.

ESTIMATION OF TOTAL TANNIN AND FLAVONOID CONTENT OF *Ixora pavetta (EIP)*

CONCENTRATION (100µg/ml)	**TOTAL TANNIN CONTENT (mg/gm of extract)**	**TOTAL FLAVONOID CONTENT (mg/gm of extract)**
50	467.21±0.071	467.21±0.351
100	486.36±0.052	486.36 ±0.06

ESTIMATION OF TOTAL PHENOL CONTENT OF *Ixora pavetta (EIP &CIP)*

TEST		TOTAL PHENOL CONTENT
Sample	**Conc. in µg/ml**	**Mg of GAE/gm of extract**
Ethanol	50	277.75±1.60
	100	260.41±0.81
RICHFRACTIONS		
Hexane	100	216.2± 0.25
Ethyl acetate	100	321.7± 0.58
Chloroform.	100	329.6 ±0.45
n-Butanol	100	237.3 ±0.32

Section:D

TLC and HPTLC studies

TLC and HPTLC studies of both EIP and phenolic rich extract(CIP) have been dealt in this chapter. TLC study revealed that EIP exhibited three spots and chloroform fraction of phenolic rich extract(CIP) exhibited five spots. Both ethyl acetate and hexane fraction of phenolic rich extract have shown two spots each and butanol fraction of phenolic rich extract has shown only one spot.

HPTLC chromatogram was recorded for both EIP and CIP .This study revealed that EIP exhibited six spots and CIP shown seven peaks at 254nm.At 366nm

EIP has shown eight spots and CIP exhibited ten peaks. This study also revealed that about eight phytoconstituents were found in EIP and ten phytoconstituents were found in CIP.

Chapter-VI

Section A

Invitro radical scavenging activity was determined for both EIP and CIP by DPPH assay method. The percentage inhibition of EIP (16 µg/mL) and CIP (8 µg/mL) were found to be 64.69% and 89.36% when compared to the standard ascorbic acid (80 µg/mL). IC_{50} value of EIP and CIP were found to be 43.14 µg/mL and 58.71 µg/mL respectively when compared to standard ascorbic acid 45.51 µg/mL. This study revealed that CIP has exhibited potent radical scavenging activity than the standard ascorbic acid. Nitric oxide scavenging activity of EIP (222.22 µg/mL) was found to be 78.25% of percentage inhibition when compared to ascorbic acid (222.22 µg/mL) 80.03 % . IC_{50} value of EIP was found to be 129.65 µg/mL when compared to standard ascorbic acid 84.65 µg/mL. This study indicates that the EIP has shown significant nitric oxide scavenging activity when compared to standard ascorbic acid.

The EIP has also been evaluated for percentage inhibition of hydrogen peroxide. EIP at 100 µg/mL has shown 76.8 % of percentage inhibition of hydrogen peroxide when compared to the standard ascorbic acid (100 µg/mL) 86.56%. IC_{50} value of EIP was found to be 59.65 µg/mL when compared to standard ascorbic acid 69.24 µg/mL. This study revealed that EIP has exhibited significant hydrogen peroxide scavenging activity activity when compared to standard ascorbic acid.

Section-B

Nephroprotective effect of EIP and CIP were discussed in this chapter. This study revealed that nephrotoxicity induced by ciplatin leads to proximal and distal tubular necrosis, predominantly in the corticomedullary region and intratubular stripe of the outer medulla.

Functional nephro toxicity indices such as blood urea nitrogen, serum creatinine, blood urea, serum uric acid, potassium and urinary sodium and protein were elevated in cisplatin treated animals when compared to control($P<0.001$). Serum protein, sodium, urinary potassium and creatinine clearance were also decreased significantly when compared to control ($P<0.001$).

At 50 mg/kg/p.o of EIP treatment ,functional nephro toxicity indices such as blood urea nitrogen, serum creatinine, blood urea, serum uric acid, and urinary sodium and protein were decreased when compared to cisplatin treated animals ($P<0.01$) and serum potassium was found to be decreased when compared to cisplatin treated animals ($P<0.05$). Serum protein, sodium, urinary potassium and creatinine clearance were elevated significantly when compared to cisplatin treated animals ($P<0.01$).

At100 mg/kg/p.o of EIP treatment, functional nephro toxicity indices such as blood urea nitrogen, serum creatinine, blood urea, serum uric acid, potassium and urinary sodium, protein were decreased when compared to cisplatin treated animals ($P<0.001$). Serum protein, sodium, urinary potassium and creatinine clearance were elevated significantly when compared to cisplatin treated animals ($P<0.001$).

At 10 mg/kg/p.o of CIP treatment, functional nephro toxicity indices such as blood urea nitrogen, serum creatinine, blood urea, serum uric acid, potassium and urinary sodium, protein were decreased when compared to cisplatin treated animals (P<0.001).

. Serum protein sodium, urinary potassium and creatinine clearance were elevated significantly when compared to cisplatin treated animals (P<0.001).

HISTOPATHOLOGICAL CHANGES:

In the control group animals showed normal tubular epithelial cells and glomeruli whereas cisplatin treated animals showed glomerular atrophy, infilteration of cells, tubular congestion. Rats treated with 50 mg/kg/p.o EIP showed regenerative changes in glomeruli and tubules. Rats treated with 100 mg/kg/p.o EIP and 10 mg/kg/p.o CIP showed normal renal parenchyma with no significant pathology.

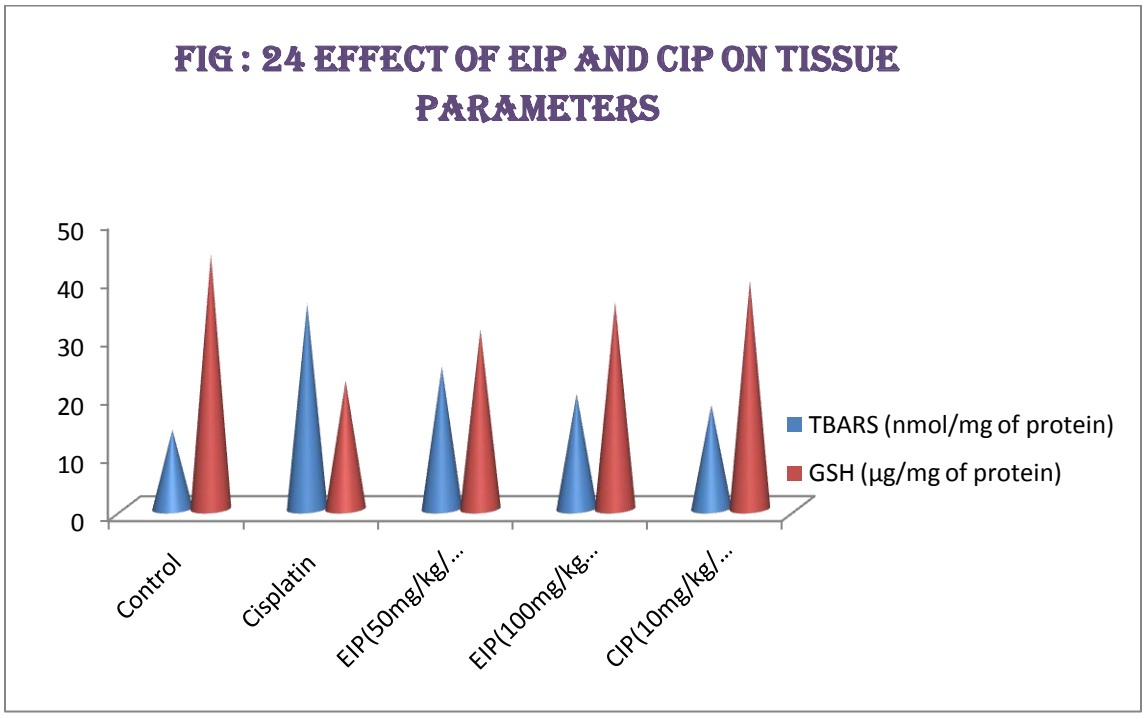


FIG : 25 EFFECT OF EIP AND CIP ON CREATININE CLEARANCE AND TOTAL PROTEIN

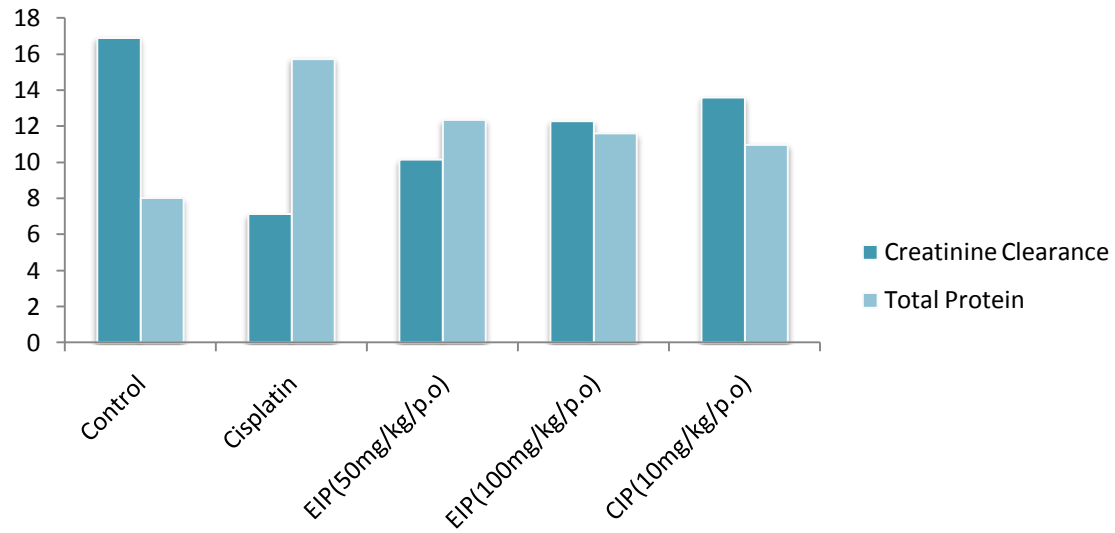


FIG : 26 EFFECT OF EIP AND CIP ON SODIUM AND POTASSIUM

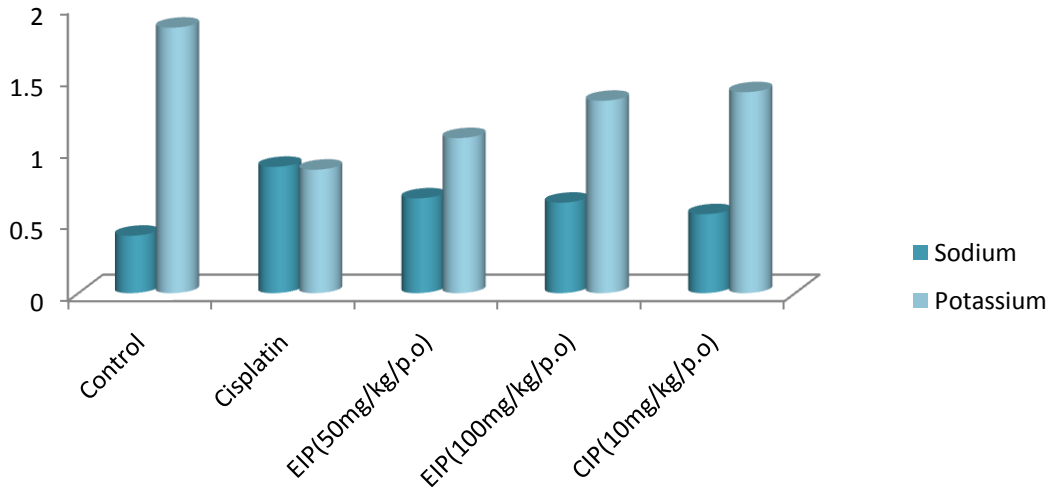


FIG : 27 EFFECT OF EIP AND CIP ON BUN AND UREA

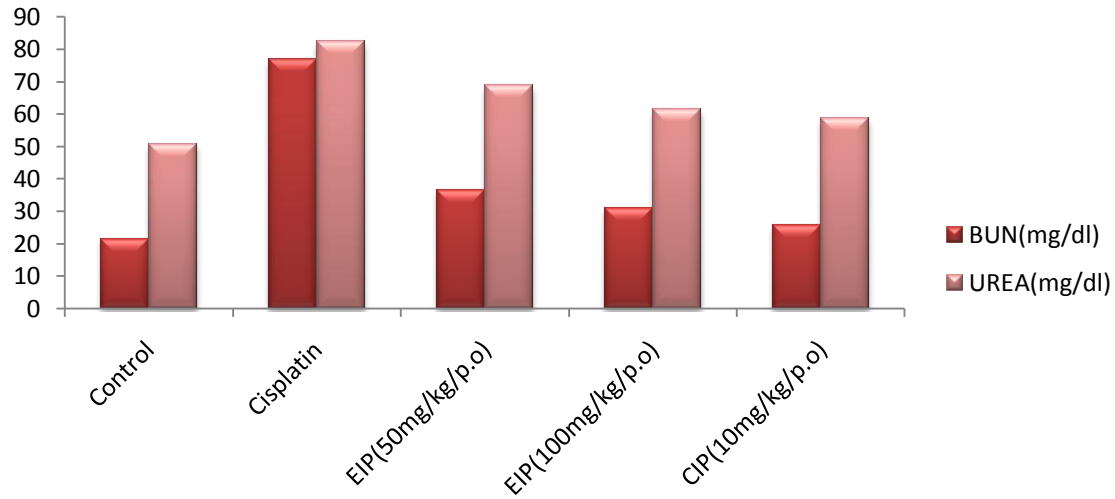
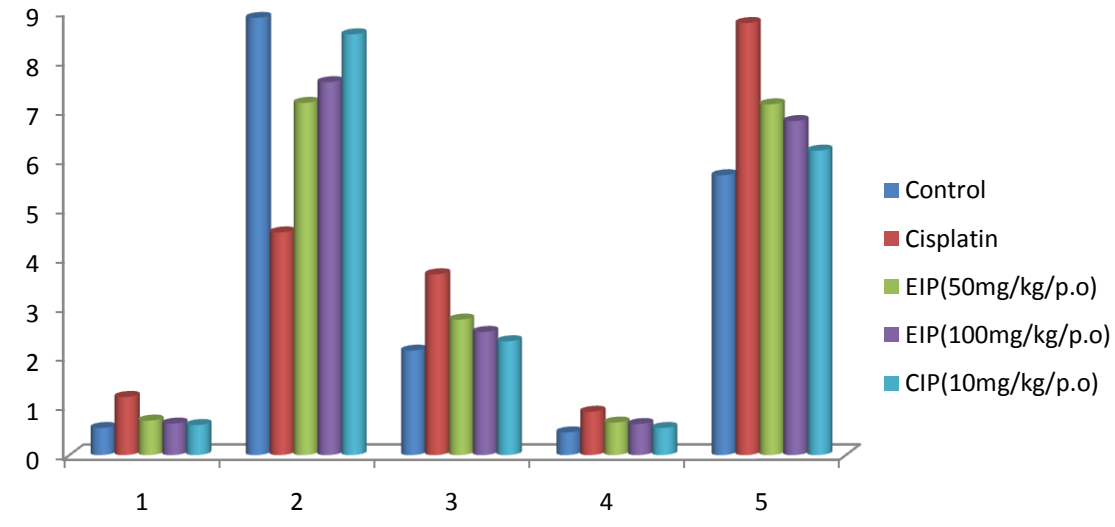


FIG :28 EFFECT OF EIP AND CIP ON CREATININE,TOTAL PROTEIN, URIC ACID,SODIUM AND POTASSIUM



Kidney plays a role in the maintenance of constant volume and composition of extracellular fluid by glomerular filtration, tubular reabsorption and tubular secretion. Kidney functions can be evaluated by different methods. Male rats are particularly suitable for evaluation of tubular lesions because the intra renal enzyme distribution is similar to that of man.

Cisplatin is a major anti neoplastic drug used in the treatment of solid tumours. Its chief dose limiting side effect is nephrotoxicity. 20% of patients develop renal dysfunction when treated with high dose of Cisplatin. Kidney accumulates Cisplatin to a greater degree than other organs and it is the major route for its secretion.

Cisplatin in proximal tubular epithelial cell is about five times of the serum concentration. The dispropotinate accumulation of cisplatin in kidney tissue contributes to cisplatin induced nephrotoxicity.In the rat cisplatin excretion occurs predominantly by glomerular filteration but very less amount by secretion. Cisplatin is accumulated by peritubular uptake in both proximal and distal nephrons.

The mechanism of cisplatin induced nephrotoxicity involved oxidative stress, apoptosis, inflammation and fibrogenesis. Highest concentration of cisplatin through necrosis of proximal tubules.Lowest concentration through apoptosis induced acute tubular injury.ROS are produced by Xanthine-Xanthine oxidase system, Mitochondria and NADPH oxidase in the cells. Cisplatin induced the production of ROS through all these pathway and produce renal injury because ROS directly act on cell components including lipids, proteins and DNA and destroy their structure.

Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity through which it produce increased free radicals and decreased anti-oxidant production. Mainly superoxide dismutase, GSH, catalase are significantly decreased. $O_2\cdot$(super oxide anion), H_2O_2 (Hydrrogen peroxide) and OH (Hydroxyl radical) damage the lipid components by peroxidation and denature of proteins. Since they are elevated in Cisplatin treated kidneys.

The results of this study showed that treatment with EIP and CIP exihibited invivo and invitro antioxidant acivity, this may be due to the presence of antioxidant phytoconstituents like phenolic compounds. EIP and CIP reduced the extent of Cisplatin induced nephrotoxicity is evidenced by the significant reduction in blood urea nitrogen, serum creatinine, blood urea, serum uric acid, potassium and urinary sodium and protein and elevate the level of Serum protein sodium, urinary potassium and creatinine clearance.

Morphological and physiological studies identified the renal tubule system as the site of maximum Cisplatin damage. Therefore protective effect of EIP and CIP would be apparent on the tubular system. The histological results revealed that glomerular atrophy, infilteration of cells and tubular congestion occur in Cisplatin treated rats. However EIP and CIP treatment ameliorates the overt changes induced by Cisplatin.

Preliminary phytochemical studies indicates the presence of phenolic constituents like tannins, flavonoids and anthraquinone glycosides. This plant has been reported for the presence of flavone glycosides chrysin. Estimation of phenolic constituents of EIP revealed the higher content of total flavonoids, total phenols and total tannins. CIP also showed the higher concentration of total phenols. Both EIP and CIP have exhibited

significant invitro anti-oxidant by DPPH, Hydrogen Peroxide and nitric oxide scavenging method. Invivo antioxidant action of EIP and CIP were corroborated by the in vivo antioxidant activity by elevation of endogenous antioxidant GSH and decreased the TBARS (LPO marker compound) in kidney tissue of both EIP and CIP treated animals.

Invitro antioxidant and nephroprotective activity may be due to the presence of phenolic constituents in these extracts. EIP and CIP were rich in flavonoids and tannins which have been ascribed for its strong antioxidant properties. It could possibly have a renoprotective effect via modulating the antioxidant system. These results revealed the beneficial effects of using free radical scavenger in modulating Cisplatin induced nephrotoxicity.

The mechanism by which EIP and CIP ameliorate Cisplatin toxicity remains to be elucidated. It may be due to inhibition of lipid peroxidation by scavenging free radicals and increasing intracellular concentration of Glutathione.

Section-C

This study was designed to evaluate the inhibiting potential of EIP and CIP on invitro calcium oxalate crystallization.

TURBIDIMETRIC STUDY:

The effect of EIP and CIP on various phases of calcium oxalate crystallization was determined by time course measurement of turbidity in the calcium chloride solution.

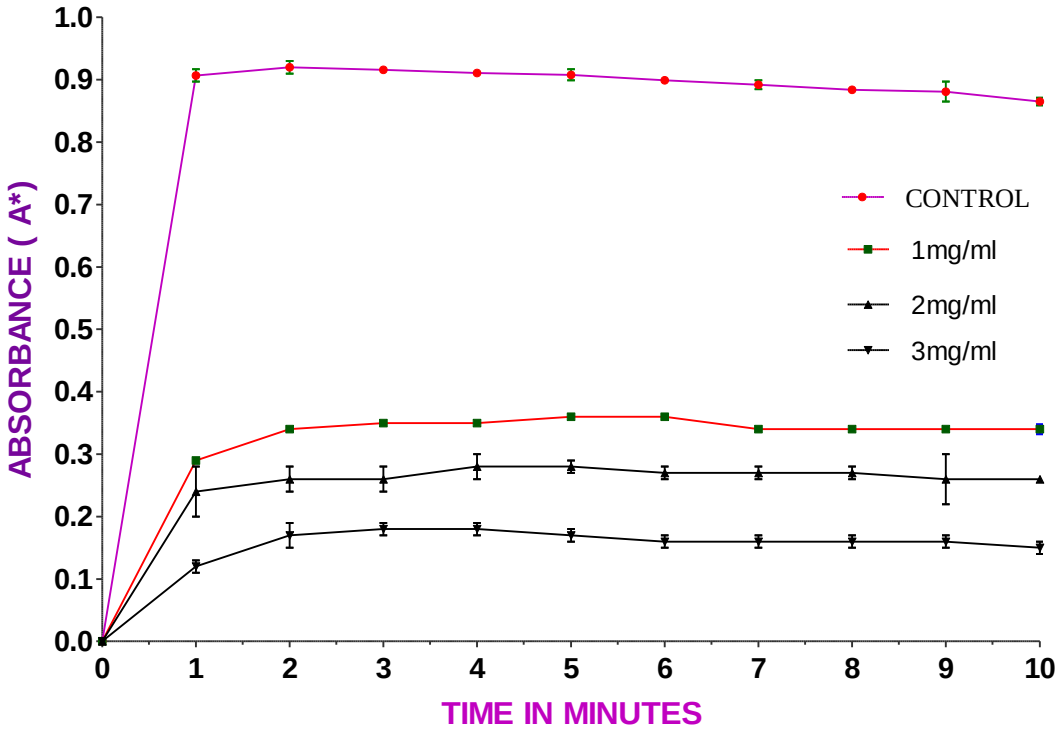
In this experiment, initial detectable increase in the turbidity after induction of the crystallisation with sodium oxalate was observed .In the control the initial steap rise in turbidity in the nucleation phase and attaining maximum, followed by a decrease in the aggregation.EIP and CIP inhibited the slope of turbidity in a concentration dependent

manner and followed by very slow decrease. Crystallisation by the addition of calcium chloride and sodiumoxalate was inhibited by EIP at 1mg/ml, 2mg/ml,3mg/ml were found to be 56.72%,83.22%,89.96%.similarly the CIP at the concentration of 100µg/ml,200 µg/ml and 300 µg/ml inhibit calcium oxalate crystallization at the percentage of 73.81%,85.24% and 88.48% respectively.

The concentration of inhibitor (EIP&CIP), turbidimetric slope relating to the curves of crystallization with and without inhibitor and percentage of inhibition were tabulated.

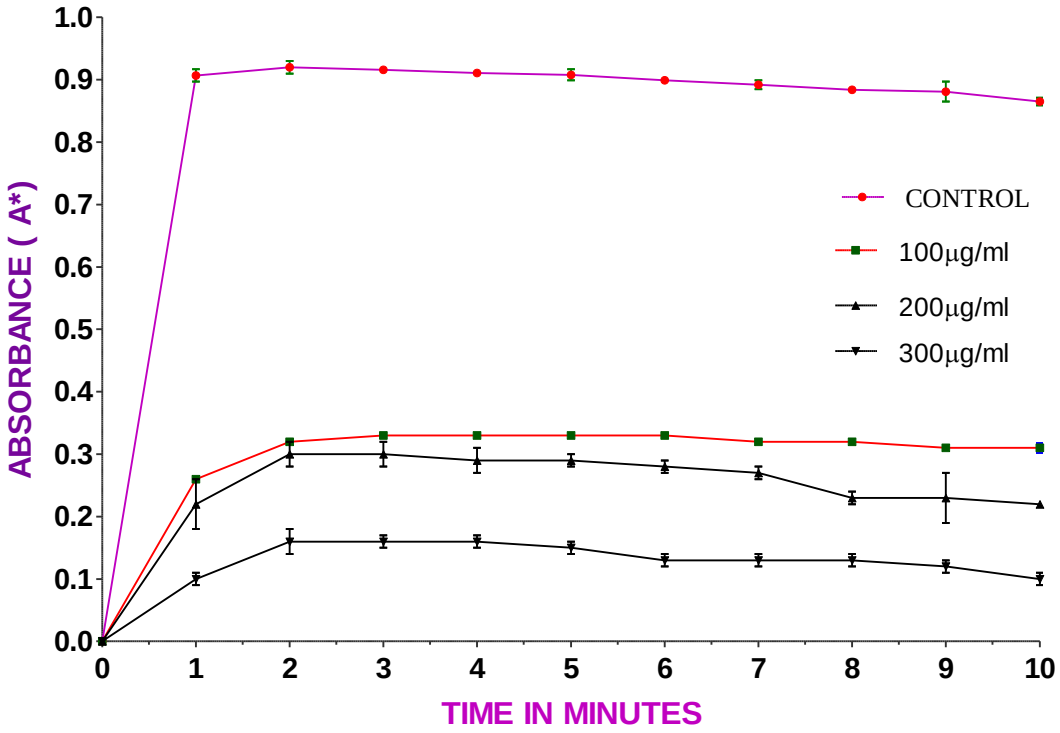
GRAPH : 1

VARIANCE OF ABSORBANCE ACCORDING TO TIME WITH INHIBITOR (EIP)



GRAPH : 2

VARIANCE OF ABSORBANCE ACCORDING TO TIME WITH INHIBITOR (CIP)



MIROSCOPIC STUDY

The photographs indicating the different stages of growth of CaOX crystals (t1&t2) with and without inhibitor were presented in figures and tables.

Crystals of calcium oxalate are primary constituent of more than 60% of the majority of human kidney stones. In vitro crystallization systems are used for variety of purposes in urolithiasis research. Since kidney stone formation is a complex process it includes super saturation, nucleation, growth, aggregation and retention within renal tubules. Initial events of nucleation of crystals occur in the first few minutes, the graphs were replotted within the first three minutes for each concentration of extract as well as control. The initial positive slope of the turbidity curve which is mainly due to increase in the particle number resulting from crystal nucleation, after a plateau is achieved in this negative slope(i.e) a progressive decrease of absorbance reflected from the decrease in the particle number due to the crystal aggregation.

In this study EIP and CIP extract inhibited the nucleation and aggregation of calcium oxalate in a concentration dependent manner. Physiological inhibitors of urolithiasis found in urine include inorganic (eg:magnesium, pyrophosphate) and organic citrate, uro epithelial glycoproteins, prothrombin fragment and glycosaminoglycons. Organic compounds adsorb to the surface of crystal and inhibit the crystal nucleation, growth and aggregation. To prevent the recurrent stone formation interference with crystal growth and aggregation are the possible therapeutic strategy.

The medicinal plants contain chemical compounds which possess an inhibitor effect in the crystallization of calcium oxalate. Calciumoxalate inhibitors prevent crystal growth and aggregation by coating the surface of the growing calcium crystals or by complexing

with calcium and oxalate. Chemical constituent like higher carboxylic acid like citrate chelates calcium and form soluble chelates and excrete through urine. Macromolecule of higher molecular weight of plant extract exerts their action similar to natural urinary inhibitors.

Several studies are carried out using microscope to validate the results obtained by the turbidimetric method. Microscopic observation revealed the extract visibly reduces the size of crystals with significant reduction of crystal number. Flavonoids, tannins, anthraquinone glycosides were present in EIP.EIP and CIP showed higher phenolic content and phenolic constituents have been reported for of urolithiasis. Hence the calcium oxalate crystallization inhibition of EIP and CIP may be due to the presence of phenolic compounds present in these extracts.

Section D

Ethanolic extract of *Ixora pavetta* and its chloroform fractions were used in the antibacterial studies by disc diffusion method against E.coli, Klebsiella spp, Pseudomonas aeruginosa and Proteus spp

Zone of inhibition of EIP at 6mg against Ecoli (20mm),Klebsiella pneumoniae(12mm),Proteus mirabilis (18mm) and Pseudomonas aeroginosa(14mm) showed that the extract has exihibited significant antibacterial activity against E.coli, Proteus mirabilis when compared to standard amikacin .

Zone of inhibition of CIP at 300µg against Ecoli(21mm),Klebsiella pneumoniae(18mm),Proteus mirabilis (20mm) and Pseudomonas aeroginosa(22mm) indicate that the extract has exihibited significant antibacterial activity against all the bacterial pathogens when compared to standard amikacin.

Both these extracts have shown significant antibacterial activity against E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabilis. But the ethanolic extract was less significant when compared the effect with its chloroform fraction. E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabilis are the chief causative organisms to produce urinary tract infection. Since the EIP and CIP shown significant antibacterial activity and it could be used for the treatment of urinary tract infections.Phenolic compounds present in both EIP and CIP may be responsible for the antibacterial activity of these extracts.

CHAPTER-VIII

CONCLUSION

Recently there has been a revival of interest on Medicinal plants. Large number of plants are constantly being screened for their pharmacological value particularly for their nephroprotectiveactivity,urolithiasis,antifertility,amoebicidal,antiparkinsonism,cytotoxic, hypoglycemic, hypotensive and anti inflammatory properties.

In this view an attempt was made to explore the plant *Ixora pavetta*.

This dissertation deals with pharmacognostical ,phytochemical evaluation and pharmacological screening of the leaves of *Ixora pavetta*.

- ❖ Pharmacognostic parameters had been determined for the leaves in order to substantiate and identify the plant for future work.
- ❖ Phytochemical evaluation of the plant including preliminary phytochemical screening was performed inorder to confirm the presence of phytoconstituents like sterols, carbohydrates, proteins, anthraquinone glycoside, flavonoids and tannins.

  Total flavanoid (24.08mg/g),total tannin(269.05mg/g) and total phenolic (269.08mg/g)contents were determined for the ethanolic extract (75%) of *Ixora pavetta* and phenolic rich extracts were prepared by means of fractionation which revealed that chloroform fraction born highest phenolic content(329.6mg/g) when compared to other fractions.TLC and HPTLC chromatogram have been recorded for both EIP and CIP.
- ❖ These extracts have also been evaluated for invitro antioxidant activity by DPPH assay, Nitric oxide scavenging and Hydrogen peroxide scavenging method.

- According to ethno medical information, leaves of this plant is used to treat kidney diseases, renal stones and urinary disorders. Inorder to confirm these ethnomedical claims both EIP and CIP have been evaluated for nephroprotective activity against Cisplatin induced nephrotoxicity in rats .This study indicate that both EIP(100mg/kg)($p<0.001$)and CIP(10mg/kg) ($p<0.001$)exert significant nephroprotective activity.Mode of action behind this activity may be due to the antioxidant potential of these extracts which is evidenced by elevation of GSH($p<0.001$) and reduction of TBARS ($p<0.001$) in the tissue parameters.
- These extracts have also exhibited inhibition of invitro calcium oxalate crystallization at 3mg/ml of EIP and 300µg/ml of CIP and phenolic compounds may be attributed for this activity.These extracts have also shown significant antibacterial activity against E.coli,Klebsiella pneumonia,Proteus mirabilis and Pseudomonas aeruginosa at 6mg of EIP and 300µg of CIP.
- Antibacterial activity may also be due to the presence of phenolic constituents present in these extracts. Hence it is suggested that phenolic phytoconstituents responsible for both invitro and invivo antioxidant activity, nephroprotective activity, invitro urolithiatic activity and antibacterial activity for UTI may be isolated and evaluated for further studies.

These phenolic phytoconstituents may serve as a lead molecule for the development of newer drugs to treat various life threatening diseases, such as renal failure, renal calculi, diseases caused by oxidative stress and urinary tract infection caused by multidrug resistant strains.

FIG 1.1 HABITAT OF IXORA PAVETTA

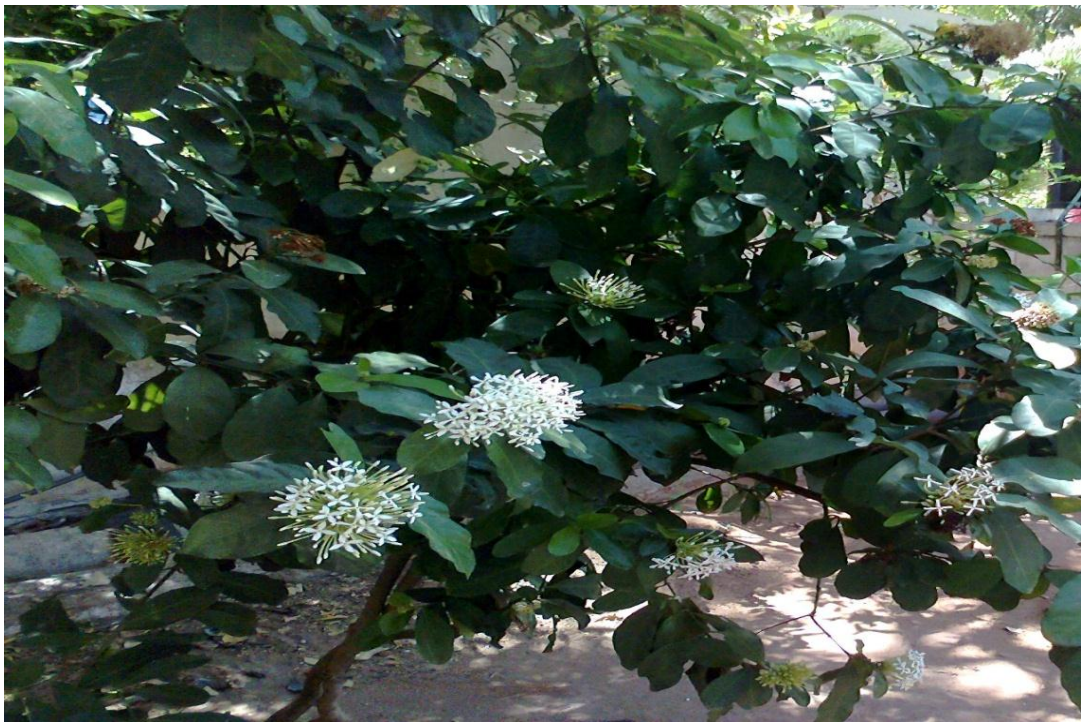


FIG 1.2 DORSAL VIEW AND VENTRAL

Fig. 2.1: T.S of LEAF THROUGH MIDRIB AND LAMINA

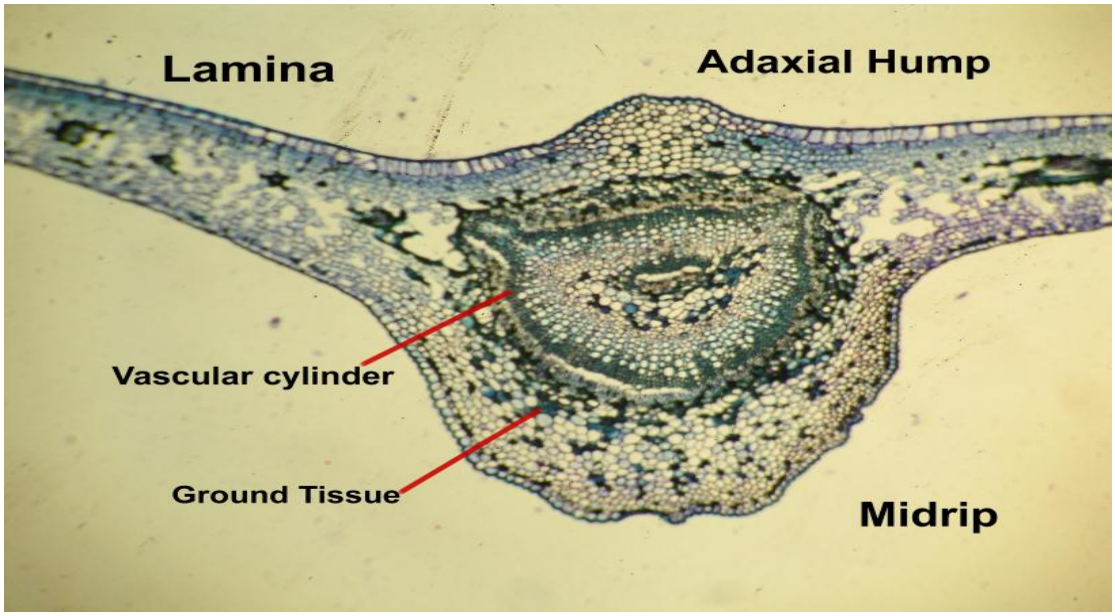


Fig. 2.2: MIDRIB SECTOR - ENLARGED

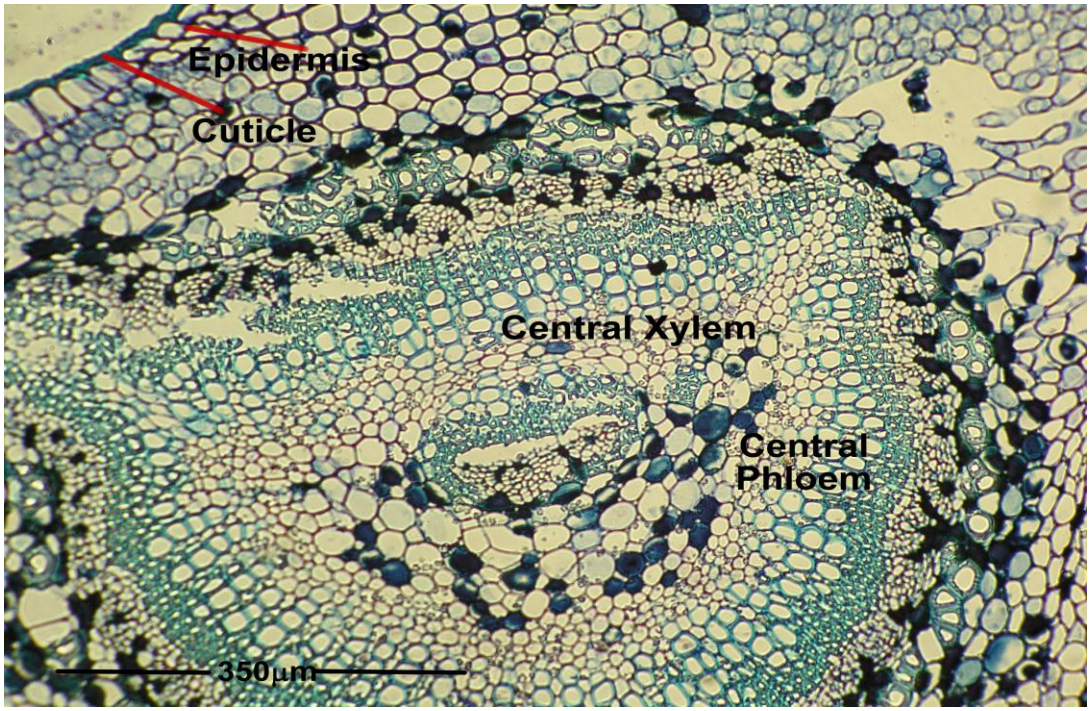


Fig.3.1: T.S OF LAMINA

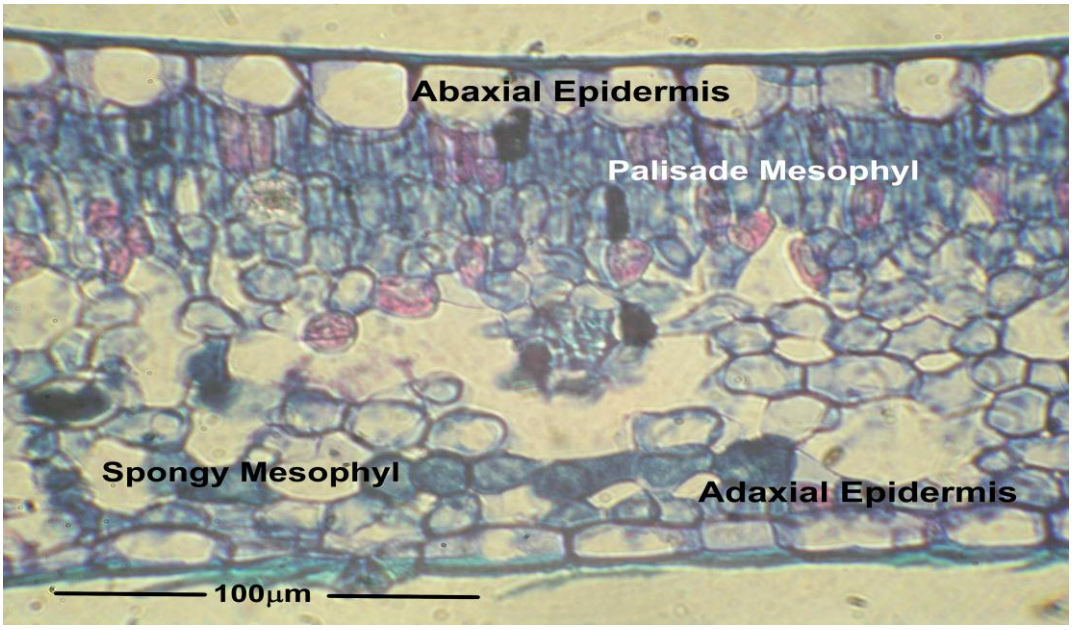


Fig.3.2: T.S OF LEAF MARGIN

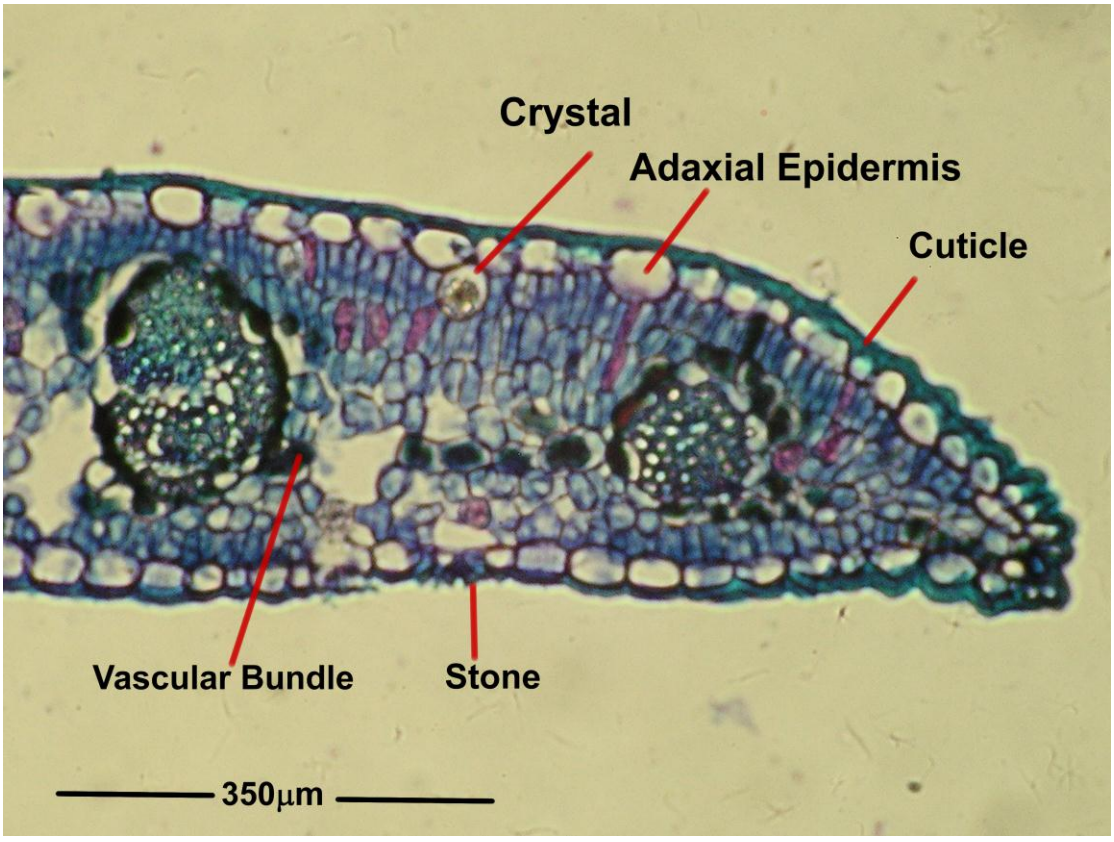


Fig.4.1: T.S OF PETIOLE

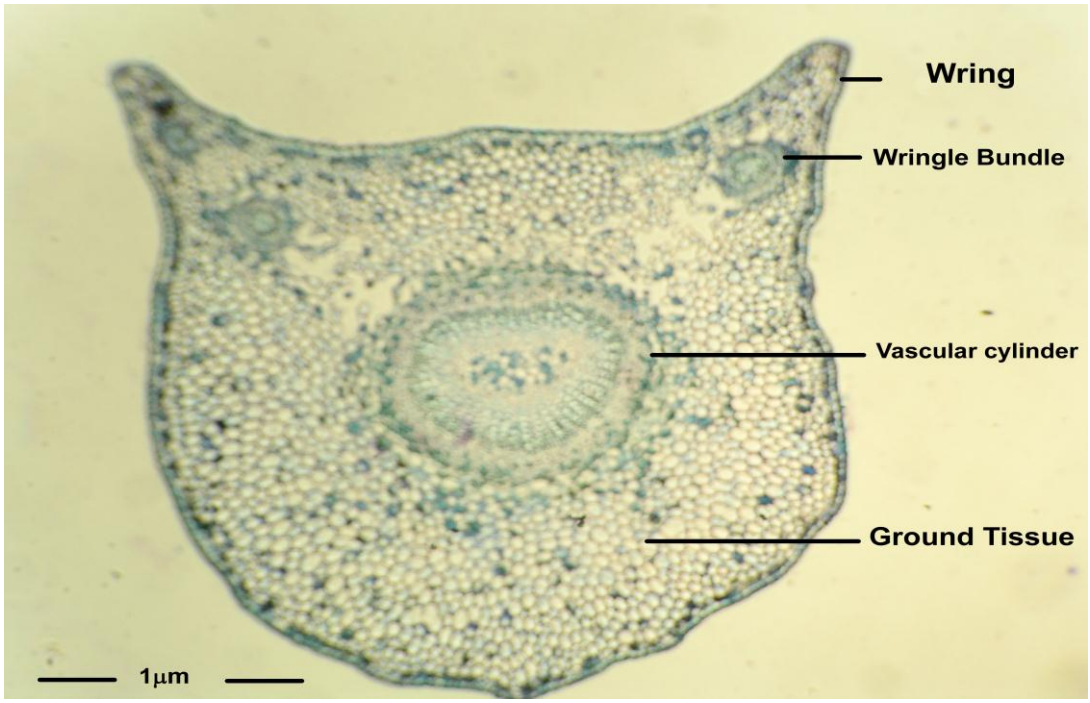


Fig.4.2: CENTRAL PORTION (VASCULAR CYLINDER) OF THE PETIOLE

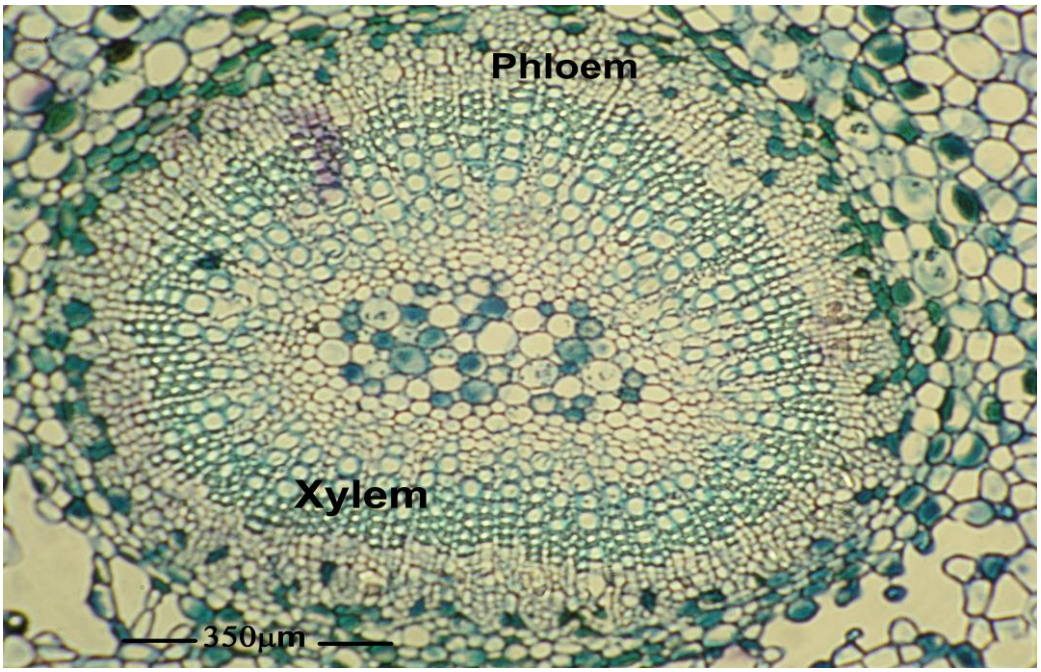


Fig.5.1: T.S OF PETIOLE WRING PORTION ENLARGED

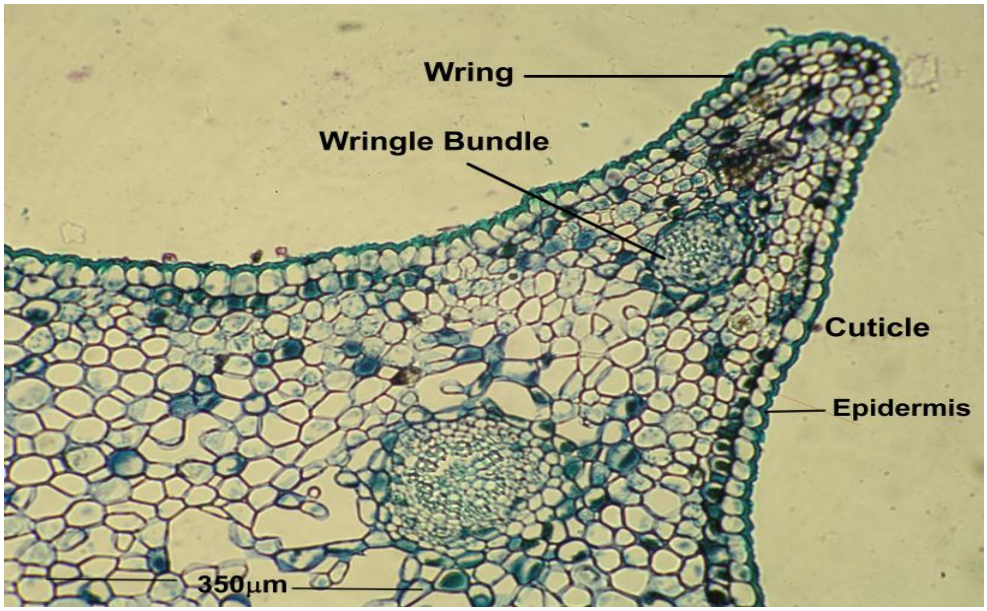


Fig.5.2: WRING VASCULAR BUNDLE ENLARGED

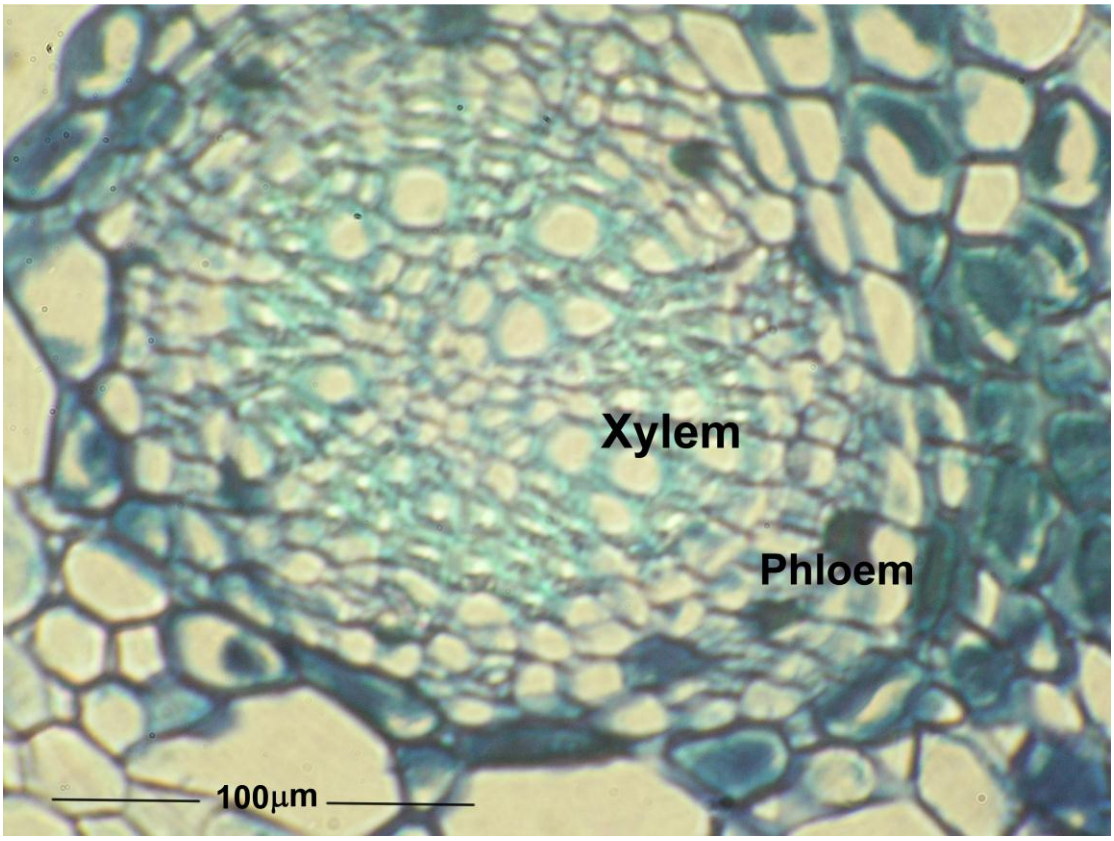


Fig.6.1: PRISMATIC CRYSTALS IN THE PETIOLE - PHLOEM

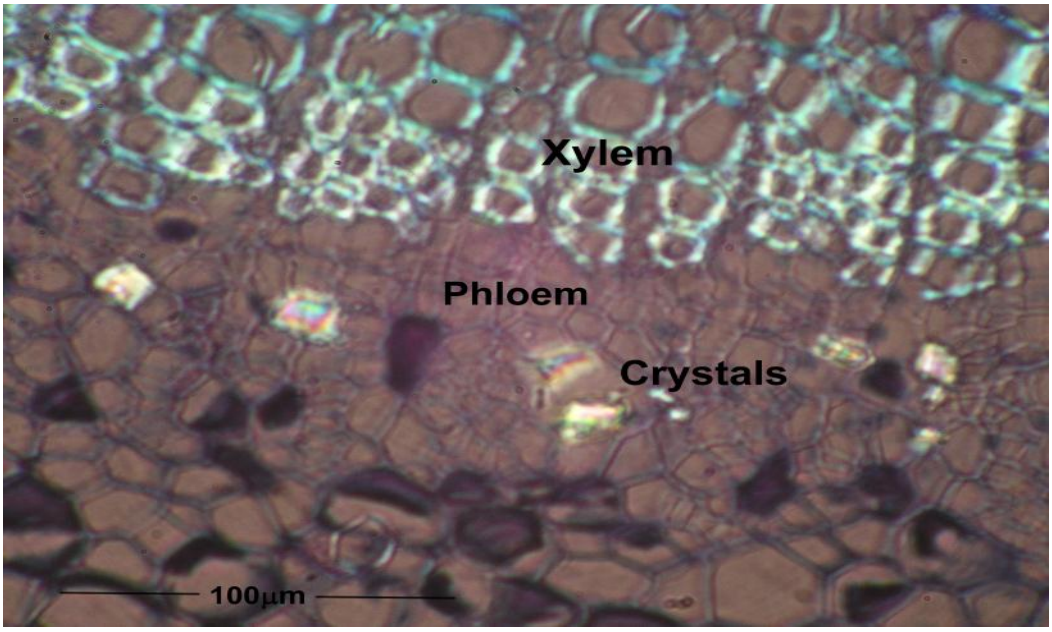


Fig.6.2: DRUSES WITH MESOPHYL OF THE LEAF

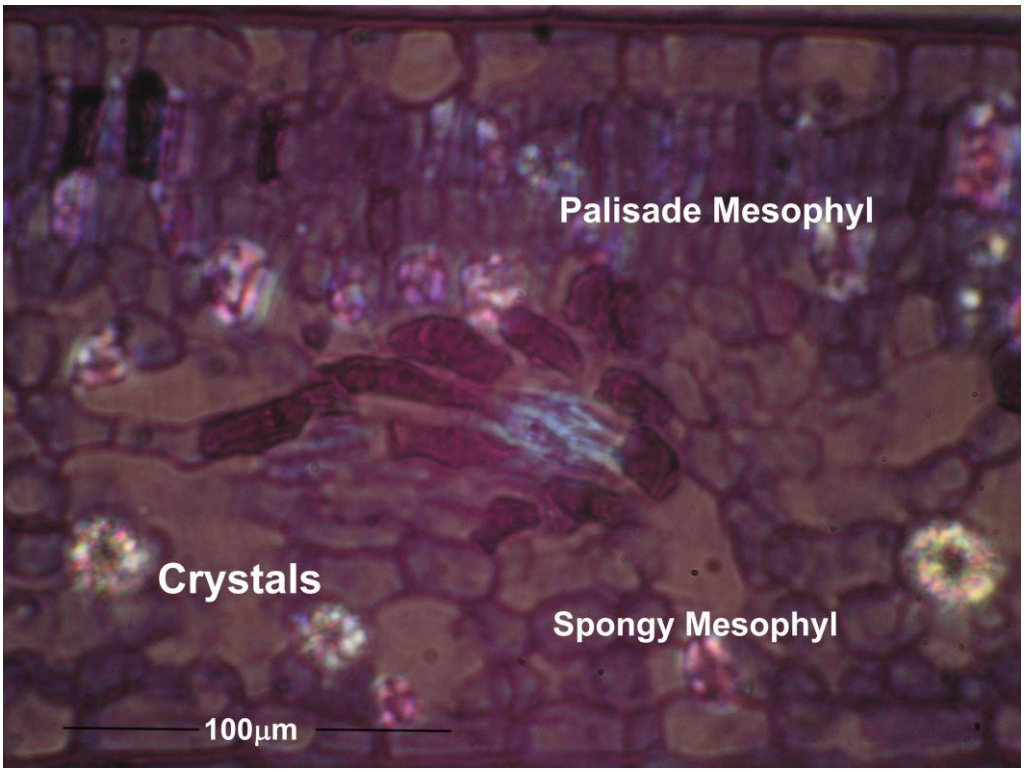


Fig.7.1: T.S OF LEAF SHOWING AN IDIOBLAST WITH DRUSE

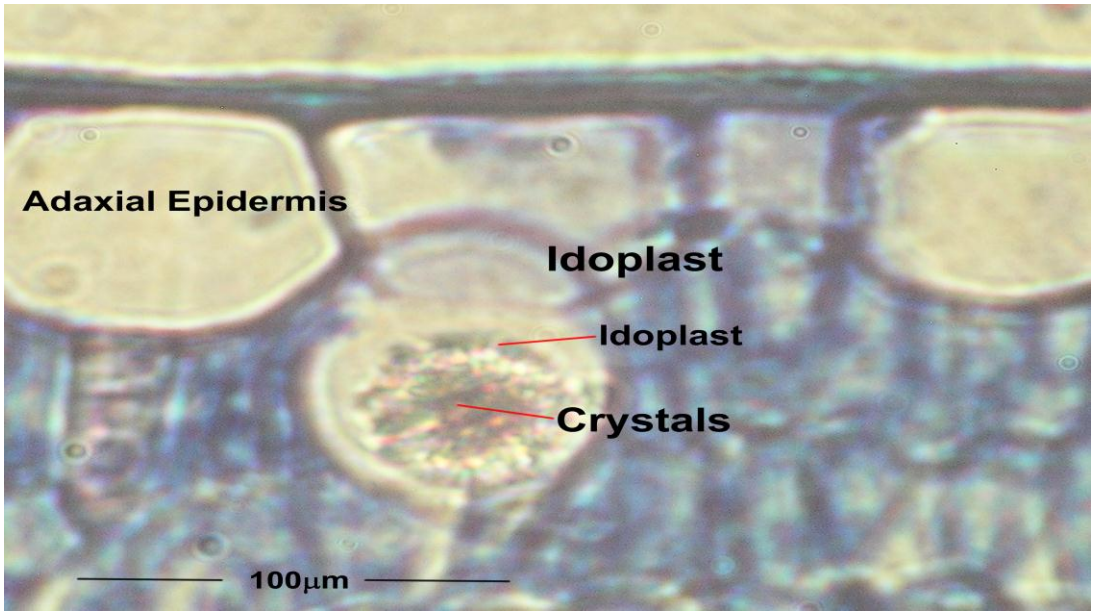


Fig.7.2: DRUSES IN THE SPONGY PARENCHYMA

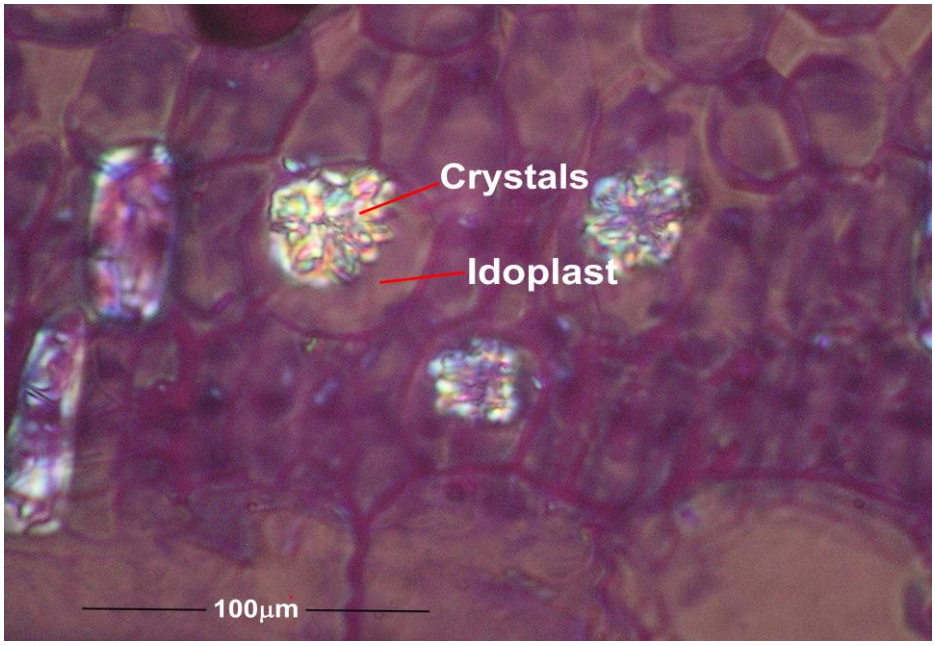


Fig.8.1: PAREDERMAL SECTIONAL VIEW OF STOMATA

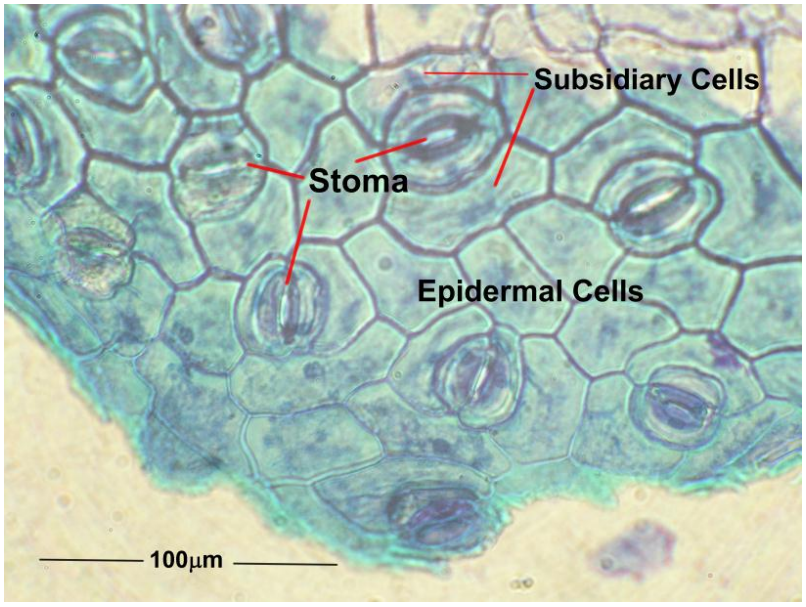


Fig.8.2: STOMATA ENLARGED

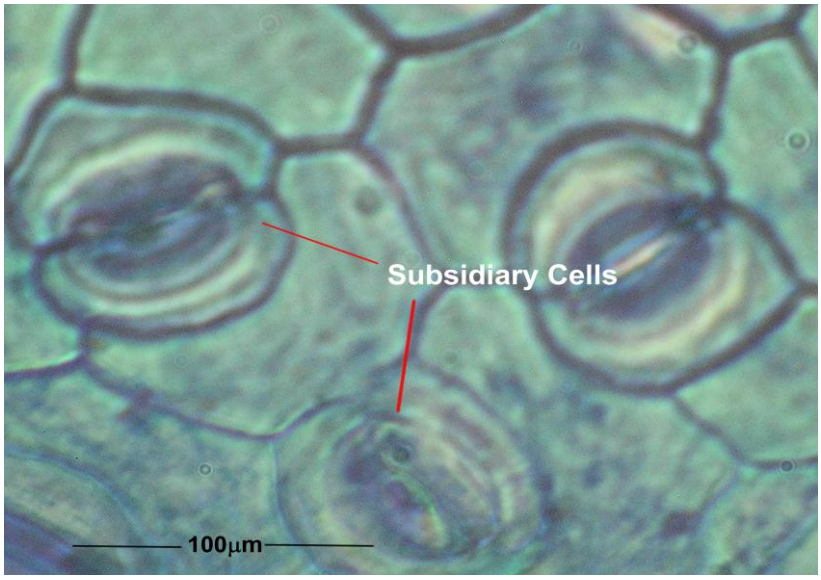
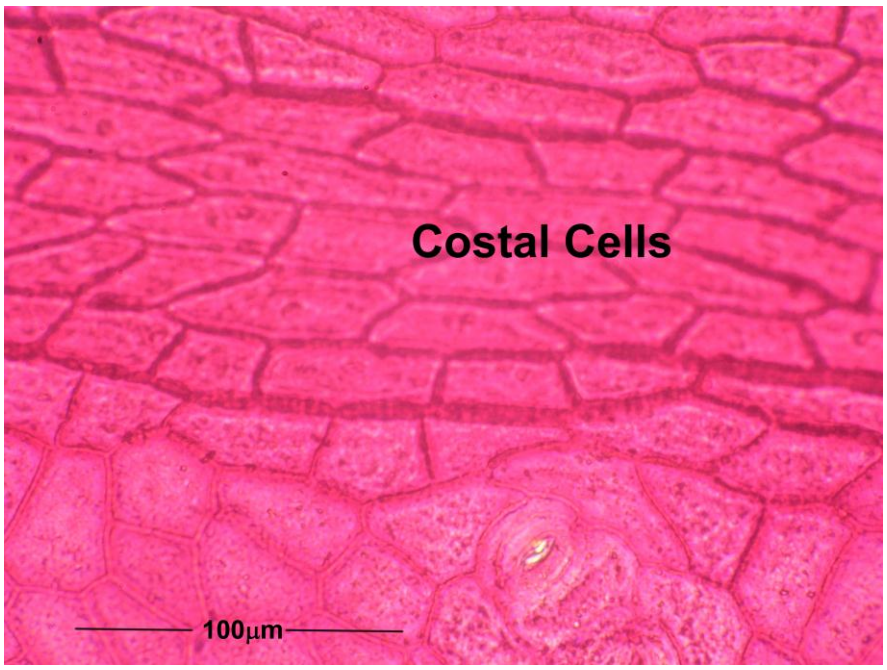


Fig.9.1: EPIDERMAL PEELINGS OF THE LEAF SHOWING COSTAL CELLS



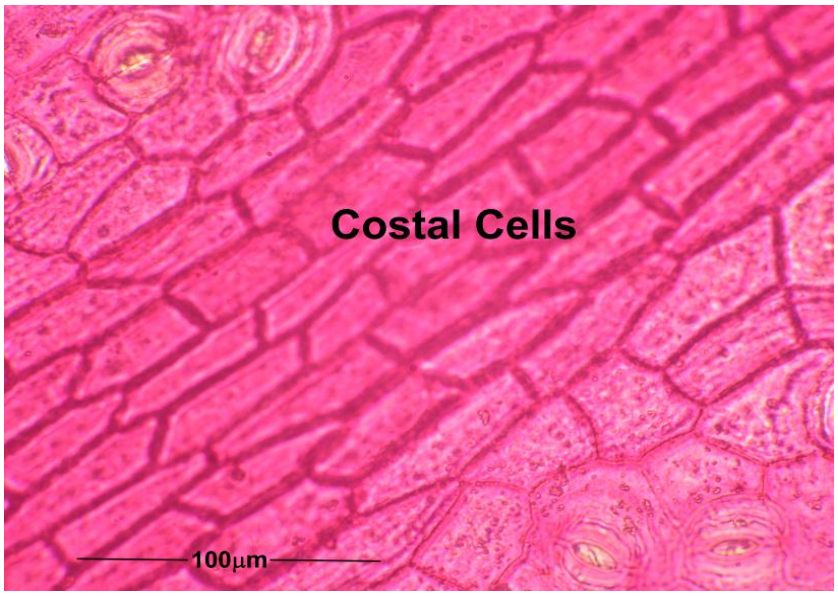


Fig.10.1: VENATION OF LAMINA

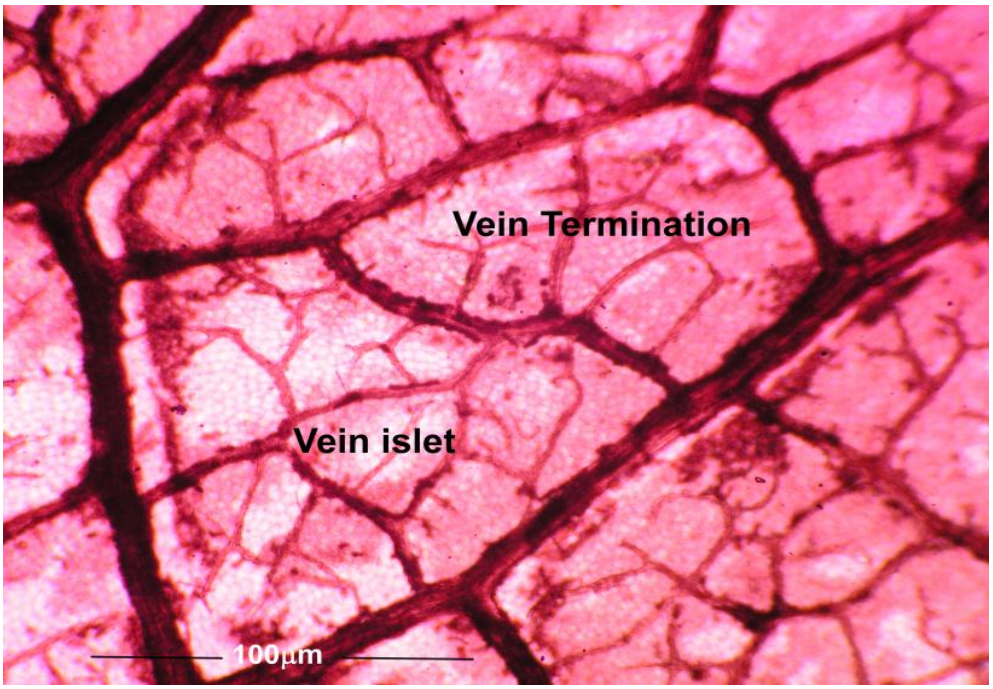


Fig.10.2: A VEIN TERMINATION ENLARGED

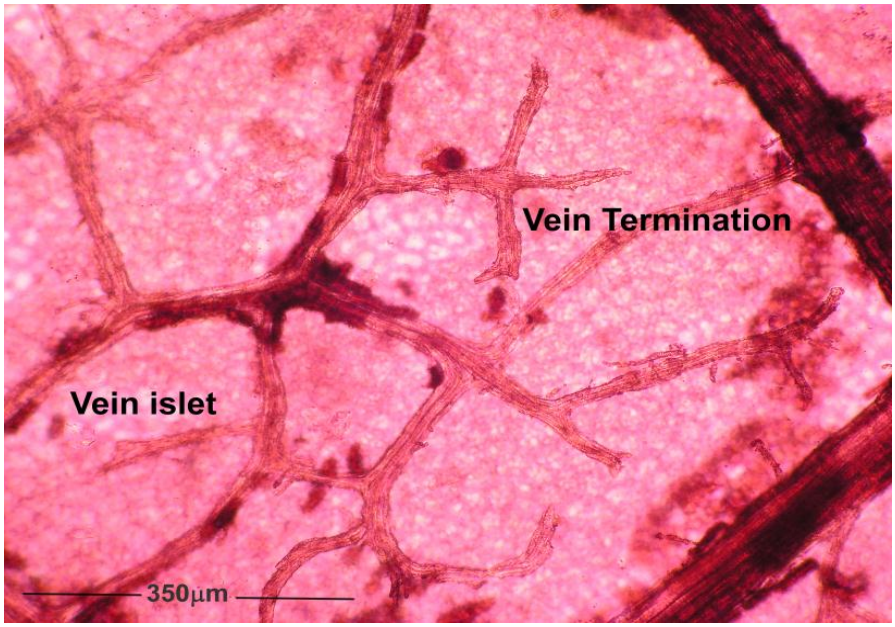


Fig.11.1: ADAXIAL EPIDERMIS

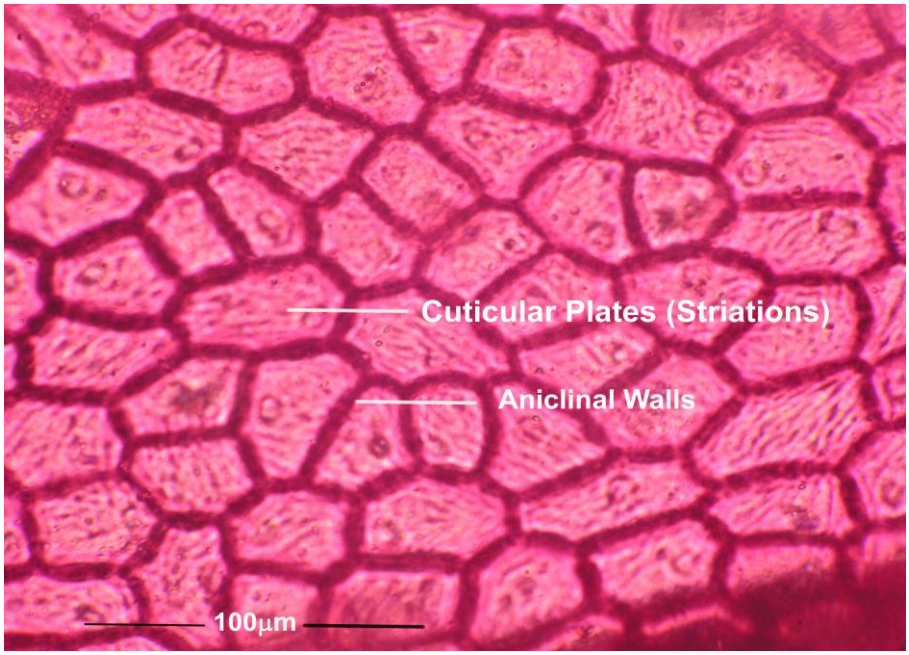


Fig.11.2: ABAXIAL CELLS WITH CUTICULAR PLATES

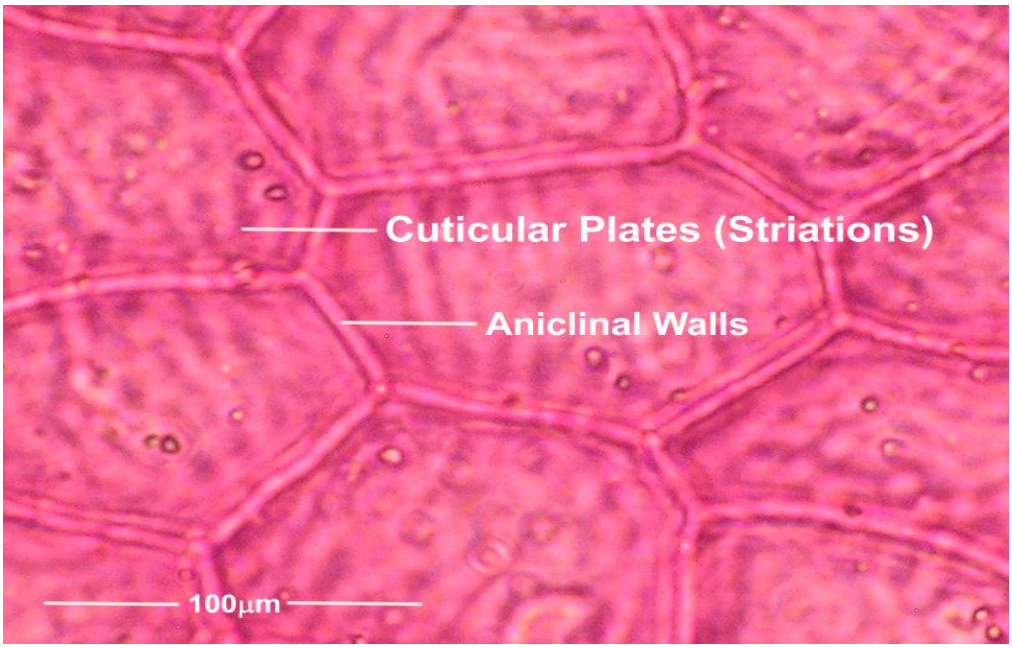


Fig.12.1: ADAXIAL EPIDERMAL CELLS AND STOMATA

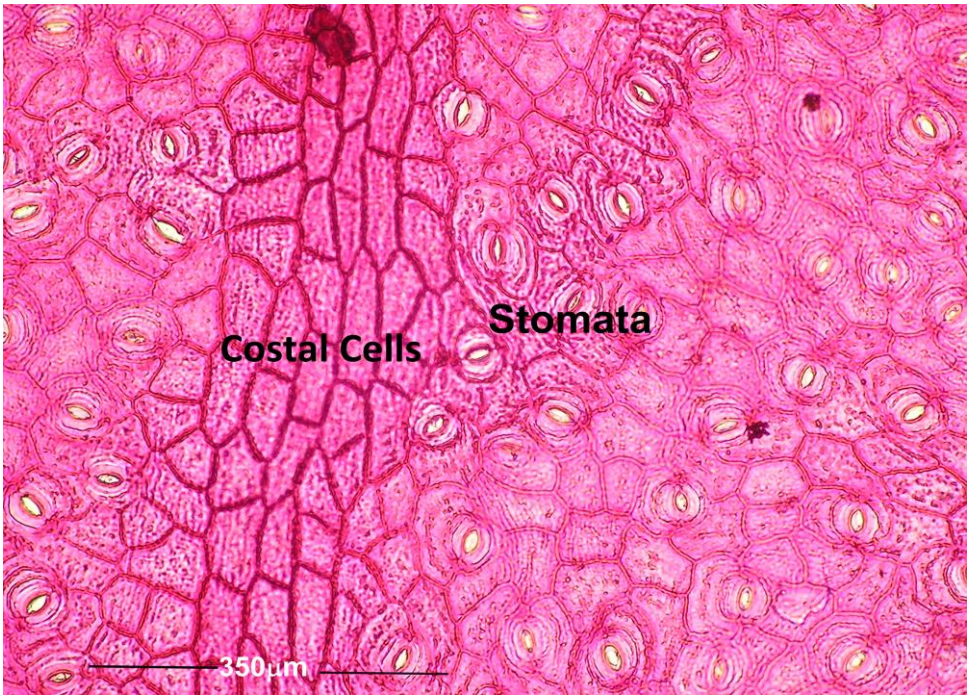


Fig.12.2: ABAXIAL EPIDERMAL CELLS WITH CUTICULAR STRIATIONS

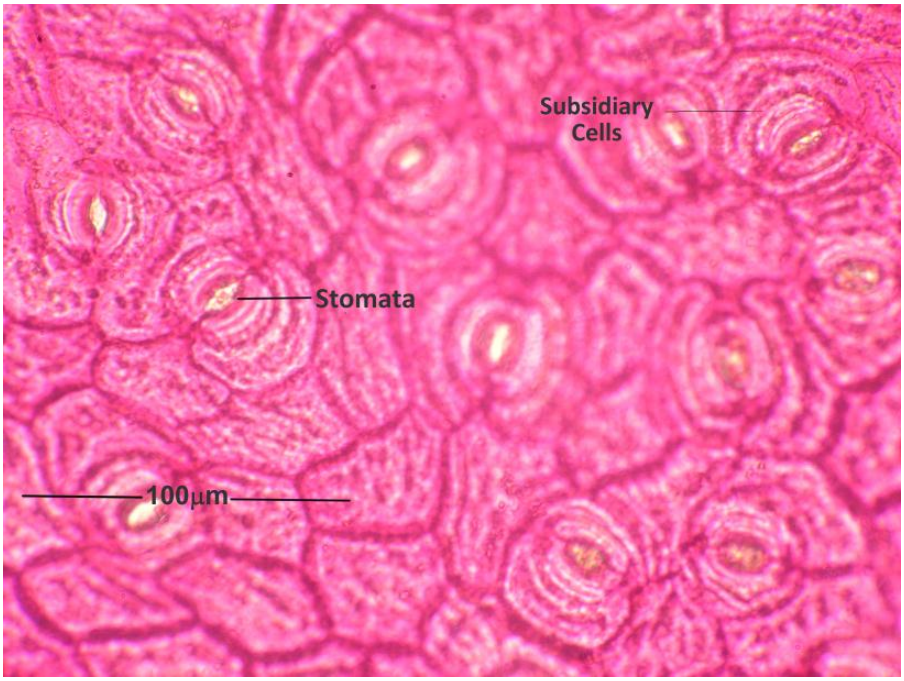
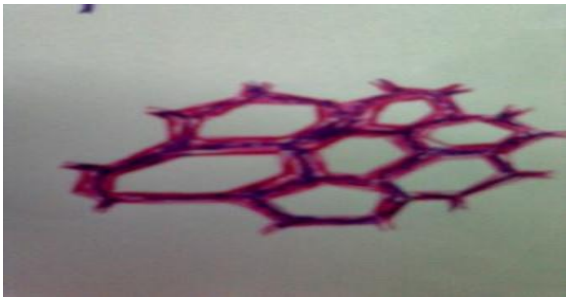
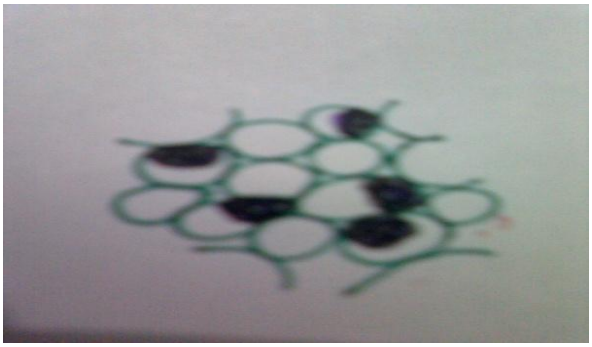


FIG:12.3 POWDER MICROSCOPY OF IXORA PAVETTA

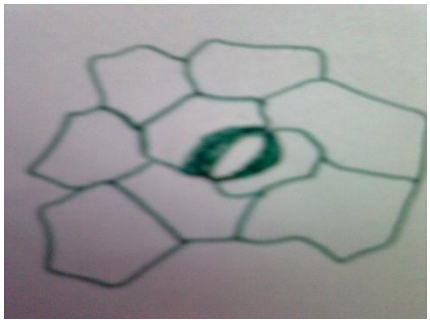
POLYGONAL EPIDERMAL CELLS WITH THICK ANTICLINAL WALLS



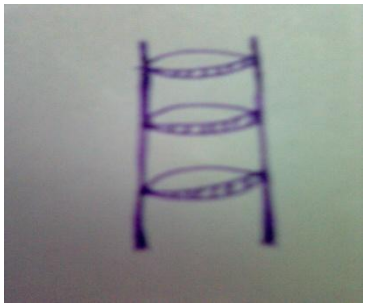
PARENCHYMA WITH TANNIN CONTAING CELLS



PARACYTIC STOMATA



SPIRAL XYLEM VESSELS



DRUSES AND PRISMATIC CALCIUM OXALATE CRYSTALS

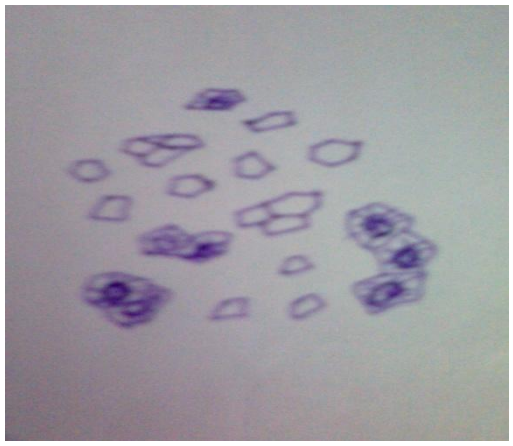
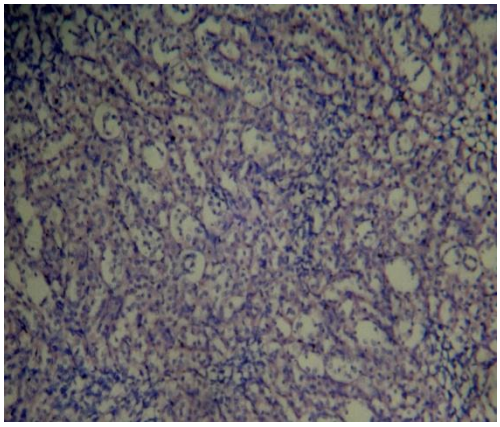
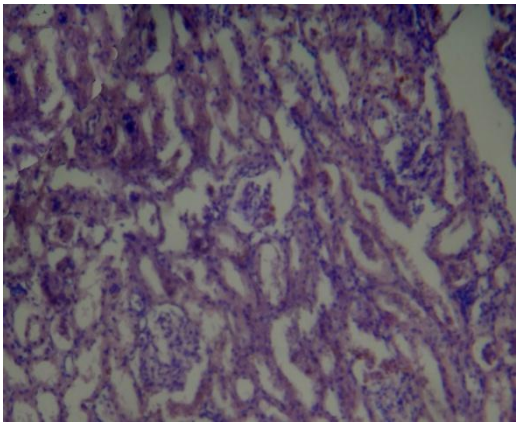


FIG: 22 EFFECT OF IXORA PAVETTA ON CISPLATIN INDUCED HISTOPATHOLOGICAL CHANGES

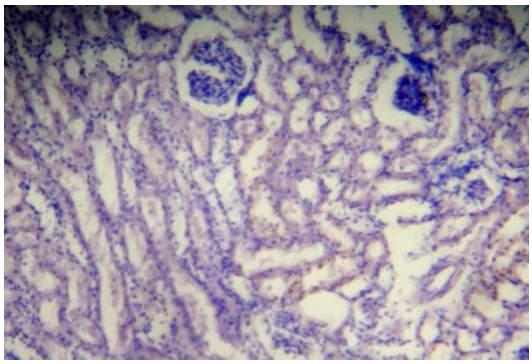
Control



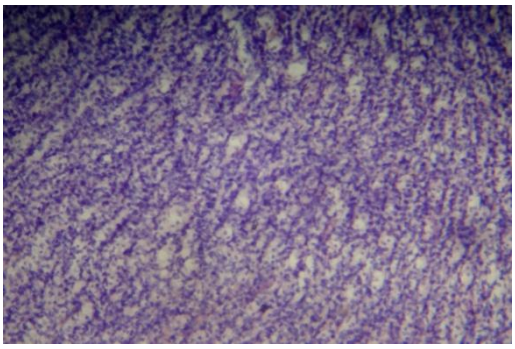
Cisplatin treated



50 mg/kg EIP



100 mg/kg EIP



10mg/kg CIP

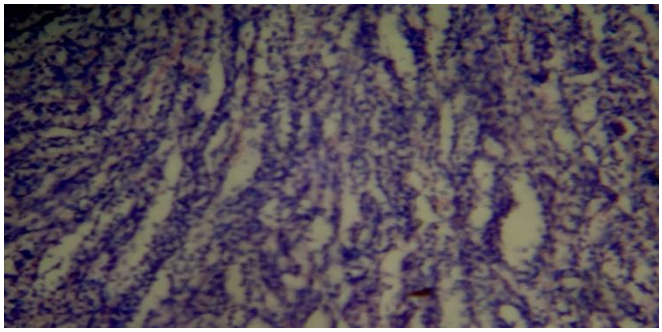
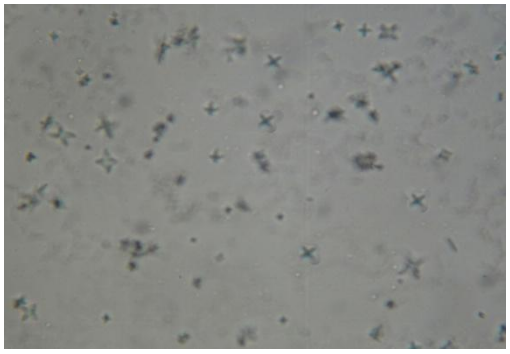
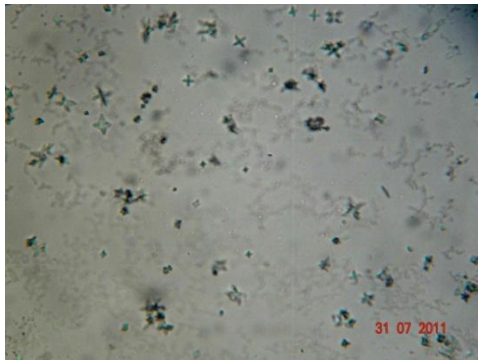


FIG: 23 PHOTOGRAPHS OF CRYSTALLIZATION FOR WITHOUT AND WITH INHIBITOR

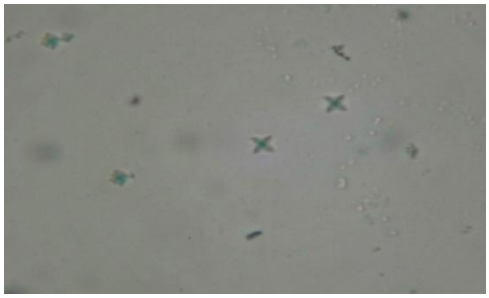
CONTROL (GROWTH)



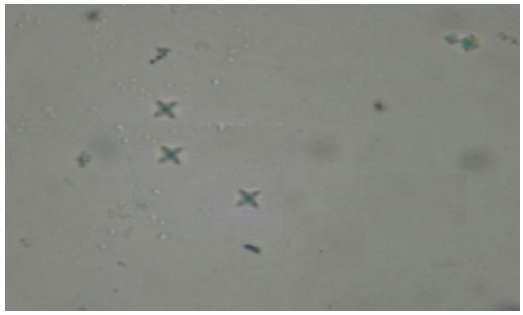
CONTROL (AGGREGATION)



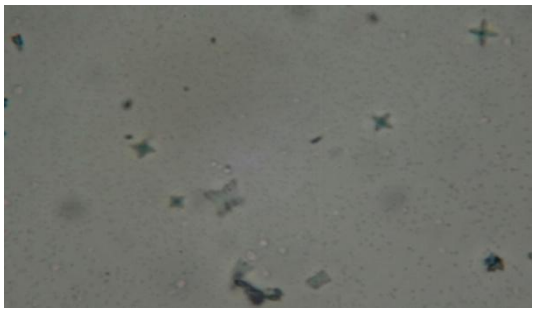
3MG/ML EIP (GROWTH)



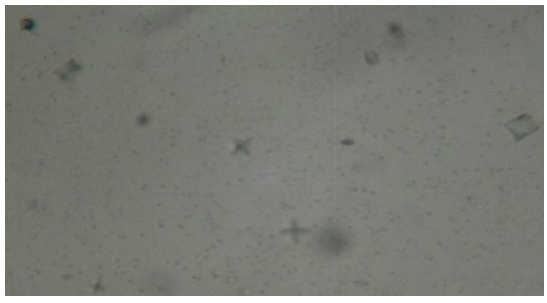
3MG/ML EIP (AGGREGATION)



300 µG/ML CIP (GROWTH)



300 µG/ML CIP (AGGREGATION)



IDENTIFICATION OF CALCIUM OXALATE CRYSTALS WITH 50 % SULPHURIC ACID

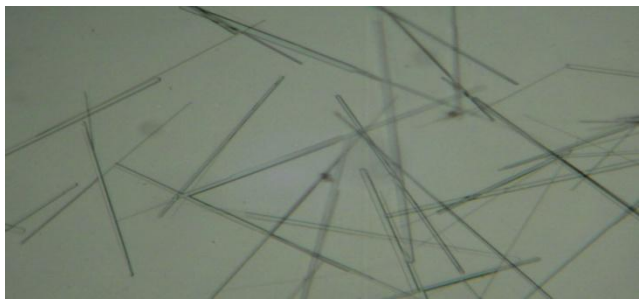


FIG: 29 Anti-Bacterial Activity of EIP against *Escherichia coli*

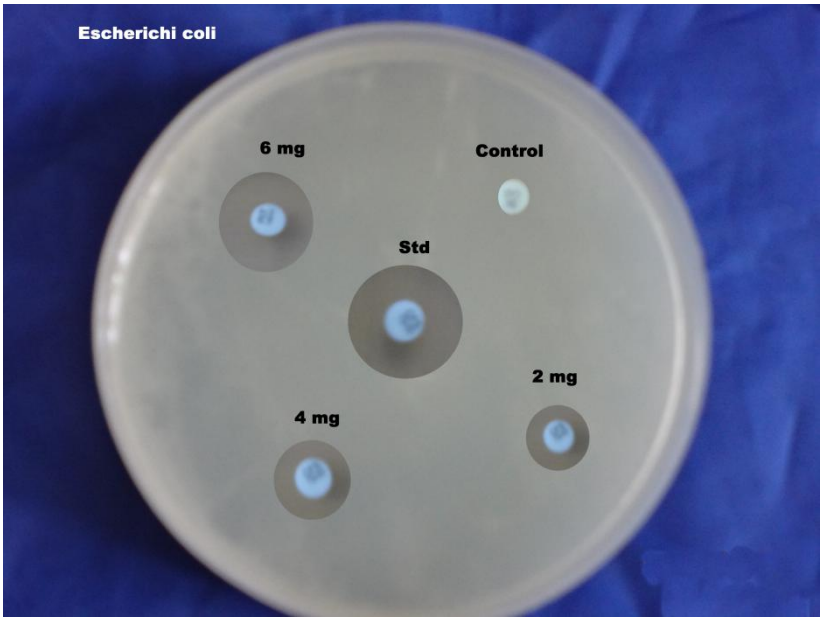


FIG30: Anti-Bacterial Activity of CIP against *Escherichia coli*

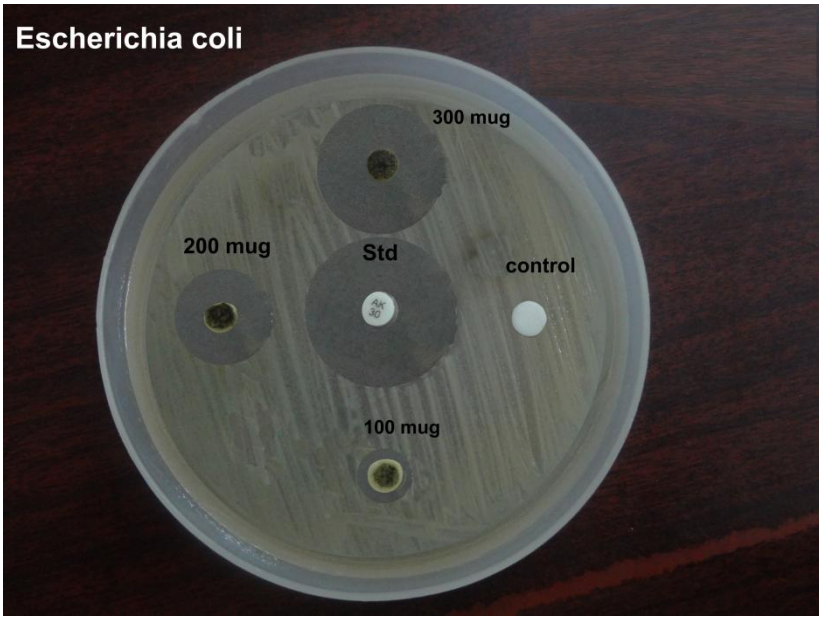


FIG:31 Anti-Bacterial Activity of EIP against *Klebsiella pneumoniae*

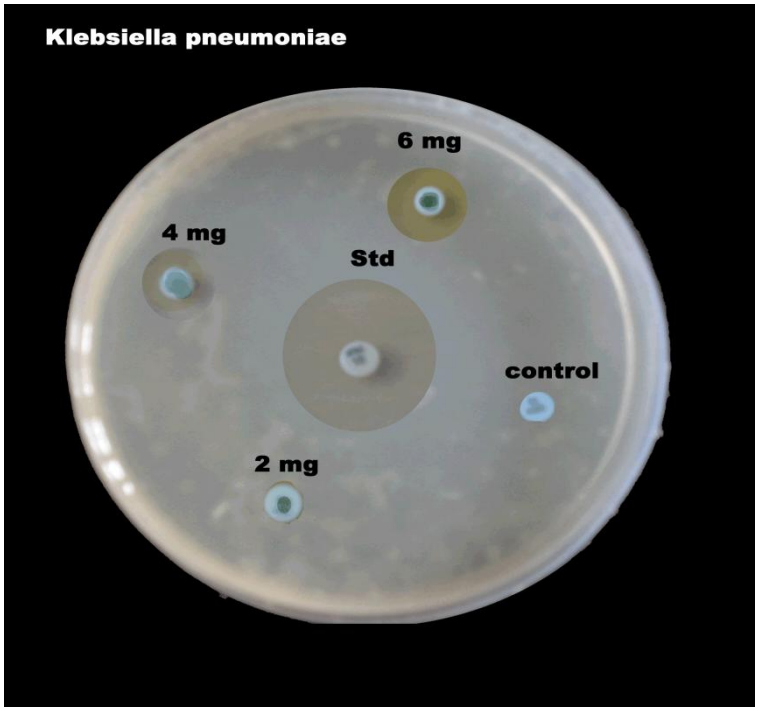


FIG: 32 Anti-Bacterial Activity of CIP against - *Klebsiella pneumoniae*

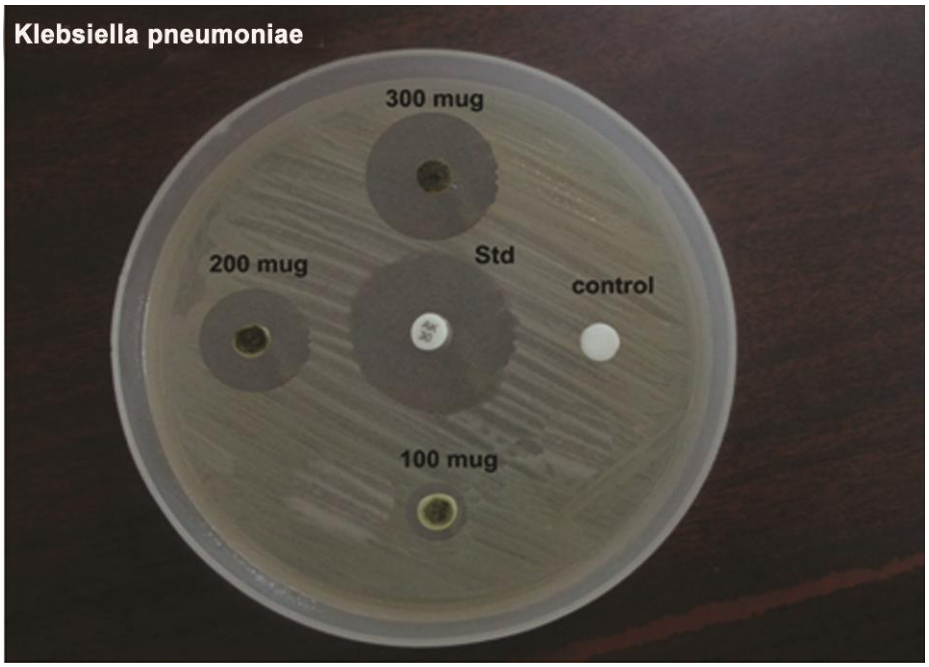


FIG: 33 Anti-Bacterial Activity of EIP against *Proteus mirabilis*

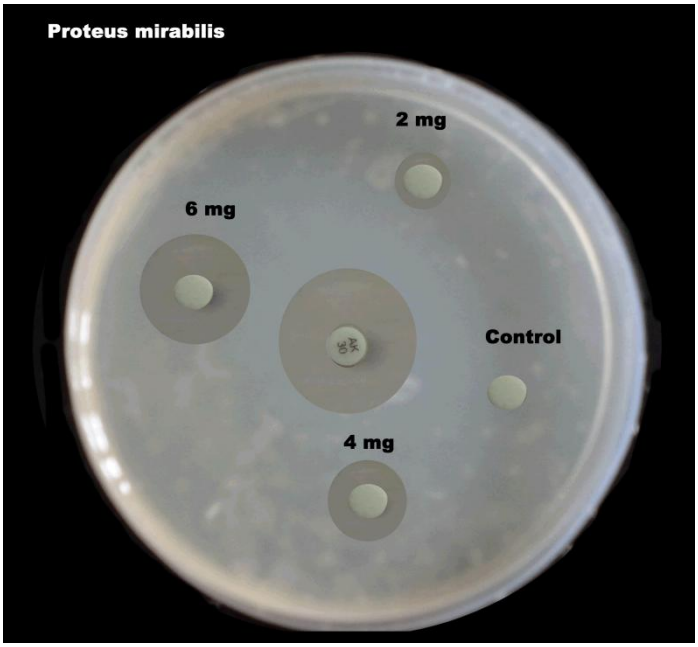


FIG: 34 Anti-Bacterial Activity of CIP against *Proteus mirabilis*

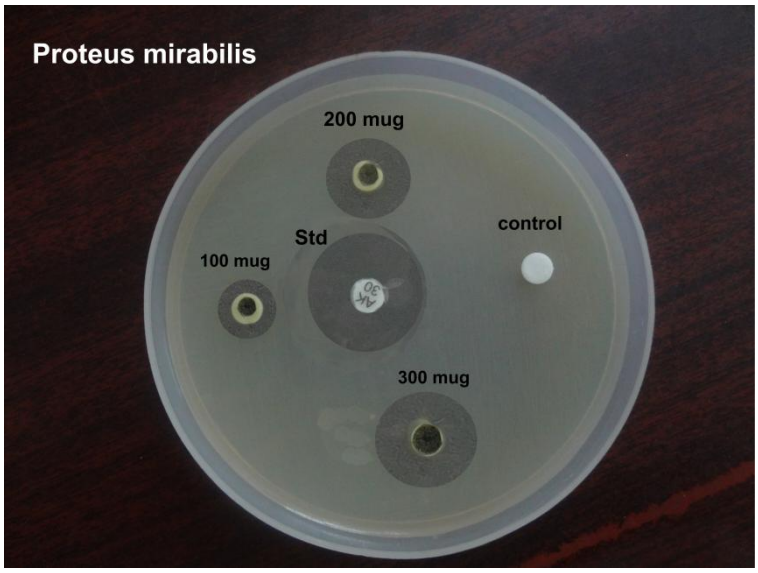


FIG: 35 Anti-Bacterial Activity of EIP against Pseudomonas aeroginosa

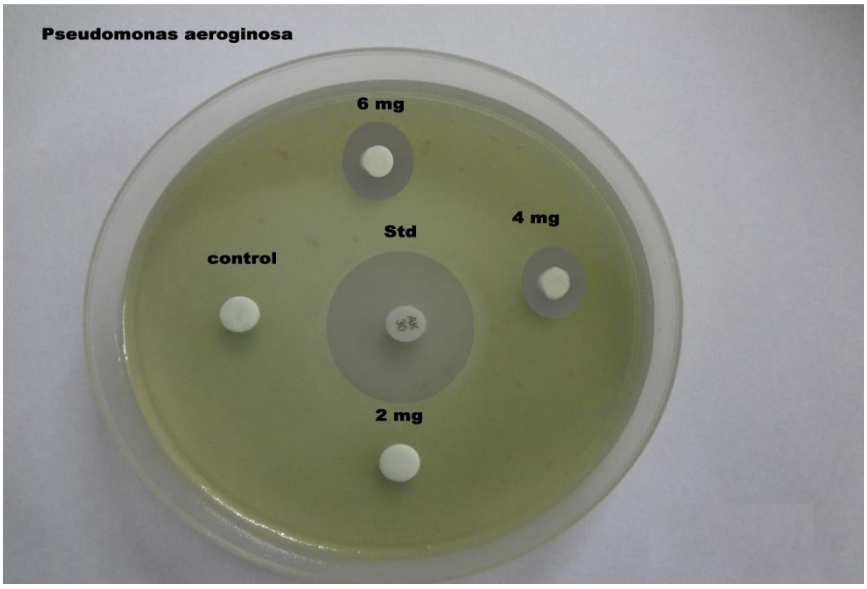
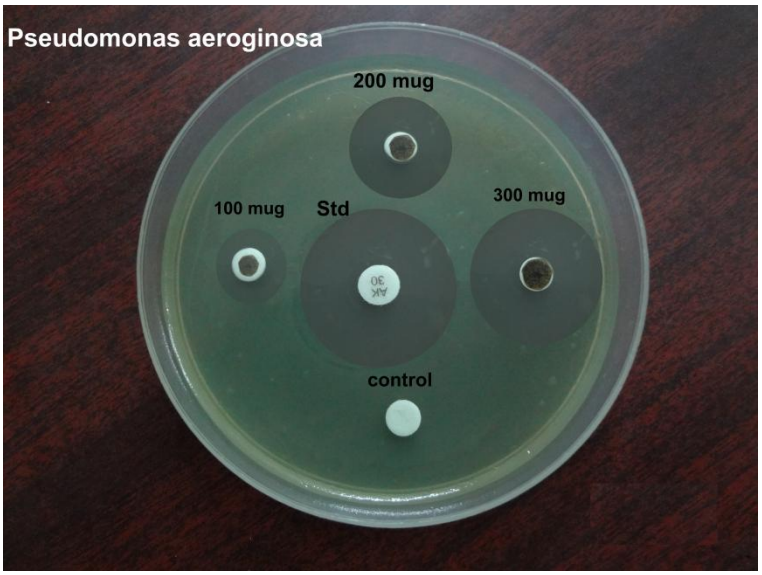


FIG: 36 Anti-Bacterial Activity of CIP against Pseudomonas aeroginosa



CHAPTER - IX

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